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A DIVISION OF TOURO COLLEGE IN FLATBUSH

Where Knowledge and Values Meet

15th Anniversary Edition

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The Lander College of Arts and Sciences at Touro College in Flatbush

Over forty five years, Touro's Lander College of Arts and Sciences in Flatbush (with separate Schools for Men and for Women) has provided thousands of aspiring high school graduates from yeshivas and seminaries with a foundation of academic excellence for professional advancement and career growth, in an environment that is supportive of the students' religious values and perspectives. Our graduates have assumed leadership roles in various professions and have strengthened Jewish communities in the United States and in Israel.

The Lander College of Arts and Sciences in Flatbush offers more than 20 majors and pre-professional options, including the Flatbush Honors Program, the Medical Honors Pathway with New York Medical College, the Integrated Honors Tracks in Health Sciences (OT, PT, PA, Pharmacy), the Fast Track Program with the Touro College of Pharmacy, and the accelerated Accounting CPA Honors program. Additionally, students may choose Honors Majors in biology, political science and psychology. Five majors are available for students interested in accounting and business, including a top-rated CPA program.

As part of their degree requirements, all students are required to complete a carefully designed core curriculum that emphasizes the development of communications skills, critical thinking and analytical competencies, computer literacy and quantitative reasoning. Enrollment in the natural sciences, notably biology, chemistry, and in quantitative fields, mathematics and computer science, continues to increase.

Faculty members have earned recognition for outstanding achievements, including Joshua November, Assistant Professor of Languages and Literature, who was selected as a finalist for the Los Angeles Times Poetry Book of the Year Prize in 2011 and was a National Jewish Book Award finalist in 2016 in the poetry category; Thomas Rozinski, Assistant Professor of Political Science, and Pre-Law Advisor who served, in 2018-2019, as Vice President of the Northeast Association of Pre-Law Advisors, and who presented several times at the Annual Meeting of the American Political Science Association; Dr. John Loike, Professor of Biology, who has published widely in the fields of bioethics and genetics; and Atara Grenadir, Assistant Professor of Art, whose work was displayed at the Architectural Digest Home Design 2016 show in New York City.

Distinguished alumni of Touro's Lander College of Arts and Sciences in Flatbush include: Dr. Ira Parness, (MD, SUNY Downstate), Chief of the Division of Pediatric Cardiology at Mount Sinai Hospital, Dr. Israel Deutsch (MD, Einstein), Director of Brachytherapy at New York-Pres¬-byterian Hospital/Columbia University; David Greenfield (JD, Georgetown), Executive Director of the Metropolitan Council on Jewish Poverty; Yossi N. Heber (MBA, Wharton), President, Oxford Hill Partners; Dr. Haim Mozes (PhD, NYU), Chair of Business and Professor, Graduate School of Business, Fordham University; Sharona Noe, Vice President and Officer, the Federal Reserve Bank of New York; Shmuel Lowenthal, CPA, Partner, DeLoitte; and Simcha Felder, CPA, member of the New York State Senate.

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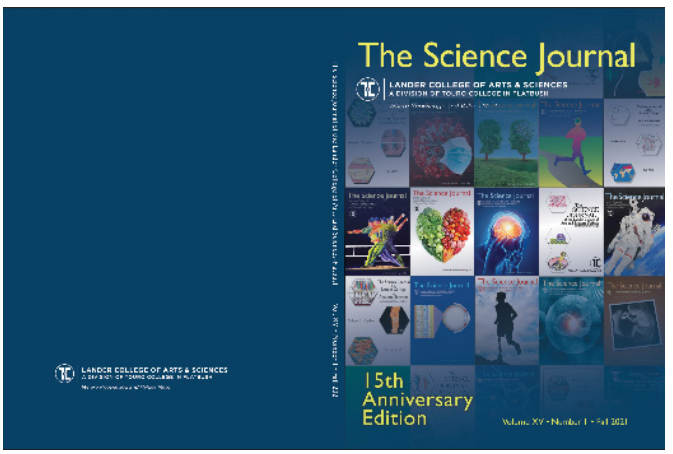

Cover picture: The cover picture was created by Professor Antony O'Hara of the Digital Multimedia Design Department, pertains to the 15th anniversary issue.

Developing Approaches Towards the Treatment of Covid-19

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Chana Weis graduated in January 2021 with a Bachelor of Science degree in Honors Biology

Abstract

In late 2019 a newly identified strain of coronavirus, SARS-CoV-2 emerged, causing a worldwide pandemic of respiratory illness, Covid-19. Covid-19 has drastically transformed the world in numerous ways. The virus is highly contagious and, in some cases, fatal. A successful cure has not yet been discovered and therefore society is urged to take proper precautions to prevent the spread of the potentially fatal virus. This paper analyzes the proper precautions that should be taken as well as the ongoing research that has been done to stop the spread of the virus.

Introduction

The novel Corona Virus, the underlying cause of Covid-19, is an infectious virus that primarily affects the respiratory tract. It was first diagnosed in 2019 in Wuhan, China where thousands of people were stricken with the virus. The virus eventually spread across the globe and was eventually categorized as a global pandemic. Symptoms of Covid-19 include fever, cough, difficulty breathing, shortness of breath, muscle fatigue, and loss of smell or taste. Recent studies revealed that Coronavirus impacts the gastrointestinal tract causing nausea, vomiting and diarrhea (Clinical Trials Week, 2020). While most people with the virus portray mild symptoms, a small but significant percentage of people develop more serious symptoms such as pneumonia which can be fatal. Elderly people, individuals with preexisting health conditions, as well as people with compromised immune systems are at the greatest risk for developing the most serious symptoms.

The virus is highly contagious and spreads, most commonly, through small droplets via coughing and sneezing. Additionally, people may catch the virus by touching a surface that is contaminated, followed by then touching their face (Mohan, 2021). Therefore, the Centers for Disease Control (CDC) enforce face coverings such as masks or bandanas to minimize transmission through droplets. The CDC strongly recommends people to properly social distance and stay six feet apart from each other to prevent the spread of the virus. Additionally, people are advised to constantly wash their hands with soap for roughly twenty seconds to rid themselves of any viral particles that they may have encountered.

Symptoms may not develop for two to seven days after coming in contact with the virus, which may make affected people unaware that they are infected, and which strongly contributes to the mass spread of the virus. Although Covid-19 is highly contagious, it is most contagious during the first three days that the patient exhibits symptoms. The incubation period for the virus is roughly two to fourteen days, and, therefore, patients who experience symptoms are strongly recommended to isolate themselves for fourteen days (Deibie, 2020). Currently, there are no proven drugs or vaccines to combat the virus and thus prevent the spread. However, over the past several months a great deal of covid-19 research has been performed to meet the global demand. Researchers have been experimenting with several anti-viral drugs that may disrupt the viral proteins and ultimately stop the virus. Additionally, there are numerous vaccines that are being manufactured and researched that can possibly immunize society and thereby stop the spread of the lethal virus.

Discussion

As noted, there is no current treatment which has proven to be effective in combatting the virus. Researchers recognize that it is impossible to kill the virus and are, therefore, working to "deactivate" the virus so that it can not spread further. Dozens of pharmaceutical companies, as well as thousands of researchers are working on producing a vaccine as well as seeking treatments and medications to help combat the virus. Vaccines typically contain an antigen, consisting a weakened or dead strain of the virus. When introduced to the bloodstream, the antigen triggers the immune system to produce antibodies towards that specific antigen. Researchers are trying several methods to produce a vaccine in hope to find a preventative cure for the lethal Coronavirus.

One approach that researchers are experimenting with is with a DNA vaccine. A DNA vaccine simply delivers genetic instructions how to build a viral protein. A DNA vaccine does not introduce the entire virus to the body, rather, it contains nucleotides that encode a portion of the virus. Upon injection, the nucleotide travels to the cell where it gets translated and a protein product is produced in the form of messenger RNA. The mRNA then assembles viral proteins which the immune system recognizes and tries to conquer. Researchers in China have used this approach and found particular genes that code for the spikes in the coronavirus, specifically genes which code for the receptor binding domain. The receptor binding domain is the unit of the virus that is found externally and on surfaces and are, therefore, the primary contributors for spreading the deadly virus. DNA plasmids are placed in the vaccine and, when injected into the body, they target the genome upon which they are translated into proteins, thereby introducing the cell to traits of coronavirus which initiates the production of antibodies. The cells then secrete the antibodies into the bloodstream which stimulate the B and T cells to act accordingly (Lee et al, 2020). The advantage of DNA and RNA vaccines is that they can be produced quicker than typical vaccines. (The usage of DNA vaccines is an emerging field and is rapidly developing.)

Another method that may be beneficial is the introduction of similar virus like particles into vaccines. These particles are not actual viruses and therefore do not cause disease. Yet, these particles prepare the immune system for what the virus proteins look like so that it can produce antibodies prior to coming in contact with the actual virus. When combatting the coronavirus, some researchers believe that by inserting some adenovirus into the vaccine, it will familiarize the cell to traits of coronavirus and thus prepare the body to fight off the corona virus when the two encounter. The adenovirus is missing one its own genes which prevents itself from reproducing and is therefore nonhazardous. When the adenovirus is injected, it places information into the cell by unraveling the gene, which then stimulates the immune system to produce antibodies. A strand of DNA that codes for coronavirus is inserted into the missing genes which trigger the body's immune response thus deactivating specific genes that are found in the coronavirus (Khadilkar, 2020). This method has been effective for the HPV vaccine that was introduced in 2006 and researchers are hopeful that this method will also be effective for combating the coronavirus.

Antibiotics are generally used to fight bacterial infections and are, therefore, ineffective for coronavirus. Accordingly, researchers are trying to create and find an effective antiviral medication. There have been several medications and treatments that have undergone trials but not yet proven to combat the coronavirus.

In September 2020 international clinical trials tried a new approach to combat the virus by using steroids. Steroids are often used by doctors to alleviate inflammation as well as to relax the immune system to prevent an overresponse. Patients who are seriously ill from coronavirus are at greater risk of dying from the immune system's overreaction to the virus, rather than from the actual virus itself (Lee et al., 2020). Accordingly, researchers hope to treat these patients with steroids to relax the body and prevent it from overreacting before the inflammation becomes serious and, in some cases, fatal. Researchers at Oxford University found that dexamethasone was extremely effective when given to critically ill patients. In addition, the World Health Organization (WHO) carried out studies using hydrocortisone and methylprednisolone on critically ill patients. The steroids were given to seriously ill patients and a surprising number of the patients did not require mechanical ventilation. Moreover, death rates were lowered with the patients who took these drugs (The Times, 2021). However, like many other drugs, steroids can cause serious side effects such as raising the body's blood glucose levels and causing confusion in the patient. Therefore, optimal doses of steroids need to be established prior to treating patients with steroids on a larger scale. Nevertheless, many researchers are confident that steroids will eventually be an effective tool in fighting the coronavirus.

Another approach to treating the coronavirus is with the drug hydroxychloroquine. Hydroxychloroquine is a less toxic derivative of chloroquine which was developed decades ago to prevent and treat malaria (Liuzzo, 2020). It is now commonly used for other diseases such as lupus and rheumatoid arthritis. Hydroxychloroquine works by regulating the immune system. The exact mechanism through which hydroxychloroquine benefits Covid-19 patients is unclear. As mentioned earlier, many patients with Covid-19 develop severe complications due to the body's aggressive immune response upon perceiving the virus. Researchers are hopeful that hydroxychloroquine can prevent the immune system from over responding which will in essence, reduce the unnecessary inflammation. Initially, when the virus first reached the United States, the Food and Drug Administration (FDA) approved the emergency use of hydroxychloroquine. However, after experimenting with the drug, researchers did not find sufficient supporting evidence that it is a safe and effective treatment for coronavirus. A clinical study using hydroxychloroquine did not demonstrate the ability to alleviate symptoms and shorten hospital stays (Liuzzo, 2020). Therefore, in June 2020 the FDA banned the usage of the drug until additional research on safety and efficiency of the drug was performed. While there is some evidence that the drug is effective, it has not been estimated that the potential benefits outweigh the risks. Some risks of hydroxychloroquine include heart disease, headaches, dizziness, and vomiting. In addition, patients suffering from malaria and other diseases, who are dependent on hydroxychloroquine, were unable to obtain the drug because it had been used widely for patients suffering from Covid-19.

Another drug that is being experimented as a potential cure is Remdesivir. Remdesivir was first developed in 2009, by Gilead Sciences to help treat patients suffering from Hepatitis C. Unfortunately, Remdesivir did not prove to be effective in fighting against Hepatitis C. In the following years, scientists experimented with Remdesivir as a potential cure for Ebola, and then again in 2020 for coronavirus (The New Nation, 2020.) Remdesivir serves as a broad-spectrum antiviral medication that is used to treat single stranded RNA viruses such as Ebola and coronavirus. This class of medication targets and inhibits RNA replication through various mechanisms (Nguyen et al, 2020). In regard to coronavirus, Remdesivir works as a pro-drug, meaning, that after it is infused, it is then

metabolized into its active form and converted into a ribonucleotide analogue, more specifically an adenine nucleotide analogue.

RNA is composed of four nucleotides including Adenine (A), Guanine (G), Cytosine (C), and Uracil (U). When coronavirus replicates, it takes these nucleotides that serve as the building blocks for the virus, and then chains them together thus allowing itself to replicate. Remdesivir serves as an adenine nucleotide analogue, and therefore inhibits viral replication. Thus, during viral replication attempts, Remdesivir is incorporated into the RNA in place of adenine. This essentially stops transcription of the viral genome by preventing the building blocks from being available to properly form the virus. Moreover, it ultimately reduces the body's viral load which gives the body an opportunity to combat the viral cells that were replicated prior to taking Remdesivir (Drugs and Therapies, 2020).

Currently, Remdesivir is in the investigational stage and has not yet been FDA approved as a safe treatment for any sort of disease. However, in May 2020, the FDA authorized the emergency use of Remdesivir via intravenous (Drugs and Therapies, 2020). Optimal doses of Remdesivir have not yet established and are under investigation.

Another approach that is being tested is the usage of convalescent plasma. When patients recover from Covid-19 their blood plasma contains antibodies that are able to fight off any future encounters with the virus. In the current situation, patients with antibodies who are eligible to donate plasma do so and the plasma is then transfused into a needy patient. The donor's antibodies may aid the patient in fighting off the virus as well as possibly shortening the length or severity of the illness. The concept of convalescent plasma has been used widely for decades to help patients recover from certain diseases such as chickenpox, measles and polio (Janice, 2020) (CotentEngine, 2020). Researchers still do not know how effective this approach will be with the coronavirus. Nevertheless, the FDA has approved the emergency usage of this treatment option. There are currently multiple studies being run that are testing the validity of this treatment. Although the evidence is not completely clear, the FDA determined that convalescent plasma has a great likelihood of improving symptoms as well as shortening hospital stays. In addition, it is estimated that the potential benefits outweigh the possible risk factors of the treatment. Risk factors include allergic reactions, lung damage and in some cases pulmonary embolism (Sanfilippo et al. 2020). These risk factors are low since donors must be completely recovered from the virus for at least two weeks prior to donating plasma. Some doctors believe that this is the most effective form of treatment when given to a patient during the beginning stages of the virus in as early as three days onset. (Newswire, 2020).

There are two categories of medicine- preventative and curative. Preventative medicine aims to promote and maintain health as well as prevent disease by living a healthy lifestyle. Vaccines fall under this category because they promote health by preventing disease and illnesses. On the other hand, curative medicine seeks to aid individuals who have already contracted a disease and have fallen ill, this includes treatment and medications. In regard to coronavirus, there has not been a successful preventative or curative treatment plan proven mainly because the virus has roughly 380 different mutations. Mutations occur through viral replication. When the virus replicates, the genetic code does not always exactly duplicate resulting in mutation. Over time, parts of the coronavirus genome have expanded into many mutations which contributes to the struggle that researchers are facing. Additionally, over time new strains of viruses evolve. Researchers have not found evidence that the different mutations and strains had significant change in how the virus affected society. However, with new data being observed and recorded researchers are optimistic that there will be a vaccine by the end of 2020 as a preventative measure towards the lethal virus.

Viruses are the most abundant biological entity on earth. They are simple nucleotides consisting of genetic material cased in a protein shell and capable of surviving only in another living cell. When a virus enters a living cell the immune system perceives the threat and begins to battle it. However, in some instances the immune system is not trained to fight off a specific germ or virus, causing the pathogen to overpower the immune system, and cause illness. Once a pathogen suppresses the cell, it replicates itself and kills the cell thus releasing particles so that it can infect more healthy cells.

Researchers believe that when facing a new virus, detection and containment are two keys that can help fight it off. Experts realize that when they are dealing with a new virus it is essential that they figure out the gene sequence so that they can create test kits so doctors can test to see whether or not patients are infected by that particular virus. After detection, containment is vital, because once a virus reaches the human population it spreads rapidly. Each virus has a different method of spreading. Coronavirus in transmittable through respiratory droplets, mainly in the forms of spray such as coughing and sneezing. Researchers are doubtful the virus will go away on its own and are, therefore, working to create lines of defense such as vaccinations as well as seeking proper treatment and medications. As discussed numerous researchers are working on producing a vaccine

that can highly limit Covid-19 and potentially eradicate it. However, vaccines need to go through strict testing and experimenting before they become available to the market. This process can take months and sometimes even years. This contributes to the struggle researchers are facing being that viruses are notorious for changing and adapting to the environment, which makes it difficult to nail down a precise vaccine. In addition, the WHO is testing many drugs that are used for illnesses, but just like vaccines this process can take a while. With all of this on the rise, society is urged to stay calm and not panic. Modern technology and further research prepare us better than previous pandemics in history. Nevertheless, the virus has had catastrophic consequences, however, if people stay vigilant and quarantined when told to do so, the spread of Covid-19 can be greatly slowed down until a cure is found.

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The Role of Immunotherapy in Treating High-Risk Neuroblastoma

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Mollie Raczkowski Graduated June 2021 with Bachelor of Science degree in Biology .

Abstract

Neuroblastoma originates from the cells in the neural crest. High-risk neuroblastoma, patients have poor outcomes even with the multi-step treatment plans, including immunotherapy maintenance treatment. Researchers in developmental biology search for unique antigens in neuroblastoma cells to utilize monoclonal antibodies (mAbs). Currently, GD2 is the most effective antigen that scientists have isolated in the tumor; these anti-GD2 mAbs are administered in the forms of Dinutuximab or Dinutuximab beta to attack the tumor. Monoclonal antibodies are currently administered in neuroblastoma instead of CAR T (that has seen success in curing different types of leukemias) due to the heterogeneity of this tumor. Although GD2 treatment has made significant strides in outcomes, there is still a high rate of relapse and treatment failure. Scientists continue to pursue further developments to help cure high risk neuroblastoma through better technology and more research.

Introduction: Neuroblastoma accounts for 15% of all pediatric oncologic deaths and is the most common extracranial solid tumor in children (Kholodenko et. al., 2018). Through understanding the tumor's origin and developmental biology, researchers have zeroed in on unique markers to utilize natural killers (NK) in targeting and eliminating the cancer. In the high-risk phase, neuroblastoma is deadly and has a high rate of relapse. Despite improvements in outcome, rates of recurrent neuroblastoma are high; in about 50% of cases treatments fail (Jabbari,2019). Currently, the final part of the treatment plan is a monoclonal antibody immunotherapy that targets the GD2 antigens on the cancer cells.

Origin

It is important to understand the developmental biology and neuroblastoma origins because its proliferation, guide tissue morphogenesis, and differentiation resemble cancer cells' progression. From studying the unique physiology seen in the neural differentiation of the sympathoadrenal lineage, researchers can apply that knowledge to treat neuroblastoma. For example, one of the backbones of maintenance therapy is isotretinoin because scientists found that the neural differentiation is driven by retinoids in vitro (Cheung and Dyer,2013).

The neural crest cells that form neurons can be divided into five functional types: vagal, sacral, cranial, cardiac and trunk cells. The trunk cells then separate into two lineages in the early stages of embryonic development – sympathetic and adrenal. When the cells migrate from the neural crest, they undergo epithelial-mesenchymal transition (EMT). Both neural crest cells and tumor cells undergo similar EMT processes and express matrix metalloproteinases (MMPs), disintegrins and metalloproteinases (ADAMs) that facilitate cell invasion and migration (Kholendenko, 2018).

The migrating neural crest progenitor cells committed to the sympathoadrenal lineage initiate their differentiation due to the signaling of bone morphogenetic protein (BMP). The cells then commit to becoming either adrenal chromaffin cells or sympathetic ganglia. Members of the MYCN family are expressed throughout this process in the migrating trunk neural crest cells and are committed to the sympathoadrenal lineage (Cheung,2013).

Neuroblastoma arises from the progenitor cells of sympathoadrenal lineage of the neural crest during development. More specifically, scientists believe that neuroblastoma may develop from this chromaffin lineage because most tumors are localized in the adrenal gland region. The expression of different transcription factors is critical for neural crest development and is upregulated in cancers including Snail, Twist, SoxE, and FoxD families. The neural crest cells are a transitional type of cell that quickly pass from multipotent progenitors to a variety of differentiated cell types. Scientists previously thought that the neural crest cells gradually lose their multipotent properties and plasticity when they reach the postmigration stage, but it has been seen that is not the case. Adult neural crest-derived cells retain the properties of stem cells and even mimic the transcriptional expression profiles of both embryonic stem cells and neural crest progenitors. These progenitor cells of the neural crest are found in many types of tissues, including skin, dorsal root ganglia, adrenal medulla, bone marrow, among other tissues (Kholendenko et. al., 2018).

Presentation of Neuroblastoma

A 2016 study showed that 50% of neuroblastoma patients presented with metastases at diagnosis (Tolbert and Matthay 2018). Most cases of neuroblastoma are diagnosed in the abdomen or arise in the adrenal medulla or lumbar sympathetic ganglia. (Cheung, 2013). The tumor can appear anywhere from the neck to the pelvis because the origin is along the paraspinal and sympathetic ganglia. The clinical presentation varies based on the tumor's primary and secondary metastatic sites (Tolbert and Matthay, 2018). The most common metastatic site is the bone marrow, at 89%; therefore, bone marrow aspirates are used for diagnosing and responding to the cancer (Shumacher-Kuckelkorn, 2020). Due to the tumor's involvement with the central nervous system (CNS) other common metastatic sites are bones and regional lymph nodes. Tumors can present at the top of the paraspinal ganglia, causing Horner's syndrome in some patients because

neuroblastoma involves surrounding nerve roots of the sympathetic ganglia. Thoracic tumors present in only 5% of patients, arising from the posterior mediastinum and paraspinal ganglia, and, often, the tumor will invade the neural foramina. The neural foramina can be invaded anywhere adjacent to the spinal cord compressing it. In the abdominal cavity, masses can cause distention with or without pain. Furthermore, extensive liver involvement can be seen in infants and can cause liver disease such as coagulopathy as well as renal and lung dysfunction due to abdominal distention. The neurologic symptoms that can accompany pelvic tumors include bladder dysfunction, constipation, lower extremity pain or weakness due to nerve root involvement. Another symptom seen predominantly in infants' metastases in the skin is painless subcutaneous nodules that have a blue hue. If the tumor metastasizes in the bone of lower extremities, the patient may limp or refuse to bear weight. Only 80% of patients who need bone marrow infiltration will present with anemia and thrombocytopenia. The common symptom of racoon eyes is caused by bone lesions in the skull, within the periorbital region, that can cause periorbital bruising. The catecholamines released can cause flushing, hypertension, or tachycardia, and in rare cases, a paraneoplastic phenomenon will secrete vasoactive intestinal peptides causing profuse diarrhea. If children present with opsoclonus myoclonus syndrome (OMS) or varying neurologic symptoms such as opsoclonus, myoclonus, ataxia, or other cerebellar symptoms, they should be tested for neuroblastoma. Fifty to eighty percent of these patients will then be diagnosed with neuroblastoma, but only 2-3% of neuroblastoma patients are affected by OMS. (OMS can be attributed to the anti-neuronal antibodies cross-reacting with the cerebellum.) (Tolbert,2018)

Stages of Neuroblastoma

The international neuroblastoma community organized neuroblastoma into four distinct stages. The first L1, is a localized tumor confined to one body compartment, which does not involve vital structures as defined by the list of image-defined risk factors. The second stage L2, is a locoregional tumor with presence of one or more image-defined risk factors. The third stage is M: a distant metastatic disease. MS is confined to children younger than 18 months - metastases are confined to skin, liver and/or bone marrow. Further classification is based on age, histologic category, grade of tumor differentiation, MYCN amplification, 11q aberration and ploidy (Tolbert,2018). Patients with low-risk classifications have a favorable prognosis with >90% survival; in contrast, patients with high-risk neuroblastoma have a 5-year survival rate that is still below 50% (Weinke,2021).

Treatment Steps Overview

Most children who present with low and intermediate risk disease respond well to treatment plans, but children with high-risk neuroblastoma need an intense "multimodal" approach as the cure rates are estimated at <50% (Khan et. al., 2021). The current treatment for high-risk neuroblastoma has three phases and lasts approximately 18 months. Induction, the first phase, is where patients receive 5–8 cycles of intensive chemotherapy and start stem cell collection to prepare for their autologous stem cell transplant (ASCT). Surgery is usually preformed towards the end of this phase (Smith and Foster, 2018). Next is consolidation, which includes high dose chemotherapy followed by ASCT, shown to be especially beneficial in neuroblastoma, unlike in most other high-risk diseases with solid tumors (Khan, 2021). Radiation therapy usually starts after ASCT recovery. The last step is maintenance, which deals with the residual disease, trying to prevent the 50% relapse rate (Smith,2018). This phase uses immunotherapy which is a combination of monoclonal antibody targeting called disialoganglioside (GD2) / Chimeric antibody 14.18 (ch14.18), GM-CSF, and interleukin-2 (IL-2), which were added to previous maintenance therapy of just administering isotretinoin. In the cases when the patient relapses or the cancer becomes refractory, the cancer is rarely cured. Salvage therapy is administered to improve symptoms and quality of life (Tolbert and Mathey, 2018).

Monoclonal Antibodies' History

B-lymphocytes are activated when a foreign substance enters the body, and antibodies are produced in response to this antigen. Monoclonal antibodies (mAbs) are the main antibodies used in immunotherapy treatment for cancer. The history of mAbs began in 1975 with future Nobel Prize winners Georges Kohler and Cesar Milstein using hybridoma technology to produce mAbs, an antibody that only recognizes a single epitope. Antibodies that are made for immunotherapy are based on the Fv's region affinity for antibody targeting and the Fc's region ability to participate in the host's immune system. The two classes of mAbs are non-conjugated: naked mAbs and mAbs that work with chemo drugs or radioactive particles that target to enable the mAbs to reach the objective. MAbs use three different mechanisms of action via targeting and binding the target cell's antigen on the cell membrane and blocking the pathways that lead to the cells multiplying. Firstly, they inhibit the factors and receptors that activate the pathways allowing cancer cell proliferation. Second, they cause antibody dependent cell-mediated cytotoxicity (ADCC), where the mAbs bind to the tumor associated antigens in the surface of target cells. Then the

Fc receptors of immune cells recognize cell-bound mAbs, followed by cross-linking of these receptors, releasing cytotoxic agents that lead to apoptosis of tumor cells. The third way is through complement dependent cytotoxicity (CDC). The mAbs bind to the antigen on the target cell, causing a "complement cascade". These complements bind an attack complex and activate the cell's complement system lyses (Kimis-Gebologlu et. al., 2018).

GD2

Scientists isolated GD2 for the mAbs to target. GD2 in its humanized forms is called hu3F8 and ch14.18. GD2 is an oncofetal differentiation antigen that was identified by comparing neuronal differentiation and tumorigenesis (Cheung,2013). GD2 is a perfect candidate for antibody therapy because it is abundantly expressed in most neuroblastoma cells, and GD2 is limited in normal cells, including peripheral nerves (Nguyen et. al., 2018). It belongs to a unique class of T cell-independent carbohydrate antigens with high density, membrane proximity, homogeneity within and across neuroblastomas, and rare occurrence of antigen loss. Anti-GD2 mAbs attach tumor cells to NK cells and rescue NK cells from being suppressed or inhibited by neuroblastoma. GD2 is also ideal for tumor-selective delivery of radioisotopes, liposomes, or nanoparticles (Cheung,2013).

Further future applications of GD2 were seen in association with tumor-associated macrophages (TAMs). These TAMS are myeloid effectors that can become polarized into type 1 antitumor or type 2 pro-tumor phenotypes. But researchers discovered that in the presence of anti-GD2 mAbs in vitro, ADCC can turn protumor M-CSF-activated macrophages into antitumor killers. Even though mAb therapy is considered a passive immunotherapy, introducing a host anti-tumor following mAb therapy might benefit long-term tumor control (Cheung,2013).

MYCN, KIR-HLA, ALK, ATRX

In addition to GD2 researchers continue to search for unique features of the tumor that can lead to a cure. Scientists specifically look at the distinctive genes and mutations seen in a lot of cases and the effect they have on the outcomes.

Only less than two percent of patients have mutations in the signaling pathways involved with the sympathoadrenal line that causes familial neuroblastoma. PHOX2B was another mutation identified that promotes cell cycle exit and neural differentiation. Though in sporadic neuroblastomas, 6-10% carry somatic anaplastic lymphoma receptor tyrosine kinase (ALK) activating mutations, while 3-4% have a high frequency of ALK gene amplification. ALK plays a role in ensuring the balance between proliferation and differentiation. (PHOX2B and ALK gene have been linked because PHOX2B may directly regulate ALK expression) (Cheung,2013). ALK is expressed only in neural tissues, and mAbs designed to recognize ALK on neuroblastoma cell surfaces have shown increased ADCC of ALK-amplified NB cells. This ALK expression is linked to the MYCN expression (Jabbari,2019).

The most common focal genetic lesion in sporadic neuroblastoma is MYCN amplification. It is a major oncogenic driver since it controls proliferation, growth, differentiation, and survival of cells in the developing CNS (Cheung,2013). MYCN is expressed prenatally in different tissues, but its expression is lost during the first week postnatally. MYCN amplification is associated with metastases, reduced T-cell infiltration to TME, and invasiveness of the tumor and its progression during induction treatment. But MYCN-amplified patients were found to have better early response to treatment, however their survival rates were unaffected. Recent studies have employed miRNAs to suppress MYC family (Jabbari,2019).

ATRX mutation is another mutation seen in some neuroblastomas. ATRX is associated with increased telomere activity that is vital for the cancer cells to survive. This high telomere activity is found in 30% of neuroblastomas at diagnosis and is predictive of reduced EFS and overall survival. There are currently no molecular therapies targeting these pathways associated with telomeres (Cheung,2013).

NK cells are capable of inhibiting colony formation of human neuroblastoma cells and infusion of NK cells into mice bearing human metastatic neuroblastoma showing improvement in OS (Venstrom,2009). NK activity is regulated by inhibitory and activating signals following engagement of cell membrane receptors with their cognate ligands on target cells (Tarek,2012).

NK cells expressing inhibitory killer cell immunoglobulin-like receptors (KIR ligand) for self- human leukocyte antigen (HLA) class I molecules are equipped with effector function, ensuring that potentially autoreactive NK cells expressing KIR for non-self HLA ("missing ligand") are not able to work when they encounter cells lacking their cognate ligand. (Venstrom,2009). Untreated NB tumors and cell lines are widely reported to have reduced to no HLA class I expression, rendering them potentially susceptible to NK killings due to lack of engagement of HLA class I–specific inhibitory KIRs (Tarek,2012).

Clinically, the "missing ligand" KIR-HLA compound genotype is a strong predictor of response and survival. When treated with anti-GD2, patients with neuroblastoma who lack one or more HLA ligand for their inhibitory KIRs respond better to treatment, have lower rates of relapse, and survive longer compared with patients who possess

all HLA KIR ligand. This suggests that the unlicensed NK cells expressing KIRs for non–self-HLA ligands are more effective in tumor eradication than the licensed NK cells expressing KIRs for self HLA. HLA class I expression on neuroblastoma cell lines selectively inhibits licensed cell activity, allowing unlicensed NK cells to mediate ADCC, showing the importance of unlicensed NK cells for the antitumor role in patients undergoing mAb therapy. This explains the "missing KIR ligand" benefit in patients with neuroblastoma. Since licensed NK cells expressing S-KIRs have higher ADCC capacity in general, rescuing licensed NK activity from class I inhibition is advantageous because it could increase response in all patients, even those with all the KIR ligands present (Tarek,2012).

In addition, the use of exogenous NK cells in the treatment of patients with NB may be potentially useful if the patient lacks class I ligands for the donor inhibitory KIRs. Augmentation of innate immunity through adoptive transfer of allogeneic NK cells or the use of agents that increase endogenous NK cell number and activity, such as IL-2, lenalidomide, and anti-CD137 antibody, may all improve NB control, particularly in the presence of 3F8. While transplantation and mAb therapy are hardly normal physiological conditions, they both take advantage of the important pool of unlicensed NK cells, previously thought to be hyporesponsive and therefore potentially less clinically relevant. (Tarek,2012).

GM-CSF, IL 2, Retinoid Acid

Dinutuximab is augmented by GM-CSF and IL-2 because they stimulate the immune response and specifically the antitumor effect. IL-2 works to stimulate NK cells, and GM-CSF activates granulocyte and macrophage cytotoxicity (Armideo et. al.,2017). Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that enhances the activity of macrophages (Jabbari,2019). IL-2 is an important component of immunotherapy because it can improve the cytolytic function of NK cells against neuroblastoma cells. Though 23% of patients receiving IL-2 suffer from capillary leaks, IL-15 has been considered as an alternative to IL-2 for combination with anti-GD2 mAbs in neuroblastoma. Furthermore, administration of IL-15 is necessary for the NKT cells, an anti-neuroblastoma lymphocyte, to survive the hypoxic neuroblastoma environment (Cheung and Dyer, 2013). The International Society of Pediatric Oncology Europe Neuroblastoma (SIOPEN) has even removed IL-2 infusions with dinutuximab beta because of the toxicities related. They suggest dinutuximab beta and isotretinoin for maintenance therapy (Ladenstein et. al., 2020). The isotretinoin is used with anti-GD2 as a differentiating agent that induces the maturation of neuroblastoma cells since GD2 is a marker on only mature neurons (Armideo et. al., 2017).

Reasons CAR T does not Work in High-Risk Neuroblastoma:

Neuroblastoma is an extremely heterogeneous disease, meaning each tumor has unique molecular, cellular, and genetic features, all affecting the tumor's response to the treatment. It can continue to metastasize or even become refractory to a specific therapy. Furthermore, scientists are unsure how heterogeneity evolves with treatment and disease progression remains unknown. Neuroblastoma has a narrow epitope range hence the T cell-based therapy is not effective; therefore, antibody-based immunotherapy is used targeting GD2, and oncofetal antigens. Unlike its restriction in normal tissue, GD2 (disialogangliosides) present on tumors that arise from the neuroectoderm (neuroblastoma, melanoma, small cell lung cancer, and sarcomas) are expressed.. Also, there are few natural antibodies opposed to neuroblastoma. Further, fighting the rare variants of somatic mutations seen in neuroblastoma contrast with the lack of mutations in adult cancers. Then the intensive use of chemotherapy further damages those T cells that were attacking the neuroblastoma. Specifically, active adaptive immunity is harder for patients with high-risk neuroblastoma because of the primary and metastatic tumor's bulk together with its rapid proliferation that overwhelms the immature immune system in children (Cheung,2013). Another technique used to gain long term control over cancers is an immune checkpoint blockade that targets the immune system by activating previously exhausted or dysfunctional T cells. These mAbs (programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) used in the blockade in adults' tumors are efficient, but in pediatric tumors, these checkpoint inhibitors have shown no significant benefit (Liu,2020).

Neuroblastoma recruits pro-tumor macrophages and silences natural killer (NK) cells. Furthermore, neuroblastoma evades T cells by downregulating or losing human leukocyte antigen (HLA) expression, but they simultaneously cause damage by re-expressing HLA to resist NK cell-mediated antibody dependent cell-mediated cytotoxicity (NK-ADCC). Once bound to GD2, anti-GD2 mAbs bind to Fc-receptors on the surface of granulocytes and neurokinin cells and eliminate NB cells through ADCC and CMC (Jabbari et. al., 2019).

Side Effects of Anti-GD2

As with all treatments when dinutuximab - using mouse

SP2/0 cells (and dinutuximab beta- In Europe) was recloned in Chinese hamster ovarian (CHO) cells), is administered, patients can experience a range of adverse effects (McKeage et. al., 2018; Ladenstein, 2020).

Dinutuximab beta was also found to be a cost-effective treatment option (Pennington,2019). Dinutuximab beta is an orphan medicine that is approved in the EU for the treatment of high-risk neuroblastoma in patients aged 12 months who have previously received induction chemotherapy, and achieved at least a partial response, followed by myeloablative therapy and SCT, relapsed or refractory neuroblastoma ± residual disease (Ladenstein, 2020). The continuous, 10-day infusion regimen appears to be associated with less toxicity than the once-daily infusion on 5 consecutive days (McKeage,2018). These side effects usually do not continue once treatment ends, but it may interrupt the treatment.

Doctors can prescribe medications during treatment to deal with some of the side effects. The most frequent of these is neuropathic pain. This appears to be caused by dinutuximab, which targets GD2, a protein that is present on the neuroblastoma cell and is also present on neurons and peripheral nerve fibers, which causes the pain (Bartholomew, 2017). The pain can be controlled to some extent by analgesic therapy, including intravenous morphine, prior to and during the infusion (McKeage et. al., 2018). Furthermore, infusing an antibody can cause severe reactions including anaphylaxis and cytokine-release syndrome (Bartholomew et. al., 2016). Since 39% of patients run a fever, acetaminophen is administered prophylactically prior to treatment (Armideo et. al., 2017). The fever may stem from a few different factors including, the IL-2 releasing pyrogenic factors, immune stimulation or the cytokines that are released when the antibody is administered (Bartholomew,2017). Patients may also require premedication of antihistamines before each infusion. Furthermore, Dinutuximab can cause hypersensitivity reactions (25%) and even more so when IL-2 and dinutuximab are administered together; this is due to immune stimulation and cytokine release (Aust Prescr,2020).

The 23% of patients that experience capillary leak syndrome (CLS) can be attributed to endothelial cell damage, a cytotoxic response of dinutuximab, GM-CSF, and IL-2. Common treatment for CLS uses furosemide, which has a higher association with hypokalemia (Bartholomew,2017). The rare side effect of a liver dysfunction can be evidenced through the elevated alanine transaminase (ALT), (23%); and aspartate transaminase (AST) (10%) along with electrolyte disturbances (Armideo,2017). A small population have ocular changes such as mydriasis and accommodation issues along with anisocoria and sluggish pupillary response; therefore, patients should be monitored for photophobia, papillary reactivity, and visual changes (Bartholomew,2017). Other common toxicities seen are infection (39%), hypokalemia (35%), hyponatremia (23%), gastrointestinal side effects (nausea, vomiting, diarrhea; 22%), hypotension (18%), hypoxia (13%), and urticaria. (Armideo,2017).

Immunosuppressive Environment:

Factors that are unique to pediatric solid tumors are the paucity of neoantigens, development of resistance, and an immunosuppressive environment. The neuroblastoma tumor microenvironment (TME) studies have identified tumor associated macrophages (TAMs) within the immunosuppressive microenvironment in neuroblastoma that specifically inhibit both innate and adaptive immune responses (Liu,2020).

Other immunosuppressive components affecting the T cells function in attacking the tumor are defects in antigen presenting machinery (APM) and low levels of MHC class I molecule displayed by neuroblastoma tumor cells that lead to decreased cytotoxic T-cell activation. Secretion of different solubles, including transforming growth factor-β (TGF-β), and galectin-1 by tumor cells, directly inhibits T cell function. Furthermore, some myeloid cells in the tumor may not fully differentiate into dendritic cells, macrophages, or granulocytes, but instead generate a heterogeneous population of immature immunosuppressive myeloid cells, MDSCs. Hypoxia-inducible factor 1α (HIF1α) is a soluble factor that promotes the differentiation of MDSCs into TAMs, creating a feedback loop to support immunosuppression. Targeting these MDSCs enhance anti-tumor immune responses in neuroblastoma, implying that MDSCs play roles in cancer-related inflammation, enhancing the tumor's progression (Liu and Joshi, 2020).

Cytokines can be used for intercellular communication; therefore, the cancer cells can use them to alter the TME according to their needs. When tumor cells release VEGF, which promotes angiogenesis within TME, it is usually associated with higher stages of neuroblastoma and its poor prognosis. IL-6 is a cytokine, that positively affects tumor growth and distant metastases while IL-10 is an immunosuppressive cytokine found in neuroblastoma. Blocking IL-10 receptors has been shown to enhance immune response to tumors and improve outcomes of treatment. Also, INF-γ, a cytokine, can also induce tumor regression by promoting ADCC (Jabbari et. al, 2019).

Traditionally the thymus eliminates T-cells through T-cell receptors (TCRs) with high affinity toward tumor (self)-antigens. T-cells with high-affinity TCRs can be eliminated or converted into regulatory T-cells (Tregs) (Jabbari,2019).

Treg cells exhibit their suppressive activity via several mechanisms including inhibition of antigen-presenting cell maturation through the CTLA-4 pathway, secretion of inhibitory cytokines such as IL-10, TGFβ, IL-35, and granzyme and perforin expression that kill effector T-cells. Studies are currently inconclusive of Treg's exact effect on patients with neuroblastoma, but they do correlate a higher proportion of Treg cells in the bone marrow and peripheral blood with MYCN amplification (Liu,2020). Since Tregs lack IL-7Rα, it can be exploited for increasing expansion and enhancing function of CAR T-cells without concomitant expansion of Tregs (Jabbari,2019).

Further challenges stem from the neuroblastoma cells. Gangliosides and sialic are highly expressed surface carbohydrates that are important for migration, adhesion, and metastasis, but are also poorly immunogenic and sometimes even immunosuppressive. Since natural anti-ganglioside antibodies are rare, it allows the neuroblastomas to survive in the circulation despite not having a complement decay accelerating factor (CD55). Myeloid suppressor cells, regulatory T cells, and it exploits protectin (CD59) to resist or suppress immunity (Cheung and Dyer, 2013).

Further Developments

Almost 80% of patients with the clinically aggressive disease do not show sustained responses to recent advances in anticancer therapy. More research is necessary to understand neuroblastoma's biology and an accurate representation of the tumor to identify agents that can be used in pediatric drug development. Additionally, since there is a low occurrence of neuroblastoma there are less clinical trials (Corallo et. al., 2020).

Current neuroblastoma studies primarily employ two-dimensional (2D) cell cultures. A three-dimensional (3D) culture would improve the research since it can reconstruct a physiologically relevant TME. Also, current models do not reflect the pediatric context of neuroblastoma, immature immune systems, differences in drug metabolism, and continuing developmental changes. A 3-D platform would also address the lack of clinical trials issue and can lead to more efficient treatment plans, hopefully reducing the exposure of pediatric patients to additional rounds of chemotherapy (Corallo et. al., 2020).

Scientists have looked at instances of spontaneously regressing neuroblastomas (NBL-4S) where an advanced metastasizing neuroblastoma that had spread to the skin, peripheral blood, bone marrow, and peripheral ganglia (but excluding the bone) suddenly regresses. This situation shows that neuroblastoma may be different and disconnected from the GD2 marker. Although GD2 is present in NBL-4S, the study showed its expression may be lower (this should infer a worst prognosis, but the exact opposite occurred). Scientists have not been able to define the immune response nature and signature of neuroblastoma rejection in NBL-4S, but they assume NK cells are involved (Rovigatti,2021).

Researchers are working on a vaccine to prevent neuroblastoma relapses. Once integrated to the cell genome, lifelong expression of transfecting genomes can detect neuroblastoma cells presenting the tumor associated antigens (TAAs). This is of utmost importance in cases of minimal residual disease. Vaccines have been directed against TAAs such as survivin, MYCN, and GD2 have been somewhat effective for this purpose. Retroviruses have been used to transfer IL-2 genes to neuroblastoma cells in mice and have resulted in sustained production of IL-2 and tumor growth control in these animal models, and neuroblastoma cells transfected with IL-1β and TNF-α by means of retroviral vectors showed tumor growth arrest in vitro (Jabbari,2019).

Neuroblastoma evades the attack of the mAbs by escaping to the CNS, which is not accessible to circulating antibodies (Cheung,2013). Studies are working to create an anti GD2 CAR T treatment to redirect T cells against GD2 (Prapa,2015). Though CAR T treatment has a long way to go until it will be effective; therefore, there is not sufficient evidence to abandon the advantages of passive immunotherapy with anti-GD2 monoclonals (Ugo Rovigatti.2021). Scientists used an anti-GD2 single-chain variable fragment (scFv) derived from a murine antibody of IgM class that was linked to the signaling domains of the costimulatory molecules 4-1BB (CD137) and CD3-ζ. The receptor was expressed in T lymphocytes. Then transduced T cells expressed high levels of anti-GD2 CAR into cultures that infiltrated the tumors and persisted into blood circulation inducing massive apoptosis of neuroblastoma cells and destroying the tumor growth. Since the preclinical had positive results, more clinical testing with this approach will be tested in neuroblastoma and other GD2-positive malignancies (Prapa et. al., 2015).

Conclusion

Cancer treatments have improved greatly throughout the years, but researchers are still looking for the best way to eliminate high risk neuroblastoma. The current treatment plans employ the use of an immunotherapy using mAbs that targets the GD2 antigens on the tumor cells. Although this treatment has toxicities while it is administered, there is an overall higher EFS rate in patients receiving it in their maintenance therapy. Sadly, the rate of relapse is still high with the GD2 treatments; therefore, researchers continue to search for better options.

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Enzybiotic Therapy as Treatment for MRSA

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Abstract

This paper reviews the antibacterial potential of enzybiotics against Methicillin-resistant Staphylococcus aureus (MRSA). Due to the increasing occurrence of antibiotic resistance, researchers are looking to make use of the natural antibacterial qualities of virus bacteriophages, viral derived lysins, and antimicrobial peptides to fight MRSA infections. The efficacy of bacteriophages, endolysins, and bacteriocins as potential antibacterial agents against MRSA was extensively researched through Touro's online library database. Each of their mechanisms of action allows them to effectively lyse S. aureus cells, by essentially disrupting the peptidoglycan in the cell wall, causing it to burst. The narrow host range of these antimicrobials causes eradication of only pathogenic bacteria while maintaining the state of normal flora. Researchers have tested the ability of bacteriophages to effectively eliminate MRSA and have experimentally created therapeutically effective phage cocktails to delay the development of bacterial resistance. Different in-vitro and in-vivo studies demonstrate the ability of endolysins to rapidly kill S. aureus regardless of their metabolic state. Truncated and chimeric endolysins are used to optimize certain endolysin properties while eliminating negative ones. The therapeutic use of bacteriocins has significantly reduced and even completely eradicated MRSA infections in rabbit and mice in-vivo studies. Additionally, bacteriocins display synergy when used along with endolysins. All areas of enzybiotics show synergy with antibiotics when both treatments are combined. Additional research must be done before bacteriophages, endolysins, and bacteriocins can be used as a new antibacterial agent against MRSA.

Introduction

One of the greatest medical discoveries was the discovery of antibiotics in the 20th century, which caused the mortality rate of patients suffering from infectious diseases to drop dramatically. Other methods of treating bacterial infections were available at that time but were discontinued after the use of antibiotics became prominent. There are two major disadvantages to the use of antibiotics, the first being that aside from killing the unwanted bacteria they also kill the beneficial ones. The second is antibiotic resistance. Antibiotic resistance occurs when bacteria evolve mechanisms that protect themselves against the effects of antibiotic drugs. With a major misuse of antibiotics globally, antibiotic-resistant bacteria are quickly increasing each year. The discovery of new classes of antibiotics has been slow and is not keeping up with the rapid increase of resistant bacteria. This is causing common infections to become untreatable and once again deadly (Matilla, et. al. 2015).

An example of this is Staphylococcus aureus, a gram-positive, round-shaped bacterial pathogen that is responsible for many infections including bacteremia, pneumonia, sepsis, and wound and bloodstream infections. It is quickly becoming resistant to more and more forms of antibiotics which are making it increasingly difficult to cure. Methicillin-resistant staphylococcus aureus (MRSA) is a group of S. aureus isolates resistant to methicillin as well as many other kinds of antibiotics. Vancomycin is one antibiotic that is used to treat MRSA however vancomycin-resistant MRSA strains have started to emerge (Jensen, et. al. 2015). The inability to effectively treat MRSA has led to a resurgence in attempts to use previously neglected antibacterial therapies to treat it. This review is aimed at researching enzybiotics as one alternative method to treating MRSA infections

Methods

This study was performed through the analysis of various original and peer-reviewed articles which were accessed from Touro's online database including Proquest, PubMed, and Plos One databases. The articles were critically read analyzed and compared to determine the efficacy of enzybiotics as a possible treatment against multi-drug resistant MRSA.

What are Enzybiotics?

Enzybiotics fight bacterial infections through the use of virus bacteriophages, viral derived lysins or antimicrobial peptides. Some advantages enzybiotics have over regular antibiotics are their different mechanisms of killing bacteria, including antibiotic-resistant bacteria. Most importantly, the use of certain enzybiotics has not resulted in new development of bacterial resistance. For these reasons, enzybiotics represent a promising alternative to traditional antibiotic use by complimenting as well as replacing antibiotics in treating bacterial pathogenic infections including MRSA.

Originally, Enzybiotics referred to designated bacteriophage enzymes provided with the ability to break down cell walls which could be used as antimicrobial agents. However, eventually, enzybiotics began referring to all enzymes that displayed antibacterial or antifungal activity.

Bacteriophages

Bacteriophages are viruses that insert their genetic material into bacteria in order to replicate. The tails of phages bind to receptors found on the surface of bacteria allowing them to inject the DNA into the bacterial cell. For virulent phages, DNA replication produces many new phages which burst from the host cell and kill it. These replicated phages now move onto the next bacterial cell and repeat the process (Thurber, 2009). Their characteristic of being

able to replicate at the site of infection and therefore be available in abundance where needed, gives bacteriophages an advantage over traditional antibiotics (Gu, et. al. 2012).

Binding to a specific receptor is required for bacteriophages to infect bacteria, making phages extremely host-specific. Due to their narrow host range, using phages to treat infections is advantageous, because phage treatment can focus accurately on the pathogen infecting human cells while not harming normal flora. For this reason, there are thought to be minimal side effects associated with phage therapy (Jensen, et. al. 2015).

Phage therapy has been used previously in the early 20th century, however, when antibiotics were discovered, research in the phage treatment ended. Now that antibiotics are beginning to fail and there is a major need for alternatives, phage therapy is being looked at as a possible option (Mattila, et. al. 2015). Although bacteria can become resistant toward phages as well, phage therapy can possibly be a greater option because of its ability to change in response to the development of resistance by target bacteria.

Nosocomial infections are infections that are contracted within a hospital environment. Transmission of these infections often occurs via hospital equipment and fomites that are not properly disinfected. This is a common way that MRSA infections get transmitted and is a major concern amongst immunocompromised patients in hospitals. Researchers have studied the ability of phages to successfully decontaminate fomites associated with nosocomial transmission. Glass coverslips were used to represent decontamination of solid surfaces and cloth from a lab coat to represent the coats worn by clinicians.

Strains of S. aureus were isolated from many sources such as human nasal swabs, hospitals, dog hair, and poultry. Isolated phages were able to significantly reduce the colony-forming units of MRSA from the surfaces of the glass and fabric. They tended to demonstrate greater lytic activity toward the MRSA strains isolated from human sources. They were able to isolate at least six different phages that displayed lytic activity against human MRSA isolates and were able to decontaminate hard surfaces as well as fabric surfaces (Jensen, et. al. 2015).

Phage Cocktails

Phage therapy isn't infallible because bacteria have been shown to develop resistance towards phages. In attempt to solve this, the use of phage cocktails was studied. Phage cocktails are when multiple phage types each possessing different host ranges are combined. Studies have shown that using this method delays the development of phage resistant variants. However, it is difficult to acquire a set of phages that are effective against all variants of a specific bacteria, and if too many different phages are used in effort to increase the host range the therapeutic efficiency of the cocktail decreases.

Researchers have studied the possibility of creating patient-specific phage cocktails containing phages specific for the infection present. Compared to pre-made cocktails, this method of tailored phage cocktails ensures that unnecessary phages aren't used. For this method of treatment to be possible, hospitals would be required to have access to a large variety of phages at all times so that when a pathogen is identified they can obtain the specific phages that are effective against it.

The probability of successfully isolating phages effective against common hospital-acquired bacterial infections on demand was experimentally tested. Researchers found that the probability of finding phages from sewage, an optimal resource of phages, varied greatly for different host bacterium. Out of a total of 117 attempts, phages for only a single strain of S. aureus were discovered. After continuing to investigate whether alternative sources would be more suitable for obtaining phages effective against MRSA, only phages for strain SA10 of S. aureus were found. This specific study concluded that creating personalized phage cocktails on demand is not possible for treating MRSA like it is for other common infections. To treat these infections using this method, pre-made wide range cocktails would have to be used. (Mattila, et. al. 2015).

Phages cocktails were also used to determine their potential synergistic ability to decontaminate fomites. The difference is that the phages used weren't required to be extremely specific. Results of this study showed that the cocktails were effective in decontaminating both the lab coat fabric and the glass coverslips (Jensen, et. al. 2015).

Combination of Phage and Antibiotic Therapy

One way to use bacteriophages to combat infections is to combine phage therapy with antibiotic therapy. The combination of both antimicrobial agents seems to be synergistic; the interactions between both antimicrobial agents create a combined effect that is greater than than each of their individual effects. Not only has using this combination therapy shown to be helpful in effectively controlling bacteria, but studies have shown that phage therapy used along with antibiotics prevents the development of resistant strains. Therefore, using methods of combined treatment of bacteriophages with antibiotics can be effective in helping to resolve the issue of antibiotic resistance (Torres-Barceló, et. al. 2016).

S. aureus is one of the most common pathogens found in diabetic foot infections. Overuse of antibiotics to treat

these infections resulted in MRSA accounting for almost half of the S. aureus isolates found in diabetic foot infections. It's estimated that at least 50% of deaths caused by diabetic foot infections are because of strains that were antibiotic resistant and therefore untreatable. One available alternative treatment option is linezolid, an antibiotic that is known to cure diabetic foot infections without causing major side effects. Researchers have attempted to used phage therapy along with linezolid to treat induced foot infections in diabetic mice. To test their synergy, they used phage MR 10 alone and in combination with linezolid.

Results of the study demonstrated that in a group of mice that received an injection of phage MR 10, the infection was completely resolved after seven days. However, greater results were observed in a group of mice that were administered both phage MR 10 and linezolid. There, the infection was also completely resolved by day seven but there were comparatively lower bacterial loads on each day when compared to treatment with phage 10 alone. This showed that phage given along with linezolid were synergistic in controlling the pathogen population. Linezolid prevented further growth of the pathogen because it is a bacteriostatic antibiotic, and phage 10 killed the already existing bacterial population (Chhibber et. al., 2013).

Endolysins

A major disadvantage to phage therapy is the ability of bacteria to develop resistance to the phages. Because of this, researchers have looked into the possibility of purifying the lysins from bacteriophages to be used separately as antimicrobial agents. Holin and lysin are two proteins that allow reproduced phages to exit the infected bacterial host cell. The holin creates pores in the cytoplasmic membrane and allows the endolysin to access the peptidoglycan in the cell wall of the bacteria. This causes water to flow into the cell, resulting in its rupture, and release of the replicated phages. Because of their properties endolysins are being studied as possible antimicrobial agents that when applied to pathogenic gram-positive bacteria attack the peptidoglycan and lyse the cell wall (Pastagia, et. al. 2013).

Cell walls of S. aureus are primarily composed of peptidoglycan, teichoic acids, and different surface proteins. Peptidoglycan is a structural polymer that is composed of glycan chains of repeating N-acetylglucosamine and N-acetylmuramic acid that are cross-linked with peptide side chains (Vacek, et. al. 2020). Peptidoglycan hydrolases are often specific to certain species and genera since their peptidoglycan structures vary. Consequently, the use of phage endolysins as antimicrobials can help provide a targeted therapeutic approach, without killing unrelated commensal bacteria. It could also be useful in avoiding the use of broad range antibiotics which often cause the development of resistance (Becker, et. al. 2009).

LysK

Phage endolysins are found to have two or three domains. One or two N-terminal catalytic domains and a C-terminal cell wall binding domain. LysK is an endolysin derived from staphylococcal bacteriophage K, a phage that has proven to kill a broad range of pathogenic staphylococci. LysK is characterized as an endopeptidase, an enzyme that breaks peptide bonds. It contains three domains, two N-terminal catalytic domains, cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) domain, an amidase-2 domain, and one c-terminal SH3B cell wall binding domain. LysK has shown to have the ability to kill MRSA without permitting bacterial resistance to develop.

In one specific study, researchers attempted to determine whether all three domains found on LysK were necessary for it to perform exolysis (lysis from outside the cell). Analysis of their activity indicated that the CHAP domain is sufficient for exolysis of S. aureus cells but it's activity was enhanced greatly when the SH3b domain was present (Becker, et. al. 2009).

Researchers have cloned and expressed LysK in Lactococcus lactis to test whether it can inhibit a range of different staphylococci species including MRSA. Results confirmed that the recombinant LysK had the ability to degrade staphylococci cell walls. It was found to be active against a variety of live staphylococci, including MRSA strains from Irish hospitals. Gram-positive bacterial strains from other genera were not affected by the lysates containing LysK, suggesting that LysK is specific to staphylococcus. These results suggest that LysK could have widespread applications as a therapeutic agent against staphylococci infections including MRSA (O'Flaherty, et. al. 2005).

CF-301

CF-301 is another example of a lysin that demonstrates activity against S. aureus. In one particular study, CF-301's activity was examined alone and in combination with standard-of-care (SOC) antibiotics. It was tested in vitro against laboratory and contemporary clinical strains of MRSA, and in vivo against MRSA-induced murine bacteremia.

CF-301 killed S. Aureus rapidly both in vitro and in vivo. Its rate of antimicrobial activity in vitro was found to be a lot faster than that of the SOC antibiotics. CF-301 began killing MRSA laboratory strains within 30 minutes in contrast to the antibiotics, which required six hours to reach the same point. The same results were true when CF-301 was used to treat MRSA-induced mice. MRSA CFU (colony forming unit) was tested in their blood prior to and post

treatment. They found that after just 15 minutes there was a large decrease in CFU and even more after an hour. This rapid killing property of lysins makes them well suitable to quickly reduce the bacterial load in infected hosts.

Aside from its activity alone, CF-301 exhibited synergy when combined with SOC antibiotics both in vitro and in vivo. The synergy between the lysin and antibiotics was assessed in three different ways. First with a time-kill assay that studies the activity of antimicrobial agents against bacteria over time. Two antibiotics, daptomycin, and vancomycin were tested alone and in combination with CF-301. Sub-MIC levels (minimal inhibitory concentration) of lysin demonstrated synergy with sub-MIC levels of the two tested antibiotics. To confirm synergy, a checkerboard assay was also used. When CF-301 treatment was combined with the two antibiotics it was more effective at killing the majority of the tested MRSA strains than when each treatment was used alone. A third method was used which further confirmed these findings by showing that when in the presence of CF-301 the MIC levels of the antibiotic majorly decreased.

Additionally, there was little to no resistance of S. aureus to CF-301 seen after treatment for 26 days, compared to high bacterial resistance of S. aureus to SOC antibiotics which were 128 and 16 times the initial MIC. When the two treatments were combined and MRSA was treated with increasing concentrations of antibiotics in the presence of sub-MIC CF-301 for twenty-eight days, there was only a 4- fold increase in their resistances. These results demonstrate that the presence of the lysin suppressed the formation of antibiotic resistance.

Mice with staphylococcal-induced bacteremia were treated with CF-301 and daptomycin together and separately, in low and high challenge models. In some of the studies, the lysin yielded a higher survival of the mice and in others the antibiotics did, but, in all the cases the survival rate yielded from the combination treatment significantly outperformed the treatments with each of them alone.

These results can have clinical implications when designing new treatments using combinations of lysins and antibiotics. Because CF-301 proved to act fast, it would quickly reduce the burden of the pathogenic bacteria, while the antibiotic would act on the remaining bacteria. Additionally, when the bacteria are exposed to small amounts of lysin, which break the bonds of peptidoglycan, it causes the bacterial structure to become more permeable which allows for the antibiotic to penetrate more easily (Schuch, et. al. 2014).

Endolysins and Biofilm Eradication

S. Aureus forms biofilms within infected tissue which help them grow and survive in the presence of antibiotics and the immune system. Biofilm infections tend to develop in patients with prosthetic objects implanted into their bodies. They are harder to treat than free-living bacteria, and even more so biofilms of antibiotic-resistant pathogens such as MRSA (Chopra, et. al. 2015a). These biofilms are difficult to destroy because of their altered metabolic activity as well as the presence of an extracellular matrix making them difficult to penetrate (Rani et al, 2007).

Researchers attempted to test the efficiency of phage lysins in eradicating old and new biofilms formed by MRSA, possessing or lacking ica-locus. Phage-borne endolysin MR-10 was tested alone and in conjunction with minocycline. First, both kinds of biofilms were treated with endolysin MR-10 alone. They found that the optimum concentrations for eradicating young ica-negative MRSA biofilm was 18 g/ml, and for ica-positive MRSA biofilms, 36 g/ml. Here, the difference in intracellular adhesion seemed to affect the optimum concentrations needed.

The effectiveness of any antimicrobial agent against biofilms is largely determined by the age of the biofilm. Young biofilm formed by ica-negative and positive MRSA can be controlled by using the antibiotic minocycline alone at high concentrations, however, once the biofilm gets older the minocycline becomes ineffective (Chopra, et. al. 2015b). Since any lysin concentration was ineffective against completely eradicating mature biofilm, minocycline was used at its highest concentrations together with endolysin MR-10, in an attempt to completely eradicate the biofilm. No significant decreases were observed when equal concentrations of endolysin MR-10 and minocycline were used. The researchers believe the reason for this is because both agents worked together on the top layers of the biofilm and did not reach the interior. It is known that antibiotics are unable to penetrate deep into biofilms because of their complex matrix structure, and since lysins are one-use enzymes it's possible that both agents bound to the same cells resulting in little activity against them.

To test this theory, the researchers studied if sequential treatment of both page endolysin MR-10 and minocycline would have positive results in eliminating older MRSA biofilm. Two sequences were studied and each had different results. First, they exposed the biofilm to endolysin MR-10 for six hours and then treated it overnight with minocycline. A decrease in mature biofilm was observed to some extent after being treated using this method. However, when the biofilm was first treated which minocycline for three hours followed by endolysin MR-10 overnight there was a more significant decrease in cell count of the mature biofilm.

The reasons for these results are as follows. Antibiotic can not penetrate the layers of the biofilm and is only effective against the active cells so when the antibiotic was used first it was able to kill the metabolically active cells which are found at upper layers if the biofilm. Since endolysins are effective against bacteria regardless of their metabolic activity and have low molecular weights, endolysin MR-10 was able to penetrate more effectively into the deeper layers of the biofilm which the antibiotics could not reach. This study provides important research that lysins have the potential as an antimicrobial agents in the eradication of MRSA biofilm. Its mode of action targets the bacteria peptidoglycan and does not require the bacterial cells to be metabolically active which is unlike antibiotics (Chopra, et. al. 2015b).

Truncated and Chimeric Endolysins

Because endolysins have modular structures, domains can be swapped or removed to create newly combined lysins that have altered catalytic activities and binding specificity. Doing so can optimize different properties lysins have and eliminate possible downsides such as low solubility and poor expression in heterologous hosts.

CHAPk is a truncated single domain lysin that has been used experimentally to eliminate S. aureus from the nostrils of artificially infected mice. It demonstrated high solubility, rapid lytic activity, and high specificity against S. aureus. A single treatment with CHAPk greatly reduced the bacteria after just one hour. Using this enzyme may be an effective way to eliminate MRSA colonization in the human nares.

The human nostril is the most frequent carriage site of S. aureus which often serves as a reservoir for the spread of the pathogen. It had been found to play a vital role in the development of S. aureus infections, particularly in immunocompromised patients. Because CHAPk has the potential to quickly reduce the reservoir, it can be valuable in the prevention and spread of life-threatening MRSA infections. This is a property that is not found in antibiotic topical treatments which often take a couple of days to effectively remove S. aureus (Fenton, et. al. 2010).

In a different study, the catalytic domains of two highly soluble E. faecalis phage endolysins were fused with the c-terminal cell wall binding domain of the staphylococcal phage endolysin, Lys87. Two different chimeric endolysins were created in hopes to solve the problem of low solubility. The combined endolysins were able to efficiently lyse 96% of the 143 S. aureus clinical isolates that were tested. Included in the clinical isolates were strains of MRSA that represented some of the most relevant MRSA epidemic clones. The MRSA strains showed to be susceptible to both of the chimeric endolysins.

Aside from showing activity against Staphylococcus, the combined endolysins showed a broadened lytic activity towards enterococcus as well. This demonstrates that engineering chimeric endolysins can be a good way of obtaining soluble and highly effective peptidoglycan hydrolyses that have a broad lytic spectrum (Fernandes, et. al. 2012).

Bacteriocins

Bacteriocins are antibacterial proteins produced by non-pathogenic bacteria that inhibit the growth of closely related bacterial strains (Farkas-Himsley, 1980). They have relatively narrow spectra of antimicrobial activity due to being directed primarily toward bacterial strains closely related to their producing strain. Bacteriocins make up a family of proteins comprised of many different types, each exhibiting different properties (Hanchi, et. al. 2017).

Lysostaphin

Lysostaphin is a bacteriocin that is secreted by Staphylococcus simulans, a gram-positive bacteria. Lysostaphin possesses the ability to lyse a staphylococcus bacterial cell by disrupting its peptidoglycan layer (Schindler, Schuhardt, 1964). The major substrate for lysostaphin is the pentaglycine interpeptide bridge (Bowder, et. al. 1965). Since this is an obvious feature of the cell wall of S. aureus, lysostaphin can selectively target it, thereby killing the bacteria. Lysostaphin cleaves the staphylococci cell wall between two amino acids in the pentaglycine cross bridge. By cleaving these amino acids the lysostaphin destabilizes the bacterial cell wall causing a loss of osmotic equilibrium which ruptures the cell (Zygmunt, et. al. 1972).

Purifying, as well as cloning the lysostaphin gene into different expression systems has led to creating a highly purified recombinant lysostaphin with high specific activity. The availability of this recombinant as a potential antimicrobial agent against S. Aureus has become of interest as new methods are being researched to combat bacterial resistance.

Durancin 61A is a bacteriocin produced by Enterococcus durans which is also being studied as a possible treatment against MRSA. It has been found to exhibit antibacterial activity when tested in vitro (Hanchi, et. al. 2017).

Lysostaphin Antimicrobial Activity in Vivo

Multiple studies have researched the use of recombinant lysostaphin in treating S. aureus. One experiment tested its ability to treat S. aureus-induced infections in mice. It was found that administering 5mg/kg of lysostaphin once a day for three days successfully cleared their kidney infections caused by MRSA and significantly reduced their liver and spleen infections. This demonstrates

lysostaphin's ability to penetrate tissue when administered intravenously. Compared to a higher dose treatment over one day, repeated administration of a lower dose of lysostaphin was found to be more effective for the treatment of S. aureus (Kokai-Kun, et. al. 2007).

S. aureus is a leading cause of bacterial keratitis which can result in irreversible damage, e.g. a loss of visual acuity or even complete blindness. Keratitis is normally treated with a topical treatment of antibiotics, such as ciprofloxacin, however more and more MRSA strains have become resistant to it. Lysostaphin was used experimentally to treat bacterial keratitis in rabbits against vancomycin, an antibiotic used to treat MRSA infections. Results demonstrate that lysostaphin is an effective treatment for experimental keratitis caused by S. aureus.

Minimal inhibitory concentrations of lysostaphin were determined for multiple strains of MRSA and were found to be many times lower than the MIC's of vancomycin. In addition, Lysostaphin successfully sterilized the rabbit corneas when treated early on in the infection, compared to vancomycin which did not completely sterilize them. When treated later on in infection, although lysostaphin did not completely sterilize the corneas, it reduced the CFU (colony forming unit) per cornea more than the vancomycin therapy did when used to treat early in the infection.

Just like endolysins, bacteriocins share the property remaining effective regardless of the metabolic status of the bacterial cells. They can kill both rapidly growing cells as well as non-dividing cells. Through experimentation, lysostaphin proved to be an effective therapy during the late stages of the staphylococcal infection when there is minimal bacterial replication. This gives bacteriocins an advantage over most antibiotics to which this is an unusual characteristic.

Infected eyes that were treated were found to be free of detectible pathological changes after being observed for seven days. Lysostaphin did not cause any conjunctival inflammation or corneal edema as did vancomycin. However, further studies are needed to determine whether there are any adverse effects caused by repeated uses of topical lysostaphin since it has the potential to cause an immune response against itself. The availability of recombinant lysostaphin can help with this issue (Dajcs, et. al. 2000).

Bacteriocin Combination Treatments

Studies have shown that there is a synergistic effect between lysostaphin and antibiotics. When lysostaphin was experimentally used with oxacillin to treat MRSA in vivo it improved its efficiency and allowed for a lower therapeutic dose to be used. Treatment with this method would also be beneficial in preventing the emergence of S. aureus that is resistant to lysostaphin (Kokai-Kun, et. al. 2007).

Researchers have also attempted to combine the treatment of lysostaphin with endolysin LysK. They did so by combining enzymes consisting of both the catalytic domains of LysK and Lysostaphin to treat infections. An experiment that combined the two enzymes demonstrated greater growth inhibition of MRSA then each enzyme showed alone (Becker, et. al. 2009).

Synergy between durancin 61A and vancomycin was observed in inhibition of growth of S. aureus ATCC 700699, a methicillin-resistant staphylococcus strain. By combining the two, each of their MIC levels were reduced drastically (Hanchi, et. al. 2017).

Conclusion

As antibiotic resistance continues to become a more prevalent medical concern, scientists are constantly searching for new effective antimicrobial therapies. Research and experimentation suggest that enzybiotics may serve as possible solutions for combating multi-drug resistant bacteria and particularly for the treatment of MRSA. Each area of enzybiotics provides different advantages over antibiotics, however possible downsides need to be taken into account.

Phage lysins seem to be a favorable way of combating MRSA infections. Their speed of bactericidal activity, low probability of affecting normal flora, as well as the small chances of bacterial resistance, gives endolysins an advantage over traditional antibiotics. Additionally, the use of phage lysins incorporates all the positive aspects of phage therapy without the negative possibility of creating resistant mutations.

Utilizing combination treatments of enzybiotics along with antibiotics is another effective method of combating MRSA. When tested, combination treatments tended to be synergistic and showed the greatest results for killing MRSA than when each therapy was used alone. Each therapy provides its own mechanism of action, so when used together, each provides its specific capability's to effectively combat the bacterial infection.

Despite the growing potential of enzybiotics, further research must be conducted as well as implementing enzybiotics into clinical trials in order to establish enzybiotics as a tried and true therapeutic option for combating MRSA infections.

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Dance Training and Delayed Maturation

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Abstract

The physiological effects of early childhood dance training are constantly being explored and adjusted. It was observed that children in training experience a higher than normal percentage of delayed maturation, both in puberty and bone development. It was suggested that the environmental conditions in which dancers practice, such as malnutrition and too much time spent indoors, as well as biological factors, contribute to the hormonal and cellular disruptions responsible for late development. Undernourishment, as is common in dancers, was found to have a direct correlation with reproductive hormone levels, including reduction in gonadotropin-releasing hormone secretion as well as lower Luteinizing hormone and Follicle-stimulating hormone levels. Low energy availability due to overexertion also plays a role in pubertal development. Furthermore, Vitamin D deficiency from lack of sunlight seems to influence dancers' bone health and development. This paper aims to present some abnormalities in dancers' growth and development, as well as explore the physiological, hormonal, and metabolic reasoning behind such a phenomenon.

Introduction

The universally practiced form of dance, known as ballet, is learned and taught by many in today's world. The biological consequences that follow those who practice it, however, may be hiding beneath the surface. In the United States today 650,000 to 10,400,000 young girls and boys in the United States study ballet in a professional dance studio. Of these students, 9,000 to 45,000 of them move on to higher education in dance training after that (Bronner, et. al. 1999). Considering the prevalence of dance in American children today, one must examine the effects, short term and long term, that intensive training has on the dancer's biological functioning. One of the major areas impacted by ballet training is the development and maturation into puberty. Perhaps the strenuous physical exertion and specific unnatural positioning of the body that ballet training often requires causes some form of malfunction in the onset of menarche, bone development, and general maturation of the body into adulthood.

Discussion

For females, puberty hits most commonly around the age of 12. This means a young girl will begin to experience many changes involving hormonal activity resulting in the development of secondary female characteristics and the start of menstruation. The two main hormones responsible for regulating the menstrual cycle are the Follicle Stimulating Hormone (FSH) and the Luteinizing Hormone (LH). FSH is released by the pituitary gland which then stimulates the ovaries to release estrogen into the bloodstream and stimulates the formation of the ovarian follicle. LH, also released from the pituitary, then takes over and encourages egg maturation and triggers ovulation, the release of the oocyte from the ovary. Low levels of LH specifically can be the result of nutrition disorders such as bulimia and anorexia. Since the ballet world encourages a thin figure, many students suffer from eating disorders leading to delayed reproductive development.

A major factor that contributes to delayed menarche and sexual development has to do with energy dispersal, specifically low energy availability. The body needs energy in order to start the process of the various developmental cycles. However, when one is overexerting oneself and therefore using more energy than the body has readily available, it uses up energy required for physical maturation. This does not necessarily relate to the amount of calories one burns when doing physical activity, but rather to the amount that the subject is pushing him or herself beyond the body's normal limits. This applies to children in professional training courses, who generally practice for at least 50 hours a week, as well as other sports that require intensive professional level training (Rigby 2012). However, ballet training specifically causes females to experience delayed development. Late maturation is defined as a girl who has not shown signs of secondary sexual characteristics by the age of 14 or has not begun menstruating by the age of 16 (Kapczuk 2017). Researchers revealed that the main hormone related to malfunction in the reproductive system is GnRH (gonadotropin-releasing hormone). When the secretion of GnRH is disrupted, LH cannot be released and therefore the regulation of the reproductive cycle is disturbed. A later study revealed that kisspeptin, a hormone directly related to metabolic functioning and nutrition, was discovered to be a positive regulator of GnRH. Undernourishment and low energy availability, as is common in most professional dancers, reduce kisspeptin production. This in-turn, causes a decrease in GnRH secretion, which slows down the entire chain of hormonal reactions that regulate the reproductive functions. An experiment demonstrated that due to a lack of sufficient energy, a group of female dancers experienced delayed menarche. Although proper sexual functioning returned after a break in their training or once they significantly reduced their training time, amenorrhea, or complete lack of menstruation, returned as soon as they restarted heavy physical activity. This study utilized the concept of energy monitoring in the brain. When there is a lack of metabolic nutrition in the brain, it needs to balance the energy among the areas in the most immediate need of energy, such as the large amount of energy used to fuel the performance of grueling dance exercises (Frisch, et al.) This takes away from energy needed for

healthy menarche development and can therefore lead to delayed onset as well as recurring amenorrhea once the high performance activity is reinstituted (Iwas, et.al 2018).

Besides the obvious effects that reproductive hormones have on adolescent maturation, other parts of the endocrine system can influence changes within the reproductive cycles as well. Stress hormones, mainly cortisol, have been shown to play a crucial role in delaying puberty. A group of carp fish who were exposed to extreme changes in water temperature three times a week and successively exhibited a delay in sexual development. Later, researchers assumed this was due to the cortisol released in the carp under stress-inducing conditions. Another experiment was executed in which one group of carp was injected with high concentrations of cortisol and the other group had their cortisol regulators "switched off". While the second group developed normally, the first group showed signs of delayed development of reproductive organs. This confirmed that high levels of stress, indicated by the increase in cortisol production, directly impact proper onset of puberty (Allsworth et. al. 2007).

A later study in 2002 tested this theory with human beings. The experiment studied 446 women under the age of 45 who were incarcerated. Many of these women in prison had suffered from highly stressful situations in their lives, including sexual abuse, stressful living conditions, or a parent who was incarcerated. Nine percent of this population of women reported amenorrhea, and 33 percent reported irregularity in their menstrual cycle. Most of the subjects had experienced some stressor or trauma as a child. The conclusion drawn from this study was that incarcerated women are more likely to exhibit irregularity in their menses cycle, which may specifically relate to the high levels of stress that the subjects faced (Thompson).

Considering the fact that young ballet dancers, as well as any child dance training professionally, is subject to a great amount of pressure, whether it be from the parents, the competitive nature of the dance world, or self-induced pressure to perform well, one can conclude that they experience high levels of stress. Recent surveys have shown that professional dancers are more likely to have a psychological stress disorder such as PTSD; 22% percent of dancers showed signs of PTSD as opposed to the average population of which 7 percent have PTSD. Stressful conditions cause the body to react in ways that then prevent the proper reproductive functions from occurring (Munoz 2004).

Another point to consider is the effects vitamin D levels have on the body. The sun is the main source of Vitamin D, and considering the fact that dancers spend a great deal indoors, it is reasonable to suggest that they may be lacking in vitamin D. Many young dancers are also undernourished, and therefore have a deficiency in many key nutrients, including Vitamin D. Vitamin D plays a major role in bone strength and development. It is involved in regulating intestinal calcium absorption and calcium resorption in the bone. Therefore, when there is Vitamin D deficiency, calcium absorption from the intestines is significantly decreased, which in turn stimulates osteoclast production, causing the bone to break down. Osteoclasts also release enzymes to break down the bone matrix, allowing the mobilization of calcium from the bone into the body's circulation. Without Vitamin D, the calcium levels in the bone and body, in general, are not sustained, and calcium begins to consistently leave the skeletal system. If this cycle of bone degradation occurs during the years of childhood development, the bone does not have a chance to be nourished and may not form properly (Mathews, et. al. 2006).

The female athlete triad has become the common term used in reference to the top three disorders in female athletes: eating disorders, amenorrhea, and osteoporosis. While the first two are most well-known in women who play all kinds of sports, osteoporosis is most commonly associated with dance-related activity, specifically ballet. Osteoporosis is directly related to one's bone mass density (BMD), since osteoporotic bones are characterized by a loss in bone density and exist as brittle networks with many holes in the bone's structure.

Bone density may be another element of maturation that is possibly delayed by early dance lessons. On the one hand, it seems that high-impact weight-bearing sports, such as football and hockey, are beneficial for bone strength. This type of activity has been shown to increase bone mass, bone density, and bone turnover, and therefore can help prevent osteoporosis later in life. This is because the tension from the muscles and tendons surrounding the bone force the bone to produce more tissue and build up bone mass and in turn increase bone health. These types of activities such as running, weight lifting, jumping rope, and strength training (i.e. push-ups etc.) involve working the body against gravity. However, the positioning and delicate movements required in ballet training seem to not be conducive for bone mass augmentation. In fact, many professionally-training dancers have reported experiencing osteopenia earlier than normal. Osteopenia, or the gradual loss of bone mass, typically begins around age 35, and is a healthy phenomenon when developed at the normal rate (as opposed to osteoporosis, which is more severe). Premature loss of bone mass appeared to be common in dancers. However, this may be due to the fact the professional dancer figure, typically one of a thin woman is prone to brittle and weak bones (Mathews

et. al. 2006). Furthermore, one might argue that although ballet is not generally classified as a weight-bearing sport, certain key leaping movements, such as grande je tes and entrechats, studied in the field of ballet mimic the gravity-defying activities of standard high impact sports. This could likely cause an increase in bone density and balance out any bone loss caused by low BMI or unnatural twisting of the limbs that grind away at the joints. In fact, a recent 3 year long longitudinal study has shown that prepubertal dancers do experience bone mass augmentation in certain areas of the body, most significantly in the femoral neck and lumbar spine regions. The hip flexors and abdominal muscles are the muscles most often used in ballet and likely put pressure on the femoral neck and lateral lumbar spine areas increasing bone tissue and bone mineral content. The subjects, however, demonstrated that the other upper extremities had not built up a significant level of bone mass when compared with the control group, suggesting that the increased BMD in the abdomen and legs were solely because of the greater weight bearing activity in those parts of the body (Turner, et. al. 2012). While this seems to support the claim that dancing can in fact improve bone strength and development, it is unclear from the ramifications of the study if the increased BMD was a direct result of the subjects' dance training. Being that the dancers ranged from ages 11-14, the bone mass augmentation could have been related to their pubertal growth spurts, in which exponential growth in the hip and leg area often occurs, as opposed to their training. Therefore, the reason behind early osteopenia and osteoporosis in dancers has yet to be completely clarified.

One specific dance move commonly referred to as "turn-out", refers to the total outward rotation of the hips required at all times during classical ballet training. Considering this isn't the natural state for one's hips to be in, this repeated movement often causes pain and degeneration in the pelvic-femoral joint. Turn-out movement can cause dysplasia to develop in the ball-and-socket hip joint so that the two bones don't fit together as they should; specifically the acetabulum socket of the pelvis and the femoral head are misaligned. Over time a dancer may begin to feel pain and deterioration. Studies have observed that ballerinas will ironically lose full range of motion, namely internal rotation and adduction, caused by overuse of the external rotator muscles (Storm, et. al 2018). Additionally, a group of 11-14 year old ballerinas were observed and shown to have a significant decrease in femoral torsion. Many of the dancers were observed to have labral tears, in which the labrum cartilage surrounding the hip joint ripped (Hamilton, et. al. 2006) .

While the effects of dance training on maturation and body health generally seem to be negative, some researchers have suggested there to be some positive effects as well. A study was conducted involving a group of dancers who were rehearsing ballet 10 hours a day over a 17 week period leading up to a professional competition. The study observed girls between the ages of 12 and 15 and attempted to monitor the impacts such training might have on the cardiac autonomic system, puberty, biochemical variabilities, as well as body mass changes. The subjects were tested before and after the 17 week period, and were found to have an increase in body mass, total protein production, and testosterone and cortisol levels. To measure the effects on the autonomic system, the subjects' heart rate variability (HRV) was observed pre and post training and overall higher HRVs were observed after completing the training period (Da Silva, et. al. 2015). Scientists compared this to a previous study that displayed significant improvement in HRV in 16 year old male gymnasts, as well as other studies which showed increased HRV in prepubertal swimmers (Tomova, et. al. 2015)). While this could possibly support the idea that intense physical exertion could improve autonomic performance, it doesn't necessarily take into account other factors that may play a role in the 17 week dance training study specifically. The two studies used as support evidence involved either post pubertal (or at the very least, the tail-end of adolescent maturation), and prepubertal subjects. The dance group experiment, however, was aimed to specifically examine the physiological effects on 12-15 year old girls, in the prime of pubertal maturation. The hormonal changes and increased growth rate that happens during these years may contribute to increased HRV and general fluctuations in the autonomic system. Furthermore, this could also explain the results showing increased body mass and height over the training period. One must consider that the subjects could have likely been in the middle of a growth spurt common to that age group, as well as experienced a general increase in body mass. Perhaps dance training augmented these factors, but there is no specific evidence of such reasoning, especially since this experiment included no control group.

Moreover, the study's results also showed that while body mass increased, the BMI for their age group declined as they continued to train. A dancer's body mass index (BMI) also relates to their delayed development. Studies found that as BMI decreases, the first onset of menarche increases. Since it is common for dancers to have a below average BMI, many will experience their first menses at a later age, demonstrating delayed development (Tomova et. al. 2015). A 2014 study observed 4030 boys from ages 7 to 19 and found that their BMI was directly related to

the timing of puberty onset. The overweight group generally experienced early puberty compared to the normal group, and underweight boys demonstrated a delay in every stage of development (Natural Institute of General Medical Sciences).

Pubertal and specifically menstrual disruption is also commonly caused by a disturbance in one's circadian rhythm. Many young dancers train for numerous hours a day, getting up before dawn and leaving after the sun has already set and spending all those hours in between in a fluorescently lit studio with mirrors instead of windows. This means their body may not be getting enough light stimuli during the day to keep their inner clocks balanced. The circadian rhythm is how the body regulates sleep and alertness based on light stimuli from the surrounding environment. It is a network involving communication between certain parts of the DNA, such as the Period and Cryptochrome genes, and various proteins. These specific genes code for proteins that play a major role in building and nourishing the nucleus in nerve cells while one sleeps. This genetic activity is significantly reduced in the daytime while one is awake. The main "clock" which regulates the body's circadian rhythm is the suprachiasmatic nucleus (SCN), referring to a group of 20,000 neurons located in the hypothalamus. This bundle of nerve cells receives light input directly from the eyes stimulating the brain to signal and inform the rest of the body as to what time of day it is (Reilly 2000). When such a regulation system is disturbed, such as spending too much time indoors without windows or having long work hours beyond the body's natural circadian clock, the body can react in many ways. One side effect observed was amenorrea. The circadian rhythm was found to have an inhibitory effect on luteinizing hormone production, causing there to be a shorter luteal phase in the menstrual cycle, and ultimately leading to amenorrhea. Studies have shown that dancers, flight attendants, and anyone who often experiences quick changes in time zones commonly display disturbances in their hormonal and menstrual cycles (Zhu, et. al. 2020). While the job description of a professionally-training dancer may not include an abnormal night/day schedule such as stewardess or world travelers, it is implicit in the method of training to practice all day in a studio with no access to sunlight and admittedly disturbing their circadian rhythms (Turner, et al. 2012).

Some treatments have been explored as to how best to prevent and treat dancers with delayed maturation. The idea of sex-steroid therapy is a common approach to late onset of puberty. These steroids influence the hypothalamus and pituitary gland to secrete growth hormones and initiate secretion of reproductive hormones. This helps individuals with low GnRH, LH, and FSH levels to mature and to be fertile in the future. An assessment involving a group of health providers revealed that 83 percent of the providers agreed that sex-steroid therapy could jump-start puberty in adolescents experiencing a delay in that area. Other providers have suggested that a method known as "watchful waiting" is ideal, in which continued observation of the subject occurs (Zhu, et al. 2020). However, this proposition may not be aggressive enough, considering how time sensitive the onset of puberty is. A doctor might be careful tracking a patient's development and decide not to introduce medication or therapy since the patient is progressing, albeit slowly. At the point in which the rate of growth is understood to not be improving further, it may be too late. There are also preventative courses of action that can be implemented to lessen the negative conditions that allow delayed puberty to occur. Since malnutrition is so common in dancers, having children eat a balanced diet with plenty of high energy foods is key to avoiding eating disorders and low energy availability. One way to counteract the negative effects of too much time spent indoors could be to instigate light therapy. This requires the subject to sit in front of a light box that emits UV rays, helping the body sleep better and experience a more consistent circadian rhythm. Vitamin D supplements can also be taken to support the buildup of bone and retain calcium levels in the skeletal system.

Conclusion

Conclusively, the physiological effects that intensive dance training has on the growing body must be carefully considered. While ultimately it is up to each individual to decide if dance training is worth all the biological long term risks, there are clearly many signs that point to negative impacts in the areas of maturation, bone development, and onset of puberty. However, one must take into account that each individual has pre-existing conditions and physiological circumstances which may influence how significantly dance plays a role. There are still many areas in which more research must be done to clarify the direct correlations between dance and development, and doctors and researchers today still strive to get a deeper understanding of this phenomenon.

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What Is the Safest and Most Effective Method of Repairing Myelomeningocele?

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Abstract

Spina bifida is one of the most common NTD's (neural tube defects) to occur during embryonic development, when the neural tube fails to close properly during neurulation.

Myelomeningocele is the most severe form of spina bifida. Characterized by an open posterior neuropore with meninges and parts of the spinal cord protruding from the fetus's body, it manifests in a variety of physical and neurological symptoms that vary both by the individual and by the state of the lesion. Until the late 1990's, the standard course of treatment was surgical closure of the lesion at birth, followed by standard protocols and treatments to treat the accompanying issues. However, once the first in-utero repair of myelomeningocele was performed in 1997, a new world of possibilities opened up. In-utero repair demonstrated distinct advantages over the standard method of postnatal repair; specifically, it reduced the likelihood of hydrocephalus and hindbrain herniation and showed significant improvement in motor and neurological function. This paper will discuss, analyze, and compare the outcomes of both the prenatal and postnatal methods of repair and discuss emerging research in the field as well as some of the inherent risks of the procedure.

Introduction

Fetal development, with all its extraordinary inner workings, is considered by many to be one of the most remarkable miracles of life—yet the more intricate processes involved, the greater the potential for damage. Neural tube defects, or NTD's, are among the possible disorders in fetal development, the most common of which are anencephaly and spina bifida. Spina bifida, in turn, is one of the most widespread birth defects, as well as the most common congenital defect of the central nervous system that is actually compatible with life (Adzick et al., 2013). As such, it is the focus of much study and intervention.

Spina bifida is characterized by an open vertebral column. In its least severe form, spina bifida occulta, the gap is merely a gap in the vertebral arches. It does not impair functioning and may never even be discovered. The most severe form of spina bifida, though, and the focus of this review, is spina bifida aperta, or open spina bifida. Commonly referred to as myelomeningocele, this is a form of spina bifida where the neural tube itself fails to close during neurulation. In cases like these, neural tissue from the spinal cord and meninges are pushed through the open vertebral arches, muscle, and skin into a sac of cerebrospinal fluid protruding from the fetus's body (Sacco et al., 2019).

The exposed neural tissue, in turn, degenerates further with increased exposure to the intrauterine environment. Thus, myelomeningocele is often considered a "two-hit" process, as damage occurs first due to the open neural tube and secondly, and more progressively, due to prolonged exposure of the neural tissue to the amniotic fluid environment. Based on the severity of the defect, the infant will be afflicted with lifelong disabilities such as impaired bladder and bowel function, paralysis, and neurological deficits, and will be at risk for hydrocephalus and hindbrain herniation (Copp et el., 2015).

The etiology is not fully understood, especially considering the various types of spina bifida; various factors are often at play in such a situation. It is understood, however, that the predominant cause of spina bifida worldwide is insufficient blood folate concentrations among women of childbearing age (Oakley, 2020) – a problem easily preventable in many cases, though folate intake is not a cure-all.

Up until the late 1990's, myelomeningocele was repaired postnatally: the defect was surgically closed at birth and the various associated health issues managed with standard medical procedures and therapies. In 1997, though, the first successful in-utero repair of myelomeningocele was performed, which paved the way for many more successful prenatal surgeries. Prenatal repair of myelomeningocele exhibits several distinct advantages over standard postnatal repair; chiefly, the fact that it actively reduces the need for shunting for hydrocephalus and results in better mental and motor function at 30 months of age (Adzick et al., 2013).

As miraculous as it may seem, prenatal surgery still comes with its own set of risks. This paper will discuss the current methods of treating myelomeningocele as well as examine new research and modern advances in the field, and evaluate the risks involved to the best course of action regarding myelomeningocele repair.

Methods

The articles and journals used in this review were found mostly on ProQuest, PubMed, and the National Institute of Health. Among the key phrases used were "spina bifida," "myelomeningocele," "in-utero repair," "stem cell therapy," and "folic acid."

Discussion of Spina Bifida

In a normally developing embryo, primary neurulation begins at the beginning of the third week of gestation. This process is characterized by the formation of the neural plate, a thickened portion of ectoderm—the very beginning of the central nervous system. Through cell division and cell migration, the neural groove is formed in the

center of the neural plate while the sides of the plate form the neural folds. These folds rise, come together, and fuse to form the neural tube; closure begins in the cervical region and extends both cranially and caudally. By the end of the fourth week of gestation, the neural tube is closed and primary neurulation is complete (Fichter et al., 2008.

The problem arises when the neural tube fails to close properly. Researchers are still uncertain as to what precisely causes this to happen, but what is clear is the outcome: an open posterior neuropore. The end result, therefore, is a neural tube defect characterized by an open vertebral arch and open meninges, fused to the skin, that forms a sac containing parts of the spinal cord (Fichter et al., 2005).

Individuals with spina bifida may experience a multitude of difficulties in various aspects of life—specifically with regard to mobility, though much of it is dependent upon the severity and location of the lesion. Though lower limb weakness, lack of sensation, or paralysis below the level of the lesion are frequent, many individuals do achieve independent ambulation as adults, approximately 57% with an L4 lesion and as many as 93% with a sacral lesion (Sacco et al., 2019).

Bladder and bowel dysfunction are common secondary conditions of spina bifida; these are normally managed with catheterization, enemas, laxatives, and the like. Some experience sexual dysfunction as well, specifically with regard to erectile dysfunction in men. In addition, leakage of cerebrospinal fluid through the spinal lesion often causes brain changes such as the Chiari II malformation which causes hindbrain herniation. The herniation impairs development of the cerebrospinal fluid pathways in the brain; this causes hydrocephalus, or a buildup of excess fluid. Hydrocephalus is typically managed with a ventriculoperitoneal shunt, though shunt complications may and do occur (Sacco 2019).

Moreover, children with spina bifida often have significant medical expenses as well as learning disabilities and lower IQ's than average, and many cannot live independently as adults (Adzick, 2013).

Historical Background

In the early 1970's, the advent of prenatal biochemical screening techniques first made it possible to diagnose neural tube defects such as spina bifida; the presence of alpha-fetoprotein (AFP) in a sample of blood or amniotic fluid was a good indicator. As the use of ultrasound technology to detect anomalies became more widespread, the use of biochemical screening techniques, though still useful in some cases, became less relevant, since sonograms are more accurate and specific (Copp et al., 2015).

Still now, the standard traditional treatment for myelomeningocele is postnatal repair and closure of the defect within two days after birth. Among other reasons, this helps avoid the risk of the open wound leading to an infection that can cause meningitis (Copp et al., 2015). The treatment includes the placement of a ventriculoperitoneal shunt to treat the hydrocephalus that will probably occur (Grivell, RM; Andersen, C; Dodd, JM, 2014). The shunt drains the excess fluid from the brain into the peritoneal cavity and needs lifelong monitoring (Adzick et al, 2013).

The main argument for in-utero repair of myelomeningocele can be made as follows. The "two-hit hypothesis" (Joyeux et al., 2018) states that a good deal of the damage caused by myelomeningocele is not due to a failure in neurulation. Rather, the development of neurological damage is progressive; that is to say, exposure of the neural tissue to the amniotic fluid in the intrauterine environment, as well as other mechanical damage, serves to exacerbate the issue and is responsible for much of the loss of function (Fichter et al., 2008). As a result of exposure to the toxicity of the amniotic fluid, the exposed spinal cord may hemorrhage and neural connections may be interrupted, leading to neural death (Copp et al., 2015). This hypothesis is supported by observations such as in cases where spontaneous leg movement was observed early on during a pregnancy, and the same leg was seen to be paralyzed or deformed later on (Grivell et al., 2014). In-utero surgical closure of the lesion, while unable to completely repair the condition, goes a long way toward preventing further damage and worsening an already unfortunate situation.

MOMS Trial

In 1997, the first in-utero myelomeningocele repair by uterine hysterotomy was performed; by 2003, more than 200 fetuses had undergone the surgery (Adzick et al., 2013). However, its efficacy was not yet proven. In 2003, the MOMS Trial, or Management of Myelomeningocele Study, was started; this was a randomized controlled trial aimed at investigating and comparing the outcomes of prenatal vs postnatal repair of myelomeningocele (Kabagambe et al., 2017).

The trial was conducted at three maternal-fetal surgery centers in the United States and went on for seven years (Grivell et al., 2014). The standardized procedure across all three maternal-fetal centers included a maternal laparotomy and stapled hysterotomy; the neurosurgical repair of the lesion was performed as it would have been postnatally (Sacco et al., 2019).

The trial was evaluated for two main outcomes, at 12 and 30 months of age. At 12 months, patients underwent

radiography and magnetic resonance imaging to determine the current state of the lesion. The outcome was based firstly on the patients surviving past birth and infancy, as well as the need for a shunt. At 30 months of age the babies were evaluated once more and given scores of infant development, specifically with regard to motor and mental development, while adjusting for the anatomical level of the lesion (Adzick et al., 2013).

The overall results were arguably and overwhelmingly in favor of the prenatal procedure. In-utero repair of the lesion reduced the need for a ventriculoperitoneal shunt by almost half and drastically improved the rate of hindbrain herniation (Sacco et al., 2019). The patients who underwent in-utero repair also demonstrated substantially better motor skills at 30 months of age—and this was despite the fact that the lesions in the prenatal group were, on average, worse than those in the postnatal group (Copp et al., 2015).

The MOMS Trial was the first of its kind, but it paved the way for other similar non-randomized studies in the years to come, many of which reported similar short-term outcomes. In addition, it was found that in cases where the procedure was performed at an earlier gestational age, the risk of chorioamniotic membrane separation, premature rupture of membranes, and premature birth increased. Therefore, it is now recommended that the procedure should not be performed before 23 weeks of gestation (Sacco et al., 2019).

New Research

In-utero closure of myelomeningocele goes a long way towards reducing spina bifida related challenges, but what it cannot do is reverse the neurological damage that has already been done. A promising course of treatment may lie in the field of stem cell therapy, which when used in conjunction with the standard course of treatment aims to improve neurological function by facilitating spinal cord regeneration and even seeking to prevent the damage in the first place (Biancotti et al., 2020.)

Type 1 stem cell therapies, which are capable of replacing damaged tissue, seem not to be the answer in the case of prenatal spina bifida repair; the ultimate goal here is to prevent the tissue from becoming damaged in the first place, and while regeneration of damaged tissue is certainly useful, it is only helpful after the fact.

Type 2 therapies, on the other hand, create an environment of protection and can minimize damage to the developing spinal cord. These therapies show more promise for in-utero treatment of spina bifida in the long run, because they seek to prevent the damage from ever happening at all (Long, C; Lankford, L; Wang, A., 2019).

Experimental results, specifically with rodent and ovine models, have already shown potential for great change. One study experimented with five different types of stem cells: human embryonic stem cells, neural stem cells, induced pluripotent stem cells, human amniotic fluid stem cells, and mesenchymal stem cells. The results indicated that mesenchymal stem cells were the best candidate for myelomeningocele repair because they were easy to obtain in large amounts, they could enable recovery from spinal cord injuries, and they demonstrated the biggest improvement in motor function (Dugas et al., 2020). Additionally, bone marrow-derived mesenchymal stem cells have the ability to specifically differentiate into the various types of defective tissue, and they were found to induce skin repair in fetuses, reducing the skin lesion area by almost 30%. Transamniotic fluid injection was found to be the most effective method of delivering stem cells (Wei et al., 2020).

However, the use of animal models has its shortcomings: the size of fetal rats and other small rodents presents difficulties in reliably and accurately performing in-utero repair. There is a risk of postoperative death, and often they do not survive long enough postnatally to examine the results properly. Larger animals have the added concern of being too expensive to work with regularly (Biancotti et al., 2020).

As of now, stem cell therapies for NTD's, specifically for myelomeningocele, are still in experimental stages; there have not been any human test subjects yet. However, promise for great change seems to lie in this relatively new field.

Maternal Mortality/Risks

As with any surgical procedure, in-utero repair of myelomeningocele carries inherent risks for both the mother and the fetus simply by virtue of opening the mother's abdomen and uterus mid-pregnancy (Grivell et al., 2014). Possible obstetrical complications associated with the procedure include placental abruption, oligohydramnios, and chorioamniotic membrane separation, as well as the risk of premature rupture of membranes and preterm delivery (Kabagambe et al., 2017). The risk of pulmonary edema and the need for a blood transfusion at birth was slightly higher in the prenatal group as well (Sacco et al., 2019). The average delivery in the prenatal group was 34 weeks while the average delivery in the postnatal group was at 37 weeks.

Additionally, 25% of the women in the MOMS trial experienced thinning and partial or complete tissue edge separation at the hysterotomy site, though none experienced a complete hysterotomy rupture. There were no deaths, but the side effects show that the procedure is not without its risks (Copp et al, 2015).

Because of this, it was necessary for the MOMS trial to

implement strict guidelines regarding maternal health prior to the procedure. Among the requirements for participation were a singleton pregnancy, a gestational age of 19-26 weeks, and myelomeningocele of particular severity as well as evidence of hindbrain herniation. Mothers had to be at least eighteen years of age and United States citizens, as well as have no contraindications for surgery (Adzick et al., 2013). Over the years, the MOMS trial criteria became standard, and most maternal-fetal research centers offering the procedure still use these criteria to determine eligibility for surgery (though some centers will not consider a body mass index of over 40 or carefully managed diabetes to be contraindications for the procedure) (Sacco et al., 2019).

Prevention

Both genetic and non-genetic causes play a role when it comes to spina bifida, or neural tube defects in general. In most instances, the cases are sporadic and do not occur in conjunction with any other syndrome, though in an approximate 10-20% of the cases the NTD is associated with a chromosomal abnormality such as trisomy 13 or 18 (Copp et al., 2015).

Maternal obesity and diabetes as well as certain anticonvulsant drugs have been shown to contribute to NTD's (Fichter et al., 2005), but the role of folic acid has been the most widely investigated (Sacco et al., 2019). Folate deficiency among women of childbearing age is currently the best-known non-genetic cause of neural tube defects (Copp et al., 2015). Therefore, the World Health Organization currently recommends that women take a supplement of 400 µg of folic acid daily from before they conceive until 12 weeks gestation (Sacco et al., 2019); however, many women do not keep to these guidelines, which has led some countries to implement mandatory folate fortification of certain basic food staples. In 2017, for instance, 59 countries mandated fortification of wheat and maize flour with folate, and the incidence of spina bifida that year was significantly reduced Sacco et al., 2019).

According to Oakley, every country should mandate fortification of food staples such as rice and flour with folate to decrease to frequency of spina bifida; inaction, he feels, is unethical (2020). This approach has validity, but it may not be practical on a global level. Additionally, ethical concerns cannot be determined by one man.

The long-term solution is unclear, but it is evident that proper folate intake goes a long way towards preventing neural tube defects.

Discussion and Conclusion

In-utero repair of myelomeningocele is still a relatively new procedure, as it has only been developed within the past twenty years. Though it has been extensively studied, researched, and documented, and though new advances in the field are constantly emerging, there is still room for extensive studies and research to further improve the process.

Nevertheless, based on the evidence from the studies that have already been performed, it appears that because of the reduced need for shunting, potential for reversal of hindbrain herniation, and vastly improved neurologic function, in-utero repair is the most effective method of treatment for myelomeningocele. True, there are inherent risks. However, as demonstrated by numerous studies, both the maternal and fetal risks are relatively low (Sacco et al., 2019). Additionally, the procedure is only performed in some of the safest settings possible in order to minimize risk.

The benefits of the procedure are evident and undeniably advantageous. It is not yet standard medical care by any means; however, with continued research, particularly with regard to stem cell therapies which seek to restore function in addition to halting neurodegeneration, in-utero repair may and possibly should become the new normal for fetuses afflicted with myelomeningocele.

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Osteoporosis: A Comprehensive Review

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Abstract

Osteoporosis is a disease of the skeleton that becomes more common with advanced age, especially in postmenopausal women. Osteoporosis increases the risk of fractures, thereby reducing the quality of life for those who suffer from it. Due to the aging population, direct costs resulting from osteoporosis are projected to reach upward of $25 billion per year by 2025. The main pharmaceuticals primarily target osteoclasts. Exercise may be an effective method of preventing osteoporosis, although more research needs to be done. More research should be conducted to explore potential ways to enhance osteoblastic activity as a method to treat and/or reverse osteoporosis. This review compares the pros and cons of major methods to treat osteoporosis.

Introduction:

Osteoporosis is a disease of the skeleton that becomes more common with advanced age, especially in postmenopausal women. The CDC reported based on the data from the NHANES (National Health and Nutrition Examination Survey) that 10.2 million adults had osteoporosis and 43.4 million had low bone mass, as of 2010 (Looker, 2015). Osteoporosis leads to an increased risk of fractures, reducing quality of life for those who suffer from it. Due to the aging population, direct costs resulting from osteoporosis are projected to swell upward of $25 billion by the year 2025 (Dempster, 2011). The goal of this review is to present the causes of osteoporosis, explain the current treatments, and weigh the pros and cons of the various therapeutics. Can osteoporosis be prevented, treated, or perhaps even reversed?

Bones are not inanimate objects that the body produces, rather bone is living tissue that continually undergoes a process called remodeling, i.e. the continuous degradation and rebuilding of the bone tissue.

Bones are composed of cells connected through a large extracellular matrix, which is comprised of 15 percent water, 20 percent collagen fibers, and 55 percent mineralized salts. The main salt is calcium phosphate, which combines with calcium hydroxide to form crystals of hydroxyapatite. These crystals continue to combine with other mineral salts to form a hardened tissue. This process, referred to as calcification, is initiated by cells called osteoblasts. Mineral salts crystallize in between and then around collagen fibers. The mixture of stiff crystalized minerals and flexible collagen provides bones with both strength and flexibility. Bone tissue is often compared to reinforced concrete. Collagen is analogous to flexible metal rods that provide support for the concrete-like mineral component (Totora & Derrickson, 2014).

There are many reasons for remodeling. Bones can buffer the amount of calcium in the blood by building more bone mass to use up excess calcium or degrade existing bone to release calcium when needed. The proper concentration of calcium must be maintained in the body, as too much calcium can cause a heart attack while too little can cause breathing to stop. There are two hormones regulating this process. PTH promotes the degradation of bone, releasing calcium, while Calcitonin promotes the deposition of bone, storing calcium.

Other factors that may affect remodeling and the rate of bone deposition include the availability of minerals that make up the bone, especially calcium and phosphorus. Vitamins, particularly Vitamin A which stimulate osteoblasts (the cells that build new bone), and Vitamin C, used in collagen production, are needed as well. Thyroid hormones (T3 and T4) from the thyroid gland promote bone growth by stimulating osteoblasts. In addition, the hormone insulin from the pancreas promotes bone growth by increasing the synthesis of bone proteins (Totora & Derrickson, 2014).

Sex hormones, including estrogen and testosterone, also affect bone growth. They are responsible for increased osteoblast activity, which is why post puberty, many teenagers experience growth spurts. As the level of sex hormones diminishes during middle age, especially estrogen in women after menopause, a decrease in bone mass occurs because bone resorption by osteoclasts outpaces bone deposition by osteoblasts. Estrogen can contribute to bone growth by promoting the death of osteoclasts. In addition, women who have smaller bones with less mass than those of men run a high risk of developing osteoporosis (Totora & Derrickson, 2014).

Bone remodeling happens in two stages. First, old bone tissue is broken down and reabsorbed into the blood via cells called osteoclasts. Then, bone deposition occurs, whereby osteoblasts deposit collagen fibers and minerals. Aside from calcium concentrations, remodeling may also be triggered by factors such as exercise, sedentary lifestyle, and changes in diet. Remodeling helps to fix injured bone and strengthen areas of bone subject to high stress. Newer bone is also more fracture-resistant than older bone (Totora & Derrickson, 2014). If the rate of degradation is higher than the rate of deposition, loss of bone mass will occur and result in osteoporosis.

Methods

The Touro library's database and Google were used to find peer-reviewed articles and papers. Search terms used included "prevention of osteoporosis", "treatment of osteoporosis", "adverse effects of osteoporosis treatments", etc. The Principles of Anatomy and Physiology 14th edition was used as well.

Discussion

Prevention

Although there are several pharmaceuticals that treat osteoporosis, treatments regimens are often poorly followed. A study of 178 patients on a course of treatment for osteoporosis found that 23% of patients did not stick to the prescribed treatment and dropped out. The study reported a number of reasons for noncompliance, ranging from expense, inconvenience of use, and fear of side effects (Segal, Tamir, & al, 2003). A large review of 24 studies on osteoporosis treatments found that "One-third to half of patients do not take their medication as directed. Nonadherence occurs shortly after treatment initiation" (Kothawala, Badamgarav, & al, 2007). As mentioned, bone is living tissue which respond to stress by strengthening itself. Therefore, applying stress through weight-bearing exercises could help to stimulate bone strengthening. A study showed that postmenopausal women who underwent back-muscle training for two years had a higher bone density than that of a control group. Interestingly, the effects were not immediately apparent, and were only evident when measured 8 years after the exercise regimen stopped. Apparently, exercise has long term, but not immediate effects (M. Sinaki, 2002). Aside from increased bone health, the impact of stronger muscles results in enhanced balance, which contributes to fewer falls and fractures. Conversely, an experiment was conducted to determine bone loss due to lack of physical stress. Ninety healthy men were placed on bed rest for 36 weeks and urine calcium concentration was measured to determine bone loss. The study found that urine calcium concentrations became elevated to 100 mg a day, showing demineralization of bone. This elevated excretion of calcium in urine continued for 36 weeks (Schneider, 1984).

How do bones react to physical stress? Bones react to physical stress through biochemical reaction resulting from mechanical stimulus. Bones contain cells called osteocytes, osteoblasts that have matured and reside within bone. Osteocytes are positioned in a way that the deformation of bone tissue is amplified by 20-100 times on its cell membrane. The deformation on the cell membrane signals that the bone is undergoing stress. This is thought to trigger a host of processes within the cell, resulting in osteogenesis. The process by which osteocytes signal is extremely complex and still not fully understood (Gusmão & Belangero, 2015). One way osteocytes can signal is through a chemical known as sclerostin, which promotes bone degradation and is coded for by the gene SOST. Mice with SOST gene deletion and humans with mutations on this gene have higher bone density. Mechanical loading has been reported to reduce sclerostin expression as well. New research is being conducted for an antibody against sclerostin to treat osteoporosis (Bonewald, 2011).

Treatments

The first line of treatment for osteoporosis is currently Bisphosphonates (BP), which disrupts osteoclastic activity. Because osteoclast are the cells responsible for bone degradation, many treatments seek to inhibit osteoclast activity. Osteoclasts degrade bone by releasing hydrogen ions, thereby creating a acidic environment. They use a ruffled border that attaches to the bone's surface. The ruffled border has crevices created by its protrusions which act as containers for the acid secreted by the osteoclast. The acid remains in these crevices, which form small pockets known as sub-osteoclastic compartments when sealing onto bone. The acidic environment causes the mineral component of bone to become more soluble, allowing bone's minerals to be absorbed by the osteoclasts.

BP has a strong affinity for calcium ions, which are found in bone, due to the presence of two phosphate groups, this results in the rapid localization of BP to bone material. Experiments using radio labeled BP has shown that BP are taken up and adsorbed in to bone primarily (Xiao-Long Xu, 2013).

When osteoclasts attach to bone that contains BP the acidic environment protonates the BP. Protonated BP has a lowered affinity for calcium ions, allowing for the release of BP into the sub osteoclastic compartment where BP is taken up by the osteoclast.

BP disrupts cell functions in the osteoclast, BP have a similar chemical structure to that of pyrophosphate. Pyrophosphate is involved in many cell processes in the osteoclast. Due to its similarity to pyrophosphate, BP is likely to interfere with any of the processes that involve pyrophosphate. It is thought that the BP inhibits prenylation of protein to the cell membrane, the lack of these proteins at the membrane results in loss of the ruffled border and prevents the osteoclast from being able to degrade the bone. This is shown as bisphosphonate-treated osteoclasts lack a ruffled border (Russell & Rogers, 1999).

Side Effects of BP

Doctors I have spoken with report that patients reported gastrointestinal (GI) discomfort while taking oral BP, and an NCBI continuing education paper for doctors states " All oral bisphosphonates have correlations with upper gastrointestinal adverse effects, including gastrointestinal reflux, esophagitis, esophageal/gastric ulcers, and

gastritis. Gastrointestinal side effects are the most common reason for discontinuation of oral bisphosphonates." The article recommends avoiding BP in patient that are at a higher risk of gastrointestinal distress (Ganesan K, 2021). A study conducted to determine the compliance of patient to osteoporosis treatment found that the major reason reported by patients for discontinuation of alendronate (a BP) was indeed GI side effects. Counter to this, a study conducted to determine if there is any correlation between alendronate (a BP) use and GI problems found no correlation of BP use and GI issues. The experiment was a randomized, double-blind, placebo-controlled trial with a mean follow-up of 3.8 years. Women were initially randomized to receive alendronate sodium, 5 mg/d, or placebo. After 2 years, the alendronate sodium dose was increased to 10 mg/d. The study did not find any significant correlation between BP use and GI problems. "The overall incidence of upper GI tract events was similar in the alendronate and placebo groups". The study goes on to suggest that GI side effects reported may be due to the higher age of osteoporotic patients (Bauer DC, 2000). The study that found that noncompliance in BP-taking patients also tracked patient adherence to Raloxifene (a different class of treatment known as a SERM), none of the Raloxifene-taking patients attributed the reason of their discontinuation of treatment due to gastrointestinal issues (Segal, Tamir, & al, 2003). This would call into question the suggestion that the gastrointestinal affects attributed to BP are really age related and not resultant of BP. Both groups were of the same population yet only the BP-taking group reported gastrointestinal issues. The study cited as well as other studies I came across that showed no correlation with oral BP use and upper GI issues were sponsored by pharmaceutical companies that produce oral BPs. These conflicting reports of gastrointestinal distress due raise eyebrows as to the potential biases in studies. Patients given intravenous BP do not report GI issues and the intravenous BPs need to be administered far less often. (Papapetrou, 2009) Both reasons make it more likely that a patient will maintain their intravenous treatment over an oral one and seem to make intravenous BP optimal.

Another method of treatment for osteoporosis is monoclonal antibodies. Osteoclasts originate from macrophages. The macrophage precursor cells have a receptor called RANK which binds to RANK ligand (RANKL) to differentiate into osteoclastic cells. Antibodies can bind to a ligand to prevent it from binding to its receptor. The antibodies bind to RANKL inhibiting their ability to bind with the RANK receptor on the macrophage precursor cells. As a result of the rate at which osteoclasts differentiate is decreases resulting in less osteoclastic cells that break up bone. (D. A. Hanley, 2012)

Antibody treatment, which circulate in the blood, can reach all skeletal sites. However, unlike BP which bind to bones and can have affects after cessation of treatment, antibodies lose their affect soon after cessation of treatment. Adverse events are rarely associated with denosumab. (Harshika Awasthi, 2018)

Calcium concentration is regulated by hormones PTH, and calcitonin. Calcitonin is produced in the thyroid gland and causes lower serum calcium concentration by acting on the renal tubules, causing them to excrete more calcium, and on osteoclasts, causing them to contract (temporarily), reducing their motility and ability to resorb bone. It also causes inhibition of carbonic anhydrase II, which disrupts the acidic environment that is optimal for osteoclast activity. Calcitonin also prevents osteoclast precursors from differentiating into their mature form. The ultimobranchial gland of salmon produces calcitonin with a different makeup of amino acids. Salmon calcitonin is a 32 amino acid, alpha-helical polypeptide that differs significantly from human calcitonin along amino acids 10-27. Salmon calcitonin is more potent then endogenous calcitonin due its difference in amino acids (Felsenfeld AJ, 2015)

Adverse Side Effects of Calcitonin

Adverse effects of calcitonin can include hypocalcemia, a dangerous condition. Since the calcitonin used to treat osteoporosis is sourced from salmon, patients who are allergic to fish can have an allergic reaction. Ten percent of patients taking calcitonin experience mild nausea that subsides as therapy continues. A meta-analysis of 21 randomized, controlled clinical trials with calcitonin-salmon (nasal spray and investigational oral forms) suggests an increased risk of malignancies in calcitonin-salmon-treated patients (4.1%) compared to placebo-treated patients (2.9%). A definitive causal relationship between the calcitonin-salmon use and malignancies cannot be established from this meta-analysis, the benefits for the individual patient should be carefully evaluated against all possible risks. (F. Cosman, 2014)

Further studies point to the questionable efficacy of calcitonin overall and show a definitive lack of efficacy in nonvertebrate fractures. This contrasts with both bisphosphonates and denosumab which both demonstrated a lowered fracture risk in vertebral, hip, and other nonvertebral fractures. (Overman RA, 2013)

Calcitonin has been shown to reduce fracture pain. The exact mechanism for its analgesic effects, is not known. There is a hypothesis that calcitonin may act on the central nervous system, and it has been used with some

success in patients with migraine pain, phantom limb pain, malignancy, Paget's disease, and other pathologies. It, however, has not been compared directly to NSAIDs in terms of effectiveness of pain relief. Regardless, Calcitonin may be helpful for pain in patients that cannot tolerate NSAIDS. (Linsey A Blau, 2016)

Post-menopausal women have low levels of estrogen, an essential hormone for bone remodeling. Osteoporosis is attributed to the diminished estrogen levels of post-menopausal women. Estrogen can inhibit osteoclasts from forming, cause osteoclastic apoptosis, as well as increase osteoblasts by inhibiting osteoblastic apoptosis (Sundeep Khosla, 2012) . An obvious therapeutic approach would be to provide hormone replacement therapy. However, hormone therapy is found to increase the risk of breast cancer (Beral, 2003) , and is therefore not widely used . Raloxifene, a drug that is an estrogen antagonist and agonist is promising drug, in bone, it behaves as an estrogen antagonist, increasing bone density, in reproductive and breast tissue it acts as an estrogen agonist. Thus, raloxifene both increases bone density and reduces risk of cancer. Raloxifene has not been shown to reduce non-vertebrate fractions and more research is necessary to determine its efficacy in non-vertebrate fractures. A meta-analysis found that "In comparison to other osteoporosis therapies, raloxifene has a lesser impact on bone mineral density, a similar effect on the occurrence of vertebral fractures, but no effect on the frequency of non-vertebral fractures. Raloxifene can be recommended for the prevention of vertebral fractures in women with osteopenia/osteoporosis who are not at high risk of non-vertebral fractures and who do not have a past history of venous thromboembolism" (Ann Cranney, 2005)

Conclusion

The most effective current method of treatment are bisphosphonates, which accumulate in bone and inhibit osteoclasts from functioning. However, many patients suffer gastrointestinal pain as a side effect. For those patients an antibody (denosumab) that prevent RANKL from signaling the osteoclastic precursor cells to mature, has also proven to be relatively effective. Other methods of treatment such as calcitonin and raloxifene while in theory look promising, proved to not be very effective in clinical trials. Calcitonin, while not necessarily very effective at reducing bone loss, may still play an important role in treating pain, especially in patients where NSAIDS aren't well tolerated. More research is needed to determine how effective exercise can be in the prevention of osteoporosis and what specific exercises, if any, would be most effective. Regardless, patients should be advised to exercise, as there is some evidence that links mechanical stress on bones to lasting improved bone mass., and stronger muscles can improve balance to further help reduce the risk of falls and fractures. Further research should be conducted to determine how it might be possible to enhance bone-building osteoblasts.

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Understanding Pathophysiology of Nonsyndromic Autism by Examining and Extrapolating from Syndromic Variants

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Abstract

Autism spectrum disorder (ASD) is a broad, heterogeneous neurodevelopmental disorder encompassing a range of presentation and severity. The common characteristics include communication deficits, impaired social skills, dependency on routine, sensitivity to environmental change, and stereotyped behavior (DSM-5, 2013). When ASD is accompanied by a host of other symptoms it is often referred to as syndromic autism. Syndromic autism is usually severe and can usually be traced to deletions or duplications on a specific gene. These monogenic disorders are by definition easier to diagnose and are good candidates for study since specific biological markers can be assessed and tracked. There have been many discoveries about monogenic autism which help provide insight into the mechanisms of disease. Nonsyndromic autism (also called idiopathic autism) is defined by the absence of additional symptoms or underlying syndromes. Although nonsyndromic autism is far more common and often manifests in a milder form of the disorder, the genes and proteins involved are much more difficult to nail down, and therapeutics are harder to discover. Family and twin studies have provided evidence that the majority of cases are due to common genetic variation (Gaugler et al., 2014).

Shifting the focus to syndromic autism has been a critical step in understanding the disease process that underlies the different manifestations of ASD. This is the key to solving the complex web of common genetic variants implicated in nonsyndromic autism.

Keywords: Autism, ASD, monogenic, syndromic, common variants, loss of function.

Introduction

Autism Spectrum Disorder (ASD) has been historically classified into two basic categories, syndromic and nonsyndromic. In nonsyndromic autism, communication and social impairment, accompanied by stereotyped behaviors represent the main symptoms observed. Syndromic ASDs are disorders wherein the autistic presentation is just one part of a broader neurological syndrome. In Phelan-McDermid Syndrome, for example, which is caused by mutations in the SHANK3 gene, the autistic phenotype is accompanied by developmental delay, intellectual disabilities, seizures, and hypotonia (Phelan et al., 1992). Another example is tuberous sclerosis complex, caused by mutations in either TSC1 or TSC2 (European Chromosome 16 Tuberous Sclerosis Consortium, 1993), wherein ASD is featured along with many other developmental and physical symptoms (Slegtenhorst et al., 1997). Many other chromosomal deletion and duplication syndromes include ASD as a feature of the disease, including Smith Magenis syndrome, Wolf-Hirschhorn syndrome, and Rett syndrome (Laje et al., 2010, Fisch et al., 2008, Hu et al., 2018).

Autism was first described in 1943 (Kanner, 1943). Even then it was assumed to be caused by an inborn (i.e genetic) disturbance. Great scientific and technological strides have been made since then and we are just beginning to uncover the molecular pathways involved. At this point, most ASD diagnoses are still made based on behavior (Lord et al., 2012), and most available therapies target symptoms and not underlying pathophysiology.

The extreme range of ASD manifestation adds a level of complexity when trying to understand its biology. Its very name indicates that there is a continuum of clinical phenotypes that make up what would seem more appropriate to call a category of neurodevelopmental disorders that are similar in symptomatology. On a genetic level, autism becomes even more complicated, as hundreds of diverse genes are found to have an association with autism susceptibility (Berg & Geshwind, 2012).

Dramatic advances over the last few years have led to an unraveling of the mystery that is ASD. The complete picture is not yet clear, but we are beginning to see coherence and cohesion within the wide range of variants and presentations and this has led to a reevaluation of the way we currently classify ASD. The newer classification will likely stratify ASDs by which biological pathway is affected. This will enable a more targeted approach to therapy and treatment, which will focus on the restoration of underlying biological issues and not just symptom control.

Methods

To gain a better perspective on the current direction of autism study, the available literature on Touro Online Library was searched using the search term "monogenic autism". Many review articles from the last 10 years were read and their information was synthesized to form a coherent overview of the latest thinking on ASD. The focus has been to highlight the differences between syndromic and nonsyndromic autism, as well as the areas in which they are beginning to converge.

Autism Etiology

The causes of autism were presumed to be genetic since it was first described (Kanner, 1943). Although there has been lots of speculation about environmental causes of autism, most of this has been thoroughly debunked (e.g. Wakefield's vaccine hypothesis). Environmental causes for ASD have been implicated only in a small percentage of cases (Newschaffer et al., 2007).

Genetic factors have been demonstrated to cause autism in many of the monogenic syndromes in which autism is commonly featured, such as Rett, ADNP, Phelan-McDermid,

and Fragile X. Additionally, epidemiological evidence from family and twin studies make it clear that genetics plays a large role in etiology. Nearly 20% of babies with an affected older sibling will develop ASD (Ozonoff et al., 2011). Twin studies show that monozygotic twins share a higher concordance rate of diagnosis for autism as compared with dizygotic twins (Hallmayer et al., 2011).

Although the case for genetic etiology of ASD is quite convincing, it has been very difficult to identify which genes account for the majority of incidence. One of the major problems with studying ASD is that its heterogeneity creates small sample sizes within each manifestation of autism along the spectrum. Much larger sample sizes are needed to locate the responsible genetic variants.

Autism Sequencing Consortium

One of the main advances in autism over the last decade has come about due to the cooperation of many different autism research centers under one umbrella group called the Autism Sequencing Consortium (ASC). The goal of ASC is to increase the power of genetic analysis by pooling resources and samples from research centers around the world. A large sample size is obtained through the consortium, allowing for meta-analysis of tens of thousands of samples. This increases the power of genome-wide association studies (GWAS) and more can be extrapolated from the data.

By February 2020, the ASC had identified 102 genes associated with ASD, of which, 31 are novel risk genes never previously identified. These genes are quite diverse and run the gamut of different cell functions. However, further analysis showed lots of connections between the biological pathways mediated by these genes (Satterstrom et al., 2020).

Understanding the Genetics of Autism

When thinking about genetic mutations there are two key classifications. The first is the frequency of the allele, namely if it is common, low frequency, or rare. The second is the potency of its effect; whether it is penetrant or if the effect is modest. Generally, the more penetrant a mutation is for a syndrome like ASD, the less frequent it is due to natural selection. Namely, someone carrying such a gene is far less likely to reproduce and thus the allele will become less common. Additionally, a variant that is rare and has a mild ASD causing effect is not part of the discussion since the sample size would have to be enormous to pick up such a rare and subtle ASD contributor.

Thus the current autism discourse is focused on the linear progression of genetic variation; there are the most common variants, which tend to have smaller effect sizes, while the rarer are highly penetrant and the likelihood of an autism diagnosis is almost certain (Manolio et al., 2009, Figure 1). Of the 102 ASD risk genes discovered by the ASC, it is clear that some have a larger impact than others. For example, there is an 80% chance of ASD in those with a SHANK3 mutation (Soorya et al., 2013), the gene deletion that causes Phelan-McDermid syndrome (Nesslinger et al., 1994). Meanwhile, there are many gene variants among the 102 genes which are prevalent in the population and this is because they only marginally predispose a person to ASD. Each of these common genetic variants individually only represents a small effect size in producing an autistic phenotype. Enough of these variations en masse seems to push the patient over a figurative threshold. Thus these common variants combine to increase the possibility of ASD (Gaugler et al., 2014).

The Focus of Autism Research

The challenge upon identifying these mutations is in mapping the variation to specific biological changes and understanding their outcome. This step is crucial in creating a model for that particular mutation. Once a model is made, the mechanics in which that gene is involved can be analyzed, which will help identify the pathogenic pathways. Eventually, experiments can be designed to try to counteract the effects created, leading to a potential treatment.

It is extremely difficult to measure the biological changes they create with common genetic variants since the effect size is often infinitesimal ((Manolio et al., 2009). Consequently, the path toward understanding and discovering treatments for idiopathic autism runs through monogenic syndromic autism (Ziats, et al., 2021). These rarer variants have major effects and are highly penetrant. Figuring out the biological conditions created by these variations is straightforward. Therefore, the potential for designing biological models–both cell and animal–is very high.

Thus, the current thinking on autism research is a move to personalized medicine. Narrowing the focus to one or several of the monogenic ASDs allow researchers to create models, discover phenotypes, and establish baseline biomarkers, all to discover treatments that can work for that particular autistic presentation. In doing so, not only can this provide relief for patients who struggle with some of the most severe forms of autism, but the hope is that these studies can help uncover the mystery of idiopathic autism, as well.

The discovery of a new drug for one small subset of ASD can help further the science in autism more broadly. Firstly, the new drug can be tested on the wider autistic population in the hope that its efficacy might not be limited to just that subset. Secondly, drug discovery

often proves or provides clarity to a particular theory of disease. For example, if we find a compound that helps to normalize biomarkers in a certain disease phenotype, this can lead to the confirmation of a previous disease hypothesis or the formation of a new one. All of these findings may offer clues for pathophysiological pathways in other disorders on the autism spectrum in addition to the one being studied.

Modeling Autism Spectrum Disorder Pathophysiology

Despite all the progress made in identifying genes and fashioning animal models, there is a lot more to uncover in ASD. We can potentially create many different models for all of the different genetic mutations but it may not be necessary. After all, the various mutations all lead to similar symptomatology, namely, the autistic phenotype. This seems to imply convergence of biological pathways at some point to create a common result (Geschwind, 2008). This is another angle that autism researchers are probing.

Systems biology is put to excellent use in connecting the various genes that have been associated with risk for autism. It has been repeatedly demonstrated that the autism implicated genes are part of networks that involve gene expression and synaptic modeling (Voineagu, et al., 2011, Gilman, et al., 2011). Different cutting edge techniques were used in these studies that all show how these genes are grouped together in performing neurodevelopmental work, particularly, in the prenatal and neonatal period. This also demonstrates a relationship among ASD risk genes that is statistically significant as well as gives evidence of their function (Chen et al, 2015).

Finally, as the power of genome-wide association studies increases, the more we can connect common genetic variants to the rare ones. From a molecular perspective, polygenic autism is more similar to monogenic autism than previously thought. This is because risk genes for syndromic and nonsyndromic converge in several different cellular pathways (De Rubeis et al., 2014). Mastering these pathways will help us to classify and treat autistic disorders in groups with the same affected pathway.

Drug Discovery for Monogenic Autism

The next step would be to create human neurons derived from patient-induced pluripotent stem cells and develop tests to measure their protein expression and function. All of these studies brought into a systems biology framework will help piece together multiple levels of anatomical, physiological, and genomic data. This will enhance the ability to test the functional, spatial, and temporal convergence of ASD genes (Dolmetsch & Geschwind, 2011).

The potential for the discovery of an effective treatment for monogenic autism is high. The reason for this is because many of the mutations discovered through GWAS are loss of function (LoF) de novo (arising in the germ cell of a parent) mutations (De Rubeis et al., 2014). The fact that autism is a result of a heterozygous LoF allele is an optimistic finding. It implies that the problem can be corrected if we can find a way to boost the remaining (i.e. functioning) allele. If it is overexpressed sufficiently, it may fully compensate for the mutated allele.

Drug Discovery is a multi-step process and takes years to go from promising compounds to clinical trials. It starts with isolating peripheral blood mononuclear cells (PBMCs) derived from syndromic ASD patients and their unaffected siblings or parents. These PBMCs are given reagents that regulate gene expression and dedifferentiate them back to pluripotency. In this state, they are called IPSCs (induced pluripotent stem cells). Then, using a retrovirus, the IPSCs are infected with a plasmid that contains certain genes which induce neuron differentiation (Yang et al., 2011).

After the induced neuronal (iN) cells are created, the goal is to find a method of distinguishing the neurons obtained from the proband vs the immediate relative which can be employed in a high-throughput assay. That way, a normal and abnormal phenotype can be defined in terms of this disorder. One potential test is to see if conductance can be altered between the two phenotypes given certain conditions. This can lead to many hundreds or even thousands of prospective drugs being tested simultaneously to see which has the potential to restore the iN cells to their normal phenotype.

If compounds that restore patient-derived iN cells to a healthier phenotype are identified, they can be studied further, used on animal models, and ultimately, a clinical trial can be conducted on actual patients. Using these steps has the potential to identify treatments that are aimed not merely toward symptom relief but the treatment of underlying biology. Additionally, the drug can be tried on other ASD variants and may shed light on the broader ASD pathophysiology.

Conclusion: Rethinking Autism Classification

Over the last decade, autism research has grown in leaps and bounds. Its heterogeneity and wide variations in phenotype make it difficult to determine etiology. However, through the use of an array of new technologies which can map the genome down to the base pair, much information has come out regarding particular genes that contribute to autism predisposition.

The wide variation in ASD phenotypes and the different genes and pathways would seem to fly in the face

of a biological convergence, yet we have encountered evidence from many studies that indicate how ASD etiologies can be organized by the intermediate pathway in which it plays a part. Perhaps as more research is done and treatments are discovered, it will become clear that ASD is a very particular manifestation that can result from many different genes that share common pathways. There is a great deal of evidence that shows convergence on intermediate biological levels which corroborate a "many genes, common pathways" hypothesis (Geschwind, 2008, Chen et al., 2015).

Therefore, a shift of focus toward the rare variants that cause syndromic autism has begun to garner results. This is because these variants represent low-hanging fruit in that they are really easy to detect and study. Normal and knockout phenotypes can be described, created in models, studied, and tested. This avenue of research can also benefit nonsyndromic autism research by helping define certain parameters and mechanisms of autism pathology.

While the historical classification between syndromic and nonsyndromic autism has been useful, it seems likely that this classification scheme will come to an end. As we start to see some convergence between syndromic and idiopathic autism on the level of molecular pathways, the various disorders will start being categorized–and treated–based on their individual pathogenic routes.

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Etiology and Associations of Oral and Oropharyngeal Cancer

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Abstract

This research paper discusses oral cavity cancer, which is one of the 11th most common cancers in the world. According to newer research, some important possible causes include smokeless tobacco, marijuana, and citrus fruits. Some research attributes it to one's genetic makeup and include exposure to Human Papillomavirus (HPV) and diagnosis of Diabetes mellitus, all in conjunction with one's age. In this research paper, each of these factors are discussed in depth, and their association with this illness is considered and debated. Review of the literature reveals that the high concentrations of nicotine found in smokeless tobacco products is deleterious to oral health. Citrus fruits have been shown to prevent oral cancer. Multiple studies reviewed implicated HPV-16 and HPV-18 as having a gross etiologic role in many cancers of the oropharynx and the oral cavity. The causes for this cancer have been previously attributed to inaccurate, or unclear factors. This paper works to identify some unfamiliar factors that could quite possibly be important risk factors for this illness. Much of this research is fairly new and requires additional studies to substantiate the causes and help raise awareness, especially in lesser developed countries.

Introduction

Cancers of the oral cavity are the 11th most common cancers in the world. About 145,000 deaths were estimated to have occurred in 2018 across all areas of the globe. Etiology of most cancers that occur in the mouth has been attributed to a complex interplay of genetic and environmental factors, as with almost all other types of cancer. Some of the more well known and preventable causes include smoking and excessive alcohol consumption. However, though the rate of cigar and cigarette smoking have trended downwards throughout the world, the rate of cancer associated with the oral cavity has steadily increased (Warnakulasuriya, 2020). Therefore, it is imperative to explore the many other, less known, risk factors that are associated with oral cancer. In the last ten years much research has been conducted regarding cancers that affect the oral cavity and new, surprising associations have come to light. Many of these associations are environmental factors that can be eliminated including smokeless tobacco, marijuana, and citrus food intake. Other associations are related to one's genetic makeup and include exposure to Human Papillomavirus (HPV) diagnosis of Diabetes mellitus, all in conjunction with one's age (Petersen, 2009).

Before one can explore the many potential causes of oral cancer, it is important to understand the types of soft tissue cancer that can be found in the oral cavity. Ninety percent of all cancers found in the oral cavity are Squamous cell carcinomas (SCC). Squamous cells line most of the oral cavity and provide a smooth and protective layer to the mouth. The other ten percent of cancers that occur in the oral cavity include Verrucous carcinomas, minor salivary gland carcinomas, Lymphomas, and many benign tumors including granular cell tumors, fibromas, and granulomas. Minor salivary gland tumors, as the name suggests, affect the many minor salivary glands that are found in the hard palate (60%), lips (25%) and buccal mucosa (15%). Subtypes of this type of carcinoma include adenoid cystic carcinoma, mucoepidermoid carcinoma and polymorphous low-grade adenocarcinoma. Lymphomas of the oral cavity develop in lymphatic tissue found in the tonsils and the posterior 1/3 of the tongue. Additionally, some of the benign tumors found in the mouth have a tendency to become malignant over time and are often excised as a precaution. The final group of soft tissue cancer of the oral cavity includes mucosal melanomas (Scully and Porter, 2001). These can often be found at the vermilion border or the hard palate and can be locally aggressive.

Dentists and oral pathologists have identified key premalignant lesions that have a strong association with SCC. These lesions include leukoplakias, erythroplakias, oral lichen planus, and oral submucous fibroses. The WHO (2018) further classifies these according to degree of dysplasia; mild, moderate, severe, and carcinoma in situ. Leukoplakias are simply defined as a "white patch or plaque that cannot be characterized clinically or pathologically as any other disease" (Van der Waal, 2015). It is important to note that these lesions or patches are not wipeable. Lesions of this nature that can be wiped away are often fungal related like Candidiasis albicans. About 2-5% of leukoplakias annually go on to become malignant. Dentists and health care providers will often biopsy these lesions and monitor them consistently for changes in color or size. One of the biggest risk factors for malignancy is the location of lesion. Lateral borders of the tongue and the floor of the mouth are known to be the most common areas for malignancy to appear. Erythroplakia is defined as a "bright red velvety patch that cannot be characterized clinically or pathologically as being caused by any other condition" (Van der Waal, 2015). These have a much higher malignant potential than leukoplakias and are mostly associated with severe dysplasia and carcinoma in situ. These will generally be excised entirely and biopsied.

The goal of this paper is to delve into the research surrounding some of the stronger environmental and genetic associations of oral and oropharyngeal cancers. The topics covered will include citrus fruit intake, smokeless tobacco use, human papillomavirus, and inflammatory bowel diseases. Systematic reviews and meta-analysis will

be utilized to understand the possible pathophysiology behind these associations, along with their prevalence, and thoughts on preventative measures.

Citrus Fruits

Oral cancer accounts for over 3% of the overall burden of cancer globally, with an incidence of 350,000 cases in 2013. Patients with oral cancer have a poor prognosis despite advances in treatment as they continue to present with late-stage disease. Indeed, the 5-year survival rate for oral cancer is only 46% for men and 54% for women. In the last 10 years, many studies have provided evidence that a regular intake of citrus fruits is associated in prevention of oral cancer. A meta-analysis discusses results that indicate that there is an inverse association between citrus food intake and prevalence of oral and oral-pharyngeal cancers.

Seventeen studies were reviewed for this meta-analysis. The authors begin the review by listing the confounders each study controlled for. These include age, sex, BMI, smoking status, alcohol use, and education levels. Most studies gathered data from participants using a food frequency questionnaire. Meta-analysis was conducted by grouping participants with an increased risk of oral cancer based on their average citrus fruit intake. Upon statistical analysis of the pooled results, it was determined that participants had a maximum of 50% reduction in risk of oral cancer based on the degree of intake. (Cirmi, et. al., 2018)

The study also goes on to outline possible mechanisms by which citrus fruits can work to combat cancer. Citrus fruits are known to have high levels of Vitamin C. Vitamin C has been proven to combat inflammation and damage to DNA structures, which can initiate cancer. Furthermore, the antioxidant properties of Vitamin C work to kill cancer cells that may be proliferating (Grosso, et. al.,2013). Another hypothesis delves into the bioactive compounds that can be found in citrus fruits. These include flavonoids, carotenoids, and limonoids. Of these three, flavonoids are known to reduce inflammation, risk of infection, and oxidative stress. Oxidative stress and inflammation can lead to oxidative damage which works to initiate and promote cancer (Ravishankar, 2013). As such, both hypotheses conclude that the components of citrus fruits provide an antioxidant environment for the oral cavity and pharynx.

Smokeless Tobacco

Another important area of study in regard to oral cancer is the use of smokeless tobacco. This includes chewing (spit) tobacco, moist snuff and other tobacco containing products that are not smoked. Many wrongfully believe that the health concerns surrounding cigars and cigarettes are caused by the 'smoke' element. Thus, they believe chewing tobacco and snuff are a safer alternative. Nicotiana rustica and Nicotiana tabacum are the two major varieties of tobacco. Nicotine, a volatile alkaloid, is the most essential component obtained from the leaves of this herb. Nicotine is one of the most stimulating and harmful substances on the market. Nicotine affects all organs, and can bind to CNS receptors, and raises brain dopamine levels, rendering it a highly addictive substance. Muthukrishnan et al states, "Chewing tobacco and other smokeless tobacco products are known to be deleterious to oral health." His group has done much research in this field showing that smokeless tobacco has more deleterious negative effects on the oral cavity, in comparison to their smoke-fueled counterpart. They further detail the main health risks associated with smokeless tobacco, namely, oral squamous cell carcinoma (SCC), verrucous carcinomas, and tobacco-induced oral mucosal leukoplakia (Muthukrishnan , 2018).

Not only do SLTs contribute to cancer risks through their nicotine content, but they can also increase this risk by the oral abrasion they cause. Many abrasives are added to SLTs in order to accelerate nicotine absorption through oral mucosa. These abrasives wear down enamel, dentin and root surfaces along with the sensitive mucosal lining. Prolonged trauma to these sites can create irritation fibromas that have an increased malignant potential (Janbaz, 2014).

To further this idea, it has been documented by WHO that the country with the highest prevalence of oral and oropharyngeal cancer in the last 10 years is India. Oral cancer incidence rates in many parts of India surpass 6 per 100,000 males, and in some areas, they are as high as 10.8 per 100,000. About one-half of all tobacco used in India is smokeless tobacco, and nearly 100 million people use smokeless tobacco products daily. A study was conducted on this subject in India from October–November 2003. Indian smokeless tobacco products that were bought from retail stores in Gujarat, Karnataka, and Mumbai, India were quantified. Each purchase's date and location, as well as the batch number, were recorded. The 32 labels that were gathered for research are items that are widely used in India. There were also five common brands of non-tobacco chewing mixtures (supari) included. Analysis of both groups of recreational chewing products concluded that the SLT products not only had high levels of nicotine, but also excessively high levels of nitrosamines (nitrates), in comparison to levels of nitrates we have in foods like cold cuts and hot dogs. Nitrosamines are known to be carcinogenic in high volumes, as so may be contributing to the risk of oral cancer along with the nicotine. (Stepanov, 2005)

To reduce the morbidity and mortality associated with the use of these drugs, immediate public health interventions are needed. It is both ironic and disheartening to realize that many third-world countries lack so much health literacy that people are choosing nicotine options that are actually worse for one's health, thinking that they are opting for the 'healthier' alternative. Ideally, advocacy campaigns to strengthen and enforce policies restricting SLT and smoking are needed in most of Southeast Asian countries, but these efforts require more resources, which many countries just cannot support at this time.

Human Papillomavirus

The Human Papillomavirus is an ancient pathogen infecting epithelial tissues in amphibians, reptiles, and mammals. Its composition includes a double-stranded DNA surrounded by a protein capsid, similar to many other viruses on the Papovaviridae family. There are more than thirty strains of this virus, of which a fourth are associated with oropharyngeal cancers. The most common low risk strains found in HPV-related oropharyngeal cancer are HPV- 6 and 11, while the most common high-risk strains are 16 and 18. The high-risk strains are mainly transmitted via frequent vaginal and/or oral sex with multiple partners and without barrier usage. The pathophysiology behind this virus is mainly a result of its oncoprotein production. HPV protein E6 has been shown to interfere with p53 mechanisms in the body and protein E7 interferes with pRb tumor suppressor protein in the host. These interruptions lead to abnormal cell growth by inhibiting the apoptosis pathways and dysregulating the cell cycle. As such, the early HPV oncoproteins E6 and E7 are responsible for the malignant phenotype.

It is important to appreciate the nuisances between HPV related oropharyngeal cancers and non-HPV related ones. The most critical difference can be seen in the detection and initial manifestations. Most HPV associated oropharyngeal cancers begin with a protruding neck mass, while non-HPV related ones often begin with symptoms of sore throat and dysphagia. Research has also shown that oropharyngeal cancers associated with HPV have a much better prognosis, but the end-term side-effects can be more drastic (Timbang, 2019). As the oropharynx regulates speech, swallowing reflexes, and airway patency, surgery or other medical treatments in the area pose substantial risk of morbidity. Standard treatment for HPV related oral and oropharyngeal cancers is currently chemotherapy, radiation and sometimes surgery. The after-effects of these procedures often wreak havoc on the oral cavity. Xerostomia, worsening periodontal condition, loss of taste, trismus and osteoradionecrosis are only some of the risks involved after treatment.

Several studies have looked into the prevalence of HPV in oropharyngeal cancers, but HPV identification rates vary widely based on the population, subsite combinations, specimen type, and detection process. HPV is more commonly seen in oropharyngeal and tonsillar cancers than in other head and neck cancers. One study provides a clear and insightful connection between HPV and oropharyngeal cancer (Herrero, 2003). From April 1996 to December 1999, the research was conducted in Italy, Spain, Northern Ireland, Poland, India, Cuba, Canada, Australia, and Sudan. Patients were recruited from cancer referral clinics, and control groups were drawn from the same clinics or nearby general hospitals that served the same populations as the case patients. Control subjects were chosen based on sex and were within a 5-year age gap from the case participant. Control participants were disqualified if they had a history of oral cavity or oropharyngeal disease.

Their methods included obtaining an interview, collecting exfoliated oral cells, bloodwork, and biopsy specimen from all case patients. HPV DNA was detected by polymerase chain reaction testing (PCR). The results indicate that HPV DNA was detected in 3.9% of biopsy specimens of 766 cancers of the oral cavity and of 142 cancers of the oropharynx. HPV DNA in cancer biopsy specimens was detected more frequently among subjects who reported more than one sexual partner or who practiced oral sex. HPV-16 DNA was found in 94.7% of HPV DNA-positive case patients. Their conclusion was that HPV, especially strain HPV-16, plays an important etiologic role in many cancers of the oropharynx and the oral cavity. However, there is still uncertainty regarding the mechanism of transmission, that requires further investigation.

In the last ten years much headway has been made in terms of prevention of HPV-related cancers of the oropharynx and oral cavity. Public health campaigns have been instituted to increase barrier contraceptive use when engaging in oro-genital intercourse. Much of this is based on the vast amount of research implicating HPV16 and 18 in cancers of the oral cavity and oropharynx. These campaigns are especially geared towards older adults who are beyond the vaccination age. In terms of vaccination, the first vaccine related to HPV was released in 2006 by the CDC and called Gardasil-4. This vaccine protects against the four main strains implicated in HPV-related cervical cancer in women. Recently a newer version of this vaccine was released, preventing against 9 different strains, and covering over 90% of strains implicated in cervical cancer. The initial roll out of this vaccine was geared towards boys and girls ages 9-26, with the optimal

administration at 11 or 12 years of age. The idea behind this was to administer the vaccine prior to initiation of sexual activity and at an age when the body has the strongest immune response. Since then, the age recommendation has increased to 45 years old, though its efficacy at this older point in life is exceedingly less.

It is important to note that the CDC has not approved the vaccine for fighting oropharyngeal cancers related to HPV. This stems from a broad lack of knowledge in the medical world regarding cancers of the head and neck. When surveyed, 15.5% of members of the Louisiana Chapter of the American Academy of Pediatrics were not aware of the link between oral cancer and HPV, and less than half knew its prevalence in this country (Mehta, 2017). One of the main reasons the CDC has chosen not to endorse vaccines for HPV related oral cancers is because this type of cancer may not develop for many years after initial infection with the virus. As such, clinical trials establishing correlation between increasing vaccine use and decreasing oral cancer rates may be exceedingly difficult. Another critical distinction between oral and cervical cancers related to HPV is detection. Papanicolaou tests for cervical cancer are administered regularly and with high accuracy. No similar test with the same acuity is available for oropharyngeal cancers. As such, this vaccine may be the strongest form of prevention.

Inflammatory Bowel Diseases

Inflammatory bowel diseases has also been associated with oral cancers. Inflammatory bowel disease (IBD) refers to two diseases, Crohn's disease, and Ulcerative Colitis, under which the gastrointestinal (GI) tract is inflamed over a long time. While the exact cause of IBD is unclear, it is caused by a malfunctioning immune system. To defend the body, a fully functioning immune system destroys infectious species such as viruses and bacteria. The immune system reacts inappropriately to environmental stimuli in IBD, resulting in gastrointestinal inflammation. There tends to be a genetic factor as well, with someone who has a family history of IBD being more likely to have this abnormal immune response. Much research has been conducted on this subject in relation to the field of dentistry to understand oral manifestations of IBD, and if there are any links between the disease and oral/oropharyngeal cancer.

One study, compared subjects with Ulcerative Colitis and Crohn's Disease in comparison to a healthy control group. The goal of this study was to understand whether IBD has an effect on the oral cavity. The case group was divided into five classes based on their medication and treatment regimen: untreated, salicylate treatment, corticosteroid therapy, immunosuppressant medications (azathioprine and cyclosporine), and biological therapy (infliximab and adalimumab). Patients who agreed to participate in the study completed a structured questionnaire that asked about their age, ethnicity, medical background, medications, smoking habits, and oral hygiene habits. Patients were also asked to mention any unusual oral manifestations they experience, such as xerostomia, halitosis, dysphagia, regurgitation, and dysgeusia, as well as other signs of oropharyngeal cancer.

The results garnered indicate a higher incidence of oral manifestations as well as oral and oropharyngeal cancer in the case group vs the control group, though statistical significance was not achieved. They also noted that the majority of complications were seen in the corticosteroid and immunosuppressant therapy groups. However, the authors state, somewhat confusingly, that the pharmacological therapy of IBD did not show a statistically significant relation with the presence of lesions of the oral mucosa or oral cancer (Laranjeira, 2015). This is difficult to understand when the untreated group did not show any signs of oral manifestations. As such, much of this data fails to serve clinical significance.

A recently published systematic review on this topic works to provide a pathophysiology behind IBD and cancers of the oral cavity and oropharynx. Their search strategy included PubMed, Embase, and Scopus articles on this topic from 1946 to January 2015, published in any language. The results of this study indicate that the connection between IBD and oral cancers are the extensive use of immunosuppressive drugs in this patient population. "Immunosuppressants may promote cancers by various mechanisms including carcinogenic mutations of cell DNA, impaired immunosurveillance of tumor cells, impaired number or function of immune cells chronically infected by Epstein-Barr Virus [EBV] or HPV, and several others (Katsanos, 2016)."

As such, prolonged immunosuppression for chronic graft-vs-host disease or IBD increases the risk of oral cancer. It is important to note that oral cancerous and precancerous lesions have been reported in patients with IBD as reported for other groups of immunosuppressed or transplanted patients. Unfortunately, there are no precautions implemented to modify this risk and there are no routine oral cancer screenings recommended for this patient population. It would behoove the medical and dental communities to collaborate on ways to manage the increased risks faced by people undergoing immunosuppressant treatment for IBD and other conditions involving immunosuppression.

Conclusion

Many lesser known and novel causes of oral cancer and oropharyngeal cancer were discussed in this literature review. The purpose of this was to broaden our understanding of the disease and consider possible etiologies that had not been associated with the oral cavity in the past. With this knowledge, we can better educate the public in preventative measures as well as encourage more routine screenings for those practicing higher risk behaviors. As the saying goes, prevention is the best form of treatment.

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What Common Factors may Influence the Success of Dental Implant?

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Abstract

The aim of this study is to investigate the common factors that may influence the success of dental implants. Addressing these factors may potentially aid experts in the field in delivering dental implants without approaching or decreasing the number of failures. Smoking, diabetes, implant maintenance, age, and implant size have significantly influenced implant success. It is suggested that patients are advised to quit smoking at least one week before surgery to minimize risk factors. Inadequate glycemic control also contributes to periodontal destruction and is associated with the severity of peri-implant complications. However, if patients maintain good glycemic control, dental implants will still have a high success rate. As a result, treating diabetic patients primarily with proper glycemic control is a safe and successful treatment option. Peri-implant maintenance treatment (PIMT) is another important component for dental implant success. Furthermore, physical, metabolic, and endocrine changes frequently occur as people become older. These changes may lead to an increased risk of osteoporosis that may cause the development of dental implant failure. Lastly, the use of inadequate implant for a certain area of the maxilla or mandible may lead to dental implant failure. This research also shows that short implants should only be utilized in exceptional circumstances, but conventional size implants should be the primary mechanism of implant delivery. As a result, the longer the implant, the better the chance of survival. Furthermore, if the buccolingual width of edentulous crest is sufficient, the use of wide implants is shown to be the best strategy for implant delivery. Having long and wide implants is established to improve the implants strength and resistance to fracture.

Introduction

There are several different methods for replacing missing teeth such as dental implants or dentures. However, dental implants have emerged as the new treatment modality for many patients and are expected to play a significant role in oral rehabilitation in the future. Conventional dentures have restricted indications and outcomes, but implant dentures have advantages in function, stability, comfort, and can replace one to all missing teeth if they are supported by healthy oral (bone quality and quantity) and overall health (Sidjaja, et. al. 2006). Dental implants are defined as surgical components that interact with the jaw or skull bone to support a dental prosthesis, such as a crown, bridge, denture, or facial prosthesis, or to function as an orthodontic anchor (Raikar, et. al. 2017). Dental implants can improve a person's look, confidence, self-esteem, improve their ability to talk and chew properly, and remove the need for complete and partial dentures (Krishnan, et. al. 2020). Over the past 10 years, 90%–95% of dental implants were reported to be successful (Raikar, et. al. 2017). Even though dental implants have a very good survival rate, a rising number of patients are developing peri-implant illnesses. Given the potential systemic consequences of chronic inflammation, it is critical to have a better understanding of peri-implant disease occurrence and risk factors to prevent or manage peri-implant inflammation. These peri-implant illnesses can cause pain, need surgical or non-surgical therapy, have significant consequences on systemic health, or result in implant failure. The future burden of peri implant illnesses must be determined for patient consent, physician decision-making, and resource allocation. Peri-implant illnesses are divided into two categories: peri-implant mucositis and peri-implantitis, both of which are infectious diseases. Soft tissue inflammation around a functional dental implant with bleeding on probing (BOP) has been classified as peri-implant mucositis, and peri-implantitis is differentiated by associated loss of supporting marginal bone past normal bone remodeling. Peri-implant mucositis is reversible, whereas peri-implantitis is more difficult to reverse (Daubert, et. al. 2015).

Prosthetic implants can fail for a variety of reasons, both mechanical and biological. Incomplete osseointegration, infection, and poor healing are the most common reasons of implant failure (Sakka, Coulthard, 2011). Osseointegration is a biological tissue healing process in which a direct functional and structural connection between organized live bone and the surface of a loadbearing implant. The direct anchorage of the implant fixture to surrounding host bone is a very important feature to affirm the reported long term clinical success of dental implants. Several factors with insufficient control can jeopardize the implant's solid anchoring to the bone tissue. These factors can be categorized as surgical (primary stability and surgical technique), tissular (quality and quantity of bone, healing, remodeling), and implantological (macrostructure, microstructure, and dimensions) (Georgiopoulos, et. al. 2007). In addition, a dentist should assess several factors to ensure that a patient is a good candidate for a dental implant treatment and that the surgery will not lead to implant disease. Smoking, diabetes, implant maintenance, age, and implant size are all possible factors that may influence the success rate for dental implant.

Methods

The literature in this research helped provide a thorough examination of the subject and enabled a conclusion to be established on the research topic. Databases including EBSCO, ProQuest, PubMed, and Google Scholar that were primarily accessed through Touro College's Online library, were extremely useful for locating essential and appropriate articles.

Smoking

Cigarette smoking has been linked to an increase of plaque formation, a higher prevalence of gingivitis and periodontitis, a higher rate of tooth loss, and increased alveolar ridge resorption in the oral cavity (Scabbia, et. al. 2001). When it comes to dental implant rejection and implant-related complications, nonsmokers have a huge advantage. About the time of implant insertion and second-stage surgery, smoking has been linked to implant failure, with smokers having a failure rate double that of nonsmokers (Gorman, et.al. 1994). Smoking may lead to problems with oral connective tissue repair, dignity, and interference with wound healing by inhibiting cellular protein synthesis and reducing the ability of gingival fibroblasts to adhere as a product of nicotine (Hoffman, 1997). The elevated amounts of fibrinogen, hemoglobin, and blood viscosity, abnormal levels of carboxyhemoglobin in blood, impaired polymorphonuclear neutrophil (PMN) leukocyte activity, and increased platelet adhesiveness have all been proposed as mechanisms through which smoking impairs wound healing (Lawrence, et. al. 1984). In a study to evaluate the influence of smoking, 2,194 implants were placed in 540 patients over a 6-year period. The overall failure rate was 5.92% which is consistent with other studies; however, when patients were subdivided into smokers and nonsmokers, it was found that a significantly greater percentage of failures occurred in smokers (11.28%) than in nonsmokers (4.76%) (Bain, Moy, 1993). Although, the authors demonstrated that implants malfunction because of smoking, there are some reports that have shown no significant differences between smokers and nonsmokers in the success of implants. A meta-analysis study monitored the performance of machined surface implants and Osseotite implants in which he was able to isolate the effect of smoking. The study showed that there was no difference observed between the smoking groups and the non-smoking group, however, there was a clinically relevant difference observed between the two types of implants (Bain, et. al. 2002). The results of this meta-analysis revealed that the risks of smoking are not represented in this group of patients who smoke around 12 cigarettes a day on average. The author does, however, emphasize that there may be a significant difference regarding implant failure between heavier smokers and nonsmokers than there are in the current sample.

Even though smoking seems to be harmful to implants, quitting smoking can significantly reduce the rate of implant failure. A smoking cessation plan was developed, and it was discovered that there was a statistically important gap in the failure rates between those who tended to smoke and those who followed the non-smoking protocol (Bain, 1996). Other studies show there was no statistically significant difference between complications and past smoking, this suggests that quitting smoking may even reduce the likelihood of complications to the level of a nonsmoker's (Levin, et. al. 2004). Since smoking has such a negative impact on implants, Bain and Moy's initial guidelines say that long periods of abstinence are needed. They recommended that the patient quit smoking at least one week before surgery to allow for the reversing of increased platelet adhesion and blood viscosity, as well as the nicotine's short-term effects. The patient can refrain from smoking for at least two months after the implant has been placed, by which time bone healing will have advanced to the osteoblastic process and early osseointegration will have occurred (Bain, Moy, 1993). Furthermore, according to certain research, the volume of cigarettes consumed is linked to a higher rate of implant failure. In a prospective study on mandibular implant overdentures found that heavy smokers (30-40 cigarettes per day) with type IV bone had a higher rate of implant failure (Fartash et. al. 1996). Furthermore, other research found that heavy smokers (>14 cigarettes a day) had slightly more marginal bone damage across implants than people who smoked less (14 cigarettes per day) (Lindquist et. al. 1996). This indicates that the higher the rate of cigarette consumption, the more likely it will lead to implant failure.

In general, smoking tends to have a greater effect on maxillary implants than it does on mandibular implants. In a retrospective analysis of over 200 implants, a gap of the success rates in smokers was observed between maxillary and mandibular implants prior to loading. The performance rates in the maxilla were impaired, but not in the mandible (Bruyn, Collaert, 1994). In addition, other research discovered peri-implantitis was slightly worse in smokers than in nonsmokers in the maxilla, but not in the mandible (Hass, et. al. 1996). Posterior maxillary bone is likely to be of poor consistency, making it more vulnerable to the negative effects of smoke. Others observed that bone loss around anterior sites was almost twice as large as bone loss around posterior (Lindquist et. al. 1997). However, it seems logical to say that since it is the region most insulated from the local influence of tobacco smoke and is, moreover, covered by the tongue, there should be lower failure rates in the posterior mandible among smokers than the anterior region. However, this is an area that needs to be looked at more thoroughly.

Diabetes

Diabetes mellitus is a chronic carbohydrate metabolism disease characterized by hyperglycemia, which reflects a disruption of metabolic balance in glucose consumption by tissues, glucose release by the liver, pancreatic, anterior pituitary, and adrenocortical hormone output liberation.

Recent studies have shown that diabetes mellitus affects any tissue of the body in some way, either directly or indirectly (Chauhan, et. al. 2019). This metabolic disease affects an estimated 15.7 million people in the United States, or 5.9% of the population (national institute of health, 1995). Diabetes occurs when the pancreas doesn't contain enough insulin (type 1) or when the body can't use the insulin it produces efficiently (type 2) (Chauhan, et. al. 2019). In the oral environment alone, diabetes has been associated with periodontitis, xerostomia, increased levels of salivary glucose, swelling of the parotid gland, an increased incidence of caries, and slower healing after surgeries leading to tissue necrosis (Rothwell, Richard, 1984). If diabetes is not managed properly, elevated levels of extracellular glucose may also form covalent bonds with macromolecules in the body (Salvatierra, et. al. 2016).

Diabetes is a crucial modifying factor in periodontitis, but its connection to peri-implant diseases has yet to be thoroughly investigated. however, diabetes may be considered one of the most encountered contraindications to dental implant therapy. Animal studies have proven that poor bone-implant healing and delayed osseointegration are linked to inadequate glycemic control (Eskow, Oates, 2017).

A study contained 200 diabetic patients and 200 non-diabetic control patients. Success occurred in 192 cases in diabetic group, while it occurred in 196 cases in the control group. The results obtained were not significantly different comparing the prognosis of dental implants in diabetic and non-diabetic patients (Chauhan, et. al. 2019). Although a study observed an association between diabetic patients and peri-implantitis, it reported peri-implantitis diagnosed in 24% of diabetic patients and 7% of non-diabetic patients (Ferreira, et. al. 2006). However, these results refer to patients with diabetes regardless of their glycemic management. Furthermore, another study reported a high risk in diabetic patients for peri-implantitis (Daubert, et. al. 2015). However, it seems logical to say that since their study only had five diabetic patients that may have influenced their statistical analysis. Therefore, high success rate is achievable when dental implants are placed in diabetic patients whose disease is under control, but patients that do not have the proper control may be susceptible to implant failure.

Studies were conducted to observe the relationship between the level of metabolic control of diabetes and peri-implantitis. When comparing poorly controlled diabetic patients to well-controlled diabetic patients, certain clinical parameters, such as periodontal disease and radiographic bone degradation, were slightly higher. Authors concluded that inadequate glycemic regulation could play a role in the modulation of periodontal destruction and may be linked to the seriousness of peri-implant complications (Venza, et. al. 2010). Other studies conclude that regardless of the level of glycemic control, type 2 diabetic patients have a significantly higher risk of peri-implantitis and marginal bone loss (Lagunov, et. al. 2019).

A systematic review investigated whether hyperglycemia/diabetes mellitus is associated with peri-implant diseases. According to the meta-analyses, the chance of peri-implantitis is around 50% higher in diabetics than in non-diabetics. Importantly, nonsmokers with hyperglycemia have a 3.39-fold increased chance of peri-implantitis relative to those of normoglycemia. In contrast, the connection between diabetes and peri-implant mucosa was not significant. Therefore, the study concluded that the risk of peri-implantitis is greater in people with hyperglycemia compared to those with normal blood glucose levels. In addition, nonsmokers with hyperglycemia have an increased risk of peri-implantitis, demonstrating that smoking is not needed to intensify the effects of hyperglycemia (Monje, et. al. 2017a). However, only 11 percent of their studies included subjects with satisfactory plaque control, the remaining 89 percent did not report any oral hygiene criteria and thus likely included subjects with low plaque control, which may have influenced their findings. In addition, only three of the experiments used in their comparative analysis omitted smokers, so smoking may have confounded the effects of hyperglycemia for implant success in the other studies.

Although this study suggests that diabetic patients with strong glycemic control may have a high success rate for dental implants, precautionary measures may increase the likelihood of a successful outcome. Before implant therapy, a comprehensive health history should be obtained by the doctor, adequately screening the candidate to ensure that they are taking their diabetic drug, and if their metabolic control seems to be inadequate, delaying implant treatment until improved control is reached is the safest option (Balshi, et. al. 1999).

Future research is required to look at the connection between peri-implant tissue health and long-term changes in glycemia and HbA1c levels. The major glycemic control parameters should be monitored not only for scientific purposes, but also for physicians since inadequate metabolic control can lead to problems such as an increased risk of infection. Under the limitations of this research, the findings suggest that implant therapy in diabetic patients with strong glycemic control is a safe and effective treatment choice.

Implant Maintenance

With the use of dental implants for teeth replacement and denture stabilization, the need for maintenance and repair is becoming more relevant in daily clinical practice. Periodontium is the tissue that surrounds and supports the teeth. If those in the field can understand the biological mechanisms of the gingiva and periodontium in normal teeth versus implants, it will demonstrate how much more critical implant tooth oral hygiene is compared to normal tooth oral hygiene. The peri implant mucosal seal is a region established to apply a tight seal to isolate the implant and the bone from bacterial plaque in the oral environment. However, unlike the periodontium surrounding a normal tooth, the peri-implant mucosal seal still lacks an effective barrier against bacterial invasion from plaque (Weyant, 1994). In addition, the vasculature in the gingival tissue that surrounds dental implants, is not as efficient as the vasculature in normal teeth, thereby, preventing the destruction of biofilms. Furthermore, the oriented collagen fibers around the implant are parallel as supposed to being perpendicular, which makes it more susceptible to bacterial invasions (Nevins, Langer, 1995). Therefore, the lack of proper oral hygiene may not only cause bacteria invasion from plaque accumulation which may lead to periodontitis or gingivitis, it can also induce the development of peri-implantitis (Kurtzman, Silverstein, 2014).

A cross-sectional study was performed on patients who were healthy and partly edentulous. 206 implants were fulfilled on 115 patients that were divided into three categories; 1) usual compliers which experienced peri-implant maintenance therapy (PIMT) at least twice a year; 2) erratic compliers which experienced PIMT less than twice a year; 3) non-compliers which didn't experience any PIMT. The study discovered that association between compliance and peri-implant condition were statistically significant. Compliance was associated with 86% fewer conditions of peri-implantitis. The probability of PIMT compliance was substantially associated with frequency of peri-implantitis (Monje, et. al. 2017b). As a result, PIMT enforcement could be the path to maintaining an inflammation-free condition that allows hard and soft tissue integrity to coexist. For instance, it was demonstrated that the failure rate of dental implants was decreased by 90 percent of patients who received routine maintenance compared to those who did not. In fact, patients who received at least one maintenance appointment on a yearly basis had a 60 percent lower failure rate than those who did not have any maintenance (Gay, et. al. 2016). In this regard, it has been stated that patients who receive regular PIMT have a lower risk of peri-implant bone loss development. To stress the importance of PIMT, in a systematic review, the long-term results of patients with periodontitis who underwent periodontal therapy and implant placement were evaluated. According to the findings, patients of periodontitis had good implant outcomes, within trials with a 10-year follow-up, implant survival was high (92.1 percent) (Zangrando, et. al. 2015). The high success rate of implant therapy in patients with periodontitis who received adequate treatment and routine periodontal care, demonstrates the significance for implant maintenance.

Regarding this, many patients remain unaware of the critical steps that must be taken to ensure proper implant maintenance. A study was performed out to assess the knowledge of oral hygiene measures in patients with dental implants. A questionnaire that involved 50 patients on a basis of assessing the awareness about hygiene maintenance for their implants. Patients who had dental implants rehabilitated were asked approximately ten questions. Patients were questioned about their brushing method, the kinds of brushes they used for implants, if they used mouthwash and floss to keep their implants clean, and if they used any other implants aids. Around 80% of patients said they are aware of the oral hygiene measures required for implants, and that they learned about them from their dentist; however, 10% of patients were unaware of the importance of hygiene measures in preserving dental implants. The findings of this survey shows that the patients in the study had a poor understanding of dental implant hygiene and its effects, and the experience of dental implant maintenance in patients is inadequate (Krishnan, et. al. 2020). As a result, dentists should be advised to provide routine dental exams and give oral hygiene tips to all patients who have dental implants. Services aimed at improving oral hygiene and implant management for implant patients are required.

Unfortunately, implant failure is associated with a lack of professional implant maintenance. It has been proposed that a professional mechanical plaque removal procedure should be programmed to avoid the formation of peri-implantitis. Disruption of the assemblage of surface associated microbial cells enclosed in an extracellular polymeric matrix must be routinely removed through self-performed oral hygiene measures. Accordingly, Peri implant maintenance compliance, experiencing at least 2 PIMT yearly has been demonstrated as a crucially essential factor for preventing peri-implantitis in healthy patients.

Age

Patients' conditions vary greatly, particularly among the elderly. Implant failure seems to be a multi-factorial problem, so it's unclear if age is a risk factor for implant placement. However, there are physical, metabolic, and endocrine changes that occur as people age, and clinicians must consider that these changes can impact implant treatment. The human skeleton accumulates bone until around the age of 30 years, at which point it begins to lose bone, causing the bone to weaken (Heersche, et. al. 1998). In addition, since diabetes and osteoporosis are prevalent in the elderly population, these conditions may influence dental implant success.

Age-associated bone loss is related due to an uncoupling of osteoblastic and osteoclastic activity, since the osteoblastic activity that creates new bone can't keep up with the osteoclastic activity that breaks down bone to rebuild it (Freemont, et. al. 2007). Furthermore, as age increases, the rate of bone healing slows down. Possible suggestions for the cause of delayed healing may include, reduction of the osteogenic stem cell numbers, a decrease in the proliferation and differentiation capability, and reduced local blood flow (Strube, et. al. 2008). An analysis was conducted to see how long it took for bone to heal and close a fracture gap for rats. By 4 weeks after fracture, young 6-week-old rats have formed bone to close the fracture gap, adult 26-week-old rats took 10 weeks, and older 52-week-old rats require more than 26 weeks (Meyer, et. al. 2004). The causes for poor bone healing may be because open wounds compress more slowly, and incised wounds develop strength more slowly as age increases. In addition to weak bone regeneration, increased aging may also cause reduced keratinization of the epithelium, a decrease in the synthesis of collagen in periodontal ligaments, and a reduction in the number of cells on the osteogenic layer of the alveolar bone, all leading to implant failure.

A prospective study was carried out with 2 groups of healthy edentulous patients to determine the influence of age on peri-implant tissues in patients treated with implant-supported overdentures in the mandible. The mean age of the younger group was 46 years, and the mean age of the older group was 68 years. After three years, the mean bone loss in the younger group was 1.2 mm, and in the older group it was 0.8 mm, but the difference was not significant. The clinical performance of implant-supported overdentures in the mandible was similarly effective in younger and older patients (Meijer, et. al. 2001). This study indicates that increased age alone is not a contradiction to implants. However, another study looked at a vast number of patients who had been operated on by an experienced surgeon and discovered that elderly age raised the likelihood of implant failure; patients over 60 years old were twice as likely to have negative results (Brocard, et. al. 2000). Furthermore, a 7-year prospective trial was observed in a private practice with the same model of implants, and it was discovered that only a limited minority of implants existed in patients over the age of 60 (Moy, et. al. 2005).

A rat study involved three age groups, 6 weeks (younger group), 12 weeks (older group), and around 2 years (old group), the young group demonstrated that new trabecular bone developed aggressively around the implant and that strong bone interaction was reached more quickly than the adult group. The old group, on the other hand, had less recently developed trabecular bone around the implant and had less bone interaction than the other groups (Shirota, et. al. 1993). The findings showed that as rats get older, the rate and amount of new bone development around implants decreased. This study demonstrates that as patients increase in age the likelihood for developing osteoporosis increases as well.

Diabetes mellitus is a serious disease that affects people all over the world. Diabetic patients get more prevalent as people get older, particularly those over 50 (Harris, et. al. 1998). Diabetic patients have poorer wound healing, greater chance of microvascular disease, a slower response to infection, and are more susceptible to periodontal disease, all of which can make implant placement more difficult (Olson, et. al. 2000). Mineral metabolism is also changed which can potentially disturb the integration process (Fiorellini, et. al. 2000). Furthermore, the time span of diabetes may affect implant performance, as an increase in diabetes duration could induce microvascular disruptions, which could lead to implant complications (Olson, et. al. 2000). As a result, implant failure is more likely to occur in elderly patients who have been diabetics for a longer time.

The reduction of bone mass and density in the body, including the jaws, is known as osteoporosis. Osteoporosis is closely linked to estrogen deficiency, so postmenopausal women are at risk for osteoporosis. The reduction in estrogen during the menopausal transition process causes more bone resorption than development, resulting in osteoporosis. There are two types of postmenopausal women. Type one or 'postmenopausal osteoporosis, in which trabecular bone loss is prevalent, resulting mostly in vertebral and wrist fractures, and Type two or senile osteoporosis, in which both cortical and cancellous bone are missing, resulting in hip fractures.

A study that examines the relationship between premenopausal and postmenopausal women and implant

failure found no evidence of a higher failure rate for implants in women over 50 relative to women under 50 or between women and men over 50 (Dao, et. al. 1993). However, according to a survey, women lost about 10 percent of their hip bone mineral density between the ages of 50 and 60, compared to just 2 percent for men. (Looker, et. al. 1998). Just like other bones in the body can decrease bone mass for postmenopausal women, the alveolar ridges have been stated to decreased bone mass in postmenopausal women as well (Humphries, et. al. 1989). Although some evidence indicates that the mandible varies sufficiently from postcranial skeletal sites, and it is therefore unclear if bone mass throughout the skeleton corresponds to bone mass in the mandible and maxilla (Boyde, Kingsmill, 1998). However, mandibular bone mineral content declines with age, and mandibular bone density was shown to be lower in elderly female subjects than in male subjects (Heersche, et. al. 1998), which indicates that postmenopausal women are more likely to develop osteoporosis even in the mandible and maxilla due to estrogen deficiencies.

According to a study that looked at jaw variations in pre- and postmenopausal women, the effect of postmenopausal estrogen status on impaired implant healing was seen in the maxilla but not in the mandible. In addition, hormone replacement therapy decreased the rate of maxillary bone loss by 41 percent. Since osteoporosis affects trabecular bone rather than cortical bone, and the maxilla has more trabecular bone composition than the mandible, the authors reasoned that the maxilla is more vulnerable to systemic osteoporosis (August, et. al. 2001). Therefore, postmenopausal women may be more likely to experience implant failure especially in the maxilla due to hormone deficiency.

Implant Size

Optimizing implant geometry to maintain a healthy stress level at the bone-implant contact is a complex issue. The use of an inadequate implant for a certain area of the maxilla or mandible may lead to dental implant failure. Dental implants come in a variety of lengths, ranging from 5.0 to 20 mm. The most frequent implant length is 8 to 15 mm, which corresponds to the length of a normal root. The diameter of currently available implants ranges from 3.0 to 7.0 mm. The implant diameter requirements are based on both surgical and prosthetic concerns.

It is not always possible to deliver dental implants of sufficient length since many situations lack having more than 8 mm of residual vertical bone height. Therefore, clinicians must choose between augmentation of the bone or the placement of short implants (Renouard, Nisand, 2006). For clinicians to prevent the use of short implants, resorbed bone should be augmented using different bone-grafting procedures. This will allow the clinician to place a longer implant. However, short implants may still be a better option than bone augmentation, since augmentation treatments can lead to extra surgical interventions, serious postoperative morbidity and complications, higher cost, and take longer before patients can chew on their implant-supported prostheses (Esposito, et. al. 2011). A study suggests that 5 mm short implants yield equal, if not better, outcomes than longer implant placed in bone one year after loading. Using the bone levels at implant placement as baseline data, there was a statistically significant difference between short and long implants. Short implants lost an average of 1 mm and long implants lost around 1.2 mm in peri-implant marginal bone levels one year after loading (Esposito, et. al. 2011). However, this study has limitations due to the small sample size, because only a few individuals had enough bone width (at least 8 mm) to tolerate implants with a 6 mm diameter. Short implants with diameters of 4.0 to 5.0 mm should also be assessed since clinicians often compensate for a lack of height by using implants with a larger diameter (Esposito, et. al. 2011). Therefore, it's logical to assume that the larger diameter of 6 mm in this study was responsible for the positive success rate of shorter implants. However, short implants with narrow diameters may lead to implant failure.

According to the findings of a systematic study, the placement of short rough-surface implants is not a less efficacious treatment modality than the placement of conventional rough-surface implants (Kotsovilis, et. al. 2009). Furthermore, a study involving 7-, 8.5-, and 10-mm implants were analyzed, and it was determined that short implants should be explored as an alternative to advanced bone augmentation operations (Neves, et. al. 2006). Another study established that when delivering 6- and 7-mm implants, short implants with a press-fit shape and a sintered porous surface geometry exhibited the highest performance (Hagi, et. al. 2004). However, other research demonstrate that short implants may be linked to decreased survival rate (Lee, et. al. 2005). According to the findings from a systematic review, short (<10 mm) implants can be successfully placed in the partially edentulous patients, though with a tendency of an increasing survival rate per implant length (Telleman, et. al. 2011). As a result, short implants should only be utilized in exceptional circumstances, but conventional implants should be the primary mechanism of implant delivery. Several presumed reasons to explain why short implants are likely to have a worse survival probability in the posterior

region may be because there is less bone to implant contact due to the smaller surface area of short implants. Furthermore, due to substantial resorption in the posterior region, a larger crown to implant ratio is created over short dental implants, which may lead to a greater implant failure rate (Telleman, et. al. 2011). In addition, it has been proposed that as the length of the surface area rises, the stress levels for a given applied load decreases on longer implants. The mechanical resistance to masticatory forces is also improved because of this (Hoon, et. al. 2005). Aside from implant length, having an implant in a threaded design rather than a smooth design may increase its surface area. This will aid in the transmission of axial tensile or compressive loads better than smooth implant types (Hoon, et. al. 2005).

Aside from implant length, the usage of implants with a larger diameter may provide numerous advantages. From a biomechanical standpoint, larger diameter implants can help provide engagement of a maximum bone and better stress distribution in the surrounding bone (Arisan, et. al. 2010). The use of wider components also enables for more torque to be applied in the placements of prosthetic component (Lee, et. al. 2005). In addition, wide diameter implants will provide an Increase bone-to-implant contact, bicortical engagement, and rapid insertion at failure locations, as well as a reduction in abutment stresses and strains. As a result, having a larger contact area improves initial stability and minimizes stress. By increasing the diameter of the implant, it is possible to improve its strength and resistance to fracture (Lee, et. al. 2005). However, wide implants are restricted due to the the width of the residual ridge and aesthetic requirements for a natural emergence profile (Lee, et. al. 2005). However, when the buccolingual width of the edentulous crest is insufficient, narrow diameter implants can be used to replace missing teeth. According to an article examining the clinical and radiographic outcome of mini dental implants (MDIs), in comparison to conventional-diameter implants, MDIs are cost-effective, have fewer complications during flapless implant placement, and can be used in edentulous arches with minimal remaining bone in a facial–lingual dimension to avoid bone graft. In addition, MDIs also has a great advantage because of its short healing time, reduced post-operative discomfort and quick restoration of mastication and aesthetics for patients throughout the healing phase (Elsyad, et. al. 2011).

Although increasing the diameter of the implant may decreases the amount of bone in the surrounding area, a recent study examined the success and survival rates of narrow diameter implants over a 10-year period, as well as peri implant characteristics and mechanical and prosthetic post loading complications. They concluded that narrow diameter implants can be utilized safely in situations only when a conventional diameter implant is not appropriate, since most of the bone loss surrounding narrow diameter implants happened within the first two years of loading and was minor afterwards (Arisan, et. al. 2010). As a result, the primary strategy should be the use of wide implants, since increasing implant diameter decreases the maximum value of Von Mises equivalent stress. Therefore, as the surface area transmitting a horizontal component of force applied to a dental implant increased, the stress distribution in the maxilla and mandible have become more effective.

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Can Robotic Exoskeletons Improve Gait in MS Patients?

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Abstract

Multiple Sclerosis (MS) is a neurological disorder that affects about one million people in the United States. The disease results from an abnormal immune response where T cells damage the myelin sheath in the central nervous system, causing scarring. The lesions can occur in any part of the brain or spinal cord, and thus affects every patient differently. One of the most detrimental effects the disease has on patients' lives is the decreased ability to walk. There has been research and treatments to manage pain and slow progression of the disease, but little progress has been made to enable MS patients to walk more comfortably. Previously, there have been gait training devices in use. However, many require intensive manual labor which causes the patient and the physical therapist to fatigue easily. Recently, Ekso, a self-supporting robotic exoskeleton, designed for patients with spinal cord damage, has been shown to improve gait and overall quality of life in patients with MS.

Introduction

Multiple Sclerosis is a degenerative neurological disease affecting the Central Nervous System. Unfortunately, it affects many younger people, mostly diagnosed in women between the ages of 20 and 50. There is often physical and emotional pain associated with the disease. There are various medications in use that can treat those symptoms and help improve the patients' quality of life (Ziemssen, 2011). However, change in natural gait is a consequence of MS that disrupts patients' daily lives. Over time, a patient's ability to walk deteriorates. Progressive worsening of gait is self-reported and is also demonstrated through data measured on various scales by researchers (Motl, et al., 2018). Robotic exoskeletons have been shown as an effective way to retrain gait. Exoskeletons are designed to mimic the movements of human limbs. They attach to the extremities and can control the position and alignment of the joints. Exoskeletons can also be programmed to provide support based on the patient's motor capabilities (Iandolo, et al., 2019). This paper discusses the pathology and musculoskeletal effects of MS and examines the effectiveness of robotic exoskeletons in improving gait of MS patients.

Methods

The information in this review is collected from various academic and scientific articles obtained from the EBSCO, PubMed, and ProQuest databases accessed through the Touro College library. Each article was analyzed in order to determine its relevance to the thesis.

Discussion:

Glial Cells and the Myelin Sheath

The nervous system is made of separate parts that work in tandem. Included is the central and peripheral nervous systems. The central nervous system is made up of the brain and spinal cord while the peripheral nervous system is composed of the nerves that connect the CNS to the rest of the body. Although they are not neurons, glia are a fundamental component of the nervous system. The glial cells espouse the neurons and enable them to function properly. Astrocytes are the most common type of glial cell. Astrocytes fill the spaces between neurons, providing support. Astrocytes also contribute to the maintenance of the chemical balance in the neuron by controlling the extracellular chemical concentration and assisting in the reuptake of neurotransmitters. There are also microglia that are involved in removing debris in the nervous system, ensuring that messages can travel without blockage. Another type of glial cell is myelinating glia. Myelinating glia are divided into two subtypes, oligodendrocytes and Schwann cells. Oligodendrocytes are in the central nervous system, while Schwann cells are present in the peripheral nervous system. These cells provide insulation to the neurons by wrapping around their axons in a covering known as myelin, forming the myelin sheath. Myelin provides insulation, allowing signals to be transported faster. There are small gaps in the myelin sheath known as nodes of Ranvier. When an action potential moves down an axon it is regenerated at the node of Ranvier in a process known as saltatory conduction (Bear, et al., 1996).

Pathology of MS

Multiple Sclerosis is a condition where the body's immune system attacks the myelin sheath. MS usually presents at first with an attack that lasts between a few days and a few weeks. It is followed by a period of relief that can last from a few months to a few years. The reprieve is a result of an autoimmune reaction. This mild form of MS usually lasts for about ten years, but varies by case. As the disease progresses, the attacks become less distinct, and the patient's condition greatly deteriorates. During the later stages of the disease, the underlying axon is damaged in addition to the myelin sheath. The axon loss in the spinal cord causes spinal cord atrophy which can make it difficult for the patient to walk and lead to paralysis (Lucchinetti, et al., 1996).

Many protein sequences on the myelin sheath are similar to microbial protein sequences. As a result, the immune system attacks the myelin in a process known as molecular mimicry. Many relapses of MS occur as a result of infection from a virus. Herpesvirus 6, influenza, measles, papilloma virus, and Epstein-Barr virus all have gene encoding sequences that are similar to the main protein structures of the myelin. Antibodies bind to the myelin instead of the

microbe. If a T-cell or B-cell is activated by the microbe, it can penetrate the blood-brain barrier (Steinman, 2001).

The diagnosis of MS is a complex process and multiple factors need to be taken into account. Firstly, there need to be lesions in the white matter of two separate parts of the CNS that developed at least a month apart from each other. All other neurological disorders with similar symptoms need to be ruled out. In addition, an MRI must be performed. The MRI will display the lesions and successive MRI scans can be a tool to help the physician track the progression of the disease. It also needs to be determined that the CNS is chronically inflamed, which can be assessed by a spinal tap. A spinal tap allows the physician to see if there are oligoclonal bands in the fluid which indicate neurological disorders. In addition, each episode of attacks needs to be distinct and last for 24 hours or more. As the disease progresses, it can be subcategorized (Garg, Smith, 2015).

MS affects the myelin of the CNS. Each part of the brain and spinal cord control different neurological functions, so the effects of the lesions can manifest in different ways. Relapsing–remitting MS is the most common form of MS. The person will have separate attacks that can last from a few days to a few months. In between attacks, there is a period of reprieve where symptoms get better and there is no neurological degeneration. The next type of MS is Secondary Progressive MS (SPMS). In a patient with SPMS, the condition worsens gradually with time and the patient does not experience any episodes of relief. Primary progressive MS (PPMS) affects a minority of MS patients. It is characterized by an unrelenting decline in neurological function. There are no relapses or remissions, but there are occasional periods where there is no significant deterioration. Lastly is progressive-relapsing MS (PRMS). PRMS is the rarest form of MS. From the beginning of PRMS, the symptoms get progressively worse. However, it is different from other types of MS because there are periods where the attacks are more intense and debilitating with no periods of remission (Loma, Heyman, 2011).

In the worst stages of MS, there are no phases of relief, as there is destruction to the axons and atrophy of the brain and spinal cord. AMPA is an ionotropic transmembrane receptor for glutamate that allows for fast synaptic transmission in the central nervous system. AMPA receptors are present on oligodendrocytes. During an MS attack, lymphocytes, brain microglia and macrophages release large amounts of glutamate, which activate the AMPA receptors and cause overstimulation. However, if the receptors are blocked, it can mitigate some of the damage from the glutamate. AMPA is not involved in the immune response, but it can protect the oligodendrocytes from being damaged. Medications that block glutamate receptors are being utilized to treat this aspect of MS (Steinman, 2001).

The Effect of MS on Gait

Since MS affects the spinal cord, the main support of the body, a patient's gait can be affected, causing difficulty walking. Damage to certain areas of the spinal cord can lead to muscle spasticity. Muscle spasticity is an increase in rigidity of the muscles. The stiffness can be mild or it can cause involuntary uncontrolled movements of the extremities. The stiffness can make straight limbs hard to bend or bent limbs hard to straighten. Since many MS patients suffer from fatigue, extreme tiredness can make coordinating movement challenging. MS patients can also suffer from weakness in the leg muscles, altering normal stride. The change in walking pattern can cause pain, furthering the difficulty of walking (nationalmssociety.org).

MS patients can also suffer from balance issues, affecting their ability to walk properly. The balance issues can be caused by many different factors, such as vision and sensory issues. A patient may have reduced visual acuity or lack proper depth perception. This can be hazardous to a patient because it affects their ability to be aware of their surroundings. MS-related sensory issues can cause numbness which results from reduced muscle spindle and joint receptor activity. This can lead to decreased sensation in the feet which compromises stability and makes it difficult to navigate uneven surfaces. There is also the vestibular impact of MS on balance. The vestibulocochlear nerve is responsible for both hearing and balance. In MS patients, the vestibulo-ocular reflex is reduced, affecting the ability to stabilize gaze, causing dizziness and unsteadiness (nationalmssociety.org).

Motor systems can also be affected by MS. Patients with MS often have decreased muscle strength and endurance. This makes it difficult for patients to use a wheelchair or scooter on their own. There are also issues with muscle control and range of motion. This can lead to an inability to anticipate changes in the environment, resulting in an increased response time. Patients may also over or under compensate with other muscles in order to make up for the decreased function. These issues can lead to uneven gait (nationalmssociety.org).

Neurological Impact of MS on Gait

A study was done with patients in the early stages of MS to assess the way gait is affected by neurological deficits. Depending on how the gait and neurological systems are impaired, gait patterns will be different among patients and even differ in the same patient over time. Disturbance of

normal gait is reported as the most debilitating symptom by MS patients. The gait of MS patients was compared to a control group of healthy people. MS patients walked slower and used shorter steps. In addition to walking less efficiently overall, there was a decrease in velocity and stride length over the course of the day as the patients became more fatigued. The patients in the study were scored on the Expanded Disability Status Scale (EDSS) scale by a neurologist. The EDSS scale measures neurological disability by assessing motor, sensory, cerebellar, brain stem, visual, and mental abilities. Gait was measured using the GAITRite Analysis System, a computerized mat with sensors that measured contact of the feet with the mat. The system combined the time and distance into a score known as the Functional Ambulation Performance (FAP) score. The system removed points for dysfunction of each leg, lack of lower limb strength, and the use of assistive devices. The FAP score was highly correlated with neurological abilities. The more neurological impairments a patient had, the less efficient their gait. The data also suggests that the impairments in upper motor neuron function lead to abnormal gait in MS. Significant changes to the FAP score would suggest a relapse in the MS (Givon, et al., 2009).

Gait Training

Conventional physical therapy involves different techniques that help patients improve gait and reduce the risk of falls. Treatment for MS involves stretching to reduce spasticity of the muscles and maintain the patient's range of motion at the joints. Exercises also work toward improving trunk control and increasing strength in the upper arms. Physical therapists also teach MS patients how to adapt to the environment and use aids such as wheelchairs and braces (Schwartz et al., 2011).

Physical therapists work with patients on balance exercises as well as strength and resistance training. In the past, Bodyweight-supported treadmill training (BWSTT) was a popular method to improve gait in patients with MS. BWSTT is based on the effectiveness of intensive and task-specific gait training. The user wears a harness which is held up and supported by the therapist. The patient has minimal support from a rope attached to the harness and walks on a treadmill, while the legs are physically guided by the therapist. However, this is extremely exhausting for both the patient and the therapist. It is also impractical for patients that have limited function of their legs (van Kammen, et al., 2016).

A study compared walking on a treadmill regularly to walking on a treadmill with body weight support in 10 healthy individuals. Normal gait pattern was disrupted when body weight support was provided. In addition, neuromuscular control of walking was affected. As gait speed increased, the differences between walking with and without support were less pronounced. It was recommended that higher speeds and less bodyweight support should be provided to patients when trying to reinstate a natural gait (van Kammen, et al., 2014).

Recently, robot-assisted gait training devices have been recognized for their ability to provide walking assistance to patients with severe gait disabilities. The robots, also known as exoskeletons, allow the patient to walk on flat ground. The parts of the exoskeleton that attach to the lower extremities are connected to a computer. The physical therapist can use the software to manually adjust the angles of the different parts in order to mimic the ideal gait of the specific patient (Berriozabalgoitia, et al., 2021).

What is Ekso?

The Ekso is a robotic exoskeleton developed by Ekso Bionics in Richmond, California. The Ekso is made of carbon fibers and steel. It functions as an exoskeleton by attaching to the thigh, lower leg, and foot. The sensors embedded in the attachments respond to input from the user's muscles. A backpack that is worn to support the torso (Lajeunesse, et al., 2015). There are motors located at the knees and hips. A spring-loaded footplate ensures that the patient's steps clear the floor. The device supports itself and does not add any additional weight to the user. The user may require the assistance of a gait aid such as a walker or cane at first, but eventually many patients can walk on their own. The advanced software allows the physical therapist to set the parameters for the level of assistance that the robot provides, including the areas of step height, step length, and swing speed. The purpose of this is to promote participation from the patient in a way that is reasonably challenging (Wee, et al., 2020).

There are three levels of assistance that the Ekso software can provide. The lowest level, named FirstStep, requires the physical therapist to initiate the step using a controller. The patient is not involved in initiating the movement. This is often used in the beginning stages of treatment when the patient is learning to use the device. In ActiveStep, the patient presses a button and the device initiates a step forward. The most advanced level is ProStep, where the device moves when the patient engages in normal walking behavior such as lifting the foot and shifting the weight forward. The Ekso is highly beneficial because it can be programmed to work with patients that have mild to severe gait issues (Thomassen et al., 2019).

A main goal of physical therapy in neurologically impaired patients is to recover gait. Walking is important

for physical and mental wellbeing. The best outcome is based on the principles of relative and repetitive training. The Ekso is relative because it allows the patient to experience a real walking position. It can be used in both indoor and outdoor environments. The Ekso training is repetitive because the patient spends multiple sessions walking wearing the device. Exercise that is high-intensity and is task-specific has been shown to promote the most progress toward recovery (Russo, et al., 2021).

Gross Motor Improvements

The trunk muscles are an integral part of maintaining proper posture during sitting and standing. Proper posture is also the first step in achieving a normal gait. Trunk muscles are activated systematically during walking to keep the body steady. Faster gait speed requires higher levels of trunk muscle engagement to keep the body stable. Engaging the trunk muscles to improve trunk function can provide many rehabilitative benefits. As opposed to other exoskeleton devices, Ekso requires the user to initiate the stepping movement by shifting their weight. This requires full body weight-bearing, which forces the user to activate their trunk muscles. Usage of the Ekso showed an increase in trunk activity both in the anterior-posterior and medial-lateral directions. The level of trunk muscle activation was much higher with the Ekso than voluntarily induced contraction. Research also suggested that the voluntary stepping promoted activity in the cortical and vestibulospinal pathways which shows the Ekso can promote neuromuscular improvement (Alamro, et al., 2018).

A study was done with 36 MS patients that required assistance when walking outside. Both the control group and the intervention group participated in weekly sessions with a physical therapist. However, the intervention group engaged in biweekly training with a robotic exoskeleton for three months. The parameters of the exoskeleton were set according to each individual patient's motor skills and joint mobility. The length of the sessions increased as time progressed, and endurance improved. The sessions were stopped early if the patient requested or if the physical therapist noticed signs of fatigue such as increased muscle tone and stumbling. At the end of each session cooling therapies were used on knee extensors and ankle plantar flexors to prevent exercise-induced hyperthermia and muscle fatigue. The study displayed that robotic gait training in addition to regular rehab sessions preserves gait speed and improves functional mobility in patients with MS. The exoskeleton did not cause increased fatigue in the participants. The control group showed an overall decline in physical function. The effects were two-fold as the symptoms did not improve, and the disease got progressively worse. The Ekso exoskeleton is a crucial tool in rehabilitation because it encourages cognitive control of body movements, specifically in the area of weight shift (Berriozabalgoitia, et al., 2021).

Quality of Life Improvements

A robotic exoskeleton can also improve the quality of life in patients with MS. The exoskeleton provides a highly repetitive and intensive gait training experience. The user is held in an upright position while walking around. The exoskeleton is portable, which allows it to be used in any environment, including outdoors. This is engaging and rewarding to the patient to be able to experience walking in a way very similar to a healthy individual. A study was done with a 51-year-old non-ambulatory patient. The goal for this patient was for her to be able to assist in transfers, to reduce the burden on her caregivers, and to improve her quality of life. Compared to other studies, the purpose here was not to promote ambulation. The patient used the Ekso robotic exoskeleton for a total of 15 sessions that occurred for one hour twice a week. The distance covered during each session was dependent on the patient's level of fatigue. After the Ekso sessions, the patient showed an increase in right knee extensor strength which made the sitting to standing transition easier. As the therapy progressed, the patient's endurance increased as well, and she was able to walk further distances. There was no change in spasticity, trunk control, or balance. However, the most notable improvement was in the patient's quality of life. Prior to using the exoskeleton, the patient was depressed and did not want to participate in social activities. After treatment, the patient expressed to the researchers how her self-confidence and self-esteem improved (Wee, et al., 2020).

Patients with impaired walking function are often confined to wheelchairs. However, wheelchairs do not promote muscle movement and can lead to muscle atrophy. In addition, the patient sitting in the wheelchair is lower than everyone that is standing, leading to feelings of inferiority. Participants reported feeling free and independent when using the Ekso. Additionally, patients that required walking aids such as a walker or a cane had previously felt unsteady while walking and feared falling. While using the Ekso, they felt supported and secure, so they were able to focus on the actual leg movement (Thomassen et al., 2019).

Conclusion

The impairment of gait is reported as the most debilitating symptom of MS. Although many gait training methods have been used over the years, few have led to significant improvements. Ekso is a new robotic exoskeleton that has recently been trialed for its effectiveness in improving

the gait of MS patients. Studies have shown that using Ekso, patients' gait and quality of life has improved. There is hope that the Ekso will become more widely available in order to provide more patients with the euphoric experience of walking again.

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