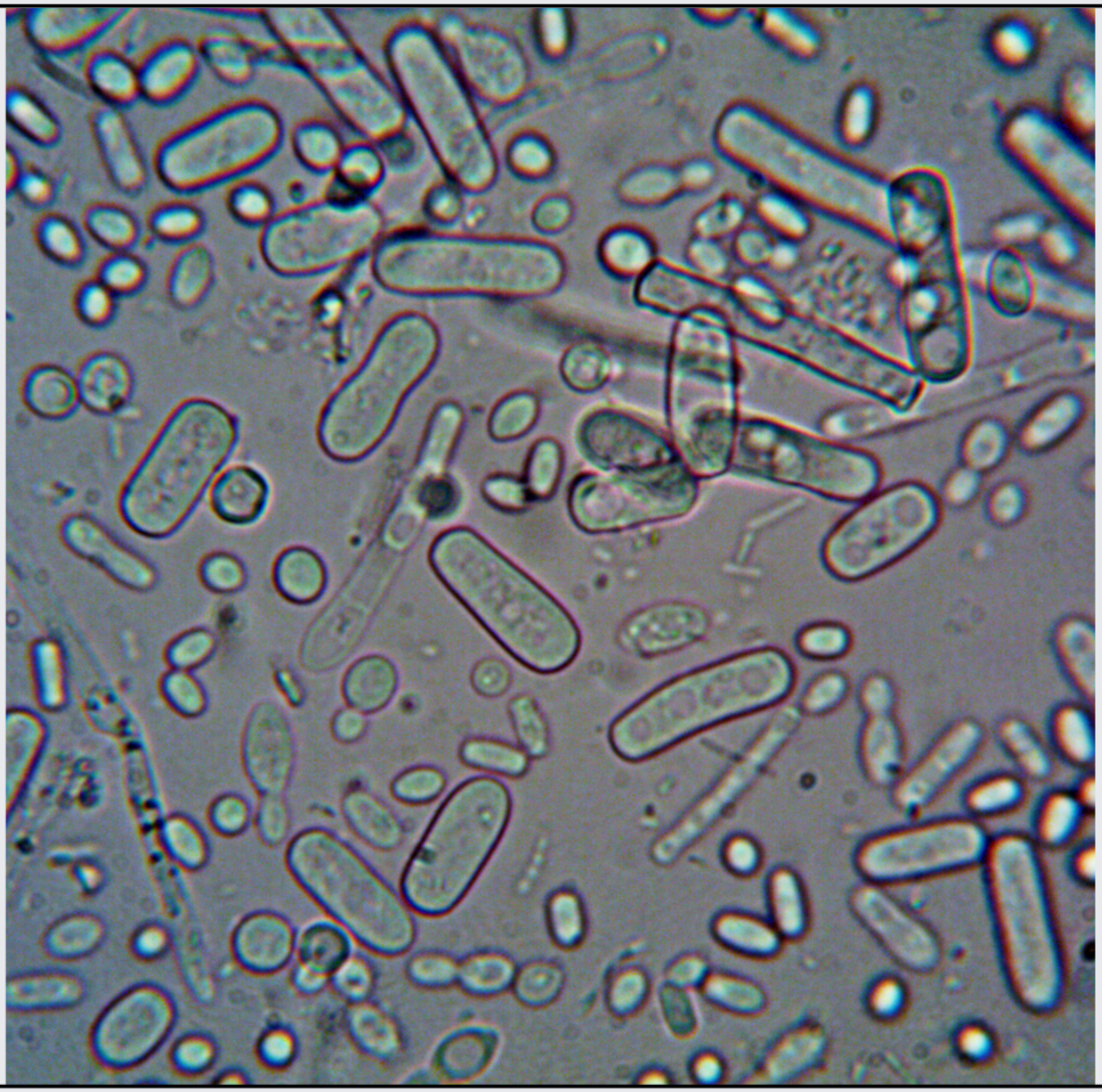
Teens in Health

Teens in Health 2022-2023 Journal



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Teens in Health is a teen led organization that aims to provide open access to biological research skill development through researching and writing articles. This session, students spent 8-10 weeks working on individual articles, ranging from immunotherapy, nanobots, and health policy. Articles vary from Literary Reviews, Experimental Proposal Research Articles, to Analytical Research Articles.

Director/Reviewer: Anika Shah
Mentor/Reviewer: Michelle To

Authors:
Aditi Venkiteswaran
Aden Lee
Alan Wang
Alex Choma Lifshitz
America Leon
Anika Shah
Badri Viswanathan
Charlotte Lungren
Christina Thomas
Dhruthi Halambi
Disha Divakar
Jibraan Saeed
Kaden Chan
Kashish Vinayak
Karen Lin
Katie Liang
Laasya Munjeti
Luana Veras
Mae-Lin Pinkstaff
Mei Peters
Michelle To
Mithra Senthil
Natalie Goldberg
Neta Gal
Peyton Higa
Saivishwateja Papaiahgari
Samridhhi Pakhrin
Subhi Karki
Thanisha Kapur
Yesh Rao

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Review Article

ADHD Brain VS. Neurotypical Brain

Aditi Venkiteswaran (author), Anika Shah (advisor), Michelle To (advisor)
Cupertino High School

Keywords: ADHD, brain size, executive function, dopamine, chemical imbalances, FPN, DMN, hyperactivity, hypoactivity

Abstract

This literary review will discuss the defining differences in the brains of people with ADHD (attention-deficit/hyperactivity disorder), a neurodevelopmental disorder, as compared to people without ADHD. While the exact cause of ADHD has not yet been determined, many believe that it is due to differences in brain size and development, chemical imbalances in the brain's central nervous system, and the impairment of certain neural networks. In a person with ADHD, certain parts of the brain are typically smaller in size and in volume, the individual often experiences lowered dopamine levels overall, and impaired neural networks can cause hyperactivity (abnormally high levels of activity) in certain regions of the brain and hypoactivity (abnormally low levels of activity) in others. This can cause impairments in the brain's structure and its function, as well as make it harder for messages to be effectively communicated throughout the brain. This directly contributes to the severity of various symptoms of ADHD, some of which include difficulty paying attention and staying focused, not being able to exhibit executive function skills (time management, planning, remembering information, etc.), and being restless, among others.

Introduction

Attention-deficit/hyperactivity disorder, commonly known as ADHD, is a neurodevelopmental disorder that affects over three million people annually in the US. It falls under the neurodivergent spectrum. Signs of the disorder typically begin to show during childhood, some of which include (but are not limited to) having trouble focusing, being forgetful, acting impulsively, and often feeling restless. While the exact cause of ADHD is not yet clear, many

believe that it is caused by abnormalities in the individual's brain structure and the way that it functions as a result. The individual's brain will take longer to fully develop, certain regions of the brain are often smaller than they typically would be, neurotransmitter levels are often much lower in the brain, and neural connections are often impaired, all of which contribute to ADHD. But how do these factors really influence the symptoms and challenges of having ADHD?

Discussion:

Brain size and volume

One of the most noticeable differences in the brains of people with ADHD actually relates to the size- the brains of people with ADHD often take a much longer time to develop as compared to the neurotypical child. This was proved in a study that took place in 2015, where MRI scans taken of individuals with ADHD showed that the volume of the amygdala, caudate, hippocampus, putamen, and intracranial volume were much lesser as compared to the brains of people without ADHD (Hoogman et al., 2017). People with ADHD often have lower overall brain volume, and certain parts of the brain often take a much longer time to develop, as compared to the development rate of a person without ADHD. The most notable structure that takes a long time to develop is the frontal lobe. The frontal lobe of a person with ADHD is typically smaller at birth and during childhood, which is a huge factor in what can cause ADHD. The frontal lobe controls things such as the ability to be organized, focusing for long periods of time, being able to make decisions, meeting deadlines, and having good memory, to name a few, all of which are weaker areas in a person with ADHD. Additionally, the cerebellum, hippocampus, and amygdala in the brain of a person with ADHD are smaller in volume, which also largely contributes to ADHD. The cerebellum is responsible for controlling movement, and because of impairment, can cause difficulty with something called a "motor response inhibition". The motor response inhibition is the ability to suppress actions that could interfere with an ongoing task (Wilkins and Nikolaidis, "How Is the ADHD Brain Different?"). Since the motor response inhibition doesn't work as it should, people with ADHD have very little control over if they can suppress their movements, and it often interferes with their ability to pay attention or follow rules. Additionally, the hippocampus controls storage of memories and learning, while the amygdala controls emotional self-control and prioritization. So as a result of having a smaller

hippocampus and amygdala size, there are difficulties in remembering and memorizing information, regulating emotional responses, and having control over behavior.

Chemical imbalances

Another abnormality in the brains of people with ADHD lies in chemical imbalances. Neurotransmitters are chemical messengers that send messages between neurons in the brain, and play a large role in helping it function effectively. One particularly important neurotransmitter is known as dopamine, which relays important information. Dopamine is associated with feelings of pleasure and executive functions, some of which include planning, time management, decision-making, and organization. While there are multiple pathways for dopamine in the brain, two of those pathways in particular are responsible for cognitive impairment in the brains of people with ADHD. The first pathway, the "dopamine reward pathway", "rewards" the individual with feelings of euphoria when experiencing something pleasurable, for instance, feeling happy when you eat your favorite food. The second pathway, the "mesocortical pathway", connects the basal ganglia to the prefrontal cortex (both of which are associated with executive function skills) of the brain. With the transmission of dopamine, this pathway helps the prefrontal cortex carry out executive functions (Wilkins and Nikolaidis, "How Is the ADHD Brain Different?"). In the brains of people with ADHD, these two pathways are considered to be disrupted, which affects cognitive and motivational functioning by reducing their effectiveness. Furthermore, because dopamine is directly associated with executive function, it's harder for the individual to get day-to-day tasks completed. This impairment is thought to be caused by an abnormal amount of dopamine transporters in the brains of people with ADHD. A dopamine transporter is a protein that stops dopamine transmission, and a high amount of dopamine transporters can cause a low amount of dopamine in the brain. Furthermore, in a research study analyzing the dopamine pathway in the brains of people with ADHD, "positron emission tomography" was used to measure the amount of dopamine receptors and markers in 53 with ADHD and 44 without; the results found that people with ADHD experienced a "reduction in dopamine synaptic markers associated with symptoms of inattention was shown in the dopamine reward pathway of participants with ADHD." (Volkow et al., 2009). This is why Adderall is typically considered the best medication for people with ADHD; as it can help to regulate the dopamine levels in the individual's brain.

Neural networks

Another fascinating thing that contributes to ADHD is impairment in certain neural networks. One of these neural networks is the fronto-parietal network, also known as the FPN. The FPN is responsible for the ability to learn how to do new tasks and making decisions. Another one of these networks is the default mode network, which is also known as the DMN. The DMN is largely responsible for making sure the brain is rested properly (Rawe, ADHD and the Brain). According to what scientists have determined to be the DMN's typical function, the network is supposed to show lower levels of activity while taking part in an activity that requires focus and attention, while showing higher levels of activity whilst daydreaming or recalling a memory. However, in the brain of a person with ADHD, the DMN doesn't function in the way that is considered to be typical of the network. Instead, the network tends to continue to show higher levels of activity while taking part in an activity that requires focus and attention. This indicates that multiple parts of the brain are active at once, which contributes to the problems people with ADHD have with paying attention (Wilkins and Nikolaidis, "How Is the ADHD Brain Different?"). Another common issue in brain activity relates to a phenomenon called hypoactivity. As previously covered, hyperactivity causes problems with paying attention because of abnormal levels of brain activity. However, hypoactivity is when parts of the brain are abnormally inactive, which can stop the brain from being able to perform executive functions and get tasks done. This occurrence was further proved in a research study conducted by taking fMRI scans of individuals with ADHD in their resting state. The results of the study showed that "ADHD was significantly associated with DMN hypo-connectivity specifically in the core subsystem," which increased the likelihood of the individual's mind to start wandering, as well as give into making impulsive choices (Broulidakis et al., 2022). This imbalance can also interfere with brain processing, which is crucial to send sensory messages throughout the body.

Conclusion

Together, the differences in brain size and development, the chemical imbalances in the brain, and the impairment of certain neural networks all contribute to ADHD by creating anomalies in an individual's brain structure and function so messages cannot be sent throughout the brain and body as they typically would be able to. This causes issues in how different parts of the brain are able to function, which directly leads back to what is arguably the biggest issue in the brains of people with ADHD- not being able to perform executive functions. This creates issues with

focusing and paying attention, planning, managing time well, controlling behavioral responses, and communicating in day-to-day activities, among other symptoms. This is crucial for people to understand and learn about as it can break the stigma that exists around ADHD and neurodiversity in general, as well as possibly improve treatments for people with ADHD in the future as we continue to learn more about how the brain works and how we can potentially reduce the severity of the symptoms of ADHD.

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Literary Review

Sickle Cell Anemia Treatment

Aden Lee (author), Anika Shah, Michelle To

Key Words: Blood Cells, Sickle Cells, Genomics, Treatments

Introduction:

Sickle cell anemia is a genetic disorder that affects the production of hemoglobin, the protein in red blood cells that carries oxygen to the body's tissues. It is characterized by the formation of abnormal, sickle-shaped red blood cells that can get stuck in small blood vessels, leading to a range of complications such as anemia, pain crises, organ damage and even early death. Sickle cell anemia is a significant public health problem, with millions of people affected worldwide. The disorder is particularly prevalent in African and African-American populations, but also affects people of Mediterranean, Middle Eastern, and Indian descent. Despite its high prevalence, the underlying mechanisms of the disorder are not fully understood, and effective treatments are still lacking. A number of studies have been conducted in recent years to better understand the pathology of sickle cell anemia and to develop new treatment options. This literary review will aim to go more in detail for the possible treatments, and treatments that the medical field is looking into to educate more on the topic of sickle cell anemia.

Hydroxyurea

Hydroxyurea is a chemotherapy drug that is also used as a treatment for sickle cell anemia. It works by increasing the amount of fetal hemoglobin in red blood cells, which helps to prevent sickling and reduce the frequency of painful episodes. In addition to its ability to reduce sickle cell crises, hydroxyurea has also been shown to have other beneficial effects on patients with sickle cell anemia. According to Platt et al., hydroxyurea can also improve the lifespan of red

blood cells and reduce the risk of organ damage. Another study by Charache et al. found that hydroxyurea can reduce hospitalizations and the need for blood transfusions in patients with sickle cell anemia. Hydroxyurea is typically taken orally in the form of a capsule. The dosage and duration of treatment may vary depending on the severity of the patient's symptoms and their response to the medication. While hydroxyurea is generally well-tolerated, some patients may experience side effects such as nausea, vomiting, or hair loss due to the decrease in bone marrow from the medicine. In addition to its use in treating sickle cell anemia, hydroxyurea is also used to treat other medical conditions, including certain types of cancer and psoriasis. Like any medication, hydroxyurea can interact with other drugs and should only be taken under the supervision of a healthcare professional.

L-Glutamine

L-glutamine is an amino acid that is found naturally in the body and is essential for the production of proteins. It is also involved in many metabolic processes and plays an important role in the immune system. In addition to treating sickle cell anemia, L-glutamine has been used to treat a variety of other medical conditions, including chemotherapy-induced neuropathy, inflammatory bowel disease, and HIV/AIDS. The exact mechanism by which L-glutamine increases the production of fetal hemoglobin is not fully understood, but it is believed to be related to its ability to reduce oxidative stress in red blood cells. Fetal hemoglobin is a type of hemoglobin that is normally only produced during fetal development, but can be reactivated in some people with sickle cell anemia to help protect against the harmful effects of sickle-shaped red blood cells. According to Niihara et al L-glutamine was well-tolerated by patients and had a low rate of side effects. Leading many to believe that L-glutamine can be an ample treatment for sickle cell anemia at reducing the pain and uncomfortableness.

Iron Blood Treatment

To prevent iron overload, patients receiving regular blood transfusions may require iron-chelating therapy to remove excess iron from the body. In addition, blood transfusions can increase the risk of infections, such as hepatitis B, hepatitis C, and HIV. Therefore, it is essential to screen blood donors and perform rigorous testing to minimize the risk of transmitting infections through transfusions. Transfusions may be used as a short-term treatment for sickle cell crises, but they are not a long-term solution. Blood transfusions can be used to treat sickle cell anemia by replacing the patient's damaged red blood cells with healthy ones. According to Adams-Graves et al. regular blood transfusions can reduce the risk of stroke and other complications in patients with sickle cell anemia.In addition to the risks of transfusion, frequent transfusions can lead to the development of antibodies that make it difficult to find compatible blood for future transfusions.

Lifestyle Changes

In addition to medical treatments, patients with sickle cell anemia can also benefit from lifestyle changes. Maintaining a healthy diet, staying hydrated, avoiding extreme temperatures, and getting regular exercise can all help to reduce the frequency and severity of sickle cell crises. It is also important for patients with sickle cell anemia to receive regular medical care and monitoring. This includes regular blood tests to check for complications, such as organ damage or infections, and to adjust treatment as needed. Patients with sickle cell anemia may benefit from working with a multidisciplinary healthcare team that includes hematologists, pain specialists, and other healthcare providers.

Bone Marrow Transplants

The bone marrow transplant process involves replacing the patient's bone marrow with healthy bone marrow from a donor. According to Kobayashi et al., bone marrow transplants can be effective in curing sickle cell anemia in some patients, but the procedure carries a high risk of complications. The donor may be a family member or an unrelated donor from a bone marrow registry. Prior to the transplant, the patient undergoes a conditioning regimen to prepare their body for the transplant. This involves high-dose chemotherapy and sometimes radiation to destroy the patient's existing bone marrow and make space for the new cells. After the transplant, the patient requires close monitoring to ensure that the new bone marrow is functioning properly and to manage any complications that may arise. Complications can include graft-versus-host disease, where the donor's immune cells attack the patient's body, infections, and organ damage. Bone marrow transplants are typically reserved for patients with severe sickle cell anemia who have not responded to other treatments. While the procedure can cure sickle cell anemia, it is not without risks. The success of the transplant depends on many factors, including the patient's age, overall health, and the compatibility of the donor. Bone marrow transplants are not widely available and can be expensive. In addition, the procedure can be physically and emotionally demanding for patients and their families. Patients may require long-term medical care and monitoring to ensure that the transplant was successful and to manage any complications that may arise.

Success and Failure in the past and future

While there have been some successes in treating sickle cell anemia, there is still much to be done. One example of a treatment that has not been successful is the use of erythropoietin, a hormone that stimulates the production of red blood cells. A study by Kastambas et al. found that erythropoietin was not effective in treating sickle cell anemia. The need for medication and

treatment is absolutely needed now, as the treatments for sickle cell anemia are generally ineffective at completely removing it unless you undergo serious operations.

In recent years, significant progress has been made in the development of new treatments for sickle cell anemia. While gene therapy has shown promising results in early clinical trials, there are other potential treatments that researchers are exploring. For instance, stem cell transplantation is another promising treatment option for sickle cell anemia. This involves replacing the patient's own bone marrow with healthy bone marrow from a donor. In a study by Kuna and Field (2018) (source), stem cell transplantation was found to be effective in treating sickle cell anemia in children. Other researchers are also exploring the use of small molecule drugs to treat sickle cell anemia. These drugs work by increasing fetal hemoglobin levels, which can help prevent sickle cell crises. In a study by Zhou et al. (2014) (source), a small molecule drug called GBT440 was found to be effective in increasing fetal hemoglobin levels in patients with sickle cell anemia. Overall, while gene therapy and CRISPR-Cas9 gene editing are promising treatment options for sickle cell anemia, there are other potential treatments that are also showing promise in clinical and preclinical studies.

Conclusion

In conclusion, the many different treatments for sickle cell anemia all aim to somehow alleviate or treat the pain or uncomfortableness from it. Although it seems as if there are not many realistic treatments, new treatments in gene therapy have shown that there is potential towards treating the ominous sickle cell disease.

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Research Article

Applications of *Vaccinia* Viral Vector in Preventive Medicine against Kaposi Sarcoma Herpesvirus

Alan Wang (author), Anika Shah (advisor), Michelle To (advisor)
University of California, Berkeley

Abstract

Viral vector vaccines involve the applications of using a weaker lab-grown virus to train the human immune system to create an immune response against a specific antigen of another virus. This in turn allows the immune system to defend against similar pathogens that carry the identical antigen so long as the immune system has a memory of infection. Kaposi sarcoma herpesvirus (KSHV), otherwise known as human herpesvirus type 8 (HHV-8), is a gamma herpesvirus capable of causing tumorous growths and skin lesions, which can lead to life-threatening conditions if the cancer metastasizes further into critical organs such as the lungs or gastrointestinal tract, leading to potentially death (American Cancer Society). Although there are no direct cures to Kaposi sarcoma, preventing initial infection is essential in order to combat viral-related tumors. Employing the usage of one of the most well-known viral vectors, *vaccinia*, is a potential method that could provide critical information on how to effectively initiate a strong immune response, as well as give insight into using new methods of immunization that can prevent viral-related cancers from spreading, while also increasing the safety of immunocompromised patients.

Keywords: DNA, herpesvirus, Kaposi sarcoma, tumor, vaccinia, viral vector, vaccine design

Introduction

Kaposi Sarcoma Herpesvirus

KSHV is a species of gamma herpesvirus, sharing the same category with Epstein-Barr virus (abbreviated as EBV or known as HHV-4). Along with EBV, KSHV holds a great concern for its oncogenetic potential, which can develop into Kaposi sarcoma (KS), characterized by cancerous purple lesions found around the skin. Other cancers, such as primary effusion lymphoma, can also develop as a result of KSHV infections. Patients that have low immune responses, such as transplant receivers or patients with HIV/AIDS, have increased susceptibility to KSHV, allowing the virus to proliferate with little immune response and subsequently increasing the chances of KS developing, which can be fatal and result in death. Unfortunately, development of KSHV vaccines are few and narrow due to the inability for the immune system to provide a strong response and the danger of using a live attenuated vaccine in humans, requiring further research and more preclinical testing before a safe method is developed.

The method of KSHV leading to cancer is unfortunately not well known enough, but is thought to be linked with its ability to tamper with the host cell's own tumor suppressor pathways. KSHV has stolen many human genes that encode for valuable proteins, such as G protein-coupled receptors and Flice inhibitory proteins (otherwise known as FLIP), along with other DNA synthesis proteins that could provide it abilities to manufacture essential viral proteins. KSHV has the ability to become latent after finding entry into a host cell's nucleus using essential integration proteins, allowing it to stay silent and evade immune detection by turning off key signaling pathways. It is still being investigated as to what fully triggers lytic reactivation in the body, but it is suspected that KSHV can be reactivated when a certain pH is met, or certain chemokines are released (Casper, et al., 2022). This leads to patients that had prior infection to KSHV without any symptoms to be "reinfected" by a secondary activation, which can become dangerous as reactivation could occur in patients that are experiencing a coinfection with another virus, such as SARS CoV-2 or worse, HIV, both which involve a change of various factors in the body due to inflammation.

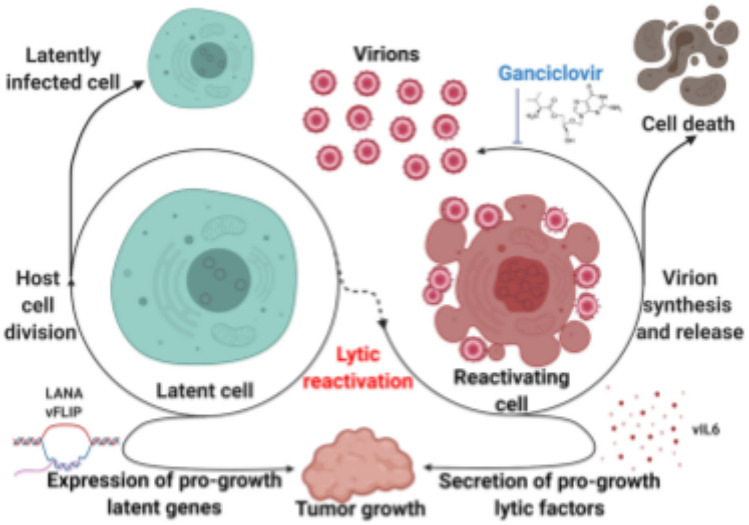

Figure 1. KSHV latency and reactivation within a host cell. LANA and viral FLIP proteins remain active during latency and will not be reactivated until an external stressor causes lytic reactivation. Additionally, gene expression can alter proto-oncogenes and tumor suppressor proteins which in turn promote tumor growth.

(Image source: Broussard, Grant and Blossom Damania, 2020)

KSHV possesses several proteins that scientists have considered to be potential target antigens for vaccine development. Unfortunately, cell-mediated responses towards KSHV are poor due to KSHV possessing many immune evasion mechanisms, making it hard to counteract after evidence of infection. Thus, preventive medicine is optimal compared to post-infection treatments, allowing the immune system to specialize in creating antibodies against KSHV. Glycoproteins of the KSHV are the most efficient targets, with notable proteins including gB, gH, gL, gM, and gN. These proteins are critical for KSHV to enter its host cell, making it a prime target for vaccine development. Due to its broad tropism, KSHV is able to infect a variety of cells including mucosal epithelial cells and lymphocytes, which can serve to be targets

for obtaining antigens for presentation to trigger adaptive immunity. Current research has observed that gB and the gH/gL complex could be potential targets to prevent KSHV infections, as it plays an integral role along with other glycoproteins to bind and target multiple variations of cells (Casper, et al., 2022). Thus, it is highly encouraged to continue investigating the potential of glycoproteins gB, gH, and gL to prevent initial KSHV infections.

Viral Vector Vaccines and **vaccinia**

Viral vector vaccines are a method of preventive medicine that aims to immunize the patient to prevent severe symptoms post-infection. Viral vectors achieve this goal by employing the use of a weakened vector equipped with the target antigen like a spike protein of a specific virus, and injecting it into a recipient to allow the immune system to produce a strong response and antibodies.

Many viruses can serve to be a vector, including adenoviruses (known for causing cold or flu-like symptoms) and lentiviruses (retroviruses similar to HIV) which have their own different applications with vaccine development. However, the highlighted vector of this article is *vaccinia*, a species of poxvirus that has been used in gene therapy when smallpox became widely spread amongst populations, and despite smallpox's eradication, *vaccinia* is still being widely used for gene therapeutics and other applications.

Vaccinia is classified as a linear, double-stranded DNA poxvirus that shares high similarities with its cowpox counterpart. Possessing a genome with about 190 kilobases in length, its high capacity allows experimentation for gene insertion of foreign materials (precisely, up to about 25 kilobases). Furthermore, *Vaccinia* has shown promise in triggering a strong adaptive immune response against an intended target antigen (Ura, Takehiro et al., 2014). Combined with the double-stranded DNA nature of KSHV, *Vaccinia* is a strong candidate for viral vector applications and deserves a closer look.

The current debate comes to whether or not a viral vector vaccine will have enough success to initiate a strong immune response. Unfortunately, even live vaccines are unable to yield enough antibody responses due to the aforementioned immune evasion mechanisms and poor detection by the human immune system. Furthermore, increased vaccination potency can result in stronger infections that may provide a higher chance of latency especially in immunodeficient individuals. However, attenuating the virus may reduce the likelihood for host cell entry, at the cost of weakened immune response with moderate or heavy adjuvant assistance, leading to lack of viral antigen presentation. Viral vectors are able to resolve this critical issue by removing entry pathway capabilities while also inducing a strong enough immune response when paired with little adjuvants that stimulate inflammation for dendritic cells to provide antigen presentation for adaptive immune response. In addition, the *vaccinia* viral genome can be heavily edited to lose all capabilities of viral entry, only to provide an alert for the immune system. Therefore,

viral vectors should be taken into consideration for its ability to modify existing viruses to perform critical dendritic cell antigen presentation.

Materials and Methods

The outline for the viral vector vaccination is to isolate a specific antigen of KSHV and recreate artificial protein samples, procure antigen samples for transfer into the target *Vaccinia*, and test the vaccine for efficacy within mice and later humans. All procedures and experimentations are purely hypothetical, and all data and results explain the ideal conditions for each step.

Clinical trials with mice will resort to Murine gammaherpesvirus-68 (abbreviated as MHV68), a mouse-related gammaherpesvirus that shares a similar genome with human gammaherpesviruses (Conrad et al., 2019). As such, vaccinations given to mice will have their own mutated glycoproteins to target, but the concept stays identical to the processes described later.

Isolating KSHV antigen

The key goal of this process is to isolate gB, gH, and/or gL glycoproteins from the KSHV, derived from a small culture of KSHV raised from human epithelial cells or B-lymphocytes. To obtain the desired antigen, the host cell must be lysed through a homogenization process. Two to three freeze-thaw procedures are most optimal, but can risk critical protein damage (Gagné, 2014). Isolation of glycoproteins from the rest of the cellular debris involves several suspension processes to obtain viable pellets, while discarding supernatants. Further chemicals and wash solutions are implemented to completely isolate the desired glycoproteins. Finally, to ensure protein yield is sufficient for testing, a gel electrophoresis is performed. Gel results should display bands at around 2 to 5 kilobases to ensure that glycoproteins are isolated (Schied, A, and P W Choppin, 1973. pg. 264). If the gel electrophoresis procedure yields a result that is not sufficient, repeat the procedure until desired results are obtained. Isolated glycoproteins serve as free antigens that can boost dendritic cell antigen presentation as a backup mechanism in case any direct detection of the viral vector fails. Free antigen presentation, however, should not be prioritized as the chance for proper antigen identification is small.

Vaccinia viral vector creation

Plasmid DNA engineering of gB, gH, and/or gL (located on the viral genome as ORF8, ORF22, and ORF46 genes respectively) of *vaccinia* can be tedious due to its recombinant potential, but is possible through a specialized promoter region that is integrated into the viral genome to trigger activation once it detects its presence in a patient's bloodstream (preferably by pH detection). Both sides of the plasmid must be flanked by homologous dsDNA sequences to the parent viral genome in order to ensure

integration is accepted at a specific site. Target promoter regions including the vaccinia virus synthetic early/late promoter region (vvSynE/L) work exceptionally well due to its ability to promote antigen production consistently throughout infection, guaranteeing abundances in antigens for detection at both early and late phases. Additionally, a helper poxvirus can pass down recombination proteins that are necessary for further replication, including fowlpox virus or Shope fibroma virus that have been enhanced to only be permissive towards *vaccinia*, it is possible to grant *vaccinia* important viral machinery to replicate efficiently without causing direct immune response towards the helper poxvirus. Thus, with the combination of proper plasmid transfers of the target genome, integration of the direct promoter region following the genome, and an assisting poxvirus, a potent viral vector that induces a strong immune response without possessing any immune evasion mechanisms is therefore created (Conrad et al., 2019)

vvSynE/L sequence:
AAAAATTGAAATTTTATTTTTTTTTTTTTGGAATATAAATA

Three letter sequence:
> vvSynE/L 13 aminoacids; Mw=1603.91Da
LysAsn***AsnPheIlePhePhePheTrpAsnIleAsn***

Three letter sequence:
> ORF46 255 aminoacids; Mw=29013.93Da
MetAspAlaTrpLeuGlnGlnThrValPheArgGlyThrLeuSerIleSerGlnGlyVal
AspAspArgAspLeuLeuLeuAlaProLysTrpIleSerPheLeuSerLeuSerSerPhe
LeuLysGlnLysLeuLeuSerLeuLeuArgGlnIleArgGluLeuArgLeuThrThrThr
ValTyrProProGlnAspLysLeuMetTrpTrpSerHisCysCysAspProGluAspIle
LysValValIleLeuGlyGlnAspProTyrHisLysGlyGlnAlaThrGlyLeuAlaPhe
SerValAspProGlnCysGlnValProProSerLeuArgSerIlePheArgGluLeuGlu
AlaSerValProAsnPheSerThrProSerHisGlyCysLeuAspSerTrpAlaArgGln
GlyValLeuLeuLeuAsnThrValLeuThrValGluLysGlyArgAlaGlySerHisGlu
GlyLeuGlyTrpAspTrpPheThrSerPheIleIleSerSerIleSerSerLysLeuGlu
HisCysValPheLeuLeuTrpGlyArgLysAlaIleAspArgThrProLeuIleAsnAla
GlnLysHisLeuValLeuThrAlaGlnHisProSerProLeuAlaSerLeuGlyGlyArg
HisSerArgTrpProArgPheGlnGlyCysAsnHisPheAsnLeuAlaAsnAspTyrLeu
ThrArgHisArgArgGluThrValAspTrpGlyLeuLeuGluGln***

Figure 2. vvSynE/L sequence (in both base pair form above and amino acid below) and partial protein sample ORF46 from KSHV type M. vvSynE/L sequence is placed upstream before ORF46. *Vaccinia* can accept up to 25 kilobases (kb) of foreign material, which translates to roughly 925 kiloDaltons (kDa) in protein size.

vvSynE/L sequence from Conrad, Steven J, and Jia Liu. ORF46 sequence from NCBI archive. Translation software from bioline.com and bioinformatics.org

A culture of these genetically modified poxviruses is raised within a non-human environment. Using poxvirus-specific viral production cells (VPCs), a culture of the modified poxvirus is created with enough viral loads to produce viable vaccines. However, due to the virus's ability to mutate even despite modifications, a purification process is necessary to ensure that the harvested culture is the pure sample derived after genetic modification. Furthermore, if the vaccine fails to create a strong immune response, conventional adjuvants may be required. The most effective and safe adjuvant available is the aluminum salts, which contains various aluminum compounds including aluminum hydroxide that induce mild inflammatory reactions but heighten immune response (CDC, 2022). Adjuvants should only be used in the case that the recipient of the vaccine has already induced a viral response towards *vaccinia* as a result

of a prior infection, such as from smallpox vaccines. However, as most viral vectors have enough potency to induce a strong immune response, the necessity for adjuvants is not critical.

Clinical Trials

As stated before, preclinical trials involving lab mice are to be performed with MHV68, a mouse-specific gamma herpesvirus that resembles human variants. However, when moving towards the human clinical trial phase, the ideal sample will require the *vaccinia* vector specific towards KSHV.

The key detection mechanism requires identifying antibody levels circulating within both the mouse and human's bloodstream. Most notably, identifying mouse IgG antibodies, monomer structure antibodies that are most often found in the blood and other tissues, is critical in order to detect primary infection success. The IgG antibody has to be specific towards KSHV gB, gH, or gL spike glycoproteins, and detection methods involve seeing antibody attachment towards isolated viral proteins procured while isolating KSHV antigens (Beatty, 2023). All mice and humans should not have prior or current infections to MHV68 or KSHV respectively, which would otherwise tamper with antibody detection.

The subject receives an initial 100 μg dosage of the viral vector vaccine with 0.5% of solution containing the viral vector and 0.2% adjuvant (if desired). A placebo shot is also administered to a separate group that contains only plasma, and should only be done within mice and not humans due to safety concerns. After a 14 day gestation period for proper antibody response, measure IgG antibody levels within the subject's blood. The general success rate should result in around 70-90% antibody concentration within an initial dose. In human clinical trials, the average whole blood volume (WBV) for adults should be 70 mL/kg, and children between 7 years old and 18 years old should be 85 mL/kg (Irbmed, 2022). Mice trials follow the same procedure for adults. A booster dosage containing 0.5% of the viral vector solution should be administered within a week after the measurement. The general antibody level count should be around 90-95% of total antibody concentration within the blood draw. Subjects that have conditions that lower blood volume should avoid participating within the trials or should consider adjusting blood draw values to ensure no adverse symptoms occur.

Phase I trials target 19-25 year old healthy individuals without prior exposure or current infection to KSHV, other herpesviruses, human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), diabetes, or any other life-threatening conditions that may affect the subject's health and clinical trial results. Additionally, recent transplant receivers should not participate in clinical trials. The expected duration of phase I trials should last around 4 months of observing antibody levels.

Phase II trials expand the audience to include 26 to 40 year old healthy individuals that also do not have the conditions listed above. The expected duration of phase II trials should last around 2 months.

Phase III trials are split into three parts. The first part expands to now include 65 year old healthy individuals also without the conditions listed in phase I. If the procedure is safe, the trials move onto part 2, where subjects can now be as young as 16 years old, with a small section of people who are immunocompromised excluding HIV/AIDS positive individuals. Finally, if part 2 is safe, part 3 now includes HIV/AIDS positive individuals and other elderly or immunocompromised individuals. With an average of 4 months per trial, the total expected time is 12 months.

Once all procedural safety reports and results are shown, phase IV includes consistent monitoring of vaccine development and observations to resolve any outstanding issues.

Hypothetical Results

The focus of the hypothetical results detail mostly at the clinical phases. All procedures prior to clinical trials are detailed individually within Materials and Methods.

The target goal for an effective vaccine is as displayed below in a sample preclinical trial involving a small population of mice. An additional note: Protein markers that bind to MHV68-specific antigen binding sites of a Fab region assist in detection of antibody quantity. The ideal efficacy should be at 90% at a minimum, with an ideal of 100%. Margins of error should be limited to no more than 1%.

> A sample of 10 mice who have never been previously exposed to MHV68 are subjected to a preclinical trial. 5 are selected at random to receive the viral vector treatment, while 5 receive a placebo shot that contains a harmless plasma solution. Antibody levels are measured within 2 weeks after injection, then a small dosage of MHV68 is injected into the mice intravenously. The mice are monitored daily for 2 weeks to see the immune response from the mice.
>
> Within a 2 week span, antibody levels are measured inside the mice through blood samples. 4 out of the 5 mice who have received the viral vector treatment produced a high quantity of IgG antibodies, with a moderate amount of IgM antibodies. The last mice produced a quantitative amount that consisted of approximately 60% of the total antibodies within the blood sample. None of the other mice that have received the placebo shot have created the ideal amount of MHV68-antigen specific antibodies.
>
> After exposure to a small amount of MHV68, mice that had been injected with the viral vector treatment have shown signs of high antibody response. Other mice that have received the placebo treatment eventually developed a high amount of antibodies, but succumbed to relative nonspecific symptoms.
>
> The nature of human clinical trials are expected to mimic this pattern but at a larger scale.

Discussion

Comparing vaccinia *to alternate vectors*

Vaccinia has been implemented in smallpox vaccines and has played an integral role in eliminating the smallpox disease worldwide. This good understanding of *vaccinia* makes it a perfect viral vector for future vaccinations. However, much like other viruses, *vaccinia* hosts a wide molecular assortment of detectable antigens, known as pathogen-associated molecular patterns or PAMPs. Most notably, viral DNA that is recognized as a PAMP can potentially cause antibodies to target *vaccinia* poxvirus over the spike proteins expressed on it. Furthermore, if an individual has already developed antibodies against smallpox vaccines that use *vaccinia*, memory B lymphocytes could already possess the equipment needed to eliminate the poxvirus and therefore reduce the chances of proper antigen presentation. Fortunately, adjuvants could attract innate immune cells like dendritic cells to phagocytize and obtain gB, gH, or gL epitopes for proper presentation. Furthermore, additional free KSHV antigens that are incorporated into the solution will potentially trigger macrophage or dendritic cell response to perform antigen presentation to naïve $CD4^+$ T lymphocytes.

Other viral vectors, including retroviruses, lentiviruses, or adenoviruses, offer variability over *vaccinia* and other poxviruses. However, retroviruses and lentiviruses are dangerous as both viruses have the ability to become oncogenic, defeating the purpose of preventing oncogenic capabilities. Adenoviruses can be compatible, but would require additional steps to modify the DNA genome to RNA, and would already possess pre-existing immunity as the virus is known for causing the common cold. Compared to *vaccinia*, its pre-existing immunity is only moderate, with variants that could bypass pre-existing immunities through mutations of viral DNA.

Using KSHV viral vector vaccines as post-exposure therapy

The concept of using a KSHV vaccine after primary infection sounds feasible on paper, but is not practical due to a variety of reasons. Post-exposure vaccine therapy is commonly used in cases of rabies, where a patient receives a vaccine to prevent a rabies infection from spreading into a patient's neurons. The immune system has major difficulties entering into the nervous system, as immune cells like neutrophils or natural killer (NK) cells possess granules and other enzymes that can cause neural damage. However, there is a caveat for rabies virus; unlike other lytic and fast replicating viruses, rabies has a slow lag phase that prevents it from being spread rapidly like other viruses. This allows post-exposure therapy within 72 hours of initial infection to activate, producing enough antibodies to effectively control the spread of rabies. In cases like KSHV, where the virus has the potential to become latent and hide within the nucleus of its host cell, post-exposure therapy may be too late for antibody production to effectively eliminate a virus before it enters latency. Stressors that can cause reactivation, similar to how varicella zoster virus (VZV) can initially cause chickenpox but later cause shingles after decades since primary

infection, can cause KSHV to reactivate itself when antibody memory is insufficient to defend against any resurfacing infections, greatly increasing the chance for oncogenesis (Beatty, 2023). Thus, KSHV as post-exposure therapy requires immediate treatment within a small window of time, when a patient may not know prior to infection due to asymptomatic symptoms, and is better off given as a preventive vaccine.

Disadvantages of vaccinia viral vectors

While the promise of using a *vaccinia* virus vector sounds nearly perfect, there are drawbacks to using *vaccinia* viral vectors. As previously mentioned, pre-existing *vaccinia* immunity can cause irregular immune responses and only kill *vaccinia* before dendritic cells can obtain KSHV-specific antigens. Furthermore, isolated spike proteins could be eliminated by macrophages and other phagocytosing immune cells, decreasing the chance for proper antigen presentation. In comparison to using live attenuated vaccines, viral vectors are much more sophisticated and costly to produce sufficient and effective yields for vaccine production, and perhaps could induce an immune response that is much weaker than an attenuated virus.

One of the biggest concerns regarding viral vector vaccines is the ability for the virus to suddenly replicate itself or mutate to become potent once more. If certain mechanisms that are turned off in the genetic sequence are reactivated or not removed, *vaccinia* has the potential to cause disease and, with it possessing glycoproteins that are KSHV-specific, expand its tropism to infect cells that would otherwise be impossible to infect. If it becomes able to replicate without proper control, an initiated immune response that can cause inflammation increases the chance of an individual developing nonspecific symptoms in most cases, like fevers, headaches, myalgia, or arthralgia. But, in the event that the patient's immune system is compromised or overreacts to such a response, a patient can experience a *vaccinia*-associated disease such as generalized vaccinia, where lesions of the skin appear at the site of injection (Mandia et al., 2017). Fortunately, the chances of a viral vector repossessing viral capabilities is extremely low, as various purification processes are performed to ensure that unnecessary mutations are removed.

Conclusion

In conclusion, a viral vector vaccine using the *vaccinia* poxvirus contains the potential to help limit the spread of KSHV, and also directly eliminate KSHV-associated cancers, including various lymphomas and most notably Kaposi Sarcoma. A detailed viral vector vaccine process includes a target isolation procedure with a sample spike glycoprotein that influences antigen presentation, a plasmid DNA transfer process that involves a vvSynE/L promoter region followed by a partial glycoprotein genome infused into a *vaccinia* virus, and several rounds of clinical trials to test for efficacy and safety. Predominantly, KSHV

is widely spread in less developed countries where sanitation conditions are poor and health practices are mismanaged, and granting vaccinations to third world countries like ones found in Africa can help limit the transmission of KSHV. Additionally, vaccines to prevent KSHV can provide herd immunity to vulnerable populations that are immunocompromised, including individuals with HIV/AIDS or transplant recipients that require immunosuppressants. While there are obvious benefits and drawbacks to the viral vector concept, modifications and new biomedical innovations can potentially improve the KSHV vaccine dramatically, and hopefully will help eliminate a deadly oncogenic virus from infecting individuals.

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Research Article

Mental Health and Exercise In Teenagers

Author: Alex Choma Lifshitz **Advisors:** Anika Shah, Michelle To, James Lucas

Keywords: Mental Health, Exercise, Adolescents, Depression, Anxiety, Stress, Sports, Psychology, High School

Abstract

This experiment is an analysis of how exercise affects mental health in teenagers ages 16-18 by using the PHQ-9, Beck Depression Inventory, and Beck Anxiety inventory. The experiment concluded that although more analysis should be done in larger numbers, teenagers who scored between 4 and 10 when combining their exercise intensity with frequency were significantly less anxious and depressed than those who scored in other ranges and that scores lower than one experienced higher anxiety and depression.

Investigation Question

To what extent does frequency and intensity of exercise affect one's mental health as seen by the amount of mental illness criteria met in the PHQ-9 questionnaire, Beck Depression Inventory, and Beck Anxiety Inventory in California high school students ages 16 through 18?

Introduction

Depression is defined as a common mental disorder that manifests as a lack of pleasure, depressed mood, loss of interest, decreased energy, and other recurring symptoms that disturb one's daily functions (World Health Organization et al.). In addition, depression has for decades remained the leading cause of disability across the world (World Health Organization et al.). Anxiety refers to various mental disorders as it is a broad classification of disorders that surround a constant state of worry or fear that disturbs one's way of life for an extended time (Oxford Medicine).

Depression and anxiety have become significant issues in adolescents, especially those ages 14-18 (FastStats - Depression). With this, it has become an issue desperately needing attention in current American Society. Suicide rates have risen by 51% due to the onset of stress from the COVID-19 pandemic which has furthered these mental obstacles, gravely increasing the population of those suffering from mental illness (FastStats - Depression).

Exercise has been heavily recommended to youth in society especially as of late with heavy campaigns by celebrities, the last first woman - Michelle Obama, pediatricians, and more due to the development of technology limiting physical activity in children. Although these campaigns have been pushing this for obesity, physical, and mental health it is important to study if there is a significant reason to push exercise onto youth for maintenance of their mental health and

whether the use of exercise should be applied in other ways for those experiencing mental health difficulties.

The Beck Depression Inventory (BDI) is an intake form used throughout the international mental health industry due to the high content validity of the assessment (Kendall). The BDI self-reporting questionnaire is consistent in properly assessing subjects and is very similar to other well-known depression diagnostic tools (Kendall).

This assessment cannot be used as a sole diagnostic tool which is why medical professionals meet with clients several times following the intake evaluation to confirm a diagnosis (Kendall). It is said that with BDI it is important to refer to subjects with high scores as "dysphoric" rather than depressed due to the limited nature of a self-reported assessment (Kendall). Another downfall of this test is the overlap it has with evaluation tests made for those suffering from anxiety. Questions on tests used to self-report anxiety or test for anxiety at intake nearly correspond/overlap as much to the BDI as the BDI does with other depression self-diagnostic tools (Kendall).

Because of the BDI's shortcomings, a second depression test will be conducted on participants to ensure more accurate results. The Personal Health Questionnaire-9 or PHQ-9 is currently considered a "golden test" for depression (Kroenke). The PHQ-9 has the validity and reliability of the BDI but adds the value of specificity that comes from using this diagnostic tool that is made mainly for screening for depression (Kroenke).

The anxiety test to be used for the experiment is the Beck Anxiety Inventory (BAI). Research has concluded that although existing measures of anxiety and depression are strongly correlated and have similar symptom patterns that may overlap, they are distinguishable. This test was made to attempt to help with this differentiation. The BAI has larger correlations of anxiety self-diagnostic tools rather than those for depression (Hewitt).

As this experiment is testing both depression and anxiety, this will not hinder the results as the anxiety assessment results. PHQ-9 test results used in co-evaluation with the BDI can be used concurrently as recommended in assessing mental illness as it allows comparison of covariation. Additionally, these tests will NOT be used as a diagnostic tool, only an assessment of the participant's mental state.

Aim

This study aims to find the level of correlation, if any, between mental health and exercise, thereby finding the ideal hours of exercise for the "best" mental health. Like most, I have seen many teenagers struggle with their mental health, especially during and after the COVID-19 pandemic. I speculate that a lack of physical activity has caused part of the increase in mental health disorders during COVID-19. I chose to conduct this experiment to combat the abundance

of adolescent mental health disorders. By doing this experiment, I hope to quantify the effect of exercise on teenagers' mental health, allowing for this research to be applied to benefit adolescents in the future.

Variables

Independent Variable - Score is given by multiplying the intensity of the exercise by their hours

a. $x<1$
b. $1<x<2$
c. $2<x<4$
d. $4<x<10$
e. $x>10$

Dependent Variable - Level of mental health criteria met (points)

- BDI
- BAI
- PHQ-9

Control Variables-

- All participants are currently attending high schools in California which ensures a similar environment
- Surveys are only sent to those ages 16-18 for participants to be of similar ages, therefore similarly cognitively developed
- The survey uses tests standardized by mental health professionals that are used in the psychology field to diagnose mental illnesses
- The survey was sent digitally in the form of a Google Form so that it is consistent and all answers are compiled for examination

Confounding Variables-

Other factors in the subjects' lives other than exercise are likely to influence the mental health of participants meaning that exercise is not a sole factor in this and results could be altered by strained mental health in those with outside factors such as a parents' divorce, death of a loved one, etc. Furthermore, many students who are involved in sports, while getting a lot of exercise, may be experiencing more pressure in sports as well as more stress from the time commitment high school sports tend to have. Some subjects may be on antidepressants or anti-anxiety medicine which can also impact the results of this experiment. Last, there is room for human error in manually measuring one's heart rate and the risk for exaggeration is also a risk as heart rates were self-reported by all subjects.

Hypothesis

Those who exercise more are likely to have fewer mental illness criteria met as exercise releases endorphins in the brain that help to lower stress and manage depression, therefore, meaning they would exhibit fewer symptoms of anxiety and depression and would score lower on the BDI, BAI, and PHQ-9 than those who do not exercise as much or as intensely.

Null Hypothesis

There is no significant difference in different frequencies and levels of intensity of exercise and the mental health criteria met by the participant.

Materials

- Consent form
- PHQ-9 questionnaire
- Beck depression inventory
- Beck anxiety inventory

* View Appendix A to view these questionnaires, along with additional questions from the survey.

Methods

The survey was compiled by combining 3 reliable depression and anxiety inventory tests that are considered accurate, reliable, and specific enough to be used professionally by psychologists to diagnose mental disorders. The experiment was advertised by contacting people in high schools in California via email or text. Participants who agreed to participate were sent a preliminary consent form that, for those under 18, were required to be signed by a guardian. This consent form informed participants of the nature of the experiment.

Participants were asked to complete the survey in a Google Form. Questions assessed different symptoms of depression and anxiety and asked how often the participants experienced these and to what extent they affected them. These questions were taken directly from the Beck Depression Inventory, Beck Anxiety Inventory, and PHQ-9 questionnaire which are all tests used by professionals to diagnose mental illness. DISCLAIMER: This survey was NOT used to diagnose mental illness, only to see the correlation between exercise intensity and the amount of mental illness criterion met. (Example criterion: Select which statement is most accurate in describing your thoughts, feelings, and/or actions in the last 2 weeks - I do not feel I am worthless(0), I don't consider myself as worthwhile as I used to be (1), I feel worthless compared to other people (2), OR I feel utterly worthless (3)).

PHQ-9 participant responses should be scored individually with answers of "not at all" receiving 0 points, "several days" receiving 1 point, "more than half the days" receiving 2 points, and

“almost every day” receiving 3 points. BDI participant responses should be scored individually with answers of no severity receiving 0 points, and adding a point as severity increases (Example: I do not feel worthless (0), I don't consider myself as worthwhile and useful as I used to be (1), I feel more worthless as compared to other people (2), I feel utterly worthless (3)). BAI participant responses should be scored individually. In the BAI, participants are asked to mark how often and severely they are affected by certain symptoms closely relating to anxiety. Answers of “not at all” received 0 points, “mild/not bothersome” received 1 point, “moderate- unpleasant at times” received 2 points, and “severely- bothers me a lot” received 3 points (For example: how often in the last 1 month have you been bothered by difficulty breathing unrelated to exercise?)

Once responses are collected via Google Forms the conductor of the experiment must view them individually per participant and add scores. Once the scores of each participant have been calculated, use the rules provided in the original tests at the top in order to classify participants which are also detailed below. BDI: total score of 0–13 is considered minimal depression, 14–19 is mild, 20–28 is moderate, and 29–63 is severe. BAI: total score of 0–7 is considered minimal, 8–15 is mild, 16–25 is moderate, and 26–63 is severe. PHQ-9: total score of 0-4 is considered minimal, 5-9 is mild, 10-14 is moderate, 15-19 is moderately severe, and a score of over 20 is classified as severe depression. A series of calculations must be completed in order to calculate a score for the subject that analyzes their exercise frequency and intensity. This involves using the Karvonen method and substituting the subject’s age and active heart rate (given in the google form) to calculate the intensity of exercise. Then multiply this number by their average hours of exercise per week to calculate this number to calculate their official score.

Safety and ethics

- Results were conducted fully anonymously by the distribution of ID codes given by a separate party to maintain the anonymity of subjects and still allow the ability to contact said subjects
- The survey did not request participants to add or subtract any exercise from their typical routine meaning no stress on the body, physical injury, or health concerns would be created by this experiment
- The consent form informed subjects of the nature of the experiment and asked that they declare that they fully understand the experiment and were willing to participate by signing

Note: COVID-19 precautions were unnecessary as the survey was digital as was most communication with subjects. Any communication with a participant in person followed all county, state, and federal COVID-19 protocols (masks, social distancing, etc.).

Data

Table 1. California high school students scores on the BDI, BAI, and PHQ-9 as well as exercise habits including frequency of exercise, resting heart rate, and training heart rate

Participent key code	BDI	BAI	PHQ-9	Hours of exercise/week	Resting HR (BPM)**	Training HR (BPM)**
0001	19	23	11	15	51	143
3144	23	37	17	1	80	116
4325	22	35	18	11	78	109
9733	2	0	3	5	66	111
0000	12	28	8	3	68	178

*A complete data table can be found in Appendix B

**As this experiment was conducted fully virtually there is no qualitative data to report.
***There is unmeasured uncertainty to the heart rates listed above due to stopwatch uncertainty, calculation uncertainty, and human error. Since it is all self-reported it is not possible to calculate the uncertainty of this or understand the impact of it in this experiment.

Processed Data

Equation 1. Intensity of exercise via Karoven method

Training Intensity = Training HR - resting HR/(220 - age - resting HR)

143-51/(220-17-51) = 0.61

Calculation 1. Intensity of exercise for subject 0001

116-80/(220-18-80) = 0.29

Calculation 2. Intensity of exercise for subject 3144

109-78/(220-17-78) = 0.25

Calculation 3. Intensity of exercise for subject 4325

*Generally the Karvonen method is used to calculate target active heart rate zones with intensity chosen by the subject. Since in this case, we are looking to find the intensity given their active heart rate we must rework the equation to isolate training intensity thereby getting the equation used above. For reference the original Karvonen method formula is as follows: Target Heart Rate= [(max HR − resting HR) × %Intensity] + resting HR. (Max HR can be substituted by 220 minus age which is what is done in the calculations above.)

Equation 2. Intensity of exercise times hours of exercise per week
Exercise intensity*(hours of exercise/week)

0.61*15= 9.08

Calculation 4. Intensity of exercise*hours of exercise/week for subject 0001

0.30*1= 0.3

Calculation 5. Intensity of exercise*hours of exercise/week for subject 3144

0.25*11= 0.73

Calculation 6. Intensity of exercise*hours of exercise/week for subject 4325

*For a table with interpreted scores see Appendix C

Table 2. r and r^2 values for the BDI, BAI, and PHQ-9

Questionnaire	r value	r^2
BDI	-0.315	0.099
BAI	-0.260	0.068
PHQ-9	-0.465	0.216

Both the r and r^2 values here are low for all tests meaning that the data collected does not indicate a strong correlation between hours of exercise and number of mental health criteria met on each of these tests.

Table 3. California high school students scores on BDI, BAI, and PHQ-9 for different treatments of frequency and intensity ranging from 0>1, 1>2, 2>4, 4>10, and >10

Intensity*hours of exercise range	Subjects' intensity*hours	BDI Score	BAI Score	PHQ-9 Score
<1	0.00	24	7	17
	0.30	23	37	17
	0.54	31	8	13
	0.47	16	12	11
	0.91	9	5	10

*Complete table in Appendix D
**Scores from intensity of exercise multiplied by hours of exercise are grouped into ranges to establish treatments of the independent variable. This is necessary to complete concise and comparable data analysis.

Equation 3. Mean Intake test scores for different ranges of intensity and hours of exercise

$$mean = \frac{sum\ of\ trials}{total\ number\ of\ trials}$$

$$mean\ BDI\ score\ =\ \frac{24+23+31+16+9}{5} = 21$$

Calculation 7. Mean BDI Intake test scores for range of <1

$$mean\ BAI\ score\ =\ \frac{7+37+8+12+5}{5} = 14$$

Calculation 8. Mean BAI Intake test scores for range of <1

$$mean\ PHQ\ -\ 9\ score\ =\ \frac{17+17+13+11+10}{5} = 14$$

Calculation 9. Mean PHQ-9 Intake test scores for range of <1

$$95\%\ confidence\ interval\ =\ \frac{2*3.29}{\sqrt{5}} = 2.94$$

Calculation 12. 95% CI of mean PHQ-9 Intake test scores for range of <1

Table 4. Mean BDI, BAI, and PHQ-9 scores for high school students in California for different ranges of scores calculated from their hours and intensity of exercise

Intensity *hours of exercise	BDI			BAI			PHQ-9		
	Mean score	Standard deviation	95% Confidence Interval (CI)	Mean Score	Standard deviation	95% CI	Mean Score	Standard deviation	95% CI
<1	21	8.38	7.50	14	13.22	11.82	14	3.29	2.94
1<x<2	11	13.82	13.82	21	19.55	19.55	6	5.23	5.23
2<x<4	10	8.11	8.16	10	14.26	12.75	8	5.48	5.48
4<x<10	12	2.89	2.89	17	7.85	7.85	9	5.48	5.48
>10	9	9.19	13.00	6	1.41	2.00	5	2.83	4.00

Data Analysis

Figure 1. Scores on Beck Depression Inventory and Beck Anxiety Inventory in relation to intensity and hours of exercise completed by California high students

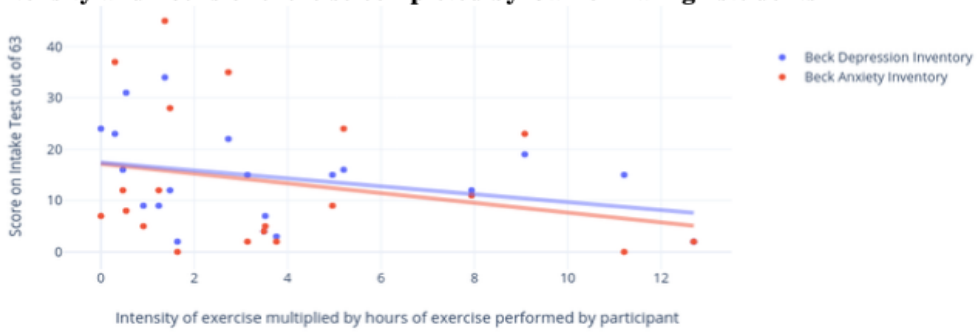


Figure 2. Score on PHQ-9 in relation to intensity and hours of exercise completed by California high school students

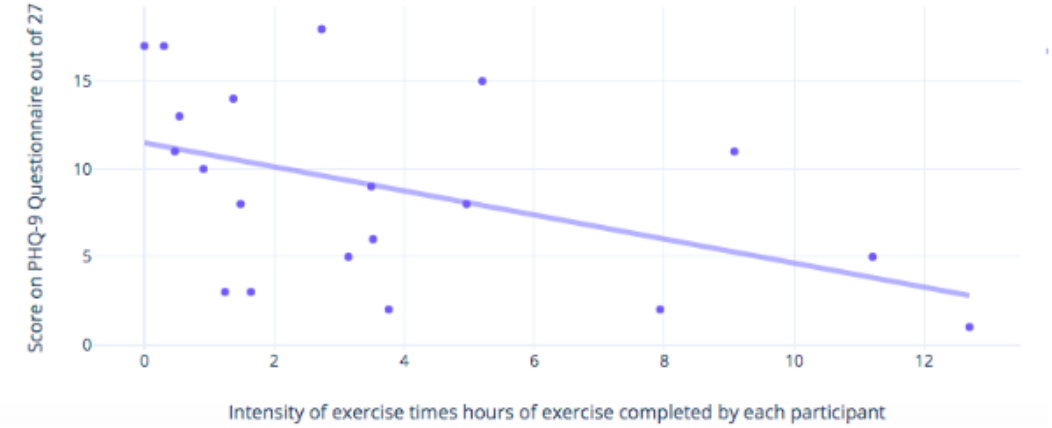
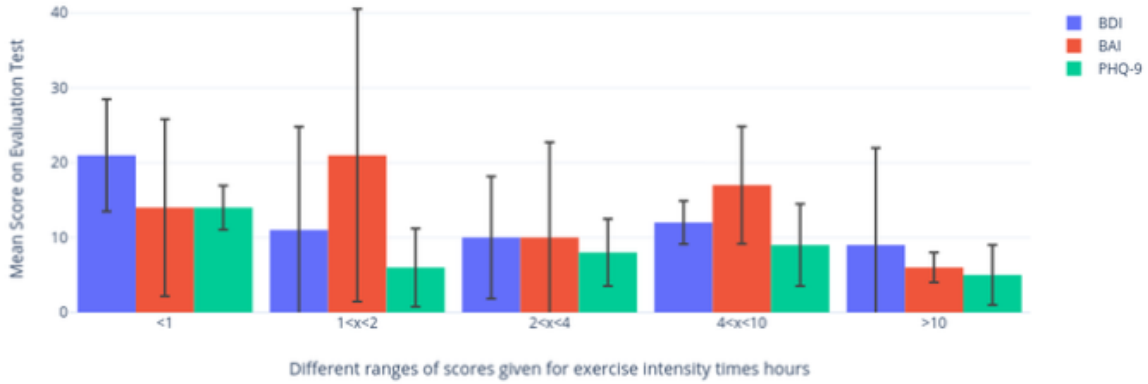


Figure 3. Mean score on BDI, BAI, and PHQ-9, in relation to intensity and frequency of exercise (by range) completed by California high school students with 95% confidence intervals



Statistical Tests

ANOVA statistical tests were run for each evaluative test (BAI, BDI, and PHQ-9) to determine whether the visual difference between the mean scores on the test was different at different ranges of exercise intensity and frequency was significant.

Table 5. BDI ANOVA Test

Hypotheses	H_0: There is no significant difference between the hours and intensity of exercise completed by a participant and their BDI score. H_1: There is a significant difference between the hours and intensity of exercise completed by a participant and their BDI score.
Alpha	0.05
F value	1.12
$F_{critical}$	3.34

F is less than F critical value at 95% significance, therefore the null hypothesis is not rejected.

This means there is no significant difference between the hours and intensity of exercise completed by a participant and their BDI score. Any differences between these are not due to chance or sampling error.

All 95% confidence interval bars did overlap; it is expected that the data is not sufficient in claiming a correlation between frequency and intensity of exercise to one's BDI score. This was confirmed by the ANOVA test. Further research must be done to verify or disprove the hypothesis. This also indicates that there is no need for a T-test.

* Statistical tests for the BAI and PHQ-9 can be found in Appendix E
** Findings showed that the ANOVA test for each had no significant difference. T-Tests found a significant difference in BAI scores between $4<x<10$ and $x>10$. Additionally a significant difference between a score of <1 to $1<x<2$ and <1 to >10 was determined.

Conclusion

This investigation is inconclusive. The ANOVA tests conducted concluded that all three evaluative tests had no significant differences between the scores people received on the test versus their scores given based on their frequency and intensity of exercise. The proceeding T-Tests performed on both the BAI and PHQ-9 tests showed several significant differences between ranges of frequency and intensity of exercise and the scores on these tests. These tests concluded that anxiety levels were significantly higher in those who scored 4 through 9 points versus those who scored 10 or more points. Additionally, the PHQ-9 T-Tests performed showed that those who scored 10+ points on their exercise habits and those who scored between 1 and 2 points scored significantly lower on the PHQ-9 than those who scored under 1 point. From this, we are able to conclude that exercise does have a significant effect on one's mental health in specific ranges. From the PHQ-9 test, it became clear that no exercise or a score between 0 and 1 caused participants to have very high scores meaning that at least some exercise is essential for maintaining stable/good mental health. Although the null hypothesis was unable to be rejected these conclusions still indicate that there is a correlation between exercise and mental health; because of this, I do suggest further testing that limits sources of error in the experiment.

Not only should a similar experiment be conducted due to the dispute between the conclusions of the ANOVA and T-Tests but the science behind this investigation seems to indicate that a retest would provide more concrete evidence that would allow the rejection of the null hypothesis and allow for the determination of a lab-based conclusion. By looking at the science behind exercise it has been seen to increase serotonin, dopamine, and other endorphin and neurotransmitters in the brain that improve mood. Therefore this investigation should be performed again taking into account the sources of error and limitations of this experiment in order to find the true effect of exercise frequency and intensity on one's mental health.

Table 6. Sources of Error and Limitations of Data

Source of Error		Explanation	Recommendation
Self report-ing of data	Hours of exercise	Participants were asked to self report their average weekly hours of exercise in the last month. However, many people - especially those who play a sport - are likely reporting the time allotted for exercise and not isolating the time they spent being physically active.	In order to prevent error due to self reporting of this information it would be largely beneficial to use a Fitbit or similar device that is able to track the participants exercise, resting heart rate, and active heart rate. This would also nearly eliminate uncertainty of the data.
	Heart rate	Human error is likely to occur when measuring one's heart rate. Although instructions were provided, stopwatch malfunction, timing issues, difficulty finding pulse, and miscounting can skew the experiment results	
Sample size		Although 20 subjects seemed like a substantial sample size, when distributing these participants into treatments based on their intensity and frequency of exercise, it became clear that a larger sample size was necessary in order to conduct a reliable experiment.	An increased sample size would support more reliable and accurate findings.
Environment controls		Outside factors such as parents' divorce, a loved one's death, new health diagnosis of themself or a loved one, etc are not accounted for. With all outside factors being uncertain it is difficult to deduce whether the conclusions of the investigation are accurate as these factors may largely contribute to the state of one's mental health which could make exercise far less effective in supporting them.	In order to account for the possible outside factors that could contribute to changing the effectiveness of exercise on one's mental health, a baseline requirement should be set for all participants. This should include background information on their health, mental health, and environmental factors that could influence the outcome of the investigation.
		This experiment chose to focus on teenagers, studying those ages 16 through 18. This could have an impact on the results as this is an age in which puberty related hormones influence the majority of emotions	Due to this it would be recommended to use a different age group such as 25 through 30 year olds as their hormone levels are more stabilized and can allow for more accurate evaluation of the

Ages	experienced by individuals. Because of this, the experiment becomes less reliable in concluding the effect of exercise on mental health since these factors were unable to be accounted for and could largely influence the effectiveness or ineffectiveness of exercise in supporting their mental health.	effects of exercise on mental health.
Max heart rate calculation	Using the equation of 220 minus the participants age is only an approximation of one's max heart rate and is not 100% accurate. Because this is not a professional experiment there are limited tools for the investigation and equipment to test for max heart rate is not accessible.	Max heart rate could be tested through use of advanced lab machinery which would make the calculation for intensity of exercise more accurate. This could be done through a VO_2 max test.

Additional suggestions for improvement:

- Create the survey through a different source than Google Forms. Although Google Forms is free, efficient, and highly useful, a lot of time and energy can be saved by finding a website capable of calculating subject scores and providing them in a table rather than individually calculating the scores for each participant's various evaluations.

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Appendix A

Beck Anxiety Inventory

Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by circling the number in the corresponding space in the column next to each symptom.

	Not At All 0	Mildly but it didn't bother me much. 1	Moderately - it wasn't pleasant at times 2	Severely – it bothered me a lot 3
Numbness or tingling	○	○	○	○
Feeling hot	○	○	○	○
Wobbliness in legs	○	○	○	○
Unable to relax	○	○	○	○
Fear of worst happening	○	○	○	○
Dizzy or lightheaded	○	○	○	○
Heart pounding/racing	○	○	○	○
Unsteady	○	○	○	○
Terrified or afraid	○	○	○	○
Nervous	○	○	○	○
Feeling of choking	○	○	○	○
Hands trembling	○	○	○	○
Shaky / unsteady	○	○	○	○
Fear of losing control	○	○	○	○
Difficulty in breathing	○	○	○	○
Fear of dying	○	○	○	○
Scared	○	○	○	○
Indigestion	○	○	○	○
Faint / lightheaded	○	○	○	○
Face flushed	○	○	○	○
Hot/cold sweats	○	○	○	○
Column Sum				

Scoring - Sum each column. Then sum the column totals to achieve a grand score.

Write grand score here _____ .

Clear Save / Print to PDF

Roche

Beck Depression Inventory — **Baseline**

V 0477 CRTN: _____ CRF number: _____ Page 14 patient Inits: _____

The BDI-II contains 21 questions, each answer being scored on a scale value of 0 to 3. The cutoffs used differ from the original: 0–13: minimal depression; 14–19: mild depression; 20–28: moderate depression; and 29–63: severe depression. Higher total scores indicate more severe depressive symptoms.

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
- 0 I do not feel sad.
- 1 I feel sad much of the time.
- 2 I am sad all the time.
- 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
- 0 I am not discouraged about my future.
- 1 I feel more discouraged about my future than I used to be.
- 2 I do not expect things to work out for me.
- 3 I feel my future is hopeless and will only get worse.

3. Past Failure
- 0 I do not feel like a failure.
- 1 I have failed more than I should have.
- 2 As I look back, I see a lot of failures.
- 3 I feel I am a total failure as a person.

4. Loss of Pleasure
- 0 I get as much pleasure as I ever did from the things I enjoy.
- 1 I don't enjoy things as much as I used to.
- 2 I get very little pleasure from the things I used to enjoy.
- 3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
- 0 I don't feel particularly guilty.
- 1 I feel guilty over many things I have done or should have done.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6. Punishment Feelings
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7. Self-Dislike
- 0 I feel the same about myself as ever.
- 1 I have lost confidence in myself.
- 2 I am disappointed in myself.
- 3 I dislike myself.

8. Self-Criticalness
- 0 I don't criticize or blame myself more than usual.
- 1 I am more critical of myself than I used to be.
- 2 I criticize myself for all of my faults.
- 3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10. Crying
- 0 I don't cry anymore than I used to.
- 1 I cry more than I used to.
- 2 I cry over every little thing.
- 3 I feel like crying, but I can't.

Roche

Inventory

V 0477 CRTN: _____ CRF number: _____ Page 15 patient inits: _____

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

0 1 2 3 4 5 6 7 8 9 10 11 12 A B C D E

Subtotal Page 2

Subtotal Page 1

Total Score

NR15645

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use "✔" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or of hurting yourself in some way	0	1	2	3

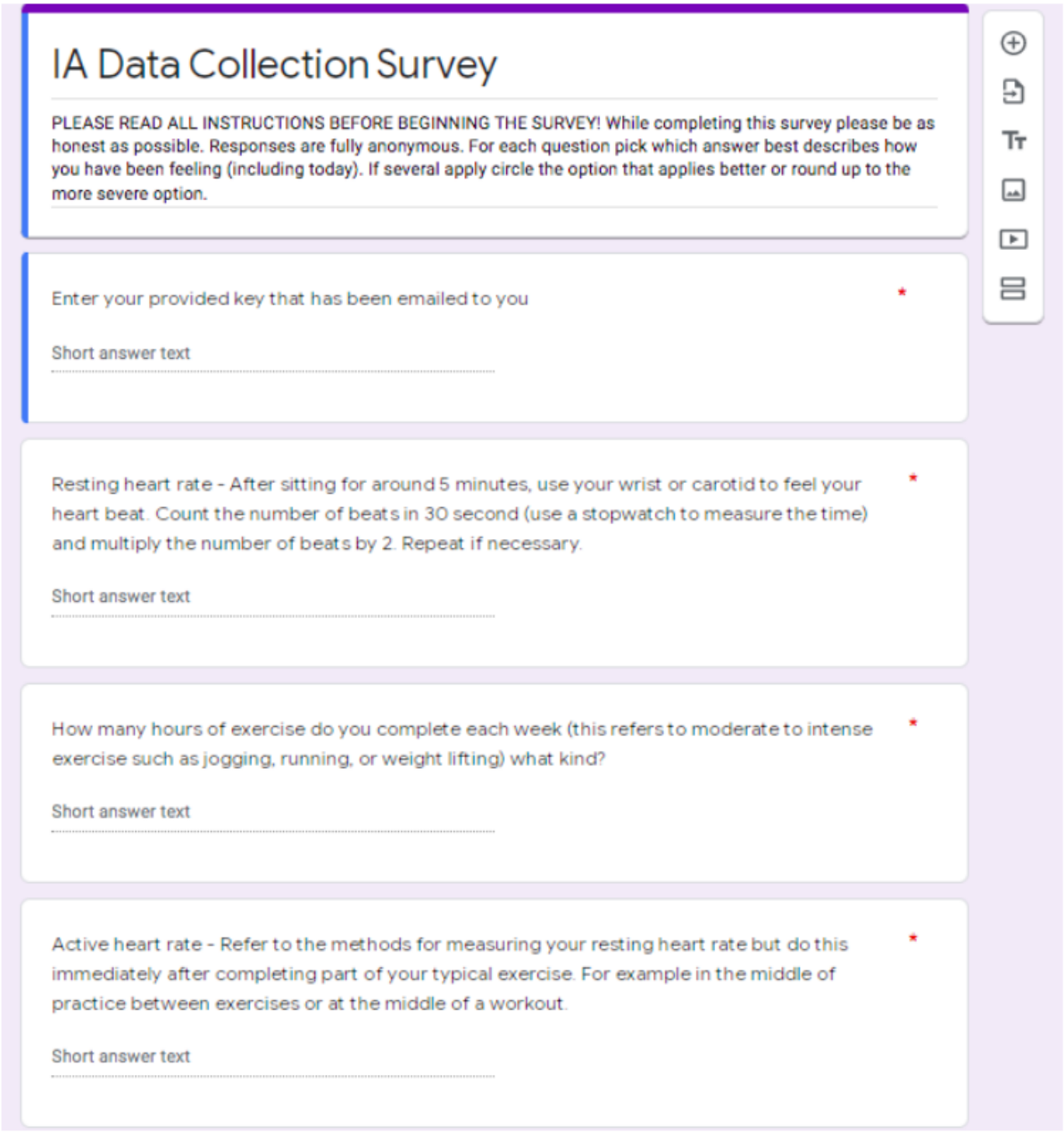
FOR OFFICE CODING 0 + _____ + _____ + _____

=Total Score: _____

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	Somewhat difficult	Very difficult	Extremely difficult
☐	☐	☐	☐

Additional questions in survey-



IA Data Collection Survey

PLEASE READ ALL INSTRUCTIONS BEFORE BEGINNING THE SURVEY! While completing this survey please be as honest as possible. Responses are fully anonymous. For each question pick which answer best describes how you have been feeling (including today). If several apply circle the option that applies better or round up to the more severe option.

Enter your provided key that has been emailed to you *

Short answer text

Resting heart rate - After sitting for around 5 minutes, use your wrist or carotid to feel your heart beat. Count the number of beats in 30 second (use a stopwatch to measure the time) and multiply the number of beats by 2. Repeat if necessary. *

Short answer text

How many hours of exercise do you complete each week (this refers to moderate to intense exercise such as jogging, running, or weight lifting) what kind? *

Short answer text

Active heart rate - Refer to the methods for measuring your resting heart rate but do this immediately after completing part of your typical exercise. For example in the middle of practice between exercises or at the middle of a workout. *

Short answer text

Appendix B

Table 7. California high school students scores on the BDI, BAI, and PHQ-9 as well as exercise habits including frequency of exercise, resting heart rate, and training heart rate

Participent key code	BDI	BAI	PHQ-9	Hours of exercise/week	Resting HR (BPM)**	Training HR (BPM)**
0001	19	23	11	15	51	143
3144	23	37	17	1	80	116
4325	22	35	18	11	78	109
9733	2	0	3	5	66	111
0000	12	28	8	3	68	178
8299	16	24	15	13	68	122
6587	34	45	14	2	65	160
9725	31	8	13	4	76	93
5436	12	11	2	15	68	140
7512	4	4	9	4.5	56	170
6042	3	2	2	10	70	120
1427	24	7	17	0	72	n/a
7216	2	2	1	18	57	160
3517	9	12	3	3	64	122
4561	15	9	8	15	64	110
4678	15	2	5	12	98	126
6174	15	0	5	18	54	148
5043	7	5	6	8	62	124
6314	16	12	11	3	90	108
25043	9	5	10	7	64	82

Appendix C

Table 8. Classification of high school students in California's score for each evaluative test paired with their score calculated from their frequency and intensity of exercise

subject	BDI	BAI	PHQ-9	Intensity of exercise times hours/week
0001	Moderate	Moderate	Moderate	9.08
3144	Moderate	Severe	Moderately severe	0.30
4325	Moderate	Severe	Moderately severe	2.73
9733	Minimal	Minimal	Minimal	1.64
0000	Minimal	Severe	Mild	1.48
8299	Mild	Moderate	Moderately severe	5.20
6587	Severe	Severe	Moderate	1.37
9725	Severe	Mild	Moderate	0.54
5436	Minimal	Mild	Minimal	7.94
7512	Minimal	Minimal	Mild	3.49
6042	Minimal	Minimal	Minimal	3.76
1427	Moderate	Minimal	Moderately severe	0.00
7216	Minimal	Minimal	Minimal	12.70
3517	Mild	Mild	Minimal	1.24
4561	Moderate	Mild	Mild	4.96
4678	Moderate	Minimal	Mild	3.14
6174	Mild	Minimal	Mild	11.21
5043	Mild	Mild	Mild	3.52
6314	Mild	Mild	Moderate	0.47
25043	Moderate	Mild	Moderate	0.91

*See **Methods** step 8 a-c for score interpretation guidelines.

Appendix D

Table 9. California high school students scores on BDI, BAI, and PHQ-9 for different treatments of frequency and intensity ranging from 0>1, 1>2, 2>4, 4>10, and >10

Intensity*hours of exercise range	Subjects' intensity*hours	BDI Score	BAI Score	PHQ-9 Score
<1	0.00	24	7	17
	0.30	23	37	17
	0.54	31	8	13
	0.47	16	12	11
	0.91	9	5	10
1<x<2	1.64	2	0	3
	1.37	34	45	14
	1.24	9	12	3
	1.48	12	28	8
2<x<4	2.73	22	35	18
	3.49	4	4	9
	3.76	3	2	2
	3.14	15	2	5
	3.52	7	5	6
4<x<10	4.96	15	9	8
	7.94	12	11	2
	9.08	19	23	11
	5.20	16	24	15
>10	12.70	2	2	1
	11.21	15	0	5

Appendix E

Table 10. BAI ANOVA Test

Hypotheses	H_0: There is no significant difference between the hours and intensity of exercise completed by a participant and their BAI score. H_1: There is a significant difference between the hours and intensity of exercise completed by a participant and their BAI score.
Alpha	0.05
F value	0.88
$F_{critical}$	3.05

F is less than F critical value at 95% significance, therefore the null hypothesis is not rejected. This means there is no significant difference between the hours and intensity of exercise completed by a participant and their BAI score. Any differences between these are not due to chance or sampling error.

Due to absence of overlap in the confidence intervals of 4>10 and >10 a T-Test must be performed to see if there is significant difference between these treatments.

Table 11. BAI T-Test: 4<x<10 and >10

Hypotheses	H_0: There is no significant difference between the BAI scores of those who scored 4>10 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise H_1: There is a significant difference between the BAI scores of those who scored 4>10 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise
Alpha	0.05
T value	3.89
$T_{critical}$	2.35

T is greater than T critical value at 95% significance therefore the null hypothesis is rejected. This means there is a significant difference between the BAI scores of those who scored 4>10 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise. Any differences between these are due to chance or sampling error.

Table 12. PHQ-9 ANOVA Test

Hypotheses	H_0: There is no significant difference between the hours and intensity of exercise completed by a participant and their PHQ-9 score. H_1: There is a significant difference between the hours and intensity of

	exercise completed by a participant and their PHQ-9 score.
Alpha	0.05
F value	2.00
$F_{critical}$	3.05

F is less than F critical value at 95% significance, therefore the null hypothesis is not rejected. This means there is no significant difference between the hours and intensity of exercise completed by a participant and their PHQ-9 score. Any differences between these are not due to chance or sampling error.

For the PHQ-9 test there is evidence of a significant difference between <1 and any of the other ranges. Because of this a T-Test is to be performed in order to see if there is a significant difference in this data.

Table 13. PHQ-9 T-Test: <1 and 1<x<2

Hypotheses	H_0: There is no significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 1>2 for their hours and intensity of exercise H_1: There is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 1>2 for their hours and intensity of exercise
Alpha	0.05
T value	2.20
$T_{critical}$	2.13

T is greater than T critical value at 95% significance therefore the null hypothesis is rejected. This means there is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 1>2 for their hours and intensity of exercise. Any differences between these are due to chance or sampling error.

Table 14. PHQ-9 T-Test: <1 and 2<x<4

Hypotheses	H_0: There is no significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 2>4 for their hours and intensity of exercise H_1: There is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 2>4 for their hours and intensity of exercise
Alpha	0.05

T value	1.80
$T_{critical}$	1.94

T is less than T critical at 95% significance therefore the null hypothesis is not rejected. This means there is not a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 2>4 for their hours and intensity of exercise. Any differences between these are not due to chance or sampling error.

Table 15. PHQ-9 T-Test: <1 and 4<x<10

Hypotheses	H_0: There is no significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 4>10 for their hours and intensity of exercise H_1: There is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 4>10 for their hours and intensity of exercise.
Alpha	0.05
T value	1.48
$T_{critical}$	2.13

T is less than T critical at 95% significance therefore the null hypothesis is not rejected. This means there is not a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored 4>10 for their hours and intensity of exercise. Any differences between these are not due to chance or sampling error.

Table 16. PHQ-9 T-Test: <1 and >10

Hypotheses	H_0: There is no significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise H_1: There is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise
Alpha	0.05
T value	4.27
$T_{critical}$	2.92

T is greater than T critical value at 95% significance therefore the null hypothesis is rejected. This means there is a significant difference between the PHQ-9 scores of those who scored 0>1 for hours and intensity of exercise to those who scored >10 for their hours and intensity of exercise. Any differences between these are due to chance or sampling error.

Literary Review

The Relationship Between Alzheimer's Disease and Sleep

America Leon, Anika Shah, Michelle To
Making Waves Academy

Keywords: Alzheimer's Disease, Dementia, Sleep, Cognitive Decline

Abstract:
Alzheimer's Disease affects millions of elderly Americans, occurring as a result of the build-up of certain proteins in the brain. Current research investigates the apparent association between sleep and the development of Alzheimer's Disease. Studies have revealed that sleep disturbances are consequences of Alzheimer's Disease, as well as potentially contributing to the progressive development of the disease. Factors like short and long sleep duration, poor sleep quality, and use of hypnotic medication can result in developing dementia and Alzheimer's years later. However, other factors including long sleep duration can also increase someone's risk. Several tests of cognition and self-reported sleep duration of participants in a study revealed that both higher and lower extremes of sleep duration can contribute to cognitive decline. Other studies point to sleep disturbance can contribute to the accumulation of beta-amyloid proteins and affect cognitive function.

Introduction:

Alzheimer's Disease is the most common type of dementia, affecting parts of the brain that control thought, memory, and language. In Alzheimer's a process called brain atrophy occurs in which neurons are injured and die, connections between neuron networks break down, and many brain regions begin to shrink, resulting in a significant loss of brain volume ("What Happens to the Brain in Alzheimer's Disease?"). The disease progressively worsens the cognitive function – memory, thinking, and ability of individuals to carry out tasks – of those it affects ("Alzheimer's Disease Fact Sheet"). Research has revealed that the disease is a result of the build-up of proteins in and around brain cells: amyloid and tau are among some of the proteins. Amyloid forms plaques around brain cells and tau forms tangles within brain cells ("Alzheimer's Disease - Causes"). Dementia is alarmingly common among older adults; 6.5 million Americans age 65

and older are living with Alzheimer's, with the risk and percentage of Americans affected increases with age. And the number of adults with the disorder is increasing. Dementia is responsible for disability and mortality, impacting not only the individuals with the disorder but the families that bear the responsibility of providing care for them–not to mention the individuals that do not have anyone to care for them. There are currently no therapies or cures for dementia. Therefore, investigating risk factors and trying to find out ways to prevent individuals from developing the disorder is incredibly important. Some people can be genetically predisposed to Alzheimer's, people with a parent or sibling with Alzheimer's are more likely to develop the disease and researchers have discovered several genes that increase an individual's risk of Alzheimer's. But external and environmental factors can also play a significant role. In fact, research has revealed that sleep deprivation is a factor in cognitive decline and the development of Alzheimer's. These studies have pointed to altered sleep amounts or poor sleep quality and sleep-disordered breathing has also been identified as a potential cause of cognitive impairment.

Discussion:

Impact of Sleep on the Risk of Cognitive Decline and Dementia

Significant sleep disturbance including shorter sleep duration and fragmented sleep, altered circadian rest/activity patterns, and elevated rates of sleep-disordered breathing are common consequences of diseases like Alzheimer's Disease and exhibited by older adults with dementia (Musiek et al.). But, investigators have examined if sleep disturbances contribute to the risk of Alzheimer's Disease through reports of insomnia symptoms, sleep duration, and sleep-disordered breathing. Other data comes from polysomnography (PSG; assessment of sleep) and actigraphy, a method of estimating sleep/wake patterns by recording movement over multiple days using a device on the wrist (Martin and Hakim). REM sleep was excluded from this article. The sleep study included participants of a mean age of 52 and cognitive impairment and dementia 18 to 26 years later in more than 2300 Finnish twins (Musiek et al.). This study found that reports of short and long sleep duration, poor sleep quality, and use of hypnotic medications more than 60 days per year were associated with lower cognitive composite scores. But sleep deprivation is not the sole risk factor for developing Alzheimer's Disease: long sleep was also associated with roughly 1.8 times the odds of developing Alzheimer's Disease. Another study of 214 Swedish adults without dementia aged greater than or equal to 75 years revealed that a decrease in typical sleep

depth or duration was associated with greater odds of dementia and Alzheimer's Disease nine years later (Musiek et al.). But external variables like depressive symptoms, physical function, respiratory problems, and pain reduce the significance of a change in sleep and could explain the results of the study better. These, along with other studies, demonstrate the association between sleep quality and cognitive outcomes. Proton magnetic resonance imaging has revealed that poorer sleep quality and sleep efficiency were associated with higher mI in the hippocampus (Musiek et al.). This suggests that sleep loss may negatively affect the hippocampus, a structure important for memory, demonstrating that sleep loss leads to neuronal damage that could result in cognitive decline. Researchers have concluded that sleep disturbance is a potential cause of cognitive decline, dementia, and Alzheimer's Disease pathology; however, more research is needed. Clinicians should learn and promote healthy sleep to prevent Alzheimer's Disease. Studies should also try to consider the impact of depression and its symptoms' association with sleep and dementia.

Association Between Sleep Duration and Cognitive Decline

Other studies dispute the association between sleep duration and cognitive decline, stating that an association between the two has not been conclusively demonstrated. This study investigated the presumed association further through a pooled analysis of two nationally representative aging cohorts, conducted in the United Kingdom and China, the English Longitudinal Study of Ageing (ELSA) and the China Health and Retirement Longitudinal Study (CHARLS) (Ma et al.). The subjects self-reported their sleep duration, the "number of hours of sleep on an average week night" and "During the past month, how many hours of actual sleep did you get at night (average hours for one night)?" to find the baseline sleep duration. Based on sleep duration (≤4, 5, 6, 7, 8, 9, or ≥10 hours), the participants were divided into 7 groups to be analyzed. Cognitive assessments in both studies were conducted to analyze memory, executive function, and orientation (Ma et al.). The performance of subjects on memory assessment tasks would determine their cognitive function to investigate whether the presumed association between sleep duration and cognitive decline is true. Global cognitive z scores were calculated according to several tests (immediate and delayed recall test, an animal fluency test, the serial sevens test, an intersecting pentagon copying test, and a date orientation test). The study revealed that the adjusted least-squares means of the global cognitive z scores among individuals reporting 8, 9,

10 or more, or 4 hours or fewer of sleep per night were statistically significantly lower than in the reference group. Further, the study also showed that the individuals who slept 10 hours or more per night had the lowest cognitive scores. This means that there is an inverted U-shaped association between sleep duration and global cognitive decline, meaning that higher and lower extremes of sleep duration could impact cognition. The research suggests that cognitive function should be monitored in people with insufficient or excessive sleep duration (Ma et al.). However, it is also true that variables like the sex of participants could affect the validity of the results. Furthermore, the study was observational, therefore, there is a possibility of reverse causality and participants could have misreported their sleep duration due to not being able to remember it (Ma et al.). This means that excessive or short sleep duration could be a result of brain impairment or cognitive decline. But, the association revealed in the study remains statistically significant. The results of the study deviate from the common belief that lack of sleep is the sole cause of cognitive decline; excessive sleep is not acknowledged or addressed as much as a concern or contributor to cognitive decline.

Sleep Disturbances Linked to Abnormal Deposits of Certain Proteins in the Brain

Other studies point out sleep disturbance as a more relevant contributor to cognitive decline than sleep duration. Beta-amyloid proteins are naturally occurring proteins, and researchers are trying to understand how the protein influences the development of Alzheimer's. In individuals with Alzheimer's, there are abnormal levels of a naturally occurring protein, beta-amyloid 42. The protein is considered toxic and clumps to form amyloid plaques that disrupt cell function ("What Happens to the Brain in Alzheimer's Disease?"). Studies suggest that sleep could clear beta-amyloid from the brain. Beta-amyloid accumulation can be tied to lower sleep efficiency, and non-REM sleep in the lower frequency domain can predict future plaque accumulation ("Sleep disturbances linked to abnormal deposits"). This study also points out that fatigue might be another important variable. A clinical trial revealed that 1 night of sleep deprivation could elevate cerebrospinal fluid levels of AB42 protein (Ma et al.). Therefore, chronic sleep deprivation and fatigue could play a connected role in affecting an individual's cognitive function. One study explored the connection between sleep disturbances and abnormal deposits of proteins that build up in the brains of individuals with Alzheimer's Disease by analyzing the brain tissue of 247 men with chronic traumatic encephalopathy (CTE) from contact sports. Some

people with this kind of head trauma have a sleep disorder that causes a disturbance during the REM phase of sleep; the disorder causes them to move their limbs and shout. The study investigated whether the REM sleep disorder would be found in people with CTE and whether the sleep disorder would be associated with the presence of accumulations of alpha-synuclein protein filaments called Lewy bodies, or with tau tangles in the brainstem region ("Sleep disturbances linked to abnormal deposits"). Family members reported that the subjects acted out their dreams while sleeping, revealing that one-third of the men with CTE had probable REM sleep disorder. Those with the sleep disorder were more likely to have the presence of Lewy bodies in their brain. Examination of the brainstem tissue, also revealed that men with probable REM sleep disorder without Lewy bodies were more likely to have tau tangles than those without the disorder, demonstrating a connection between contact sports, sleep disorders, and tau tangles ("Sleep disturbances linked to abnormal deposits"). Additional research analyzes the presumed connection between sleep disturbances and brain impairment further. For instance, an additional study measured non-REM sleep slow-wave activity and sleep quality, and tracked the development of beta-amyloid plaques in nine men and 23 women between 70 and 80 years old. Their initial sleep efficiency was measured and in the one to six years that followed, they received PET scans to track amyloid plaque accumulation. Analysis of this data revealed that participants with lower slow-wave brain activity developed amyloid plaques faster than those with higher slow-wave activity. And, those with the lowest sleep efficiency had the greatest increase in beta-amyloid plaque accumulation ("Sleep disturbances linked to abnormal deposits"). Although more research is needed to solidify a relationship between cognition, the accumulation of amyloid beta and tau, and sleep, several studies have revealed that there is an association between sleep disturbances, particularly during certain sleep stages that can impact cognitive function and contribute to eventual cognitive decline, Dementia, or Alzheimer's Disease.

Conclusion:

Research and studies reveal that there are associations between cognitive decline as a consequence of insufficient and extreme sleep duration and sleep disturbance. Therefore chronic sleep deprivation can impair cognitive performance. Sleep disturbances and sleep disorders can also contribute to the build-up of proteins that research has revealed contribute to cognitive

decline and can predict the development of Dementia and Alzheimer's Disease. Sleep patterns vary from person to person. Individuals, especially older adults, should try to limit periods of long-term fatigue and short/excessive sleep duration. However, it is true that factors other than sleep contribute to cognitive decline, Dementia, and Alzheimer's Disease. Additional studies are needed to further examine and prove the association.

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Literary Review

CRISPR and its Applications in Colon Cancer

Anika Shah (author)
San Mateo High School

Keywords: Cancer Research, Colon Cancer, CRISPR, Genome Editing

Abstract:
CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats – Cas9) is an innovative technology that enables researchers to interrogate the function of genes through precise editing of genes. In recent years, researchers have utilized this technology to assess the function of all genes in a system through the use of CRISPR-Cas9 screens. These screens are a powerful technique that enable the unbiased interrogation of gene function in various model systems and organisms. These pooled screens employ a predefined set of genetic perturbations, called sgRNAs, which are introduced into a pool of cells. This pool of cells each with their own genetic perturbation are then grown in competition with each other. After a period of competition, the effects of each perturbation are assessed through the sequence-based counting of each specific mutation, or sgRNA. CRISPR-Cas9 screens have revealed numerous molecular pathways that may confer resistance or sensitivity to various biological challenges. This review will describe how these techniques have advanced our understanding of colon cancer and will demonstrate how these screens can be modelled and optimized *in silico* using computational simulation tools. It will further describe how these screens have been applied to living organisms and provide insight into how these may advance our understanding of various biological mechanisms and organ function.

Introduction:

CRISPR screening has proven to be a promising method for the future of medicine, with still a lot more to come. Essentially the pooling of a multitude of cells in order to find target genes that respond well to a particular drug or infection, CRISPR screening is conducted with a uniform method in most screens. Traditionally this is done by constructing a pool of oligos, which essentially contains single stranded DNA complexes that code for the sgRNA of a target gene, as well as contain specific genetic sites so that a lentivirus plasmid can be cloned from the single stranded DNA[1]. Once these lentivirus plasmids are designed, each with a different genetic makeup, they are injected into a plate of target cells. The RNA is then reverse-transcribed allowing it to amalgamate with the target cell's DNA. Conversely, a new CRISPR screening method is becoming more and more prominent when researchers have already narrowed down the genes they want to test and there isn't a wide abundance of possible target genes. Here, researchers use a CRISPR gRNA library where there is one gRNA per cell, and all the cells are

[1] Bock et al., "High-Content CRISPR Screening."

separated by wells. The gRNA is introduced into the cell through a variety of methods such as electroporation, lipofection, or viral infection. Upon introduction of these vectors, the gRNA becomes integrated into the cell's DNA. Once the new genes are integrated into target cells, these cells are placed in a competitive cell culture, in order to see which genetic combination has the most selective survival in this specific cell culture. Cell growth and expression can be measured with fluorescent markers and dyes, allowing researchers to see which cells which survived, and which genes were responsible for giving the cells a selective advantage over the others. Thus, both of these processes have various applications, such as those in colon cancer and other life-threatening diseases, as researchers are able to discover genes that are responsible for a cancer cell's growth, and are able to start targeting those genes in future treatments. Results of these CRISPR screens can be portrayed through heat maps and characteristic curves, allowing for more data analysis and plausible future experiments. Overall CRISPR screening serves as an adequate first step in fabricating a set of experiments that eventually produces a drug that is able to target a problematic gene.

History of CRISPR

The history of CRISPR dates back to over 20 years ago, in 1987, where the CRISPR-Cas9 function was found in various bacteria and archae.[2] However, there wasn't much information circulating surrounding DNA sequencing, limiting possibilities of CRISPR at the time. As the years progressed, and more knowledge about the DNA sequence grew, researchers were finally able to understand the purpose of the CRISPR-Cas9 system in bacteria and archae alike. They were able to realize that the combination of CRISPR and Cas9 proteins could essentially provide immunity to the organism. However, researchers noticed abnormalities in these organisms, such as uncounted for repetition of certain sequences.[3] As a product of this, multiple researchers in the early 2000s continued to study the function of the CRISPR-Cas9 system, and its possibilities. Makarova et. al came to the conclusion that CRISPR could be used to essentially silence genes in organisms that coded for the required Cas9 protein.[4][5] This was experimented on a cleave experiment, where target DNA was cleaved by crRNA-tracrRNA-Cas9. After the Cas9 bound to the target DNA, due to its REC lobe that recognizes the nucleotides, it was able to unwind the DNA. Then the HNH and RuvC domains of Cas9 broke the DNA, allowing the process to essentially silence a target gene. This experiment shed light on many experiments to come, paving the way for one of the most influential and efficient methodologies of biomedical research: CRISPR-Cas9 Screens.

Discussion:

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[2] Ishino, Krupovic, and Forterre, "History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology."
[3] Jansen et al., "Identification of Genes That Are Associated with DNA Repeats in Prokaryotes."
[4] Makarova et al., "Evolutionary Classification of CRISPR–Cas Systems: A Burst of Class 2 and Derived Variants."
[5] Makarova et al., "A Putative RNA-Interference-Based Immune System in Prokaryotes: Computational Analysis of the Predicted Enzymatic Machinery, Functional Analogies with Eukaryotic RNAi, and Hypothetical Mechanisms of Action."

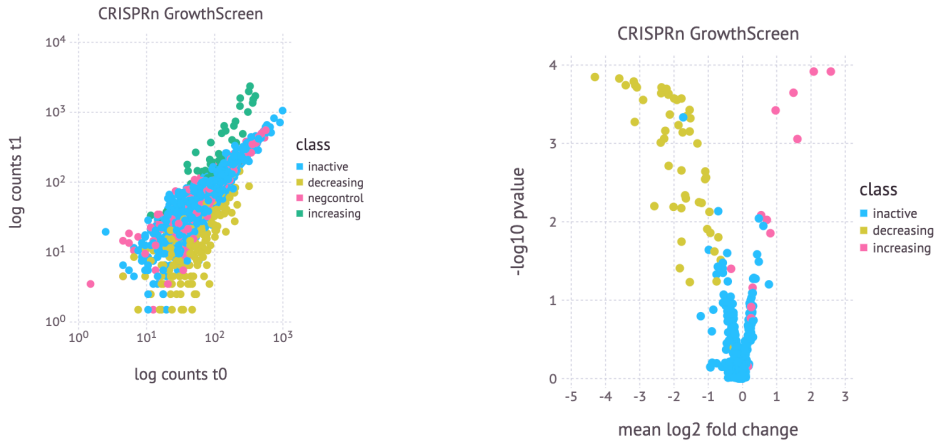
CRISPulator allows Researchers to Conduct In-Silico Screens to Optimize future Lab Results

As technology in this up and coming world advances, scientists from all over are able to harness advanced technology to visualize and assist them in their research. Developed by Tamas Nagy and Martin Kampmann, and written by Tamas Nagy, CRISPulator is a new and running innovation, which allows researchers to digitally design CRISPR screens in advance. It allows researchers to simulate the parameters of CRISPR screens in a digital setting. Despite CRISPR screens' utmost potential, they can become extremely costly and timely, making CRISPulator extremely helpful and viable. Researchers are able to run their desired numbers through the program in their terminal, and see which combinations give them the best results. CRISPulator generates visuals with different colors and methods of presentation so that researchers can view where a potential CRISPR screen may take them, and where different parameters may benefit and harm them.

Running Crispulator

Running CRISPulator requires a series of steps to download. After adding Julia to the computer's PATH, researchers must download Crispulator, and then run their screens through the Crispulator directory. Then, researchers can edit the parameters of a CRISPR screen and the program will provide them with two images. Edits include changing the number of genes, the number of sgRNAs, and whether the screen is a growth screen or FACS[6] screen. A growth screen measures the cell's ability to grow with or without certain genes. However, this type of screen isn't sufficient for all situations, so researchers also opt for FACS screen, where they screen based on fluorescent activity and use different colors to analyze the activity of a certain gene or gene pathway.

With a growth screen of 500 cells, 5 sgRNAs per cell, a transfection[7], selection, and infection value all of 100, CRISPulator was able to generate two images (Fig. 1).



[6] Menasche et al., "Fluorescence Activated Cell Sorting (FACS) in Genome-Wide Genetic Screening of Membrane Trafficking."
[7] Chong, Yeap, and Ho, "Transfection Types, Methods and Strategies: A Technical Review."

Figure 1: An in silico model of a CRISPR growth screen. a) A comparison between initial time point and end time point of the pooled sgRNA (n=500) in the modelled CRISPR screen. b) statistical significance of the difference.

CRISPulator has now given us a breakdown of how each sgRNA will react, as each point represents a different sgRNA. On the image on the left, it is color coded to express whether or not they are targeting a positive phenotype or negative phenotype. The negative control (pink) points represent the sgRNAs that are simply controls, and aren't for a specific target gene. For the image on the right, the image shows researchers calculations for gene phenotypes, and each dot represents a gene. They aim to signify how far each gene is away from the "wild-type" through a volcano plot.

With a FACS screen of 19114 cells, 6 sgRNAs per cell, and transfection, selection, and infection all values of 100, CRISPulator generates two graphs (Fig. 2) that look very different from the previous screen.

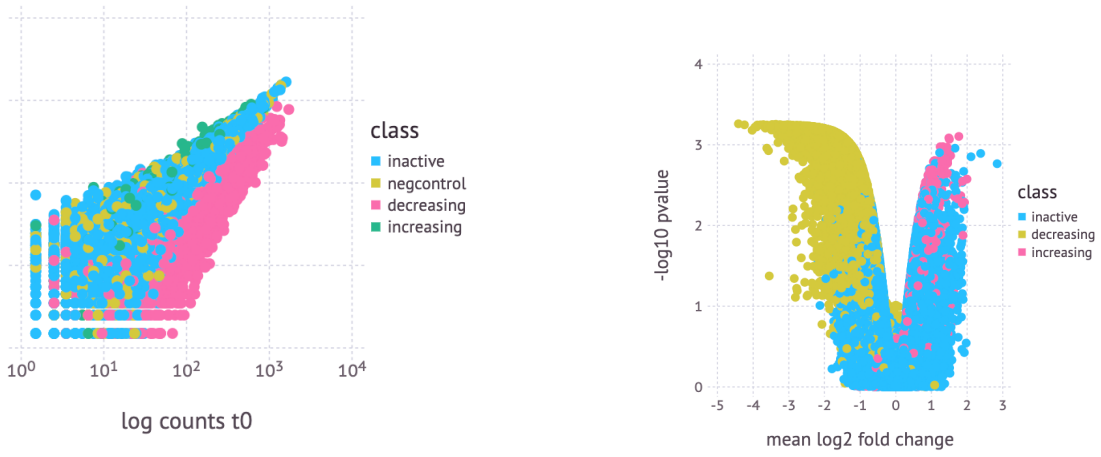


Figure 2: An in silico model of a CRISPR Facs screen. a) A comparison between initial time point and end time point of the pooled sgRNA (n=19114) in the modelled CRISPR screen. b) statistical significance of the difference in terms of genetic DNA targets.

These images contain a visibly higher amount of spots, and a more dense looking graph. This is because both the amount of cells was increased, as well as the amount of sgRNAs. If there are more sgRNAs, then there are more dots on the graph on the left. Additionally, if there are more sgRNAs, that means there are more target genes, which is why the graph on the right is also denser, as that graph indicates genetic phenotypes. Because FACs screens measure fluorescence

levels, researchers can use CRISPulator to determine the top and bottom quartile of the cell population, as this is ideal for a FACS screen[8].

Lastly, we conducted a growth screen, with 19114 cells, 4 sgRNAs per cell, and transfection, selection, and infection all values of 100 (Fig. 3)

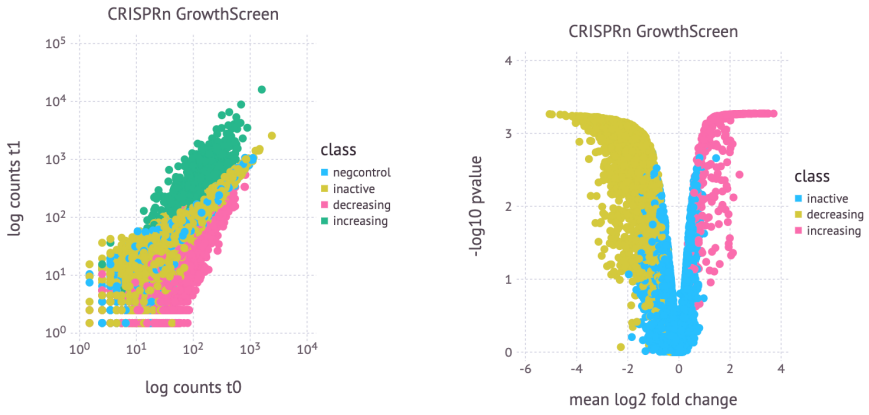


Figure 3: An in silico model of a CRISPR growth screen. a) A comparison between initial time point and end time point of the pooled sgRNA (n=19114) in the modelled CRISPR screen. b) statistical significance of the difference in terms of genetic DNA.

The graphs here look slightly different, as this screen measures the growth of the cells. Growth in-silico screens allow researchers to determine the strength of positive phenotypes, which can be rare in growth in vitro screens. Researchers can then edit the numbers to optimize the amount of positive phenotypes in growth screens , as they can use these graphs to analyze the results.

In Vitro Crispr Screen:

The overall methodology of a CRISPR screen follows a complex 4 step process. The initial step is to select an organism from which the samples will be collected, whether that be human tissue, animal tissue, or plant tissue.[9] A multitude of cells that are extracted from the selected organism creates the cell culture that the CRISPR screen will be performed on. Once the cells have been chosen, they are then usually genetically-engineered, through a plasmid, mRNA, or a protein, to transduct the CRISPR-cas protein, as this increases the efficiency of the CRISPR screen. It is imperative to check the efficiency of the cell's ability to express the CRISPR-cas protein at this stage, which can be done by (FLAG-tag) immunoblot[10] which tests the flow of these new proteins. At this stage, if an vivo experiment is being performed, the cells will be transplanted back into the organism where they will be screened, in order to test the

[8] Nagy and Kampmann, "CRISPulator: A Discrete Simulation Tool for Pooled Genetic Screens."
[9] Miles, Garippa, and Poirier, "Design, Execution, and Analysis of Pooled in Vitro CRISPR/Cas9 Screens."
[10] Dong and Kantor, "Lentiviral Vectors for Delivery of Gene-Editing Systems Based on CRISPR/Cas: Current State and Perspectives."

effects of a drug on the organism itself. In an in vitro screen, where the screen will be performed on the cell culture outside of the living organism, the next step is to determine target genes and sequences in the cells' genomes. Looking at the genome in its entirety, researchers determine which genes they want to target with the sgRNAs, sometimes all of them in the genome, and sometimes a select handful. After the target genes are selected, a pool of sgRNAs must be ready to distribute within the cell culture. This is done by first creating a pool of oligos, customized single-stranded DNA strands that correlate with the cells' targeted genes. In these engineered oligos, it is crucial to include positive and negative controls, as the screen will not be seen as accurate without them. Once these oligos have been engineered with not only the target gene's sequence, but specific genetic sites that allow cloning into a plasmid, they are then cloned into plasmids that contain lentiviral genes. This is crucial because these lentiviral-gene-containing plasmids are used to produce lentiviruses, which are able to efficiently carry these genes that have been artificially engineered into the cell culture due to their ability to stay intact when being transported through the nuclear membrane.[11] Once these lentiviruses have been produced, they are now ready to be infected into the aforementioned cell culture. The sgRNAs will detect DNA that correlates with their nucleotide, allowing them to attach to the target genes. Once attached, the addition of tracrRNA will complete the complex by binding the Cas9 protein to the target DNA.[12] The Cas9 protein is a protein that is able to cut the target gene out, allowing for the screen to begin. An extremely important factor to consider when infecting the cell culture is the multiplicity of infection[13] (MOI), which is usually set in between 0.3 to 0.5.[14] This number is the number of virions, or essentially the gRNAs, delivered to each cell. This means that if a screen is testing 123,411 gRNAs, a screen with a 0.2 MOI would require around 617,055 (123,411 divided by 0.2) cells, ensuring that each virion is delivered to a cell. [15]Another factor that is considered when determining the amount of cells to screen is representation. When the screen is running, it is possible for certain cells to die due to uncalculated reasons, which could greatly alter the results. To avoid this, the amount of cells that are injected is increased, so that small blips in the data don't have as heavy of an influence on the actual data. Therefore, instead of 617,055, we multiply the amount of cells by 100, giving us overall 61705500 cells. Once the cell culture has been infected with the lentiviruses, which because they are in RNA form, they must be reverse transcribed into DNA, in order for CRISPR to begin. Once CRISPR begins, the goal of the screen is to determine a cell's ability to grow with or without a certain gene.

The cell culture can be examined at different time stamps, examining the progressions of growth or death in cells with different genomes. Examinations can be conducted through multiple methodologies with various complexities, usually following a 5 step process. Beginning

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[11] Durand and Cimarelli, "The Inside Out of Lentiviral Vectors."
[12] Liu et al., "Delivery Strategies of the CRISPR-Cas9 Gene-Editing System for Therapeutic Applications."
[13] Gutiérrez Serafín et al., "The Multiplicity of Cellular Infection Changes Depending on the Route of Cell Infection in a Plant Virus."
[14] Shojaei Baghini et al., "Optimizing SgRNA to Improve CRISPR/Cas9 Knockout Efficiency: Special Focus on Human and Animal Cell."
[15] Wan et al., "Genome-Scale CRISPR-Cas9 Screen of Wnt/β-Catenin Signaling Identifies Therapeutic Targets for Colorectal Cancer."

with data processing in in vitro CRISPR screening pools, where sequencing reads of the cell culture are processed into matrices, a digital and more accessible method of holding the data. These matrices now include data on each gRNA being tested in the CRISPR screen, which are formed using various digital programs. Following data processing is quality control, which ensures that the collected data is reliable and accurate. This factors in the amount of current sgRNAs compared to the initial, consistency of results, expected behavior by positive and negative controls, and high sgRNA representation. After having collected data and ensured its accuracy, the next step involves ranking each sgRNAs phenotypical effect on the cells, factoring in components such as time-stamps as to when the screen was examined. After the ranking process is finished, researchers are now able to decipher which genes are most relevant to a cell's proliferation and thus determine possible gene targets for drug treatments. Researchers can then compare these results to other screens' results to find commonalities and points of comparison. The last step to data analysis for CRISPR screens is presenting the data visually, in order to better overall understanding for a larger audience. Visual interpretations include graphs, heat maps, and volcano plots, all of which assist in finding patterns, consistencies, and outliers which can help determine the viability of a CRISPR screen.

Indirect In Vivo Screens Start to become Efficient in Determining the Effects of an Absence of a Gene in a Living Body

Although in Vitro screens are used abundantly, they are limited by the fact that they are still only being done in a plate, rather than in an organ. Oftentimes, the results found in vitro screens differ greatly from the same experiment conducted in a living body, such as mice. Due to this inhibition with in vitro screens, researchers usually prefer doing at least one in vivo screen alongside their in vitro screen. Indirect vivo screens are specifically useful for cancer screens, as a lot of cancers inhibit or weaken an organ, which is a combination of various cells rather than a group of one type of mutated cell. The initial methodology of an in vivo screen is similar to an in vitro screen, where cells are taken from a specific organ of a specific biological model (such as a mouse). The MOI and representation are usually the same as they would be in an in vitro screen: a screen with 123,411 sgRNAs would have the same MOI of 0.2 and a representation multiplier of 100.[16] After cells are collected, oligos are engineered with desired DNA targets, including the DNA that the researchers want to prevent replication of, as these oligos will then form lentiviral plasmids with gRNAs. However, once these gRNAs are inserted back into the collected cells, the process veers from the in vitro process. These cells are now inserted back into the living body the cells were originally collected from, so that researchers can study how the engineered cells react in a complex organ system. This screen is given the name "indirect" because rather than inserting the engineered DNA into the animal, the cells are removed from the animal and then inserted back in with the engineered DNA. In cancer CRISPR screens, the engineered cells that survived will likely form a tumor, whereas the cells that had a non preferable DNA makeup due to the sgRNAs will likely die out. This allows researchers to dissect the tumor and examine the cells that have survived in the tested organism. The cell that is responsible for the tumor's

[16] Kuhn, Santinha, and Platt, "Moving from in Vitro to in Vivo CRISPR Screens."

injected sgRNAs will likely become the next target for future tumor supressors, in order to inhibit such tumor from starting in the first place. Despite its strengths, indirect vivo screens are not able to conduct all types of screens, as the cells used in indirect screens must be transplantable, meaning they are able to be transplanted in and out of the cell. Additionally many of the organisms that contain these transplantable cells are immunocompromised, thus bringing into picture ethical considerations.

Direct In-Vivo Screens are able to Further the Progress of In-Vitro CRISPR Screens

As a result of some of the limitations of indirect vitro screens, researchers have started conducting direct in vivo screens. As done with prior screens, the first step is to create a sgRNA library, with single strands that correlate with the soon to be tested target genes. The sgRNA is then used to create the lentiviral plasmids. The lentiviral plasmids are then injected into the organ of choice, such as a liver. The sgRNA is able to assimilate throughout the organ by entering the various cells. After a sufficient period of time, the levels of sgRNA are then examined within the organism. Researchers are now able to determine which sgRNAs concentration went up, meaning the cells with that specific sgRNA were able to survive and replicate, and which sgRNAs concentration went down, meaning the cells with that specific sgRNA were not able to survive and had started to die out. One recent discovery using direct vivo screening was the screening of a mouse's liver that aimed to discover information about the mouse's regulation of heptocyte fitness. Using a targeted and direct vivo screen, researchers induced the mouse liver with sgRNAs from lentiviruses,[17] allowing these single guide RNAs to integrate within each and every liver cell. Because a mouse's liver cell is covered in hepocotytes[18], there were lots of DNA targets that the researchers' sgRNAs could target. Once the sgRNAs had assimilated throughout the cells' DNA, researchers could observe which hepocotyte cells were surviving and which were not, leading to the finding of positive and negative regulators of hepatocyte fitness.

Science Advances Identifying DLD1 as a therapeutic vulnerability in colon cancer.

Colorectal Cancer is one of the world's leading causes of cancer related deaths, making the disease an important disease to study. A mutated WNT pathway accounts for the majority of colorectal cancers, due its overactivation. When the WNT pathway is overactivated, there is an excessive amount of **β**-Caratenin in colon stem cells, leading to the development of "colonic polyps", and thus the development of carcinomas. When there is an excess activation of **β**-Caratenin, the expression of various targets are activated through transcription factors. These targets, such as *cMYC*, *AXIN2*, *ASCL2*, *LGR5*, and *CD44* regulate the proliferation of colon stem cells, making them targets for current researches to prevent the over expression of **β**-Caratenin.[19]

Researchers initially used CRISPR Cas-9 Screening to produce colon cancer cell lines that they could then perform the actual screen on. Through the screen, researchers hoped to evaluate the activation status of Wnt/**β**-Caratenin in colon cancer cells in the DLD1 line, rather than finding a specific gene that regulated it. Using a lentiviral 7× TOP-dGFP mCherry vector

[17] Keys and Knouse, "Genome-Scale CRISPR Screening in a Single Mouse Liver."
[18] Mendoza et al., "Isolation and Culture of Mouse Hepatocytes and Kupffer Cells (KCs)."
[19] Wan et al., "Genome-Scale CRISPR-Cas9 Screen of Wnt/β-Catenin Signaling Identifies Therapeutic Targets for Colorectal Cancer."

where a green fluorescent protein is regulated through 7× TOP-dGFP, allowing researchers to determine the **β**-Caratenin levels through the fluorescence of the cell. Vector mCherry acted as a fluorescent control, allowing researchers to compare the fluorescent glow of the proteins regulated through 7xTOP-dGFP with the ones of mCherry. Results showed that the lack of **β**-Caratenin led to a lack of GFP Signaling and hence a lower fluorescent glow in comparison to that of the mCherry vector. This demonstrated that the DLD1 was indeed a good and effective line with Wnt/**β**-Caratenin activity.

Now that the researchers had the selected cell line of study (DLD1), scientists conducted a whole genome CRISPR Cas-9 screen. They injected the Brunello library of sgRNAs, with 76,411 sgrnas) to target 19,114 human genes of the DLD1 line.[20] They wanted to determine what **β**-Caratenin regulators were able to inhibit cell growth in a colorectal cell line. They collected GFP levels, similar to the first step, in order to determine **β**-Caratenin activity. After the 7 day mark of injecting sgRNAs, they assessed which sgRNAs had resulted in an increase or decrease of GFP levels, with 5% highest GFP being classified as GFP-high, and 5% lowest GFP being classified as GFP-low. After the checkpoint at the 7 day mark, the cells continued to grow until the 21 day mark to test cell viability. Whichever cells were still alive served as indicators for which regulators resulted in sustained cell growth. The results showed 497 genes that were vital to DLDI proliferation in colon cancer cells. In addition, the transcription factor TCF7L2 was shown to be a bona fide negative regulator of DLD1. However, researchers also discovered the lack of TCF7L2 resulted in little to no review, thus making it an unreliable target gene[21]. However, they had now found a viable number of target cells they could further study to possibly produce/release a treatment.

To further test their results, researchers treated two [reporter] DLD1 cell lines with **β**-Caratenin[22]. They also knocked in cassettes with dGFP and red fluorescent protein so that they could visibly compare the levels of dGFP, as the transcription of **β**-Caratenin endogenous was parallel and relative to the transcription of dGFP.[23] This part of the experiment confirmed that the addition of a **β**-Caratenin sgRNA[24] led to the transcription of a desired target gene, that were determined with the previous CRISPR screen. Researchers now tested if inhibiting this target gene produced significant results. They did this by knocking in a cassette of dGFP into the mCYC and then used JQ-1 to inhibit the transcription of mCYC, the target gene. When this transcription was inhibited, the cell like RKO showed a reduction in GFP levels.

Now, scientists took the 2 DLDI cell lines as well as the RKO cell line to perform a general CRISPR screen in efforts to find genes that are required for both **β**-Caratenin transciptional output and growth, as these genes would be targets for newest treatments and

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[20] Sanson et al., “Optimized Libraries for CRISPR-Cas9 Genetic Screens with Multiple Modalities.”
[21] Wenzel et al., “Loss of the Nuclear Wnt Pathway Effector TCF7L2 Promotes Migration and Invasion of Human Colorectal Cancer Cells.”
[22] Sebio, Kahn, and Lenz, “The Potential of Targeting Wnt/β-Catenin in Colon Cancer.”
[23] Campbell et al., “A Monomeric Red Fluorescent Protein.”
[24] Hu et al., “CRISPR/Cas9-Induced β-Carotene Hydroxylase Mutation in Dunaliella Salina CCAP19/18.”

drugs. The results of this CRISPR screen found 476 genes that were unique to the cells with **β**-Caratenin, in comparison to the **β**-Caratenin inactive cells.

The Importance of the MAPK Pathway was determined in Colon Cancer cells Using in vitro screens

Another major mutation found in many colon cancer cases are mutations in the KRAS gene, which has the ability to activate the MAPK pathway.[25] Previous studies have tried to repress MEK, using MEKi. However, MEKi treatments in clinical trials proved to be inefficient, as inhibiting MEK resulted in various negative effects. For example, the inhibition of MEK resulted in the activation of RTKs, which could restart the MEK pathway despite the efforts to inhibit it.[26] Hence, researchers performed a CRISPR knockout to identify oncogenes that improve MEKi resistance in colorectal cancer cells.[27] They also targeted MEK and PLK kinases to determine what type of effect it would have in colorectal cells, both in vitro and in vivo. They used the cell line HCT116[28], which had a medium drug resistance to MEKi treatments. They utilized a representation of 500, thus multiplying the amount of sgRNAs by 500, as well as an MOI of 0.3. Their final number of cells was a grand 2 million, which they then infected with a lentivirus system from the human GeCKO library[29]. These cells were also infected with AZD6244 for seven days, so that researchers could determine which cells were proliferating, and thus producing AZD6244. The active genes in the cells that were producing AZD6244 were considered "candidate genes" that were responsible for MEKi resistance in the RTK Pathway. One of the specific genes they found was GRB7, where the knockout of GRB7 in a cell resulted in a lethal effect in the MEKi of that cell, meaning GRB7 plays a crucial role in MEKi resistance, making it a future drug target[30].

Conclusion:

Through in silico screens, in vitro screens, and in vivo screens, CRISPR-Cas9 Screens have proven to be a powerful tool in detecting gene targets in cancers, specifically colon cancers. From single stranded DNA, to lentiviral plasmids, researchers are able to insert sgRNAs attached to Cas9 proteins into cells and cut target genes out, a renowned and developed process. This allows researchers to evaluate how cells react with the addition or removal of different genes: a meaningful discovery for cancer research. Researchers carefully evaluate the number of cells they should use in their culture, in comparison to the number of sgRNAs they are testing, using methods of evaluation such as MOI and representation. Recently, researchers have discovered

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[25] ZHANG and LIU, "MAPK Signal Pathways in the Regulation of Cell Proliferation in Mammalian Cells."
[26] Roberts and Der, "Targeting the Raf-MEK-ERK Mitogen-Activated Protein Kinase Cascade for the Treatment of Cancer."
[27] Zhan et al., "MEK Inhibitors Activate Wnt Signalling and Induce Stem Cell Plasticity in Colorectal Cancer."
[28] Ma et al., "Anti-Cancer Potential of Polysaccharide Extracted from Hawthorn (Crataegus.) on Human Colon Cancer Cell Line HCT116 via Cell Cycle Arrest and Apoptosis."
[29] Sanjana, Shalem, and Zhang, "Improved Vectors and Genome-Wide Libraries for CRISPR Screening."
[30] Han, Shen, and Guan, "The Grb7 Family Proteins: Structure, Interactions with Other Signaling Molecules and Potential Cellular Functions."

target genes to improve hepacyte fitness, as well as target genes for countering the growth of cancer in colons. The limits of CRISPR screens are indefinite, and possibilities wide, as further advancements in CRISPR screens could ultimately lead to more and more cures for one of the deadliest diseases: cancer.

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30. Han, Shen, and Guan, “The Grb7 Family Proteins: Structure, Interactions with Other Signaling Molecules and Potential Cellular Functions.”

Review Article

Meta-Analysis of Leukocyte Diversity in Atherosclerotic Mouse Aortas

Badri Viswanathan (author), Anika Shah (advisor), Michelle To (advisor)

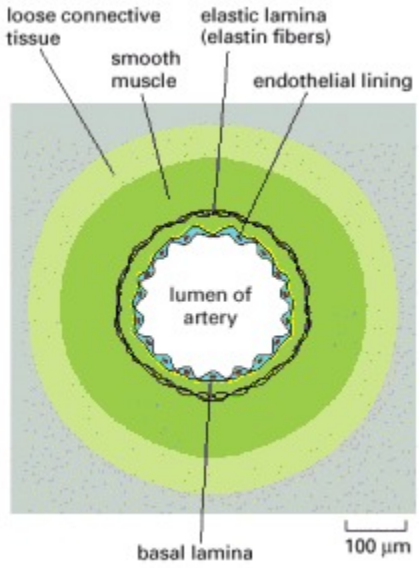
Hillsdale High School

Keywords: Atherosclerosis, Aortas, Leukocytes

Abstract

Atherosclerosis is a blood vessel-related condition in which blood flow is prevented because of blockage in the blood vessels. This can result in heart attacks or strokes occurring, which can cause death. The immune system is connected with the development of atherosclerosis, and by understanding the types of white blood cells involved in atherosclerosis, researchers can find better ways to modify lifestyle to prevent this disease.

Summary



Left: Normal blood vessel cross-section

Atherosclerosis is a disease that involves the hardening of blood vessels. In blood vessels, a layer of cells called the **endothelium** acts as a fence around the blood flowing through. This helps move the blood in the intended direction.

When an individual has high blood pressure or smokes, for example, their endothelium can get injured. This can be due to higher pressure on the vessel or exposure of the endothelium to poisons.

The following injury causes **platelets**, which help the body stop bleeding, as well as different types of white blood cells, or **leukocytes**, to attach to the site. When this happens, growth factors get secreted into the innermost layer of the vessel, causing the endothelium to become open to certain substances. “Proinflammatory agonists such as thrombin, vascular endothelial growth factor (VEGF), and platelet-activating factor, by binding to [endothelial] receptors, disorganize IEJs, leading to increase in endothelial permeability” (Sukriti).

These growth factors affect small junctions in the endothelium. Normally, these junctions only allow minimal passage into the endothelium. However, when growth factors are secreted, these junctions can’t function optimally, so more molecules can penetrate the layer. This secretion allows LDL, a type of cholesterol that leads to negative effects, to come and reside in the innermost layer of the vessel. “The LDLs start to accumulate in the **intima** [innermost layer], and go through a biochemical modification… This further encourages things to go through the wall. Most notably, we start to see monocytes going through,” (Barton). When LDLs go through that biochemical modification, they begin to release **antigens**, toxic agents. This is significant as it kickstarts a massive immune response. **Monocytes** are another type of white blood cell, and when they enter the intima, they start to feed on the LDLs, resulting in bigger cells that are filled with LDL fat. This buildup of white blood cells and fats begins to clog the blood vessels, leading to the blood not being able to flow optimally.

In this study, the atherosclerosis-related immune response was studied in the **aortas** of mice.

The purpose of this study was to fully comprehend the scope and diversity of the immune response in atherosclerosis. By understanding what cells are triggered and how they contribute to the issue, finding more effective ways to treat heart disease may become easier. “A distant goal is to translate findings in experimental atherosclerosis into prevention and therapy strategies for people with cardiovascular disease” (Zernecke)

For this study, a brief introduction to the immune cell types is necessary.

Types of Immune Cells

T-cells are versatile white blood cells that find a condition and differentiate into a type of immune cell needed for a response. **CD4 T cells** help activate a large immune response. **CD8 T cells** help attack viruses and try to cause the death of cells that are infected. **Natural killer T cells** release chemicals that target and kill cells. This can also cause inflammation, which is extremely detrimental in the case of atherosclerosis, as inflammation can further clog blood vessels. **Natural killer cells** kill all cells that are not recognizable by the body. **B cells** are cells that make antibodies, or molecules that help fight infections. **Neutrophils** are the "main defenders against bacterial and fungal infections" (Zernecke). When atherosclerosis happens, some of these are found in the plaque that builds up in the blood vessels. Monocytes, as covered earlier, play a major role in atherosclerosis, but aren't found often in normal blood vessels. **Macrophages** are the immune cells that play the biggest role in atherosclerosis. They first develop from monocytes, and begin consuming LDLs and becoming larger. This results in cells that clog the blood vessels.

Results

In this study, it was found that macrophages were most prevalent in the mouse aortas with atherosclerosis, which is accurate based on the way the development of atherosclerosis occurs. However, interestingly, "macrophage frequencies increase[d] with the duration of [high-fat diet] in Ldlr–/– mice and with Western diet in Apoe–/– mice" (Zernecke). This shows that there is an association between the Western diet (consisting of processed foods) and atherosclerosis.

Discussion

This paper is extremely insightful as it shows how the cardiovascular system and the immune system are intertwined, and how unhealthy habits that increase our LDLs and fats can result in a massive immune response that can harm our bodies. Additionally, this paper places an emphasis on how we can modify our actions to reduce our chances of atherosclerosis, as by reducing our intake of processed foods and fats, we can reduce macrophage buildup in our blood vessels.

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Literary Review

Past, Present and Future Implementations of AI in Healthcare

Charlotte Lungren, Anika Shah (advisor), Michelle To (advisor)
Palo Alto High School

Abstract:

The paper discusses the integration of artificial intelligence (AI) in healthcare. It includes past, present and possible future applications while also touching on the challenges with successfully integrating AI in hospitals. The different types of AI applications in healthcare include, machine learning, natural processing language, and physical robots. AI is already being used in hospitals to help doctors make accurate and efficient diagnoses and treatments, and AI has a lot of potential to be implemented in radiology, surgery, oncology, pathology, and primary care. However, data quality and availability and ethical concerns regarding patient data usage and human decision making replacement pose significant challenges to the widespread adoption of AI in healthcare.

Introduction:

As technology advances, the integration of Artificial Intelligence (AI) in various industries is becoming more common. The Turing test, also known as the imitation game, created in 1950, tested whether computers were capable of human level intelligence, and even to this day no computer has passed. Since the Turing test, there has been almost exponential improvement in AI developments. The first use of AI in healthcare was MYCIN, which was an AI program created in 1970 which was used to identify blood infection treatments (Xsolis, 2021). Despite these promising early advancements, AI did not significantly advance for several decades, particularly in healthcare. It was not until the advancements in computing power and availability of massive amounts of digital healthcare data that AI began to demonstrate significant capabilities broadly, including healthcare. Along with these advancements, the integration of AI in healthcare is becoming increasingly prevalent and it is clear that AI has the potential to greatly improve the accuracy and efficiency of diagnoses and treatments. The current, past and potential role of AI in healthcare, along with the challenges it might pose prompts the question: will AI be able to fully replace doctors in the near future?

Discussion:

Types of Applications:

There have been many different applications of AI in healthcare and it is continuing to develop and help doctors efficiently achieve more accurate results. Pranav Rajpurkar, a biomedical professor at Harvard thinks that, “the role of doctors will change. If you break down their current role, and take out the parts that require certain input of data, I think that is what we can expect machines to replace. Humans are just not able to perform computational tasks with data as well as AI can.” Machine learning, a subtype of AI, entails the use of an AI algorithm to examine data to learn useful patterns that can then be applied to new data. In January 2020, the first ever AI-designed drug, entered clinical trials. DSP-1181 is a serotonin receptor which is currently being investigated as a possible treatment for obsessive compulsive disorder (Wills, 2022). Additionally, natural language processing, the process of using a machine to interpret text and language, can be used to analyze patients' medical records and is improving patient care. NLP can identify patients who are at high risk for developing certain conditions, allowing doctors to intervene early and prevent the spread or worsening of the disease (Akash, 2023). Health providers are also able to use NLP programs to extract relevant information from a patient’s history, and develop a personal treatment plan. Physical robots are also starting to be implemented in hospitals. Initially, robots were solely used for delivering medical supplies, but modern versions can now be used to help humans manage hospitals and perform surgeries. For example, the *da Vinci* Surgical System is a surgery bot that has 3D high-definition controls that a surgeon can wear to make tiny, exact incisions that human hands wouldn’t normally be able to do (Case School of Engineering, 2022). Along with minimizing surgical errors, through robots like the *da Vinici*, hospitals are also using robots to prevent hospital acquired infections. These infections are most commonly caused because hospital rooms can’t be cleaned with 100% sterility between patients, so to prevent them hospitals are using Xenex. Xenex is an automated robot that can disinfect an entire room in just a few seconds by using full spectrum UV-rays to kill microorganisms and bacteria. AI has a lot of potential to further develop in this field, through making humanoid doctors, physicians that would help around in hospitals. Artificial intelligence robots could also be used as surgeons that could perform minor surgeries without much supervision of a human. This would help increase not only the accuracy of the surgeries but it would reduce some of the stress on the actual doctor. Robotic process automation is a type of computer program that is cheaper than other applications of AI. It is most commonly used to record early authorizations, update patients’ medical records, and extract data when combined with other technologies like image recognition. While all different forms of AI are being used in hospitals across the country, the ultimate decision making authority and the responsibility of the patient’s health is still up to the doctor. There are still many ethical, legal, and regulatory issues that must be addressed before AI can be more widely adapted. Thus, AI is not currently replacing doctors, but rather working alongside them to help improve the quality and efficiency of health care.

Current AI Effects, and Applications:

Every year roughly 400,000 patients suffer preventable medical harm, and 100,000 patients die because of human errors (Deloitte, 2020). These deaths are normally linked to incomplete medical histories and too large caseloads. AI is now starting to be implemented to predict and prevent these errors faster than any healthcare professionals. AI is able to generate accurate solutions to problems by listening to a patient's symptoms and health concerns while also looking through the patient's past medical history and looking for patterns. These solutions not only reduce the doctor's workload, they also tend to be more efficient and accurate than a human decision would be. AI is currently largely wide spread across different medical professions. Currently, Mayo Clinic is using AI to help its radiologists in multiple locations across the US. AI is being used to efficiently diagnose patients based on images, blood tests, and genomics. By scanning blood tests, AI can detect cancer and other deadly hematologic disorders. These types of diagnosis applications are the most popular in hospitals. In immunoncology and neurology, AI is helping to create and develop new medicines. AI not only helps with the procedural aspects of healthcare, it also reduces the workload of doctors and manages patient flow by quickly organizing and updating documents. AI is helping doctors to be proactive rather than just reactive.

The Future Of AI:

The future of AI in medicine has a lot of potential, especially in radiology, surgery, oncology, pathology and primary care. "I think that anything that involves having full access to data is something AI will have the greatest impact on because everything that we can't observe we can't use as evidence. (Rajpurkar, 2023). In the near future AI will also be able to analyze scans such as MRIs and CTs to identify patterns and abnormalities that the human eye would normally miss. AI is also developing to be able to personalize a patient's medical plan based on their needs, history and preferences. AI would be able to analyze large amounts of data at one time and search for patterns that humans wouldn't catch. The development of intelligent surgical robots could be used to perform complex surgeries on the brain or the heart. By using AI to analyze real time data through imaging and data scans, robots would be able to provide valuable insights and perform more precise surgeries, and limit small human errors in these important surgeries. Pathology is another field where AI could have a huge impact. "In pathology one of the biggest challenges is that the slides are not digitized, but as soon as that digital transformation happens, that's going to be another area where the machine can just do so much more than pathologists can do in the workflow" (Rajpurkar, 2023).A huge amount of lives could be saved by the elimination of human error. Wearable applications like AI equipped glucose monitors, and smart watches could save up to 313,000 lives by alerting the patient before a condition becomes serious (Deloitte, 2020). Around 42,000 lives could be saved by AI monitoring patient's vitals in hospitals and AI's analysis in imaging could save around 41,000. Not only will AI be able to save thousands of lives, it also will save billions of

dollars and shave hours off of doctor's time. Another future implementation of AI would be in the form of an app. If getting healthcare advice was as easy as just opening an app on your phone, it would greatly improve healthcare accessibility for many patients. “I’d love for AI solutions to be as simple as installing an app. I think that it would greatly change the expectation of what people are used to with healthcare, and that is what I am most looking forward too” (Rajpukar, 2023).

Current Challenges:

One of the biggest challenges with using AI in healthcare is data quality and availability. The data needed for an AI program must be accurate, complete, and representative of the targeted population. This type of data is extremely rare due to patient confidentiality and the inconsistency of data samples. Another consequence of incomplete data quality is accidental biases. Biases occur when the data samples are not reflective of the full population which causes the programs to make unhelpful or inaccurate diagnoses. Without unbiased data sets, AI would not be able to accurately help all types of patients. Additional concerns with AI in healthcare are the ethical questions regarding the use of patient data and the potential for AI to replace human decision making. The most logical solution isn’t always the more ethical one, and that line is crucial in the healthcare system. For example, in some cases a minor surgery would be the most logical solution for treating a minor condition, but it may be more invasive and risky than less effective treatment methods like medication. It is up to the health care provider to make decisions with patient input, not solely based on the risk and benefit of the different treatment options. Another common decision doctors have to make is whether to prescribe a patient with opioid medication for chronic pain. Opioid medication relieves the patient's pain, but is highly addictive and can have serious side effects. AI might prescribe opioids because they would solve the immediate problem without considering the later risks of addiction. A recent study done by Pew Research Center showed that 60% of Americans would not feel comfortable if their health care provider relied on AI, even though the majority thought it would lead to better health outcomes for patients overall. Human compassion and emotions are another crucial part of healthcare services that AI has not been able to replicate. All humans have some relatively similar experiences that shape who they are and allow them to develop a sense of empathy. This is what makes it easier for patients to confide in their healthcare provider, and AI doesn’t yet have the capability to understand human emotions which is what makes people weary of AI doctors. It is crucial that a doctor can report bad news and be understanding of the complex emotions that a patient or a patient's family might be expressing. They also need to be able to support the patient as needed in a way that would be hard for AI to do. Human interactions are a major part of healthcare, and will be one of the hardest parts of trying to fully implement AI in hospitals.

Conclusion:

AI has a huge potential to completely reshape daily life both within healthcare and outside of it, even if it doesn't seem that way right now. "I think that lots of hospitals have not been heavily implementing AI because there is not a very straightforward incentive to do so. If you think about the digitization policy in 2009 that gave a reward for hospitals who digitized, and a penalty to the ones that didn't, that policy is what encouraged all hospitals to move in a certain direction. I think what's stopping us from doing that with AI, is we really don't have concrete evidence that says if we have AI, these specific outcomes will be improved" (Rajpukar, 2023). While, AI is not currently able to replace doctors, and won't be able to in the near future, it has helped assist doctors and lessen their workload. Once we are able to tackle the main challenges that AI developments are currently facing, practically anything will be possible.

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Literary Review

Rise in Antibiotic Resistance

Christina Thomas (author) Anika Shah (advisor) Michelle To (advisor)
Milpitas High School

Keywords: Antibiotic Resistance, Antimicrobial Resistance

Abstract:
Antibiotics, which have been used to cure bacterial infections and diseases, have been under threat of losing their ability to fight off bacteria. This is because certain strains of bacteria are able to adapt and resist the effect of the antibiotics. Some types of bacteria, such as *E. Coli*, are more resistant to antibiotics. This is a part of the larger pattern of antimicrobial resistance. This pattern of antibiotic resistance is more prevalent due to many factors, such as the mismanagement of prescribing antibiotics, the lack of new research into developing new ones and the overuse of them in general. Lack of functioning antibiotics will mean that old diseases, which were once eradicated due to the use of antibiotics, will return and harm a larger part of the population. This review will focus on three different research experiments related to antibiotic resistance; some overall conclusions are that antibiotic resistance can occur with just about any antibiotic, and that multidrug resistance is a common phenomenon among certain strains of bacteria.

Introduction:
Antibiotics and antibacterial drugs are used synonymously, as antibiotics are medications that fight conditions caused by bacteria by either killing them or making it harder for them to grow and multiply (CDC). They tend to aid white blood cells to help fight bacterial infections and can multiply using binary fission, which is when cells grow twice as long before splitting up into two (Cornell). The first use of Penicillin was to cure Streptococci, a deadly bacteria that has caused rheumatic fever, strep throat, and scarlet fever in humans; now it is used for all types of bacterial infections (“How”). However, the overuse of Penicillin has caused bacteria to adapt and become resistant to the medication. This trend has been shown among other antibiotics as well. Some of this is due to the overuse of antibiotics for treatment not related to bacterial infections as well as unauthorized prescriptions (“New”). While increasing the dosage has worked in the past, bacteria have now become resistant to larger doses, thus diminishing the value of antibiotics.

Discussion:
Harvard Medical School and Technion-Israel Institute of Technology

A team from Harvard Medical School and Technion-Israel Institute of Technology studying antibiotic resistance created a two-by-four petri dish (known as the Microbial Evolution and Growth Area, or MEGA) and placed agar on it to allow the bacteria, *Escherichia coli,* to grow (Pesheva). The scientists also divided the petri dish into sections, each with different amounts of the antibiotic trimethoprim, with the outside sections free of any medication. Afterwards, they recorded the process of the bacteria adapting and overcoming the medication for a span of two weeks by using a camera, which they placed on the ceiling. What occurred was that bacteria would spread until they reached a certain dosage of the antibiotic. While the dosage would kill most of the bacteria, a small group of them was able to adapt, resist the antibiotics, move on, and spread out; this was a sign of the mutations that the bacteria were creating to survive. As the bacteria spread through higher doses, new mutants were created to overcome the bacteria. By the end of ten days, the mutants were able to overcome the highest concentration of antibiotics on the petri dish. The team also tried this experiment with a different antibiotic, ciprofloxacin, and saw similar results as to how the bacteria adapt to resist the antibiotics. The researchers concluded that the experiment does provide an example of how bacteria is overcoming medication. Their most significant conclusion from this experiment was that the bacteria create stronger mutants from weaker strains as a way to adapt, and that these "stronger" mutants may be more dependent on the proximity of the antibiotics rather than their actual strength.

One weakness that can affect how quickly and successfully include: geography, bacterial evolution, size, and space. For example, an increase in temperature leads to an increase in bacteria growth and antibiotic resistance. This may be the reason why more people who live near the equator, or in hot and humid climates, die from bacterial infections, compared to those who live in cooler climates (Burnham). Another weakness is that a lab may not be able to accurately represent an environment where bacteria encounter antibiotics. This may be because a lab has a limited amount of equipment, and tries to prevent interference from other confounding variables. Outside the lab, each environment may have different antibiotics with different strengths, thus leading to a slower or faster process of antibiotic resistance. Nevertheless, the experiment is significant because it shows the general process of how bacteria adapt to resist antibiotics.

Novo Nordisk Foundation Center of Biosustainability and Technical University of Denmark

Another experiment related to antibacterial resistance was later published in the Frontiers Microbiology Journal. The researchers used adaptive laboratory evolution experiments (ALE) to observe the different strains of the bacteria and their tolerance to certain antibiotics (Jahn, Leonie J, et al.). Unlike Harvard's experiment, which used one main antibiotic and another antibiotic as a way to retest the theory, this experiment tests antibacterial resistance using three different antibiotics for its main experiment: amikacin sulfate, piperacillin sulfate, and tetracycline hydrochloride. The point of the experiment is to compare the

genotypes (genetic composition of living things) and phenotypes (observable characteristics when the genotype of the living thing interacts with the environment) of the bacteria, *Escherichia coli*, after the bacteria have interacted with each of the antibiotics in four different sections; each section increases in concentration of antibiotics in fixed intervals. They found that the ability for bacteria to adapt was drug-specific, which may have to do with how the antibiotic interacts with the bacteria; amikacin sulfate and tetracycline hydrochloride attack the ribosomes in the bacteria, while piperacillin sulfate attacks the cell wall. In addition, they found that mutations tend to become extinct quicker when antibiotics are suddenly introduced rather than gradually. A lot of the strains towards the end of the experiment tend to be rather strong mutants, as they have adapted from the previous strains' shortcomings. Another finding is that genotypes of different strains end up overlapping even though the extremity of the factors differed. Also, these final strains of bacteria tend to be resistant to other types of antibiotics; this concept is known as cross-resistance.

Similar to the Harvard Medical School antibiotic research experiment, one major weakness of this experiment is that it does not properly replicate natural occurrences. Since it is an experiment, many factors have been controlled and limited, when in the natural world, there are many confounding variables; this may lead to events that may not occur in the natural world. For example, from the ALE experiments, some strains from the experiment did not occur in real life. Nevertheless, this experiment does show a basic pattern as to how antibiotic resistance occurs and confirms the observation from the Harvard Medical School's experiment by concluding that bacteria can resist many types of antibiotics, not just one specific type of antibiotic.

Beijing Children's Hospital, Capital Medical University, and National Center for Children's Health

A team at the Beijing Children's Hospital conducted an experiment related to antibiotic resistance by using the E-test, where they put antibiotics onto a plastic strip with Mueller-Hinton agar (Wu et.al). The antibiotics include: amoxicillin, cefuroxime, amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, cefoperazone- sulbactam, meropenem, gentamicin, ciprofloxacin, and sulfonamides. Then, they took one-hundred *E. Coli* strains from different fluids (such as blood and sputum) from hospitalized newborn children (neonates) and used the E-test to observe which antibiotic the *E. Coli* was most resistant to. What they observed was that the E. Coli strains were most resistant to amoxicillin, then cefuroxime and ceftriaxone. They also concluded that 26% of the *E. Coli* samples were multidrug resistant, or that they were able to resist many types of antibiotics.

One weakness about the experiment is regarding the E-Test. An E-test is especially useful for antibiotic resistance experiments because it is reliable, simple and accurate (Marroki, Bousmaha-Marroki). It is also an easier way to interpret the quantitative results. But, the limitation is that the test cannot be used for many types of antibiotics. This may limit the results of how *E. Coli* may react

with other types of antibiotics. Nevertheless, the results of this experiment are similar to the results of the other experiments, which are that antibiotic resistance does occur and that multidrug resistance is present, especially among the *E. Coli* strains. Another weakness is that, like the previous experiments, this experiment does not properly replicate a natural environment. But, it does continue to prove the pattern of antibiotic resistance as well as the idea of multidrug resistance.

Why use E. Coli?

In all of the experiments mentioned above, *Escherichia coli,* or more commonly known as *E. Coli,* is used. This is because *E. Coli* is a well known bacterium that causes a lot of diseases for people of different ages (Wu et al). It is a Gram-negative pathogen and is a highly drug resistant bacteria, which has caused many public health issues across the globe. By figuring out how *E. Coli* interacts with antibiotics, scientists and researchers will be able to explore new drug possibilities that bacteria are unable to adapt and resist quickly.

How does Antimicrobial Resistance Occur?

Antibiotic resistance is similar to antiviral resistance, as they are both examples of antimicrobial resistance, where viruses, bacteria, parasites, and fungi resist medication that is designed to eradicate them ("Antiviral"). This is mainly due to mutations in the microbes. Bacteria adapt so that they are not affected by current dosages. Viruses mutate so that they do not respond to medication. New viral mutations are more likely to spread across populations and cause new epidemics; one example of this is influenza, where the virus mutates rapidly, resulting in annual vaccine shots to prevent illness. Darwin's theory of natural selection may explain why bacteria may do this; that is, some strains have traits that allow them to survive nature's trials. Through mutations, these adapted traits get passed on; other mutations that are unable to continue their lineage eventually become extinct. However, the research from Harvard's experiment does add to this theory, stating that the stronger mutants are further away from the antibiotics, which means that they will be able to survive a bit longer.

Arising Problems due to Antibiotic Resistance

Due to antibiotic resistance, Penicillin, once a life-saving medicine, is now rendered useless. This is because of the overuse of antibiotics. For example, many people do not know when to take antibiotics, as they do not know the difference between viral and bacterial infections; as a result, they may consume antibiotics for viral infections, which will be ineffective for this particular case ("New"). When they do not see any difference in terms of care, they may take a higher dosage. This leads to bacteria in the body adjusting to the dosages and being able to adapt and resist them. To add to the first example, doctors may misdiagnose a patient's condition and give antibiotics for a condition that is not related to bacterial illnesses. Doctors and pharmacists may over-prescribe medication, or give higher doses than necessary, which can lead to the bacteria adapting to the medication. In addition, antibiotics have been used for

agriculture as they promote the growth of livestock and prevent animals from being infected (Ventola). Research has shown that it promotes growth and creates more agricultural products. However, when people consume meat, these antibiotics are transferred from animals to humans. Due to the high amount of antibiotics used for agricultural purposes, the bacteria become resistant, which will then affect the consumers as well as the environment around the animals. Also, animals release their waste into the environment, leaving the microorganisms in the neighboring areas to be susceptible to resistant bacteria, which could reduce their population. Lastly, pharmaceutical industries have stopped exploring new antibiotics as they are not as profitable, thus slowing down antibiotic research and reducing the types of medication that can be used to fight bacterial infections and diseases. This is due to many reasons: the short periods of use, the low cost to buy them (thus lessening the amount of profit), and the fact that doctors are more hesitant to prescribe them to patients.

The problem with antibiotic resistance is how crucial antibiotics are and how their importance is fading away at a fast rate. In 1962, problems from antibiotic resistance to Penicillin were starting to arise when less than a decade before, it was successful in terms of treating soldiers and those who had serious infections (Ventola). Antibiotics were a crucial step to get rid of diseases such as typhus and cholera. Without antibiotics, it is more likely that these diseases, which were prevalent in the nineteenth and twentieth centuries, will return and be as fatal as they were before the invention of antibiotics. Without antibiotics, life expectancy will decrease, as more people, especially those without access to quality healthcare, will die. This can include people from minority backgrounds, rural or remote areas, poor economic backgrounds, or countries lacking proper healthcare infrastructure.

While it can harm the body, antibiotics are also used in anti-cancer therapies, as they support the immune system and can reduce cancer metastasis, where cancer cells spread to other parts of the body. Other medical uses for antibiotics include treating “chronic diseases such as diabetes, end-stage renal disease, or rheumatoid arthritis; or [those] who have had complex surgeries such as organ transplants, joint replacements, or cardiac surgery” (Ventola). If there is no progress in discovering new antibiotics, then the antibiotics used for these surgeries may be out of use as a way to combat antibiotic resistance. However, this will mean that people who need antibiotics for other benefits (such as for these complex surgeries) have a hard time obtaining them, which can cause their procedures to be more painful and complicated.

Conclusion:

In conclusion, multiple teams of researchers have showcased how antibiotic resistance happens, and how powerful bacteria are when it comes to overcoming high doses of antibiotics. With this knowledge, this may mean that any antibiotics that have been used for a long time are useless. One solution is that the federal government should fund antibiotic research to incentivize researchers to find cures and new

developments. Antibiotic research should be promoted among companies and research universities. Also, stricter laws should be passed to make sure that healthcare workers do not over prescribed doses of antibiotics. Regulation is important as a lack of regulation is causing antibiotics to become useless. One addition should be that courses about antibiotics and how to not over prescribe them should be mandatory so that healthcare professionals are more knowledgeable about the decisions they make. This measure is not just important to protect patients' lives but to also preserve the long-term use of antibiotics. Overall, direct measures must be taken to protect future generations from bacterial infections.

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Literary Review

The Respiratory System Analogy

Dhruti Halambi (author), Anika Shah (advisor), Michelle To (advisor)
Fremont High School

Keywords: Breathing, Respiratory System, Analogy, Oxygen, Gas Exchange, Affinity, Heart

Abstract

When we breathe in and out, there are a lot of components at play. This paper will be explaining the respiratory system through an analogy involving a molecule of oxygen going on a "vacation." In this scenario, the check-in counters, security checkpoint, and pathways leading to the gate are our pharynx, tracheas, and bronchi, respectively. The check in gate is our alveoli, and walking to planes (red blood cell) imitates diffusion. The path the plane takes, the control center, and the arrival airport are all part of the circulatory system, as they are our capillaries, heart, and receiving blood cells. Similarly, when the airplane flies back to its home airport, it is like the carbon dioxide returning to the lungs, and returning out of the airport is like the gas being exhaled.

Introduction

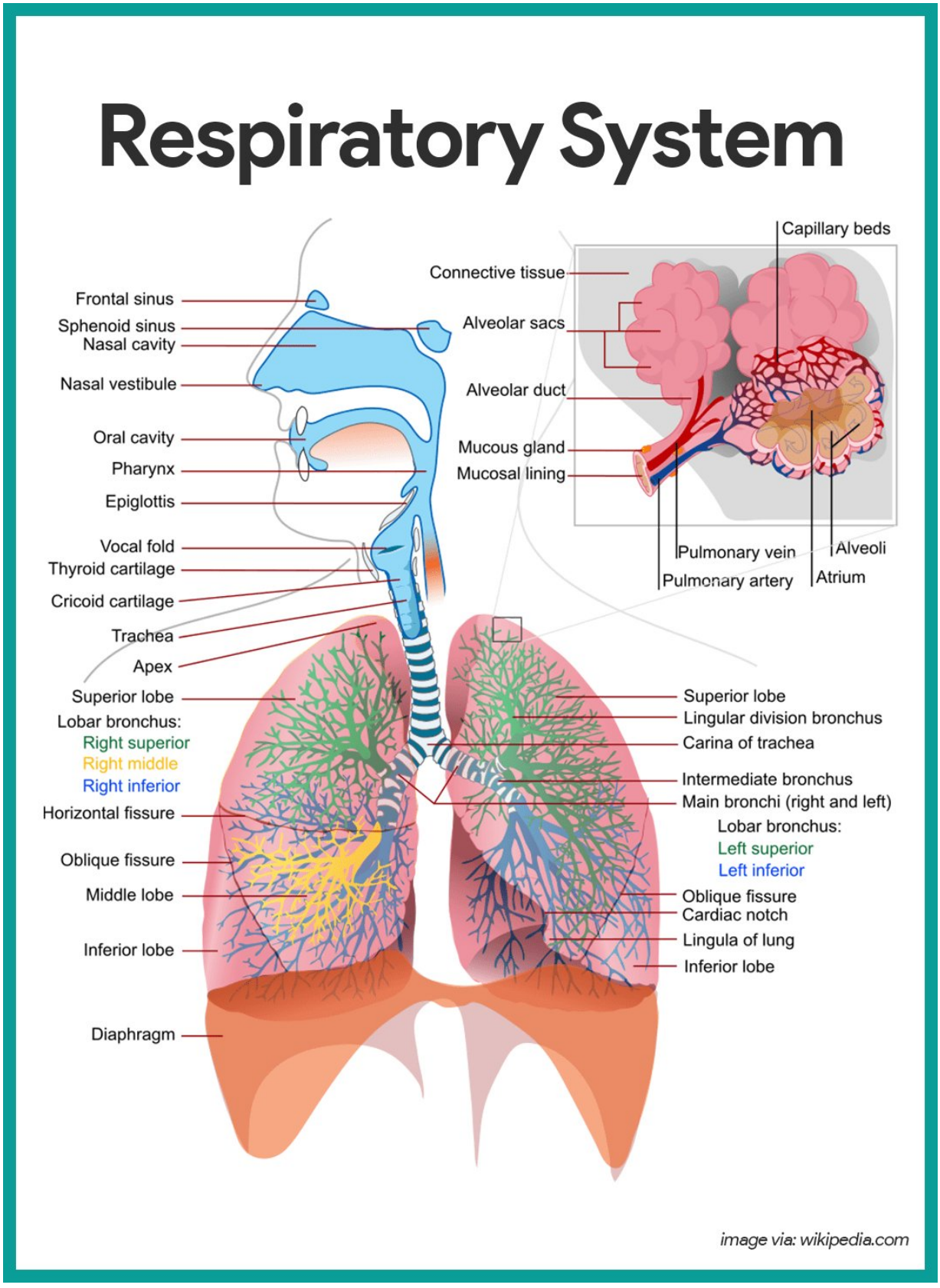
Breathe in. Breathe out. Deep breathe in. Deep breathe out. This process of inhalation and expiration, aka respiration, is something that we have done about the first 10 seconds since we were born (Villines). We have learned to do it unconsciously, upside down, while sleeping, and in total, we breathe in around 13 pints of air every minute (Ferris). However, as symbiotic to life as it may be, do you really know how this process that we do 17,000 times a day actually works (Villines)? In short, it is the respiratory system, a function of our lungs, brains, nose and mouth that is behind respiration. However, it is actually so much more than that. It is the function of the heart, the alveoli, the bronchioles, the windpipe, the pharynx, larynx, orthopharynx, arteries, veins, the list goes on and on. At first glance, the physiology and conduction of the respiratory system may seem overwhelming and unnatural, but all it takes is a simple analogy to understand this beautiful process. This paper aims to combine, comment on, and discuss the research done by other articles into one overarching analogy that accurately simplifies the process of respiration.

For our analogy, take a minute to imagine Mal E. Kuhl, a molecule of O2, more commonly known as oxygen (Oxygen is commonly found bonded to one other oxygen in order to ensure stability), getting

ready for a vacation (he is one rich molecule!). This analogy will be following Mal on his journey to Hawaii, while connecting every step of the way to the respiratory system, using the information provided by various research articles.

Reviews

Of: ***Respiratory System: Functions, Facts, Organs & Anatomy***



^of https://nurseslabs.com/respiratory-system/

The first piece to the respiratory system is the oral and nasal cavity. These are the only two places where air enters and exits the respiratory system. They are separated by a hard palate, a bony plate that can be felt at the roof of the mouth, and a soft palate, nearer towards the back of the throat. Then we have the pharynx, otherwise known as the throat. This is a tube that starts at the end of the soft palate, and ends at the start of the trachea. There are three parts to the pharynx: The nasopharynx, which is where the nasal cavities drain, the oropharynx, which extends from the uvula and contains the epiglottis, which is a cartilaginous structure that acts as a sifter to block food from getting in the larynx (our voice box). Lastly, the laryngopharynx, which contains the larynx, a triangular shaped hollow structure in which air passes through to the glottis to make noise, the glottis, which has vocal chords that tighten and relax following the rush of air.

The trachea serves as a pipe to connect the pharynx to the lungs, and is made up of 16-20 cartilaginous and muscular rings that propel the air to the bronchial tubes. The bronchial tubes are, in fact, tubes that split the air into the right lung, and the slightly smaller left lung via the primary bronchus. The bronchi are lined with hair like projections called cilia, that clean up debris and microbes out of the airways. The primary bronchi divides into secondary and tertiary branches, called the bronchi, which terminate at the end of smaller branches called bronchioles, into the alveolar sacs.

The alveolar sacs contain the alveoli, which are tiny air sacs in the lungs where the exchange of oxygen and carbon dioxide takes place. At the outside of each alveoli, forming a ‘web,’ we have the alveolar capillaries which are a layer of blood vessels which transport our oxygen and carbon dioxide.

Of: ***Animal Gas Exchange and Transport | Organismal Biology***

Now once the air enters the alveoli, gas exchange occurs. We have around 300 million alveoli in each lung, totaling to around 75 meters squared of lung surface area. Gas exchange happens primarily through diffusion, which is transportation driven by a concentration gradient (high concentration of gas drives down to low concentration of gas in an attempt to equalize distribution).

The alveoli are thin-walled sacs that can hold all the air we breathe in, and then diffuse them out to the capillaries. When the tiny alveoli fills with oxygen, the oxygen will diffuse through the alveolar wall (made of one layer of squamous epithelial tissue), through the pressure gradient, and into the capillary wall (one layer of capillary endothelium tissue). The capillaries are a network of blood vessels that distribute blood to every part of the body possible (I like to call this inside-out diffusion, as the gas is moving from the inside of the alveoli to the outside).

In each red blood cell in the capillaries, there are several hundred million hemoglobin molecules. Hemoglobin molecules are proteins, made up of 4 protein chains, called Alphas and Betas. In the center

of each chain, there is a heme, which is a ringlike organic compound and an iron 2+ atom. The oxygen binds to the iron atom in a covalent bond. As the oxygen bonds, the hemoglobin molecule changes its shape, and as it changes shape, it gets easier and easier to bind the other three atoms.

Once the red blood cells have reached their destination, the hemoglobin proteins have a decreased affinity (want) for oxygen, as carbon dioxide takes over and forces the hemoglobin to drop off the oxygen. The oxygen diffuses through the capillaries, into individual cells, which use the oxygen in aid of cellular respiration, which is how our body stays alive. The out product of cellular respiration is CO2, which diffuses through the cells, and enters the capillaries. From the capillaries, the CO2 have a couple different options: They can either be dissolved directly into the blood (around 5-7% of CO2 does it), replace the oxygen in the hemoglobin molecules, and ride back up through veins (around 10% of it), or be carried as part of the bicarbonate buffer system. The bicarbonate buffer system takes the CO2 dissolved within the red blood cells, converts them to hydrogen ions and bicarbonate ions (with the addition of water molecules floating around in the blood), using the carbonic anhydrase enzyme. The hydrogen ion binds to the hemoglobin, forcing any remaining oxygens out, and rides up to the lungs, along with its bicarbonate ion counterparts.

The carbon dioxide containing red blood cells get pumped to the capillaries surrounding the alveoli. Here, the presence of oxygen molecules takes over the red blood cells, allowing the hydrogen atoms to dissociate, bind to the bicarbonate atom, and become carbon dioxide, with the help of the Carbonic anhydrase enzyme.

This carbon dioxide diffuses out of the capillary walls, into the alveolar walls (outside in diffusion), up the bronchioles, through the bronchi, up the trachea, through the pharynx, and out the nasal and oral cavities.

Of:* *How the Heart Works & Pumps Blood Through The Human Body

Now, you might be wondering what role does the heart play in this? Doesn't the heart help with circulation? Yes, it does. Remember when oxygen molecules bonded the heme groups in the red blood cells, and then flowed out through the capillaries, and then somehow ended up at different parts of our body?

It is a bit more complicated. The heart pumps the blood to different parts of the body that it feels it needs, after receiving signals from the brain. But first, let us learn about some quick heart terms: our circulatory system is responsible for pumping oxygen-containing blood through a system of blood vessels, which "are elastic, muscular tubes that carry blood to every part of the body" (WebMD). The three main types of blood vessels are arteries (which carries blood away from the heart), veins (which carries blood to the heart), and capillaries. The arteries and veins start off large, near the heart, and then branch into smaller

and smaller channels as they stray further away from the heart. This is where the capillaries come in, as they connect arteries and veins at points of gas exchange.

The heart is made of muscle, it is this muscle that contracts, creating that pumping motion that allows the blood to be distributed. The heart is a four-chambered, hollow organ. The septum, a muscular wall, divides it into left and right sides, and each side is further divided into a top chamber called the atria, and the bottom chambers, called the ventricles. The tricuspid valve separates the right atria and right ventricle, and the mitral valve separates the left atria and left ventricle. These valves open and close, like double doors, and they prevent blood from flowing in the wrong direction.

The pulmonary circulation loop consists of the pulmonary artery, which connects the heart to the lungs, and carries away de-oxygenated blood, and the pulmonary veins, which carry oxygen rich blood to the heart.

So now that we know all about common terms regarding the heart, how does this all work? Let us start off with the oxygen rich blood cells in the pulmonary capillaries (the capillaries right outside the alveoli). These oxygen rich cells flow to a pulmonary vein, and end up pouring out at the left atrium. The atrium contracts, the mitral valve opens, and blood flows from your left atrium into your left ventricle. When the ventricle is full, the mitral valve shuts. Then the ventricle contracts, and dumps all of its blood to the aorta, which is the major artery that branches out and delivers the blood to different parts of the body. The whole gas exchange, ATP producing, CO2 creating process happens, and now, the deoxygenated blood flows on veins, which ends up merging into the inferior vena cava (brings blood from abdomen and lower), and the superior vena cava (blood from head and arms), which end up entering the right atrium of the heart. As the atrium contracts, the tricuspid valve opens, and blood flows from the right atrium into your right ventricle, and once the ventricle is full, the tricuspid valve shuts. The ventricle then contracts the blood into the pulmonary artery, which delivers the deoxygenated blood to the lungs, where gas exchange occurs, CO2 is expelled, oxygen rides on, and the cycle continues

Discussion

Introduction

Now we know all the terms. It may seem like a word dump, but this discussion will tie it all together, following Mal E. Kuhl on his vacation, and I promise, it will make a lot more sense. A disclaimer to note, this analogy oversimplifies certain areas: for a better, more in depth explanation, please check out other articles elsewhere.

Here are a couple things to note about Mal and his vacation. First off, he doesn't know where he is going. All he knows is that he wants to take a break from just floating around, and actually experience something

in his life. He booked this trip for the spontaneousness of it, and when the plane takes off, that is when the captain of the plane will know the destination. Also, Mal's people do not like to be alone. They like to go in groups, sit in groups, travel in groups, for them, it is all about surrounding yourself with people. Finally, Mal's world has got some weird terms: they call you Oxygen if you are sad, and Carbon Dioxide if you are happy. So as of right now, Mal is an oxygen, as he is getting bored of life.

Inhalation

It's a bright and cloudless day in SF. Mal is cruising down the freeway in his Uber to get to the airport. He is so excited for his journey, and he cannot wait to see what Hawaii is like!

At the airport, (which will serve as the overarching term for the main components of the respiratory system), there are 2 entrances. There is the international entrance (located on the ground floor, also known as our oral cavity), and there is the domestic entrance (second floor, also known as our nasal cavity). The international terminal is separated from the domestic terminal by a floor (which is known as our hard palate, and our soft palate). When his taxi turns at the exit to the airport, he instructs the driver to enter in through the domestic entrance (that is where his ticket tells him to go). The car stops at the loading zone, Mal steps out, collects his luggage, says bye to the driver, and enters the airport via the nasal cavity. Mal is officially in our respiratory system!

From here, he continues on down a little, until he reaches the check in counters. There, on the signs, he sees that his check in counters are on the international floor. So, from there, he takes an escalator down to the international terminal (going down to the nasopharynx), and finds the check in gate for his airline, RISPA Tory AirLines. At the check in gate, they do two things. First, they check his ID and passport, to make sure that he is a valid citizen, and that he is not impersonating someone else. In our respiratory system, this is the work of the oropharynx. It has the epiglottis, which opens and closes to make sure that only oxygen enters our respiratory system, blocking away food. After that, then, they print out his plane ticket, stamp it with the gate that he has to go to board, and give him the go to move on! In our respiratory system, this would kind of be our laryngopharynx. Its main job is to let the air pass through it (resulting in noise, speech) and it uses the glottis (which would be the computer in this case), to do so.

So, Mal stands in line at the ticket counter, they check his passport (he is through to oropharynx!), and then they give him his plane ticket, and send him off! (he is through the laryngopharynx!)

Then Mal has to go through the security checkpoint, otherwise known as the trachea. It is quite long, and connects the upper part of the respiratory system (aka check in) to the lungs. Also in the trachea, goblet cells and cilia, (security guards) secret mucus (metal detectors, x-ray machines) to expel foreign particles, (unwanted substances, like alcohol). Now, after the security checkpoint, the airport splits into a left wing

and right wing (the left and right Bronchi). Each wing goes down a bit, till it splits into terminals, and this distance is known as the secondary bronchi (or the lobar bronchi). The terminals then branch out into piers and this distance is also known as the tertiary bronchi (or the segmental bronchi). Finally, from the piers to the actual, singular gate, the distance is known as the bronchioles, the gate waiting room being an alveoli. (Note, around 10,000 alveoli connect to one bronchiole, so that would mean that there would have to be 10,000 waiting rooms in one gate, but that would be impractical, so we are going to simplify it to be 1 alveoli per bronchiole). But enough about the airport. Let us talk about Mal. Mal has to get to gate L12r, which is located in the left wing, terminal 1, pier 2, gate r. So, he goes through the security checkpoint (the trachea), turns to the left wing (following the right bronchi), turns at terminal 1 (this distance is known as the secondary bronchi), walks to pier 2 (aka follows the path of the tertiary bronchi), and then runs all the way to gate R (going down the bronchioles), to arrive at his plane's waiting room (alveoli). Now here is where the vacation begins.

Gas Exchange

Well, first of all, he sees so many other oxygen molecules fill up the waiting room with him. It looks like it's going to burst! So, the flight attendants decide to start boarding. They say, "Good morning ladies and gentlemen. On behalf of RISPA Tory AirLines, it is my pleasure to welcome you aboard flight 10,000 with service to a Surprise Location. At this time, I would like to inform you about the boarding process. When your boarding group is called up, line up by the ticket counter. When it is your turn at the ticket counter, please present your passport, and wait for approval. Then, walk to the double doors, go through them, walk down the ramp, and arrive at the plane. At this time, I would like to welcome all members in First Class to counter number A. That is, all First Class members to Counter A…" And so the boarding behind. But what does this mean for us? Well, the checking in at the ticket counter is what oxygen molecules do when they diffuse through the alveolar walls, and going through the double doors is equivalent to diffusing through the capillary walls. If the plane is 1 red blood cell, then walking down the ramp is equivalent to the oxygen molecule finding its host red blood cell. (Note: in reality, all the oxygen molecules in 1 alveoli do not fill up only 1 red blood cell. It's more like hundreds of them. So, if we were being 100% accurate, there would be hundreds of planes at the end of one ramp, but that wouldn't be realistic, so we will leave it at that.) Now, back onto Mal. He waits for business class to be announced (he is a classy molecule), checks in at the counter (diffuses through the alveolar wall), walks through the double gates (diffuses through the capillary wall), walks down the ramp, until he gets in the plane (the red blood cell).

Oxygen Affinity

And then, we have the seat selection. The plane is made up of 2 columns of 4 seat-rows each (it is a very wide plane). Let us call each row of each column of 4 chairs a Hemoglobin Molecule. Now, remember how hemoglobin molecules are made up of 4 protein chains? Each protein chain is 1 seat. Also remember how each protein has a heme group, which contains an iron atom that the oxygen bonds to? Let's call the cushion of each seat a heme group. 4 cushions, 4 heme groups, 4 seats, 4 protein chains, = 1 hemoglobin protein. When the people arrive, they will bond with the softness of the chair cushion, and they will sit on it. (This is how the oxygen bonds to the iron in the heme group: It is attracted to it!) Now, remember how Mal's people do not like to be alone? Well, that applies in planes too. They wait for someone else to sit in an empty row, and then, they will sit right next to them. It is always hard for one molecule to bond first, but once he does, it is easier for the rest. (This idea that it is harder for the first oxygen molecule to bond than the others is called oxygen affinity). So Mal scans the plane over for groups of chairs that look like there are other people in it, and one catches his eye. He finds one in the middle that has 2 open seats, so he walks over there, puts his luggage in the overhead compartment, squeezes into his seat, examines the softness of the cushion, and finding that it matches his taste, he sits down. He is now binded to an iron atom in a heme protein which is part of a protein chain which makes up a hemoglobin molecule which is what all red blood cells contain.

Oxygen Transportation

Soon after, the plane begins to taxi. It backs out of its gate, turns onto the tarmac to the runway, and after a few minutes of taxiing, it arrives at the runway, and waits for further instructions from the control tower. But what does this mean for us? Well, the control center, for us, is the heart. So, another name for the route which the oxygenated red blood cell (plane) travels from the lungs (airport) to the tarmac, is called a pulmonary vein, which if you remember, is the route through which oxygenated red blood cells travel from the lung to the heart.

Now, the control center does its job. Remember how the plane, as of right now, doesn't have a destination? The control center is going to change that. Here is how the control center works: There are 4 groups in the control center, which each have a different function. The Left Atrium group, or the group in the topmost left corner, takes in signals from planes departing, and collects information about the passengers, etc. It then gives all of these signals to the Left Ventricle, (through a usb drive nicknamed "Mitral Valve") or the bottom most left group, which picks a destination that they think the people might enjoy, conjures up a air route for them to follow, and sends them off through the Artery Routes. (there are two types of air routes = arteries, which carry a plane to its destination, and veins, which bring back the plane to its home airport). The opposite happens for arrivals. When a plane arrives, following the vein routes, the signal goes to the right atrium (top right section), they collect information about the flight, put

them on a USB drive called the tricuspid valve, then pass it on to the Right Ventricle group (bottom right), which gives the plane a gate to park at (taxi route is called the pulmonary artery), but more on that later. So, the signal of flight 10,000 RISPA Tory AirLines reaches the control center, the left atrium collects all the info regarding the plane, sends it to the left ventricle through the USB drive (mitral valve), and the left ventricle, after careful consideration, decides to send the plane to…HAWAII (our right hand)!! So, to summarize, our red blood cells reach the heart, collect in the left atrium, go through the mitral valve, down the left ventricle, and are on their way to be pumped to our right hand through Arteric Routes.

Gas Exchange

The flight was pleasant. Mal, our oxygen molecule, rides through the world, and arrives at the beautiful island of Kawaii. He gets out of the plane, walks out of the double doors (diffuses through the arterial wall), and checks out at the check out gate (diffuses to the capillaries), and is in Hawaii! This is equivalent to red blood cell arriving at the hand, unbinding its oxygen molecules in its hemoglobins due to decreased affinity (everyone is getting up from their chairs, because they have reached their destination, and nobody wants to stay on the plane), and then diffusing them into the capillaries of the hand to be sent out to individual cells in the hand to make into ATP.

Hawaii is a beautiful land. Mal has so much fun driving down by the coast, admiring the waves, basking in the sun, gazing at the lush forests, surfing, and hiking. He loves Hawaii. So much so, that he can now be labeled as a Carbon Dioxide: A happy molecule! (remember, oxygen = sad molecule, carbon dioxide = happy molecule). This process of converting the oxygen to carbon dioxide, in our body, is called Cellular Respiration. We take the oxygen that we breathe in, send it down to parts of our body that need it, and in turn, they convert the oxygen (with the help of other materials) to energy, called ATP, which they can use to perform their functions. The outproduct of this chemical reaction conducted in our cells is carbon dioxide. So now, Mal, our dear old oxygen molecule, has gone through cellular respiration (has had fun at hawaii) and now is Carbon Dioxide!

Carbon Dioxide Transport

Sadly, his time in Hawaii is over. Even the sun sets in paradise. Mal has to go back now, but he is happy knowing that he got to experience hawaii. He has got a couple ways he can travel back. The first one, is well, he doesn't go back. He might just want to move to Hawaii, and dissolve into Island Life. (5-7% of CO_2 molecules do this). He also might want to ride back on the red blood cell hemoglobin molecule, binding to the heme just like he did when he was an oxygen, traveling on the veins back to the heart, and then the pulmonary artery to the lungs to be exhaled out, the opposite of the whole arrival system (go via plane, 10% of CO_2 molecules do this, and when they do, it is called carbaminohemoglobin). The third,

and last way he could return, is definitely a little complicated (so much so that the following analogy will only scrape at it, so make sure to research more if this is something you are interested in!). It is called the bicarbonate buffer system, and it is when the Carbon Dioxide undergoes more reactions to split up into a hydrogen ion, (which rides up on the red blood cell), and bicarbonate, which follows the red blood cell up through acid-base homeostasis

So here is how it looks for Mal. While in Hawaii, he met a friend who can fly a helicopter. Now, Mal really wants to go back in this helicopter, but there is a weight limit. He has to split up his luggage, and send the luggage on the plane. (The plane has the same heme/red blood cell format as it did before, his luggage bonds as a H+ ion, and he becomes bicarbonate). He follows the plane on the arteric routes, because he doesn't want to lose his luggage (acid-base homeostasis), and once in the airport, he meets up with his luggage, and goes back to being carbon dioxide. (Most carbon dioxide splits up and travels this way).

That is a lot of options, but it looks like Mal really wants to go in that helicopter. So that is what he does. He splits up, leaves his luggage at the airport, becomes bicarbonate, follows the plane through the bicarbonate buffer system, and travels through the vein routes, until he reaches the control center of the SF Airport, aka the heart.

Expiration

If you remember how the heart, aka the control center works, the planes signals reaches the right atrium, which transfer all the data of the helicopter on a usb stick (called the Tricuspid valve), and send it to the right ventricle, which takes that information and verifies it, sending it the gate of the plane that has his luggage, then allowing the helicopter to land near the plane. Mal is (sadly) home! He meets up with his luggage near the ramp, diffuses through the capillary and alveolar walls (goes down the ramp, through the double doors, checks out, etc), and ends up in the alveoli (waiting room gate). Following the same path he took when he went into the airport, he exits out of the alveoli into the bronchioles (gets from the waiting room to the gate), goes down the tertiary, secondary, and right bronchi (goes out of the gate to the pier then to the terminal), and goes to immigration (trachea). Carbon dioxide follows the exact same path oxygen does, just it goes the other way out.

Mal is almost back! At immigration, (He doesn't have any check in baggage, so he skips baggage claim) they check his passport, check his luggage, etc (he passes through our pharynx), and it all looks good! Mal is one good molecule. Still thinking about his wonderful time in Hawaii, Mal tightens his backpack, saunters his way out of the airport (through the nasal cavity, because he is arriving domestically, of course), and goes out into the world!

Conclusion

Now you know how the respiratory system works. It's a magical symbiosis of so many components doing their work, I find it a great wonder on how something so complex and vast can be conducted day to day, effortlessly. To summarize, first air enters in our lungs, and goes down all our pharynxes. This is symbiotic of Mal entering the airport, and checking in. Then, air goes down the trachea, and through the branching bronchioles till it reaches its alveoli. This is like Mal going through the security check, and walking to the waiting room of his gate. Then, the oxygen diffuses out of the alveoli into a red blood cell in the capillaries, binding to a heme group. This is like Mal going into the plane and finding a seat. Then, it goes to the heart, which pumps it to a location in the body. This is like the control center telling the plane where to go, and then sending it off. Once the oxygen reaches its destination, it unbinds, goes through the process of cellular respiration to produce carbon dioxide, and rides back to the lungs in one of three different ways. This is like Mal having so much fun in Hawaii, he becomes carbon dioxide instead of oxygen, but then he has to go back home. Then, they go back to the heart, get pumped to the lungs, diffuse through to the alveoli, and then go back out the way they came in, getting exhaled at either the nasal cavity or oral cavity. This is equivalent to Mal reaching his airport, the control center telling him the gate at which to stop at, him getting out, walking to immigration, going through immigration, and walking out of the airport.

It is a lot. It took me a week of watching youtube videos, surfing through hundreds of articles to understand it a little bit. Which is why I created this article. I hope you have at least a general, vague idea on how our respiratory system conducts itself, without having to go down the internet hole. That being said, this analogy very barely skims the surface of the respiratory system, so if there is a part that is confusing you, a part that you want to know more about, or a part that you feel wasn't mentioned here, surfing the internet is perfect for that.

So, the next time you take a breath in, take a minute to imagine Mal E. Kuhl entering your "airport", going down your trachea, binding to a red blood cell, riding to Hawaii, or wherever else in the world you take him to!

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Research Article

Liposomal Drug Delivery for Pediatric Glioblastoma

Disha Divakar (Author), Anika Shah (Advisor), Michelle To (Advisor)
San Marin High School

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Abstract

Pediatric glioblastoma is now the leading cause of death from cancer in children yet the treatment options are narrow and challenging. Today's treatment can be ineffective and the options are hard to pick from. The challenge is that the child's brain is developing and sensitive. So, the typical treatment of toxic chemotherapeutic drugs or invasive surgery for tumor resection leads to long-term complications. Liposomal drug delivery has shown to be a promising solution. It can directly deliver the drug temozolomide to specific cancer cells rather than harming healthy brain cells. Liposomes effectively cross the blood-brain barrier so the drug is administered more effectively and actually makes it to the brain, by crossing the blood-brain barrier, and target cells. The use of a temozolomide delivering liposome is a game-changer for these children. It takes away the painful decision-making, the risks on the developing brain, and the life-altering damage that could happen.

Introduction

Pediatric Glioblastoma is a challenging, fast-paced, and devastating brain cancer that leaves children in lots of pain with the treatment available today. We struggle to fully get rid of the tumor, minimize side effects, or effectively stop the disease. It makes it more challenging because these children's developing brains are so toxically harmed by the chemotherapy, radiation, and invasive surgery we have today. This troubling situation calls for the need to search for a new solution and a nanoparticle-based treatment plan shows promising results. Pediatric glioblastoma is the 2nd most common tumor in children and the most aggressive kind of brain tumor. It is a malignant brain tumor starting in astrocytes; those are brain cells that help the central nervous system store energy and support neurons. The symptoms of pediatric glioblastoma usually involve headaches, seizures, and cognitive issues. Once diagnosed, the general life expectancy is around 15 months as it is fast-paced, resists treatment, and can also

tend to be caught late. The location of the tumor is generally in the supratentorial part of the brain. This is the section that holds key elements of the brain including the cerebrum, lateral ventricle, third ventricle, choroid plexus, pineal gland, hypothalamus, pituitary gland, and optic nerve. This is the major part of the brain and so the treatment which includes resection surgeries and radiation therapy proposes a serious threat to the whole brain and in turn the functions of the whole body. Today's treatment challenges are what is calling for a more innovative approach in the first place. To start, the diagnosis and treatment itself can be very challenging due to the difficulty in identifying both where as well as how much the glioblastoma has infiltrated the brain. Because of this, even surgery cannot fully tumor the tumor. It's very difficult for doctors to identify where the cancerous vs. normal parts of the brain are because of a "gradient of cells". Also, trying to catch it early is not an option due to the above reasons and there are no diagnoses or therapeutics for early-stage glioblastoma. This causes diagnoses to generally be at the dangerous stages making treatment even more difficult. This all means that the cancer has to be treated *very* aggressively and is very harsh on the body from the intense radiation, chemotherapy, and many surgeries as the tumors aren't always fully found or can be recurring. To continue, the cytotoxic/cytostatic drugs used in the treatment cause detrimental effects on the healthy cells around them due to the lack of targeted therapy. So, when it is pediatric, it is very hard to make a treatment choice for children due to the concerns of intense radiation and toxic drugs on the child's developing brain. The use of nanobots for pediatric glioblastoma will significantly improve treatment options. By definition, nanoparticles are matter that exists on a nanometer scale. 1 nanometer is a billionth of a meter, and to give you some perspective the width of a DNA strand is about 2.5 nanometers. Due to their incredibly small size and various qualities, they are a revolutionary technology in the scientific, and medical world. Common types of nanoparticles are micelles, liposomes, dendrimers, and carbon nanotubes. Nanobots function at the scale of biological processes and molecules within our bodies. Meaning, they can directly interact with the specific molecules and DNA in our body and can travel to such small areas that other technologies can't make them uniquely useful. On top of this, nanoparticles come in millions of shapes and forms allowing scientists to be able to design them to their needs. For example, they can mimic white blood cells, different bacteria, and sperm, or even carry drug loads or biological markers on them. On top of this, nanoparticles can access regions of our body that we aren't able to, such as the Blood Brain Barrier. Nanoparticles are extremely useful for

preventative, diagnostic, and therapeutic medicine. They help with imaging and early diagnosis by using biomarkers for scans and detecting changes in tissues at a cellular level. They are very helpful with treatment, especially drug delivery due to their ability to non-invasively and directly administer medicine to the specific area/tissue. (Gao et al.) They are able to be engineered with specific attachments or ligands that will connect them to the appropriate cells. This eliminates side effects and makes treatment work better and more efficiently due to its precision and target. Adding on, liposomes are a specific type of nanobot and are composed of lipid bilayers with an aqueous internal component. They show success in drug delivery due to their biodegradability, low toxicity, biocompatibility, ability to load hydrophilic and hydrophobic drugs, and ability to cross the blood-brain barrier. (Dutta) They are positively charged and with ligands that are specific to the BBB, they can easily get through using endocytosis. They are essentially artificial vesicles. In the case of pediatric glioblastoma, they would noninvasively remove the cancerous cells and maintain the healthy brain cells due to their ability of targeted therapy and ability to cross the BBB. To explain, the blood-brain barrier is a system of blood vessels and tissue that protect and prevent the brain from specific substances.(National Cancer Institute) It's like a wall that will only let certain molecules in. This close-knitted barrier only allows in small molecules, fat-soluble molecules, some gasses, and molecules like glucose that go through transporter proteins. This makes it a challenge for treatment as it is very difficult to get medicine or drugs directly to the brain. It lowers the efficacy of the medicine. This obviously poses a major concern for the treatment of glioblastoma because cancer requires aggressive, intense, and targeted therapy without something like the BBB in the way of it. So, by using nanoparticles, we can expect directed and efficient drug delivery. In the past, researchers have attempted to use nanoparticles to attempt and treat different types of cancers and tumors. One experiment was a paclitaxel albumin-stabilized nanoparticle formulation to treat metastatic breast cancer. 100 mg/m^2 was provided to the patient in a schedule consisting of 3 weeks on and 1 week off. 39 patients went through this testing. Paclitaxel albumin-stabilized nanoparticle formulation also known as Abraxane is FDA-approved and is used in breast cancer, pancreatic cancer, as well as non-small lung cancer. (Paclitaxel Albumin-Stabilized Nanoparticle Formulation, Gemcitabine, and Bevacizumab in Treating Patients with Metastatic Breast Cancer). Doxorubicin is an anti-cancer drug that is known for posing cardiac toxicity. But when encapsulated in a liposome, the toxicity was reduced by 50%. This is due to liposome's ability for site-avoidance delivery.

Materials and methods:

(Results and procedure are purely hypothetical, they were not performed)

The objective of this proposed treatment is to effectively treat cancer cells and protect the healthy parts of the brain. The experiment being conducted is trying to achieve a successful temozolomide-delivering nanobot that successfully reduces the side effects of today's treatment. The reason for using a liposomal nanobot is that it can cross the BBB. Also compared to other nanobots for drug delivery, they work very well due to their biocompatibility, biodegradability, and lesser toxicity. They help prevent side effects as they are great at avoiding toxic exposure to other tissues. So, the needed resources for this experiment are a liposomal nanobot, temozolomide, and a sample of gliomas. First, the liposome was derived using the ether injection method. (Akbharzadeh et al.)In this method, the solution of lipids dissolved in diethyl ether was slowly injected into an aqueous solution of the temozolomide under reduced pressure. Transferrin will also be a ligand attached to the liposome to help the liposome precisely bind with the cancerous cells in the brain and enter the BBB. This is because cancerous cells have overexpressed transferrin receptors. The fact that it has this ligand attached eliminates the issue of harming the healthy and developing brain with treatment. Liposomal nanobots were made with the construction of a phospholipid bilayer that includes cholesterol to help stabilize the membrane. To prevent its detection by the immune system, it was coated in PEG(polyethylene glycol) which is a hydrophilic polymer that will help avoid interaction with blood components. The liposomes measure a length of 30 nm. Then, 150mg/m^2 of temozolomide is split between multiple liposomes for the correct dosage. It is inserted into the liposome by including it in the lipid film as it is a lipophilic drug. The temozolomide was loaded into the liposome actively. To do this, a pH gradient was created, an acidic solution next to a normal solution, which let the unionized temozolomide travel into the lipid bilayer and become ionized which causes it to stay there trapped. To increase the shelf life of the liposomes, they will be freeze-dried rather than suspended. The liposomes are stored in containers at 46 degrees Fahrenheit to preserve both the liposome and the temozolomide.

As for the experiment, the dependent variable is the glioma samples and young mice and the independent variables are the course of treatment we administer (the traditional way vs. the use of liposomes). First, a patient-derived glioma xenograft was used in the mouse models. This

is when a tumor sample is obtained from a patient, then used to make a cell line, which is then placed into the mouse's brain. (Noriyuki Kijima and Yonehiro Kanemura.) The mice are younger to test the effects on the developing brain as the focus is on pediatric glioblastoma. Mice will be treated with the temozolomide carrying liposome and to observe the difference, we will examine the old way of temozolomide administration on a young mouse and glioblastoma sample of the same grade. They were intravenously administered the treatment every day for 14 months. The intention of the treatment is to reduce and hopefully kill brain tumors. MRI scans were then used to track the reduction of the tumors.

Results:

Because this experiment was hypothetical, this article will use knowledge and hypothesis. After 6 months, the tumors decreased fully or mostly when treated with the liposome model. After a year, researchers would observe and check the progression of the tumors in the mice and hopefully see a decrease in size as well as no other toxicity to healthy cells and abilities. The way of evaluation would be through MRI testing. On the other hand, the mice and samples treated with the regular administration of temozolomide would show to have recurring tumors, and cognitive imparities from the toxicity, and the mice often passed away due to ineffective treatment. A few mice may not survive even with the new treatment, which most likely resulted from errors made in the liposome or the condition of the mice. Errors in the testing may be the dosage level administered or a malfunction of the liposome from manufacturing issues such as leakage from the bilayer. The way this data was collected was through cancer imaging and screening. This involves the use of periodic MRI scans. MRI (magnetic resonance imaging) scans use magnetic fields to show images of the soft tissues in the brain. (Newman)The machine is shaped like a tunnel. The scans can then be evaluated by a radiologist or the doctor to record the difference in the tumor growth. Neurotoxicity can be tested through evaluating cognitive abilities.

Discussion:

The results of the liposome model show an increase in the treatability of pGBM along with protective abilities on the developing brain. We can see that the liposome was successful in direct and targeted treatment as well as in reducing toxicity to healthy tissue. These results help provide

a new and very modern option to treat pediatric Glioblastoma. We were able to successfully determine a better way to treat it**.** A limitation of this liposomal drug delivery is that there can still be a chance of the patient becoming resistant to the temozolomide. To combat this researchers can try to experiment with a better drug for the liposome to carry or perhaps a ligand that can be attached to kill the cancer cells even more effectively. To add on there are some disadvantages of liposomes that researchers should look at and try to better. They include accidental leaking of the drug, a short half-life, and low solubility. These disadvantages are mostly manageable by taking some extra time in the manufacturing process. For example, shelf life can be extended by freeze-drying and oxidation can be prevented by doing all manufacturing without the presence of oxygen. Also, liposomes are high-cost production due to the raw materials needed which poses a limitation both to the consumers and producers. Another thing people can research in the future is perhaps the use of liposomes for diagnostic imaging as there is still no way to detect the early stages of glioblastoma. Liposomes can carry or function as markers and contrast and help show where the cancer is located. The development process of a child's brain is crucial to their life and lasts with them. With the current treatment options, we have for pediatric glioblastoma, it's an incredibly tough choice to make. Not only is cancer on our minds, but the life and future of that child are also permanently affected. That's why it's crucial to develop a therapy that will prevent the life-damaging side effects of cancer treatment. By making use of new advances like nanotechnology, pediatric glioblastoma will be able to be managed and treated effectively while protecting the precious future and state of the child's brain and body. Cancer is the leading disease-caused death among children and brain cancer is one of the top reasons of death among this. This shows that the need for better treatment is critical. These findings open up the doors to more productive and less detrimental treatment for children with glioblastoma or any cancer even.

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Literary Review

Cardiovascular Disorder Linkage

Jibraan Saeed (author), Anika Shah (advisor), and Michelle To (advisor)

Keywords: Sudden Cardiac Arrest, Ventricular Fibrillation, Arrhythmia, Cardiopulmonary Resuscitation, Heart, Cardiovascular System

Abstract

The cardiovascular system in humans is responsible for the flow of blood, which carries oxygen and nutrients, throughout the body. It, like all other systems in the human body, is prone to developing disorders, many of which can be fatal. Sudden Cardiac Arrest (SCA) is a disorder where the heart loses function due to a malfunction in electrical signaling within the heart, and it often results in death. SCA prevalence is higher and aftereffects are often worse in women as opposed to men, largely due to societal gender concerns, such as the fear of injuring a woman when administering cardiopulmonary resuscitation (CPR). Because SCA is caused by an electrical malfunction, it is possible that other cardiovascular diseases that cause ventricular fibrillation (the electrical malfunction), such as Heart Valve Disease, Coronary Artery Disease, and Heart Attacks, are all linked to one another, and more importantly, to SCA.

Introduction

Diseases in the Cardiovascular System

Cardiology is the study of the cardiovascular system and diseases that affect it. The human body's cardiovascular system is responsible for the delivery of oxygen and nutrients throughout the body via red blood cells which are pumped by certain structures. The system includes a couple of defining components: the heart, blood, arteries, veins, and many more. This system is intricate, with the average human heart pumping roughly 2,000 gallons of blood daily, and with nearly 60,000 miles worth of blood vessels in the average adult. The heart itself is divided into four chambers, the right and left atria and ventricles. Valves throughout the heart and its vessels ensure that oxygen-rich and oxygen-poor blood flows the correct way. Countless capillaries carry oxygenated blood to where it needs to go in the body. Arteries move blood away from the heart, veins move blood to the heart, pulmonary vessels exchange blood between the heart and the lungs so they can receive oxygen, and systematic vessels exchange blood between the heart and the body.

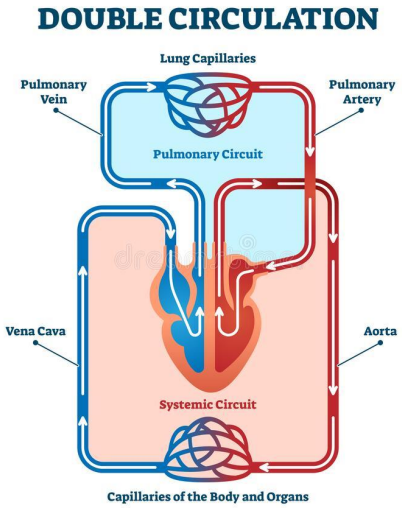

Image from: https://www.dreamstime.com/illustration/pulmonary-circulation.html

With such an intricate system, disorders like arrhythmia, valve failures, and countless others can occur at any point in time. Arrhythmia is an irregularly ordered or paced heartbeat. It usually stems from a malfunction of electrical signaling in the heart. Although often benign, more disorders are prone to development as a result of arrhythmia. Valves are structures in the heart that ensure the correct direction of blood flow. In the case of valve failure, for whatever reason a cardiovascular valve is not functioning and blood is flowing in the wrong direction. This can result in oxygenated blood and deoxygenated blood getting mixed up and as a result, cardiovascular failure. Articles that have identified sources, proposed solutions, and also suggest that such diseases may be linked to one another are presented in this text.

The many cardiovascular diseases in humans are linked because one of them can cause another, resulting in a domino effect that could potentially result in total system failure, and thus death.

It is important to review this topic because heart diseases are the current leading cause of death in the United States. Researching how they are linked can help us minimize if not eradicate damage done to the heart, so society is better protected against this terror.

Body

What SCA is

Sudden cardiac arrest (SCA) is the cause of 20% of all deaths in developed countries. Sources from all across the world indicate death rates of up to 100 deaths per 1000 people purely from SCA. Additionally, more than 50% of all coronary heart disease deaths are caused by SCA, meaning the arrest led to the development of additional complications in the heart, which eventually died from another disease.

As for the disease itself, SCA is when an irregular heart rhythm, stimulated by a mixup in electrical signals, causes heart inactivity. Unless immediately given therapy, someone who experiences SCA will

stop breathing, blood will stop being pumped throughout their body, and they will die. Visible symptoms include someone suddenly collapsing, not breathing, without a pulse, and unconscious. Patients who have recovered from SCA describe the feelings before as chest discomforts, shortness in breath, heart palpitations, and physical weakness; however, it occurs without warning or prediction. Aftereffects include permanent brain damage due to the lack of oxygen rich blood flowing throughout the body.
The actual cause for SCA is very complex. Risk increases as people age, but the incident is more commonly seen in males than females at any given age, although women are more severely impacted. Racial differences also potentially contribute to SCA frequency. Hispanics have a significantly lower incidence than non-Hispanics in the US, with a mere 7.5% makeup of SCA occurrence. African Americans, whose occurrences make up 24.7% of out-of-hospital cardiac arrests, have a significantly higher incidence and mortality than other racial and ethnic groups. Many factors have been observed to see if there was a specific demographic cause for the frequency of SCA, but none was found. What researchers did find, however, is that markers like fatty acids and cytokines have been related to SCA. Many current methods, such as carefully analyzing T-Wave alternans, are being implemented to further examine the exact causes, if any, of SCA.
After many trials and different experiments, researchers were able to conclude that heart failure symptoms can be managed using ACE inhibitors and beta blockers. Studies indicate that the drug eplerenone, a blood pressure medicine, has been pretty useful in preventing SCA. Preventing SCA as of now is still very challenging, but is being researched all across the world and constantly tampered with. No solution for SCA has emerged from the current studies, but organizing the issues is society's first step, so progress has been made.

Why Women are Less Likely to Survive SCA Than Men

Researchers in the article hoped to find out why women are more severely impacted by SCA in the long term. Sudden Cardiac Arrest (SCA) kills almost 6 million globally in a year. Symptoms start with chest pain, dizziness, nausea. It occurs quickly and can be lethal just as quick, so the typical primary treatment is instant CPR administration until further help arrives. Aside from CPR, AEDs can also be used on an SCA victim. SCA is unpredictable and therefore hard to provide long term solutions or preventions for. SCA is not to be confused with a heart attack, where an artery becomes blocked and the heart is left devoid of blood, leading to a failure. Rather, in SCA, the heart's electrical system malfunctions. Electrical signals from the brain and spinal cord control most organ communication and function. In the event that the heart's electrical signaling is flawed and it stops working, no pumping of blood will occur, meaning the rest of the body will not receive any oxygen and nutrients that the cardiovascular system is responsible for pumping, and eventually the organism will die.

Researchers concluded a couple of reasons, social and medical, for why women are more severely impacted. One of these reasons is that women who experience sudden out-of-hospital cardiac arrest (OHCA) were less likely than men to receive CPR from a bystander, largely due to gender concerns. Another observation was that women who were eventually resuscitated had lower chances of living regardless.

Anatomically speaking, it is not entirely known why women suffer worse symptoms and are more likely to die from OHCA, but results of social observations and responses from bystanders to interviewer questioning provide more of an answer. After an OHCA occurrence, researchers would ask first responders as well as the people who happened to be present at the site of occurrence about the scene. The conclusion: people generally feel afraid to perform CPR on women for fear of injuring them in the process. Gender concerns hinder a female OHCA victim's chance of receiving CPR and further treatment, which eventually can be fatal.

Researchers emphasize spreading awareness for this issue so all people receive the proper treatment and in due time in the event of an emergency, and came to their conclusions by examining cases of SCA all across the nation, both in hospital cases and as soon as the arrest occurred.

Disorders Associated with SCA Occurrence

Although people with no known heart disorders are still prone to SCA, there are a few CV disorders that are believed to be linked to SCA. All instances of SCA stem from irregular heartbeat. For example, the human heartbeat normally follows a pattern 1234, 1234, 1234. SCA occurs when the heartbeat deviates from its normal pattern (for example, 1234, 1423, 1243). The cause for this irregularity is usually a mixup in electrical signals from the part of the brain responsible for autonomous heart function. There are a couple of disorders supposedly linked to SCA. These include Coronary Artery Disease, a Heart Attack, Cardiomyopathy, and Heart Valve Disease. Coronary Artery Disease is when buildup in arteries leads to restricted blood flow. This could be related to SCA because when blood flow is minimal, the heart may spontaneously malfunction. Heart attacks usually follow as a result of Coronary Artery Disease, and when they do occur, they can trigger ventricular fibrillation, which is the irregular heart rhythm that is seen in all SCA occurrences. Thus, SCA, Coronary Artery Disease, and Heart Attacks could all be linked. Cardiomyopathy is when the heart is enlarged and the walls all get stretched. This stretching and scarring of the heart could also potentially result in ventricular fibrillation. Heart valves are responsible for ensuring the correct flow of deoxygenated and oxygenated blood. Heart Valve Disease results in incorrect flow of blood, which could cause the heart to spasm and replicate the effects of ventricular fibrillation. This all goes back to the initial idea of a "domino effect" with CV disorders. Heart Valve Disease, and Heart Attacks can result in the irregular rhythm in hearts which results in SCA, and Coronary Artery

Disease can result in a Heart Attack. This relates to the initial articles on what SCA is and how women are more affected than men. Since a linkage between Heart Attacks, Coronary Artery Disease, and Heart Valve Disease to SCA is possible, researchers could observe one phenomenon and observe impacts in either sex and observe similarities and differences in other phenomena. Methods to combat initial causes for ventricular fibrillation could emerge through further research on the other disorders that lead to potential causes for the irregular rhythm. Fighting Coronary Artery Disease in the first place could prevent not only SCA, but also the permanent brain damage mentioned in the first article that often occurs as a result. A real world application to this proposition would be conducting experiments and observations on not just SCA patients, but Heart Valve Disease and Coronary Artery Disease patients as well. Closely monitoring levels of LDL and HDL as well as levels of oxygen-rich and oxygen-poor blood in different chambers of the heart could give insight on the exact amounts of each substance resulting in ventricular fibrillation and consequently, SCA.

Preventions

Although currently there are no known cures for it, there are many ways to prevent not only SCA, but all general cardiovascular disorders. Since the vast majority of cardiovascular problems arise from plaque buildup in blood vessels, maintaining a healthy lifestyle is vital for preventing such problems. There are two main aspects to consider when trying to keep this healthy lifestyle: diet and lifestyle. When it comes to dieting, avoiding certain foods that result in high blood cholesterol is the smartest move. There are two kinds of blood cholesterol to consider: LDL and HDL. LDL, commonly known as the “bad cholesterol” is what tends to build up in blood vessels. HDL, the “good cholesterol,” helps regulate LDL levels in the human bloodstream. Typically, eating foods with high content of saturated fats will result in high LDL levels. Saturated fats can be found in dairy, fatty meats, and cured meats. As for lifestyle, certain daily habits can help keep blood flowing efficiently in the body’s vessels. Daily exercise, even a short daily walk, can help prevent cardiovascular complications. The body’s movement and increase in heart rate keeps blood flowing and moving to all parts of the body. Daily exercise, in whatever form, can even break down blood vessel buildup of cholesterol and other fatty materials that block them. Aside from daily exercise and a balanced diet, more actions someone could take to prevent cardiovascular disorder development include avoiding smoking, drinking in excess, and anything that increases mental anxiety, since anxiety is often linked with an abnormally high heart rate.

Conclusion

The cardiovascular system is extremely intricate and highly prone to many disorders. It is important to study these disorders because heart problems are the current leading cause of death in the world, the

second being cancer. SCA, sudden cardiac arrest, is a major cause of death within the “heart problems” category. It is the unexpected loss of function in the heart, resulting in a lack of blood flow and eventually total system failure. It occurs after an electrical disturbance in the heart which causes the heart to beat in an irregular manner. The exact cause of this irregular rhythm, known as ventricular fibrillation, is unknown. Women are far more severely affected in the long term by SCA, because the immediate treatment is the administration of CPR, which people are afraid to perform on women for fear of injuring them. Women are also more prone to experiencing SCA, the exact anatomical reason for which is still unknown. It is known that SCA occurs after ventricular fibrillation, which means that the disorder could be related to other cardiovascular disorders. A buildup of cholesterol can lead to a Heart Attack, which can cause ventricular fibrillation. The incorrect flow of oxygen-rich and oxygen-poor blood can also cause ventricular fibrillation. Thus, Coronary Artery Disease, Heart Valve Disease, and Heart Attacks all could be linked to SCA occurrence. Studying and working to prevent other stimuli of ventricular fibrillation could prevent and provide further insight on SCA.

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Literary Review

Modeling Breast Cancer

Kaden Chang (author), Anika Shah (advisor), Michelle To (advisor)

Keywords: Breast Cancer, Biological Modelling,

Abstract

The development of a new model to predict the individualized breast cancer (BC) risk and life expectancy of women over 55 years has been reported. The new model was created using data from the Nurses' Health Study (NHS) and the Black Women's Health Study (BWHS). The model predicts 10-year non-BC death, and it includes 21 variables such as age, body mass index, physical function, comorbidities, alcohol, smoking, age at menopause, and mammography use. The final BC prediction model included age, BMI, alcohol and hormone use, family history, age at menopause, age at first birth/parity, and breast biopsy history. The c-index for predicting 10-year non-BC death was 0.790 in NHS and 0.768 in BWHS, while the c-index for predicting 5-year BC risk was 0.612 in NHS and 0.573 in BWHS. This new model may help inform shared decision-making around mammography screening.

Introduction

Breast cancer is a disease causing uncontrolled dividing of abnormal cells in the breast. Having over 200000 cases in the US per year, breast cancer is second in deaths caused by cancer in women. There are two types of breast cancer. Invasive ductal carcinoma, where cancer begins in the ducts and then spreads into the other parts of the breast tissue. Invasive lobular carcinoma, where cancer begins in the lobules and then spreads into the other parts of the breast tissue or even other parts of the body.

Recently, a new competing risk model has been created for predicting the probability of breast cancer death with non breast cancer deaths. A competing risk model is a type of survival analysis that predicts the probability of an event in a sea of events. In this case, the model predicts deaths from breast cancer to deaths from non breast cancer. This model will be used for helping women who have high probabilities of non breast cancer death eliminating their need for screening for cancer, while also helping older women understand their breast cancer risks.

Methods

Creating a model requires a lot of data. First was a study from the NHS of 121,738 female nurses who were white aged 30-55 years in 1976. Second was from the Black Women's Health Study(BWHS), which had information on 59,000 black women aged 21-69. Both studies retrieved information about the woman's lifestyle and medical history through mail. In the end, a sample from each was used to create the model(83,330 from NHS and 17,390 from BWHS).

Note: The NHS study was used because women had stopped using menopausal hormone therapy to match with current health practices, had follow ups, and also included function assessments.

The causes of death, for both studies, were taken from state-issued death certificates, the National death Index, family and friends, and the post office. Over 98% of the deaths were identified. Deaths caused by cancer were confirmed to be accurate.

60 other death causing risks were used to improve the model. Health, physical functions, comorbidity, age, age of menopause, and long of parent's lives were factors included. The socioeconomic factor was not included because the team did not want to have a model basing life expectancy on socioeconomic status.

After the model was finished, it was tested to measure its accuracy. The Royston and Altman methods were used to predict if the probabilities were accurate and how the model identifies results.

Calibration of the model was done by measuring the ratio of expected survival to observed survival at 5 and 10 years. Discrimination was tested by calculating the model's c-index and using risk factor regression coefficients.

Results

From the NHS study, with 55,553 participants who were labeled as non-hispanic white, 3.1% developed breast cancer, 0.3 died from breast cancer, 20.1% died from other causes. In the validation group, 27,777 participants were used with characteristics less than or equal to the characteristics of the women used in the study.

The BWHS has 17,380 participants. It was predicted that women, who were in this study group, are more likely to die from breast cancer compared to the other group. This was due to the group having participants who were younger, different races, were likely to have mammograms and breast biopsies,

higher BMI, and younger age of menopause. After systematizing both groups by age, it was found that both had similar non-breast cancer death rates.

Out of 961 of the top non-breast cancer death models 281 of them had the highest c-index of 0.789. It included taking in 20 inputs (age, BMI, alcohol use, cigarette use, function, mobility, walking pace, age at menopause, and 12 diseases), which were all variables in the top 281 out of 961 models. Mammography was also in the model since it predicts breast cancer death, a competing risk of non-breast cancer death. This change produced a better c-index 0.795 compared to 0.778 which was produced from including 6 mortality risk factors.

There were no changes to the c-index from removing follow-ups of participants over 90 or by using non-self reported diagnosis. Regulating for age, led to the model having a small decrease in performance.

PHR (Public Health Records) was used to evaluate the final non-breast cancer death model. The PHR c-index was 0.796 for predicting 10 year non-breast cancer deaths and had risk hazard factors with a >3% difference to the model. This shows that the model performs as well as PHR does.

Examining the final model

Regression coefficients were used to predict 5-year breast cancer risk in the training and testing group of each study. All risk factors before were used including non-breast cancer deaths and breast cancer. The c-index of the NHS study was 0.603 and the study from BWHS produced a c-index of 0.556. After removing other factors that affect mortality(cigarettes and comorbidities), the c-indexes for NHS and BWHS increased to 0.611 and 0.566. Then the model's efficiency was further increased by changing variables:BMI and alcohol consumption into continuous variables instead of categorical variables. Other factors from previous models like months breastfeeding, having a grandmother with breast cancer, and age at menarche were also added into the model. They had no effect on the model's efficiency.

The final breast cancer prediction model had a c-index of 0.612 in the NHS test group and 0.573 in the BWHS test group.

Discussion

This model was created to predict breast cancer risk and non-breast cancer death to assist older women for their breast cancer screening decisions.

C-statistics or cordance statistics are measures of the accuracy of a logistic regression model. The number ranges between 0-1, with C-statistics below 0.5 representing poor models and C-statistics above 0.5+ representing better models.

The non-breast cancer model predicted accurately on both studies. There was only a slight over prediction in the BWHS study due to inaccessible data. The model produced a similar c-statistic 0.57 compared to other research on breast cancer prediction. This means that the model predicts with medium accuracy and gives similar results as other research models.

The model for predicting 5-year breast cancer risk proved to have a high accuracy with some discrimination in the model. It compares well with the Gail and Tyrer-Cuzick breast cancer prediction models and shows similar c-statistics of <0.60 at participants of >70 years old.

Some limitations for the model are mammographic density, inclusion of polygenic risk scores (PRS), and data on Asian and Hispanic populations. The data lacked information on these categories so the model has such limitations.

Conclusion

A model has been created to predict breast cancer death and non-breast cancer death to be used in a competing risk model in clinical use. It will help young women with a high risk of non-breast cancer death determine if they should take a screening or not. It will help older women by approximating their breast cancer risk. Before publishing the model, the current model will be used to compare with existing models such as BCRAT and Tyrer-Cuzick to further examine the model and also test on other studies.

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Acknowledgments

Division of General Medicine and Primary Care, Department of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA, USA

Mara A. Schonberg, Emily A. Wolfson & Long H. Ngo

Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

A. Heather Eliassen & Bernard A. Rosner

Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Harvard School of Public Health, Boston, MA, USA

A. Heather Eliassen & Bernard A. Rosner

Slone Epidemiology Center, Boston University, Boston University School of Medicine, Boston, MA, USA

Kimberly A. Bertrand & Julie R. Palmer

University of Hawaii Cancer Center, University of Hawaii at Manoa, Manoa, HI, USA

Yurii B. Shvetsov

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Literary Review

Results of Chemotheraphy Mix-Ups

Kashish Vinayak, Anika Shah (Advisor), Michelle To (Advisor), Tennyson High School

Keywords: Cancer, Pancreatic Cancer, Breast Cancer, Chemotherapy, Prescription, Drugs, Treatment, Mixups

Introduction:

Abstract

Pancreatic Cancer is very rapid uncontrollable cell growth, which begins in the pancreas, located behind the lower region of the stomach. The pancreas releases enzymes that assist with digestion and hormones that help to regulate the metabolism of sugars. However, pancreatic cancer affects the pancreas in the way that DNA begins to replicate in clusters to create a tumor which is very dangerous tissue that spreads into the bloodstream eventually. A procedure called chemotherapy, which can prevent, stop, or slow the growth of cancer cells, is used. Chemotherapy was founded by two Yale assistant professors experimenting with nitrogen mustard on lymphoma. This shows that there are many different kinds of cancers. There are also specific medicines used for their respective chemotherapy treatments, but why? What would happen if pancreatic cancer would be switched with a different treatment for breast cancer treatment? All of my research will be conducted by using articles and research by professional oncologists and medical studies, and will be about what the result is of mixups in chemotherapy drug treatments.

Discussion

Probability and Occurances of Mixups

“Chemotherapy Errors: A Call for a Standardized Approach to Measurement and Reporting” is an article that is all about the recklessness that can occur with chemotherapy. In summary, chemotherapy administration in the United States is an unregulated process, and safety assessment remains voluntary. A detailed review of safety event reports at Massachusetts General Hospital (MGH) revealed that chemotherapy errors continue to occur and are safety hazards in the delivery of oncologic care. Classification of Chemotherapy Medication Errors says that mix ups occur with the wrong patient, drug, time, route, or rate. Although counts of these events or event rates are not well suited for measures of quality because of the voluntary

nature of the reporting process, oncology practices should undergo voluntary review or attest to having a mechanism to internally report events, review root causes, and develop plans for improvement. This relates to my research question since this means that there are many instances where mix-ups in chemotherapy occur. This is important to bring to light because there are many innocent lives who're passing away due to mix-ups that can be avoided. Considering that 3% of cancers are pancreatic, there have to be studies or mishaps that have occurred between pancreatic chemotherapy and breast cancer.

Frequency of Mixups

"Prescription errors in cancer chemotherapy: Omissions" supersede potentially harmful errors is another article about chemotherapy treatments being mixed up. A total of 4253 prescribing errors was found in 1500 prescriptions, which is such a high number. Redesigning the hospital systems while making use of modern technology may help in reducing prescription errors and eventually reducing medication errors in the long run. In this study, a high frequency of omissions or incomplete prescriptions as well as potentially harmful prescription errors have been found, which is mainly due to time constraints and lack of adequate attention by the prescribers. The errors were then analyzed to see symptoms that were harmful and not harmful based on the results of harm that happened after the mix-up occurred. This means that these mix-ups between pancreatic chemotherapy and breast cancer are extremely dangerous, and cause high harm. This implies that the different kinds of chemotherapy aren't compatible since they target different cells.

Outcomes of Mixups

"Pancreatic Cancer: Types of Treatment" is an article that has a focus on pancreatic cancer. It lists the different kinds of cancer treatments, including cancer. This article highlights that there are different treatments that can be used at once to optimize treatment. The key piece of information that could be extracted is the specific cancer treatment medicines used for pancreatic cancer which includes: Capecitabine, Fluorouracil, Gemcitabine, Irinotecan, Leucovorin, Nab-paclitaxel, Nanoliposomal irinotecan, and Oxaliplatin. These are all examples of pancreatic cancer chemotherapy drugs. On the other hand, breast cancer has Ixabepilone, Eribulin, Anthracyclines, liposomal doxorubicin, Vinorelbine, Capecitabine, and Gemcitabine. There is some overlap between the treatment medicines, so with a potential mix-up, there is a chance of safety, otherwise, there will be very bad outcomes for the patient that will most likely end in their

condition progressing in the danger zone. This danger zone only occurs when chemical pathways, from the mixed up chemotherapy drugs, affect important organs, and harm cells with important functions.

Conclusion:

Findings

With all of this research combined, conclusions can be drawn about the commonalities of cancer treatments being mismatched often. Discussed was the reasons behind these often mismatches because it's absolutely fatal to the patient if chemotherapy drugs are mismatched. This doesn't add up since fatalities are caused by carelessness, however, it was also researched that there are some overlapping drugs. However, that doesn't do much justice since there is a miniscule likelihood that the patient recieves the same dose that's needed. This means harm will still be done. This is why further root-cause analysis is needed to learn about how mix ups can stop, and how to create a safety net when mix ups occur. Finding a solution for mixups where patients with pancreatic cancer get the chemotherapy treatment of a breast cancer patient, would save many from fatality.

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Literary Review

Odontophobia

Karen Lin, Anika Shah, Michelle To
Palo Alto Senior High School

Keywords: DFA, dentistry, odontology, dentophobia, odontophobia, anxiety, fear, phobia

Abstract

Vicariously learning through media and traumatic experiences of others has propelled the prevalence rate of dentophobia to rapidly increase. This article discusses the symptoms, complications, and treatment options for those who are suffering from this dental fear. The most common symptoms include postponing dental appointments and insomnia or crying before a visit to the dentist. To diagnose this condition, one would have experienced these symptoms for at least half a year. Treatments for odontophobia include cognitive behavioral therapy, psychotherapy, and relaxation techniques.

Introduction

Odontophobia, dental fear, is a widespread psychological disease recognized by the World Health Organization, affecting approximately 20% of the world population (De Stefano, 2019). A phobia is an exaggerated fear or anxiety disorder related to a specific event or circumstance (Cleveland Clinic). Since 1960, research has proven that this condition has been more prevalent as the environment in the dental office can be stress-inducing. It can affect a patient's well-being by provoking sleep disorders and aggression. In this literary review the history, experience, and therapies for odontophobia are assessed.

Discussion:

Definition & Symptoms

Odontophobia, also known as Dentophobia or DFA (short for dental fear and anxiety), is a type of anticipatory anxiety known as dental anxiety. This condition was not acknowledged and recognized by the WHO until recently. DFA can be fear of the dentist, the dental clinic, oral treatment, or dental instruments. The term was first coined in 1946 by Isador Coriat and defined as "an excessive dread of anything being done to the teeth," eventually leading to the

postponement or procrastination of dental prophylaxis (Coriat, 1946). While this is the primary symptom for most victims, other symptoms include shivering, rapid heart rate, restlessness, and squeamishness. An example of a sign of DFA would be an infant shaking and sweating in the dental chair. They could be saying something irrational such as “The periodontal probe is choking me to death!!” or “I can’t breathe when I bite the articulation paper!” Because this is a type of anxiety, there can be some more severe symptoms, such as panic attacks, insomnia, and breakdowns.

Causes & Factors

The adult population has a higher percentage of fear—36% (Cleveland Clinic, 2022) of dentists compared to children. The discrepancy could be attributed to the fact that the risk of dental caries is lower in childhood due to less gingival recession and stronger immune systems. For many, their fear of dentists begins in their youth and stems from anxious patients' inaccurate memory of pain experienced during treatment. A study by psychiatrist Gerry Kent suggests that, “there was a closer association between remembered and expected pain than there was between remembered and experienced pain” (Kent, 1985). Thus, dentophobia is more than just a negative experience; it is present because of their catastrophic thinking, exaggerating their fear, and pessimizing their visit. Another study by Themessl-Huber confirms the significant correlation between child and parental dental anxiety. Therefore, children indirectly learned and acquired anxious behavior and response to dental treatment. But what could be so bad about fearing the dentist? There is a vicious cycle of fear and anxiety about dentistry, avoidance of dental care, and the deterioration of dentition. As a result, a study by Berggren demonstrates that these victims not only have aggravated oral health, but they also feel guilty, shameful, and inferior as their “long period of avoidance [has propelled the need for] more invasive treatment which has the potential to reinforce” odontophobia and avoidance even more (Drews, n.d.).

Complications & Treatments

When discussing complications, it is always necessary to examine treatments for this disorder as this problem and its effects have only continued to worsen. According to periodontist Deva Priya Appukttan, there are multiple psychotherapeutic interventions including, but not limited to, relaxation and breathing techniques, biofeedback, cognitive behavioral, exposure, and hypno-therapy, acupuncture, positive reinforcement, and guided imagery (Appukttan, 2016). Biofeedback is a technique where you learn to control some of your body functions. Exposure

therapy or desensitization is where a patient is gradually exposed to the environment. Positive reinforcement is a technique provided by the dental staff, requiring them to reassure their patients by smiling and modulating their voices. The most accepted and traditional method is common therapy, which involves the modification of negative cognitions. Recently innovation has made significant progress in helping victims cope with anxiety by introducing laser dentistry, a more comfortable alternative to dental treatment. Not only is laser dentistry more cost-effective and efficient, but it can also perform a wide variety of procedures in fewer visits than traditional treatments and procedures. According to Healthline, LASER can minimize blood clotting and damage to surrounding tissues, detect premature dental diseases, and heal wounds faster (Healthline, 2019). While there can be disadvantages to this technology, such as the risk of gum and tooth pulp injury, for a patient of dentophobia, the idea may seem less scary than traditional procedures as many procedures with LASER don't require anesthesia or drills. Thus it is possible that the DFA of many patients will be treated with this new technology as the risk of damage is still small.

Conclusion

Odontophobia, Dentophobia, or Dental Fear Anxiety (DFA) is a prevalent, paralyzing condition in America and internationally. The most common cause is the acquired behavior of fear of dentists. While one can be intimidated by dental procedures, dental instruments, the dental staff, or the environment, the WHO classifies this condition as a type of anxiety and phobia. The condition has become even more widespread in recent years due to the influences of media and myths. Surprisingly, stories of exaggerated experiences can remarkably affect one's cognition of the dentist, heightening their fear of receiving prophylaxis. Symptoms of DFA include rapid heart rate, trembling, panic, apprehension, restlessness, and irritability. Other behaviors consist of canceling or postponing periodontal exams to mitigate and relieve the anxiety; however, in doing so, complications can occur, such as worsening oral health and toothache, which may have hurt less if the victim were to go to the dentist and seek treatment. Patients of Dentophobia are fortunate to have a gamut of treatments to alleviate their fear and anxiety. Most therapy treatments can significantly reduce the condition in a couple of weeks or months (laser dentistry being the most promising). Technology and innovation will continue to cure society of this anxiety and fear shortly.

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Literary Review

The Effect of Alcohol on Our Body

Katie Liang (author), Anika Shah (advisor), Michelle To (advisor)

Keywords: Alcohol, AUD, Physical, Mental, Brain, Therapy

Abstract

In this literary review, we will be exploring how alcohol can lead to physical health problems in our bodies. Learning how alcohol can impact our blood pressure with the result of unbalanced hormones. Additionally, talking about how our body breaks down ethanol into acetaldehyde causing DNA to be damaged, leading to cancer. We will also be exploring mental health issues such as depression and sleep disorders. Another thing we'll dig deeper into is how our brain changes when we overuse alcohol. Finally, this article will examine the different treatments for alcohol, going over the pros and cons.

Introduction

Alcohol is an addictive liquid that contains ethanol. Alcohol can be known as a drug or an addiction. Alcohol use disorder (AUD) is an uncontrollable ability to stop drinking alcohol. College students and over 40 million people ages 18+ have alcohol use disorder (AUD). Many youths also suffer from this disorder. Alcohol can have a temporary relieving effect. But in reality, it has an extremely powerful impact on mental & physical health. In this review, we will discuss how alcohol causes an increase in blood pressure. We will also be discussing how alcohol can lead to cancer through DNA damage. Though, due to the overuse of alcohol, there have also been studies on how alcohol can affect our mental health and sleep. Another topic we'll talk about is how our brains change over time when we consume an enormous amount of alcohol. Finally, we will be discussing the treatments for alcohol use disorder (AUD). There are many treatments available such as medications, behavioral treatments, and mutual-support groups. This review will discuss the pros and cons of these treatments and how they work.

Discussion:

Physical Effects

There are severe physical effects from consuming alcohol that can cause harm to one's body. One main physical effect of alcohol is high blood pressure. Blood pressure is the amount of pressure in blood vessels. For example, normal blood pressure is considered less than or equal to 120/80 mm Hg but anything greater than or equal to 130/80 mm Hg is considered high. When we consume alcohol, it

changes the different balance of hormones in our body such as renin, vasopressin, cortisol levels, and calcium. Blood levels of renin will increase by drinking alcohol, which constricts the blood vessels and decreases how much fluid the body eliminates. This combination of higher fluid levels and smaller blood vessels increases blood pressure (Christine Richardson). Alcohol also reduces how much vasopressin the body makes. Vasopressin is an antidiuretic hormone that causes the body to hold onto water, leading to dehydration. Antidiuretic hormone is a hormone that helps the kidneys control the amount of water and salt in the body which helps control blood pressure and the amount of urine that is made. Another potential mechanism is the increase in cortisol levels. Cortisol is a hormone that regulates the body's response to stress and regulates metabolism, immune function, and inflammatory pathways. Alcohol consumption increases the amount of calcium in the blood vessels. That increases the sensitivity of the blood vessels to compounds that constrict them, leading to increased blood pressure.

Another physical effect caused by extreme misuse of alcohol is cancer. Cancer can severely impact someone's life. It causes a lot of barriers for them. When we consume alcohol, our body breaks down the ethanol into acetaldehyde, which damages our DNA and prevents our body from healing the damage. DNA is the cell's "instruction manual" and when it is damaged, a cell can lose control and create a cancer tumor. When the body can't process the acetaldehyde fast enough, it can build up and cause irreversible DNA damage, leading to cancer. Alcohol can affect hormones which can lead to breast cancer in women. Consuming an enormous amount of alcohol can cause circulating oestrogen levels to rise in our bodies. Alcohol can also cause cancer by altering the cells in the mouth, throat, and oesophagus, making them easier for other carcinogens to be absorbed and spread.

Mental Effects

Mental health is equally important as physical health, consuming alcohol can lead to many things like depression, anxiety, sleep disorders, difficulty regulating emotions, and alcohol use disorder. These are five ways that alcohol can affect our long-term mental health and mood. When these conditions are mixed with alcohol, it's associated with worsening symptoms, mood swings, impulsive behavior, etc. One main mental effect out of the five outcomes that alcohol causes on our mental health is depression. Depression is having long-term sadness and lack of interest in activities. Alcohol is a depressant that affects our brain's natural level of happiness chemicals like serotonin and dopamine. Although you'll feel an initial 'boost' the night before, the next day you will be deficient in these same chemicals which may lead to feelings of anxiousness, down, or depression. When you regularly drink too much, it makes you feel depressed. Alcohol affects the chemistry of the brain, increasing the risk of depression. Hangovers can create a cycle of waking up feeling ill, anxious, and guilty. Additionally, another mental effect alcohol

causes is sleep disorders. Low amounts of alcohol can decrease sleep quality by 9.3% (Pacheco, Danielle). Moderate amounts of alcohol decreased sleep quality by 24% (Pacheco, Danielle). High amounts of alcohol can decrease sleep quality by 39.2% (Pacheco, Danielle). Alcohol has sedative effects that can induce relaxation and sleepiness, but excessive consumption can lead to poor sleep quality and duration. Alcohol is absorbed into the bloodstream from the stomach and small intestine, and its effects depend on the person's age and body composition. The relationship between alcohol and sleep has been studied since the 1930s, and those who drink large amounts of alcohol before bed are more likely to experience sleep disruptions and decreases in sleep quality. Losing quality sleep doesn't help us grow or improve as a human.

Brain Changes

With long-term drinking, not only our body changes over time but our brain does the same. Alcohol interferes with the brain's communication pathways, making it harder for the brain areas controlling balance, memory, speech, and judgment to function (National Institute on Alcohol Abuse and Alcoholism (NIAAA). Long-term heavy drinking causes alterations in the neurons, such as reductions in their size (National Institute on Alcohol Abuse and Alcoholism (NIAAA). Adolescent brains are more vulnerable to the negative effects of alcohol than adult brains, resulting in long-lasting changes in brain structure and function (National Institute on Alcohol Abuse and Alcoholism (NIAAA). Therefore, alcohol-induced blackouts can occur more often when a person drinks enough alcohol to block the transfer of memories from short-term to long-term storage in the hippocampus. That's why alcohol overdose is a serious condition that can lead to permanent brain damage or possibly death. As individuals continue to drink alcohol over time, progressive changes can occur in the structure and function of their brains, which can compromise brain function. Advanced technology such as magnetic resonance imaging (MRI), diffusion tensor imaging (DTI), positron emission tomography (PET), and electrophysiological brain mapping are providing valuable insight into how alcohol affects the brain's structure and function. Long-term heavy drinking may lead to the shrinking of the brain and deficiencies in the fibers (white matter) that carry information between brain cells. MRI and DTI are being used to assess the brains of patients when they first stop chronic heavy drinking and again after long periods of sobriety, to monitor for possible relapse to drinking. Studies using PET imaging have detected deficits in alcoholics, particularly in the frontal lobes and cerebellum, which are responsible for learning and memory. PET imaging is allowing researchers to visualize the damage that results from heavy alcohol consumption and analyze alcohol's effects on various nerve cell communication systems. PET is a promising tool for monitoring the effects of alcoholism treatment and abstinence on damaged portions of the brain. Electroencephalography (EEG)

records the brain's electrical signals, which are magnified and graphed as brain waves. These methods are exceptionally helpful for scientists and researchers to figure out how alcohol is affecting our brains.

Treatments

There is currently no cure for alcoholism, though there are many methods to treat it. The two main treatments are therapy and medications. Cognitive behavioral therapy is one type of therapy for alcoholism. This type of therapy helps you change your thinking and behaviors that lead to drinking. Motivational enhancement therapy is another type of therapy to treat alcoholism. This type of talk therapy uses our internal strengths to encourage and motivate change. Both therapies can help you change your thoughts about drinking alcohol. Change is something that we individuals have to do, therapies can only guide us. Other ways to treat alcoholism are medications such as Acamprosate, Disulfiram, Naltrexone, etc. Acamprosate (Campral®) is a medicine that helps rebalance chemicals in the brain. Disulfiram (Antabuse®) is a medicine that if you drink alcohol, this medicine will cause unpleasant effects so you will stop consuming alcohol. Naltrexone (Revia®, Vivitrol®) is a medicine that works by decreasing the craving for alcohol. These three medications are all approved by the FDA to treat alcohol dependence. The cons of taking any of these medications are nausea, dizziness, headache, sweating, and chest pain (*Effective Health Care Program)*. Since there is no cure for alcoholism, people can only rely on these methods to stop drinking long-term alcohol. Technology is advancing year by year so in the future, there might be a possible medication to stop the side effects of alcohol. For now, there are many programs that help combat AUD such as the Substance and Mental Health Service Administration (SAMHSA), National Treatment Network (NTN), and many more (Crest View Recovery Center).

Conclusion

The consumption of alcohol can only harm our bodies. What alcohol industries are doing is only feeding risks into our bodies. More than 140,000 people die from excessive alcohol use in America each year (*CDC*). Alcohol leaves severe physical effects on the body, such as increased blood pressure, decreased vasopressin, increased cortisol levels, and increased cancer risk. Leading to cancer by damaging DNA, affecting hormones, and altering the cells in the mouth and throat. Alcohol’s long-term effects on mental health, such as depression, anxiety, sleep disorders, difficulty regulating emotions, and alcohol use disorder. When alcohol interferes with the brain's communication pathways, making it harder for the brain areas controlling balance, memory, speech, and judgment to function. Though, advanced technology such as MRI, DTI, PET, and EEG are providing insight into how alcohol affects the brain's structure and function. Finally, therapy and medications are the main treatments for alcoholism, but there is no cure.

Therefore, in conclusion, communities who want their students, people, etc. can try consuming other liquids such as water or milk.

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Literary Review

Alzheimer's Disease

Laasya Munjeti (Author), Anika Shah (Advisor), Michelle (Advisor)
Windemere Ranch Middle School

Keywords: Alzheimer's, Age, Brain, air pollution, treatments

Abstract

The management of Alzheimer's disease has been a challenging and important topic for many years. As we have learned more about the causes of the disease and the number of people affected has increased, researchers have looked for new and innovative treatments. This review will analyze the treatements and new links to Alzheimer's disease. After being studied, air pollution seemed to have an effect on older adults which made these adults more likely to develop Alzheimer's disease. In addition to the potential link between air pollution and Alzheimer's disease, other treatments for the disease have been explored. The current treatment options for Alzheimer's disease are limited and primarily aimed at managing symptoms. Cholinesterase inhibitors and memantine are the most commonly used drugs for Alzheimer's disease, but their effectiveness is limited and does not address the underlying cause of the disease. Researchers are currently exploring new treatments that target the underlying pathophysiology of Alzheimer's disease. These treatments include those that aim to reduce inflammation, oxidative stress, and the accumulation of amyloid plaques in the brain. Gene therapy directed at neurotrophins is also under investigation as a potential treatment.

Introduction

Alzheimer's disease is a devastating condition that affects millions of people worldwide, and currently, there is no cure. As the population ages, the prevalence of Alzheimer's disease is expected to increase, making it a pressing public health concern. In recent years, researchers have made significant progress in understanding the underlying causes of Alzheimer's disease, and in developing new treatments and preventative strategies. In this literary review, we will examine three articles that explore different aspects of Alzheimer's disease, including the potential link between air pollution and the disease, the impact of chronic stress on cognitive decline in older adults, and a new approach to treating Alzheimer's disease using an old drug called quinacrine. This review will highlight the complexity of Alzheimer's disease and the need for continued research to better understand the disease, develop effective treatments, and identify preventative strategies.

Discussion

Link between air pollution and Alzheimer's disease

The article reports on a new study that suggests that there may be a link between air pollution and an increased risk of Alzheimer's disease. The study, which was conducted in the United States, analyzed data

on over 3,600 older adults and found that those who were exposed to higher levels of fine particulate matter (a type of air pollution) were more likely to develop Alzheimer's disease than those who were exposed to lower levels.The article explains that previous research has suggested that air pollution may have a negative impact on brain health, but this is one of the first studies to specifically investigate the link between air pollution and Alzheimer's disease. The study's findings suggest that reducing exposure to air pollution may be an important way to prevent or delay the onset of Alzheimer's disease.The article notes that air pollution is a major public health issue that affects people around the world, and that efforts to reduce air pollution may have a range of other health benefits beyond reducing the risk of Alzheimer's disease. The article concludes by highlighting the need for further research to better understand the relationship between air pollution and brain health, and to identify effective strategies for reducing air pollution and protecting public health. The study is significant because Alzheimer's disease is a devastating condition that affects millions of people around the world, and currently, there is no cure. The findings suggest that air pollution, which is a preventable risk factor, may contribute to the development of the disease. The study's authors note that the results are correlational and do not prove that air pollution causes Alzheimer's disease. However, the study used sophisticated statistical methods to control for other factors that could contribute to the development of the disease, such as age, sex, education, and smoking status. This strengthens the case for a causal link between air pollution and Alzheimer's disease. Reducing air pollution is a complex issueWe that requires action from individuals, governments, and industry. The article notes that measures to reduce air pollution could include improving public transportation, increasing the use of clean energy sources, and regulating emissions from factories and vehicles. In addition to reducing the risk of Alzheimer's disease, these measures could have a range of other health benefits, including reducing the risk of heart disease, lung cancer, and respiratory problems.

Current treatment approaches

The article titled "Alzheimer's disease: Review of current concepts" provides an overview of Alzheimer's disease (AD), its pathogenesis, and current therapeutic approaches. The article begins by describing AD as a progressive and debilitating disease that affects memory, cognition, and behavior. It is characterized by the deposition of amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), and neuronal loss. The article then discusses the various hypotheses that have been proposed to explain the pathogenesis of AD. These include the Aβ hypothesis, tau hypothesis, oxidative stress hypothesis, and the cholinergic hypothesis. According to the Aβ hypothesis, Aβ peptides accumulate in the brain, leading to the formation of plaques, and eventually, neuronal death. The tau hypothesis, on the other hand, suggests that abnormal phosphorylation of tau protein leads to the formation of NFTs, which disrupt neuronal function and eventually lead to cell death. The oxidative stress hypothesis proposes that oxidative damage to neurons is

a key factor in the development of AD. Lastly, the cholinergic hypothesis posits that the loss of cholinergic neurons in the brain leads to memory impairment and cognitive decline. The article then describes the current treatment options for AD. These include acetylcholinesterase inhibitors (AChEIs) such as donepezil, rivastigmine, and galantamine, which are used to improve cognitive function and behavior. The article also discusses memantine, an N-methyl-D-aspartate (NMDA) receptor antagonist, which is used to treat moderate to severe AD. Other therapies, including antioxidants and nonsteroidal anti-inflammatory drugs (NSAIDs), have also been investigated for their potential to treat AD. The article goes on to discuss the limitations of current therapies and the need for new approaches. One promising area of research is the development of disease-modifying therapies that target the underlying pathogenesis of AD, such as Aβ antibodies, tau aggregation inhibitors, and secretase inhibitors. The article also discusses the importance of early diagnosis and prevention strategies in the management of AD.

New treatment approaches

The article reports on a recent study that may offer a new approach to treating Alzheimer's disease (AD). The study, conducted by researchers at the University of Cambridge, found that a drug called lithium chloride, which has been used to treat bipolar disorder for decades, could help reduce the formation of amyloid plaques in the brains of mice with AD. The researchers tested lithium chloride in mice that had been genetically modified to develop AD-like symptoms. They found that the drug reduced the number and size of amyloid plaques in the mice's brains, as well as improved their cognitive function. The study's findings are significant because current treatments for AD, such as acetylcholinesterase inhibitors and memantine, only target symptoms and do not address the underlying pathology of the disease. The researchers believe that lithium chloride may work by inhibiting an enzyme called glycogen synthase kinase-3 beta (GSK-3β), which is known to play a role in the formation of amyloid plaques. While the results are promising, the researchers caution that more research is needed to determine the safety and efficacy of lithium chloride in humans. They also note that the drug has potential side effects, such as kidney and thyroid problems, that need to be carefully monitored. The article provides context on the prevalence and impact of AD, noting that an estimated 6 million Americans currently live with the disease, with that number projected to increase in the coming years. It also highlights the challenges researchers face in developing new treatments for the disease, including high costs and low success rates in clinical trials. The study's lead author is quoted as saying that the findings "open up a new avenue for drug discovery for Alzheimer's disease." The article notes that the research team is now planning to conduct further studies to better understand how lithium chloride works and to test its safety and effectiveness in humans. Overall, the article offers a hopeful glimpse into a potential new approach to treating AD, while acknowledging the challenges and uncertainties that remain.

Conclusion:

In summary, the three articles reviewed in this literature analysis shed light on distinct aspects of Alzheimer's disease, highlighting the significance of ongoing research to enhance our understanding of the disease, develop effective treatments, and identify preventative strategies. The first article emphasizes the potential association between air pollution and Alzheimer's disease, emphasizing the need to decrease air pollution to prevent or delay the onset of the disease. The second article discusses various current treatment approaches This article also describes the adverse impact of chronic stress on cognitive decline in older adults and suggests that interventions to decrease chronic stress may promote cognitive health. Lastly, the third article presents a novel approach to treating Alzheimer's disease using an old drug called quinacrine. Although further research is needed to establish the effectiveness of quinacrine in treating Alzheimer's disease in humans, the study's findings suggest a promising new avenue for treatment development. Collectively, these articles underscore the complexity of Alzheimer's disease and the urgent need for ongoing research and action to address this critical public health issue.

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Literary Review

Precision Cancer Medicine

Luana Veras (author), Anika Shah (advisor), Michelle To (advisor)
Monte Vista High School

Keywords: Cancer, Cancer Genomics, Precision Health Medicine

Abstract

This essay discusses the emerging field of cancer genomics, which involves using the human genome to identify genetic differences that may cause cancer. Cancer is a genetic disease caused by alterations in the cell's DNA that can lead to the formation of tumors. Precision cancer medicine, derived from the study of cancer genomics, seeks to identify and treat cancer more specifically. Through clinical trials, such as IMPACT, treatment plans can be specified for individual patients, demonstrating a significant step forward for cancer treatment. Cancer genomics has the chance to drastically increase our understanding of cancer biology and improve cancer treatment outcomes.

Introduction

The study of cancer genomics is a fairly new field. It involves using the human genome in order to identify differences in the DNA that may cause cancer for humans. Caused by mutations or alterations in the cell’s DNA, cancer is a genetic disease. These alterations can cause proliferation of the cells and formation of tumors. Precision cancer medicine is derived from the study and is now working to identify and treat cancer more specifically. Researchers are trying to figure out if this new form of treatment will be able to efficiently treat all types of cancer. By observing new clinical trials such as IMPACT and recognizing the advantages of precision health, cancer genomics will become the best way to treat cancer.

Discussion

Cancer Genomics

Cancer genomics is an exciting and rapidly growing field of research that focuses on the study of genetic changes in cancer cells. Scientists in this field are interested in identifying the genetic

mutations that occur in cancer cells, how these mutations arise, and how they contribute to the development and progression of cancer. The ultimate goal of cancer genomics research is to develop more targeted and effective treatments for cancer patients. By analyzing the genetic makeup of tumors, researchers can identify specific molecular pathways that are involved in the development and growth of cancer cells. Mutations in the oncogenes cause them to become permanently activated leading to uncontrolled cell growth, or proliferation. On the other hand, mutations in tumor suppressors can inhibit them and allow tumors to grow as well. Moreover, through gene expression profiling, scientists can see which genes are turned on and off in cells, allowing them to identify the specific cancer causing genes. Gene expression is a tightly regulated process where genetic information from the nucleus is encoded in a gene which is used to make a gene product such as a protein or an RNA molecule. It is here where mutations can occur creating cancer. This information can be used to develop drugs that target these pathways and block their activity, ultimately leading to the death of cancer cells. Additionally, cancer genomics research has the potential to improve cancer screening and diagnosis, as well as the development of new cancer prevention strategies. With the rapid advances in technology and the increasing availability of large-scale genetic data, cancer genomics is poised to revolutionize our understanding of cancer biology and improve cancer treatment outcomes.

Precision Health

Using cancer genomics, a new form of treatment emerged: precision health. Precision health, according to Sam Gambhir, a professor and researcher at Stanford University, is the best way to treat cancer. This treatment involves analyzing a patient's genetics, lifestyle, and environment to develop personalized treatment plans that are tailored to their unique needs. Through genomic sequencing, technologies can identify mutations that contribute to the patient’s disease. It analyzes the patient’s and the cancer’s DNA in order to find the alterations leading to cancer. Precision health also takes into account the patient’s lifestyle including their diet, exercise, and stress levels, which can all have large impacts on their lives. The patient would wear tracking devices and take surveys in order to obtain this information. Additionally, certain environmental factors such as air quality and exposure to toxins are considered when identifying a patient's treatment. As described above, cancer can be developed in many ways and precision health can help manage this. By doing so, precision health can improve patient outcomes and reduce the toxicity and side effects associated with traditional cancer treatments. Precision health recognizes

that each patient's cancer is unique and requires an individualized treatment plan. By analyzing genetic information, doctors can develop targeted therapies that are designed to attack the specific mutations driving the patient's cancer. This approach can lead to better outcomes and fewer side effects as treatments are tailored to each individual patient. Furthermore, precision health is not limited to genetic analysis but also includes lifestyle and environmental factors. By understanding a patient's environment and lifestyle, doctors can develop treatment plans that take into account their individual needs and circumstances. This approach can lead to better outcomes and a more personalized approach to cancer treatment. In addition, precision health emphasizes the importance of collaboration and data sharing in advancing cancer research. By sharing data and resources, researchers can accelerate progress in this field and ultimately improve patient outcomes. Precision health also recognizes the importance of early detection in improving cancer outcomes. By analyzing genetic information, doctors can identify patients who are at high risk of developing cancer and develop screening programs to detect the disease early when it is most treatable. It is one of the most effective ways to treat cancer as it recognizes the uniqueness of each patient's cancer and tailors treatment plans to their individual needs. By analyzing genetic information, lifestyle, and environmental factors, doctors can develop targeted therapies that lead to better outcomes and fewer side effects. Furthermore, precision health emphasizes collaboration and data sharing, which can accelerate progress in cancer research and ultimately improve patient outcomes.

Clinical Trials

The clinical trial IMPACT, or the Initiative for Molecular Profiling and Advanced Cancer Therapy, looks to establish cancer genomics as the best way of treating cancer by analyzing the genetic makeup of patients' tumors and tailoring treatment plans to their unique needs. The trial recognizes that cancer is a disease of the genome and that analyzing genetic information can provide valuable insights for developing personalized treatment plans. By doing so, the trial hopes to improve patient outcomes and reduce the toxicity and side effects associated with traditional cancer treatments. The IMPACT trial represents a significant step forward in cancer treatment by recognizing the importance of genomics and personalizing treatment plans to individual patients. By analyzing genetic information, the trial hopes to develop more effective and targeted treatments that can improve patient outcomes and quality of life.

Limitations

However, this trial is far from perfect. One feature is that it focuses only on patients with advanced cancer. As of right now, the future results of this study will leave people with early-stage cancer with nothing. Moreover, there is no control group for this study meaning that there is no way to see the effectiveness of the results. This study only includes people using precision health and there are no subjects using regular chemotherapy. IMPACT also struggles to make a large impact due to its small sample size. This makes the results faulty making this trial less likely to become a new form of medicine. As any new trial might be, IMPACT is expensive and time-consuming. Many people suffering from cancer may not have the money to treat their loved ones with precision health and will continue to do chemotherapy. Finally, IMPACT fails to address that there are other contributors to cancer other than genes. Lifestyle and environmental factors may also contribute to cancer. Precision health is capable of addressing this issue, however, there are few studies showing its success. All of these factors affect the success of this trial and if they were modified it would help to perfect precision health medicine. IMPACT has lots of potential, but there are other trials that need to be made to justify precision health medicine.

Conclusion

In the end, this is a single trial and many more are underway to test the theories made by scientists on precision health. Precision health is a new discovery due to cancer genomics might allow us to treat all types of cancer in a less toxic, more specific way. Through trials like IMPACT, we can begin to develop new forms of treatment based on precision health. There are so many ways this treatment can grow and with a little more research, we can perfect it. On the path that we are on, we could create a new form of medicine that is the most efficient way of treating cancer.

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Literary Review

Antibiotic Alternative Development For Livestock

Mae-Lin Pinkstaff, Anika Shah, Michelle To

Keywords: Antibiotics, Resistance, Alternatives, Livestock

Abstract:

This article offers a literary review on two research articles that address the global antibiotic-resistant crisis we live in and propose alternatives to the development of new antibiotics to address this prevalent problem. In *A Call for Antibiotic Alternatives* by Thaddeus Stanton and *The Antibiotic Resistance Crisis* by C. Lee Ventola, the authors attributed the root causes of antibiotic resistance to the overuse of antibiotics in the livestock in an effort to keep them healthy in unsanitary conditions. These strains of antibiotic resistant bacteria are then transmitted to humans when the animals are consumed due to the food chain effect. Proposed solutions are to better maintain the living conditions of farm animals through the development and deployment of technologies of early detection of the bacteria in these livestocks and to start a coordinated approach to advance alternatives to new antibiotics to prevent the spread of the illnesses.

Introduction

Antibiotics have been around for hundreds of years with the mother of all antibiotics, penicillin, arguably being the most important breakthrough in the medical field in the 1920s. Since then, many lives have been saved everyday due to antibiotics. This type of medicine works by destroying or inhibiting growth of bacteria in your body by interfering with their process. However, as long as antibiotics exist, so will antibiotics resistance. It is still prevalent today mostly due to over prescription and overuse of antibiotics. These overuse of antibiotics in humans, animals and plants have imposed substantial health, social and economic burdens globally. In order to tackle this enormous problem, a coordinated global approach to control has been urged by scientists and researchers. Developing effective antibiotic alternatives for agricultural applications to reduce transmission of antibiotic resistant bacteria from livestocks to humans is one of these leading proposals.

Discussion:

Resistant Bacteria in Animals Affect Humans

In *The Antibiotic Resistance Crisis* research article by C. Lee Ventola, the author stated that antibiotics are widely used as growth supplements in livestock in both the developed and developing world. An estimated 80% of antibiotics sold in the U.S. are used in animals, primarily to promote growth and to prevent infection. Treating livestock with antimicrobials is said to improve the overall health of the animals, producing larger yields and a higher-quality product. The antibiotics used in livestock are ingested by humans when they consume food. The transfer of resistant bacteria to humans by farm animals was first noted more than 35 years ago, when high rates of antibiotic resistance were found in the intestinal flora of both farm animals and farmers. More recently, molecular detection methods have demonstrated that resistant bacteria in farm animals reach consumers through meat products. This occurs through the following sequence of events: 1) antibiotic use in food-producing animals kills or suppresses susceptible bacteria, allowing antibiotic-resistant bacteria to thrive; 2) resistant bacteria are transmitted to humans through the food supply; 3) these bacteria can cause infections in humans that may lead to adverse health consequences. The agricultural use of antibiotics also affects the environmental microbiome. Up to 90% of the antibiotics given to livestock are excreted in urine and stool, then widely dispersed through fertilizer, groundwater, and surface runoff.

Antibiotic Alternative as the Key Solution

In *A call for Antibiotic Alternatives* research article by Thaddeus Stanton, government scientists with the US Department of Agriculture (USDA) proposed national strategies to develop effective antibiotic alternatives for agricultural applications. The dramatic decrease in the discovery and release of new antibiotics while extensively drug-resistant and pan-resistant (resistant to all therapeutic antibiotics) strains of these bacteria have been identified in human and veterinary clinics pose grave concerns. The author noted that since the 1990s, both the decline in attractiveness of the antibiotic market and consolidations within the biopharmaceutical industry have resulted in a 75% decrease in the number of companies with large antibiotic R&D efforts resulted in only fewer than 2% of drugs in clinical development by the 15 largest drug companies were antibiotics in 2004. Lack of profitability led to the significant decrease in development of new antibiotics by large pharmaceutical companies with R&D facilities. Additionally,

government safeguards and regulatory requirements prolonged the market release of the new antibiotics and added to the development costs. Because of the lack of the development of new antibiotics, the antibiotic resistance problem resulting from the extensive use and misuse of antibiotics for humans, animals, and plants will continue. USDA Microbiologists Tom Casey and Mark Rasmussen, and an Iowa State University colleague, Jacob Petrich invented a fluorescence detector to reduce animal-to-human transmission of antibiotic-resistant bacteria. The detector monitors, in real time, fecal contamination of animal carcasses in meat processing plants. Since fecal contamination is a significant source of foodborne pathogens and spoilage bacteria in meat products, the detection will lead to timely implementation of measures to prevent and reduce the spread of fecal transmitted bacteria. This will in turn reduce the antibiotic use in farm animals.

Solutions to the Antibiotic Crisis

In *A call for Antibiotic Alternatives* research article by Thaddeus Stanton, the author presented the significant effect of this invention due to the food chain effect. As meat is a significant, often preferred, protein source in human diets, this invention will therefore reduce the transmission from meat to humans of antibiotic-resistant foodborne pathogens and non-pathogens. The invention of early detection of fecal bacteria addresses the pathway of the bacteria in livestocks to human infection at a very early stage, therefore, is an effective intervention. The outcome of the invention could lead to future development of new antibiotic alternatives by private and public research partnership. The author argued that seeking alternatives to new antibiotics for animal health is important because the decades-long debates and impasse over agricultural antibiotic use and human health were exacerbating the problem. The early detection and prevention approach is much more preferred than the development of new antibiotic therapeutics. The author continued to urge for nationally coordinated, interdisciplinary, multi-agency (human and animal health) efforts to encourage research on promising antibiotic alternatives and to consider all physical, chemical, immunological, genetic, and biological approaches with fast tracking of the most promising. Cooperative research should be encouraged between public institutes and private corporations to confirm, define, and improve the efficacy of existing non-antibiotic health products for animals.

Conclusion

Researchers propose a coordinated approach to tackle the development and spread of the antibiotic resistance in bacteria because of the enormous economic and social costs due to the

spread and treatment of the antibiotic resistance infections. The article proposes several methods to control antibiotic resistance, one being controlling antibiotic uses and seeking alternatives to antibiotics such as better hygiene regulation in food production. Investing in better sanitation infrastructure in places where food production takes place as well as imposing restrictions and enforcement on routine use of antibiotics for humans and livestocks would reduce the need to use antibiotics and curtail excessive use. Encouraging research and development in seeking alternatives to antibiotics to prevent and protect against infectious diseases. In conclusion, a multidimensional globally coordinated approach to address the antibiotic resistance issue is called by the authors of the two articles.

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Research Article

Data Management for Clinical Trials

Mei Peters (author), Anika Shah (advisor), Michelle To (advisor)
Design Tech High School

Keywords: Healthcare, Clinical Trial, Clinical Data Management System

Abstract

The data management process for clinical trials varies for different research groups, as the needs and objectives of every group are different. But for the most part, every group notes the advantages and disadvantages they discovered regarding their chosen method of organizing their data. Throughout this research paper, common advantages and disadvantages from multiple previously conducted clinical trials, as well as the Coursera course "Data Management for Clinical Research," will be listed and briefly discussed. Afterward, this paper will describe a possible data management process that's primary objective is to help create a data organizational tool that is professional and user-friendly.

Background Information

Artificial intelligence and machine learning hadn't been widely utilized in clinical trials up to this point, as researchers were concerned that applying these tools would result in "considerable threats of privacy problems, ethical concerns, and medical errors," according to Health IT Analytics. But recently, Clinical Data Management (CDM) systems such as Oracle Clinical One, IBM Clinical Development, Castor EDC, and TrialKit have been used by thousands of clinical trials as a way to provide researchers with quality data while complying with any protocols or regulations. This system also allows clinical trial stakeholders such as collaborating organizations and sponsors to track every step of the trial's data organization process.

Three of the primary data collection tools that researchers utilize are questionnaire surveys or patient-reported data, hospital medical records, and biological material, according to "Commonly Utilized Data Collection Approaches in Clinical Research", an article written by researchers Saczynski, McManus, and Goldberg. This article states that the major disadvantages of patient surveys are patient validation, study representative credibility, and differences in responses based on contact format. Creating a data organization tool that automatically records the participant's name, email, and signature would eliminate the first issue. A few disadvantages of hospital care records are believed to be the inconsistency of the

collected data, incomplete information, and overall, the patient's background information isn't properly obtained. A CDM system would notify researchers of cells with missing information and would follow up with patients in order to obtain a completed background scan.

Objectives

The primary objective is to understand the various impacts, both positive and negative, of different types of CDM systems. Since many researchers and healthcare specialists are still skeptical about clinical trials, it is important to maintain a professional and easy-to-understand data management system. A system containing error-free data will additionally make a huge difference when analyzing participant results and understanding what needs to be altered in a research group's treatment.

Materials and Methods

This study explores and tests what features make up the best data management system when organizing patient data for clinical trials. The first portion of this study compares two documented clinical trials, one that was successful and one that wasn't. I will primarily be looking for the step-by-step procedure of each study, in order to fully understand what was and wasn't taken note of, as well as what key data points made the studies more or less efficient and effective. The combination of these studies and the information from the Coursera course will assist me in developing a possible data management system.

Studies

One study that I explored was one Pharmaphorum conducted with the US Tufts Center for the Study of Drug Development (CSDD), their objective being to determine the impact of clinical data management on drug development. They discovered that although researchers organizing a clinical trial are given approximately 60 days between submitting their study's protocol and starting Phase l, many researchers fail to meet this time frame due to the slow process of designing a database. And even if they start their trial on time, they are likely to experience challenges throughout the study due to delays in data management tasks. A more technologically advanced method of clinical data management is necessary, as more researchers increase their trial's sample size and seek for more complex results in their data.

Another clinical trial that was conducted by researchers at Zogeniz was regarding a treatment called Fintepla, which was created with the aim to assist in anti-seizure therapy for individuals with Dravet syndrome. This treatment was denied two rounds of testing solely because in 2019, the FDA received an

“incorrect version of the clinical dataset” (Yanick Millet), and then in 2021, the dataset that was submitted contained missing information, which prevented the FDA from completing the reviewing process.

How can these results be made more definite? This study’s goal is to determine the impact of proper data management and artificial intelligence on clinical trial outcomes.

In addition to analyzing what could make a clinical trial successful versus unsuccessful, I participated in a Coursera course called Data Management for Clinical Research. I learned about the Electronic Data Capture (EDC) fundamentals, how to plan a data strategy for a prospective study, how to collect data through surveys and questionnaires, and how all of these concepts would be implemented in a real-life study. In this course, researchers chose to use REDCap as their primary EDC software system; this system provides its users with case report forms that consist of questions/prompts that are easy to answer for patients, and it also provides auto-variable coding, which automatically turns multiple choice fields into code. For instance, if a field asks for the patient’s education and the options are ‘No High School’, ‘High School’, ‘Some College’, and ‘College Degree’, this form would transform these responses to 0, 1, 2, and 3, respectively. Similarly, when coding categorical variables on Google Sheets, Excel, etc., it is important to never reuse a number for different values. For example, if a researcher comes across missing data, a widely used option is to have Patient Doesn’t Remember = 888 and Patient Will Not Disclose = 999.

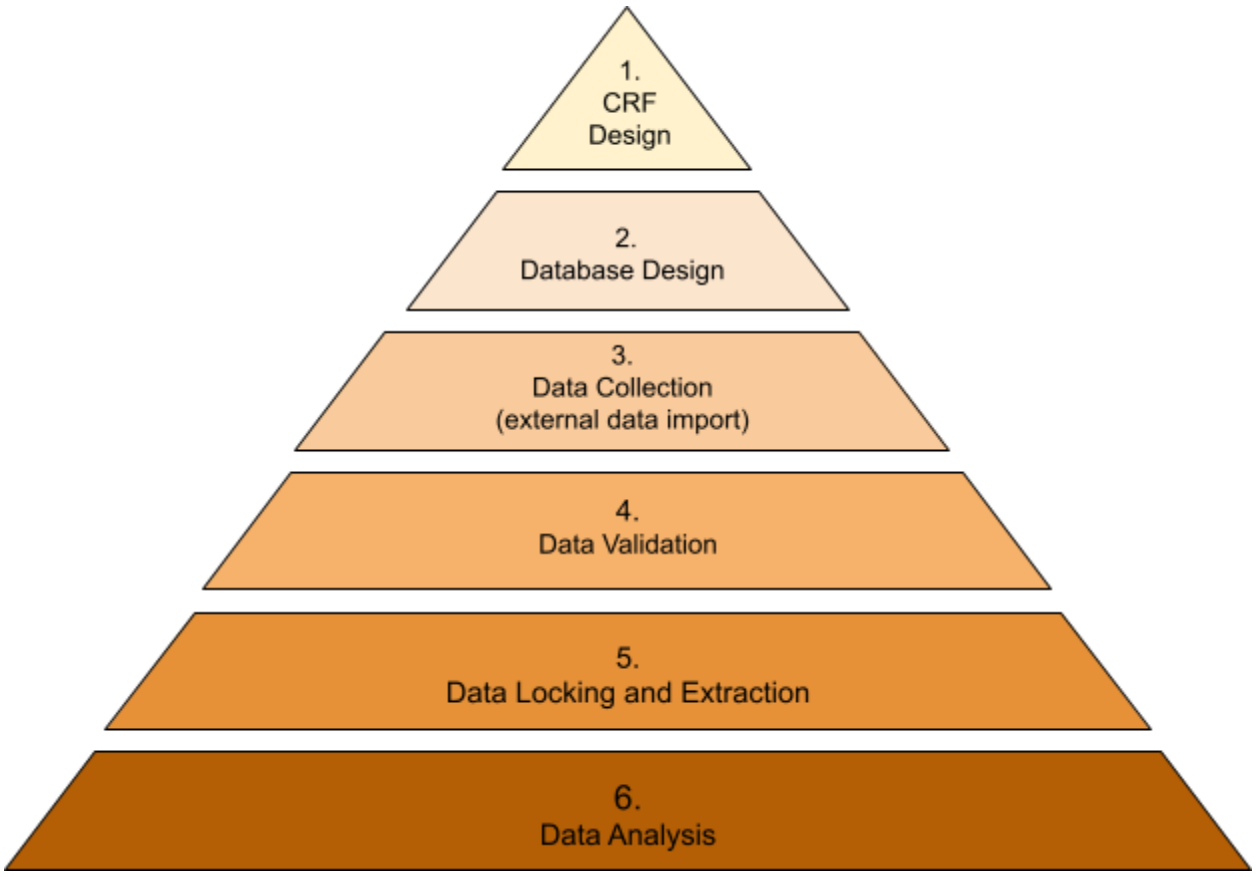
This form of baseline questionnaire allows researchers to collect important information on their patients such as age, gender, race, etc. without having to read through open-ended responses. Certain prompts such as age, weight, and height would require a more specific response, but in general, this method allows both researchers and stakeholders to more efficiently analyze the data. After collecting the patient’s initial information, it is important to keep track of how much and how often they receive the treatment. This can be done using the same method of assigning numbers to possible responses. These various variables should be sorted into a key that provides the meaning of each number, as well as an explanation in needed. This use of variables allows systems such as REDCap to compare data and perform further statistical analysis, making it more structured and reliable than manual analysis performed by researchers.

Another important topic in data management is patient confidentiality. Since this is one of the biggest issues with implementing AI, it is necessary to create proper security safeguards for accessing patient data, especially when patient identifiers, such as name, phone number, and social security number, are collected; HIPAA identifiers are good starting points when identifying confidential fields in a dataset. For

individuals interested in learning the fundamentals of data management for a clinical trial, this course discusses in detail what one should and shouldn't do.

Discussion

While examining numerous clinical trials and articles about proper data management, I created a diagram that contains a possible outline of making a CDM system with few disadvantages.



1. ***CRF Design*** - Recently, most clinical trials use Electronic Case Report Forms (eCRFs), as they allow for quicker data collection and better quality information. An eCRF can be summed up into 6 sections: demographics, vital signs, basic measurements, medical history, lab exams, and adverse events. This form has to be specifically designed for each clinical trial as every trial's protocols and needed information differs to some extent. The CRF may also contain surveys or questionnaires that are sent to the patients in order to collect more personal and open-ended responses in addition to their medical data.

2. ***Database Design*** - A chart or key that provides users with all of the information they need as to what each variable stands for and to help users understand whether certain prompts were categorized together. Overall, the database design creates an easy-to-understand system for defining the relationships between variables.

3. ***Data Collection*** - This step consists of collecting the patient's information from their health records, medical devices, patient-reported outcomes, and any other sources of information that may be needed for the trial.

4. ***Data Validation*** - This step is important, as it makes sure that all of the inputted data is accurate, consistent, and comprehensible. A few ways to perform data validation is through electronic edit checks which are embedded into eCRFs, Source Data Verification (SDV), which is the process of comparing CRF results with the patient's medical records, and code checks, which detect any errors in numerical data and categorical data.

5. ***Database Locking and Extraction*** - This step solely consists of researchers finalizing the data they collected for their clinical trial and preparing it for the next step, data extraction. During this step, the data is submitted to stakeholders in order to get it processed for statistical analysis.

6. ***Data Analysis*** - Statistical analysis of the data through models and predictions allows for determining any significance of the clinical trial's proposed treatment. Analysis methods are chosen based on the trial's sample size, its variables, and its primary objective. Some examples of analysis models are descriptive analysis, inferential analysis, predictive analysis, and prescriptive analysis. Descriptive analysis interprets and summarizes the collected data, making it easier to understand. Inferential analysis draws significant connections from the data and determines relationships between certain variables. Predictive analysis compares the data to previous data and predicts future trends. Lastly, based on the data, prescriptive analysis determines the best course of action.

Conclusion

Although the designing process of a proper CDM system requires extensive research, as some of the patient questions need to be fabricated from scratch, it is made easier with the help of data analysis systems such as REDCap and various machine learning algorithms. Additionally, it is crucial to utilize an

electronic CDM system, as it makes it easier for researchers and stakeholders to access the data, as well as detect any missing or incorrect data that requires reaching out to the patient. Recent technological developments have made algorithm-based predictive analysis more reliable, which has moreover made CDM systems more popular. So clinical trials that do not use an electronic CDM system are left with less sound and error-free statistical analysis methods, which may lead to more time constraints as well as issues when the data is examined by the FDA.

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Literary Review

Using Photons' Properties to Study Meat

Mass Spectroscopy and Magnetic Resonance Imaging for the Cultured Meat Industry

Author: Michelle To

Abstract

Cultured meat production is limited by current practices to distribute nutrients to cells, resulting in necrosis. Moreover, due to limited interest, the current ways to monitor the growth of sarcoplasmic and myofibrillar muscle tissue require invasive techniques. For example, using matrix-assisted laser desorption/ionization (MALDI) spectroscopy, a commonly-used technique to study stem cells, muscle tissue must be pulverized and mixed with acidic solution (Zaima et al). This would not be ideal for a meat production company who would want to conserve as much tissue as possible to sell. As a result, a non-invasive technique to monitor the fat, muscle, and water composition of meat would be economically beneficial, allowing both researchers and producers to monitor the growth and quality of meat. In this review, we will cover the current techniques used to study cultured meat from stem cells as well as other traditional imaging techniques. Finally, we will refer to these techniques and propose further research that can create cost-effective, specific, and noninvasive ways to promote cultured meat production.

Introduction: Current Non-Invasive Techniques to Image Stem Cells

When trying to find a non-invasive technique to image cells, one is limited to light with wavelengths longer than UV rays. To image cellular processes, low-energy near-infrared light (NIR) has lower phototoxicity and is able to penetrate through tissue up to 1 centimeter (Chen et al.). Since it has a longer wavelength, it has less reaction activity and becomes a good candidate for imaging slices of tissue. Most often, NIR spectroscopy is paired with some fluorescent molecule that is excited at a narrow wavelength. Upconversion nanoparticles (UCNPs) are also used to excite NIR light, emitting UV light that can activate light-sensitive protein-ion channels on genetically engineered cells, allowing us to image GM cells. Particularly in imaging cultured meat, NIR light might become important in studying cell death and nutrient distribution. Traditionally, imaging has been developed to monitor proxies for cell death (important for cell

culture research), like caspase activation, phosphatidylserine externalization, DNA fragmentation, and other pH changes. Measuring apoptosis-associated caspase activity is the most common way to measure apoptosis. A probe can be developed to link a fluorophore to caspase, which activates apoptosis (Zhang et al.). In monitoring cell apoptosis, then, future research will want to focus on replicating a similar technique to find cleavable caspase-3 substrate peptide, DEVD (Asp-Glu-Val-Asp), or intracellular glutathione (GSH).

One of the current most promising non-invasive imaging techniques is synchrotron infrared spectroscopy (SR-FTIR), which creates images to differentiate nucleic acids, proteins, fatty acids, and carbohydrates (Qian et al.). Synchrotrons work by very strongly accelerating protons into a circular motion. This method is currently novel, and it may not be cost-effective for a budding industry for cultured meat. However, in the future, it may be interesting to see a similar technique using pulsing light to excite proteins like caspase-3 substrate peptide as proxies for measuring cell death in cultured meat and measuring how well nutrients are circulating. As a result, current cost-effective imaging techniques use fluorescent agents as markers, meaning they are more invasive; however, less invasive techniques are limited by cost.

Corpus: Functional MRI

This leads us to find a technique already being used in a different field that could similarly produce the macronutrient specificity and noninvasiveness from SR-FTIR. Functional MRI has been used almost exclusively on the brain, using magnetic fields to measure oxygen levels as proxies of brain activity. In traditional fMRI, deoxygenated hemoglobin (in blood cells that are not carrying oxygen) versus oxygenated hemoglobin correspond to changes in different T2 values, which are magnetic resonance signals that are different from the main magnetic field produced by the MRI machine (Gruber et al.). Oxygenated hemoglobin are diamagnetic, so this means they often lineup perpendicular to the magnetic poles, while deoxygenated hemoglobin will line up parallel. As a result, the blood-oxygen level dependent, or "BOLD" signal, is a signal of where the deoxygenated (shorter T2) and oxygenated (longer T2) hemoglobin is in the brain. The fMRI thus takes many images along different T2 values and weights the final image along shorter to longer T2 values.

Traditional fMRI is thus a similar non-invasive technique to SR-FTIR that can differentiate between different proxies for cell activity and nutrient distribution. Excitingly, functional MRI may also be used on muscles. In muscle functional MRI, the T2 relaxation time value is also compared to the water in the muscle, which could be a proxy for muscle activation due to exercise (Cagnie et al.). Unlike brains, it is not clearly understood how T2 values might correspond to oxygenation and perhaps cell apoptosis. However, we *do* know that electrical activity can also be an essential proxy for cell apoptosis, since disintegrated muscle cells will no longer contract. While EMGs (electromyography) might not be efficient in measuring the electrical activity in deep muscle cells, there might still be hope with MRIs detecting electrical activity. Nonetheless, research on brain MRI can still be applied to cultured meat research. Recently, researchers implanted a very small electronic device (ImpACT) into the brain, acting as a miniature radiofrequency coil in an MRI, which changes the magnetic signal when it detects electrical activity from neural action potentials (Hai et al.). While they originally tested the ImpACT under a very strong magnetic field (9T), they also tested under a clinically relevant magnetic field (3T) and found that the ImpACT could detect 15 mV and greater.

With larger ImpACTs, the magnetic field from the ImpACT was much larger than the main magnetic field when there was no input. With 500 mV input, it is a better ratio (from 1.1 to 1.3). But after 300 mV, the nonlinear relationship makes a confounding factor for large ImpACTs measuring greater electrical signals. While they originally tested the ImpACT under a very strong magnetic field (9T), they also tested under a clinically relevant magnetic field (3T) and found that the ImpACT could detect 15 mV and greater. The most important limitation might be implanting the electronic device with other devices, but this shouldn't be an issue with cultured meat. As a result, muscle-tailored ImpACT devices and fMRI become a promising non-invasive and relatively inexpensive technique to measure nutrient distribution and cell growth.

Discussion

Compared to conventional techniques used to study stem cell growth, fMRI seems to be more translatable into mass production of cultured meat. Unlike traditional mass spectroscopy techniques, which are more invasive, fMRI does not require the meat to be pulverized, keeping its integrity to be able to be sold to consumers. And different from current state-of-the-art

non-invasive spectroscopy, fMRI is a much more widely-used technique, meaning it would be more accessible to facilities to study, improve, and scale up. As a result, using fMRI, producers are able to monitor the inner growth of cultured meat from the outside, even being able to differentiate the fat and muscle content to fine-tune the taste and texture of meat.

Moreover, using current research on ImpACT devices, additional research can measure the electrical activity in the meat, which could be a secondary measure of cell neurosis, meat structure, and muscle strength. Currently, the amplitude of EMG readings are in between 0-10 mV, prior to amplification, so this novel implementation needs to be improved to be able to measure lower amounts of electrical activity (and perhaps for measuring electrical signals from neurons). As a result, research on ImpACT devices are promising projects to be funded and be up-scaled. By funding ImpACT devices, confirming their noise differentiation techniques, fine-tuning ImpACT sensitivity, and increasing ImpACT robustness, this can be used to not only improve the cultured meat industry but even individual neuron research, which would be beneficial for research on degenerative muscle diseases, neurodegenerative diseases, and even cardiovascular reactivity.

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Literary Review

Childhood trauma and associated risk factors for bipolar disorder

Mithra Senthil (author), Anika Shah (advisor), Michelle To (advisor)
Carondelet High School

Keywords: Bipolar Disorder, Childhood Trauma, Abuse

Abstract:

For my literature review research, I am focusing on bipolar disorder and how childhood trauma can take effect later on getting diagnosed with the mental disorder. I found common themes when reading each article and have sectioned my paper into the following: the clinical presentation and diagnostic criteria of bipolar disorder, features and definitions of childhood trauma, how childhood trauma and the development of bipolar disorder relate, how childhood trauma and bipolar disorder affect adults, risks of developing the bipolar disorder if one's parents had diagnosed bipolar disorder, and finally therapies and treatment options that can mitigate the clinical features of bipolar disorder. Finally, it will conclude with a discussion of the next research steps to better understand the nuances and intersectionality of trauma and bipolar disorder.

Introduction:

Bipolar disorder (BD) is a psychiatric disease that affects around 45 million people worldwide. [1] Many patients diagnosed with BD have endorsed suicidal thoughts from time to time. Studies have shown that researchers estimate that between 25% and 60% of individuals with BD have attempted suicide and 4% to 19% have died of suicide.[2] In addition, people faced with mental disorders have challenges in many ways. Firstly, patients not only struggle with the disorder and disability itself but also have to deal with the stigmatized stereotypes given to people with disorders. Many job opportunities are taken away due to a person's mental disorder and the thought they couldn't handle the pressure and workload given. Some stereotypes include acting childlike, and being irresponsible, their actions should be controlled, and that people with mental disorders should be feared.[3] Childhood trauma (CT) has been shown to elevate the risks of BD symptoms, including rapid cycling, suicide attempts, substance misuse, mood episodes, and early age of onset. Thus, analyzing early risk factors for BD development, such as childhood trauma, is a good starting point to help mitigate these negative effects on quality of life.

Symptoms of Bipolar Disorder:

Mania, hypomania, and depression are all symptoms that can occur when a person has bipolar disorder. A person going through a manic episode will receive mood disturbances where they have an abnormal amount of energy, mentally and physically, that usually lasts a week. Some can experience separation from reality, like hallucinations and delusions.[4,5] A hypomanic episode is a milder version of mania. A person going through a hypomanic episode can experience elevated

mood, increased self-esteem, and a decreased need for sleep. A hypomanic episode can last at least four days. Some symptoms of mania and hypomania can include: abnormal increase in confidence, self-esteem, talkativeness, decrease in sleep, risky behavior, and can cause a medical emergency.[6,7] A person going through a depressive episode can experience a depressed mood or loss of interest or pleasure in life. To be diagnosed with a major depressive episode, a person has to have depressive symptoms continuously for about two weeks. Symptoms can include sleep issues, loss of energy, and a depressive mood overall.[4] In some cases, people can have a mix of symptoms involving mania and depression all at one time.[8]

Depending on the type of BD an individual has, the type of symptoms they have may vary. There are three different conditions involving bipolar disorder, which include BD type 1, BD type 2, and cyclothymic disorder.[9] A person diagnosed with BD type 1 can have at least one manic episode with or without a depressive episode. This type has the most severe symptoms, such as intense manic episodes which sometimes require hospitalization. BD type 2 consists of depressive and manic episodes which alternate and are typically less severe. The cyclothymic disorder comprises brief episodes of hypomania and depression.[10]

Childhood Trauma and Development of Bipolar Disorder:

Childhood trauma (CT) plays a major role in the development of BD. CT is when a person has or had been exposed to traumatic events during their childhood. Many studies have demonstrated that patients with bipolar disorder have experienced some kind of childhood trauma in their life. Childhood trauma can affect cognitive functioning that might decrease the ability to cope with later stressors, thus being a major risk factor in the development of mental disorders.[11] A case-control study assessing 206 patients with BD compared to 94 controls using the Childhood Trauma Questionnaire shows that multiple traumas are more frequent in patients with BD than in controls.[12] In another study, it was shown that BD patients were 2.6 times more likely to report CT than controls.[13] Cross-sectional and longitudinal studies have shown children who have experienced both CT and BD symptoms are at an increased risk of developing BD.[14] CT could trigger an altered developmental pathway, as seen by brain imaging abnormalities. [11] Thus, CT is very important and is a crucial risk factor in the development of BD. CT affects every child differently, negatively affecting their health and well-being. With that being said, if a child isn't given the help he/she needs after experiencing trauma, it can severely impact their life and can cause the development of mental disorders, suicide attempts, and changes in behaviors. In this research review, we will be covering the aspects of how CT plays a role in the development of BD, the risks involving the development of BD, how CT and BD affect adults and children, and the available treatment and therapy options.

Epigenetic effect of childhood trauma on BD development

CT, along with environmental and genetic risk factors, plays a huge role in the development of various mental health conditions, including BD. Children who are experiencing trauma during

neurodevelopmental stages early in life show symptoms of BD and are at an increased risk of developing BD later on.[14] The development of BD is often due to an interplay between the genetic factors, environmental risk factors, and other yet to be identified factors. Studies have shown that children of parents with diagnosed mental disorders are at an increased risk of developing BD, due to increased genetic and familial risks. Some psychological and biological symptoms linked to CT are emotional difficulties, cognitive deficits, altered neural function, altered circadian neuroendocrine, and immune response markers.[14] Other studies have demonstrated that impact of exposure to an environmental stressor on mental disorder phenotype is conditional on the individual's genotype. One such example is that a functional polymorphism of the COMT gene is associated significantly with the Childhood Trauma Questionnaire (CTQ) total score, particularly with regard to the symptom of perceived dissociation.[15] Additionally, the low-activity Met allele of the brain-derived, neurotrophic factor gene and the allele of the apolipoprotein E gene, with accompanying sexual abuse, has been shown to be associated with reduced memory test scores.[16] Another study that investigated whether CT mediates age of onset of BD showed that a greater number of traumatic events was related to earlier ages of BD onset. Environmental factors due to CT can also change the trajectory of BD via influence on gene expression, further described in Table 1.[17] In summary, the interplay between genetic and environmental factors due to CT are important to study, due to the increased risk of developing BD.

Child and adolescent experiences

A child with BD can experience various episodes including mania, depression and a mix of both. Adolescents in particular experiencing manic episodes might have short tempers, be happy for a long period of time, talk fast, have sleep issues, find it hard to stay focused, and conduct risky behavior. Adolescents experiencing depressive episodes might be sad for a long period of time, complain about the body hurting, an excess amount of sleep, have little energy and might have suicidal thoughts as well.[18]

The rapid cycling of symptoms in children diagnosed with BD occurs at crucial developmental stages of their lives. This can deprive them of the opportunity for more normal developments cognitively, emotionally, and socially.[19] Children diagnosed with BD can also develop other problems like substance misuse and develop other psychiatric disorders like ADHD, trouble concentrating and anxiety. Extreme behavioral changes can also take effect when the child is going through mood episodes. When in a manic state, they might take extreme risks harming themselves and when in a depressive state, they might run away from home and have suicidal thoughts.[18] If the child doesn’t get the correct treatment, their daily life, school and relationships become difficult for them to handle. Even though their episodes are shorter, they can alternate between mania and depressive episodes throughout the day. A sixteen-year-old diagnosed with BD at fourteen wrote about their experience: “I had mood swings that were the worst anyone could have ever seen. My poor parents thought I hated them, but really I was sick and didn't even realize it. But now I am on medications for my disorder and I live a pretty normal life. My family

and friends support me, and they, along with my therapist, have helped me get to the point where I am today. I just want other teens to know that even though it is hard at times to be bipolar, things will get better.[20] While a child is going through a manic episode, it is hard for them to focus in class, which makes them not do well in their classes. Lack of sleep and associated fatigue additionally poorly affect the student as well. The University of Michigan's health department suggests that reducing academic stress is important in children experiencing severe episodes. They also suggest the child receives extra support, such as tutoring, to additionally decrease stress.[21] Children and adolescents diagnosed with BD are more likely to develop subsequent problems cognitively, socially and emotionally.

Difficulty and implications of childhood diagnosis of BD

There has been controversy over how BD in children might be overdiagnosed and result in overuse of prescribed medication because of the earlier documented onset of BD. Appropriate assessment methods need to take place and assessment should focus on obtaining an accurate family history of BD.[22] Mark Zimmerman, a psychiatrist at Rhode Island Hospital in Providence shares his thoughts of BD being overdiagnosed. He starts by saying how half of the patients diagnosed with BD never had their diagnosis confirmed.[23] Even though some patients experience BD symptoms, they could be accompanied by other criteria factors like hyperactivity. Another reason is that there are too many medications for BD, which is why physicians have the tendency to diagnose patients because they feel comfortable treating them. For children specifically, medical professionals find it difficult to recognize and diagnose a child with BD because their symptoms don't fit precisely to the symptom criteria for adults.[24] In the US a survey showed that there was a misdiagnosis rate of 69% mainly towards patients with major depressive disorder and BD.[25] With there being too many treatment options in BD, it is easy for a doctor to be "overconfident" in diagnosing a patient with BD if they are only showing one type of symptom correlated with BD.

Parental factors playing a role in the development of childhood BD:

It has been demonstrated that there is a higher risk of childhood development of bipolar disorder if parents themselves have been diagnosed with BD, lending credence to the role that genetics play in the development of BD.[12,26,27] Studies have shown that children of parents diagnosed with BD in particular, relative to other psychiatric disorders, have a higher risk of getting diagnosed themselves with BD. However, even if parents have been diagnosed with other mental disorders, how the parents then express themselves can moderate how a child with BD expresses their aggressive trait.[28] A study has found that fifty-six percent of children of bipolar parents reported mood symptoms compared to 9% of children of control parents.[29] The genetic factors can increase the risk of developing BD, especially in adolescents.

Additionally, negative parental behavior, including marital conflict, emotional abuse of children, and child neglect, can be contributing factors to the childhood development of BD. It has been hypothesized that poor parental bonding may have a negative impact on mental health. Many BD patients have experienced affectionless and low-care parents growing up.[30] Relatedly, studies

have correlated the relationship between poor parental marital adjustment and risk for the development of BD in their children.[31] Marital conflict, beyond adjustment difficulty, may be even more detrimental for children of bipolar parents since these children have been found to show more distress than other children.[31] Potential risk factors for BD have been identified in studies of children with depressed mothers. Risk factors may include inadequate living conditions, being a single caregiver, having an abusive partner, early parenthood, low education, and substance use.[32] Additionally, severe childhood trauma and abuse occurred in about half of patients with bipolar disorder; this may lead to more severe symptoms and earlier age of onset.[28] Sexual and emotional abuse have been thought to contribute to the acceleration of illness complexity.[28] This may occur through a lack of a secure emotional base, resulting in the development of an insecure attachment style.[30] The negative impacts from abuse and subpar support networks could help explain why childhood trauma survivors with BD often experience poorer clinical outcomes, accompanied by the increased risk of symptoms including relapse, rapid cycling, and more extensive suicidality.

Potential risk factors for BD have been identified in studies of children with depressed mothers. A study in Ireland where they took 49 patients from a mental health outpatient service showed that high rates of CT were present in 74% of patients with BD and 82% of patients with depression.[33] They were able to find how CT and poor parental bonding with the mother figures caused higher rates of interpersonal difficulties and extreme episodes.[33] Interpersonal difficulties are associated with high relapse and recovery rates through the lack of social relationships, support networks, and coping styles.[33] A child experiencing the psychological unavailability in their parents may have a similar experience to actually losing your parents. CT survivors are shown to experience poorer clinical outcomes and are at a higher risk of having a relapse and a harder time recovering from mental illnesses.[33] The lack of a secure emotional base for the child can add to the development of insecure attachment style, like vulnerability and viewing the world as a threat.

CT and BD affecting adulthood:

Many studies have been performed demonstrating that the effects of CT, due to emotional abuse, neglect or even physical abuse, can carry on into one's adulthood. CT subtypes, like abuse and neglect, are risk factors associated with the worsening of BD and have caused suicidal behaviors, substance misuse, and early age of onset.[28]

Association of emotional neglect/abuse with development of BD

Emotional neglect and/or abuse has been the most studied subtype found in research. Delfina Janiri, et al (year) evaluated how different types of CT are associated with BD 1 and BD 2 compared with healthy controls. CT of emotional neglect/abuse, defined as dysfunctional attitudes of parents or to inadequate emotional responses, was found more frequently in patients diagnosed with BD 2. Interpersonal sensitivity, defined as "the ability to accurately assess others' abilities, states, and traits from nonverbal cues," [34]has been linked to patients with BD 2 as

well.[35] Additionally, prior emotional abuse was found in both BD subtypes.[35] Another study examined 100 patients at an academic specialty center for the treatment of bipolar disorder. They identified that half of the patients had a history of severe CT, and severity of CT was associated with early ages of onset. Patients who had experienced emotional abuse had greater likelihood of BD with substance misuse and rapid cycling.[28] A study took 72 individuals with BD during euthymia and assessed them through the CTQ and Wechsler Abbreviated Scale of Intelligence. The results showed that 54.1% of the individuals had a history of emotional abuse and neglect.[28,36]

Studies have also shown that patients whose CT involved emotional neglect performed worse at emotional recognition than those without a history in recognizing anger.[30] A study that took 141 patients with BD had their history of mental illness as well as clinical characteristics assessed.The study showed that an increase in emotional abuse was associated with earlier age of onset of BD. The data shows that the higher the CT, the less likely a patient was to get optimal hospital care, perhaps due to lower social functioning (i.e. childhood trauma negatively affects future interpersonal relationships).[37] A research study taken in North-East England and New Zealand took 60 outpatients with BD treated for depressive episodes and 55 controls and assessed them using the Childhood Trauma Questionnaire (CTQ). The results showed that there was a higher rate of CT in patients with BD 1 & 2 compared to controls. This can conclude that CT may trigger an altered developmental pathway. Emotional abuse showed significance in the assessment and may therefore be considered as a potential contributor to the development and causes rapid cycling and suicide attempts as well.[38] Emotional abuse/neglect is a key subtype of CT and has been shown in many patiens with BD.

Relationship between prior sexual abuse and BD

Emotional neglect/abuse has been the most studied risk factor for BD, however other forms of CT, such as sexual abuse, have preliminary evidence as risk factors. CT of sexual abuse has been reported in one of every five women and one in every thirteen men with BD. Sexual abuse is reported more by women.[28] Janiri et al, demonstrated that BD 1 patients are more likely to have phased sexual abuse than healthy controls.[35] A study from Mongolia observed 20 different studies and found that across ten countries 3407 patients with BD reported high rates of CT of sexual abuse.[12] In a study conducted by Tina Du Rocher Schudlich et al, found an association between sexual abuse and family environemt. It also causes more severe and frequent manic episodes and reports of higher rates of hospitalization.[39] Studies have shown correlation between sexual abuse and suicidal behavior. A nonclinical sample showed that patient who had delt with sexual abuse were eight times more likely to repeat sucidal attempts while neglect showed no difference.[28] Even though the subtype has been understudied, many patients with BD have experienced CT of sexual abuse, supporting the fact that CT is a risk factor associated with BD.

Relationship between prior physical abuse and BD

The relationship between physical abuse and BD has also been understudied, however there are some studies examining physical abuse as a risk factor. CT of physical abuse has been reported more by males than females. A study in New Zealand found that CT of physical abuse is the main predictor of drug use.[12] Physical abuse has been associated with a negative family setting, more severe manic/depressive episodes, self harming, increased risk of suicidal behavior, developing PTSD and substance misuse. It correlates to mood lability and frequency of episodes as well. Physical abuse has also been related to a family history of depression.[39] Like sexual abuse, physical abuse as well has been understudied, but patients of BD have shown to have experienced CT of physical abuse, making CT a major risk factor associated to BD.

Therapies, treatments, and outcomes:

Behavioral therapy

There are several different treatments, such as behavioral therapies and medications, that can be used to treat patients diagnosed with BD, with the main treatment goal being to decrease likelihood of relapse. There are six main types of therapies for BD: family-focused therapy (FFT), interpersonal and social rhythm therapy, cognitive behavioral therapy (CBT), and group psychoeducation. FFT involves the person diagnosed with BD and their family members. The therapy usually lasts for about 12 sessions; the first few sessions educate the patient and family on aspects of BD such as symptoms, signs, how to prevent relapses, and what the family can do to help the individual with BD. Later on, the sessions cover communication, problem solving skills, and discussion of family conflicts. Studies conducted by UCLA and University of Colorado have shown that patients who go to FFT while also continuing medication have milder symptoms, less severe episodes, and better functionality in the span of 1-2 years, compared to pre-interventional baseline. [40,41] Interpersonal and social rhythm therapy focuses on stress arising from relationship difficulties, in addition to disruptions in daily activities caused by BD. This therapy can help reduce the mood cycling related to patient interpersonal relationships, as well decrease mood cycling via the establishment of a more daily routine (e.g. consistent eating, socializing, and sleeping schedules).[42] CBT can either be a solo or group therapy that focuses on the patient's relationship between their thoughts, feelings, and behaviors, and how these can affect their emotions. The therapy is used to help turn negative thoughts, feelings, and behaviors into a positive way of responding. This therapy is also useful for identifying methods to adjust daily tasks relative to mood state, such as rewarding oneself when one is depressed or not overreaching when one is in a manic state.[40,41] Dialectical behavior therapy (DBT) is a subset of CBT that involves both individual and group therapies. DBT is a skills-based approach that teaches mindfulness, distress tolerance, healthy ways to cope with stress, improve relationships with others and many more coping mechanisms. Finally, group psychoeducation which is a group therapy where people get together and talk about their story while supporting and relating to each other. It is a highly structured session that involves both educational and skill training

sessions.[40] There are many different therapy options a person diagnosed with BD can be treated to, so depending on the person and how severe their episodes and symptoms are can determine which therapy treatment is right for them.

Medical treatments

Along with psychiatric therapies, there are medications that doctors can prescribe to help decrease symptoms, as well as lessen frequency and severity episodes. The medications may include mood stabilizers, antipsychotics, antidepressants, anti-anxiety medications and sometimes combined antipsychotics and antidepressants. Mood stabilizer medications help control manic and hypomanic episodes. Historically, lithium has been the most common mood stabilizer, used since the 1970s to help prevent recurrent episodes of mania or hypomania. More recently, valproate (Divalproex) has become first line mood-stabilizing treatment due to longer time to recurrence of manic episodes.[43] Antipsychotics are given when mood stabilizing medications have not helped; sometimes these can be given in combination with other medical therapies. Currently, the first line antipsychotic is quetiapine (Seroquel), a dopamine type 2 and serotonin type 2 antagonist, which also has been demonstrated to increase the time between recurrent manic episodes.[44] In addition, BD can be accompanied by depressive episodes, particularly in type 2 bipolar disorder; depressive symptoms can be treated with adjunct antidepressant medication. However, this concomitant use in patients with BD is controversial, since antidepressants can trigger manic episodes[45,46,47]. Historically, the combination of olanzapine (antipyschotic) and fluoxetine (antidepressant, selective serotonin reuptake inhibitor) had been prescribed as treatment for bipolar major depression. However, newer antipsychotic monotherapy, such as lurasidone (Latuda) have been shown in early trials to demonstrate efficacy in treating both bipolar symptoms and depressive symptoms without adjunct antidepressants.[48,49,50] Like therapies, depending on the symptoms and episodes the patient is facing contributes to the prescription of medicine they are given.

Discussion:

Childhood trauma associated with clinical characteristics in bipolar disorder has been shown to elevate the risks of rapid cycling, suicide attempts, substance misuse, mood episodes, early age of onset, and can affect cognitive functions. People with BD can experience various symptoms depending on if they have BD 1, BD 2, or cyclothymic disorder, with symptoms ranging from manic episodes to depressive episodes. Children experiencing CT during neurodevelopment, along with environmental and genetic risk factors, have increased risk of developing BD, in turn depriving them of opportunities for more normal cognitive, emotional, and social development. Medical professionals find it difficult to precisely diagnose children with BD since their symptoms don't fit the same symptom criteria found in adults, causing controversy over overdiagnosing patients for BD. Many studies have shown that children of parents diagnosed with BD have elevated risk of developing BD; additionally, BD patients have reported that they had experienced poor parenting in the past like marital conflict, emotional abuse and childhood neglect, in turn affecting their adult behavior, thinking, and actions. While there are many

medication and treatment options for BD to prevent symptom relapse, based on type of BD, there are also effective therapy options, including group sessions, family sessions and individual sessions. Importantly, literature review has demonstrated a strong link between childhood trauma and future development of BD, identifying a key time point to both observe for and intervene in the event of childhood traumatic events to prevent BD development.

CT is one of the more modifiable risk factors linked to BD, but it has been understudied which is why the importance of understanding the nuances of their relationship for both diagnosing patients with BD and the prevention of BD is important. Why is early identification of CT-related BD important? Suicide rates have been recently declining, which is good because it shows that more people are reaching out for help. Even with Covid-19 impacting mental health with the loss of so many lives and the high rate of unemployment, researchers from The Journal of the American Medical Association found a 5.6% decrease in suicide rates. Experts say that there is a possibility of an increased focus on mental health during the Covid pandemic, giving people the opportunity to take a step back and reevaluate their lives, which could help with the decreased number of suicides.[51] In 2014, researchers from John Hopkins found that patients who had attempted suicide had a decline in the possibility of attempting it again after therapy.[52] But, many studies have shown that suicidality is a huge factor in patients with BD (BD). Two studies took individuals diagnosed with BD and showed that about 20.5% to 60% of individuals had attempted suicide and 4% to 19% had died by suicide.[2, 53]. Thus, early identification of BD risk factors, such as CT, can mitigate severe BD symptoms resulting in suicide, along with mitigating negative effects on their health and well-being. Additionally, evidence has shown consistently that along with childhood trauma there is a higher risk of developing bipolar disorder for children who have BD-diagnosed parents. Some studies have even shown various subtypes of CT can be linked to BD 1, BD 2, or cyclothymic disorder depending on the type of CT they have experienced. Thus, there is a clear time point during which healthcare providers should screen for trauma, in addition to more rigorous study designs incorporating the effects on development of BD with early supportive intervention in the case of CT.

There have been a few limitations that may have impacted the accuracy of results across the literature reviewed. Firstly, some of the older patients may not be able to recall their experience of CT, and subsequent effect on development of BD, resulting in uncontrolled recall bias. Additionally, most studies reported either presence or absence of CT and effect on BD development; thus we still do not understand the role of CT severity, and CT subtype severity, on subsequent BD symptomatology. Most of the studies reviewed either only focused on a certain subtype of CT or a type of BD, thus limiting the scope of each finding. Many of the studies were also conducted in North America, with fewer studies having global representation, limiting the conclusions that can be drawn with regard to cultural effects either promoting or mitigating the increased risk of BD development with CT. With regard to studying resultant effects of CT on BD symptomatology, many studies did not have specific inclusion criteria that limited the scope

of BD treatment options, thus resulting in greater heterogeneity of the patients and their therapies being taken at the time of the study. Future multicenter global initiatives with both adolescent and adult enrollment for longitudinal cohort studies, with both CTQ positive and negative study members, accompanied by yearly surveys of symptomatology/BD episodes/BD therapies utilized would enable better tracking of individual variation in CT subtype effect on BD type symptoms, in addition to drawing more accurate conclusions about how specific interventions may benefit or harm patients' cognitive, emotional, and social function. Additionally, with collection of family history and pertinent medical history, such an initiative could better account for studies examining risk factors for BD development, including more nuanced relationships between CT and BD.

Conclusion:

The field is still in early stages as to how childhood trauma epigenetics contributes to BD development. Further research is needed in how best to accurately diagnose patients with BD as opposed to other psychiatric disorders that may be BD mimics. BD lacks pathophysiologic indicators and tests that would provide a standard for diagnosis, making it mainly a clinical diagnosis. To help prevent overdiagnosis, medical professionals should carefully examine the patient's symptoms, as well as gather information on their family medical history, history of CT, and prior medical conditions. Using this information, risk scoring systems and/or diagnostic questionnaires for BD more accurately be designed. With further research into subtypes of CT and effect on BD, more nuanced diagnostic criteria may be developed in the future, informing more individualized and perhaps more effective BD therapies.

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Literary Review

The Immune System and Its Ability to Fight Off Cancer

Natalie Goldberg (Author), Michelle To (advisor)
The College Preparatory School

Keywords: Immune system, Cancer, Antigens, T cells, Natural killer cells, Checkpoints, PD-1, Immunotherapy, Signaling pathways, Cell types, Cancer-specific antigens

Abstract

The immune system is a complex network of cells, tissues, and organs that defends the body against invading pathogens and abnormal cells, including cancer cells. This paper provides a brief overview of how the immune system recognizes and eliminates cancer cells. Specifically, we discuss the role of cancer-specific antigens in triggering an immune response, the importance of T cells and natural killer cells in targeting and killing cancer cells, and the role of immune checkpoints in regulating the immune response to cancer. We also highlight the potential of immunotherapies that target these pathways to enhance the immune system's ability to eliminate cancer cells. Overall, a better understanding of the mechanisms underlying the immune system's response to cancer has the potential to lead to more effective cancer therapies.

Introduction

Cancer is one of the deadliest diseases that has plagued humanity for centuries. It is a complex and multifactorial disease that arises from the uncontrolled growth of abnormal cells in the body. The immune system is the body's defense against cancer, and it plays a critical role in preventing cancer from developing and spreading. In this literary analysis, we will examine the various ways in which the immune system fights off cancer.

Discussion:

Immune System Functions

The immune system is a complex network of cells, tissues, and organs that work together to defend the body against harmful pathogens, including cancer cells. The immune system is divided into two main branches: the innate immune system and the adaptive immune system.

The innate immune system is the body's first line of defense, and it is responsible for detecting and eliminating foreign invaders, such as bacteria and viruses. The adaptive immune system is a more specialized branch of the immune system that is responsible for recognizing and targeting specific pathogens, including cancer cells. One of the ways in which the immune system fights off cancer is through the process of immunosurveillance. Immunosurveillance refers to the immune system's ability to recognize and eliminate cancer cells before they have a chance to develop into tumors. The immune system accomplishes this through the use of various immune cells, including natural killer cells, macrophages, and dendritic cells.

Natural killer cells are specialized immune cells that are capable of recognizing and killing cancer cells. They do this by recognizing certain proteins on the surface of cancer cells that are not present in healthy cells. Once they have identified a cancer cell, natural killer cells release chemicals that cause the cancer cell to undergo apoptosis or programmed cell death.

Macrophages are another type of immune cell that plays an important role in fighting off cancer. Macrophages are responsible for engulfing and digesting foreign invaders, including cancer cells. They do this by recognizing certain proteins on the surface of cancer cells and engulfing them, a process known as phagocytosis. Dendritic cells are specialized immune cells that are responsible for presenting foreign antigens to other immune cells. They do this by engulfing foreign invaders, including cancer cells, and presenting them to other immune cells, such as T cells. This process helps to activate the adaptive immune system and promote an immune response against cancer cells.

How the Immune System Targets Cancer

In addition to immunosurveillance, the immune system also fights off cancer through the use of immune checkpoints. Immune checkpoints are molecules on the surface of immune cells that help to regulate the immune response. They do this by either activating or inhibiting immune cells, depending on the signals they receive. One of the most well-known immune checkpoints is programmed death-ligand 1 (PD-L1), which is expressed on the surface of cancer cells. When PD-L1 binds to its receptor on T cells, it sends a signal that inhibits the T cell's ability to attack the cancer cell. This process is known as immune evasion and is one of the ways in which cancer cells are able to avoid detection and destruction by the immune system.

Immunotherapy is a type of cancer treatment that works by blocking immune checkpoints and activating the immune system to attack cancer cells. One of the most promising immunotherapy

treatments is checkpoint inhibitors, which are drugs that block the interaction between immune checkpoints and their receptors. By doing so, they allow T cells to attack cancer cells more effectively and promote an immune response against cancer. It is a relatively new and promising approach to cancer treatment that has shown great potential in clinical trials. One of the main advantages of immunotherapy is that it targets cancer cells specifically, leaving healthy cells unharmed. This is in contrast to traditional cancer treatments, such as chemotherapy and radiation therapy, which can damage healthy cells along with cancerous ones.

Immunotherapy

Immunotherapy works by either stimulating the immune system to attack cancer cells or by blocking the signals that cancer cells use to evade the immune system. For example, some immunotherapy drugs work by targeting immune checkpoints, which are molecules on the surface of immune cells that regulate the immune response. By blocking these checkpoints, immunotherapy can activate the immune system to attack cancer cells more effectively. Another advantage of immunotherapy is that it can provide long-lasting benefits. In some cases, patients have experienced a complete response to immunotherapy, meaning that their cancer disappears completely and does not come back. However, immunotherapy is not without its drawbacks. One of the main challenges of immunotherapy is that it can be expensive and difficult to manufacture. Additionally, not all patients respond to immunotherapy, and it can have side effects, including fatigue, skin rash, and diarrhea. Despite these challenges, immunotherapy has shown great promise in the fight against cancer. It has already been approved for the treatment of several types of cancer, including melanoma, lung cancer, and bladder cancer. As researchers continue to explore the potential of immunotherapy, it is likely that this approach to cancer treatment will become increasingly common in the years to come.

Conclusion

The immune system plays a critical role in fighting off cancer through the processes of immunosurveillance and immune checkpoints. Through the use of specialized immune cells, such as natural killer cells, macrophages, and dendritic cells, the immune system is able to recognize and eliminate cancer cells before they have a chance to develop into tumors. The immune system plays a critical role in the fight against cancer, using a variety of mechanisms to detect and eliminate abnormal or cancerous cells. While there are still many challenges to

overcome in developing effective immunotherapies for cancer, our understanding of the immune system's role in cancer biology is constantly evolving, offering new hope for the future.

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Review Article

Using Whole Genome Sequencing to Diagnose Acute Myeloid Leukemia

Neta Gal (author), Anika Shah (advisor), Michelle To (advisor)

Palo Alto Senior High School

Keywords: Leukemia, Whole Genome Sequencing, Cytogenetics, Acute Myeloid Leukemia

Abstract

Leukemia is a cancer that occurs in the blood and is caused by the rapid growth of abnormal blood cells. The disease has four different subtypes that affect different types of blood cells and have varying symptoms. Currently, leukemia is diagnosed with routine tests such as blood and bone marrow tests. Additionally, molecular and genetic testing play a crucial role in the diagnostic process, because genetics can provide insight into what course of treatment is best for the patient: such as predicting how a patient will respond to chemotherapy, or if the patient needs a stem cell transplant. The most common type of genetic testing for leukemia is cytogenetic analysis, but it has some limitations such as the need for viable cells. Whole genome sequencing is a different diagnostic method that can solve these limitations because it only requires a sample of DNA. Researchers are currently studying and comparing whole genome sequencing to cytogenetic analysis, to see if whole genome sequencing should be standardized in diagnosing leukemia. A recent study in 2021 shows great promise for whole genome sequencing in the diagnostic process for acute myeloid leukemia, but this method has a long way to go before it can be present in everyday care.

Introduction

Leukemia is a type of cancer that affects blood cells. It typically develops in the bone marrow, which is where blood cells such as white blood cells, red blood cells, and platelets are made (MedlinePlus). Leukemia occurs when DNA changes in bone marrow cells lead to an abnormal amount of blood cells (usually white blood cells) being produced. The dysfunctional blood cells

are called blasts and they can multiply quickly and overcrowd the bone marrow, making it difficult for healthy blood cell production (Pfizer).

Leukemia has different subtypes based on the speed of cell proliferation and the type of blood cell that is affected (Chennamadhavuni et al.). When leukemia is considered acute, it has a fast growth of blasts with symptoms that progress rapidly. On the other hand, chronic leukemia has a smaller degree of blasts and tends to have more chronic symptoms that worsen over time. There are two blood cells that can be affected: Lymphocytes and Myleoids. Lymphocytes are white blood cells in the immune system such as T cells, and myeloid cells are white blood cells such as neutrophils and monocytes (National Human Genome Research Institute). Overall, the four subtypes of Leukemia are acute myeloid (or myelogenous) leukemia (AML), chronic myeloid (or myelogenous) leukemia (CML), acute lymphocytic (or lymphoblastic) leukemia (ALL), and chronic lymphocytic leukemia (CLL).

Current Diagnostic Methods for Acute Myeloid Leukemia

The first tests done in the diagnosis process are getting a physical exam, CBC blood tests, and bone marrow tests (Yale Medicine). A routine physical exam is when symptoms of leukemia could potentially be observed, such as swelling of the lymph nodes. Taking a blood test is useful for diagnosis because it reveals any abnormalities in red and white blood cells and platelet levels, such as having an abnormally high or low complete blood count (The Leukemia & Lymphoma Society). Bone marrow testing is another significant diagnostic method because it can not only diagnose acute myeloid leukemia but also assist with treatment options, depending on the discoveries from the bone marrow sample. Bone marrow testing involves taking a biopsy from the hip bone and running different molecular and cytogenetic tests on it, such as flow cytometry, fluorescence in situ hybridization (FISH), and other molecular profiling techniques (Percival, Mary-Elizabeth et al). This type of testing will help further identify the subtype of AML and the best course of treatment.

Cytogenetics

Cytogenetic analysis is crucial for diagnosis because it detects chromosomal and genetic abnormalities that are associated with AML, such as mutations on the FLT3, NPM1, KIT, and

CEBPA genes (Kayser and Levis). The type of chromosomal abnormalities that a patient has can determine many things such as the severity of the disease, reaction to chemotherapy, risk of relapse, and the need for a hematopoietic stem cell transplant (Medeiros et al). Different chromosomal abnormalities place patients in different classifications or risk categories. One example is the World Health Organization (WHO) classification of myeloid neoplasms and leukemia, which is commonly used and includes eight different subtypes based on genetic abnormalities.

Disadvantages of Cytogenetics

Currently, detecting chromosomal abnormalities for AML is mostly done using conventional metaphase cytogenetic analysis, such as karyotyping and chromosome banding analysis (Duncavage et al). This process has limitations though, such as limited resolution and sensitivity and the requirement of viable cells. Cytogenetic evaluations can also be unsuccessful due to an insufficient number of metaphases to test on, or issues with sample processing and specimen handling. In one study, 220 out of 1,623 patients' cytogenetic reviews were rejected (14%), because of these same issues such as inadequate processing and having too few metaphases to analyze (Medeiros et al). Cytogenetic analysis is an essential tool in the diagnostic process for AML and has made a huge impact on the way that physicians diagnose and treat patients with AML. However, cytogenetics has some downfalls and it is frustrating to see patients have their diagnostic process delayed due to a rejected cytogenetics report. That is why alternative diagnostic methods are currently being considered, such as targeted sequencing, next-generation sequencing, or whole genome sequencing.

Discussion

Whole Genome Sequencing

Whole genome sequencing (WGS) involves sequencing the entire genome in one test to detect pathogenic variants for diseases (Nisar et al). Understanding the specific gene mutations of a disease can allow for the creation of personalized treatments and help us understand how a disease will progress (Yale Medicine). Sequencing costs and data storage requirements have been

continuing to decrease over the last decade, which makes WGS more realistic and plausible to use as a diagnostic tool.

Lately, researchers have been looking into using WGS as a diagnostic method for AML because it is rapid, precise, and has the possibility of providing a broader diagnostic yield than cytogenetic analysis (National Cancer Institute). A recent study published in 2021 obtained genomic profiles for 263 patients with AML and compared streamlined whole genome sequencing to two other types of methods: conventional cytogenetic analysis and targeted sequencing. Results from the study showed that WGS identified the same amount of chromosomal abnormalities as cytogenetic analysis, and was even able to find new genetic information for 24.8% of the sample (Duncavage et al). It also helped classify patients with inconclusive cytogenetic reports into their correct risk categories, which may have altered their treatment options. This study was significant because it showed an approach that only requires one test, as opposed to the multi-test process that is currently being used to diagnose and classify patients into risk categories. Furthermore, WGS provided results in multiple days and the costs were fairly equal to genetic testing.

A huge advantage of WGS is that, unlike conventional genetic testing for AML, it only requires DNA and not living cells. In conventional testing like karyotyping, there are many reasons that could cause the test to be inconclusive. It mainly stems from the requirement for viable living cells, and any mishandling or inadequate processing can lead the cytogenetics review to be rejected. This frustrating detail prolongs the diagnostic process and with WGS, it could be avoided, since WGS only takes a small sample of DNA from the patient's cancer cells.

What's Next

Even though this study showed a lot of promise, WGS is not ready yet to be implemented in everyday practice. The researchers in the study found some limitations such as that WGS did not detect small genetic changes like targeted sequencing, which is a sequencing method that focuses on only a panel of genes (Bewicke-Copley et al). There's also uncertainty about whether or not WGS helps improve the lifespan of patients.

Fortunately, there are some precision medicine trials for AML that are in the works that could help. Master Screening and Reassessment Protocol (MSRP) for the NCI MyeloMATCH Clinical Trials is a large trial sponsored by the National Cancer Institute (NCI) that will test existing and new treatments for AML (U.S. National Library of Medicine). Currently, the estimated start date for the study is February 28th, 2023 and the end date will be May 12, 2032. A different precision medicine trial called Beat AML Master Trial is estimated to finish in November 2023 (ASH Clinical News). This trial started in 2016 and is sponsored by The Leukemia & Lymphoma Society. 500 patients who are newly diagnosed with AML are enrolled in the study and based on their genomic profiles, they will be receiving personalized therapies and treatment.

Conclusion

Whole Genome Sequencing has a long way to go before it can be solely used to diagnose Acute Myeloid Leukemia. This approach has advantages compared to standard genetic testing and cytogenetic analysis, but it is a reasonably new concept to be applied to leukemia. More trials should be performed to compare whole genome sequencing and current diagnostic tests to confirm the differences between the two and to see if whole genome sequencing is significantly better. Additionally, even if whole genome sequencing is more efficient, it should not fully replace cytogenetic analysis, but be given as an option for patients along with the current diagnostic process. This way, patients can choose the diagnostic journey that they are most comfortable with.

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Literary Review

Chimeric Antigen Receptor T Cells: The Next Generation of Cancer Treatment

Peyton Higa (author), Anika Shah (advisor), Michelle To (advisor)
Hillsdale High School

Keywords: Cancer, immunotherapy, chimeric antigen receptor T cell, CAR T, axicabtagene ciloleucel, healthcare

Abstract

Developments in cancer treatment since 2011 have skyrocketed after the release of the first immunotherapy: chimeric antigen receptor (CAR) T cells. This novel therapy became very popular after it proved to have extreme potential in clinical trials. This literary review has assessed whether CAR T treatment could become the main mode of medicine for cancer by evaluating its emerging downsides and benefits. This article has been compiled through analysis of a variety of reliable sources and databases. One drawback of the treatment is that a large portion of CAR T immunotherapy patients experience cytokine release syndrome or neurotoxicity [9]. While reversible, both situations produce side effects that range from general sickness to coma [9]. In addition, CAR T is extremely expensive and has no potential for being sold off-the-shelf because it is personalized to each patient [15]. All considered, CAR T treatment's efficacy surpassed that of standard care, proven in the Yescarta stage-3 clinical trial of axicabtagene ciloleucel (ZUMA-7) [2]. Considering all aspects of this innovative treatment, it is unlikely CAR T immunotherapy will replace other treatments. However, seeing how new generations of CARs are being engineered, it is entirely possible that its downsides could be resolved.

Introduction

An estimated 39.5% of people will be diagnosed with cancer in their lifetime [3]. This makes it one of the leading causes of death worldwide, second only to cardiovascular disease [11]. Cancer is a disease where abnormal cells divide uncontrollably and damage the rest of the body [13].

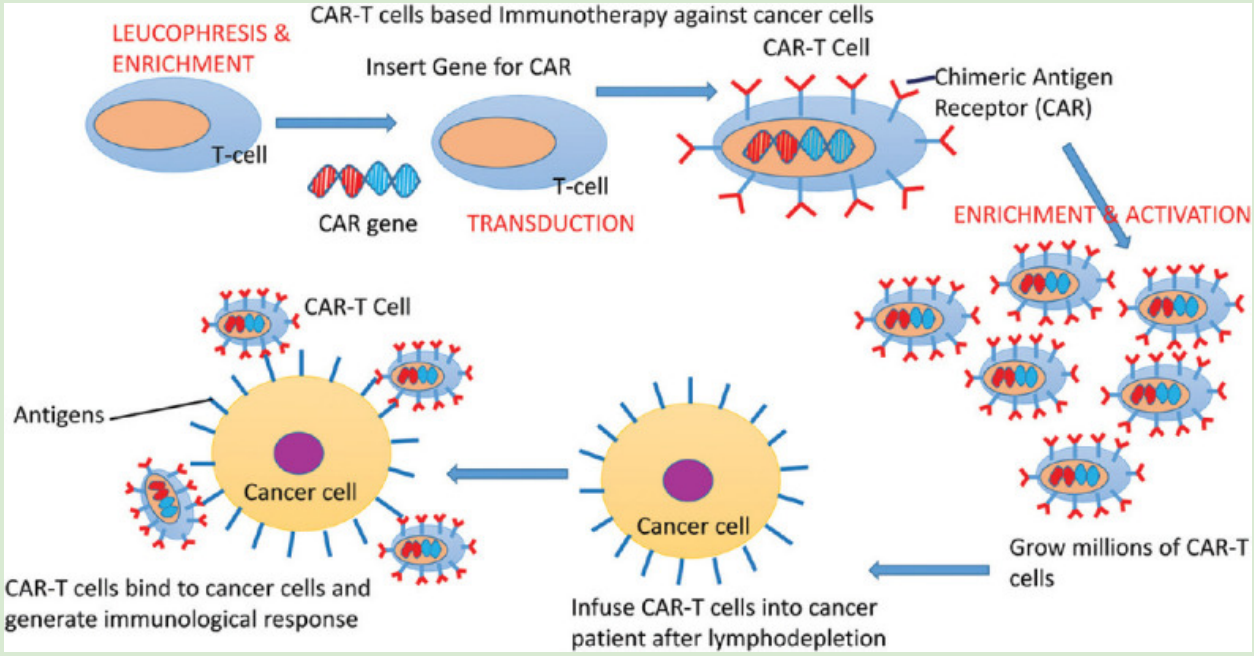
Cell division and death are normal processes that healthy cells undergo, however, cancer cells ignore chemical signals that regulate this cycle. These cells may clump together to form cancerous tumors, which invade nearby tissue and distant parts of the body to form new tumors through a process called metastasis [13]. In the past, chemotherapy, radiation therapy, and surgery were thought to be the only treatments for cancer– until 2011, when the first immunotherapy for cancer was approved [7]. This groundbreaking medicine, called chimeric antigen receptor T cells (CAR T), was a decade in the making [7]. It introduced the idea of personalized treatment that altered a patient's immune system to be able to fight against cancer [4]. This literary review aims to evaluate whether immunotherapy will replace other treatments entirely. Considering how it is used, its side effects, the cost, and its effectiveness, CAR T cells have immense potential and could prolong the lives of millions.

Discussion:

How CAR T Immunotherapy Works

CAR T cell therapy was the first FDA-approved immunotherapy [7]. A vital component of the immune system, T cells, are white blood cells meant to recognize and attack harmful or foreign particles in the body. Cancer cells go undiscovered by T cells, allowing them to proliferate and damage the body [4]. During treatment, T cells are "retrained" to recognize cancer [4]. This is done by altering its DNA so the T cell presents a structure called a chimeric antigen receptor (CAR) [4]. The shape of a protein, such as a receptor, determines its function, so adding a new receptor would introduce a new function to the cell. In this case, the new function would be to bind to antigens presented by cancer cells. When the CAR T cells are transferred back into the patient, they are able to bind to cancer cells, which then activates them to release chemicals that destroy cancer cells [4]. In order to engineer the CAR T cells, a sample of the patient's blood is drawn and the T cells are separated [12]. Then, the cells are "retrained" by introducing new DNA by means of a viral vector like lentivirus [12]. Vectors are used as vehicles to transport genetic material into a new cell [12]. This process, called transduction, allows the DNA coding for the CAR to be inserted into the genome of the T cell [12]. Subsequently, the viral vector is washed out and the T cells are activated by culturing them with growth factors [12]. The CAR T cells are cultured and grown until they reach numbers effective as a clinical dose [12]. Then, they are frozen while the patient receives a chemotherapy treatment called "lymphodepletion"–not to kill

the tumor, but the patient's own T cells [12]. This allows the population of engineered CAR T cells to expand once they are thawed and re-enter the body [12].



The Downsides of Immunotherapy: Side Effects

Although this new treatment shows promise, there are drawbacks. Cytokine release syndrome (CRS) is one major toxicity arising from treatment that nearly all patients of CD19 CAR T treatment experience, and symptoms start with fever, stiffness, general discomfort, and appetite loss [9]. In severe cases, it causes organ failure, hypoxia (oxygen deficiency), or hypotension (low blood pressure) [9]. The altered CARs functioning simultaneously with intracellular signaling domains causes an increase in the cell's capacity for cytokines once activated by a cancer antigen [9]. Consequently, the CAR T cells expel a larger amount of cytokines [9]. Cytokines are chemical signals that regulate the growth and actions of other cells in the body [9]. Immune effector cell-associated neurotoxicity syndrome (ICANS) is another condition on treatment that may follow CRS [9]. Also referred to as neurotoxicity, it is a disease that alters and impairs brain function [9]. Early symptoms include word-finding difficulty, confusion, speech impairment, difficulty understanding language, impaired fine motor skills, and drowsiness [9]. In severe cases, it may induce seizures, motor weakness, cerebral oedema, and coma [9]. Both CRS and ICANS are reversible [9].

The Downsides of Immunotherapy: Logistics

In addition to biological roadblocks, the widespread use of CAR T is unlikely due to production efficiency, cost, and narrow range of effectiveness. CAR T immunotherapy is personalized medicine for each individual. This means that it has to be prepared differently for each patient, so it has no off-the-shelf potential [15]. Despite the fact that cancer affects an enormous amount of people, CAR T treatment is available to very few. The total cost that companies spend to administer CAR T ranges from $373,000- 475,000 [5]. Monitoring the disease status after infusion can add an extra $79,000-85,000 [5]. As mentioned previously, cytokine release syndrome affects a large number of CAR T patients, and CRS treatment can be $30,000-56,000 per patient [5]. All things considered, the total cost of CAR T treatment for patients with severe CRS can accumulate up to $500,000 [5].

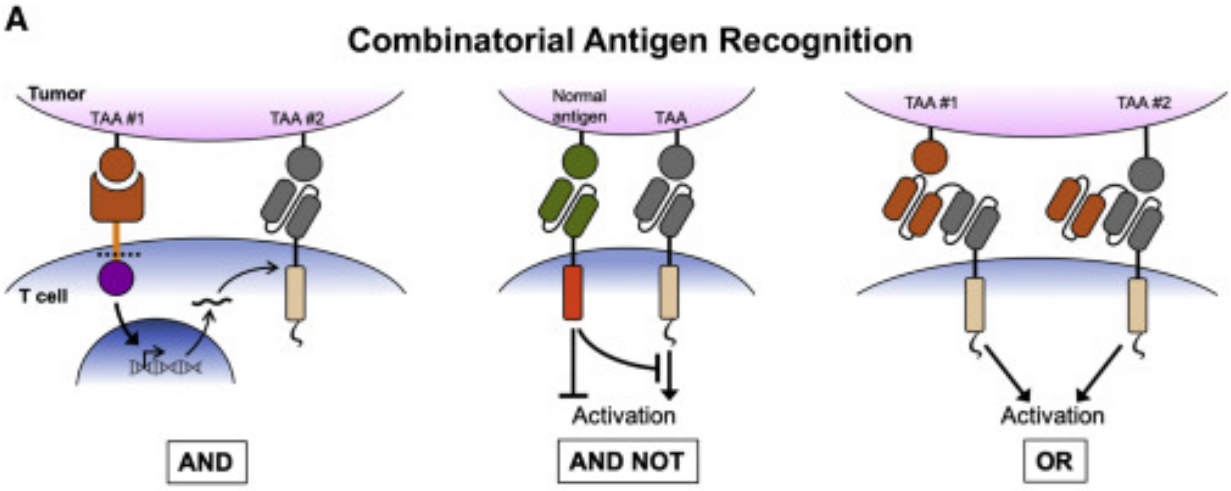
The Evolution of Immunotherapy: Effectiveness in Clinical Trials

Newer versions of CAR T are in testing, and prove to be extremely effective. CAR T treatment proved to be superior to the standard care in a study of patients with relapsed or refractory large B cell lymphoma [2]. The first-line treatment for patients with this cancer is chemoimmunotherapy, a combination of chemotherapy and immunotherapy [2]. When the first line of care is not effective or stops working, doctors administer a second-line treatment. For relapsed or refractory large B cell lymphoma, the second-line treatment is chemotherapy with autologous stem cell transplantation [2]. So far, the US Food and Drug Administration has approved three CAR T cell drugs: axicabtagene ciloleucel, lisocabtagene maraleucel, and tisagenlecleucel [12]. Axicabtagene ciloleucel (axi-cel) is a CAR T medication sold by Yescarta, which has shown immense potential in phase-3 trials [2]. Before getting approved by the FDA, treatments must submit data from clinical trials, which are conducted in phases [10]. Each stage has a different purpose, with phase 1 and 2 trials aiming to test a drug's safety and effectiveness [10]. A phase-3 trial measures its performance by comparing it to a standard treatment [10]. The prognosis is poor for patients after first-line treatment, so in the study titled the "ZUMA-7" trial, axi-cel was compared to the standard care for second-line therapy [2]. During the study, 180 patients received axi-cel and 179 received standard care [2]. In the 24 months following treatment, the event-free survival (meaning time passed after cancer treatment where no complications occur that the treatment was intended to prevent or delay) was 41% in axi-cel

patients and 16% in standard care patients [2]. 83% of axi-cel patients and 50% of standard care patients showed a response to the treatment [2]. These points, along with many others brought up by the trials, were testament to how CAR T cell therapy is growing more effective. The fact that the axi-cel treatment produced better results than the standard care shows its potential to overtake other types of cancer treatments in the future.

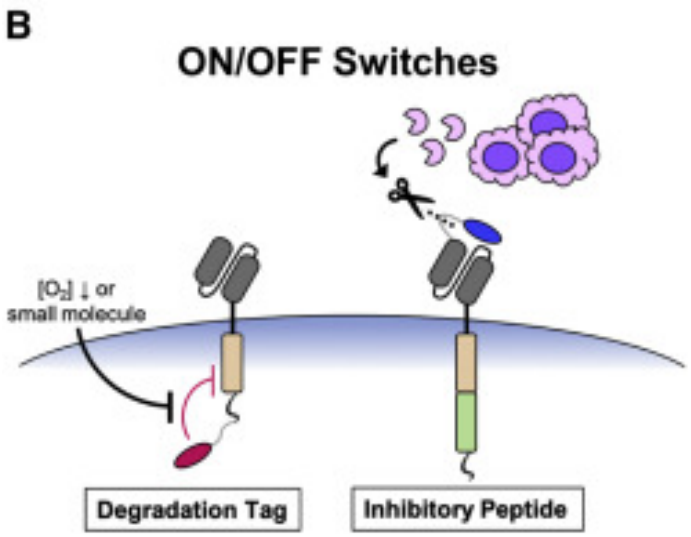
The Evolution of Immunotherapy: Improving Efficacy

There are many adaptations to CAR T cells that may improve its efficacy. Three methods include combinatorial antigen recognition, engineering an "on/off" switch, and adaptor-mediated activation. These three alterations increase the target specificity of CARs, regulate its presence to reduce toxicity, and help overcome tumor heterogeneity (genetic diversity in tumor cells that may allow it to go unnoticed, even by engineered T cells). In combinatorial antigen recognition, CAR T cells are programmed to recognize multiple antigens to produce different reactions [6]. This technique uses three different Boolean logic gates: AND-gate logic, AND-NOT gates, and OR-gates (pictured below) [6].

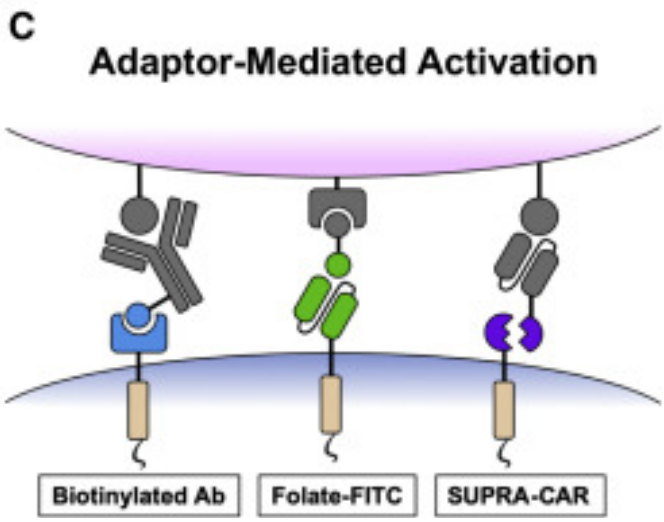


AND-gate logic requires two different antigens to produce a response [6]. Having a more specific stimulus decreases the risk of CAR T cells damaging healthy tissues that present an antigen that is also on the tumor cells. After an antigen triggers tumor-associated antigen #1 (TAA#1), the cell presents a CAR, and if that CAR recognizes a second antigen (TAA#2), it will trigger a response [6]. Additionally, CAR T cells using AND-NOT logic use iCAR, an inhibitory

receptor that blocks CAR function when activated by an antigen found on a healthy cell [6]. CAR T cells that express OR-gate logic can use two different CARs on one cell, and the activation of either receptor will trigger a response [6]. ON/OFF switches are another method used to increase the safety of CAR T treatment. Unlike combinatorial antigen recognition, this method focuses on mitigating symptoms such as neurotoxicities and CRS, rather than raising its specificity [6]. The default state of CARs are always "on," but the ON/OFF switches regulate the amount of functional CARs on the surface of the engineered T cells [6].



The third way of improving the efficacy of CARs is adaptor-mediated activation. With this alteration, it is easier to manipulate when and how the T cell responds. In this method, adaptor CARs bind to tumor-specific adaptor molecules [6]. The adaptor molecules are administered externally to "label" the tumor cells [6]. Thus, *any* cells marked with the adaptor molecule can induce an anti-tumor response. This way, a range of antigens could be targeted instead of just one, like the conventional CAR T cell. The molecule is administered externally, so that when it is eliminated from the body, the adaptor CARs no longer interact with the tumors [6]. The flexibility of adaptor CARs allows doctors control when to turn "on" and "off" the adaptor CAR. This increases the safety of CAR T cells because it mitigates the risk from side effects such as CRS or other toxicities, as well as the fact that treatment can be re-initiated in the case of a relapse.



Conclusion

It is undeniable that CAR T cell immunotherapy has and will continue to produce groundbreaking results. Considering the side effects that it induces, as well as the measures engineered in newer generations to mitigate these toxicities, it is likely that CAR T cell treatment will become safer for patients. However, due to the fact that it is extremely cost-inefficient, it is unlikely that it will be overtaking chemotherapy and other treatments, at least not anytime soon. No matter how much potential it has to outdo other cancer therapies, it would not matter if only a few patients are able to afford it.

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Literary Review

Leukemia Cancer in Children: The Role of Hazards/Environmental Factors in the Diagnosis of Leukemia

Samriddhi P. (author), Anika Shah (advisor), Michelle To (advisor),
Summit Tamalpais High School

Key Words: Childhood Leukemia, Cancer, Acute Lymphoblastic Leukemia (ALL), and Acute Myeloid Leukemia (AML).

Abstract:

Leukemia Cancer is one of the most common types of cancer that occurs in children. It is a type of blood cancer that starts when cells in your body start to grow and begins to form in your body just like any other type of cancer. Leukemia Cancer is a type of cancer that forms in white blood cells. White blood cells fight infections. Current research has been endeavoring to discover if early environmental chemical exposures and exposures to risky hazards both indoors/outdoors from an early age play a role or significant connection to why children develop Leukemia. Factors such as solvents, tobacco smoke (smoking), pesticides, and other detrimental factors are taken into consideration in research to see if these components influence childhood Leukemia (National Library of Medicine: Childhood Leukemia: A preventable disease). Taking these factors, researchers have researched each factor to see if children who grow up in these environments have a higher risk of developing Leukemia cancer. In this review article, we will be looking more into the research and discussing more of the experiments that have been conducted. The goal of this review is to dive more into the data collected in these experiments and discuss the downsides, and other connections. Remember that these are real research done by different researchers, and we're trying to find if environmental factors play a role in contracting childhood Leukemia.

Introduction:

The two main cases of Leukemia are acute lymphoblastic leukemia (ALL), which affects lymphocytes and acute myeloid leukemia also referred to as (AML), which affects blast cells. Another form of Leukemia is called Chronic Myelogenous Leukemia (CML). This type of Leukemia is rare, less common in children, and is a developing type of Leukemia. Leukemia cells are caused by white blood cells that fill up in the body and rapidly produce the white blood cells. When unnecessary blood cells are formed this

causes your body's immune system to be weak making your cells fragile, and difficult to fight diseases. When your body consists of more white blood cells then red blood cells you are at a high risk of having Leukemia. This all happens in the blood marrow, which is the area in your body where blood is formed. When this happens, the good blood cells in the children's body get blocked and become unhealthy, and eventually get weaker. Leukemia can also spread into other parts of your body such as the brain, chest, liver, and other areas in the human body. You must take immediate action, take important care, and precautions, and measure your child's health if you find out that you are diagnosed with Leukemia Cancer. As scientists and doctors are learning more about this type of blood cancer, initially more treatments have been discovered from as early as the 1950s as a result more and more treatments have been accessible to children diagnosed with Leukemia. It is dominant that we learn more about Leukemia Cancer as it is one of the most common forms of cancer in children and has a diagnosis rate of around 25.1 percent % (Childhood and Adolescents Blood Cancer Facts and Statistics - Leukemia & Lymphoma Society). Several different treatments are available for your children to treat Leukemia, if your child is diagnosed; it is important that you know your options, and choose the treatment best for them (based on stage and risk level). In this case, make sure to reach out to your child's doctor, do research, and have a good understanding of your options before sticking with treatment. Although we will not be talking about treatments, you need to know that there are treatments, and Leukemia is curable and can be treated when you take action. Childhood Leukemia has a survival rate of 90% (National Library of Medicine: Childhood Leukemia: A preventable disease). Now that this article has discussed about Leukemia cancer, the different types of Leukemia (ALL, AML, CML - both common/uncommon types of cancer), the diagnosis rate, and the survival rate we will get more into research on Leukemia and talk about how big of a role environmental factors play in contributing to attracting Leukemia in a child's body. Although, children who suffer from genetic conditions and other issues are indeed more likely to have a higher risk of developing Leukemia. In this article, we will look at research outside of genetics, outside of family/parental history, outside of health issues, and instead see how simply the environment and the hazards a child grows around can influence a higher risk of developing Leukemia than a child who grows up in an environment without these factors. The research we will be looking into is, "Childhood Leukemia: A preventable Disease" by the National Library of Medicine.

Rationale:

A child's factor and thus the conditions they grew up under can play a huge role in their health. A child that grows up in a toxic household with a family that smokes in the household is most likely to develop diseases and develop health problems from an early age compared to a child that grows up in a household with no smoking. Factors like smoking and the use of other chemicals, and household conditions can also

play a role in your children's health or even be a reason why your child has developed Leukemia. It is important to be careful about these factors when raising a child, as these factors will give an individual's child lifelong problems and health issues. Make sure to stay aware of the environment that your child is raised under as it can be a cause of the development of childhood Leukemia.
Leukemia Cancer can also occur outside of environmental factors, and there are also many causes outside of environmental factors so if your child does have symptoms make sure to do blood tests, and other medical procedures to detect if you have cancer.

Objectives:

The main objective is to discuss Leukemia Cancer, specifically Leukemia Cancer that occurs in children and how different environmental factors impact this type of cancer, and any new things scientists are researching in Childhood Leukemia. This review is intended to inform the audiences about childhood Leukemia and to look deeper into past research conducted by researchers that looks into hazards that might nurture early childhood Leukemia cancer. We will look into the possible different causes of Leukemia, and why exposure to these hazards may introduce children to be detected with Leukemia at such an early age. The research looks at conditions in the environment before pregnancy, during, and after conditions, looks really into the different factors children were exposed to, and collects data.

Discussion:

***Research on "hazards/risk factors/environmental factors that possibly can cause childhood Leukemia"*Methodology:**

Duration and Location: An epidemiologic study focusing on Leukemia in children and if activation of certain risk factors causes children to have a higher chance of being diagnosed with Leukemia. This research was published in November 2016 by Pediatrics.

Subjects: The subjects of this research are both parents (measuring/observing their environmental conditions and exposures during, before pregnancy, and preconception, and children (observing their living conditions after birth). This research wasn't research that was done in person all at once but rather a collection of experiments and findings conducted by different researchers.

Materials: The materials used in this research were three research done in the year 2016 and published through the Gov database to further connect how environmental factors from as soon as preconception to before pregnancy can influence the diagnosis of childhood Leukemia. This first research on pesticides

was done using dust samples and geographic information (National Library of Medicine: Childhood Leukemia: A preventable disease).

Data Analysis: All three of the conducted research had different sources of exposure. The first source of exposure in the first research was the exposure of pesticides, the second exposure (2nd research) was smoking tobacco, and last the final research's source of exposure was solvents and chemicals such as paint. All these exposures can negatively affect your child's health and as we look into each research we will unwrap the data that was collected in each of these research.

The effects of exposure to Pesticides in childhood Leukemia

Firstly, we will look into the first research that was done regarding exposure to pesticides. Most people spray pesticides in their homes to get rid of insects, pests, and for other reasons. But, what people don't know is the consequences of pesticides. Pesticides consist of heavy chemicals that take not months but years or in some cases even longer to clear out. Pesticide exposure is harmful because they are full of toxic materials that are connected with cancer, and other major health issues. It is important even as adults to avoid the usage of pesticides as they have been shown to result in many effects. It is also important not to use these toxic sprays when conceiving a child as evidence has shown that exposure to pesticides during pregnancy can affect both the mother and children. In the research, the researchers were trying to search if pesticides in the house both in both indoor and outdoor sites influence Leukemia. In the chart, we can see that each of these data comes from different sources, is conducted by different people, and there are several sources that this information comes from. The type of Leukemia that is tested for in this first research includes the two more common types of Leukemia which include: Acute Lymphoblastic Leukemia (ALL), and Acute Myeloid Leukemia (AML). This specific research also looks at the "period of exposure" that the children were exposed to pesticides and the source of exposure which are Maternal Occupation, Paternal Occupation, and Residential. The reason why the exposure to Pesticides was being tested in this research was that pesticides are full of toxic chemicals that affect your and your children's health even years after exposure. When children come in contact with pesticides they can get serious illnesses and are more likely to face health issues compared to adults since they have a lower immunity and health capacity. Since they are so young, their body is still growing and sensitive so when coming in contact with chemicals like pesticides their body reacts fast. According to Childhood Leukemia: A preventable disease, states "Use of pesticides in and around the home is of particular interest because of young children's hand and mouth contact with surfaces potentially contaminated by persistent pollutants, including pesticides." This shows how pesticides are a major component of the force that influences Leukemia. In this research, it was found from different sources of information that chemicals such as

Dichlorodiphenyltrichloroethane (DDT) were found in people's homes ever after decades since it was banned in 1972. DDT is an extremely harmful chemical that was banned in the US because of its potential human health-related risks. This shows how strong these pesticides are that they still stayed in the resident's homes (National Library of Medicine: Childhood Leukemia: A preventable disease). Furthermore, this study of 10,000 Leukemia cases reported a finding that households exposed to these such harmful pesticides were more likely to have children that developed childhood Leukemia and because of this it raised the proximity of Acute Lymphoblastic Leukemia (ALL), and Acute Myeloid Leukemia (AML)(National Library of Medicine: Childhood Leukemia: A preventable disease). Another discovery that scientists found in this research is that maternal occupational exposure and preconception exposure also expanded the risk consisting of ALL and AML. It is also shown that exposure to these dangerous pesticides in parents can also affect children, transmitting the exposure to their children. Such exposure to chemicals including DDT, pyrethroids, and other toxic chemicals have shown to attract and lead to the development of ALL. Altogether, this first research has shown that exposure to pesticides and such chemicals affects the development of Leukemia. Like every experiment, this research has downsides and flaws because we don't know how these tests were done, and since these researches were done worldwide we don't know if all the researchers used the same methods and tactics. Since we don't know everything about how the experiment was conducted we would have to look into each research used and search if it includes the implementation of any biases in the first place before even guaranteeing it to be 100% true.

TABLE 1

Selected Meta- and Pooled Analyses of Pesticide Exposure and Risk of Childhood Leukemia Subtypes

Source	Source of Exposure	Period of Exposure	ALL		ANLL/AML	
			No. Studies	OR (95% CI)	No. Studies	OR (95% CI)
Van Maele-Fabry, 2011 ([32]) (MP)	Residential (insecticide)	Ever	5	2.11 (1.80–2.48)	3	2.30 (1.53–3.45)
		Pregnancy	4	2.22 (1.87–2.64)	2	3.13 (1.45–6.75)
		After birth	2	1.78 (1.12–2.84)	n/a	n/a
Bailey, 2015 ([19]) (PO)	Residential	Preconception	12	1.39 (1.25–1.55)	9	1.49 (1.02–2.16)
		Pregnancy	12	1.43 (1.32–1.54)	9	1.55 (1.21–1.99)
		After birth	12	1.36 (1.23–1.51)	9	1.08 (0.76–1.53)
Van Maele-Fabry, 2010 ([33]) (MP)	Maternal occupation	Ever	4	1.34 (0.70–2.59)	2	2.68 (1.06–6.78)
	Paternal occupation	Ever	3	1.09 (0.75–1.60)	2	0.73 (0.19–2.76)
Bailey, 2014 ([17])(PO)	Maternal occupation	Pregnancy	12	1.01 (0.78–1.30)	5	1.94 (1.19–3.18)
	Paternal occupation	Preconception	12	1.20 (1.06–1.38)	8	0.91 (0.66–1.24)
Bailey, 2014 ([17]) (MO + MP)	Maternal occupation	Pregnancy	n/a	n/a	9	3.30 (2.15–5.06)
	Paternal occupation	Preconception	14	1.23 (0.99–1.53)	n/a	n/a

ANLL, acute nonlymphoblastic leukemia; CI, confidence interval; MO, meta-analysis of original data; MP, meta-analysis of published data; OR, odds ratio; n/a, not available; PO, pooled analysis of original data.

Metayer, Catherine, et al. “Childhood Leukemia: A Preventable Disease.” *Pediatrics*, vol. 138, no. Supplement_1, American Academy of Pediatrics, Nov. 2016, pp. S45–55. Available at: https://doi.org/10.1542/peds.2015-4268h.

The effects of exposure to tobacco in childhood Leukemia

Another research that was done in this review was done concerning exposure to tobacco smoking. Tobacco Smoking for many adults is the leading cause and accountable for cancer so the purpose of this second environmental factor was to see if there was any finding that connected Tobacco smoking with the diagnosis of childhood Leukemia. It is shown that smoking also influences Leukemia cancer in adults. Aside from this, many factors impact or trigger the risks of cancer, and smoking is one. Now we will see if this is the same for children. We’ll see in situations where a parent or guardian smokes at home or even outdoors, children have a higher chance of contracting Leukemia. The purpose of this research is that researchers here were trying to see if children exposed to smoking in their households at an early age even before and during pregnancy had a higher risk of contracting Leukemia and other health issues. The usage of tobacco has been shown to make a person’s immune system weaker, and because of this, it can be hard for someone to fight disease and even influence cancer types such as AML and ALL. In this research, not enough evidence was found that smoking leads to childhood Leukemia, and until today this is still a controversial topic. Research is still being done trying to see if these environmental factors connect to childhood Leukemia. This is not the only environmental problem that is said to influence childhood Leukemia but if found connections with smoking it is a big deal because it explains how dangerous smoking in an environment consisting of children can be. Just like the first research, this research is done in the same way and follows the same processes. It comes from different sources but the difference here is that the sources used here are farther back and thus older (11-12 years back). It is a better option to use research that is not too old so that it consists of more advanced information. Therefore, this is a flaw of this second research. As discussed earlier every research has its flaws and limitations so we must look more into it and do our research of what has been researched to see if we come across problems with data and basics like that. Lastly, another finding of this research is that researchers found that when parents were involved in smoking both beforehand of birth, and after children were more likely to be affected. More evidence needs to be found to determine whether parental smoking of tobacco has a direct connection with increasing the risk of Childhood Leukemia, and more data still needs to be found to be able to know if there are connections between these two factors.

TABLE 2

Selected Meta-analyses of Published Data of Tobacco Smoking and Risk of Childhood ALL

Source	Source of Exposure	Period of Exposure	No. Studies	OR (95% CI)
Liu, 2011 ([52])	Paternal (yes/no)	Preconception	13	1.25 (1.08–1.46)
	Paternal (highest exposure index)	Preconception	10	1.38 (1.11–1.72)
	Paternal (yes/no)	Pregnancy	8	1.24 (1.07–1.43)
	Paternal (highest exposure index)	Pregnancy	4	1.28 (0.93–1.76)
	Paternal (yes/no)	After birth	7	1.24 (0.96–1.60)
	Paternal (highest exposure index)	After birth	6	1.33 (1.00–1.78)
Milne, 2012 ([53])	Paternal (yes/no)	Preconception	10	1.15 (1.06–1.24)
	Paternal (≥20 cigs/day versus no)	Preconception	7	1.44 (1.24–1.68)
Klimentopoulou, 2012 ([49])	Maternal (yes/no)	Pregnancy	20	1.03 (0.95–1.12)

CI, confidence interval; OR, odds ratio.

Metayer, Catherine, et al. "Childhood Leukemia: A Preventable Disease." *Pediatrics*, vol. 138, no. Supplement_1, American Academy of Pediatrics, Nov. 2016, pp. S45–55. Available at: https://doi.org/10.1542/peds.2015-4268h.

The effects of exposure to Paints and Solvents in Leukemia

The final research that was done in this review was trying to find if exposures to paints and solvents in the household in early childhood influenced the risks of developing ALL and AML leukemia. This research included the same methods as the past two pieces of research and involved the same periods of exposure which were during pregnancy, after, and conception, and the amount of time in months before conceiving a child. Paint is extremely toxic during pregnancy because it contains heavy chemicals and petroleum-based chemicals that affect children's health. It is hazardous and pregnant women are told to stay away and if not limit their exposure to pain and other related solvents. It is toxic and evidence has shown that environmental exposure to paints and solvents can increase a child's risk of Leukemia. In this research, it has been shown that Leukemia specifically: Acute Myeloid Leukemia (AML), the chances of detecting AML type of leukemia arise when a child is exposed to Paints and Solvents in their household. In the text, it states, "1.2- to 1.4-fold increased risks of childhood ALL associated with these exposures" (National Library of Medicine: Childhood Leukemia: A preventable disease). This ratio just shows how paints and solvents are harmful to have around. This and further evidence have proven that when exposed to paints and solvents in the home, AML and even at times ALL types of Leukemia are triggered. We can see the connection between how environmental exposures to Paints, Solvents, and other petroleum-based

products influence the risk of developing Leukemia cancer. Some downside of this experiment is we don't exactly know how each different research was tested. We aren't told how these tests were done, and if it were simply people telling the researchers if they have or not have been exposed to these factors. We have a limitation of information and since this research comes from many different people we don't know if they used the same methods or various methods. Also, we don't know if we're receiving biased information since we aren't told a lot about how each of these experiments was conducted, where they took place and other basics like that. We do know that all these experiments are fr. m 8 to 9 years ago, so the data should be pretty up to date. All in all, this research overall does have limitations and lacks the basic information of where the data comes from, and suffers in aspects like that. We also need to consider that these are not the only factors that risk the diagnosis of Leukemia and that many more factors can influence childhood Leukemia. Factors such as a child's diet, nutrition, and the atmosphere or place where they live (pollution) are examples of some other factors that can risk the increase of Leukemia.

TABLE 3

Meta- and Pooled Analyses on Exposure to Paint, Solvents, and Petroleum Products and Risk of Childhood ALL

Study	Source of Exposure	Period of Exposure	No. Studies	OR (95% CI)
Zhou, 2014 ([50]) (MP)	Solvents; any sources; maternal	Pregnancy	7	1.25 (1.09–1.45)
	Petroleum, any sources; maternal	Pregnancy	7	1.42 (1.10–1.84)
	Paint; any sources; maternal	Pregnancy	7	1.23 (1.02–1.42)
Bailey, 2015 ([16]) (PO)	Paint; residential; any users	12 mo before conception	2	1.00 (0.86–1.17)
		3 mo before conception	5	1.54 (1.28–1.85)
		Pregnancy	8	1.14 (1.04–1.25)
		After birth	4	1.22 (1.07–1.39)
Bailey, 2014 ([18]) (PO)	Paint; occupational; paternal	12 mo before conception	12	0.93 (0.76–1.14)
	Paint; occupational; maternal	Pregnancy	11	0.81 (0.39–1.68)

CI, confidence interval; MP, meta-analysis of published data; OR, odds ratio; PO, pooled analysis of original data.

Metayer, Catherine, et al. "Childhood Leukemia: A Preventable Disease." *Pediatrics*, vol. 138, no. Supplement_1, American Academy of Pediatrics, Nov. 2016, pp. S45–55. Available at: https://doi.org/10.1542/peds.2015-4268h.

Conclusion:

The purpose of this literature review is to link how early childhood factors such as environmental exposures can play a prominent role in your children's health and to explain how comprehensive

Leukemia Cancer is. The purpose of this is not just to notify you of Leukemia, but also to make you aware of the different factors that can affect your and your family's health. It is to alert you to take immediate action if you do have these exposures in your home, and rather notify you of the consequences. Many parents aren't aware of this and environmental exposures aren't much discussed. It is so important that you do your research before pregnancy so you are aware of the conceivable risks and detriments that may affect your child beforehand. When informed about these environmental factors, we are to better know how to keep our children safe from Leukemia and reduce chances of having hazards of contracting Leukemia to mothers' preconceptions and during pregnancy. With these exposures in mind, we hope to see reduced numbers of Leukemia and the future days and more research done to find and better understand how environmental exposures increase the risks of childhood Leukemia. While other factors of Leukemia are not discussed in this review, make sure to do your research on other causes of Leukemia as there is not only one cause of this widespread disease. If you, yourself do have children and know that your home is exposed to the factors discussed in this article then take urgent action, and get rid of these exposures from your children as soon as possible. To be safe, you can take extra precautions to keep your children and home away from such harmful exposures. Childhood leukemia is preventable when you know about it and make specific efforts to keep it away from a child. I hope that this article informed you about Leukemia and alerted you on how environmental factors can play a big role in threatening a child's health, and you learned how to better keep your household safe.

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Literary Review

A Brief Overview of the Evolution of Medicine and Healthcare

Saivishwateja Papaiahgari (author), Anika Shah (advisor), Michelle To (advisor)

Keywords: Medicine, Healthcare, Injury, Treatment, Patients

Abstract

The birth of medicine was a key moment in the rise of civilization that represents mankind's first resistance to nature's pull on human life. Medicine, with its accumulated value and services to humanity, slowly evolved into a sacred field that could not be entered and practiced freely. The first man to consolidate a picture of medicine for the general public was the great Hippocrates, Greek doctor and scientist, as he created a revolutionary prescription of a prototypical aspirin and established the need for a scientific approach to medicine (Hajar). His many feats earned him the right to be the namesake for the oath through which doctors are sworn into the medical field today. However, between 460 BCE and the present year, 2023 CE, so much time has passed that many of the once revolutionary feats that famous physicians from the past initially performed have become an everyday occurrence. Furthermore, medicine has slowly incorporated other interactions than the initial purpose of a patient receiving treatment for an ailment or injury. The purpose of this essay is to collectively analyze and provide insight on the evolution and branching that have occurred in the medical field over the period of time since medicine's initial conception. Furthermore, it aims to hypothesize (in a limited manner) how medicine will evolve and subsequently map out the directions and paths that it will follow in the future.

Discussion

Past

It can be said that the main motivation behind the creation of the medical field was to ensure a certain quality of life for mankind. After all, in a world unfamiliar to mankind, for which there was no prior knowledge of how to comfortably live, survival was undoubtedly the essence of their motivations. As such, humans began to adapt to their environments, developing appropriate weaponry for the sake of hunting for food and rudimentary clothing to shield them from the elements. As time passed, humans evolved, following the agricultural revolution that allowed them to lead a sedentary lifestyle. Eventually, they learned living collectively would increase their survival chances against the elements and their fellow inhabitants of the lands. These small groups formed the foundation for modern society; as they continually expanded and grew, they became intellectually and technologically advanced, eventually building themselves up from mere villages to civilizations, cities, and nations. However, with these great changes came equally pressing consequences that needed to be addressed, the frontrunners being diseases and injuries. At that time, diseases and injuries were largely chalked up to being “the wrath of a god”, “part of life”, “misfortune”, or some other similar belief, as the concept of a disease was incomprehensible to people who had never seen a precedent for them (Underwood et al.). Their belief was that some higher power was doing something to tear away a piece of the afflicted person (physical, spiritual or both). However, despite their beliefs in their chosen higher power, they did have a method to remedy the conditions caused by diseases. In order to make the affected person (or people) whole again, they would perform various rituals, incantations, and other things associated with witchcraft. In fact one of the more widely seen techniques is “trepanning”, a process in which a two-point-five to five centimeter hole is created in the skull

(usually around the forehead) for the purpose of allowing the disease to find an escape path from the body (Underwood et al.).

According to Underwood and colleagues' findings, this method was found to be used in several locations around Europe and unexpectedly in Peru. The widespread nature of the technique could be attributed to a prehistoric ancestral technique that was shared by the common ancestors of the people from the two geographic locations. This could be explained by the theorized migration of a certain proportion of the population across a hypothetical land bridge that once existed. The techniques' similar development indicates that despite the geographical differences the purpose of the technique would have been the same: "treatment". This idea of "treatment" is what Ajai R. Singh, M.D. explains as "palliation", the first stage of medicine's development and the progenitor of its evolution in the future (Singh). Singh finds palliation to be something that is quite common today. Palliative care, he says, is the standard practice in modern medicine, in which the doctor controls the spread of the disease while decreasing the amount of discomfort that the patient(s) feel(s) in the process (Singh). This theme is concurrent with the practice of trepanning in Ancient Europe and the Ancient Americas, where the root cause is assumed to be "treated" by placebo practices that could have, in fact, been harmful to the receiving party. Despite the fact that current palliative care is more medication-based, which is nowhere near as invasive as cutting a hole in a person's skull to "provide a path of escape for a curse", the use of drugs to curb the presence of symptoms and side effects can have long-term consequences that ultimately lead to a failure of a bodily system and subsequently death. Even if the fact that medical care and lifespan have greatly improved across the initial times of medicine's conception, it cannot be denied that palliative care should not be a prominent method of healing any longer. It simply avoids fixing the root problem in order to satiate a patient in the short term

without having a clear plan for tackling the long term ramifications of the disease that the patient will experience. Palliative care is only suitable for providing comfort to a patient who has reached the terminal stage of their illness, helping them to live out the rest of their lifespan unhindered.

Present

While palliative care has indeed been a standard practice since olden times, in more recent times there has been a large shift towards "cure" and "prevention" as defined by Dr. Singh (Singh). Cure and prevention are two opposite but equally important sides of the spectrum of modern, science-based medical treatment. Cure refers to attacking the root cause of a disease and eliminating it for good so that it doesn't relapse/reoccur, while prevention refers to taking actions and making decisions beneficial to preventing the acquisition, passage, and spread of a disease. Cure and prevention, while ultimately reaching the same end goal of removing the presence of a disease through some course of action, take different approaches to tackling the root cause of the issue. Compared to the straightforward approach of a cure, a prevention is more oriented towards finding an alternative path around the root cause itself. While it may seem to be similar to palliative care, prevention is not the same. Prevention, by assessing the situation and identifying the possible alternative paths around a root cause for disease, essentially tackles the disease itself at the root level by establishing methods by which acquiring the disease has a chance so low that the probability of mass spreading is brought very low, ideally close to zero. The biggest reason for the large shift towards cure and prevention, despite palliative care's substantial remaining component in modern medicine, is a result of the Scientific Revolution. At the heart of the Scientific Revolution, there was one single sentiment: "I think; therefore, I am". The brilliant

mind of Renee Descartes was able to associate mankind's whole existence with their ability to think for themselves; in other words, the ability to think is the essence of humanity. From that one spark of a thought, a prairie fire of creativity began to erupt, starting a golden era of scientific and cultural development across the world, an occurrence from which medicine also reaped a load of benefits. Once humans began to view the world through a more secular lens, they also began to look at disease scientifically. They began to start breaking diseases down into their causes, which at first, they discovered, was mainly due to unsanitary living areas due to lifestyle. However, they soon realized that living organisms could be the catalysts for diseases of destructive proportions. Sanitation and antibiotics thus became two of the most touted medical milestones representing the two sides of the modern medical spectrum, prevention and cure, respectively. Another of the most famous inventions was the vaccine, created in order to help patients build immunity by injecting a nonviral/dead strain of a disease. The vaccine itself is practically the representation of the broad spectrum of modern medicine as it ecompasses both the cure and the prevention. Vaccines stimulate the body into producing the correct biomolecules to combat a disease should it be acquired, thus providing a certain level of immunity to the disease. In addition to that, a vaccine is like a preventative measure that is intended to provide a patient with that required immunity before they ever contract the disease. What this provides to the realms of prevention and cure is the ideal blend of the two, possibly indicating a path for the future development of the medical field. However, the shift did not occur exclusively in the practices of medicines. The field itself has expanded to take in other aspects such as technology and business. Business in particular has become such a large component of the field that an untrained bystander can barely distinguish between the two. The term "healthcare" draws its definition from the business aspect of the medical field, as healthcare is simply any medical

service that demands reparations from the person receiving care (Sawyer). This ideology of taking money for treatments started long ago but has since reached its peak in the current age, with a national GDP contribution of 4.3 trillion dollars or "18.3 percent of gross domestic product" ("NHE"). This shift of attitude towards monetary reparations can be thus linked to the standardization of palliative care within modern medicine, as not treating the root cause of an issue but simply relieving some of its symptoms forces patients to return for palliative treatments, allowing for a source of revenue until the patient either ends treatment or cannot continue it.

Concluding Remarks and Hypotheses

While there are some issues to fix with the current state of medicine, the field has already started to move towards the right directions. Cure and prevention are both highly promising as guidelines for medical development, however, it is reasonable to predict that, no matter what, prevention will be used to deal with the diseases of the past, and cure will be used to deal with diseases of the future, in an endless cycle from now until the end of humanity's existence. After all, as humanity develops, we will gain exposure to newer, more deadly diseases that cannot be combated by current means and medicines. As such, the same cycle, cure to first breakdown and initially defeat the disease, then prevent to enact countermeasures that limit the effectiveness of the disease's cause, will always be humanity's order of action against those new diseases. In the immediate future, prevention will always take precedence, as cure can only take its mantle once new diseases are uncovered, and the short-term is similar in that sense to the long term. As humans continue to develop, there is no telling what the future holds; however, the medical field has its two sturdy weapons "cure" and "prevent" to rely on.

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Literary Review

CRISPR Technology

Subhi Karki (author), Anika Shah (advisor), Michelle To (advisor)
Castro Valley High School

Keywords: CRISPR, gene, Cas9, PASTE

Abstract

CRISPR is a genome editing tool made up of a Cas9 enzyme and a strand of RNA. The RNA guides the CRISPR to a targeted location on the gene sequence. Then the Cas9 enzyme can delete genes and insert new ones in its place, providing gene regulation that can get rid of any defective genes that could be causing genetic diseases. With further research on this technology, the possibilities are endless. Already, new technology is being created such as the PASTE technology created by MIT researchers. It does the same thing but on a bigger scale. It has the ability to change many more nucleotide bases with the use of serine integrases.

Introduction

CRISPR is a new genome editing tool that has continued to be developed during the past ten years. It is made of a Cas9 enzyme and a guide RNA. It has the ability to delete genes and replace them with new ones. In order to further the research behind CRISPR, it is vital that more people are aware of how it works. Although many people are wary of the technology, spreading awareness about it will ease their fears. I will explain why CRISPR is so effective, proving its need in today's medicine despite some of the backlashes it faces. CRISPR is the next step in furthering our medicine in order to cure incurable diseases.

Body

New tools for engineering human genome, broadly defined

The wide variety of CRISPR tools allows us to better understand the role that the genome has in the area of disease development. These tools enable us to control genes by controlling processes such as transcription and translation. The dCAS9 molecule, an important part of CRISPR, is used for gene expression regulation as stated above. It directly changes the RNA sequences in cells by changing the nucleotide sequences. This includes insertions, deletions, and substitutions. Gene expression regulation would also include promoting or inhibiting the transcriptions of gene sequences. By changing the nucleotide sequences or preventing sequences from being transcribed, CRISPR could be a solution to genetic mutations caused by repeats such as Huntington's disease by fixing those repeats. Huntington's

disease is caused by a repetition of CAG bases. Manipulating the CRISPR tool to be able to delete or replace those repeats could be able to provide a treatment or cure for this disease, helping so many lives. Moving on, there is another part of CRISPR tools called CRISPR-GO which is used to manipulate the 3D structure of the genome from within the nucleus (Stanford Qi Lab). Changes in structures cause a change in function, and genomic researchers want to further study the extent of the impact on function. These researchers also believe that CRISPR will help them better understand how other factors affect genome function such as epigenetic modification, DNA looping, nuclear domain, and more. New methods for treating diseases by starting with the genes are already coming out. Researchers are engineering cells to sense extracellular inputs and use them to control gene expression in order to have therapeutic functions. An example of this is the ChaCha device which adds dCas9 effectors to the GPCR function in the human body in order to control gene expression. Being able to control gene expression means that technology would be able to determine which genes are "on" and active and which genes are "off" or inactive. This means that disease-causing genes could be turned "off", preventing them from damaging the body. While this research overview article by Stanford Medicine did provide a good general overview of CRISPR tools, I would have preferred there to be more examples of work that was being done in order to give a better idea of how this works.

Gene Editing - Digital Media Kit

The CRISPR-Cas9 system is the most common genome editor, despite there being a few other tools available such as the zinc-finger nucleases or the meganucleases. CRISPR stands for clustered regularly interspaced short palindromic repeats. It is an RNA-based system, which allows it to be more flexible by being able to target more sites. The Cas9 protein is guided by RNA guides, called CRISPRs, to the specific gene that the scientist is trying to target. This process is similar to the process of translation, where DNA is run through the ribosome until it is recognized. The Cas9 enzyme will cut out the targeted gene, and then new DNA can be inserted into that section. A sgRNA (the RNA guide) will recognize the nucleotide base pair of the targeted DNA sequence. This activates the Cas9 enzyme, bringing it to the targeted sequence where it can then perform the removal of that gene. There is a wide scale of impacts that result from this, ranging from a single base pair change to the regulation of gene expression levels. The benefits are endless: new medicine, new ways to treat genetic diseases, and even the ability to replace damaged tissues or organs. Scientists have already started studying ways to inactivate pig viruses in order to bring about the possibility of using pig organs for human bodies (NIH). Another area being researched is modifying yeast cells in order to create biofuels that could lead to improved strains of agricultural crops. I think that all these innovations give a good reason why it is so important to do further research on CRISPR because it could result in medical techniques no one knew were possible, like animal organs in

human bodies. However, the CRISPR tool faces lots of backlash due to ethical concerns. For that reason, gene editing in human embryos is mostly restricted because of the possibility that the embryos could die and the fact that no one really knows the long-term effects of editing the genome in such an early stage (NIH). Nonetheless, Chinese scientist He Jiankui used CRISPR-Cas9 to create the first gene-edited babies in 2018. He had stopped the copy of a gene in an embryo in order to prevent HIV from surfacing in the babies. This received lots of outrage from scientists who felt it was morally wrong especially because no one knew how those children would be as they got older. A report by the international commission stated that gene editing in embryos should only be done as a last resort for a serious monogenic disease, or a disease only caused by one gene. Certainly, there would be a plethora of regulations and criteria that would need to be met beforehand. Although I understand why there is so much moral concern over doing gene editing in humans, I feel we will have to get there eventually because it is the next step in curing these diseases. We can't let the fear of the unknown hold us back. Had we done that our whole experience, society would have never come this far, rather it is the risk that took us to this point and it is the only risk that will allow us to keep growing.

New CRISPR-based tool inserts large DNA sequences at desired sites in cells

A new possible technique based on the CRISPR tool was started by MIT researchers, and it's called PASTE. It has the ability to insert genes as long as 36,000 base pairs into a variety of human cells, and this could help with the treatment of diseases caused by long strands of mutated genes such as cystic fibrosis because it would be able to fix many base pairs at once. CRISPR-Cas9 is a group of molecules from the bacterial defense system. Researchers had the idea to combine it with enzymes called integrases, which are used by viruses to infiltrate bacterial genomes with their own genetic material. Before this idea, Cas9 would be guided by an RNA strand to a target location that would be cut by the Cas9 molecule. DNA repair processes would begin to repair that area, resulting in the deletion of a small section of the gene. New DNA could be introduced into the gene as the repair is happening, acting as a sort of replacement for the deleted gene. However, this introduction of new DNA would result in double-stranded breaks, causing chromosomal deletions or rearrangements that could harm the cell. Also, this process would only work in dividing cells because they are the cells with the active DNA repair process. It is because of the problems that MIT researchers looked at integrases, which would prevent the double-stranded breaks but still carry out the insertion of new DNA. They used serine integrases, which can insert DNA as long as 50,000 base pairs and are programmed to target specific genome sequences, where they will insert their DNA cargo at those specific locations. The difficulty comes in changing the target location of the integrases. However, combining the integrases with CRISPR-Cas9 makes it easier to change the target location. This is what PASTE is, Programmable Addition via Site-specific Targeting

Elements. The Cas9 enzyme will cut at a specific site in the genome, and the landing site for the integrase can be inserted at that site. There wouldn't be any double-stranded breaks because the DNA strand would only be added one at a time since the landing site is 46 base pairs long. Now the integrase can go to the new landing site, and add its huge cargo of DNA. In a multitude of studies, scientists were able to use the PASTE tool on 9 out of 13 different genes, including liver cells and T-cells. Although there were different ranges of success (5%-60%), there were only a few unwanted indels (insertions or deletions), however, they were not on as large of a scale as before because of the no double-stranded breaks (Trafton). The wide range of success can be due to a variety of factors. Different methods are required in order to change the DNA, what works for one may not work for the other because all the genes are different and located in separate areas. Personally, I believe that this is a good sign because more studies can be done in order to figure out how to increase success rates and decrease unwanted indels. Furthermore, I am sure that those ranges of success could be higher for some types of cells than others, which means we could already be making huge progress on certain tissues.

Conclusion

All of the articles go over how the CRISPR tool works to delete and substitute parts of the DNA. They help the reader understand why gene editing can help provide solutions to many genetic diseases that scientists have struggled with. I liked the article by MIT the best because they introduced a new tool that combined with the CRISPR tool, which shows how building off of this technology is already allowing us to progress with medicine. There are ethical concerns with the CRISPR tool, especially when it comes to humans because it is something that could have detrimental effects. However, science is all about pursuing the unknown, so it shouldn't be something that stops us because so many people out there could have their health improved drastically with the use of the CRISPR tool.

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Research Article

Effect of the Subarachnoid Lymphatic-like Membrane on Alzheimer's

Thanisha Kapur (author), Anika Shah (advisor), Michelle To (advisor)
Saint Francis High School

Keywords: SLYM, Subarachnoid Lymphatic-like Membrane, Alzheimer's, Microglia, Meningeal Layers, Brain

Abstract

This paper and research article examines the effect of the Subarachnoid Lymphatic-like Membrane layer that was a newly discovered anatomy in the brain on the brain disease of Alzheimer's. It also compares the function of the SLYM to different meningeal layers in the brain which help to protect and filter out its toxins.Through the isolation and extraction of the meningeal layers and microglia from the baby mice, it was found that the microglia have a very big effect on the function of the brain and the SLYM. The meningeal layers and the microglia protect a brain's central nervous system from trauma injury, such as a blow to the head by acting as a shock absorber. Furthermore, they anchor the central nervous system and keep the brain from moving around within the skull. If the meningeal layers stopped functioning properly, it would hinder the SLYM from fully filtering out toxins and the cerebrospinal fluid that causes Alzheimer's. This experiment aims to find the purpose of the SLYM in combating Alzheimer's, as well as how it compares to/works with other parts of the brain that also have an impact on Alzheimer's. Through this research article, by analyzing the purity of the mice microglia and meningeal layers, it was found that the SLYM's function and its effectiveness in combating Alzheimer's is affected by the purity of the microglia and the other meningeal layers in the brain.

Introduction

The Subarachnoid LYmphatic-like Membrane (SLYM) is a recently discovered anatomy in the brain, which acts as a protective barrier and a platform from which immune cells can monitor the brain for infection or inflammation. The tissue is a thin membrane encasing the brain that keeps newly made cerebrospinal fluid (CSF) – which circulates inside the brain – separate from "dirty" fluid containing cells' waste products. The SLYM is morpho- and immunophenotypically similar

to the mesothelial membrane lining of peripheral organs and body cavities, and it encases blood vessels and harbors immune cells. Functionally, the close apposition of SLYM with the endothelial lining of the meningeal venous sinus permits direct exchange of small solutes between cerebrospinal fluid and venous blood, thus representing the mouse equivalent of the arachnoid granulations. The functional characterization of SLYM provides fundamental insights into brain immune barriers and fluid transport (Beinlich, et al.). This new membrane discovered in the brain is a fourth membrane; the other three (pia mater, arachnoid, and dura mater) have already been discovered previously and researched upon extensively (Wilson). This new membrane was found by researchers from the Center for Translational Neuromedicine at University of Rochester and the University of Copenhagen. The first author of this study initially started this research after positing that a mesothelium, which is a layer known to line organs in the body, might exist in the central nervous system. Because the SLYM's discovery is relatively new, there have been no further studies released on the topic; however, many researchers are focusing on the possible implications and the role that the SLYM can have on brain diseases such as Alzheimer's (Charuchandra). Although the new membrane is very thin and delicate, the SLYM is a tight barrier, and allows only very small molecules to transit; it seems to separate "clean" and "dirty" CSF. This separation ability allows the influx of fresh CSF while flushing the toxic proteins associated with Alzheimer's and other neurological diseases from the central nervous system. This research paper is geared towards studying the role of the SLYM in preventing diseases such as Alzheimer's.

Materials and Methods

This experimental design used samples from mice's brains in order to study and extract samples of microglia, which is involved in recognizing Alzheimer's disease. All materials and methods below are borrowed from BioTechniques and its study on simplifying the procedure for the isolation of primary murine microglia. The materials are as follows: Heat-inactivated fetal bovine serum to use as a growth supplement for the in vitro cell culture of eukaryotic cells, Cell culture of murine eukaryotic cells; Dulbecco's modified Eagle medium with 4.5 g/l glucose, l-glutamine and sodium pyruvate; phosphate-buffered saline (PBS) without calcium and magnesium to support the growth of the mice; trypsin–ethylenediaminetetraacetic acid solution; and 75-cm^2 cell culture flasks were purchased from Corning (NY, USA).

Penicillin–streptomycin–amphotericin B solution and bovine fibronectin solution were purchased from MP Biomedicals (OH, USA) and used to properly sanitize the brain after extraction. Before starting all experimentation, lab safety equipment and procedures should be followed. The researchers and the team should wear safety goggles, gloves, and a lab coat to prevent contamination and be safe. First, the culture media, which are mediums that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory, were prepared by adding 10% fetal bovine serum and penicillin–streptomycin–amphotericin B to the Dulbecco's modified Eagle medium and then filtering the mixture through 0.22-μm filter cups. Rat anti-CD11b (clone M1/70) labeled with fluorescein isothiocyanate (FITC) and mouse anti-GFAP (clone 1B4) labeled with Alexa Fluor® 647 were purchased from BD Biosciences (NJ, USA). Hoechst 33258 DNA stain and Alexa Fluor 488 phalloidin were purchased from Thermo Fisher Scientific (MA, USA). The FITC-tagged Aβ1–42 peptide was obtained from Bachem (CA, USA). Trypsin from porcine pancreas, trypsin inhibitor, DNase I and lipopolysaccharides from *Escherichia coli* O111:B4 were purchased from Millipore Sigma (MO, USA). Procedures were performed in a sterile environment (Scott, Witt, Schober).

The 75-cm^2 flasks were prepared by the addition of 5 ml poly-l-lysine solution (0.1 mg/ml in sterile water) and then incubation at room temperature for 30 min. The poly-l-lysine solution was removed, the flask washed once with 5 ml sterile water, and then dried before use. In this protocol, primary microglia were isolated from mouse pups ranging from P0 to P3. Two or three pups were removed from cages, and body temperature was maintained with a heating blanket. The brain was extracted using forceps and submerged in 30 ml cold dissection medium (Hanks' balanced salt solution with calcium, magnesium, glucose, 10 mM HEPES and penicillin–streptomycin–amphotericin B) and washed for 2 min to remove blood cells and monocyte contamination. The murine (mouse's) brain was then placed on weigh paper and the hemispheres were separated using a razor blade. Each hemisphere was then carefully rolled across the weigh paper to remove the meningeal layers. The hemispheres were rolled until meningeal layers were completely removed. The brain tissue was separated into small pieces on the weigh paper using forceps and then any remaining blood particulates were removed. The tissue pieces were placed in a 50-ml conical tube with 5 ml cold dissection medium to ensure tissue hydration. Once all the tissue was in the conical tube, the volume of dissection medium

was increased to 30 ml, 1.5 ml trypsin solution (2.5%) was added and then the 50-ml conical tube was gently mixed. The tube was then placed into a 37°C water bath for 15 min. During the 15-min period of incubation, the tubes containing the trypsin and brain tissue were inverted once per minute to facilitate digestion. Next, 1.5 ml of trypsin inhibitor (1 mg/ml) was added and mixed gently for 1 min; then 750 µl of DNase I (10 mg/ml) was added, gently mixed and then centrifuged for 5 min at 400 × g at 20°C. After centrifuging, the supernatant was removed and replaced with 5 ml of culture medium at 37°C. The cell pellet, which is used to isolate the microglia cells, was resuspended using a 1-ml transfer pipette and then transferred to a 15-ml conical tube. The 15-ml tube was centrifuged for 5 min at 400 × g at 20°C. After centrifugation, the supernatant was removed and replaced with 5 ml culture medium at 37°C. The cell pellet was resuspended using a 1-ml transfer pipette and then transferred into a T-75 cell culture flask (pretreated with poly-l-lysine) containing 10 ml of culture medium, making a total volume of 15 ml in the flask (Scott, Witt, Schober). The flask was placed into a 5% CO2 cell incubator at 37°C. After 5 hours of incubation, the mixed cell population had settled on the bottom of the flask and attached. Tissue debris and apoptotic cells settled on the top surface of the attached mixed cell population. The next day (day 1), the culture medium was removed and replaced with 15 ml fresh culture medium, and the flask containing the mixed cell population was incubated for an additional 4 days. On day 5 the culture medium was replaced. On day 6, microglia, which we want to isolate to examine its purity, were visible on top of the mixed cell layer and ready for harvest. At this point of the procedure, the microglia were weakly attached to the underlying mixed cell monolayer. To remove the microglia from the rest of the cell mixture, the flask was tapped on the edge of a bench top three times. After tapping, the flask was visually inspected for the detached microglia. Additional tapping may increase microglia detachment but may lead to contamination of the microglial cells with other cell types (Wilson). Alternatively, the microglia may be detached in a more reproducible manner by placing the flask on a rocker or shaker. The culture medium containing the detached microglia was harvested, pelleted by centrifugation at 800 × g for 5 min and resuspended in 2 ml culture medium. Alternatively, the medium can be carefully removed from the flask and replaced with a small volume of fresh culture medium before tapping; the detached microglia are harvested in the small volume and can be used without the need for centrifugation. The isolated microglia can be seeded into a new culture dish,

added to coverslips for fluorescent staining and microscopy procedures or analyzed by flow cytometry.

Data Analysis

The data analysis will be used to find the purity of the isolated cells so that their susceptibility to being affected by Alzheimer's later on can be deduced. Flow cytometry analysis was used to assess the purity of the isolated microglia. For purity measurement, 100 µl isolated microglia were placed in 1.5-ml microcentrifuge tubes, then 1:100 dilution rat anti-CD11b labeled with FITC and 1:100 dilution mouse anti-GFAP labeled with Alexa Fluor 647 were added and the tubes were incubated for 30 min at 37°C with 0.01% Triton™ X-100 from Thermo Fisher Scientific (MA, USA). After incubation, the tubes were removed and 50 µl was sampled from each tube by flow cytometry to measure the mean fluorescence intensity. For the 30-min phagocytic activity assay, 100 µl of isolated microglia were placed in 1.5-ml microcentrifuge tubes, 100 nM FITC-labeled Aβ1–42 peptide was added, and the samples were incubated for 30 min at 37°C. The samples were analyzed by flow cytometry to measure the mean fluorescence intensity. After incubation, the plates were tapped to isolate the microglia, and the cells were analyzed by flow cytometry (Scott, Witt, Schober).

Another way that the microglia was analyzed was using the epifluorescence microscopy, which performed isolated microglial nucleus and actin staining and FITC-labeled Aβ1–42 peptide uptake experiments. For this, 18 × 18 mm glass coverslips were coated with 50 µg/ml bovine fibronectin for 1 hour at 37°C and then washed once with sterile PBS (Scott, Witt, Schober). The fibronectin-coated coverslips were immediately placed in a 35-mm dish and the microglia, suspended in culture medium, were added. For microglial nucleus and actin staining, the cells were incubated for 24 hours to allow attachment and spreading to the fibronectin, then fixed in PBS containing 4% paraformaldehyde and 0.1% Triton X-100 for 60 min, and finally washed in water. The coverslips were blocked in 1% bovine serum albumin solution for 20 min and then stained with Hoechst 33258 DNA stain and Alexa Fluor 488 phalloidin for 20 min at room temperature. The coverslips were washed with water for 20 min and mounted onto glass slides. The nucleus and actin images were acquired with a Leica DMi8 inverted microscope and fitted with a 63× oil immersion lens and 12-bit charge-coupled device monochrome camera. For the

FITC-labeled Aβ1–42 peptide uptake experiments, isolated microglia were added to 35-mm culture dishes, peptide was added and incubated for 24 hours, and the microglia were imaged with a 10× dry lens. All images were acquired and processed using Metamorph imaging software (Scott, Witt, Schober).

For the flow cytometric analysis, an event threshold was set at 750,000 forward scatter height and a gate was drawn around the intact cell population in the forward scatter height versus side scatter height plots. All experiments were repeated a minimum of three times. In the 24-h FITC-Aβ1–42 peptide uptake experiments measured by flow cytometry, the data were normalized, averaged and analyzed by one-way analysis of variance and the multiple comparison post hoc test (Charuchandra). In this simplified procedure, the removal of the meningeal layers was easily achieved by rolling the brain hemispheres on paper rather than removal under a stereomicroscope using forceps (which is the typical procedure implemented to do this). After trypsin digestion on day 0, the mixed cell population was added to a standard tissue culture flask or plate. On day 6, the microglia were ready for harvest by tapping the flask on a bench top. The isolated cells could be used directly in microscopy staining procedures or other assays. Through this experiment, the researchers attempted to assess the purity of the isolated microglia by measuring the expression of CD11b and GFAP because expression of CD11b is a definitive marker of brain microglia. Although the experiment was not implemented in real life, projected results based on the hypothesis are below: consistently among repeated procedures, the isolated cells would display homogenous appearance in culture and stained >90% CD11b-positive and <1% GFAP-positive.

Additionally, it is suspected that purity would be high; despite that, the total yield per mouse would range from 50,000 to 100,000 cells, which is lower than other reported and previously attempted procedures. Tapping the flask to detach the microglia is a crucial step in the procedure and may need optimization to achieve the desired balance between purity and yield. Additional tapping may increase the total microglia yield; however, the increased yield may be at the expense of purity. Other factors, such as the presence of colony-stimulating factor 1 in the culture medium after isolation, may promote microglia proliferation, thereby increasing the cell numbers available for experimental purposes (Scott, Witt, Schober). To further ensure cell

numbers for experimental purposes, performing peptide uptake experiments at 24 hours on both the isolated microglia and microglia among the mixed cell population would be beneficial. The predicted observation after 24 hours would be that the peptide fluorescence at both concentrations are associated. In the mixed cell population experiments, peptide and lipopolysaccharide were added on day 6 of the procedure. After a 24 hour incubation period, the microglia were isolated by gentle tapping and then analyzed for FITC fluorescence by flow cytometry (Wilson). Both the low (0.83 ng/ml) and the high (8.3 ng/ml) lipopolysaccharide concentrations should increase peptide uptake above the control because the microglia were harvested on day 6 and immediately incubated in suspension with indicated concentrations of fluorescein isothiocyanate-labeled Aβ1–42 peptide for 30 min and analyzed by flow cytometry.

Discussion

In conclusion, this research article details a simplified and low-cost procedure for isolating mouse microglia which can be performed in any lab with standard cell-culture equipment. The isolated cells were of high purity and were functional in peptide uptake assays and response to lipopolysaccharide. This means that the isolated cells were pure and thus could be used as a good comparative analysis when comparing the toxic-CSF filled isolated cells and the pure ones and examining their susceptibility to Alzheimer's. The beginning of the isolation procedure (day 0) requires a few hours of work, and then the remaining days of the procedure require little effort – only medium changes and centrifugation of isolated cells before analysis. In addition to its simplicity, the procedure has other advantages (Scott, Witt, Schober). We recommend removing the meningeal layers by rolling the brain hemispheres on weight paper rather than using forceps under a dissection microscope, which is more tedious. Microglia can be used in experimentation while in isolation or when they are among the mixed cell population. After treatments in the mixed population, the microglia can be isolated by tapping. The single-step purification is particularly useful because purified microglia attached to glass or plastic surfaces are difficult to remove without mechanical cellular damage. We chose newborn pups due to the greater number of brain microglia; however, although not tested directly, we believe our procedure is extendable to adult brains. The only serious limitation of this procedure is that purity is gained at the expense of overall yield. Nevertheless, this simplified procedure can be useful to many labs with a research interest in neuroinflammation, neurodegenerative disease or basic microglia biology.

The purpose of removing the meningeal layers of the mice's brain is in order to examine its relation to Alzheimer's disease and see how it would compare to the role of the Subarachnoid Lymphatic-like Membrane in preventing Alzheimer's disease. Through this experiment, the results found were analyzed through different techniques to assess the purity of the meningeal layers. The purpose of doing so was to compare the purity of meningeal layers in the human brain and see the differences between pure and impure meningeal layers as well as how effectively this allows the brain to protect itself against Alzheimer's (Wilson). The preliminary research that discovered the anatomy of the Subarachnoid Lymphatic-like Membrane also describes the function of SLYM in mice. Discovery of the SLYM opens the door for further study of its role in brain disease. For example, the researchers note that larger and more diverse concentrations of immune cells congregate on the membrane during inflammation and aging (Michaud). Furthermore, when the membrane was ruptured during traumatic brain injury, the resulting disruption in the flow of CSF impaired the glymphatic system and allowed non-central nervous system immune cells to enter the brain. These and similar observations suggest that diseases as diverse as multiple sclerosis, central nervous system infections, and Alzheimer's might be triggered or worsened by abnormalities in SLYM function. They also suggest that the delivery of drugs and gene therapeutics to the brain may be impacted by SLYM, which will need to be considered as new generations of biologic therapies are being developed (University of Rochester Medical Center).

Ruptures in the brain cause blood to spill into the surrounding tissue (called a hemorrhage). Hemorrhages happen between the skull bone and the outermost membrane layer, the dura mater. The dura mater is also what the meningeal layers are known as (Michaud). Therefore, it is clear that the meningeal layers staying pure and free from things such as brain hemorrhages is crucial in allowing the SLYM to properly function and protect the brain. It is also very important to note that small cerebral bleeds are frequently observed in brains of patients with Alzheimer's disease, which means that it is much more common for the meningeal layers to be affected and stop functioning properly if a brain is prone to Alzheimer's disease. As a result, the role of the SLYM is essential in stopping this from happening in the first place. Both the dura mater (the outermost

membrane layer) as well as the SLYM are two very important parts of the brain that work hand in hand with each other in order to protect it from diseases such as Alzheimer's (Charuchandra).

Research in the future on this matter would definitely be to see ways that the SLYM can be protected or even enhanced in functioning so that brains that are already in later stages of Alzheimer's or even ones that are just beginning to show symptoms can have a possibility of being saved. The membrane is immunophenotypically distinct from the other meninges, and is comparable to the membrane lining of peripheral organs and body cavities (Michaud). SLYM envelops blood vessels and houses immune cells. The number of immune cells in the membrane increases in response to acute inflammation and natural aging, according to the researchers. In addition, the membrane forms a barrier to solutes above three kilodaltons, including tau and beta-amyloid. When a break in the SLYM occurs in the event of a head injury, this could explain why head trauma increases the risk of Alzheimer's, according to the researchers (Wilson). Moreover, if waste products from brain cells are not properly disposed of, this can play a role in brain diseases. For example, accumulations of the proteins tau and beta amyloid are thought to be involved in the development of Alzheimer's (Charuchandra). That is why the removal of waste products, which are released during the metabolism of brain cells, is important to keep the brain healthy.

As the mechanisms of microglia in neuroinflammation, neurodegeneration and neuropsychiatric diseases become increasingly understood in detail, microglia will be the target of future therapies. A demand for preclinical cellular models is thus expected to increase as well. The use of primary microglia is not intended to replace cell lines, but will provide a path toward a more physiological system. Simplified procedures for primary cell isolation will facilitate an increase in laboratory participation in these much-needed research areas and still produce great discoveries and innovations (Charuchandra). In conclusion, the role of the Subarachnoid Lymphatic-like Membrane is super important in regards to diseases such as Alzheimer's because its primary function is to separate the toxic and clean Cerebrospinal Fluid. Furthermore, it is a thin yet tight and strong barrier, which explains why injury to the brain causes a break to the SLYM, and thus, increases the risk of Alzheimer's. The SLYM works in tandem with a couple other parts of the brain, such as the microglia and the meningeal layers (dura mater). These

meningeal layers essentially keep the brain pure and free from brain hemorrhages as well as anything that has to do with causing toxic CSF coming into the brain. After that, the SLYM would filter out the toxins from the CSF and further keep the brain protected. Essentially, through this research paper, it was concluded that the SLYM plays a huge role in Alzheimer's and its role has some significant connections to the roles of different parts of the brain such as the dura mater.

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Literary Review

The State of US Healthcare: An In-Depth Analysis

Yesh Rao (author), Anika Shah (advisor), Michelle To (advisor)
San Mateo High School

Keywords: Healthcare, Covid-19, Single Payer, Public Option

Abstract

The present state of research into varying forms of healthcare is limited if existent. Discussions are limited to the state of different healthcare policies and their overall effects but have not effectively attempted to compare proposals in regards to US applicability. This article aims to bridge that gap and build off existing pieces of research to effectively produce concrete analysis on various forms of healthcare. Irrespective of one's opinion on the status of healthcare, there is no denying the current system is in need of change. Via analyzing proposals in Germany, Switzerland, Canada, and even the United States, this paper discusses respective benefits in conjunction with the harms. The goal of this paper however is not to *determine* the best healthcare policy as that answer will inevitably not arise at any point in time but rather to discuss what forms of benefits we might see as a result of

Introduction

1.11 million, the amount of deaths in the United States from Covid 19. This catastrophically high number highlights what should have been an already apparent truth in the US: status quo health care and pandemic response is unsustainable. While many scholars reach the same conclusion, being the necessity of reform, the question of what to implement is yet to reach a conclusion, even among experts. This paper thus aims to analyze a variety of different nations, their health care policies, and their respective pandemic response to dissect numerous different potential alternatives to the current United States health care model. While health care policies play a role in nations' economic vitality as well, this paper will prioritize the consequences in relation to quality of care. This includes but is not limited to pandemic preparedness, doctor-patient trust, wait lines, presence of innovation, and of course, accessibility. The nations to be analyzed within

this paper include Germany, Canada, and The United Kingdom, due to their diversity in health care standardization. This includes single payer health care, the National Health Service, and private options. To limit ambiguity, this article will some important terms that will be used throughout the course of this paper. Single Payer health care refers to a system of health care in which the government funds all health care providers and reimburses them, usually funded through an increased tax on constituents of a nation. The National Health Service, present in Britain, is a great example of single payer but differs in that even the providers of health care are government affiliated. Within a single payer model, health care providers themselves can still be private corporations but under Britain's NHS, the entire process is nationalized under government control.

Discussion:

The State of Healthcare

First, analyzing the defective nature of status quo healthcare in the United States, we can look to Allison Galvani's recent study analyzing healthcare's impact on Covid-19 preparedness in the United States. GalvanShe finds that 26.4 percent of lives lost during Covid in the United States would likely have been prevented if universal healthcare was guaranteed (Galvani 3). This number is corroborated by her analysis of the US population in which 14 percent of US adults report they would not seek care even if they noticed common symptoms related to Covid-19. Now, these numbers provide us with alarming insight into how fundamentally flawed status quo healthcare is. The privatized nature of healthcare turns it into a commodity, rather than a guarantee. This deters broad swaths of the US population from seeking care under the impression that access to care will have to come at the expense of having food on the table, or being able to pay rent. The question then becomes, why is healthcare inaccessible and what is resulting in the absurdly high population of uninsured citizens. This is answered by Patrick Drake of the KTF's recent paper which used data from the American Community Service to ask American adults the reason they are uninsured. Out of the uninsured adults surveyed, 64 percent reported the reason was due to the cost of healthcare being unaffordable (Drake 1). Access to basic necessities such as health should never impose a dichotomy on individuals yet in the United States, it does. Now, many point to the existence of cost-sharing documents as a potential solution to the overwhelmingly high costs of health care that exist in the United States. Cost sharing, under the

Affordable Care Act, allows low income individuals to pay a "small" out of pocket cost in exchange for the government paying the remainder. In essence, this splits the cost of healthcare between families and the government to reduce the overall cost. While in theory this makes practical sense, there is an arduous and painstaking literary process required to interpret cost sharing applications that deters low income families from signing on. There are 8.1 million Americans with incomes between 11,770 and 29,425$, being the eligibility requirement to qualify for cost sharing, but a recent study found that only 5.9 million are currently signed on. This means that 2.2 million, or 29 percent, of low income families are currently denied access to health care due to the overwhelmingly difficult process required to apply for cost sharing (Carpenter 4). Carpenter's analysis required using American Health Census data from databases that tracked a) healthcare enrollment rates, b) cost sharing usage among low income populations, designated by Census data and c) geographic proximity's impact on healthcare usage. From here we see that while the Affordable Care Act attempts to equalize health care and provide affordable options for low income families, still health care remains broadly inaccessible within the United States.

Quality of Care

Next, it is important to view this in terms of quality of care. Yes, particular populations may be denied health care in the status quo but the question turns to whether or not an alternative means of health of care can effectively respond to these qualms. Proponents of privatized health care often argue that scaling up health care to a universal standard results in "overuse" of health care in which the healthcare system gets overwhelmed. To this, we can look to Canada as a glowing case in study of the effects of universalizing health care. In 2016, Paul Geyman published a study in which he analyzed health care data provided from the CDHC over the last 25 years starting from the 1970s. His analysis attempted to answer a simple proposition: does single payer healthcare require overusage? The results were clear as day. In analyzing Canada's system, being single payer, he found that there was just a 5 percent increase in health care usage, almost all of which being primary, necessary care (Geyman 7). This has broad implications on the way we interpret single payer health care. First, it corroborates Galvani's study results in revealing that implementation of single payer does result in at-risk patients getting necessary care. Second, it reveals that there is no "overheating" effect of single payer that results in too many patients using

the system at once. In effect, the increase in health care usage is taken up by patients who are in desperate need of the system which at the end of the day, is the goal of health care. Analyzing Canada, it is important to go a little further. Proponents of maintaining the status quo of health care often point to the increased wait times that arise as a result of single payer health care. This seems to be the most stringent criticism that exists of any alternative health care system. There is weight to this argument given that as an institution, Canada has suffered from longer wait times however this conflates cause with correlation. Andrew Torrance in 2017 published a study in which he compared United States health care to that of Canada through viewing the infrastructural capacities of both systems. He analyzed trends in health care related to the total revenue spent on healthcare and how relatively accessible both systems were. His aim in the study was to discover whether implementation of healthcare in the United States would result in significant rationing (aka wait times) in the same manner that it did in Canada. His conclusion was that the Canada example is non-telling of how the United States single payer would operate. In Canada, the total amount of money spent on health care procedures is not even half as much as that of the US. As a result, the money directed towards paying for different procedures is far lower meaning that the supply is in turn exponentially lower which is what results in patients needing to wait to get access (Torrance 6). Additionally, the analysis of single payer in Canada often attempts to reach a monolithic decision while ignoring valuable complexity. Colleen Flood and Bryan Thomas in 2017 conducted a study in which they compared the wait times in Canada for non-essential procedures against the more essential ones such as surgery, or radiation. They additionally chose to separate Canada into different provinces in order to yield a more accurate result of what Canadian wait times look like across the board. They found that across all provinces for radiation therapy, 9 out of 10 patients were able to garner access in a time frame of less than a month which is the expected time frame for radiation therapy (Flood and Thomas). Flood and Thomas study along with Torrence's point towards a brighter conception of single payer health care. While often singled out as an unrealistic, overly radical system that is bound to reproduce the harms it attempts to solve, in actuality it has yielded empirical results and criticisms seem more directed towards the infrastructure of a nation, rather than the health care itself.

The Public Option

Moving from the single payer model, many have advocated for the usage of a public option as the middle ground between private and public care. The public option health care system has been utilized in different ways by many different nations most notably, Germany. The method holds as follows: health care is best when there is both a public and private option. Public health care can thus be taken advantage of by those in desperate need of public services to guarantee some access to health care and the private system of insurance can still be maintained. Public option essentially splits health care into two separate sectors: the private and public. Rather than imposing a dichotomy in which individuals have to access insurance to receive health care, there is a choice that presents both options to individuals to ensure there is some level of universal access. In theory, this sounds like the perfect middle ground and has been actualized by some nations. Germany, for example, has competing private health insurance systems for those that can afford it or for those that have their health care covered by employers. They simultaneously offer a public option for those that have neither and have seen meaningful results. Most notably, results have come in the context of costs. Chase Madar, a New York attorney in health care analyzed the year by year change in percent of American GDP dedicated to health care. As of 2017, that number was 18 percent. Close to one-fifth of the total GDP is spent just on our health care industry by funneling money into private insurers that prioritize monopolization over access. Germany in contrast, as Madar finds through his statistical analysis of spending in relation to GDP, directs just 11 percent of their GDP on health care (Madar). Now, money alone may not be the sign of a positive health care system unless it can transfer into noticeable tangible results. The European Observatory on Health Systems and Policies, who conduct an annual report on European nations and their health care trends through the years, find that German life expectancy has risen to 81 years as of 2020. This is a positive, 3 year trajectory and one of the top average life expectancies across the globe (European Observatory). Another positive trend beyond just life expectancy, is the accessibility of the system. The total out of pocket costs that individuals are forced to pay in the German system to fund health care is just 13.6 percent. Taking low cost sharing numbers with high life expectancy displays the vitality of the German trend and is needed insight into the alternative potentialities of health care. Many view the American system as being the only “realistic” option for health care yet seemingly this is a view from nowhere as there quite literally is no real empirical backing that justifies this proposition.

Still however, criticisms of the "public option" exist and cannot be dismissed. The Congressional Budget Office in 2017 conducted a study in which they scored the proposal of a public option proposal based on the way it could be added to the Affordable Care Act which lays out the scripture for effective health regulation in the United States. The CBO's analysis, as described by Adam Gaffney, scores the program immensely low, finding that the 28.6 million who are currently uninsured "would not budge" (Gaffney 5). We cannot only view this CBO report as a point to consider, but *the* point to consider. The CBO did not estimate that a large portion of uninsured people would remain uninsured, they estimated that almost *all* uninsured people would remain without access to health care. The CBO additionally analyzed the potential applications of public option specifically in a US setting making their results far more applicable to the infrastructural capacity. For individual patients, this would have a variety of implications. First, patients would be unable to differentiate between different forms of care thereby deterring any *and all* services. When patients are unaware of differing options, they are deterred from securing care meaning all in all harms persist. The second potent criticism of the public option relates to admin costs. Before delving into studies, the importance of administrative costs must be made clear. Administrative costs occur when healthcare providers have bureaucratic struggles with managing payments from numerous different insurers. While this may seem like a small concern, for rural hospitals the absence of a standardized payment system can eat close to 80 percent of healthcare budgets. Not the provision of health care, not the payment of workers, but the managing of funds from different providers eats up 80 percent of costs. Dr. Woolhander studied at the CUNY school in public health administration and attempted to analyze the effects of the public option on administrative costs. While a single payer system would cut administrative costs down to 0, as the system in it of itself centralizes payment directly from the government meaning there is just one outlet, the public option still maintains the numerous different providers that small hospitals have to manage. Woolhander finds that a public option would forgo "84 percent of the administrative savings available through single payer" (Woolhandler 6). Woolandler and Gaffney offer the most insightful criticisms of a public option that seem to follow a common line. While theoretically perfect, the idea of dividing health care into an option seeking a middle ground suffers from the *worst* of both worlds rather than the best of both.

Switzerland's Model

The final alternative to health care this paper will analyze is the Swiss model. Switzerland maintains what many like to refer to as the "universal non universal model". They offer a set of 82 different private insurance options of which some include basic plans and others include supplemental treatments of higher care. Here is the catch, while each option is *private* in nature, it is **compulsory** that each person purchases one of the treatment plans that are offered. Thus, the system is universal in that all individuals are mandated access to healthcare as a law but non universal in that accessing said health care requires purchasing it. In theory, this system seems like the inverse of the public option: totally undesirable. It would seem ridiculous to propose a system that compulses individuals to sign onto health care, yet in reality, it is substantially different. As described in paragraph 1, the problem with American health care is not *just* the cost of health care but the financial illiteracy that deters many Americans from signing on to cost sharing alternatives and the perceptive risk that the process is too time consuming. When individuals are given a choice between accessing health care or not, if the costs are even perceived to be too high then most will choose to obviate from signing on to health care. This is exactly why within the United States, the uninsured rate is so high as individuals are inclined to believe they will survive without healthcare and thus avoid paying the fee. Switzerland, takes away this choice. A recent PBS study analyzed two Americans who traveled to Switzerland with the sole goal of analyzing their health systems and both reported needing to pay 16 percent of their income on purchasing a health care plan. (PBS 3) This seems repressive but that same PBS study found that Switzerland has the lowest unavoidable death rate being the death rate of individuals that could be avoided by health coverage. In comparison, the United States has the highest of any nation. Yet still, the obvious criticism of this system is obvious for a reason: healthcare is too expensive. Switzerland's population and infrastructure may be well equipped to handle a system of such compulsion, but whether the United States is, remains unknown. To date, no quantitative assessment has evaluated the potential usage of Switzerland's system in the United States and until then its potential applications are limited.

Conclusion

The purpose of this paper is not to find or evaluate an objective solution to the problem of healthcare in the United States. On the contrary, what becomes obvious is the relative success of

alternative options across the globe and the, put simply, failure on the part of the United States to respond to emerging health concerns. Switzerland, Germany, and Canada are examples of nations that have proposed three separate models of health care. The compulsory system in Switzerland, public option in Germany, and single payer system in Canada have all yielded both positive and potentially concerning results in one way or the other but what is important is their prioritization of access over monopolization. These systems have all out performed the United States in their intent which albeit matters less than actual results, is a great measure of the future potentials of any system. The United States alternatively, fails both in intent and results. When evaluating the application of any other health care system and its potential in the United States, there are various angles that must be accounted for as this paper displays but status quo health politics seem to operate under a facade that genuinely deems American health care as efficient. Breaking out of this mindset and confronting the failure of American health care is necessary to actualize change and ensure equitable access and cost savings which at the end of the day, is not debatable.

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