The Science Journal of the Lander College of Arts and Sciences

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Volume 14
Number 2 *Spring 2021*

2021

Full Issue: Volume 14, Number 2, Spring 2021

The Science Journal

LANDER COLLEGE OF ARTS & SCIENCES
A DIVISION OF TOURO COLLEGE IN FLATBUSH

Where Knowledge and Values Meet

Volume XIV • Number 11 • Spring 2021

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

Over more than four decades, Touro's Lander College of Arts and Sciences in Flatbush (with separate Schools for Men and for Women) has provided cohorts 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. 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.

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, reflecting the career interests of pre-medical, pre-dental and health science students, as well as of students interested in technology fields.

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-Presbyterian 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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Vol. XIV · Number II · Spring 2021

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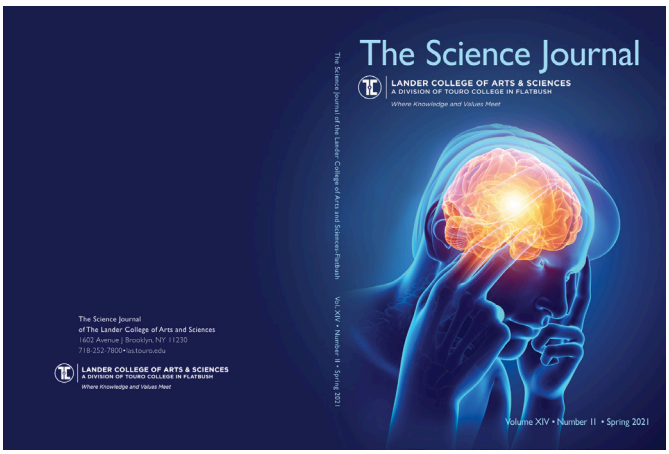
Table of Contents



Cover picture: The cover picture was created by Professor Antony O'Hara of the Digital Multimedia Design Department, pertains to the article "Migraine Triggers" by Adina Jeidel.

Migraine Triggers

Adina Jeidel

Adina Jeidel graduated in January 2021 with a Bachelor of Science degree in Biology.

Abstract

Migraines are a painful and life-interrupting disease which strikes around 23 million Americans every year (Goadsby et al., 2017). Not enough studies have been done to help the public fully understand migraines. Details regarding the causes and pathophysiology of migraines continue to be analyzed by physicians and scientists, as no theory has been fully confirmed regarding a migraine's concrete path. The goal of this scientific review is to provide an overview for the main triggers of migraines, in reference to recent clinical investigations, and to understand why they might cause patients to be more prone to having a migraine attack upon encountering these triggers. Additionally, topics including the uncertainty whether migraineurs should avoid their triggers or learn to live with them, and the pathophysiology behind the triggers will be explored in this review. Some physicians suggest staying away from known triggers, while others say to allow one's self to be exposed, as a means of getting the sensitized central nervous system (CNS) used to the triggers and to make the CNS aware that these stimuli are, in essence, not harmful.

Migraines have been affecting people's lives for over two thousand years and are said to be the 6th leading cause of disability, according to the World Health Organization (WHO) (Goadsby et al., 2017). Of the 23 million Americans that suffer from migraines, women are predominantly affected (Silberstein et al., 1999). The ratio of 3:1 women versus men getting migraines is most likely due to hormonal changes and leads to an understanding of the reason migraines usually begin in females at puberty and can last until the age of 35-45, according to the WHO. Migraineurs claim that their daily lives are impacted by their frequent migraines, with 50% of them having 1 or more migraines per month (Silberstein et al., 1999).

Methods

Through the usage of databases such as Google Scholar, Touro Library, and the National Center for Biotechnology Information (NCBI), scholarly articles and peer-reviewed journals were obtained and reviewed for inclusion purposes. Each article was analyzed and verified, before assuming validity. Some key phrase searches included "migraine triggers," "hormonal migraine triggers," and "caffeine as a migraine trigger."

Introduction

Migraine is a neurological disease that is not understood very well. It is more than just a headache and can be debilitating and life-altering at times. According to the International Classification of Headache Disorders, a headache is only classified as a migraine if it fits the description of a "recurrent headache manifesting in attacks lasting 4-72 hours. Typical characteristics of the headache are unilateral location, pulsating quality, moderate or severe intensity, aggravation by routine physical activity and association with nausea and/or photophobia and phonophobia."

Many scientists have tried taking a deeper look into what exactly causes a migraine, what transpires in the brain while a migraine is occurring, why some people are more prone than others, and how to successfully treat migraine patients. From the studies that have been done to relate what can trigger a migraine, very few have been successful and completed in their entirety. However, scientists have gained some knowledge over the past century based on diary studies, clinical questionnaires, and patient surveys. The most common triggers are known to be caffeine, stress, sleep deprivation, a woman's menstrual cycle which involves hormonal changes, and some foods (Marmura, 2018). This review will give an overview of those triggers and explain some possible reasons why these triggers will cause a migraine headache.

What is a Migraine?

Migraines can be broken down into 4 phases. A migraine begins with the premonitory phase, followed by aura (if present). It then proceeds into a migraine headache, and finally, the postdrome phase. Although there is a listed sequence of events, most migraineurs say that many of the phases overlap each other. For example, although exhaustion is associated with the premonitory phase, a migraine patient typically feels tired throughout the duration of the migraine (Goadsby et al., 2017).

The first phase is named the premonitory phase, and this occurs around 24-48 hours prior to the headache. Many people, including some doctors, believe that the migraine begins with a headache. However, contrary to the public's belief, some scientists admit that the headache phase of a migraine is already well into it. The premonitory phase notifies the patient of an upcoming headache. It can be observed as tiredness and having a hard time concentrating, as well as a feeling of irritability. The hypothalamus, which controls homeostasis, is said to be involved in this phase, due to altered homeostatic functions such as thirst, nausea, and frequent urination, which are observed during this phase. Studies done using positron emission tomography (PET) have shown increased blood flow to the hypothalamus during the premonitory phase, indicating a connection between the two. Seventy two percent of migraineurs can recognize when a migraine is coming, according to these premonitory symptoms (Goadsby et al., 2017).

Aura, which is found in one of three migraineurs, can present itself in numerous ways- mainly sensory and visual. Although symptoms play out differently in each patient, a sensory aura usually involves a patient feeling numb in one body part or limb. A visual aura can be characterized as visual hallucinations with flashing lights. Aura is correlated with cortical spreading depression (CSD), as

a result of experimental discussion. CSD is defined as a depolarization of glial cells across the visual cortex, followed by an electrical or chemical wave spreading across the brain (Goadsby et al., 2017). Animal studies have shown that CSD causes changes in blood flow as well as a release of neuropeptides involved in vasodilation and inflammation which is linked to migraine pain. CSD is also known to activate the trigeminal nociceptive pathways (Cui, Kataoka, & Watanabe, 2014).

The migraine phase is characterized, as stated above by the World Health Organization, as unilateral pulsating pain accompanied by photophobia or phonophobia, and very often nausea. Migraineurs report this pain as moderate to severe and tend to crawl into bed at this point.

The postdrome phase is very similar to the premonitory phase. It involves neck stiffness, drowsiness, and difficulty concentrating. In fact, scientists are unsure whether these symptoms persist throughout the entire migraine malady, or if they reappear after the headache phase. Interestingly, many migraineurs attribute these symptoms to the medication they took to relieve their headache (Goadsby et al., 2017).

Trigeminal Path

The trigeminal nerve is one of twelve cranial nerves. Its function is to supply sensation to parts of the head including the face and mucous membranes. This nerve is connected to many blood vessels throughout the brain, and it originates in the brainstem. Upon receiving signals from the meninges, which in turn releases neuroinflammatory mediators, the trigeminovascular system is activated and can cause a migraine headache (Goadsby et al., 2017). Scientists do not know why this signal occurs, and they continue to investigate its source. The release of inflammatory substances such as prostaglandins or serotonin, can cause the blood vessels near the end of the nerve to swell, which causes head pain (Professional, 2018). These inflammatory substances cause the surrounding tissue to be sensitive to otherwise normal activities, and therefore pulsations, which under normal circumstances are not felt, are perceived as throbbing pain in a migraine. In turn, anything that would cause one's heart rate to climb, and increase the rate of blood flow, will be perceived as pain during a migraine. Examples include exercise and physical activity (Amin et al., 2018). This may be the reason why migraineurs avoid daily physical activities during their attacks.

Symptoms

One of the countless symptoms related to migraine headaches, is known as allodynia. This refers to having pain from regular daily activities, such as brushing their hair, laying their head down on a pillow, scratching their scalp, or wearing contact lenses. This pain is perceived as a result of these small stimuli, through sensitization of migraine pain pathways (Moy & Gupta, 2020). Two thirds of migraine patients experience this symptom together with migraine attacks. This aspect of migraine pain is usually attributed to the more severe headaches and can be a pointer leading to the diagnosis of chronic migraines. Proven by studies that measure the brain signals during a migraine, sensitized thalamic neurons are said to be the cause of this overreaction towards normally harmless stimuli (Goadsby et al., 2017).

Photophobia

Photophobia is described by patients when light becomes bothersome and too bright. When most people are not bothered by this light, migraineurs can become aggravated by it. For example, in a study done, non-migraineurs were able to endure light until it reached the intensity of a sunny day, yet the migraineurs already reported pain and discomfort at the level of an overcast, rainy day (Rossi & Recober, 2015). Studies show that light can activate nociceptive thalamic neurons and worsen migraine pain. This is true even if the patient is blind yet has an intact optic nerve (Goadsby et al., 2017).

Triggers

Many ideas and hypotheses have been reported regarding the connection between migraine pain and triggers. A trigger is anything that causes a migraine to be more likely to occur. Migraines can be brought about by anything that directly or indirectly initiates vasodilation, activation of the trigeminal pathway or the brainstem, and cortical spreading depression. Dietary triggers can cause migraines by releasing norepinephrine and serotonin, which can act as vasodilators. Additionally, migraine attacks are more likely to occur when the migraine threshold is diminished by a trigger, which makes it easier to initiate an attack (Nowaczewska et al., 2020).

Many migraineurs cannot point to one specific thing that most likely causes their migraines, but rather a combination of a few factors (Nowaczewska et al., 2020). In fact, when asked what a possible cause of their migraine is, migraineurs most often cannot answer that question point blank, and are only able to identify some triggers when given a list of options. Confirming migraine triggers is almost impossible with the methods used most often such as diary studies, surveys, and questionnaires. This demands participants to think retrospectively and they very often confuse their thoughts with common migraine

misbeliefs, as opposed to actual symptoms that they've had. Electronic diaries seem to be the best method of recording data, as contributors can mark down their symptoms as they are experiencing them, and it is less likely for them to get confused (Nowaczewska et al., 2020).

Although some scientists believe that there are actual triggers that can bring on a migraine, others say that this is only a confusion of premonitory symptoms. They claim that a migraine has already begun 24-48 hours before the onset of pain (during the premonitory phase), and factors that others believe to be triggering the migraine, are only a consequence of premonitory symptoms. Some of these symptoms include food cravings, inability to stay focused and to concentrate, and fatigue. So, for example, when one feels tired as a result of this premonitory symptom in his already existing migraine, he is inclined to drink coffee or another caffeinated drink, and later feels the pain that is characterized as a migraine headache. This causes the patient to believe that coffee, or any other caffeinated food item, generates migraine headaches for him, when, in reality, it was only a cause and effect of his premonitory symptom. In the face of this confusion and difference of opinion amongst the scientists, the general public still believes that some factors, whether endogenous or exogenous, can cause one to be more prone to migraines (Goadsby et al., 2017).

A study was done in the Headache Outpatient Department of the University Medical Center at Hamburg-Eppendorf that proves this point, in which around 1000 participants were asked to highlight their so-called triggers and to relate the time interval in which it took to perceive head pain. While analyzing the results, 38.5% of the migraine patients claimed to have symptoms starting at the earliest 6 hours before the headache, and many of the presumed triggers were very closely related to leading premonitory symptoms. For instance, those who claimed flickering or bright light was a trigger for their migraine, also mentioned having photophobia during their premonitory phase. It is very likely that the flickering and bright lights were confused as a trigger and were in fact only early signs of a looming migraine. Researchers say that some 'migraine triggers' do have the ability to prompt a migraine, depending on which phase it is perceived (Schulte, Jürgens, & May, 2015).

If a migraine is said to be due to sensory neurons malfunctioning, then each individual's trigger threshold can be different, and even within one individual there can be different threshold levels during independent migraine attacks. It would therefore be clear, that triggers will only cause a migraine to materialize if the trigger reaches the sensory threshold. So, an external stimulus like an environmental factor or stress will only induce a migraine if it is above the threshold level during that specific time (Schulte, Jürgens, & May, 2015).

Caffeine

Caffeine has a dual job in migraine headaches. On one hand, caffeine is said to be beneficial to many migraineurs as it can lessen their symptoms or the length of their migraine headache. In fact, many migraineurs will choose their medication based on whether or not caffeine is included in its makeup. Yet, on the other hand, caffeine is said to be a migraine trigger, as well as being reported to cause headaches upon withdrawal. Coffee, tea, and other soft drinks all contain caffeine and have been listed as migraine triggers in around 10% of the population (Nowaczewska et al., 2020).

According to the Association of Migraine Disorders, when people enjoy consuming this substance to boost their energy, they tend to overdose. Their bodies then become dependent on having their daily coffee and no longer produce the same results as they have gotten accustomed to. They then try to consume more caffeine, hoping that a larger dose will increase the benefits, and run into a nasty cycle. If one day, they miss a coffee, they will most likely experience a caffeine withdrawal headache. This can occur after drinking coffee for as little as seven days. Caffeine withdrawal must be done gradually to avoid a migraine headache. Therefore, migraine patients are advised to be consistent with their amount of caffeine intake, and not to drink beyond 200 mg a day, about two servings of a coffee (this amount varies depending on how one makes their coffee) (Nowaczewska et al., 2020).

In a recent caffeine withdrawal study done in Norway, 80 participants were told to terminate their daily caffeine intake and replace it with either a capsule filled with caffeine or a placebo, identical looking. This study was randomized, and participants did not know which capsule they received. They divided the capsules of caffeine/placebo into smaller doses, to enable the participants to continue their way of life (i.e. having a few cups of coffee scattered throughout the day). Many of the participants dropped out before the end of the study due to withdrawal headaches. The study ended with nine participants, of which seven had intense migraines upon withdrawal from their usual intake of caffeine. When these participants continued with this daily dose for some time, they ceased to have migraines. However, one participant chose to reintroduce caffeine into his diet and consequently suffered from a migraine attack (Alstadhaug, Ofte, Müller, & Andreou, 2020).

Caffeine reduces the amount of urinary magnesium

by acting as a reabsorption minimizer. Magnesium is important in managing chronic pain as well as migraines. Therefore, if someone has a reduction of magnesium in their urine due to excess intake of caffeine, they will be more prone to having a migraine attack. Additionally, caffeinated drinks such as coffee can cause a person to need to urinate which can lead to dehydration, another well-known migraine trigger (Nowaczewska et al., 2020).

Sleep deprivation and Malnourishment

When looking at nuclei in the hypothalamus before and during a migraine attack, scientists noticed that they were more active than usual. These nuclei are involved in activating the trigeminovascular system and are recognized as migraine triggers. The hypothalamus regulates homeostasis regarding food and energy systems, circadian rhythm, and salt balance. Therefore, if someone skips a meal or is malnourished, or if they have constant interrupted sleep or reduced amounts of rest, they can be more likely to have a migraine attack. A study done on fasting revealed that 50% of fasting participants had a migraine headache (Goadsby et al., 2017). In fact, during Ramadan and on Yom Kippur, many fasters report having migraine headaches. An additional reason why fasting can cause a headache is due to caffeine withdrawal, as fasters miss their daily coffee.

A person's diet causes them to intake different amounts of needed vitamins and elements. Some foods will cause inflammation, release of nitric oxide, and vasodilation. A study conducted in Rome reported that increased consumption of whole-grain bread and pasta, thereby decreasing one's intake of white bread, was associated with fewer migraines and a significantly smaller amount of people needing medication to help with migraine pain (Hindiyeh et al., 2020).

Stress

Stress is another factor of disrupted homeostasis that can trigger a migraine. It seems that migraineurs are more susceptible to changes in the environment. When a migraineur who has enhanced sensitization of brain stem pathways experiences stress, it can be misinterpreted as an unwanted intruder and can cause a migraine attack (Goadsby et al., 2017). A study reviewed a migraineurs stress levels over a period of time, and indeed, stress levels were high in the days leading up to the migraine (Schulte, Jürgens, & May, 2015).

Menstrual Cycle

There is a 3:1 ratio of women getting a migraine versus men. This is most likely due to a woman's menstrual cycle, which is reported to be the cause of more than 60% of migraines in women (Silberstein et al., 1999). By their early 50's, almost half of the world's women will have experienced at least one migraine headache (Moy & Gupta, 2020). Although it is unconfirmed due to lack of successful studies, menstrual migraines seem to be due to estrogen withdrawal at the end of the menstrual cycle. Results of studies done, in which women were given estradiol, shows that their migraines were delayed until their estrogen levels dropped. In addition, women who were given gonadotropin-releasing hormone to help with in-vitro fertilization reported migraines as an aftereffect of a rapid estrogen level descend. When looked at from the other side, high levels of estrogen are shown to lessen the likelihood of migraines, like in women who are post menopause or in their second or third trimester of pregnancy (Sacco, Ricci, Degan, & Carolei, 2012). Towards the end of a woman's menstrual cycle, she has low levels of estrogen and serotonin. This causes her trigeminal nerves to stimulate the production of substance P and calcitonin gene-related peptide (CGRP). These two substances cause vasodilation and sensitization to the trigeminal nerve, both of which are thought of as migraine triggers (Moy & Gupta, 2020).

Genetics

While an average individual will not be affected by triggers, migraineurs, because of their sensitive protein receptors, will see these triggers as an enemy and cause an attack. The fact that some people are more prone to migraine headaches leads doctors to believe that there is some genetic factor (Nowaczewska et al., 2020).

Those who are taking medications like a hormonal contraceptive, can be more prone to migraines as their estrogen levels fluctuate drastically. (Moy & Gupta, 2020) Other medications that can cause migraines are those that contain high doses of caffeine and can lead to a caffeine withdrawal migraine (Nowaczewska et al., 2020).

Treatments

Although caffeine is most often known as a migraine trigger, it can also be used to treat migraines. Caffeine can act as a vasoconstrictor which can lessen the migraine's effect and is said to relieve head pain by 40% when combined with other drugs like acetaminophen and aspirin. However, patients should be sure not to overuse this method as it can lead to a caffeine withdrawal headache if one consumes too much over a short period of time (Martin, 2019). Additionally, during a migraine, it is understood that adenosine binds to specific receptor molecules, causing a widening of blood vessels. Caffeine, as a

vasoconstrictor, can help bring the blood vessels back to their original size, thereby reducing the risk of having a migraine (Shapiro & Cowan, 2017).

Sleep is another well known treatment. Patients report sleep as having a therapeutic effect on headache pain. Therefore, doctors try to choose sedative medication when deciding what to give the patient to aid in healing the migraine (Vgontzas & Pavlović, 2018). Other treatments include taking analgesics like acetaminophen, aspirin, and Excedrin. Treatments are specific to each patient and their distinct migraine symptoms and should be discussed with a physician.

Discussion

Many migraineurs have been advised by physicians or by medical personnel to steer clear of objects or situations that usually cause them to experience a migraine. However, studies have shown that avoiding common triggers can cause sensitization and an increased likelihood to have a migraine when one is later, perhaps unwillingly exposed to those triggers. Additionally, when people are constantly avoiding certain situations or specific foods, it can get frustrating and induce stress. This can be detrimental as stress is considered to be a migraine trigger as well. Furthermore, if we believe that migraines can be a consequence of sensory signals in the central nervous system overreacting to so-called 'triggers', getting the CNS used to these triggers and teaching it that they are not harmful, can be beneficial to those who suffer from migraines. Therefore, the public is recommended to continue their usual lifestyle, with these 'triggers' included in their daily living, so long as one does not yet feel the beginning of a migraine (Nowaczewska et al., 2020). However, all physicians will agree, that once a migraine patient feels the onset of the ailment, he should immediately do anything he can to avoid more pain, including staying away from things he knows can exacerbate his pain. Additionally, each individual is recommended to follow his/her migraine adventure with their physician, as each case is unique in its own way.

Conclusion

Scientists have not come across enough information regarding migraines to know if it is better to avoid triggers, or to learn to live with the pain. More investigation needs to be done before a clear decision can be made. Researchers should try to find migraineurs who will not drop out of the study before the end (due to headache pain), as a means to discovering more information on migraine headaches and their pathophysiology. To avoid the bias of migraineurs reporting triggers through a questionnaire, more investigations should be done via electronic diary, so the patients can report triggers as they are happening. This report includes data that helps the public better understand migraine triggers and their side effects.

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Is LSVT BIG an Effective and Realistic Treatment of Parkinson's Disease?

Elisheva Erlbaum

Elisheva Erlbaum graduated with a Bachelor of Science degree in Biology, January, 2021 and is accepted to Touro School of Physical Therapy.

Abstract

Parkinson's Disease is one of the most common neurological disorders which disrupts the everyday lives of millions of people. This disease is categorized by the loss of dopamine in the brain, specifically the Substantia Nigra. People diagnosed with PD suffer from motor and cognitive impairments. While there is no one treatment which can completely cure the disease, there are many treatments available which can help alleviate symptoms. However, most of the treatments cause many negative side effects. Recently, a new physical therapy treatment, LSVT BIG has begun to show its effects. This revue describes the many factors and treatments of Parkinson's disease and explains the newly discovered treatment of LSVT BIG.

Introduction

Parkinson's disease (PD) is a progressive neurological disorder which disturbs one's motor cortex, thereby affecting one's mobility. This disorder affects over one million people in the United States alone and more than ten million across the entire world (National Parkinson's Foundation, n.d.). Unfortunately, this disease slowly worsens from the time one is diagnosed. Even more, PD has a large range of indications and effects. While many with Parkinson's disease have similar symptoms, no two individuals experience the illness in exactly the same way. If you see someone shuffling down the street taking small steps or shaking their hands quickly and repeatedly in their lap, you can suspect that this person is suffering from Parkinson's Disease. What causes this neurological disease, and what can we do to slow the progression and treat the symptoms?

Methods

The information stated in the following paper was acquired through the Touro College Library, which grants its students access to many useful databases such as ProQuest, Ebsco, PubMed and others.

Pathophysiology

Patients with Parkinson's Disease are lacking in dopamine, which is normally produced in the substantia nigra part of the brain. The thalamus must have access to the motor cortex in the brain, specifically to the basal ganglia, in order to produce movement. Within the basal ganglia there are two pathways which have opposite effects on movement. The direct loop promotes movement, while the indirect loop inhibits movement. In a healthy person, the dopaminergic pathway maintains the balance between the other two pathways, enabling the thalamus to have control over the motor cortex and over the person's movements. This dopaminergic pathway works by exciting the direct pathway, while inhibiting the indirect pathway to promote movement. This happens in two ways. First, the substantia nigra sends dopamine to the striatum which excites the striatum and causes it to inhibit the globus pallidus. Thus, the globus pallidus is unable to obstruct the thalamus. Second, the substantia nigra sends dopamine directly to the globus pallidus, inhibiting it, so it cannot prevent the thalamus from controlling the motor cortex. In those with PD, much of the dopamine in the substantia nigra is exhausted. Consequently, the globus pallidus is excited and inhibits the thalamus. Thus, the thalamus has little control over the motor cortex (Singh, 2018). See Figure 1.

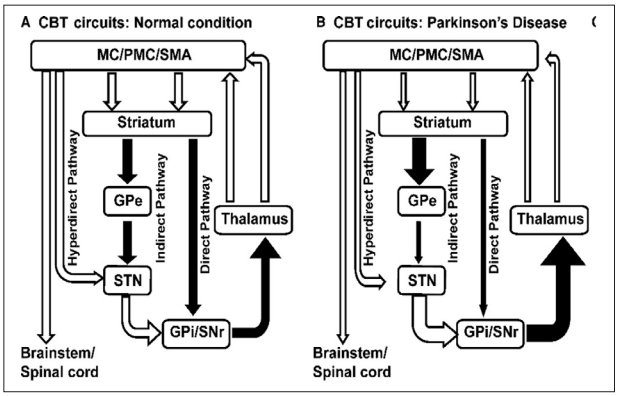

Figure 1

Figure 1: GPi = Globus Pallidus internus, GPe = Globus Pallidus externus, SNpr = Substantia Nigra, STN = Subthalamic Nucleous, MC = Motor Cortex, PMC = Pre Motor Cortex, SMA = Supplementary Motor Cortex The arrows thickness represents the amount of increase and decrease of neuronal actions. In the healthy person, the arrows of the direct and indirect pathways which are related to dopamine (explained above) are significantly thicker than the arrows in the PD patient. Furthermore, the Globus Pallidus internus has the power to inhibit the thalamus in the PD patient as demonstrated by the thickness of the arrows (Singh, 2018).

The lack of dopamine causes akinesia, bradykinesia and other motor deficits which are so commonly found in patients with PD. As the levels of dopamine decrease, the levels of Acetylcholine (Ach) increase. The increased Ach is what causes the symptoms of tremors and rigidity in PD patients. In other words, the loss of the dopaminergic system leads to disinhibition of the cholinergic system (Armstrong & Okun, 2020).

Another major marker for Parkinson's Disease is the existence of the Lewy Body. The Lewy Body is a "neuronal inclusion" which mainly contains a cluster of a-synuclein proteins (Bridi & Hirth, 2018).

Causes of Parkinson Disease

PD is believed to be an idiopathic disease being that scientists are unable to find one definitive cause of this Parkinson's disorder. However, there are factors that increase one's likelihood of developing Parkinson's disease. First, there are those who have a genetic predisposition. The most established or proven genetic cause of Parkinson's is mutations in what is known as the Parkin gene (Park2) (Shadrina, et al., 2007) or the PINK1 gene (PARK6) as they both inhibit normal mitochondrial function (Seirafi, et.al. 2015). Additionally, people who experience a head injury are at a greater risk for developing PD. High milk and dairy intake and exposure to pesticides can also increase one's risk for developing PD. Interestingly, research has found that there are certain steps one can take in order to reduce his/her risk of developing the disease, such as cigarette smoking and consumption of coffee or other caffeinated drinks (Ascherio & Schwarzchild, 2016).

Symptoms of Parkinson's Disease

PD is a progressive disorder, meaning there is no cure and the disease slowly worsens over time. Most people consider PD a disease which only affects movement, however this is not the case. Rather, Parkinson's causes a large range of symptoms and affects different systems in the body. Individuals suffering from PD perceive the world as much smaller than healthy individuals. Therefore, many of the symptoms are correlated to this viewpoint. For example, when a PD patient shuffles, one thinks that s/he is walking normally. (Fox, et.al. 2011).

Motor Symptoms

There are two groups of symptoms caused by PD. First, and more well recognized, are motor symptoms. Up to 80% of dopamine in the Substantia Nigra is depleted before these motor symptoms become noticeable. However, in order to be diagnosed with PD, people must demonstrate some symptoms. First, they must exhibit slow movement and low amplitude of everyday movements (shuffling of gait), otherwise known as bradykinesia. Then, they must also display one additional cardinal sign of Parkinson's, which include rigidity, akinesia or unable to initiate movement, lack of balance or tremors. As mentioned above, about 80% of PD patients suffer from limb tremors, usually concerning the hand, which is caused by increased levels of Ach. Furthermore, several years after initial symptoms, 25% -60% of patients with PD exhibit frozen movements, while 40% – 80% of PD patients have swallowing issues and 25% drool. Additionally, more than 50% suffer from speech impairments including quiet and hurried speech (Sveinbjornsdottir, 2016). Additionally, those with PD display a masked expression, also known as bradykinesia of the face. This happens because when a person is suffering from Parkinson's Disease, all movements slow down including facial movements. The delay of the facial muscles is what causes PD patients to lose their ability to express themselves via facial expressions (Bologna, et al., 2013).

Non-Motor Symptoms

Contrary to popular belief, Parkinson's is not just a movement disease, rather it affects non-motor abilities as well. Even before the motor symptoms start and the person is diagnosed, patients may experience a variety of non-motor symptoms. These symptoms can be divided into three sections: autonomic function disturbances, sleep disturbances and neuropsychiatric symptoms (Sveinbjornsdottir, 2016).

Autonomic Function Disturbances

Those with PD often experience rapid drops in blood pressure causing dizziness, visual troubles and weakened cognition which can ultimately cause loss of consciousness. Furthermore, the movement of the gastrointestinal tract slows down. This can cause overstuffed feelings, gastric retention and constipation. Constipation occurs in over 75% of PD patients. Also, those with Parkinson's suffer from an inability to control their urinary frequency and urgency. About 60% of PD patients display frequent nocturia, needing to wake up several times throughout the night to use the bathroom (Sveinbjornsdottir, 2016).

Sleep Disturbances

Roughly two thirds of PD patients suffer from an assortment of sleep disorders. This can be due to physical or cognitive limitations of the disease itself, or due to the different medications and treatments which are being used to treat the patient's symptoms. The most common ailment is fractionated sleep. Studies show that PD patients sleep lighter and have regular interruptions throughout the night. This fractionated sleep can also be a result of other PD sleep disorders such as frequent nocturia, difficulties with moving in bed and nocturnal tremors. Depression caused by Parkinson's can also disturb a patient's sleep throughout the night. Additionally, about

50% of people diagnosed with Parkinson's undergo extreme daytime sleepiness. Some say that this is somewhat caused by the dopaminergic drugs. Even more, 27-32% of PD patients kick and smack while dreaming and others uncontrollably jerk while sleeping (Sveinbjornsdottir, 2016).

Neuropsychiatric Symptoms

Many with PD suffer from various Neuropsychiatric Symptoms. The earliest indications consist of executive functioning issues, visuospatial disfunction, weakened speech fluency and memory loss. Additionally, in more than a third of PD patients experience hallucinations and illusions. Also, as the disease progresses some develop paranoia, particularly feeling suspicious towards their spouse. Moreover, dementia is also common in those with Parkinson's. Other common Neuropsychiatric symptoms include depression and anxiety. One third of PD patients have clinically diagnosed depression and 17% were confirmed to have a major depressive disorder (Sveinbjornsdottir, 2016). All symptoms, whether they are movement related or not, adversely affects the lives of PD patients.

Unified Parkinson's Disease Rating Scale

For any disease, when testing a new treatment, or tweaking a current treatment, there must be a way to measure the success. Specifically for Parkinson's disease, there are different ways of measuring the severity of PD symptoms, thereby displaying the treatments progress. Most frequently used is the Unified Parkinson's Disease Rating Scale (UPDRS). This rating tool is used to determine the severity of the PD patient's symptoms. It is divided into four sections which evaluate the symptoms' severity on a 0-4 scale. First, UPDRS I measures non-motor daily behaviors such as sleep, mood and brain activity. Second, UPDRS II assesses the patient's motor abilities for daily living, such as speech, chewing, handwriting, walking and drooling. Third, UPDRS III is the section which includes motor evaluation such as rigidity, gait and tremors. Last, UPDRS IV calculates treatment complications such as pain (Kleiner-Fisman, et.al. 2010).

Brief Overview of PD treatments

Although there is not a single cure for PD, there are different treatments which target specific symptoms of the disease. This includes both motor symptoms and non-motor symptoms. Furthermore, there are three types of treatments used to improve PD patient's way of life. There are medicinal treatments, surgical intervention and physical therapies.

Drug Therapy

Many of the medicinal treatments for Parkinson's Disease patients target the dopaminergic pathway, working to increase the dopamine in the patient's brain. While some medicines work better than others, no drug cures symptoms completely. The most commonly used drug is levodopa which increases the dopamine in the brain. Primarily, levodopa targets the movement disorders of PD. Although levodopa is still considered the most effective treatment, about 40% of patients develop motor complications after 4-6 years of levodopa treatments. There are other drugs taken to combat the side effects of levodopa (Dong, et.al. 2016). Another commonly used drug is amantadine. Many clinical trials have proved that amantadine can lessen the dyskinesia side-effect of the levodopa drug. It was also found to reduce the severity of freezing gait. Thus, improving PD patients' abilities to perform daily activities. Additionally, there are less frequently used drugs such as anticholinergic drugs. These drugs help by restoring the balance between dopamine and acetylcholine. Anticholinergic drugs are mostly used at the onset of the disease, when the primary symptom is tremors. The use of this type of medication is very limited, as there are many side-effects which some believe outweigh the benefits (Dong, et.al. 2016). Side-effects include decreased cognitive function, especially in older PD patients, constipation, blurred vision and tachycardia (Lertxundi, et al., 2015).

Surgical Treatments

There are many surgical interventions which were proven to be beneficial for PD patients. One such method is called Deep Brain Stimulation (DBS). This surgery places electrodes at certain places in the brain which control movements. They are either placed in the subthalamic nucleus (STN), the globus pallidus interna (GPi), or the ventralis intermedius (VIM) nucleus of the thalamus. DBS improves patients' movement abilities and may lessen tremor severity. Yet, unlike other pharmaceutical drugs, DBS has demonstrated little to no side-effects (Chou, Grube, & Patil, 2012, p. 15). Another surgical treatment is cell transplantation. This treatment has proven to be effective on motor symptoms of PD. This works as the transplanted stem cells can "infiltrate and integrate with diseased tissue, differentiate into dopaminergic neurons to replace damaged cells and reconstruct neuronal circuits to restore nerve function (Li, 2012)."

Physical Therapy

A major issue with medication for PD patients is that the effectiveness of the medicinal drugs decreases over time.

As a result, the creation of different exercises and physical therapies have also proven to be an effective way of combating Parkinson's disease. First, basic exercises lessen the motor and even non-motor symptoms of those with PD. Exercise increases mitochondrial respiration and arouses neuroplasticity. Furthermore, recent clinical trials recommend aerobic exercises. They claim that these exercises improve gait, physical performance and balance. They also state that they impact fatigue, depression and improve cognitive ability. Additionally, a Chinese exercise called Tai Chi, through breathing exercises combined with slow movements, has improved the balance of PD patients (Dong, et.al. 2016). Finally, LSVT BIG, a relatively new approach to physical therapy in PD, has begun to show its effectiveness.

What is LSVT BIG?

LSVT Big was created based on the success of a treatment called LSVT Loud. First introduced in 1995, LSVT Loud was proven to be an effective way of improving the strength and loudness of the voice of PD patients. LSVT Loud is a treatment which focuses on how one feels and sounds when talking loudly. Unlike many speech treatments which target many different speech systems, LSVT Loud primarily focuses on increasing movement in the respiratory and laryngeal systems. Currently, after conducting many studies and experiments, LSVT Loud was proven to be a successful treatment (Fox, et.al. 2011). After the success of LSVT-Loud, in 2005 LSVT-BIG was first introduced (McDonnell, et al., 2017).

LSVT-BIG was created to combat the hypokinesia or reduced amplitude movements which interfere with the everyday lives of people with PD. The goal of the treatment is to revise the way the patient perceives movement performance. LSVT BIG therapy includes four consecutive, one on one, 60-minute sessions per week in addition to one (on treatment day) or two (non-treatment days) 15 to 20-minute home sessions per day. When performing any action, patients are told to conduct large amplitude movements. The beginning of each out-session includes seven whole-body exercises such as reaching in more than one direction, weight shifting and stepping. Next, the patient works on five every-day movements, such as shifting positions, rolling over in bed and any others that the patient requests to improve. Third, the patient practices walking with larger amplitude, while standing straight. At the end of the session, the patient works on more complicated tasks which are directly related to his/her goals and abilities. Later in the treatment, this final segment would increase in difficulty by either adding an additional task or by enhancing the existing mission (Isaacson, et.al. 2018).

Experiments Conducted

To prove the effectiveness of LSVT-BIG, there were numerous studies and experiments done on all different types of people at all stages of Parkinson's disease. One such experiment performed, compared the results of 41 patients involved in general exercise to 43 patients participating in the LSVT-BIG program. After sixteen weeks, they found the motor function of the LSVT-BIG patients to have improved significantly more than the other 41 patients. Additionally, it was found that those participating in the program increased their speed of moving. Another study found that LSVT-BIG helped decrease response time when prompted (McDonnell, et al., 2017).

Another study was done to see if LSVT-BIG also impacted the duration of a patient's 'off time,' meaning the time of day where one feels at his/her low. Eight patients participated in the study. Before starting treatment, each patient was required to report their 'on' and 'off' durations for three days. Then they were evaluated under the UPDRS III scale on motor ability. Then they began treatment. The changes were measured every four weeks by ministering different tests. The results proved that LSVT-BIG therapy does lessen the duration of 'off' time as it did significantly improves the UPDRS III scores (Ueno, et al., 2017).

In addition, a non-controlled study was performed which examined the effects that LSVT BIG had on eighteen PD patients. It was found that after just four weeks of therapy, the participants experienced a 12-14% increase in walking and walking rate (Fox, et.al. 2011).

A different blind study was done which took sixty people suffering from Parkinson's disease and randomly assigned them to one of three therapies, LSVT BIG, Nordic Walking in a group setting, or regular exercise with no therapist. After four months of treatment, the average improvement of the scores on the UPDRS III on patients receiving LSVT BIG therapy was 5.05, while the scores of those undergoing Nordic Walking or domestic training worsened. This amount of scaled motor increase clinically proves that LSVT BIG does improve the symptoms of PD patients (Fox, et.al. 2011).

Another case study included twelve idiopathic PD patients who underwent LSVT BIG therapy treatment. There were also two control groups: one with eight PD patients who did not undergo any physical therapy and the other included fourteen healthy individuals who experienced the same experiment as the case study. The PD LSVT BIG patients were given sixteen sessions, four sessions per week for four weeks. Each session was an individualized meeting with a licensed LSVT BIG therapist and lasted one hour each morning. Each appointment began with

using the patient's entire body including "BIG" amplitude exercises and "repetitive multidirectional movements." The second half of the meeting included daily activities according to what the patient wished to focus on such as buttoning shirts and putting on a coat. The results showed the gait speed and the step length of patients undergoing LSVT BIG increased. Furthermore, the LSVT BIG patients displayed better functional mobility as they were able to transition from sitting to standing faster and more often. The LSVT BIG patients' improvements were significantly greater than the PD control group (Flood, et al., 2020).

Each of the experiments described above prove the effectiveness of LSVT BIG therapy. There were countless of other case studies conducted whose results were similar to the outcomes stated previously. Thus, it is safe to conclude that LSVT BIG is an effective way of treating Parkinson's disease.

Conclusion

Parkinson's Disease is a neurological disorder which affects tens of millions of people across the world. Primarily, the disease is associated with progressive loss of dopamine in the brain. People with PD are affected in many different areas of life including motor skills and cognitive abilities. Unfortunately, researchers have not found one perfect solution to completely cure Parkinson's Disease. Rather, there are only palliative solutions, treatments which either ease the symptoms or slow the progression of the disease however, doctors don't often give patients medication for two reasons. Often, many of the commonly used medicinal treatments can cause difficult side effects. Secondly, as time progresses, the impact the medicine has on the body weakens as the patient becomes accustomed to the drug. Therefore, doctors and therapists are beginning to use more non-invasive treatments such as exercise and Physical Therapy. Recently, there has been a new treatment, LSVT BIG, which targets the amplitude of the PD patients. This treatment has continuously proved itself effective and worthwhile. Every Parkinson's patient should have the opportunity to undergo LSVT BIG treatment.

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Effects of Soy Isoflavone

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Abstract

Soy, which contains a form of phytoestrogen known as isoflavones, impacts the biological activity of humans at all stages. There are many aspects to consider when determining whether soy is beneficial. Since the hormone estrogen plays a significant role in maintaining the biochemical and homeostatic conditions of an individual, it follows that the disruption of estrogenic levels can be detrimental. There are many hormone- dependent diseases that can be linked to one's diet, and the possibility of utilizing phytoestrogens, such as soy, to prevent or control hormonal irregularities is compelling. This paper explores the effects that phytoestrogens, specifically soy, can have at various stages of hormonal progression.

Introduction

Recently, soy has been garnering attention for its estrogen-like qualities. One group of chemicals found in soy are phytoestrogens, a non-steroidal class of estrogens. Of the numerous phytoestrogens, the study of the role of isoflavones has been isolated because of their high concentration in soy. Crucial to understanding soy's "estrogen-like" qualities is understanding isoflavones' chemical makeup. In soy specifically, isoflavones present as glycosides, which are bound to sugar molecules. When the isoflavone glycosides are digested and released, the isoflavone presents as aglycones. This includes compounds such as genistein, daidzein, and glycitein.

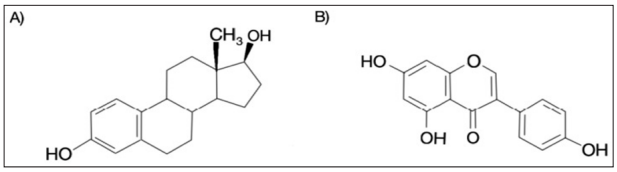

Figure 1. Chemical structures of 17β-estradiol (A) and genistein (B) (Thangavel, 2019)

Structurally, these isoflavones are similar to a form of estrogen, 17β-estradiol, as shown (fig, 1). The phenolic ring on the isoflavones is a crucial part of its structure which chemically allows the phytoestrogen to attach to estrogen receptors, thus promoting "estrogen-like" activity. Once the isoflavone is bound to an estrogen-receptor complex, the phytoestrogen can act as an estrogen agonist or antagonist. Based on various factors, such as the concentration levels of the phytoestrogen and endogenous estrogen, the specificity of the receptor, and the particular tissue that the phytoestrogen is targeting, the soy will either act as an estrogen inhibitor or stimulant. This holds promise as a physiologically beneficial method of regulating hormonal abnormalities. Dietary estrogen has shown potential in alleviating postmenopausal symptoms, reducing the risk of breast cancer, and bone resorption. However, soy is dosage- dependent, and a high intake of soy in infancy may lead to some physical signs of sexual maturation at a young age. Soy has also been a contributing factor for both very early and late menarche. There is a concern for early maturation, as it can cause hormone- related cancers such as breast cancer. Therefore, the consumption of soy that leads to the binding of isoflavones to sex-hormone binding globulins (SHBG) necessitates much research to determine all possible outcomes (Setchell, 1998).

Methods

The following research is based on the analysis of numerous articles from various databases, that include but are not limited to The Touro College Library online, PubMed, and ProQuest. The National Center for Biotechnology (NCBI), and BioMed Central (BMC) were additional sources of data as well.

Discussion

Infants

Soy- based infant formula (SBIF) has been used for over 100 years. SBIFs were developed as an alternative to cow's milk-based formula for infants that were lactose intolerant or required vegan replacement. Recent data has shown that SBIF is used by approximately 20%-25% of Americans. SBIF has a high concentration of isoflavones; the concentration levels vary from 32 to 47 mg isoflavones/L of formula. According to research, "infants fed SBIF are exposed to a 6–11 fold higher level of isoflavones on a body weight basis than adults. Additionally, circulating isoflavone levels of these infants were 13,000–22,000 times greater than circulating levels of 17-β-estradiol." (Dinsdale et al., 2010) The effect of an infant consuming and absorbing this high of a dosage of an estrogenic-like substance can have long-term consequences. This is because SBIF is primarily used from birth to one years old, which is a stage of development that is "particularly sensitive to dietary and environmental compounds." However, it is exceedingly difficult to track soy formula's precise long-term effects on reproductive health. In order to so, all participants must be monitored before reaching puberty and all through the child-bearing years; this would allow the possible differences in reproductive health and organ development to be determined. Since that would require long-term compliance, a retrospective approach has been taken to analyze the effects on those who consumed SBIF. However, as a retrospective study, there are the limitations of recall bias and environmental differences, such as smoking, physical activity and history of diseases, which make it difficult to determine

the accuracy of the results. No reports were given of long-term adverse effects for the male infants fed SBIF. At six months of age, female infants were reported to have increased vaginal cell maturation. There did not seem to be any vaginal discharge or breast development that was out of the ordinary. However, when children at two- years -old were still fed SBIF, there was an increase in the pervasiveness of breast tissue development in these children. Furthermore, the study reported that formula feeding in general, whether soy- or cow's milk-based, can cause greater ovarian volume, an increase in the numbers of ovarian cysts per ovary, and lower testicular volume.

On the other hand, SBIF and soy in general seem to prevent breast cancer. High levels of isoflavone enhance differentiation of the mammary glands, which leads to greater protection against chemically-induced mammary cancer. This was proven through a study in which genistein treatment was given, resulting in "fewer terminal end buds and advanced development and ductal elongation." As the least mature terminal ductal structures, it follows that terminal end buds are the most susceptible to possible carcinogens. Therefore, reducing the number of terminal end buds can lead to lower incidences of breast cancer. This is due to the fact that, "Part of the terminal end bud differentiates according to each estrous cycle, giving rise to alveolar buds that consist of lobule structures that are more mature and less susceptible to chemical carcinogens." This treatment of genistein increased the number of lobules, which shows that there is a potential protective effect against mammary cancer. The exact mechanism in which genistein affects mammary gland development is unclear; however, according to these findings, genistein clearly displayed estrogenic-like behavior (Dinsdale et al., 2010).

Table 1

Exposure	No. (%)	≤10 years	11 years	14 years	≥15 years
		rRR (95% CI)	rRR (95% CI)	rRR (95% CI)	rRR (95% CI)
Soy formula					
Ever	1,066 (4)	1.21 (0.94-1.54)	0.95 (0.78-1.15)	1.17 (0.98-1.40)	1.28 (1.06-1.56)
None	27,027 (96)	1.00	1.00	1.00	1.00
Breast fed					
Ever	12,961 (41)	1.01 (0.92-1.11)	1.00(0.93-1.08)	1.03(0.96-1.10)	0.96 (0.89-1.04)
None	18,478 (59	1.00	1.00	1.00	1.00

Relative Risk Ratios for Early and Late Menarche in Association with Early-Life Exposures in Women Aged 35 to 59 years at Baseline in the Sister Study, 2003-2009 (n = 33,501) (D'Aloisio et al., 2013)

Further studies show that high soy exposure during the prenatal and infant stages can cause early onset puberty, specifically menarche. Early menarche (≤10-11 years old) is just one of the factors that are considered when determining whether a female reached puberty; however, it is the most commonly used benchmark. There are many issues associated with early development, such as adult obesity, type 2 diabetes, metabolic syndrome, and other markers of cardiovascular disease. A study was done to determine the various factors involved in early menarche, soy being among them. The Sister Study enrolled 50,884 American and Puerto Rican women between the ages of 35-74. The study was interested in finding whether certain early-life exposures are related to the health conditions mentioned above. The authors conducted interviews that included a comprehensive family medical history. Anyone that did not have a mother to relate the early life events was excluded. The study combined those that said they "definitely" or "probably" had feeding exposures into one category, and those that responded that they "definitely did not" and "probably did not," into another category. Race/ethnicity, maternal age menarche, birth weight, birth decade, and childhood family income were also factored into the results. Results showed that, "The frequency of early menarche (≤11 years) was 20%, with 7% reporting menarche at 10 years of age or younger. The frequency of late menarche (≥14 years) was 24%, with 10% reporting menarche at 15 years of age or older."

The table above shows that SBIF can cause both early (≤10 years old) and very late menarche (≥15 years old). Although age at menarche is not the only sign of pubertal development, it is a key component; therefore, these studies are an important resource to tracking pubertal development. These findings are consistent with animal data that proves that the dose of genistein impacts whether early or late puberty will occur. "Mice administered a higher dose of genistein had a delayed vaginal opening (marker of puberty), while mice given a lower dose had an accelerated vaginal opening. Neonatal administration of genistein to mice has also produced other alterations in reproductive characteristics, including changes in estrous cycles, early reproductive senescence, and decreased fertility." In other words, the effects of neonatal administration of

genistein were greater than when genistein was administered to older mice. This makes sense considering that soy formula delivers a high dosage of its estrogenic components to infants "per unit body weight." Additionally, genistein is highly digestible and has been found in high concentrations in infants' urine and plasma. Thus, maintaining that the genistein can influence the estrogenic-like effect on these infants (D'Aloisio et al., 2013).

Furthermore, while there is no evidence that SBIF directly effects reproduction, SBIF does exert its influence on the menstrual cycle. Women who were given soy formula as infants reported prolonged menstruation and more discomfort. With all this data, the question of whether or not soy formula is safe remains. Many countries have regulations on the use of SBIF. For instance, in Europe, SBIF is a prescribed product. However, despite these regulations, there is a large percentage of infants each year that are fed SBIF (Dinsdale et al., 2010).

Male Reproduction

Considering isoflavones' estrogenic qualities, it follows that there can be estrogenic effects on males that consume high concentrations of soy. There are not many cases reported of soy stimulating feminizing effects on men, such as gynecomastia. However, a study was done on an instance of hypogonadism and erectile dysfunction that was linked to high intake of soy. A 19-year-old male that was recently diagnosed with type 1 diabetes began experiencing complete loss of libido and erectile dysfunction. Until the previous year, he had been in perfect health. He stated he had a heterosexual preference, was sexually active, had satisfactory libido, denied any history of orchitis or undescended testicles, had normal testicular size, and body hair pattern. Additionally, he denied any androgen abuse, hormonal medication, drug abuse, or psychiatric disorders. He had never had a sexually transmitted disease, no visual or muscular mass and strength changes, and no headaches. The one notable difference in his lifestyle was that he had taken on a vegan diet, which included high levels of soy (360 mg/day), due to his recent diagnosis of diabetes. A lab assessment revealed that he had low free and total testosterone levels, with increased levels of dehydroepiandrosterone (DHEA). His symptoms began after he started his vegan diet, which included products such as soy milk, soy cookies, tofu, soy sauce, soy nuts, and soybeans. Beforehand, he had been consuming the average American 2,000- kcal diet. After stopping his vegan and soy diet, his symptoms improved over the course of 12 months. These improvements were consistent with the gradual normalization of his testosterone and DHEA levels. The table below shows the progression of the patient's levels of total and free testosterone, as well as the levels of DHEA normalizing over a period. The insufficient blood levels of free testosterone, which causes hypogonadism and erectile dysfunction, normalized after the cessation of the soy diet, indicating that the high levels isoflavones may have been the cause.

The correlation between testosterone and soy may be explained by research on animals. "Animal studies have shown that isoflavones can bind to the estrogen receptor expressed by testosterone-producing Leydig cells, thereby affecting Leydig cell differentiation and testosterone production, which ultimately leads to reproductive toxicity." Therefore, consuming high levels of isoflavones can stimulate similar bioactivity in males. Another possibility is that the estrogen- like isoflavones can cause a negative feedback mechanism that interferes with the DHEA conversion to testosterone. As the DHEA converting enzymes 3-b-hydroxysteroid dehydrogenase and 17-b-hydroxysteroid dehydrogenase are inhibited through the isoflavones genistein and daidzein present in soybean, the negative feedback is facilitated. The inhibition is what can lead to a decrease in testosterone. Another possible way in which the isoflavones decreased the free testosterone levels can be through the increase of the sex hormone-binding globulin (SHBG), which is produced by the estrogenic activities of isoflavones. The increase of SHBG leads to more binding of the SHBG with the total circulating testosterone, which means there will be less free testosterone; this also further explains why the total circulating testosterone levels were within normal range (table 2). However, because there were missing SHBG measurements during the vegan diet, this hypothesis cannot be confirmed. There were nine more studies done in which the highest level of isoflavone concentration given was 139 mg/d, as opposed to the 360 mg/d given in this study. As this was the only study done that there was clearly a testosterone decrease with

Table 2

Serum Concentration	Normal ranges	Day 0: cessation of vegan diet	Day 15	Day 158	Day 724
Total testosterone (ng/dL)	260–1000	339	344	361	463
Free testosterone (pg/mL)	50–210	35.5	41.4	57	86.8
Free testosterone (%)	1–2.7	0.95	1.2	1.6	1.9
Unconjugated DHEA (ng/dL)	180–1250	1976			714
Estradiol (pg/mL)	7.6–42.6			19	30
Progesterone (ng/mL)	0.2–1.4			1.7	1.5

(Partial) Hormone profile at cessation of vegan diet and during 2-y follow-up (Siepmann et al., 2011)

the consumption of soy, researchers speculate that there is an "isoflavone intake threshold" for symptoms to occur. There is also the possibility that since the composition of soy products has evolved over the years, there might have been differences in the soy products of the previous studies. While further studies are necessary, this case report successfully indicates that a high consumption of isoflavones is related to hypogonadism and decreased free testosterone (Siepmann et al., 2011).

Menopause

Phyto-estrogens that are administered in an environment that has a high concentration of endogenous estradiol have an antagonist effect on the estrogen receptors. In that case, the isoflavones act as an inhibitor. However, when there is a decrease of endogenous estrogen, such as hypogonadism and menopause, an administration of isoflavones will stimulate estrogenic bioactivity (Casini et al., 2006). Menopause symbolizes the end of a women's reproductive life and is characterized by the cessation of menstrual periods for 12 consecutive months, which normally occurs between 45-55 years of age. Aside from the cessation of menstrual periods, there are additional symptoms, such as hormonal disturbances, hot flashes, night sweats, sleeping disorders, vaginal dryness, joint pain, mood swings, reduced bone density, and cardiovascular disease (Sunita et al., 2011). This mainly occurs because the menopause transition results in estrogen deficiency. Estrogen regulates appetite, cholesterol levels, carbohydrate and lipid metabolism, and protects bone. Therefore, when a woman is estrogen- deficient, the possibility of obesity, osteoporosis, and cardiovascular disease is more prevalent. To combat the hormonal imbalance, there are various forms of hormone replacement therapies (HRT). However, there are a host of issues with the with the HRT available, because the treatments available can cause diseases such as thromboembolism, uterine hyperplasia, uterine cancer, increased risk of breast, ovarian, and endometrial cancers, coronary heart disease, and stroke. Therefore, in more recent years women have been turning toward more natural methods of HRT, such as soy, to alleviate menopausal symptoms. Genistein promotes an inhibitory effect on many of the common menopausal symptoms. "It should be noted that genistein acts on various molecular pathways to emulate the effects of estrogens, without being known to elicit any life-threatening adverse effects." There have been studies done to determine how effective this form of therapy may be; however, this is still a relatively recent discovery and there are many aspects that need further exploration (Thangavel et al., 2019).

The Asian diet is heavenly concentrated with soy, relative to the European and American diet. Statistically, only 20%-25% of Asian postmenopausal women experience hot flashes, whereas 70%-80% of European and Latin American postmenopausal women complain of hot flashes. Since these hot flashes are one of the main postmenopausal symptoms that women seek HRT for, researchers were compelled to discover what caused Asian women to have a lower incidence rate (Thangavel et al., 2019). There are a few studies that have reported the effectiveness of genistein in treating hot flashes. A study was done on Japanese women specifically, comparing soy intake to incidences of hot flashes. This study was conducted over six years, in which it was shown that the consumption of soy reduced the hot flashes considerably. Even at the lowest concentration of isoflavones (75.2- 115.9 g/day), the hot flashes were lower, but the response rate was definitely more successful in the women that consumed higher levels of soy (Nagata et al., 2001). Another study was done on menopausal women and found that the "administration of 30 mg of genistein for 12 weeks reduced hot flashes by 51% (9.4–4.7/day), whereas the placebo group experienced only a 27% reduction (9.9–7.1/day)." (Braxas et al., 2019) Furthermore, a randomized trial was conducted in which hot flashes were alleviated by the consumption of 54 mg/day over the course of a year. The mechanism in which genistein can inhibit the hot flashes is unclear. However, researchers speculate that the genistein diffuses through the cell's lipid bilayer "due to genistein being an effective ER (estrogen receptor) modulator." This will set off the mRNA synthesis and production of tissue-specific proteins (Crisafulli et al., 2004). While there is no definite conclusion as to why genistein inhibits hot flashes, it seems to be a successful method based on the results of the numerous studies above.

Another effect that occurs because of estrogen depletion is the change in metabolism, such as the slowing down of the body's ability to metabolize carbohydrates and lipids. Consequently, women in the menopause stage are susceptible to weight gain. Genistein was found to stimulate a metabolic rate of carbohydrates and lipids to help prevent weight gain. This occurs because genistein regulates adipose tissue by restricting lipogenesis and enhancing lipolysis in adipocytes (Szkudelska et al., 2007). Furthermore, phytoestrogen treatment "reduced obesity markers, such as total cholesterol and low-density lipoprotein (LDL) cholesterol." These are common markers that are associated with obesity and cardiovascular health (Jayagopal et al., 2002). Additionally, another study reported that after 6 to 12 months of 54 mg/day of genistein treatment there was a significant reduction in triglycerides, total cholesterol, and

LDL-cholesterol (Squadrito et al., 2013). Conclusively, soy treatment can potentially improve cardiovascular health and prevent obesity.

There seems to be a positive correlation between a soy rich diet and the inhibition of cancers, specifically breast cancer. SBIF can inhibit breast cancer as well. Most of the studies compared the association of soy-rich diet with reduced breast cancer in Asian women, compared to those in the Western world. In one case, an Asian woman who migrated to a Western country showed an increased risk for cancer. The risk of breast cancer increases with age, and this form of cancer has become one of the most common forms of cancer affecting women. As the common forms of HRT are known to increase the risk of developing breast cancer, natural methods are a compelling substitute. Genistein, the form of isoflavone that is the most abundant in soy, has shown anticancer properties in preclinical trials, thereby opening the option of attempting clinical trials. Since this bioactive compound, soy, induces apoptosis in various cancer cell lines, such as HepG2 and Hep3B, in- vitro studies have proven the efficacy of genistein as a promising chemotherapeutic agent against cancer. Table 3 summarizes some of the common menopausal symptoms and how genistein can alleviate the symptoms (Thangavel et al., 2019).

Table 3

Symptoms/Disease	Genistein Effects
Vasomotor	Reduction of hot flashes, night sweats, and sleep disturbances frequency; as well as depression symptoms and memory loss
Cardiovascular	Reduction of myocardial necrosis, macrophage and serum levels of TNF-α, severity of atherosclerosis, and myocardial infarctions incidence
Obesity	Reduction of serum concentration of total cholesterol, LDL, triglycerides, and HDL
Diabetes	Reduction of fasting glucose concentration, insulin resistance, and improves glycemic metabolism
Cancer	Reduces the incidence of breast, hepatocellular, lung, gastric, and ovarian cancer
Stress responses	Improves 5-HT metabolism, stabilizes MAO activity, and improves turnover ratio of 5-HIAA/5-HT

Abbreviations: 5-HIAA: 5-Hydroxyindoleacetic acid; 5-HT: serotonin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; MAO: monoamine oxidase; TNF-α tumor necrosis factor alpha.

Effects of genistein on menopause symptoms and some related diseases.

In addition to the diseases mentioned above, osteoporosis is another disease that postmenopausal women are prone to. Since estrogen has bone- protecting properties, it follows that a lack of estrogen puts menopausal women at risk of developing osteoporosis. Soy has been reported to reduce the risk of osteoporosis in peri- and post-menopausal women. A study was done examining the effects of a heavy soy diet on the overall bone density and strength of postmenopausal women. Eighty-seven women were eligible and were assigned to consume soy or control foods daily for one year. "Bone mineral density (BMD) and bone mineral content (BMC) of the whole body, lumbar (L1-L4), and total hip were measured using dual energy x-ray absorptiometry at baseline and after one year (Arjmandi et al., 2005)." Additionally, urine and blood markers of bone metabolism were assessed. Of the eighty-seven women, sixty-two completed the one-year long study. Results showed that whole body and lumbar BMD and BMC decreased in both the soy and control groups. However, the total hip BMD and BMC did not change in either group. "Both treatments positively affected markers of bone formation as indicated by increased serum bone specific alkaline phosphatase (BSAP) activity, insulin-like growth factor-I (IGF-I), and osteocalcin (BSAP: 27.8 and 25.8%, IGF-I: 12.8 and 26.3%, osteocalcin: 95.2 and 103.4% for control and soy groups, respectively)." (Arjmandi et al., 2005) Neither group had any effect on urinary deoxypyridinoline excretion, which is a marker of bone resorption. However, according to epidemiological data, populations with soy rich diets, such as the Asians, do have a lower incidence of osteoporotic fractures. On the other hand, there are numerous factors that contribute to skeletal health; therefore, the credit of the lower rates of fractures in these populations cannot be fully attributed to soy consumption. There are a number of animal studies that did show how isoflavones positively influenced BMD. However, when it comes to human studies there are a limited number of studies that examined the effects of soy on bone. A study was set up in which the identity of the individual treatments was only revealed after the analysis was complete, to keep the results as accurate as possible. Compliance of the participants was measured based on whether participants recorded the amount of soy they consumed and if they returned the uneaten food to the study site to be tallied. This study did not show that soy protein alone can substantially prevent bone loss. However, another study did indicate that a consumption of 40 mg/day to 80 mg/day for a year resulted in positive increases in BMC of the hip of the women who are at least four years post menopause. Additionally, these women were of low body weight or had low levels of dietary calcium. In the study

done in which there was no substantial effect of soy on reversing bone loss, most of the participants were four years post-menopause; however, many did not have low body weight or calcium intake. Therefore, it is possible that the differences in these factors are what created the discrepancy of the observed results of the bone (Chen et al., 2004). As far as which form of isoflavones is the most beneficial when it comes to affecting the bone, according to studies genistein is the single isoflavone with the greatest effect. A study demonstrated that genistein at a dose of 54 mg/d combined with HRT increased BMD in early postmenopausal women. Genistein was proven to increase the BMD of the femoral neck by 3.6% and the lumbar spine by 3.0%. HRT increased femoral neck and lumbar spine BMD by 2.4 and 3.8%. The researchers speculated that the genistein "reduces bone resorption markers" and "enhances new bone formation parameters." As a result, there is a bone mass net gain (Morabito et al., 2002). According to the circulating sex hormone levels that were assessed after examining the changes in the participants after a year, the soy did not produce any estrogenic effects. However, the soy did decrease SHBG concentrations, which increases the availability of circulating estrogens. Therefore, the soy did affect estrogen levels, even though it may not have directly exhibited estrogenic qualities. However, to confirm this, it would be necessary to measure the endogenous estradiol that is circulating. While there are certainly a number of studies that show correlation between bone protection and soy, there are still too many factors that require closer examination (Arjmandi et al., 2005).

Conclusion

Many soy- related articles are published annually; however, there are still numerous questions that require further exploration. Questions include whether SBIF is indeed harmful or not, how effective of an HRT is soy when it comes to menopausal symptoms, whether the effect of isoflavones on bone is transitory, and whether soy isoflavones and lower doses of antiresorptive agents can prevent postmenopausal bone mineral loss. In the United States, advisory groups gave their recommendation regarding the safety of soy infant formula. "The North America Committee on American Academy of Pediatrics recommends soy formula only for infants with galactosemia or hereditary lactase deficiency and mentions that soy formula might be useful for families wishing to avoid formula containing animal products." (Adgent et al., 2018) While high consumption may not be advisable for infants, Asian postmenopausal women with soy rich diets benefit from the isoflavones as shown by the studies mentioned above. The efficacy of soy and its isoflavones as an alternative to HRT and as a natural estrogenic source for treatment has yet to be determined.

Although the biological effects of soy hold potential, there are limitations, such as low bioavailability and low biological estrogenic activity. This has limited the clinical applications of genistein to some extent. However, the components of soy are promising in terms of being a natural source of preventative care.

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The Relationship Between Autoimmunity and Polyautoimmunity

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Abstract

Autoimmune disease refers to a systemic immune response by the body against its own healthy tissue and cells. This results in various non-specific and systemic inflammatory processes that evolve into more than 100 individual diseases. Numerous biological similarities exist between the different pathophysiological pathways, including biochemical cascades and inflammasome mediators. This paper aims to investigate whether contracting one form of autoimmune disease can lead to the development of polyautoimmunity and multiple autoimmune syndrome. Scientists have identified chronic levels of high stress as a contributor to higher levels of C-reactive protein and several immune modulating interleukins, which can lead to both autoimmune and polyautoimmune processes (Steptoe et al., 2007). Immunologists and virologists have established how viruses can lead to autoreactive immune activity through molecular mimicry and epitope spreading. These same processes are found in all forms of inflammation and may explain the connection between undiagnosed adult-onset celiac disease and multiple autoimmune syndrome. Many genetic pathways have been identified as drivers of both individual autoimmune conditions and specific inflammasome mediators that could be responsible for familial based autoimmunity and specific subtypes of multiple autoimmune syndrome. Collectively, these studies underlie and illustrate a direct connection between several contributors that definitively link autoimmunity with polyautoimmunity.

Introduction

Autoimmunity is defined as the misdirected immune response by an organism against its own healthy tissue and cells. Encompassing as many as one hundred individual diseases, autoimmune disease affects nearly 23 million Americans of all ages and has been referred to by many as the next frontier in the battle for longevity (NIH 2012). Some of the more common manifestations of autoimmunity include Crohn's disease, colitis, systemic lupus erythematosus, Sjogren's syndrome, celiac disease and type 1 diabetes. The specific mechanisms and causes of these diseases are still the subject of investigation, some of which will be explored in the following discussion. It is widely recognized that autoimmune disease is on the rise across nearly all age groups and social categories (Dinse et al., 2020). The reason for this increase is unclear, though many believe that environmental conditions as well as behavioral factors may be to blame.

As the general rate increases, an important question arises. Does contracting one form of autoimmune disease raise the risk of developing another? Since many symptoms of autoimmune disease overlap with one another, perhaps a connection between them exists. For instance, Sjogren's, Behcet's and celiac disease all present with aphthous ulcers in the mucosal lining of the oral cavity. Even though overlapping symptoms clutter the differentials and make a definitive diagnosis more elusive, these commonalities may demonstrate a connection between the various diseases. In addition to overlapping symptoms, many autoimmune diseases share specific pro-inflammatory cytokines such as IL-1, IL- 6, and TNF-alpha, which trigger the release of inflammation markers like CRP, Fibrinogen, and Haptoglobin (Castro, Gourley, 2010). Another study found that specific CD4+ T lymphocytes responsible for misidentifying healthy tissue as foreign were found in 3 separate autoimmune diseases (Christophersen et al., 2019). Using mass cytometry and RNA sequencing, the CD4+ T lymphocytes were first identified in celiac patients but were later linked to Systemic lupus and multiple sclerosis. While only a correlation, the findings may explain why some immune modalities are more susceptible to make mistakes and whether contracting one disease such as celiac raises the risk of more activity.

Since there are multiple drivers of disease, the investigation into polyautoimmunity and how its diseases are linked will need to focus on several factors, including the environment, hormones, and genetic predispositions. Perhaps the environment and exposure to chemicals and pollution are interfering with a healthy immune system? Maybe bacterial diseases and viruses are propelling the immune system to turn on itself in multiple ways? Lastly, can specific genes be contributing to patterns that have been established between specific conditions such as celiac and type 1 diabetes?

Methods

The research reviewed in this article was obtained using several online databases and search tools, including Pubmed, JSTOR, and Google Scholar. Information was also accessed through the Touro College Libraries database using EBSCO and ProQuest.

Discussion

Mechanisms of Autoimmunity

Before exploring the polyautoimmunity connection, it is important to first analyze and define the autoimmune response itself. Over the last several decades, scientists have formulated several explanations to illustrate the specific pathways and mediators responsible for a typical immune reaction in the body's tissues. Researchers isolated and identified one such pathway using cytometry and RNA sequencing on tissue derived from inbred C57BL mice. Upon initial activation, CD4+ T cells produce cytokine tumor necrosis factor (TNF), which engages its

TNF receptor on mononuclear phagocytes (MP). This leads to the synthesis of pro-cytokine interleukin (IL)-1B. Concurrently, CD4+ cells also express membrane-bound Fas-Ligand (FasL), a subgroup of the tumor necrosis receptor family, which activate death receptor Fas signaling in nearby macrophages, leading to the induction of the inflammation process. This eventually results in Caspase-8-dependent-pro-IL-1B cleavage. Caspase-8 is believed to regulate inflammation through the modulation of mRNA expression in inflammasomes (Gurung, Kanneganti, 2015). The researchers concluded that the inflammasome-independent cytokine interleukin 1B was believed to be responsible for the systemic inflammation found in general autoimmune responses. Consequentially, when tumor necrosis factor receptors or Fas signaling was inhibited in the population, the mice were protected from cell-driven immunity (Jain et al., 2019).

These cytokine interleukins (IL-1B, IL-18, IL-6) have been identified in further studies to be a central player in innate immune responses due to their interaction with inflammasomes. Broadly speaking, cytokine interleukins have been found to be present in almost every immune reaction. However, what makes each reaction unique and specific to individual tissues and organ systems is the type of inflammasome involved in the response (Rathinam et al., 2012). Inflammasomes were first discovered in 2002 as a prominent component of type 2 diabetes and systemic gout. As a type of macrophage, these inflammasomes are a key player in cytosolic surveillance, constantly scanning the interstitial space for signs of tissue injury or infection. The human genome has been found to contain twenty-three Nod-like-receptor (NLR) nucleotide-binding and oligomerization domains (Nods), many of which codify and assemble into inflammasome complexes. Each of these different complexes interacts with and control for the release of cytokine interleukins in response to various bacterial pathogens, viruses, and even cancer (Rathniham et al., 2012). In a healthy individual, these specific pathways of inflammatory signaling are kept in check in order to respond only to the invading microorganism. When this process breaks down, autoimmunity develops. It is important to keep these unique pathways and cellular components in mind when investigating polyimmunity and the link between individual diseases.

Stress and Poly-Autoimmunity

During the last several decades, significant research has been directed on the role that stress plays in the development of autoimmune disease. Various forms of both short-and-long-term stress exacerbate already existent conditions such as rheumatoid arthritis (RA). One twelve-week study involving forty-one women suffering from RA found a significant relationship between even minor stress and joint pain (Zautra et al., 1997). However, these studies were unable to provide an association between chronic stress and the initial manifestation of diseases. Researchers focused on four common pro-inflammatory cytokines in patients with increased levels of acute stress, IL-1B, IL-6, TNF-alpha, and C reactive protein (CRP), and cortisol. One meta-analysis examined 30 studies in order to determine the effects that stress had on these robust effects for higher levels of IL-1B, IL-6 and TNF-alpha while the results for CRP were mixed (Steptoe et al., 2007). It is possible that these higher levels of interleukin exasperated by stress leads to multiple autoimmune conditions being contracted. One 2018 study aimed to investigate the link between stress and developing autoimmunity. The retrospective cohort study followed 1,171,104 people, 106,464 of whom were diagnosed with a stress-related disorder. The results showed that the stressed patients were almost three times more likely (95% CI, 9.2 per 1000) to develop some form of autoimmunity versus the non-stressed cohort (95% CI, 2.99-3.25 per 1000). In addition, patients were also more likely to develop multiple autoimmune conditions, especially when they did not seek any treatment for anxiety (Song et al., 20 Though this study did not investigate why stress causes autoimmune disease, the correlation clearly indicates that stress plays a prominent role in the development of multiple autoimmune conditions and more research will be needed to establish why this occurs.

Viruses and Polyautoimmunity

There are two main hypotheses proposed to explain the connection between viral pathologies and autoimmunity. One coined "molecular mimicry" by Robert Fujinami in 2006 refers to the misrecognition of an antigen by a memory B-cell. The belief is that immunological memory is achieved after initial microbial and viral infection by a host-pathogen. Antigens from the initial infection stimulate humoral immunity and the production of plasma cells and memory B-cells. If these antigens reappear during the secondary response, they will reactivate the plasma B-cells to proliferate and then destroy the invading pathogen. If an organism's self-antigen mimics the viral antigen in some way, then a secondary response is likely to occur against the body's own tissues (Kim et al., 2007). There are three identified ways that this might occur. The first is when the original antigen and the self-antigen share identical amino acid sequences in their primary protein structure. A second possibility is when secondary and tertiary structures mimic each other, such

as with similarities regarding polarity, hydrogen, and disulfide bonding. A third type of molecular mimicry exists when one antibody becomes capable of recognizing two or more antigens with completely different structures (Cunningham 2009). Researchers now believe that this third mechanism is the result of individual T-cells exhibiting receptors for both foreign and self-antigens. When a foreign insult triggers the T cell, it then moves to attack healthy body tissue as well. This third mechanism has consistently been linked to multiple autoimmune diseases or polyimmunity (Cusick et al., 2012). For instance, studies have linked Herpes simplex virus to stromal keratitis and type 1 diabetes, streptococcus to rheumatoid myocarditis and poststreptococcal autoimmune disorder, as well as many others (Munz et al., 2009).

Another hypothesis that explains how viruses generate autoimmune disease is that a virus induces a systemic and non-specific activation of the immune system that eventually leads to an overexcitability state and autoreactive immunopathology (Munz et al., 2009). Known as the "bystander activation," this line of reasoning stems from the fact that, similar to stress, those already suffering from autoimmune conditions tend to fare worse with their symptoms whenever they are infected with a virus. Scientists believe that the pro-inflammatory environment in the body initiates a release of self-antigens from infected tissue. The antigens are then mistakenly taken up by major histocompatibility complexes (MHC) or antigen-presenting cells (APC), which then present these self-antigens to autoreactive T cells that have already migrated to the damaged tissue (Fujinami 2006). This can sometimes lead to a concatenation of events known as "epitope spreading," where these T cells begin attacking other self-antigens that mimic the previous one, further inflaming the tissue and releasing even more self-epitopes. This phenomenon has been observed in multiple sclerosis, autoimmune encephalomyelitis and myasthenia gravis and has been linked to viruses such as EBV, rotavirus and cytomegalovirus (Smatti et al., 2019). It is important to note growing evidence that viruses may also play a protective role in preventing autoimmunity by activating regulatory immune responses that work to suppress inflammation and inhibit cytokine interleukins. In addition, scientists still do not know whether higher viral loads or compromised immune systems during the infection period, raise the risk of autoimmunity.

This second idea regarding how bystander activation and molecular mimicry leads to multiple autoimmune syndrome (MAS) illustrates how inflammation leads to more inflammation. This concept may explain the essence of another important phenomenon. For many years, doctors have emphasized the importance of treating previously diagnosed autoimmune conditions and disorders in order to improve a patient's prognosis and quality of life. These treatments have included the use of biologics, corticosteroids, dietary modifications and lifestyle changes. Scientists have long believed that inflammatory processes that are left unchecked can lead to the development of coronary artery disease and even cancer. Now, researchers have discovered another important reason to aggressively treat these immunological processes: untreated autoimmunity increases the risk of developing further autoimmune conditions. In 1999, researcher Andrea Ventura postulated that for individuals with celiac disease, the later the age of diagnosis, the greater the risk of developing a second autoimmune condition as well as cancer (Ventura et al., 1999). For example, those who were diagnosed between the ages of four and twelve had a 14% risk of developing another condition, while those over twenty years of age had a 34% risk. For each one-year increase in age at diagnosis, the chances for developing another disease increased by 1.1% regardless of gender or weight. In his study, Ventura explained that the longer the duration of exposure to gluten, the higher the prevalence of other autoimmune conditions developing. Further studies by other researchers revealed that 15% of untreated celiac patients eventually developed type 1 diabetes and that 26% percent developed autoimmune thyroid disease such as Hashimoto's thyroiditis and Graves' disease (Lauret, Rodrigo, 2013). These studies were significant because most of these patients were asymptomatic and prior to that, many questioned the importance of adhering to a strict gluten-free diet. In a later paper published in the Journal of Pediatrics, Ventura advocated for the strict use of a gluten-free diet to reduce and even prevent the manifestation of secondary autoimmune activity, after he demonstrated that celiac patients who followed a strict gluten-free diet for two years were more than 50% less likely to develop other autoimmune conditions. (Ventura et al., 2000). Additionally, other studies also reported similar findings regarding Crohn's disease and colitis. In one study, patients were found to be 2.5 times more likely to develop a co-morbid inflammatory disease within five years after an initial diagnosis (Bernstien et al., 2005).

While the absolute reason for this phenomenon hasn't yet been identified, many hypothesize that the prolonged state of inflammation, caused by high concentrations of glutamine and proline found in the protein fraction of rye, wheat, spelt and barley contribute to other diseases. Specifically, these residual peptides and amino acids initiate a cascade of interleukins (IL-1B, IL-6 and IL-18) that trigger the proliferation of other inflammatory markers, including inflammasomes and C-reactive protein (CRP). Higher levels of CRP have been implicated in coronary

artery disease as well as rheumatoid processes. In addition, specific phenotypes of CD4+ helper T-cells that are activated in celiac disease are of the same subtype involved in some of the other conditions such as systemic lupus and multiple sclerosis (Christophersen et al., 2019). (Though these specific T-cells comprise less than 2% of all the Helper T-cells in the body, they are disproportionately responsible for the identification of most self-antigen responses that take place within these inflammatory pathways.) This phenomenon seems to be related to the mechanism of molecular mimicry and dual T cell activation discussed earlier, which takes place in response to viral infections. However, in this scenario, an initial autoimmune condition takes the place of the virus and contributes to a set of circumstances where the body continuously mistakes healthy tissues as foreign and as a threat. It is clear from these studies that untreated or resistive autoimmune processes have a direct effect on the risk of further developing other forms of chronic inflammation which may lead to the manifestation of other autoimmune conditions.

Genetics and Polyautoimmunity

Upon examination, autoimmune diseases appear to manifest in clusters. Though Ventura investigated all forms of autoimmunity, he reported that individuals with celiac were much more predisposed to developing type 1 diabetes and thyroid disease versus ulcerative colitis or Addison's disease. Rheumatoid arthritis has been linked to Sjogren's syndrome, while systemic lupus erythematosus has been linked to multiple sclerosis. Indeed, so specific are these clusters that investigators have now classified these groups into three clinical subtypes (Cojocaru et al., 2010). Of note, each of these subtypes contain at least one skin condition and one connective tissue disease.

I. Type 1 is comprised of polymyositis, thymoma, giant cell myocarditis and myasthenia gravis.

II. Type 2 consists of rheumatoid arthritis, Hashimoto's, scleroderma, and Sjogren's syndrome.

III. Type 3 is the largest subgroup and includes Addison's disease, type 1 diabetes, vitiligo, psoriasis, autoimmune hemolytic anemia, dermatitis herpetiformis, idiopathic thrombocytopenic purpura (ITP), Sjogren's syndrome, myasthenia gravis and systemic lupus erythematosus.

Patients diagnosed with having more than one autoimmune condition are said to have polyimmunity, while patients diagnosed with more than two are classified as having multiple autoimmune syndrome (MAS) type i, ii, or iii. These classifications are not absolute; however, they provide clinicians and scientists with an understanding of what to look out for and how to treat each patient based on their specific classification. Researchers have discovered that families also seem to develop these illnesses as a group. For example, a mother may develop Addison's disease while a daughter is diagnosed with lupus and a grandson has vitiligo (Cardenas-Roldan et al., 2013). This occurrence seems to confirm that in addition to the environment, genetics play a broad and important role in the development of multiple autoimmune syndrome which will be discussed.

Scientists employed genome-wide association study (GWAS), genome scans and RNA sequencing to investigate common genetic etiologies that contribute to autoimmune diseases and polyautoimmunity. They identified loci responsible for the human leukocyte antigen (HLA) region on DNA that contribute to autoimmunity. The researchers also identified loci shared between the most common autoimmune conditions which included IL23R, OLIG3/TNFAIP3 and IL2RA. The study revealed that type 1 diabetes was genetically associated with rheumatoid arthritis and that Crohn's disease was linked with ulcerative colitis. It also underlined how some diseases seem capable of developing without any genetic links, particularly systemic lupus erythematosus (SLE) and systemic sclerosis. This would suggest that not all forms of autoimmune disease are caused by genetic heritability and phenotype. Rather, genes play a sizable role in the broader picture of how autoimmunity manifests (Ramos et al., 2011).

The Major Histocompatibility Complex (MHC) gene has been investigated as an important factor in autoimmunity. Located on chromosome 6, the locus codes for cell surface proteins found on numerous lymphocytes as well as receptors found on almost every living cell in humans. Scientists performed a genome-wide association study (GWAS) on the MHC gene which compares the allele/genotype frequency between individuals that have been affected by disease and those who have not. The study visually confirmed that the MHC locus is a major predicator in most autoimmune conditions (Kochi, 2016). The most common MHC genes include HLA-A, HLA-B and HLA- DP/DQ/DR. Since these genes play a crucial role in the adaptive immune response, perhaps it is possible to surmise that MHC genes are involved in the formation of autoimmunity in general. However, this does not explain why one patient develops Crohn's disease and one Hashimoto's disease. The development of a specific disease or condition can be caused by a single allele, yet this does not prove that a single allele can cause polyautoimmunity. For example, rheumatoid arthritis has been linked to multiple HLA-DRB1 alleles such as *01:01,

*04:01, *04:05 and *09:01 while Ankylosing spondylitis is known to be caused by the allele, HLA-B27 (Newton et al., 2004).

Researchers have identified a mutation in the genetic factor PTPN22 as an important driver of multiple autoimmune syndromes. PTPN22 is responsible for encoding lymphoid-specific tyrosine phosphatase, a critical regulator of T-cell receptor signaling pathways. A single nucleotide missense mutation involving PTPN22 has been linked to rheumatoid arthritis, type-1 diabetes and systemic lupus erythematosus. FC receptors (FcRs) on the surface of immune cells recognize immune complexes comprised of autoantigens and autoantibodies. After the immune complex binds to the receptor, FcRs release Src and Syk family kinase which leads to antigen uptake, presentation and secretion of interleukins and proteins that initiate an immune response. Tyrosine phosphatase encoded by PTPN22 inhibits the release of Src and Syk kinase, thereby regulating the FcR immune response in dendritic cells. In one study, patients who had a C1858T missense polymorphism in PTPN22, were found to have higher levels of Scr and Syk kinase as well as higher instances of type 1 diabetes. In another study, bone marrow derived dendritic cells of wild type mice (PTPN22-/) were compared with dendritic cells from knockout mice with PTPN22-/-. The PTPN22-/- dendritic cells had higher levels of T cell proliferation and activity (Clarke et al., 2018).

Studies examined innate immunity to determine if genes responsible for the initial immune response play a role in autoimmune development. In innate immunity, macrophages and dendritic cells express toll-like receptors which recognize patterns on virus antigens and then activate type 1 interferons responsible for immunomodulatory and antiviral responses. These interferons or cytokines bind to receptors on white blood cells which activate a cascade of secondary messengers which interfere with the further proliferation of the invading virus. A subgroup of the interferon proteins, the type 1 interferon, has been implicated in myositis and systemic lupus erythematosus. Research demonstrated that patients with lupus displayed higher levels of type 1 interferons at diagnosis when compared with individuals without symptoms of disease (Padilla, Niewold, 2016). Additionally, genome wide association studies for systemic lupus erythematosus discovered multiple gene and loci sites that were directly linked with type 1 interferons and its associated signaling pathways. These included IFIH1, IRF5, IRF7, TLR7, IRAK1 and TYK2. Other studies using GWAS identified these loci sites in other autoimmune conditions. For example, type 1 diabetes, psoriasis and Addison's disease have been linked to IFIH1. Interestingly, these three diseases are all included in the type 3 subgroup of multiple autoimmune syndrome (MAS) described earlier. Similarly, the IRF5 loci was correlated with primary biliary cirrhosis, Crohn's disease, colitis and rheumatoid arthritis.

Patients with lupus who presented with the IRF5 risk haplotype also had higher levels of signal transducer and activator of transcription 4 (STAT4). STAT4 is a transcription factor involved in the differentiation of T-helper 1 (Th1) cells. Researchers believe that increased levels of STAT4 may increase the activity and proliferation of these T cells leading to autoimmunity (Kochi Yuta, 2016). STAT4 is activated by type 1 interferons and is responsible for the synthesis of IFN-y, another interferon type. This discovery highlights how one gene factor can drive multiple different components that lead to disease. IRF5 produces excess interferons that may exacerbate an autoimmune response in the innate immune system, while simultaneously activating STAT4 which is involved in adaptive immunity. Also noted in the literature, was that lupus patients who presented with the PTPN22 mutation discussed had reduced levels of TLR7 induced type 1 interferons, possibly indicating heterogeneity of disease expression (Wang et al., 2015). These studies clearly indicate that genetics play a role in both innate as well as adaptive immunity and that there are several overlapping genetic variances that contribute to the manifestation of multiple autoimmune conditions.

Studies performed using genome-wide association studies (GWAS) identified and linked individual genes with specific autoimmune conditions. Researchers investigated whether one gene can be responsible for general autoimmunity encompassing a broad number of non-specific autoimmune conditions. One study focused on the ITGAM gene, a genetic region responsible for the alpha-chain subunits found in a cell surface receptor of neutrophils and monocytes named Integrin-aMB2. A variant at exon 3 (rs1143679) within the ITGAM gene was thought to up-regulate the binding capacity of Integrin-amB2 which would increase the susceptibility to multiple autoimmune syndromes. However, SNP genotyping, statistical and meta-analysis of the gene region confirmed that there was no significant association between systemic sclerosis, scleroderma, rheumatoid arthritis and the ITGAM gene. Only systemic lupus erythematosus was found to be directly correlated with the re1143679 mutation found in the population (Anaya et al., 2011). Still, researchers have identified a general autoimmune contributor using GWAS. As previously discussed, pro-inflammatory cytokines play a major role in the development of autoimmune conditions. Researchers studying the origins of celiac disease discovered an association between celiac

disease and the receptor for Interleukin-23 (IL-23). Two subunits (p40 and p19) within IL-23 are found within the IL-12 and IL-1B receptors as well. (IL-12 receptors are composed of p40 and p35 heterodimers while IL-1B contains p19 dimers.) Additionally, the p19 (IL23R) subunit on IL-23 is located just 3'downstream to the p40 subunit (12RB1) on IL-12. This highlights just one of the many connections between cytokinetic interleukins and autoimmune disease. Indeed, the IL-23 receptor has also been linked to psoriasis, rheumatoid arthritis, Crohn's disease and multiple sclerosis (Tang et al., 2012). Other diseases have been linked to specific interleukins as well. IL-2 and IL-2R were found to have a direct connection with psoriasis, Gaucher disease, rheumatoid arthritis and type 1 diabetes while IL-21 was linked with multiple sclerosis as well as inflammatory bowel disease. The studies did not investigate or confirm whether contracting one of the autoimmune conditions raised the risk of developing another. However, it seems plausible that if genetic and environmental conditions caused one disease, they could lead to a second one.

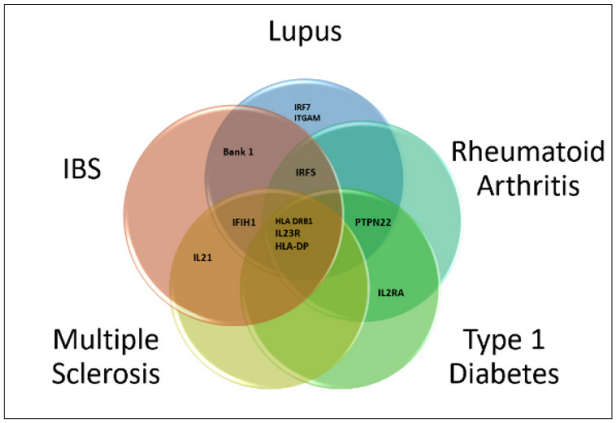

Figure 1 illustrates the various linkages between differentially expressed genes and five common autoimmune conditions.

Researchers at the Benaroya Research institute attempted to map the many genetic connections between each autoimmune disease in order to determine how one may lead to the other. They began by investigating why patients with Down syndrome are 15-100 times more likely to develop some form of autoimmunity, many of whom develop polyautoimmunity as well. Additionally, these disorders are varied, affecting both endocrine and non-endocrine systems. Diseases commonly seen include thyroid disease, type 1 diabetes, scleroderma, Sjogren's syndrome, systemic lupus erythematosus, autoimmune hemolytic anemia and celiac disease. A biorepository of blood and tissue samples was established using tissue extracted from the Down syndrome population as well as from their healthy siblings. Since Down syndrome is caused by trisomy 21 (HSA21) and is genetic in nature, researchers focused on the genetic drivers in these patient's immune responses and composition. They also investigated whether one gene could be the driving force behind all these illnesses. The data identified a gene called dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (DYRK1A) as a possible driver of autoimmunity. Previously, this gene was linked to the cognitive decline and neuro-developmental conditions found in most patients with Down syndrome (Feki, Hibaoui, 2018). Patients with Down syndrome express an excessive amount of DYRK1A as a result of the extra chromosome HSA21, leading to a buildup of proteins that lead to memory deficit and motor abnormalities. Scientists hypothesized that DYRK1A produces excess proteins that prevent the body from shutting off unnecessary inflammatory immune responses leading to autoimmune disease.

In a separate study, researchers worked to identify substances that could increase the number of Treg cells, white blood cells that suppress other immune cells, in order to prevent autoimmunity. The scientists believed that by increasing Treg cells while maintaining or even inhibiting T helper cells, a novel therapeutic approach to autoimmunity could be found. Using Similarity Ensemble Approach (SEA) and chemoinformatic methods involving 3,100 substances, the team isolated a chemical called B-carboline alkaloid harmine and discovered its ability to reduce autoimmune activity in induced inflammatory reactions in vitro. Further studies demonstrated that harmine acts in part by inhibiting DRYK1A which led to differentiation of Treg cells. More importantly, when DRYK1 was inhibited, T1 helper cell and T17 helper cell differentiation remained constant or was reduced. The team then tested harmine's impact on Treg and T helper cells in vivo, using three separate subtypes of inflammation; type 1 diabetes, colitis and asthma. The three inflammatory diseases were chosen for their T cell properties, diverse genetic backgrounds and distinct tissue types. When Foxp3 mice were induced with type 1 diabetes, the mice developed diabetes approximately 10 days after injection. When the mice were induced under TregHarmine conditions, onset was delayed by a minimum of seven days. Next, when mice with ulcerative colitis were injected with TregHarmine, mucosal inflammation in the intestines was significantly reduced. Finally, researchers induced Foxp3 mice with airway inflammation using intratracheally administered ovalbumin. When these mice were treated with Harmine, a significant suppression of inflammation was noted (Kohr et al. 2015). These findings benefit the hypothesis that the overexpression of DYRK1A in patients with Down

syndrome may contribute directly to the development of autoimmune disease by increasing differentiation of T1 helper cells, while simultaneously preventing the expression of anti-inflammatory Treg cells. This might explain the broad spectrum of disorders seen in Down syndrome since helper T cells and Treg cells are involved in almost all autoimmune disorders. Additionally, as a DYRK1A inhibitor, B-carboline alkaloid harmine should be further investigated as a therapeutic for Down syndrome patients with autoimmune conditions. Another potentially important question is whether inhibiting DYRK1A can also decrease some of the neurological symptoms seen with Down syndrome cases, since DYRK1A has also been associated with the mental retardation and cognitive delay that is prevalent in these cases.

GWAS was able to identify and establish a substantial link between genetics and autoimmune disease. In addition, many of these genes have also been linked to multiple autoimmune syndromes and illnesses. However, just because an individual is genetically predisposed does not guarantee the development of one or several autoimmune conditions. Epigenetically, these genes can be regulated due to several mechanisms and several epigenetic processes have been linked to autoimmune disease. These include DNA methylation, post-translational histone protein modifications, chromatin remodeling and RNA regulation of gene expression. DNA methylation involves the addition of a methyl group to carbon 5 on the cytosine molecule of cytosine phosphate-guanosine resulting in 5-methylcytosine. The methyl groups occupy the major groove of DNA, thereby silencing the gene by blocking proteins and transcription factors from forming transcription complexes on the DNA helix. DNA demethylation can also occur through the enzyme-catalyzed removal of cytosine methyl groups. Researchers studying systemic lupus erythematosus were able to identify specific epigenetic processes by focusing on the development of peripheral blood mononuclear immune cells of patients affected with lupus and then comparing them to their identical monozygotic twin siblings. The results identified approximately 50 instances of DNA hypomethylation in the gene regions of the lupus patients. Absolute cause for the hypomethylation was not identified, although possibilities that were mentioned include UV exposure, age, viruses and environmental stress (Surace and Hedrich, 2019). More research is needed to investigate DNA methylation in other autoimmune conditions as well as other immune cell types.

Histone modifications occur via covalent posttranslational alterations of amino acids at the N-terminal end of histone proteins. These changes include adding or removing a phosphate, acetyl and methyl group that transforms the chromatin structure, making the DNA unavailable for transcription. A similar concept called chromatin remodeling occurs when ATP-powered protein complexes attached to chromatin, alter, remove or transfer the nucleosome to another part of a chromosome. Scientists explored the genetic links of Cryopyrin-associated periodic syndrome (CAPS), a rare autoimmune disease that consists of three phenotypic processes: Muckle-Wells syndrome, neonatal-onset multisystem inflammatory disease and familial cold autoinflammatory syndrome. The results of one study revealed a down-regulation of numerous genes which included the histone proteins SUMO1 and HIST2H2AC as well as histone deacetylase enzymes HDAC1/2 (Surace and Hedrich, 2019). However, a second study involving CAPS demonstrated excessive levels of DNA demethylation which results in an epigenetic up-regulation of genes as the driver of the inflammatory process (Tormo et al., 2017). These studies illustrate the immense complexity of histone modifications and epigenetics in general. Though the two studies appear to contradict each other, it is highly plausible that epigenetic events not only vary considerably between diseases but even between individuals as well. Still, these studies were limited in scope and design and more research is needed to fully understand the epigenetic connection to CAPS and how different events drive the genetic expression of inflammatory processes.

RNA regulation involves the repression of gene expression through long and short noncoding RNAs (ncRNA). Long ncRNA's are silent and lack an extended open reading frame required for translation. Short ncRNA's include micro-RNAs (miRNAs), short interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs). MicroRNAs are transcribed as precursor molecules 80-100 bases long and after hydrolytic cleavage are processed to contain 20-23 bases. The shortened span allows them to interfere with DNA methyltransferase thereby inhibiting DNA methylation. During the CAPS studies mentioned earlier, researchers were able to identify a direct association between miRNA and upregulation of the STAT4 gene responsible for CD4+ T helper 1 cell proliferation. In addition, STAT3 and STAT1 were also upregulated, resulting in increased cytokinetic activity, specifically IL-6 and IL-17A (Surace and Hedrich, 2019). Since epigenetics contain many factors, it is difficult to determine the exact role it plays in polyautoimmune disease. However, epigenetic processes have been identified in some of the universal genes described earlier. For example, the ITGAM gene that contributes to multiple autoimmune diseases contains approximately 40 sites of DNA methylation and

histone modifications. Though scientists are unsure of the specific effects and links for these sites, many roles and possibilities exist. As science continues to explore the complex concept of gene expression and epigenetic factors, researchers will surely uncover many other associations between multiple autoimmune diseases and epigenetics.

Conclusion

Research has identified several risk factors for developing polyautoimmunity. It is clear from the data that the presence of one autoimmune disease raises the risk for developing further illnesses. Chronic and acute forms of stress facilitate autoimmune processes by raising inflammatory markers in the bloodstream. Though viruses and bacteria typically prime the adaptive immune system for future responses, molecular mimicry, bystander activation and epitope spreading can direct a vulnerable immune system to turn on itself. Furthermore, untreated and protracted forms of inflammation might lead to autoimmune disease through bystander activation or similar forms of immune dysfunction. Genetic processes involving interleukins and inflammasome signaling, regulate the many components responsible for managing a healthy immune system. Mutations and hereditary factors involving these genes can directly contribute to multiple autoimmune diseases. Lastly, epigenetic elements driven by DNA methylation and hypomethylation of cytosine bases as well histone modification and RNA regulation can lead to the development of multiple autoimmune diseases, though the exact connection is not yet fully understood. The data does not establish absolute causation of any one contributing factor for developing polyautoimmunity; however, each additional variable enhances the risk over time. Patients who are genetically predisposed to autoimmune disease and are chronically stressed or have contracted multiple viruses in their lifetime, have a significant risk of developing an autoimmune disease. If inflammation levels of the initial disease are not managed, patients face an increased risk of developing another condition. It is important for physicians and healthcare professionals to be aware of their patient's genetic profile, susceptibility and history to determine the probability of further autoimmune development. Communication among the healthcare team is essential since patients may have multiple doctors treating several conditions, each without knowledge of the patient's complete history. Finally, through more research into the causes and associations of polyautoimmunity and multiple autoimmune syndrome, doctors will be able to better diagnose, treat and eventually prevent this abstruse and perplexing condition.

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What is the Underlying Cause of Infantile Colic?

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Abstract

Infantile colic (IC) is an important area of current research due to the extreme distress it causes parents and their infants. It is vital that a cause is isolated so that treatment can be found because IC is a risk factor for child abuse. In this paper, two major theories were posed to elucidate the underlying cause of IC—the gastrointestinal model and the neurological model. The gastrointestinal model suggests that IC stems from issues such as an immature gut. The neurological model suggests that infantile migraines are the causative agents of IC. Both theories supply correlational evidence but are subject to scrutiny because they are incomplete. A third theory, the fourth trimester theory, is suggested to fill in the gaps found in the two major models. Due to the novelty of this area of research, additional studies, such as genetics studies are suggested for future IC research.

Introduction

Infantile colic (IC) is defined as recurrent periods of crying, fussing, or irritability, for a minimum of three hours per day, three days per week, for at least three weeks per month. This syndrome is unique to infants between approximately three weeks to three months of age. The symptoms persist even though the infants are well-fed and do not present with any other signs of illness (Levinsky, et al 2020). IC leaves parents feeling helpless and pediatricians lack success in treatment of the phenomenon.

Although there is insufficient supporting evidence, IC has long been assumed to have a gastrointestinal (GI) etiology. Attempts to treat IC have included GI drugs treatments such as simethicone, probiotics, dicyclomine hydrochloride, and proton pump inhibitors. In drug studies, it was determined that treatments did not cause better outcomes than placebo. Other treatments have included diet modification. Diet modification treatments have not been successful, and pediatricians do not believe that there is sufficient evidence to recommend this to parents (Gelfand…, et al 2016).

More recently, it has been hypothesized that the underlying problem in IC is neurologically based. Amy Gelfand, a pediatric resident, noticed that many infants in the NICU who presented with IC had mothers who suffered from migraines. This theory suggests that IC is a premature form of a migraine. Studies conducted have confirmed that the symptoms, such as cyclic vomiting, are consistent with those of an adult migraine.

If IC is indeed neurologically based, treatment with acetaminophen—which is known to be a safe treatment of other infantile illnesses—would be a potentially effective treatment. Acetaminophen is used in children as young as 4 years of age for migraine disorder treatment (with and without aura). Studies recommend against treatment with triptans, a class of medications commonly used to treat migraines, as there is no known evidence of its safety for infantile use (Gelfand…, et al 2016).

The aim of this meta-analysis is to review the existing literature to determine if there is a basis for IC being caused by neurological phenomenon.

Methods

To gather evidence-based research for this meta-analysis, scholarly search engines, such as EBSCO and ProQuest were used to sort and gather all relevant research and publications available on infantile colic. Google Scholar was utilized as well.

Discussion

The Gastrointestinal Basis of Infantile Colic

Gastrointestinal distress has long been considered the cause of infantile colic. Physicians have postulated that gas, an immature gut, and an unbalanced gastrointestinal microbiome serve as the culprit. IC typically subsides after three months of life and is benign in nature (Qubty et al…, 2016). Supporters of a gastrointestinal etiology suggest that since it is well known that newborns suffer from a sensitive and underdeveloped digestive system, it is the likely cause of IC. Problems, such as swallowing air during feedings can cause painful gas in newborns which can lead to the uncontrollable crying spells. In addition, reflux, a condition in which stomach acid or bile ascends from a baby's stomach, is often cited as a possible cause.

A study which fed the fecal matter of babies suffering from IC to mice led to the presentation of visceral hyperalgesia in the mice. Visceral hyperalgesia is a condition in which the nociceptors of the internal organs', specifically the intestines, are activated and amplified (Qubty et al…, 2016). The study suggests that perhaps infants suffering with IC experience pain related to digestive processes. This is credible because visceral hyperalgesia is present in other GI disorders, including irritable bowel syndrome (IBS).

The bowels of infants' lack a diverse microbiome. Specifically, species of bifidobacteria and lactobacilli, which act as anti-inflammatory agents in the gut, are missing. On the other hand, species such as proteobacteria, which produce gas and inflammation are present in higher numbers than adults. Therefore, scientists can infer that infants have a higher propensity of GI inflammatory syndromes that do adults. Based on this evidence, it would be reasonable to infer that IC can have a gastrointestinal basis.

The GI basis of IC falls short in a few areas. Firstly, while babies suffering from reflux should exhibit crying as a symptom, it is unlikely that crying should be the only

manifestation of the dysfunction. Therefore, reflux as an isolated cause is unlikely. Secondly, if IC is a disorder of the digestive tract, it would be reasonable to assume that breastfed and formula fed babies would be affected differently. However, this is not the case. Both types of feeding options have similar rates of IC. Additionally, altering maternal diet in breastfed babies does not pose significant improvement in crying spells (Qubty et al…, 2016). Changes to partially hydrolyzed formula for formula fed babies, a transition known to help reflux, does not relieve IC either. This further indicates that diet and reflux are perhaps not the causative agents. Lastly, drugs that reduce GI distress, such as probiotics, simethicone (a drug that reduces surface tension and breaks down gas bubbles), dicyclomine hydrochloride (an anti-spasmodic agent), and proton pump inhibitors (PPI) which aim to reduce the crying spells, do not reduce the symptoms of IC. This poses the obvious question—if IC is caused by a gastrointestinal cause alone, then why do drugs known to help with those issues not help?

The Neurological Basis of Infantile Colic

Alternatively, physicians have wondered if perhaps there is something else entirely that is causing the distress. Based on Dr. Gelfand's observations of the relationship of maternal migraines and IC, a longitudinal study was initiated and replicated by other researchers and physicians, to unveil the potential relationship between migraine disorder and IC.

Efforts to reduce the symptoms of IC using methods such as shaking and shushing babies in brightly lit rooms have only exacerbated symptoms. Using the migraine-based approach this would make sense, as methods such as shushing and shaking would only worsen the headache. However, treating the colic in a way that migraine sufferers prefer, such as being placed in a cool, dark room and being put to sleep, have reduced IC significantly (Gelfand…, et al 2016).

A study was published in 2015 that related IC with migraines. In this study, 1267 infants were assessed for IC and 13% were IC candidates. Of the infants participating, 787 of the infants were followed to the age of 18 years and reassessed for migraine disorder. Of the infants studied, 16% had developed a diagnosable migraine disorder, and 22% of those had a history of IC. Interestingly, IC was the only statistically significant predictor of migraine disorder in these adults. Moreover, migraine sufferers only developed migraine without aura (aura is described as visual sensations, such as flashing light, that precede neurological events.) There was no statistical correlation between IC and migraine with aura (Sillanpaa, 2015). This is important because migraine with aura and migraine without aura are correlated with different genetic mutations. Therefore, perhaps there are migraine genetics that are significant in IC. This would be an excellent hypothesis to study in future research of IC.

An earlier cross-sectional survey was done in 2012. This survey followed 154 mother-infant pairs of which 14% of the infants were determined to have IC. To allow for more accuracy in the self-reporting process, the infants were re-screened at two months when IC is at its worse. Maternal migraine disorder conferred a 2.6-fold likelihood of having a baby with IC (Gelfand et al…, 2012).

Another study conducted in 2013 compared 208 children with migraine disorder to 471 controls. The study found that 72.6% of children with migraine had a history of IC compared to the 26.5% likelihood of IC history of the control group (Romanello et al…, 2013).

In a study conducted, children ages 6-18 who were admitted for migraine disorders were screened for a history of IC. The positive correlation was significant ($p=.001$). Such a correlation was not found for other types of tension-type headaches ($p=.10$) (J, G. M…, et al 2013). This is important because it isolates migraine headaches as the only type of headache related to IC. As mentioned previously, there is plenty of genetic research available for migraine disorders which can provide more insight on potential genetic mutations related to IC.

Furthermore, there is reason to believe that IC is maternally inherited. A survey conducted in 2019 asked parents if their infant has cried for at least three hours a day for a minimum of three weeks. A follow up question asked if the parent had a known migraine disorder. Of the respondents, 827 mothers responded yes to the initial question. Of those respondents, 33.5% had a migraine problem. Of these mothers, a frequency of 15 or more migraines a month corresponded to a higher likelihood of IC. In the same study, 592 fathers responded yes to the initial question. However, paternal history of migraine disorder was not correlated to higher probability of IC. This was especially true if the infant was a female, suggesting that being female was protective (Gelfand…, et al 2019). This information strongly suggests that the relation between IC and migraine disorder is potentially maternally inherited. This study is important because while it is commonly known that migraines are genetically inherited, it was not known that it is specifically inherited maternally.

A recent cross-sectional historical study looked to compare pediatric headache types to a history of IC. The study included 219 patients between the ages of 3-18 years. Prevalence of a positive history of IC was compared for children with migraine and other types of primary

headaches. The results showed an association of IC to pediatric migraine but not to other types of pediatric headaches. The study included 132 females and 87 males. The mean age was 12.8 ± 3.48 years. Migraine headaches were diagnosed for 170 patients (77.6%). Other types of primary headaches were diagnosed in 49 of the patients (22.3%). There were 51 patients with a history of IC. Of these patients, 45 were in the migraine group. The statistical significance in the rate of colic for the migraine group was extraordinarily strong (p=.0196), building on the hypothesis that migraines and IC are related (Levinsky et al…,2020).

In a study conducted in 2013, IC was determined based on parental responses and physician diagnosis. The study included 208 children—66 with aura, 120 with tension headache—and compared them to 471 controls. The prevalence of IC was 72% in children with migraine—70% with aura and 74% without aura. The results showed a 33% prevalence of IC for those with tension type headaches, and 27% for the controls. Interestingly, a pulsating quality of headache pain was significantly more common for those with colic than those without. This study confirmed the association on between childhood migraine headache and IC (F, B. S et al…, 2013).

A meta-analysis conducted in 2019 included seven large studies looking at the relationship between IC and migraine headaches. The study found an increased incidence of infantile colic history for migraine groups (p=.05) than for those with tension type headaches (p=.51) (Zhang et al…,2019).

Conclusion

The causative agent of IC is yet to be fully elucidated. However, there is promise in the neurologically based theory of IC. The main critique of the studies on the neurological basis of IC is that many of them include too few patients. Additionally, many of the results are based on surveys which are subject to inaccuracy due to self-reporting. Lastly, the neurological basis of IC fails to include tangible and measurable elements that the gastrointestinal basis of infantile colic addresses, such as bacterial imbalance in the gut.

The gastrointestinal basis of IC has many components which make it a reasonable causative agent of IC, but it is similarly incomplete. Many of the studies are based on trials preformed on mice. The results of such trials cannot be simply assumed to be identical in human infants. Additionally, the fact that drugs aimed to reduce gastrointestinal distress are ineffective poses a suspicion regarding its accuracy. This can be because gastrointestinal distress is not the only cause of IC.

Many physicians suggest that perhaps it is a combination if the two theories. There may even be more causes that we are currently unaware of. More research on the topic of IC is warranted to make definitive statements. However, based on the studies cited it can be said with certainty that there is a correlation relationship between IC and migraine disorder.

Another theory has been posed by a group of pediatricians consulted on this meta-analysis. This theory suggests that the "4th trimester", or the first 3 months of life, is a sensitive time for the newborn. The infant is familiar only with the quiet, warmth, and darkness of the amnion. These conditions are comfortable for the baby. The harsh transition from womb to the bright and often noisy external world is the suggested causative agent of IC. Perhaps the reason that colic subsides while swaddled in a quiet and dark room is due to the infants' comfort and recognition of a familiar and safe space. This theory perhaps serves as the missing part to the gaps found in the gastrointestinal and neurological basis if infantile colic.

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The Riddle of the Fetal Allograft

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Abstract

The immunological paradox of nurturing a fetus with paternal antigens poses some perplexing questions. Peter Medawar, an immunologist, asked at a lecture, "How does the pregnant mother contrive to nourish within itself, for many weeks or months, a fetus that is an antigenically foreign body?" Researchers have since then struggled to answer this question. The research on this topic has led to a few general hypotheses that try to explain this phenomenon. The downregulation of T cells toward paternal alloantigens is an accepted hypothesis. Another hypothesis discusses the significance of the decidua and its ability to impair dendritic cells, which are potent antigen presenting cells and critical in initiating an immune response (Ehrlbacher, 2010). Mechanical barrier and cytokine-shift hypotheses also attempt to explain the "riddle of the fetal allograft." Research is ongoing as there is no one clear answer to this query. Some of these hypotheses have flaws in them while others don't explain enough in regard to the safety of a woman and her fetus. There is one hypothesis that appears to hold the greatest significance in understanding the maternofetal relationship and the successful births of millions of children each year: local active suppression in the decidua.

Introduction

Most living organisms have some form of an immune system that protects it from foreign entities. Humans have a complex immune system that protects us on a daily basis. The notion that an antigenic foreign entity can exist within a human for many months and develop is astonishing. However, this occurs daily with millions of pregnant women across the globe. More than fifty years ago, Peter B. Medawar raised the question of how a semi-foreign transplant with paternal antigens can survive the immune system. Seventy years later, a few hypotheses and the resultant research is available for analysis. This paper will explore and evaluate these hypotheses.

Methods

Information discussed in this paper was compiled from various published articles, taken from Touro's database, including Proquest Science, EBSCO, and PubMed, or Google searches. This paper compares and contrasts the various hypotheses explaining the immunological paradox of a pregnancy and evaluating which are the most siignificant.

Discussion

The Immune System

The main purpose of the immune system is to identify non-self from self and defend the body against non-self proteins, viruses, bacteria, fungi, parasites, tumors and other pathogens. Substances that the body doesn't recognize as its own can activate the immune system. These are called antigens. Proteins on the surfaces of bacteria, fungi and viruses are examples of antigens. When these antigens attach to special receptors on the immune cells (immune system cells), a whole series of processes are triggered in the body. Once the body has come into contact with a disease-causing germ for the first time, it usually stores information about the germ and how to fight it. Then, if it comes into contact with the germ again, it recognizes the germ and can initiate a quicker attack. The body's own cells have proteins on their surface as well: self proteins. Sometimes the immune system mistakenly thinks that the body's own cells are foreign cells. It then attacks healthy, harmless cells in the body. This is known as an autoimmune response (IQWIG 2020).

As soon as the body recognizes a "non-self" entity the immune system will set out to remove the pathogen from the body. The immune system consists of many parts that work together to defend the body against invaders. Two separate, but interacting defensive systems that provide an array of defensive weapons are innate and adaptive immunity. An innate response, neutralizes invading pathogens before they can harm the body. For example, in a wound, white blood cells known as macrophages engulf invading microorganisms as well as release cytokines, signaling proteins, to activate other parts of the immune system. Natural killer cells are an example of immune cells that destroy any pathogen in its path with no need for prior exposure to the invader (Mor, 2007). Adaptive immunity is a more specific response toward a pathogen and is acquired. Activated T and B lymphocytes are specialized white blood cells that are involved in dealing with specific antigens. Antigens (Ag), molecules on foreign organisms that contain distinct epitopes or sites that can initiate an immune response, activate B cells and T cells that "remember" the specific antigens thereby reacting quicker and more vigorously toward the pathogen (Sela, 1998).

The Initial Stages of Pregnancy

After an egg is fertilized it forms a blastocyst that is implanted in the uterus. The sphere consists of an inner cell mass, which becomes the fetus after 8 weeks of conception, and an outer layer that forms the trophoblasts (Mor, 2007). Trophoblasts aid in implantation within the uterus. Syncytiotrophoblasts are multinucleated trophoblasts that form finger-like projections reaching into the mother's bloodstream thereby forming the placenta and aiding in nutrient and gas exchange. With this, understanding the conceptual framework of reproductive immunology is redefined. The trophoblast cells are the only part of the differentiating blastocyst that interacts directly with the mother's immune system. The embryo itself—and

the fetus to which the embryo gives rise—has no direct contact with maternal immune cells. As a result, the real puzzle is not why the mother's immune system tolerates the fetus, but why it tolerates the trophoblast cells (Colbern, Main, 1991).

Proposed Hypotheses

Downregulation of T cells

One study attempted to prove the hypothesis of downregulation of T cells. They discovered that macrophages, an important immune cell involved in antigen presentation, can disable killer T-cells. This, in turn, will prevent the T cells from attacking any object that is recognized as non-self (Anonymous, 1999). In order for this to occur, the syncytiotrophoblasts in the placenta produce an enzyme known as indoleamine 2,3-dioxy-genase (IDO). The function of IDO is to inactivate tryptophan, an amino acid required by T cells to destroy a foreign object (Munn, 1999). In 1990, Andrew Mellor, a colleague of Munn, concluded that IDO inhibits a mother's T cell response towards a genetically different fetus. Miscarriages occurred in the absence of IDO. (Munn et. al., 1998).

In another study he and his colleagues conducted experiments to prove their hypothesis. They used two groups of pregnant mice; one group had been bred to genetically identical fathers of the same strain while the second group was bred to fathers from a genetically different strain (Munn, 1998). The experimenters then embedded time release-capsules consisting of either 1-methyl tryptophan, which is an IDO inhibitor, or a control substance underneath the skin of the pregnant mice. Results showed that only the mice carrying genetically different fetuses that had been given the inhibitor rejected their fetuses (Munn, 1998). Interestingly, the embryos developed normally until inflammatory cells migrated to the implantation site and caused hemorrhaging around the embryo. Munn proposed "the mother is rejecting the placenta and eventually the embryo chokes off and dies" (Munn, 1994). From the data collected, they concluded that after implantation, an embryo starts making connections with the mother's blood supply. Sequentially, syncytiotrophoblasts will start producing IDO, destroying tryptophan and suppressing the maternal immune system (Munn, 1998).

To support the above hypothesis, researchers showed that the antigen receptors of maternal T lymphocytes that recognize paternal alloantigens are specifically downregulated during pregnancy, reducing their ability to initiate an immune response against the fetus (Simpson, 1996). During pregnancy, a semi-allogeneic fetus survives despite the presence of maternal T cells specific for paternally inherited histocompatibility antigens. A mouse transgenic for a T cell receptor recognizing the major histocompatibility (MHC) antigen H-2Kb was used to follow the fate of T cells reactive to paternal alloantigens. In contrast to syngeneic and third-party allogeneic pregnancies, mice bearing a Kb-positive embryo had reduced numbers of Kb-reactive T cells and accepted Kb-positive tumor grafts (Tafuri et. al., 1995). T cell responsiveness was restored after delivery. Thus, during pregnancy maternal T cells acquire a transitory state of tolerance specific for paternal alloantigens.

However, other researchers have reservations about this hypothesis. In "Pregnancy Reconceived", Mor argues that if the maternal immune system is suppressed, exposure to any pathogen would be fatal (Mor, 2007). With evidence of Kb-positive tumor grafts growing in mice (because of its commonality with paternal alloantigens and the risk of a pathogen) it would be nearly impossible for a mother and its fetus to survive. Research showed that presence of immune cells at the implantation site is not associated with a response to the 'foreign' fetus but to facilitate and protect the pregnancy. Therefore, the immune system at the implantation site is not suppressed, on the contrary it is active, functional and is carefully controlled (Mor, 2010).

Mechanical Barrier

Another hypothesis explains that the fetal tissue is unrecognizable as "nonself" by the mother's immune cells due to a mechanical barrier (Mor, 2007). Syncytiotrophoblasts around the fetus contribute to the mechanical barrier between the uterus of a pregnant mother and the rest of her body. This trophoblast-immune interaction includes three stages. Stage one is attraction, the trophoblast cells secrete chemoattractants that will signal immune cells to migrate to the implantation site. The area of the uterus in which the trophoblasts invade is referred to as the implantation site. Stage two, recruitment and/or education, the trophoblasts produce cytokines that regulate the differentiation of immune cells. Upon completion of these two steps, the response can take place. In this final stage, the immune cells from stage two respond to different signals (Swain, 2013). After completion of all three stages, the mechanical barrier is formed. This in turn prevents the movement of activated T cells from the periphery to the implantation site and enables antigens that are inside the barrier to be undetected by the mother's immune system.

In conflict with this hypothesis, researchers have found that the barrier between fetal and maternal is limited at best- fetal cells can be found in the maternal circulation and vice versa, indicating that there is only a partial physical separation between mother and fetus. Furthermore, although placental trophoblasts have reduced antigenicity and attenuated expression of MHC genes, an array of

transplantation antigens is clearly expressed (Fernandez et. al., 1999). A study showed that women produce antibodies and exhibit lymphocyte reactivity against fetal human leukocyte antigens (HLA) (Hunt et. al., 2003). This forces us to recognize that the immune system is not ignorant of, but instead recognizes and responds to these antigens.

Cytokine Shift Hypothesis

The immune system can generally be divided into the innate and adaptive immune system. The former is a nonspecific system providing immediate defense against pathogens, while the latter is more targeted, consisting of T and B lymphocytes. Although communication between these lymphocytes exist, B cells and their antibodies mainly contribute to humoral immunity, whereas T cells primarily provide cell mediated immunity (Abrams, Miller, 2011). T helper cells (CD4+) form a subset of T cells and can be further subdivided, depending on their cytokine production, into T helper 1 cells (Th1) and T helper 2 cells (Th2). Th1 cells secrete interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) which are proinflammatory cytokines, whereas the Th2 cells secrete interleukin 4 (IL-4), IL-10, and IL-13, anti-inflammatory cytokines (Sykes et. al, 2012). A mutually exclusive interaction exists between the Th1 interleukin, IFN-γ, and the Th2 interleukin, IL-4. IL-4 is the dominant factor for promoting growth and differentiation from the Th0 to the Th2 subtype, and directly inhibits the development of the Th1 cells (O'Garra and Arai, 2000). IFN-γ indirectly promotes Th1 differentiation by upregulating the IL-12 receptor whilst inhibiting the growth of Th2 cells (O'Garra, 1998).

Pregnancy is a complex immunological state and this hypothesis suggests that a shift towards T helper 2 (Th2) protects the fetus. When a foreign object enters the body of a woman who is not pregnant, Th1 cells will secrete proinflammatory cytokines that will signal for a cel-mediated response to occur. However, according to the cytokine-shift hypothesis, the balance of Th1 and Th2 will go towards the secretion of cytokines by Th2, resulting in a suppressed inflammatory response (Mor, 2007). Additionally, inhibition of the production of TNF-β and IFN-γ is aided with IL-4 secreted by Th2 cells. The production of IFN-γ, TNF-β and IL-2 are believed to be damaging to pregnancy. In an experiment studying pregnant mice, these cytokines were injected into the mice and caused fetal loss (Koch and Platt, 2003).

Many studies have been conducted in an effort to better understand this cytokine shift. In an experiment conducted by Sykes, et. al. (2012), subjects included pregnant women at 28 weeks and term (prelabour and in labour) and nonpregnant women of child bearing age. The experiment consisted of the isolation of Peripheral Blood Mononuclear Cells (PBMCs). In this study, they employed flow cytometry to examine the effect of stimulation by the mitogen PMA/ionomycin on cytokine production at different gestational stages of pregnancy and during labour (see Figure 1). Th1 cytokine profiles of CD4 positive cells were assessed for intracellular IFN-γ and TNF-α and compared to levels of nonpregnant controls. The percentage of peripheral T cells producing IFN-γ in response to stimulation reduced in pregnancy from 10.7% in nonpregnant women to 6.7% at 28 weeks, 5.1% at term, and 5.6% at term in labour (Sykes, et. al. 2012). Additionally, there was a reduction in the proportion of TNF-α producing cells, although not significantly, from 20.6% in nonpregnant women to 14.5% at 28 weeks, 15.8% at term and 13.3% at term in labour. Overall levels of Th1 cytokine production (expressed as mean fluorescence intensity), in the CD4+/IFN-γ+ or CD4+/TNF-α+ cells, remained consistent throughout gestation. The Th2 cytokine, IL-4, was similarly assessed in CRTH2 positive cells. While PMA/ionomycin stimulation did not increase the percentage of IL-4 expressing cells, the mean fluorescence intensity of IL-4 was significantly increased in samples collected from women at 28 weeks (39.3,) and at term (39.4,) compared to levels of nonpregnant controls (37.1) (see Figure 1). Levels of IL-4 in term labouring samples were consistent with non labouring samples (37.1). The ratio of the IFN-γ:IL-4 producing cells reduces during pregnancy, due to the suppression of the Th1 rather than the promotion of the Th2 cytokine production (Sykes, et. al. 2012).

Many researchers agree that cytokines play a crucial role during pregnancy. Koch and Platt mutually agree that a Th2 response is necessary for the fetus to survive in the womb. Results from an experiment with mice showed that there was a 20-50% rate of fetal loss due to a lack of Th2 cytokine production. Furthermore, they applied this idea to humans and suggested that irregularities with Th2 cytokine response may lead to miscarriages. However, the researchers advise for additional investigation in order to validate the cytokine-shift hypothesis.

Local Active Suppression of Decidua

In addition to the general suppression of a mother's immune system, researchers have found that an important role in the maternofetal interaction is the local active suppression by cells in the decidua (Chaouat, 1990). The decidua is the thick layer of modified mucous membrane which lines the uterus during pregnancy and is shed with the afterbirth. This lining allows for nutrition and gas exchange before the placenta is functional (Mizugishi, et.

al. 2007). Despite its importance for embryogenesis, the development and function of the decidua remains very poorly understood. "On the one hand, we are pursuing the possibility that decidual tissue impairs the overall function of dendritic cells" (Erlebacher, 2010). Critical for initiating T cell-mediated immune responses within lymph nodes, these cells are the most potent antigen-presenting cells. The research team has discovered, in a mouse model, that the onset of pregnancy causes the genes that are responsible for recruiting immune cells to sites of inflammation to be turned off within the decidua. As a result of these changes, T cells are not able to accumulate inside the decidua and therefore do not attack the fetus and placenta. Informatively, they revealed that the implantation of an embryo changes the packaging of certain chemokine genes in the nuclei of the developing decidua's stromal cells. The change in the DNA packaging permanently deactivates, or "silences," the chemokine genes. Consequently, the chemokines are not expressed and T cells are not recruited to the site of embryo implantation (Erlebacher, 2010). Therefore, local active suppression versus systemic suppression aids in the prevention of foreign objects attacking the mother's immune system and fetal rejection (Chaouat, 1990).

Also of note, the observed change in the DNA packaging was an 'epigenetic' modification, meaning a modification that changes gene expression without the presence of a heritable gene mutation. This explains the mechanisms of fetal-maternal immune tolerance as a modality for limiting the trafficking of activated T cells. They concluded that the decidua appears as a zone of relative immunological inactivity due to the fact that the cells that typically secrete the chemoattractants to bring the T cells to sites of inflammation are inhibited from doing so in the context of the pregnant uterus. (Nancy, et. al., 2012). Inappropriate regulation of this process, Dr. Erlebacher explained, could cause inflammation and the accumulation of immune cells at the maternal-fetal interface, which could lead to complications of human pregnancy, including preterm labor, preeclampsia and spontaneous abortion.

While this hypothesis holds the most significance and the least flaws it still needs to be adjusted. A study showed that removing macrophages (an immune cell) caused pregnant mice to miscarry (Mor, 2007). This, along with the antibodies produced, as mentioned above, highlights the immune system activity that exists among the maternofetal relationship.

Conclusion

The phenomenon of a pregnancy and what it entails is astounding. Attempting to understand the intricacies of the maternofetal relationship forces us to realize the vastness of this topic and how much more research is needed to unravel this complex "riddle". With many theories being investigated, it appears that the hypothesis that holds most credence in explaining how a fetus with foreign antigens survives the mothers immune system is the local active suppression within the decidua. Nevertheless, amongst these hypotheses, there is more research that needs to be done concerning the role of the immune system in pregnancy. Pregnancy is complex and is divided into stages. Many researchers believe that labour, which brings the pregnancy to completion, is an immune response. There seems to be the need of a balance between the immune system activity versus inactivity depending on the stage of pregnancy.

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Rapamycin and Metformin in Treating COVID-19

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Abstract

The SARS-CoV-2 virus has spread rapidly, resulting in a global pandemic. There is a great need for an effective drug cocktail therapy to combat Acute Respiratory Distress Syndrome (ARDS), a major cause of death due to COVID-19. The two drugs examined are metformin, an antidiabetic medication, and rapamycin. Rapamycin is often prescribed for transplant patients as it has an immunosuppressive effect. The aim of the investigation was to determine the efficacy of metformin and rapamycin in treating COVID-19, and to examine what an effective protocol would look like. These two drugs both inhibit mTOR and can reduce the body's auto-immune response, which destroys bronchial cells via cytokine storms. Both drugs have a long history of clinical use and have sufficient evidence of efficacy. They possess antiviral properties and downregulate inflammatory markers, making them excellent candidates for further study, both individually and in combination. Rapamycin has been shown to reverse markers of aging and can help repair organ damage. Importantly, metformin can help negate the toxic side effects of the potent rapamycin, while still preserving the positive effects of the compound. Metformin also has been shown to aid those who are at risk of developing ARDS due to comorbidities such as diabetes or hypertension. As such, using metformin as a preventative therapy, either alone or with small doses of rapamycin, may be warranted in patients at risk (Hussain et al, 2020) (Malhotra et al, 2020).

Introduction

The SARS-CoV-2 virus's lethality can be largely owed to Acute Respiratory Distress Syndrome (ARDS), a major cause of death due to COVID-19. The lungs become inflamed and are heavily damaged as fluid from the blood vessels leaks into the lungs, obstructing breathing and depriving the organs of adequate amounts of oxygen. This results in high morbidity and mortality rates. The overall percentage of mortality in patients with ARDS was 40 percent, making the disorder of primary importance in the search to reduce deaths in the patient population (Tzotzos et al., 2020).

The ability to repurpose drugs is vitally important to combat novel pathogens and can shorten the amount of time and money required to develop an effective protocol. Novel viruses often use elements of central pathways that are commonly used by other viruses. Therefore, drugs that have been previously developed can prove efficacious, as they target the same pathway. Additionally, the drugs that are currently in use have already been proven to be effective and safe, which allows their immediate use until another, more pathogen-specific, drug can be developed. Furthermore, modeling the drug after an existing therapeutic agent can shorten the amount of time and energy required to develop a new agent, as portions of the previous drug can be used as a starting point. This can all add to an advantage that may save countless patients' lives as they wait for a new drug to be developed (Husain et al, 2020).

One of the more interesting drugs proposed as a therapeutic involves the use of metformin, an anti-diabetic medication. This drug has been touted for its anti-inflammatory effects and seems to have a positive effect on the mortality rate of patients suffering from COVID-19, even though the exact mechanism of action remains contested. Possible theories range from its ability to act as a strong base, disrupting the viral envelope, to its ability to up-regulate the ACE-2 enzyme, the critical binding site for the spike protein that serves as the virus' entry point (Scheen, 2020).

Another potential drug being offered as therapy is rapamycin. Rapamycin first gained recognition as an anti-rejection medication for transplanted organs. It suppresses the immune system, resulting in less inflammation, and has a protective effect, shielding the patient from ARDS. Rapamycin can ensure that even if a cure for the virus remains elusive, we can mitigate the negative physiological effects of the illness on the patients. It would therefore be advantageous to examine the differing protocols involving metformin and rapamycin for their efficacy in treating COVID-19 generally, and ARDS specifically, in vulnerable patient populations (Husain et al, 2020).

Methods

Several databases (PubMed, Medscape, clinicaltrials.gov) were systematically reviewed for the relevant literature. Papers concerning the effects of metformin and rapamycin were studied extensively, both in relation to COVID-19 as well as other illnesses.

Discussion

Metformin is a drug that is generally used in the treatment of type 2 diabetes as well as other metabolic conditions. Its widespread use and its minor side effects have led to it being christened the "aspirin of the 21st century." It was originally introduced as an anti-influenza medication before being used for its effects on glucose reduction. It works primarily by decreasing the production of hepatic glucose while simultaneously increasing the action of insulin on tissues, combatting a major factor of type 2 diabetes. Metformin did not achieve widespread use in the United States until 1995, yet by the year 2017, it was the fourth most prescribed medication in the United States. Even though the exact mechanisms of metformin are unknown, there are multiple proposed mechanisms of action, including inhibition of the respiratory chain in complex 1 of the mitochondria and lowered production of cyclic AMP. Cyclic AMP is the major activating factor for

protein kinase A, an essential part of the second messenger pathway involved in hormonal regulation. This greatly assists the uptake of glucose, preventing insulin resistance, the primary cause of type 2 diabetes. Insulin resistance is caused by an overproduction of insulin, which causes the bodily tissues to require more of the molecule to uptake glucose, resulting in the increasing inability of glucose to migrate into the resistive tissue (Sharma et al, 2020).

The SARS-CoV-2 virus initially binds to the angiotensin-converting enzyme 2 (ACE2). The virus uses its spike protein (S) region to bind to the enzyme, forming a complex that allows the virus entry into the cell. After gaining entry to the host, the virus will downregulate the ACE2 receptor, leading to excessive inflammation, including cardiovascular damage and ARDS. The inflammation is due to ACE2/Ang (1-7)/Mas acting within the Renin Angiotensin Aldosterone System (RAAS) as the anti-inflammatory element to counteract the ACE1–Ang II pro-inflammatory arm. The inflammatory arm causes elevated systemic blood hypertension and inflammation. Metformin is hypothesized to be able to counteract the downregulation of the ACE2 receptor through stimulation of the AMPK/mTOR pathway. The AMPK/mTOR pathway is thought to be involved in increasing the upregulation and stability of ACE2, by phosphorylating ACE2 Ser680. (Malhotra et al, 2020). Although clinical evidence in vivo is required, there is strong evidence that Metformin upregulates ACE2, from studies with human umbilical vein endothelial cells (HUVECs) and human embryonic kidney 293 (HEK293T) cells (Zhang et. al. 2018b). Additionally, phosphorylation decreases ubiquitination, extending its half-life. Furthermore, the phosphorylation of the ACE2 enzyme by a large negative phosphate group would change its 3D conformation and sterically hinder the binding complex that the virus uses for entry. In this way, metformin can decrease the ability of the virus to enter the host and reduce mortality through its upregulation of the ACE-2 receptor (Malhotra et al, 2020).

There is evidence that by upregulating ACE-2, Metformin can exhibit a protective effect on the cardiopulmonary system through the AMPK pathway. AMPK activation often leads to ACE2 conformational changes leading to SARS-CoV-2 having a greater difficulty binding to the receptor (Malhotra et. al. 2020). A study found that in animal models "Transgenic mice overexpressing the phosphomimetic ACE2 S680D exhibit less damage in pulmonary vasculature under injurious conditions" (Zhang et al., 2018). Metformin has also been shown to have a healing effect on the pulmonary system in lipopolysaccharides in animal models (Jian et al., 2013).

The glucose-lowering effects of metformin seem to have a significant effect on survivability. Hyperglycemia has been labeled a major risk factor in various Chinese studies studying pulmonary illnesses. (Chen et al, 2015). A study of patients with diabetes and COVID-19 found that patients with well-controlled blood glucose levels had a much better prognosis and overall reductions in mortality (Crouse et al, 2020). As such, metformin's proven ability to aid in the treatment of type 2 diabetes is important for diabetic patients that contract COVID-19. Another study has demonstrated that there is a direct correlation between the SARS-CoV-2 virus and diabetes, leading to speculation that the virus can enter the cells of the pancreatic islets where there is an expression of the ACE2 enzyme. This leads to damage to the pancreatic beta cells, which produce insulin, leading to transient type 2 diabetes mellitus (Yang, 2010). This would indicate that the regulation of diabetes is of primary importance for the treatment of COVID-19. Metformin's previously stated effects on insulin sensitivity would make it an ideal candidate.

Two retrospective studies of COVID-19 patients with diabetes from China in early 2020 found that Metformin could provide benefit. In a study of diabetic patients, the mortality rate for patients on metformin was lower than the group that did not receive the drug. This was despite the metformin patients having higher levels of fasting glucose on admission. The length of the hospital stay was the same between the two groups. (Luo et al. 2020). The SARS-CoV-2 and Diabetes Outcome study in France initially showed that patients who were given metformin before admissions had a lower mortality rate after seven days (Cariou et al, 2020). However, multiple confounding factors needed to be accounted for, which once considered, rendered the findings no longer significant.

In a retrospective analysis studying whether Metformin had any favorable effects 1,213 type 2 diabetic patients with COVID-19 in 16 hospitals in Hubei, China were examined. They excluded confounding variables such as eliminating patients with a glomerular filtration rate of less than 30ml/min or who had cirrhosis. Patients exclusively using insulin were also eliminated. They looked at patients treated with metformin and other drugs compared to just other diabetic medications. They used propensity score matching to adjust for confounding variables and used a Cox regression model to account for the changes in clinical conditions throughout the patients' stay. They discovered that the length of stay and the 28 days all-cause mortality rate were both unchanged, though metformin was associated with higher levels of acidosis (Lui et al, 2020). Metformin had a protective effect, guarding against heart failure and the body's various inflammatory responses. Markers of heart failure,

inflammation, and cardiac injury were examined, and all were found to be lower. With these data taken together, according to the results of the study, Metformin exhibits a positive effect on cardiac failure and inflammation (Cheng et al., 2020). This was particularly true in severe cases but did not translate into an overall reduction in mortality. The studies' discovery that Metformin does not influence mortality was contrasted by alternate findings in which researchers had found an improvement in patients using Metformin (Luo et al.2020). It is possible that since in the Luo study, patients on insulin were included in the non-Metformin group, there was a confounding variable that biased the results.

The poor outcomes of diabetic patients can be explained by several factors. Patients with diabetes generally live with greater levels of overall inflammation daily, putting their already inflamed tissues at an increased risk of destruction of the bronchial epithelium by the body's immune system, resulting in ARDS (Azar et al, 2020). Elevated levels of glucose in patients with diabetes is an additional risk factor as high blood glucose levels have been shown to depress the immune system (Ceriello et al, 2020). These reasons make metformin a viable candidate for the treatment of diabetes in COVID-19 patients, particularly as its glucose-lowering effects can help mitigate the negative role of diabetes (Kow et al, 2021). Additionally, there seem to be positive effects independent of the reduction of blood glucose. Various biochemical mechanisms are involved in Metformin's suppression of inflammatory cytokines, which would aid in the prevention of an overwhelming immune response on the part of the host (Lui et al, 2020).

Metformin's can play a role in the reduction of inflammation in patients either with or at a high risk of contracting ARDS (Acute Respiratory Distress Syndrome). Metformin has proven to be effective at diminishing cytokine storms, an immune response that damages the body's cells. It does this by inhibiting interleukin 1α and 1β, important kinases for pro-inflammatory action. The standard measure of systemic inflammation is the neutrophil to lymphocyte ratio, which is used as a marker. Tracking these markers showed an overall reduction of inflammatory cytokines. In a follow-up study of nondiabetic patients with heart failure, it was demonstrated that metformin suppressed plasma cytokines (Cameron et al, 2016). This makes metformin a viable choice in the prevention of inflammation and the formation of ARDS.

The pathway that metformin uses in its anti-inflammatory effects is by the inhibition of tumor necrosis factor-α–dependent IκB (IkappaB kinase) degradation. In an experiment, researchers treated mouse hepatocytes with metformin. They found that "Metformin treatment for 3 hours suppressed TNFα-induced degradation of the NF-κB negative regulator IκB while modulating AMPK and mammalian target of Rapamycin signaling in a dose-dependent manner" (Cameron et al, 2016). Metformin also inhibited signaling downstream by inhibiting the cytokines that are normally produced and activated, 5' AMP-activated protein kinase (AMPK). AMPK is an enzyme that increases glucose uptake and is often thought of as a negative regulator of inflammation. In the same vein, IL-1β (interleukin-1-β), and IL-6 (interleukin-6) which are both markers of TNF-α–dependent inhibition, were greatly reduced (Cameron et al, 2016).

A 30-day study examining the mortality of nursing home patients found that metformin was associated with significantly less mortality (Lally et al, 2020). Additionally, a retrospective analysis was made of claims made by the United Health group's Clinical Discovery database. This study analyzed the records of enrollment of COVID-19 patients across all 50 states, as well as the various pharmacy claims and laboratory reports. They found that when metformin was given to adults with type 2 diabetes mellitus or obesity there was a marked decrease in mortality exclusively for women, with men receiving no benefit. This finding partially confirms the proposed theory that metformin would be beneficial based on its known effects of decreasing levels of tumor-necrosis-factor α (TNFα). TNFα is of particular importance as patients with COVID-19 have shown to have remarkably high levels of it in their lung tissue. Patients with diabetes also have high levels of TNFα, as diabetes has been shown to further elevate levels. Metformin also promotes the upregulation of anti-inflammatory cytokine, IL-10. Additionally, The study found that TNFα inhibitors were associated with reduced mortality (Bramante et al, 2020).

This study, while interesting, has several noticeable flaws, including the obese and overweight patient samples and the lack of strength associated with retrospective analysis. Additionally, patients may have been prescribed Metformin previously, due to its prevalence, which may have gone unreported. It is important to stress that although a helpful effect was observed, these were individuals with other comorbidities such as T2DM and obesity. The protective effect may be much less pronounced with a patient group that has a lower risk factor and absence of these comorbidities.

Another factor in analyzing the results of the study is the difference in the sensitivity required to activate mast cells between males and females. Mast cells are an early indicator for the SARS-CoV2 immune response. Females exhibit a far greater increase in TNFα than their male

counterparts. Metformin would therefore exhibit a greater positive effect in females, as they are naturally predisposed to a more severe reaction that can be inhibited by the drug. Women and men differed in their cytokine responses even though the levels of the ACE2 receptor were equal (Bramante et al, 2020).

Rapamycin or Sirolimus is a potent anti-transplant rejection drug that can suppress and inhibit mTOR. mTOR is a serine/threonine-protein kinase that is composed of a two-part protein complex named mTORC1 and mTORC2. mTORC1 is the complex that is sensitive to rapamycin and other more common factors such as oxygen, glucose, and various amino acids. mTORC2 is insensitive to rapamycin and acts as an effector of insulin/IGF-1 (Insulin-like growth factor-1). The proteins s6, p70S6K and 4E-BP1 are the point of control for many cellular functions when phosphorylated by mTORC1. This controls protein synthesis and the cell's self-destruction mechanism known as autophagy. mTORC2 is mostly required for other kinases such as Protein Kinase B. One of the reasons that the mTOR pathway is so vital is that it regulates pivotal moments in the life cycle of a cell including metabolism, transcription, proliferation, and eventually, cell death. This has led mTOR, and by extension, rapamycin to be often studied in the examination of aging, also known as cellular senescence (Husain et al, 2020).

Rapamycin first rose to prominence when it was discovered to block the immune system generally, and T cell proliferation specifically. Additionally, mTOR is involved in the cellular division cycle, playing a role in the transition of the G1 to the S phase. Rapamycin as an inhibitor blocks the cell cycle. This would suggest that mTOR can serve as an important chokepoint in mitigating the spread of the virus as it could block the proliferation of infected cells (Husain et al, 2020).

In numerous studies examining the mortality of patients with COVID-19, it was found that that a primary factor in the occurrence of infectious diseases was blood Vitamin D concentration. In a study of elderly patients, Vitamin D helped reduce the inflammatory response in the upper respiratory epithelium and lowered the risk of developing intense symptoms (Grant et al , 2020). Vitamin D has also been shown to interact with the ACE2 enzyme, limiting the virus's entry into the cell, as this serves as the entry point for the SARS-COV-2 virus. Evidence would suggest that the mechanism of action for the positive effects of Vitamin D is the suppression of the mTOR pathway through multiple mechanisms. One of these mechanisms involves a regulator known as "regulated in development and DNA damage response 1 (REDD1), a suppressor of mTOR activity", which is stimulated by the 1,25(OH)2D form of Vitamin D (Husain et al, 2020).

In all, it would be reasonable to suggest that the numerous benefits of Vitamin D are achieved through the inhibition of mTOR and can therefore be stimulated directly by an mTOR inhibitory agent such as rapamycin.

The anti-aging effects of Rapamycin can be of great use as well, with the drug being shown to extend the life of mice significantly (Harrison et al, 2009). But, importantly for the treatment of COVID-19, Rapamycin has been shown to rejuvenate damaged tissues such as damaged cardiac cells and increase the vitality of hematopoietic stem cells (Guarda et al, 2004). It may be that as proteins are continuously synthesized, they acquire damage and various misfolding that inhibit their function and are the prime indicators of age-related diseases. The domain that is affected by rapamycin is mTORC1. Inhibiting mTORC1 reduces protein synthesis and causes the cell to induce autophagy, thereby recycling the damaged components. COVID-19 has been proven to affect the elderly population at an increased rate, with higher mortality being attributed, making rapamycin a potential anti-aging drug that can mitigate the negative effects of age-related vulnerabilities (López-Otín et al, 2013). Additionally, it might go some way in aiding with the reversal of organ damage that is a hallmark of critical cases of COVID-19.

mTOR regulates metabolic processes that help to serve as signaling for anabolic (building) and catabolic (dismantling) processes in the cell. mTOR inhibition has been shown to protect against high fat-induced obesity in mice by regulating the breakdown of glycogen and other processes involving glucose. This could make rapamycin useful in mitigating the negative effects associated with insulin resistance and obesity in COVID-19 patients, which has been proven to result in negative health outcomes (Saxton et. al. 2017).

Acute respiratory distress syndrome (ARDS) is caused by the breakdown of upper respiratory epithelia that results from a cytokine storm that destroys the body's tissues. This can lead to multi-organ failure that can often be fatal. The various cytokines that are released are "IL-2, IL-7, IL-10, MCP-1 (monocyte chemoattractant protein), MIP1A (Macrophage Inflammatory Proteins) and TNF-α (Tumor Necrosis Factor-α)". Rapamycin's primary effect as an immunosuppressive drug can decrease the levels of the cytokines in the body, making it a useful tool for dealing with cytokine storms (Costela-Ruiz et al., 2020). Rapamycin's targeting and inhibition of a wide variety of cytokines make it an ideal drug for the suppression of harmful immune responses, making it potentially more useful than the drug Tocilizumab and other monoclonal antibodies that merely target individual cytokines.

Rapamycin's inhibition of the mTOR pathway remains

very promising not only for its pleiotropic effects on the cell's regulatory mechanisms but also for its anti-immune properties. It is important to mention, however, that rapamycin's side effects can be rather unpredictable. This makes it necessary to monitor the effects of rapamycin in each patient and to educate them about the potential negative side effects. There has been a proposal to examine upstream mutations in mTORC1 signaling to determine ideal candidates for therapeutic interventions using rapamycin. This would help mitigate the negative side effects experienced by members of the patient population.

T-cell senescence is prevalent in long term infections and cancer and is a state of T-cell dysfunction. Cytokine storms can play a role in inducing T-cell apoptosis and necrosis, leading to overall lower T-cell counts. Patients with COVID-19 have been observed to have lower CD4+, CD8+, and total T-cell numbers which are all implicated in lowering the survival rate of patients with the illness. Even when the CD4+ and CD8+ cells are present in severely ill COVID-19 patients, they exhibit less function overall and are unable to secrete perforin, granzyme, and IFN-γ, all of which are cytotoxic molecules. The senescent markers PD-1 and Tim-3 are also present at higher levels. Cells that are senescent release certain cytokines and molecules that are indicative of the cell's status and are known as the "senescence-associated secretory phenotype (SASP)". Rapamycin, being an mTOR inhibitor, can suppress SASP and by extension the cytokine storm that results from T-cell senescence. Therefore, administration of Rapamycin in the early phase of the cytokine storm might prevent the emergence of a severe form of COVID-19 through the downregulation of SASP (Omarajee et al, 2020).

Rapamycin has a history of being beneficial regarding respiratory infections, and has been shown to reduce the recovery time in H1N1 and SARS patients. study that examined 38 patients with H1N1-induced pneumonia, reported that Rapamycin was associated with positive outcomes in the overall prognosis of patients and shortened their time on a ventilator. It also was associated with significantly increased viral clearance, lower rates of hypoxemia, and reduced multiple organ dysfunction. Both H1N1 and SARS-CoV-2 activate mTOR, leading to lung inflammation, fever, and other intense immune reactions. Rapamycin may provide a significant benefit by inactivating mTOR and therefore IL-1β secretion, the mediator of inflammation (Wang et al., 2014).

Rapamycin is currently undergoing phase two clinical trials to determine its efficacy in a 30-patient sample that seeks to improve clinical outcomes in COVID-19 patients (NCT04341675). The study will last for 14 days with 2mg being given orally daily. The primary outcome is to determine the proportion of patients that do not need advanced respiratory support by 28 days. The secondary outcomes involve tracking changes in lymphocyte concentrations as biomarkers as well as the proportion of patients requiring general respiratory support.

There have already been numerous studies done on the use of rapamycin and metformin in the treatment of a plethora of cancers and tumors, including pancreatic and breast cancers (Faria et al, 2019; Amin et al, 2019). These studies have determined the combination to be safe and effective, with the combination able to target slightly different pathways than any of the two drugs alone. Rapamycin can cause glucose intolerance and insulin resistance if taken long term. These effects may be mitigated by metformin, due to metformin causing increased insulin sensitivity in tissues. The ITP (Intervention Testing Program) reported that the effect of both rapamycin and metformin on longevity when taken together was far superior to the effect of each drug alone. It should be noted that in mice the combination of the two drugs did reduce the effective concentration of rapamycin in females and metformin in both sexes. The final concentrations were still within the range to be clinically useful (Strong et al, 2016).

In a study conducted on mice, examining the effects of metformin on glucose, the animals were given both rapamycin and metformin. Interestingly, in contrast to studies that had been conducted previously, the researchers found that metformin did not inhibit mTOR and stopped rapamycin from inhibiting mTOR in the liver. The researchers theorized that metformin could alleviate the disfunction in gluconeogenesis that was found in other mTOR mutant mice (Kim et al, 2020).

Metformin and rapamycin would seem uniquely suited for COVID-19 patients with obesity and diabetes. These patients can be aided by glucose mitigation of metformin but can also cycle lower doses of rapamycin for the potent mTOR effects. In a study of rats being given rapamycin, the researchers attempted to see if they could mitigate the increased hepatic gluconeogenesis caused by rapamycin administration by combining it with metformin. The female mice in the study exhibited significantly lower gluconeogenesis, thereby implying that the metformin served to remove the harmful side effects of rapamycin (Weiss et al, 2018). Here, as in our previous discussion, the effect was sex-specific for many of the reasons outlined previously.

In a study examining metformin and rapamycin on the proliferation of pancreatic cancer cell growth, the optimal therapeutic dosage was determined to be (20 mmol/l) Metformin + Rapamycin (200 ng/ml) in vivo. The study discovered that the combination was vastly more effective than monotherapy at inhibiting mTOR (Zhang et al, 2018).

Conclusion

In all, given the evidence that metformin reduces the mortality and morbidity in diabetic patients, it should remain a drug of primary importance in the treatment of COVID-19 in diabetic patients. The effects of the drug on insulin resistance can play an important part in reducing the immunosuppression found in diabetic patients due to the abundance of glucose in the bloodstream. It should be noted that the guidelines urging concern about metformin inducing acidosis in patients was specifically applicable to patients already in multi-organ failure. Additionally, even though the prevalence of acidosis was higher in patients that were given metformin, it did not affect the mortality rate(Cheng et al, 2020). As such, the positive effects of metformin would make it an excellent candidate for further study, not only in patients with preexisting comorbidities but as a possible therapeutic given its anti-inflammatory properties. Metformin's significant effects on the RAAS system can make it an important agent in the reduction of inflammatory cytokines that are the hallmarks of COVID-19.

Regarding metformin in the treatment of patients with COVID-19 and diabetes, it should be noted that many of the studies are retrospective and examine patient outcomes using statistical analyses. They seek to determine the efficacy of metformin in mitigating the detrimental effects of diabetes on COVID-19 patients. The positive effects of metformin previously noted, there is a paucity of data of metformin being given to COVID-19 patients without diabetes. Many of the studies specifically look at the outcomes of patients who were previously given metformin to control diabetes before the virus and were then examined to determine the beneficial effects specifically on patients with diabetes and COVID-19. But for such a widely prescribed drug such as metformin, it is shocking that there exists so few clinical trials for individuals without diabetes as a comorbidity. Many papers propose that metformin's anti-inflammatory effects could be of use to the wider population to reduce the negative immune response, but as of the date of this paper, there have not been any clinical trials examining the exact outcomes of the drug on a non-diabetic patient population.

There may be several reasons for the lack of clinical trials, among them, that even if metformin does show some benefit in its ability to prevent an overzealous immune response, other drugs currently being used or examined in the treatment of COVID-19 are even more effective through a similar mechanism. Tocilizumab, for example, has already been shown to help in CAR T-cell-induced cytokine release syndrome, which bears many similarities to the cytokine storm that can occur in COVID-19. A common side effect of Tocilizumab, however, is an elevation in blood pressure, which has proven damaging in COVID-19 patients (Jones et al, 2010). Therefore, it would be efficacious to conduct a thorough study of metformin, given its ability to be tolerated by a wide subset of the population and its proposed benefits in its ability to downregulate the immune system.

Additionally, many of the monoclonal antibody therapies that are being examined specifically exert their effects on one target, while metformin has been shown to operate across many different pathways. Metformin lowers inflammation and oxidative stress, all the while enhancing the immune system of patients.

Rapamycin can be a dangerous compound that must be handled with caution due to its ability to act as a potent inhibitor of mTOR. While mTOR inhibition can be beneficial in the reduction of inflammation, it is important not to inhibit the pathway completely. A study examining the effects of rapamycin on elderly patients discovered that total inhibition of mTOR stops the function of T cells and leads to complete immunosuppression. While some suppression of the immune system is favorable due to its effect on cytokine storms, the body's natural defenses must remain viable. As such, the dose of rapamycin must be carefully monitored to be safe and effective. In that same study, they determined that the most effective dose at promoting a healthy immune response in elderly patients was the lowest dose of rapamycin followed by a flu shot (Mannik et al., 2014). This has a great deal of significance as of early 2021, due to the availability of the mRNA COVID-19 vaccine currently seeing widespread use. The use of rapamycin followed by a vaccine should possibly be explored as a method of enhancing immunity in vulnerable patient populations. This could maximize the effectiveness of a protocol involving not only the currently available vaccine, but also carries implications for other protocols involving vaccinations.

The combination of rapamycin and metformin used in pancreatic cancer has been proven to be safe and effective. The dose used was the optimal dose to lower the expression of mTOR in pancreatic cells, and it stands to reason that this dosage would work in other cases where mTOR should be partially inhibited, such as in COVID-19. Rapamycin inhibits mTOR directly, while metformin does it through the AMPK pathway (Zheng et al, 2020).

The dose of rapamycin administered must be carefully controlled to avoid negative side effects. Metformin can temper some of these effects, which can include insulin resistance and glucose intolerance, through its ability to promote insulin sensitivity. Rather than administering the two drugs as a cocktail, risk can be assessed by the

administration of each drug separately. This would allow a thorough examination of the differing effects while still allowing metformin to counter the deleterious side effects of rapamycin.

An important point in discussions concerning the treatment of viruses is the health of the host. It has been demonstrated that immunocompromised patients and those with significant comorbidities fare far worse than patients without these complications. Metformin has the dual role of not only directly combatting the virus, but also reinforcing the immune system of the host. The ability to reduce comorbidities (e.g.: diabetes) should not be treated flippantly. A major factor in the morbidity rate in the United States has been a large proportion of the population with high blood pressure and obesity (Azar et al, 2020). These conditions have been proven to have significant negative effects. If metformin can provide a mechanism to reduce these additional comorbidities, the patient's health outcomes could be vastly improved.

Taking metformin to prevent an extremely negative reaction to COVID-19 should be considered. Being that metformin is a mild mitochondrial toxin that blocks mTOR, it would be a useful preventative drug. mTOR inhibitors have been shown to block viral action and express innate antiviral gene expression. In previous studies, mTOR inhibitors were effective against other coronaviruses. Prescribing metformin to vulnerable patient populations as a preventative drug may grant the benefits of mTOR inhibition without rapamycin's toxic side effects (Benedetti et al, 2020).

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What are the Possible Causes and Effective Therapeutic Approaches of Preeclampsia?

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Abstract

Preeclampsia is a complication of pregnancy primarily characterized by hypertension and proteinuria and affects other organs as well. The underlying causes are not yet fully understood. However, it is suggested that angiogenic factors of the placenta, genetic factors, a malfunctioning immune system, and oxidative stress all play a role in causing preeclampsia. Currently, the only definitive cure known for preeclampsia is delivery of the baby. Management of the condition includes taking preventative measures as well as drugs such as labetalol and MgSO4. This paper analyzes mechanisms of preeclampsia and determines the possible causes and most effective ways to manage the condition.

Introduction

Preeclampsia (PE) is a multisystem complication that occurs during pregnancy and is primarily characterized by elevated blood pressure and abnormally high levels of protein in the urine. The disorder manifests itself after the 20th week of gestation and can lead to serious complications, possibly even fatality of the mother and the baby. It can also cause long-term health conditions. The disease has a worldwide prevalence of 5-8% of pregnancies and currently ranks as one of the leading causes of maternal and perinatal morbidity (Pennington et al., 2012). In addition to hypertension and proteinuria, afflicted patients may undergo other symptoms including edema of the face and hands, headaches, dizziness, decreased urination, nausea, and vision changes. Furthermore, 10-20% of women with severe cases of PE can develop a potentially lethal condition known as the HELLP syndrome, characterized by the fundamental features of "hemolysis, elevated liver enzyme levels, and low platelets." If PE progressively worsens, it can turn into eclampsia, where the elevated blood pressure causes the mother to experience seizures and may result in a coma (Preeclampsia. 2016).

The exact etiology of PE remains unknown. The purpose of this review is to determine the possible causes of PE and the most effective therapeutic approaches available to manage the condition.

Methods

This comprehensive review was conducted based on critical analyses of data collected from various databases accessed through Touro College's online library, such as ProQuest and PubMed. The National Center for Biotechnology (NCBI) website was also a useful tool in providing additional sources. Key words and phrases used to retrieve data include "mechanisms of preeclampsia," "causes of preeclampsia," and "management of preeclampsia."

Discussion

PE can be classified into an early onset and late onset form. Women suffering from early-onset PE are diagnosed before their 34th week of pregnancy and symptoms of low birth weight and intrauterine growth restriction will manifest. Conversely, the late onset form expresses itself after the 34th week and its cause is generally associated with various maternal conditions including obesity, diabetes, and chronic kidney diseases. Regardless of the subtype, it is evident in all cases of PE that the placenta plays a central role in its pathophysiology (Lisowska et al., 2018).

In normal pregnancies, an increase in blood flow to the uterus will occur to ensure sufficient supply for the intervillous spaces and overall proper fetal development. Cytotrophoblasts invade deep inside the maternal spiral arteries to establish a vascular network and remodeling subsequently takes place to form high-capacity blood vessels. This mechanism is defective in the placentas of individuals destined to develop PE. In such cases, the cytotrophoblasts fail to entirely convert from their proliferative epithelial form into their invasive endothelial form. As a result, remodeling of the spiral arteries is greatly hindered and the restricting maternal vessels will lead to placental ischemia (Rana et al., 2019).

Another identifying feature found to be associated with the pathogenesis of this disorder is elevated levels of an antiangiogenic protein called soluble fms-like tyrosine kinase (sFLT1) in the placenta (Roberts & Bell, 2013). This was furthered studied on non-human primates. A group of animals was induced with uteroplacental ischemia (UPI) while a Sham group of animals was not. The concentration of plasma sFLT1 of both animal groups was measured over a two-week period and the results were compared. As seen in Figure 1, the UPI animals showed a significant increase in sFLT-1 over time, whereas the Sham group's sFLT1 levels remained roughly unchanged (Makris et al., 2007). This experiment shows how reduced blood flow directly increases the amount of circulating sFLT1 in the plasma.

SFLT-1 is a soluble receptor that binds to the angiogenic factors, vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and inhibits them from interacting with their receptors, Flt1 and Flk1. When they cannot bind to their receptors, the angiogenic factors are incapable of carrying out their function of promoting the growth of new blood vessels. Therefore, the presence of sFLT1 inhibits the growth of blood vessels. In PE, the excessive amount of sFLT1 abnormally constricts the mother's blood vessels, leading to hypertension, in addition to affecting the function of various other organs. The constricted

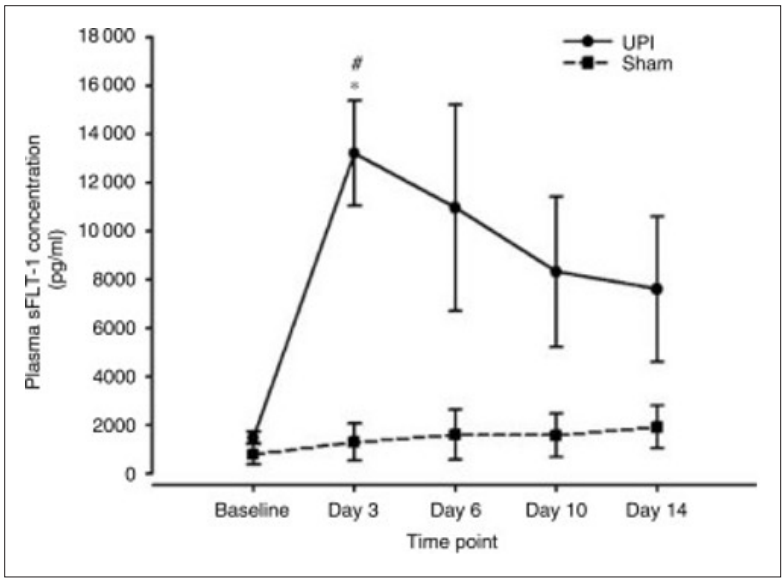

Figure 1: The concentration of plasma sFLT1 levels are significantly elevated in the UPI group in comparison to the sFLT1 of the Sham group (Makris et al., 2007).

blood vessels are also responsible for causing the kidneys to release proteins into the urine (Roberts & Bell, 2013).

Etiology of PE

The causes of placental malformation are still being researched and currently remain unclear. It is understood, however, that anything causing oxidative stress to the placenta is likely to cause PE. Oxidative stress is when there is an imbalance between reactive oxygen species (ROS) and antioxidants which disrupts metabolism and cell signaling in aerobic organisms. Several cell compartments can produce ROS, such as the mitochondria, endoplasmic reticulum, and nuclear membrane. They produce O2•– anions as a byproduct of auto-oxidation during the electron transport chain. ROS can also be produced as a result of arachidonic acid metabolism. Oxidative stress regulates the transcription factors NRF2 and FoxO which control the expression of genes that detoxify oxidizing molecules. Thus, the imbalance causes an interruption in the detoxification process and can lead to structural and physiological damage to DNA, RNA, proteins, and lipids. (Auoache et al., 2018). Oxidative stress is known to cause endothelial cell dysfunction which can contribute to PE (Duhig et al., 2016).

Risk Factors for PE

Several principle risk factors were found to be associated with the onset of PE. The presence of certain medical conditions can make a woman more prone to developing PE. Hypertension has strongly proven to be correlated with an increase in one's risk of developing PE. To be diagnosed with hypertension, there must be a "systolic blood pressure greater than or equal to 140 mmHg and/or diastolic blood pressure greater than or equal to 90 mmHg" (Hinkosa et al., 2020). A retrospective study of the databases from two hospitals identified 362 women who had chronic hypertension that required treatment prior to their pregnancies were analyzed. The data revealed that the percentage of superimposed PE in these women was 23.2%, a percentage notably higher than that of the general population. (Lecarpentier et al., 2013).

Autoimmune diseases like systemic lupus erythematosus and antiphospholipid antibody syndrome, diabetes type 1 or 2, and chronic arterial hypertension are other conditions classified as high-risk factors for PE (Mayrink et al., 2018).

Other risk factors known to be linked with PE include nulliparity, maternal age over 35, multifetal pregnancy, prior case of PE, chronic kidney disease, prior stillbirth, and a pre-pregnancy BMI over 25. Data was extracted and pooled from 92 studies regarding the relative risk of developing PE for women with each risk factor. Pooling of the data is helpful in bringing about results that are as reliable as possible.

Genetic Factors

Currently, there are no identified genes that are directly responsible to cause the disorder. However, because clustering of PE among families is common, it is suggested that there must be a genetic etiological component involved. Women with a first relative that developed PE have a three to five-fold increased risk of getting it as well (Hansen et al., 2018). There is also research suggesting that paternal genes may be a contributing risk factor for the development of PE as well. Women who became pregnant from a man that has had a previous partner with preeclampsia were seen to be at a slightly higher risk for PE (Galaviz-Hernandez et al., 2018).

Mother's Immune Responses

PE is thought to be a disorder that may be due to malfunctioning of the mother's immune system. The syncytial surface of the placenta sheds particles, ranging from large deported multinuclear fragments to sub-cellular components, and it is suggested that these particles contain proteins that trigger an inflammatory response. In PE, the number of circulating particles present increases, indicating a relationship between excess particles and the pathology

of the disease. Thus, preeclampsia is characterized by an exaggerated inflammatory response (Palei et al., 2013).

Furthermore, complications of the maternal immunity mechanisms can be a causing factor of PE. During pregnancy, it is essential for the mother's regulatory CD4 + T cells to interact with the uterine natural killer cells in order to recognize and accept the fetal antigens and enable placental growth. Failure of this process leads to a spontaneous miscarriage, while a partial failure leads to poor placentation and dysfunction of the placental perfusion and chronic immune activation that stems from the placenta. Women with PE were found to have decreased levels of circulating regulatory CD4 + T cells. Additionally, they have increased levels of T helper 17 cells, cells that are also upregulated in various autoimmune disorders (Palei et al., 2013).

Possible Complications of PE During Pregnancy

When symptoms of PE become severe, it can lead to dangerous complications for both the mother and the fetus, including fatality. Severe PE is when one or more of the following conditions is present: blood pressure of 160 mmHg or higher for systolic and 110 mmHg diastolic on at least two occasions that are at least 6 hours apart, at least 2 g of proteinuria in a 24 urine specimen or on two urine samples collected at least 4 hours apart, oliguria of less than 500 mL in 24 hours, cerebral or visual issues, pulmonary edema, pain in the epigastric or right upper quadrant, fetal growth restriction, a persistent and severe headache, or medical issues involving acute renal insufficiency, hepatic hematoma, and HELLP syndrome (Nankali et al., 2013). It is said that 25% of PE cases are classified as severe (Minire et al., 2013).

The mother may develop HELLP syndrome, which causes mortality in 25% of affected women. This can bring about a variety of complications that can affect many different organ systems such as the central nervous system, renal system, respiratory system, and liver. Complications include stroke, cerebral edema, retinal blindness, pulmonary edema, laryngeal edema, jaundice, renal failure, liver failure, HELLP syndrome, and eclampsia. Globally, preeclampsia and eclampsia are responsible for 10-15% of all maternal deaths (Nankali et al., 2013)

As for the fetus, the baby is often deprived of receiving a sufficient amount of blood and does not get the oxygen and nutrients it needs. Development under such conditions will be hindered, and the baby will have fetal growth restriction, causing it to be very small at birth. Often, these babies will need to be hospitalized for a period following their birth. A stillbirth can happen if the placenta separates from the uterine wall, causing the mother to heavily bleed. This is more likely to happen if the mother has a more severe case of PE, including the HELLP syndrome (Preeclampsia research at the NICHD.2012). Infants whose mothers had PE during their pregnancy also have an increased risk of developing some long-term health conditions due to the lack of proper development, such as "learning disorders, cerebral palsy, epilepsy, deafness, and blindness. Later on, they may be at risk for diabetes, congestive heart failure, and hypertension. Infant death is also a possible occurrence (Preeclampsia research at the NICHD.2012).

Management Options for PE

Because of the severity of the complications that preeclampsia may cause, women should take several precautions to help lower their risk of developing the disorder. Antiplatelet drugs, primarily low dose aspirin, are useful preventative agents as they reduce the risk of PE by 19% as well as decreasing the risk of stillbirth or neonatal death by 16% (Duley et al., 2006). Aspirin is effective because it reduces platelet aggregation, but it is also a risk of in utero cerebral hemorrhage (Atallah et al., 2017). It is advised that women at high risk should begin taking this before 12 weeks until 36 weeks of gestation (English et al., 2015).

Calcium supplementation during pregnancy also appears to reduce the risk of hypertensive disorders in pregnancy, including PE. A group of 579 women were assigned as a placebo group and a group of 588 women were given calcium supplementation. When comparing the percentage of hypertensive disorders of pregnancy in the placebo and calcium groups of women, the calcium group had a significantly lower risk of hypertensive disorders, particularly after the 28th week of gestation. There seem to be no side effects associated with calcium supplementation (Belizan et al., 1991).

There is also some research suggesting that antioxidant supplementation of vitamins C and E may be useful in lowering one's risk of PE. These agents are thought to prevent ROS from inflicting oxidative damage and overall restoring the redox equilibrium. This therapy aims to ultimately prevent endothelial cell dysfunction, which is an important pathological feature of PE (Aouache R. et al., 2018). However, there are studies that show contradictory results about the effectiveness of these supplements on reducing risks of PE. To test their outcomes related to PE, women who were 9 weeks to 16 weeks pregnant were examined until delivery. 5,088 women were given daily antioxidant supplementation of vitamins C and E, whereas 5,066 women served as a placebo group. The vitamins did not have much of an effect on the risk of developing PE. Based on such findings, they do not seem to serve any significant preventative purpose (Preeclampsia

Research at the NICHD 2012).

Early and accurate detection of the disorder is important in order to provide immediate optimal management. Therefore, closely monitoring changes in pregnancy, especially for women who classify as high-risk, is recommended. Regularly assessing blood pressure can catch any hypertension which is a good indicator of PE (Mayrink et al., 2018). Consistent urinary analyses should be performed in order to check for proteinuria, another big marker of PE. Monitoring growth often is useful in identifying signs of fetal growth restriction as well (English et al., 2015).

It is certain that the only definitive cure for PE is delivery. The decision to deliver the fetus prematurely is often based on two factors, estimated fetal weight and the severity of the disorder. If the mother is experiencing "uncontrolled severe hypertension that is not responding to therapy, eclampsia, acute pulmonary edema, abruptio placentae, subcapsular hepatic hematoma, or thrombocytopenia <50,000/mm3 ", then that indicates a need for immediate delivery (Uzan et al., 2011). Nevertheless, there are various ways to help control the condition during the pregnancy. Premature birth of the fetus is almost always inevitable, since the mother will most likely be unable to survive the full pregnancy period due to her destructive conditions. However, it is ideal to allow the baby to develop for as long as possible to improve neonatal outcomes. Mainly, it is important to allow the fetal lungs to mature. Prolonging treatment for as much as possible will result in the most beneficial outcome for the fetus (Le et al., 2019).

If blood pressure reaches the level of 160/110 mmHg or higher, antihypertensive treatment is a necessity as this is considered a medical emergency. Oral antihypertensive medications such as labetalol, methyldopa, and nifedipine should be administered. If the oral therapy fails to elicit a response, intravenous medication such as a labetalol infusion or hydralazine should be given. However, the commencement of treatment may result in a drop in blood pressure that can affect uteroplacental circulation and cause fetal distress; hence, the dosage of these drugs should be titrated gradually (English et al., 2015).

Magnesium sulfate (MgSO4) is a drug that aids in preventing eclampsia. The drug should be administered to women at high risk for eclamptic seizures. It has also been shown to lower the risk of cerebral palsy in the offspring. MgSO4 does have some adverse effects and can lead to paralysis, an absence of reflexes, a lower respiratory rate, and arrhythmias. Therefore, patients will need to have their pulse, respiratory rate, and reflexes continuously monitored in the time following. In the case that toxicity does occur, it can be corrected by giving the patient calcium gluconate (English et al., 2015).

Conclusion

PE is a disorder that is not fully understood yet. Due to the limited knowledge of its precise mechanisms, it remains difficult to determine the exact etiology of the disorder. However, the reviewed research articles were all consistently in agreement that women with the disease have a common pathophysiological finding. Elevated levels of sFLT1 are present in the placenta, which suggests the disease is largely due to dysfunctional angiogenic factors. The inability of the mother's angiogenic factors to bind to their correct receptors causes her blood vessels to constrict and leads to manifestations of PE (Roberts & Bell, 2013). This seems to be the major factor behind the disorder.

PE does not have any known cures; the only way to successfully stop the disease would be to terminate the gestation period and deliver the baby. The goal would be to manage and prolong the pregnancy for as long as possible in a way that takes both the mother's and fetus' safety into account (Uzan et al., 2011). After reviewing the various treatment options available, it seems that a combination of therapeutic agents would be the most viable approach for PE. Taking low-dose aspirin is a useful protecting drug as it reduces platelet aggregation (Atallah et al., 2017). Anti-hypertensive medication is essential to control the mother's blood pressure in cases when it reaches 160/110 mmHg or higher. The use of anti-hypertensives can lead to a sudden drop in blood pressure that can cause fetal distress, so it is important to responsibly monitor women's blood pressure when they are on these medications. Additionally, MgSO4 is important for preventing or treating seizures in eclampsia (English et al., 2015). Side effects that may present include paralysis, an absence of reflexes, a lower respiratory rate, and arrhythmias. However, the drug is vastly advantageous, and monitoring the patient and regulating drug administrations will eliminate most of the harm it can cause (English et al., 2015). Knowledge of the various factors that put women at high risk for PE is also useful for preventative care. Women should have their blood pressure monitored to check for hypertension, and urinalyses monitored to look for proteinuria, two major symptoms of PE. (Mayrink et al., 2018). Taking these steps can help in early detection and management of PE.

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Lupus and the Effects on Pregnancy

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Abstract

Systemic Lupus Erythematosus is a disease that manifests in many different ways. The cause of lupus still remains elusive. However, many of the pathologies associated with the disease as well as the disease process have been described. The pathophysiology of the disease as well as its effects on specific patient groups will be discussed below. More specifically, Systemic Lupus' effect on pregnancy with current diagnostic and treatment modalities will be the focus of this paper.

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that is common and affects around 400 per 100,000 people in certain populations. SLE mainly affects African or Hispanic women, especially during their reproductive years. The telltale sign of SLE is the production of autoantibodies, specifically antinuclear antibodies (ANAs). The most common method for detecting these ANAs is indirect immunofluorescence, which can recognize antibodies that bind to nuclear antigens, such as DNA, RNA, and proteins. Besides ANAs, patients with SLE have a variety of other autoantibodies that fight against red blood cells, platelets, and lymphocytes. The causes of SLE, like most autoimmune diseases, are unknown. However, genetic, environmental, and immunological factors seem to play a role (Kumar, et. al. 2018). Fatigue, joint pain, and rash are the most common symptoms of SLE. SLE is unique because of the disease's ability to appear and disappear called flares. Flares are known to occur during rapid hormonal changes, as occurs in pregnancy. For women with SLE, pregnancy is a major concern. Pregnancy is a high-risk time for SLE patients because flares during pregnancy may be related to increased irreversible organ damage (Ateka-Barrutia, Khamashta, 2013). The risk of flare during pregnancy depends on the disease activity 6-12 months before conception; the risk is higher in those who have had repeated flares preconception. Therefore, women living with SLE who are thinking about conceiving should consult their rheumatologist prior to conceiving, so that they can monitor disease activity to ensure the best outcome for mother and baby.

Methods

The data in this paper was compiled using Pubmed, ProQuest, and Google Scholar. PubMed and Proquest were accessible through Touro College's Online Library system. Key terms used to search were: "SLE pregnancy", "SLE", "hydroxychloroquine", and "autoimmune diseases".

Discussion

Pathogenesis of Systemic Lupus Erythematosus

The principle deficiency in SLE is the failure of the mechanisms that maintain self-tolerance. Although the cause(s) of the lack of self-tolerance remains unknown, there are several genetic, environmental, and immunological factors that seem to play a role.

There is a lot of evidence that suggests a genetic predisposition to SLE. Family members have a higher risk of developing SLE, and even twenty percent of uninfluenced first-degree family members have autoantibodies. The concordance of SLE in monozygotic twins is twenty-five to fifty percent and around five percent in dizygotic twins, which suggests that genetic factors play a crucial role in the predisposition of SLE (Kumar, et. al. 2018). The above said indicates a polygenic inheritance of the disease- it is approximated that at least 4 susceptibility genes are required in order to develop SLE (Schur, 1995).

Studies reveal that SLE susceptibility involves human leucocyte antigen (HLA) class II gene polymorphisms. In patients from different backgrounds, a relation between HLA DR2 and DR3 with SLE is a regular finding with an odds ratio for developing SLE of roughly 2 to 5 (Pisetsky, 1997). Which means, a person with HLA DR2 and DR3 are two to five times more likely to develop SLE than a person without HLA DR2 and DR3. HLA class II genes have also been linked to the presence of some autoantibodies, for example: anti-Sm, anti-Ro, anti-La, anti-nRNP, and anti-DNA antibodies. HLA class III genes, specifically those "encoding complement components C2 and C4", are major contributors in the development of SLE. Patients with homozygous C4A null alleles, regardless of their background, have a higher risk of having SLE. Additionally, SLE is connected to inherited deficiencies of C1q, C1r/s, and C2 (Mok, Lau, 2003). A reduction in complement activity could encourage SLE susceptibility by damaging the neutralization and removal of foreign and self-antigens. "When the antigen burden overwhelms the clearance capacity of the immune system, autoimmunity may ensue" (Mok, Lau, 2003). Other polymorphic genes have been linked to SLE such as: tumor necrosis factor alpha, interleukin six, the T cell receptor, CR1, Fc gamma RIIA and Fc gamma RIIIA, immunoglobulin Gm and Km allotypes, and heat shock protein seventy (Sullivan, 2000). Nonetheless, in majority of cases, consistent results were not reported in studies of patients from different ethnic backgrounds.

Although genetic factors play a crucial role in regard to the predisposition towards SLE, the start of the disease stems from environmental triggers. Infectious agents, such as bacterial DNA/endotoxins and retroviruses, may cause particular reactions by molecular imitation and disrupt immunoregulation. Viruses might set off or induce a flare in lupus by harming tissues to release autoantigens,

triggering B cells, and inducing SLE by molecular imitation. But, viral findings have not been consistent in the tissues of people with lupus (Herrmann, et. al. 1996). Therefore, there is not enough evidence to defend that any one infectious agent causes SLE. Certain diet choices, like alfalfa sprouts and high intake of saturated fats, influences the making of inflammatory mediators. Alfalfa sprouts contain L-canavanine which has been associated with the development of SLE-like symptoms (Prete, 1985). Procainamide, hydralazine, chlorpromazine, isoniazid, phenytoin, and penicillamine are drugs that alter "cellular responsiveness and immunogenicity of self-antigens" (Mok, Lau, 2003). Procainamide and hydralazine are aromatic amines or hydrazines, and they can cause an SLE-like disorder (Adams, Mongey, 1994). Ultraviolet light may aggravate SLE in many patients. UV light can trigger inflammation, promote cellular apoptosis, and induce tissue damage. Exposure to the sun's light causes and aggravates SLE. Exposure of skin to ultraviolet light changes the location and/or chemistry of DNA, Ro, and nRNP antigens, and also amplifies their immunogenicity. Recent studies have shown that ultraviolet light causes the apoptosis of human keratinocytes, which brings about the development of clusters on the exterior of dying cells, that hold nuclear and cytoplasmic antigens. This supplies a method for the exposure of self-antigens to the immune system and evokes autoimmunity (Mok, Lau, 2003).

To summarize: UV light and other environmental factors cause the apoptosis of cells. Insufficient removal of the nuclei of these cells leads to a large burden of nuclear antigens. Underlying abnormalities in B lymphocytes and T lymphocytes are accountable for flawed tolerance, and, as a result, self-reactive lymphocytes live on and stay functioning. Said lymphocytes are activated by nuclear self-antigens, and antibodies are made to fight the antigens. Complexes of the antigens and antibodies stick to Fc receptors on dendritic cells and B cells and might be engulfed. The nucleic acid elements engage toll-like receptors (TLRs) and trigger B cells to create more autoantibodies. TLR stimuli also trigger dendritic cells to make interferons and other cytokines, which intensifies the immune response and induces more apoptosis. The overall result is a "cycle of antigen release and immune activation resulting in the production of high-affinity autoantibodies" (Kumar, et. al. 2018).

Autoantibodies

The number one hallmark, and the number one concern, of lupus is the production of autoantibodies. These antibodies attack the patient's own molecules found in the cytoplasm, nucleus, cell surface, and soluble molecules like coagulation factors and IgG. Antinuclear antibodies are found in more than ninety five percent of SLE patients; anti-double stranded DNA (ds-DNA) and anti-Sm antibodies are specific for SLE and not found in patients with other autoimmune diseases, making them very important in the diagnosis (Tan, et. al. 1982). ANAs can be divided into 4 groups: antibodies to DNA, antibodies to nucleolar antigens, antibodies to nonhistone proteins bound to RNA, and antibodies to histones. Additionally, many other autoantibodies are found in patients with SLE. These autoantibodies attack lymphocytes, platelets, and red blood cells. Thirty to forty percent of SLE patients have anti-phospholipid antibodies. These patients have complications secondary to excessive clotting (Kumar, et. al. 2018).

Symptoms of SLE

SLE is a chronic disease, meaning that the disease is long lasting, in this case specifically, the disease waxes and wanes. The symptoms and the effects on daily life of SLE vary, however, there are some that were seen in nearly all patients. A group of patients were selected from 6 rheumatology practices that were spread across the United States between May and July 2014. These patients were between the ages of eighteen and seventy-five and had a clinical diagnosis of lupus. Ninety eight percent of patients reported they felt fatigue, ninety three percent reported joint pain, eighty eight percent reported a rash, eighty percent reported swelling of feet, legs, fingers, or hands and joint stiffness. Because SLE is chronic it has major effects on a patient's daily life and activities. Sixty one percent of those interviewed had difficulty with housework, thirty nine percent had difficulty driving and sleeping, and twenty two percent had difficulty caring for children. Sixty two percent of the patients who participated in this study were not working outside the home; ninety one percent said that this was caused by SLE (Mathias, et. al. 2018).

Hormonal Effects on SLE

Lupus is primarily a female disease; it is "characterized by a 9:1 female to male ratio of disease incidence" (Weckerle, Niewold, 2011). Generally, SLE occurs between puberty and menopause, the reproductive age range (15:1 ratio). Occurrence of SLE before puberty and after menopause is uncommon. Furthermore, patients with a hypergonadotrophic disorder, namely Klinefelter's Syndrome, are prone to lupus as well. From these observations, it is assumed that endogenous sex hormones play a major role in lupus (Mok, Lau, 2003).

Epidemiological studies show a connection between the use of exogenous estrogens and the emergence of

lupus. The Nurses' Health Study revealed that hormonal replacement therapy and the use of oral contraceptive pills have an association with an increased chance of developing SLE (Sanchez-Guerrero, et. al. 1997). Lupus improvement was observed in patients who had gone through menopause or an oophorectomy. Conversely, lupus flares mainly occur during hormonal changes, such as pregnancy, exogenous estrogen administration, puerperium, and ovulation during IVF (Mok, Wong, 2001). Many patients exhibit disease flares during the second half of their menstrual cycle, this has been attributed to the mid-cycle estrogen surge. Additionally, patients who develop lupus after the age of fifty were reported to have a milder disease and less significant organ involvement. All these observations are helpful in explaining and understanding why pregnancy for SLE patients is extremely difficult.

Pregnancy and SLE Pregnancy

Healthy, normal pregnancy causes the body to go through many physiological changes; these changes may influence rheumatic disease expression. Most organ systems go through some level of change during pregnancy. The glomerular filtration rate goes up by fifty percent during a normal pregnancy. Subsequently, women with preceding proteinuria might be expected to have a noticeable rise in urinary protein excretion in the 2nd and 3rd trimesters. There is also an expected thirty-fifty percent elevation in intravascular volume; women who have cardiac or renal compromise might not endure this well. Additionally, blood counts are usually different during pregnancy. Anemia is usual due to hemodilution, and in eight percent of uncomplicated pregnancies there is an occurrence of thrombocytopenia. The chance of venous thromboembolism increases by fivefold during normal pregnancy, because of the prothrombotic state that pregnancy creates along with compression by the expecting uterus and venous stasis (Sammaritano, 2016).

In normal pregnancy, the mother's immune system is altered in order to ensure fetal health and survival: immunoglobulin secretion rises, cell mediated immunity decreases, and pregnancy-specific proteins work to inhibit lymphocyte function (Branch, Wong, 2014). General immunosuppression would reduce maternal resistance against infection, so instead, there is an activation in the maternal immune system during pregnancy of immune-modulatory molecules and immunocompetent cells (Ostensen, Clowse, 2013). Cytokines and chemokines manage these immunocompetent cells with T helper cells; cytokines are an important factor in supporting successful pregnancy. The Th1/Th2 cytokine shift is a crucial immunological change that occurs during pregnancy. Th2 includes numerous interleukins which trigger antibody synthesis and humoral immunity. In pregnancy a prevalence of the Th2 response might be anticipated, and since lupus is predominantly a Th2-mediated disorder, aggravation of the disorder is more likely (de Jesus, et. al. 2015).

Pregnancy is high-risk for women with SLE, because disease flares during pregnancy have been linked to organ damage. Therefore, it is recommended that every woman with lupus should receive a preconception evaluation which should assess organ damage related to lupus, medications, and disease activity. If a patient is taking medications for lupus that have adverse effects on pregnancy, it is suitable for the patient to change to a lower risk medication (Flint, et. al. 2016). Additionally, for the best pregnancy outcome and for the mother's safety, it is advised that women with SLE should conceive during a time of inactive disease. Disease flares during pregnancy have been linked to disease activity six to twelve months before conception. Lupus flare within 6 months prior to conception has been linked to a significant rise in the chance of flare during pregnancy and a fourfold increase in pregnancy loss (Clowse, 2007).

Pregnancy for women with lupus has been linked to: risk of flare, preeclampsia, hypothyroidism, stroke, preterm birth, hypertension, pre-gestational diabetes, caesarean section, placental deficiencies leading to intrauterine growth restriction (IUGR), pregnancy loss, and even death. The Danish National Registry stated that maternal complications were found in fifty percent of lupus pregnancies (Jakobsen, et. al. 2015). A recent study observed thirteen thousand five hundred and fifty-five SLE pregnancy deliveries. Twenty five percent of SLE pregnancies were delivered preterm, meaning the pregnancy was shorter than thirty-seven weeks (Yan Yuen, et. al. 2008). Six to thirty five percent of babies were born small for gestational age. One in five lupus pregnancies ended in pregnancy loss (compared with one in ten from controls), with a four to six-fold increased likelihood of stillbirths compared with controls (Clark, et. al. 2003). Disease activity within six months before conception has been linked to an increased rate of fetal loss. Patients with anti-dsDNA antibodies have the highest risk for preterm birth and pregnancy loss. Patients with lupus have a three to four-fold increased chance of developing preeclampsia. Antiphospholipid antibodies are found in thirty-forty percent of SLE patients and have been linked to negative obstetric outcomes. Women with aPL antibodies have an increased chance of developing IUGR, preeclampsia, preterm birth, and fetal loss (Smyth, et. al. 2010).

The PROMISSE study observed three hundred and eighty five women with lupus and found that fifteen percent of

them experienced a mild flare, whereas five percent experienced an extreme flare. Sixty percent of women with active SLE prior to conception experienced flares during pregnancy, however, only ten percent of women with inactive SLE prior to conception experienced flares during pregnancy (Buyon, et. al. 2015). A study conducted in Sweden observed five hundred and fifty one first singleton births to patients with lupus and assessed their outcomes in comparison to the general population. This study included twelve thousand eight hundred and forty seven normal pregnancies, one hundred and ninety eight pre-lupus women, sixty five women who were first diagnosed with lupus zero-two years after giving birth, and one hundred and thirty three women who were diagnosed two-five years postpartum. Compared to those who were diagnosed with lupus two-five years after their first pregnancy, those with lupus during their first pregnancy, or diagnosed soon after, had the highest risk of poor clinical outcomes. Twenty six percent of women who were diagnosed with lupus zero-two years after giving birth had preeclampsia during pregnancy, thirteen percent of women who were diagnosed two-five years after giving birth had preeclampsia during pregnancy, and sixteen percent of women who had lupus while pregnant had preeclampsia; while only approximately five percent of women without lupus had preeclampsia (Arkema, et. al. 2016).

Over the last forty years some of the adverse pregnancy outcomes have improved. A study compared lupus pregnancies from forty years earlier to their current pregnancy group, which consisted of eighty three pregnant women. The rate of pregnancy loss decreased dramatically from forty percent to seventeen percent, compared with the general population rate of sixteen percent. On the other hand, the preterm delivery rate did not change dramatically. It only dropped from thirty seven percent to thirty two percent versus nine to twelve percent preterm delivery rate of the general population (Clark, et. al. 2005). A Norwegian study analyzed pregnant women with connective tissue diseases, including lupus, over the last four decades. Although maternal and fetal complications were more prevalent in lupus patients compared to the general population, the number of births did increase and the rate of C-sections, low birth weight infants, and preterm births decreased (Wallenius, et. al. 2015).

Effects of SLE on the Baby

Complications during pregnancy can impact fetal and neonatal outcomes. There is an increased chance of preterm delivery, preeclampsia, fetal loss, and low birth weight babies in women with lupus. When maternal autoantibodies, aPL antibodies, anti-Ro/SS-A and anti-La/SS-B antibodies are present there are more precise risks. The presence of aPL antibodies has been commonly linked to prematurity and intrauterine growth restriction. Anti-Ro or anti-La antibodies are found in around thirty percent of SLE patients. These autoantibodies can cross the placenta by active transport between the sixteenth and thirtieth weeks of pregnancy. Babies who are born to women with anti-Ro/SS-A and anti-La/SS-B antibodies have an increased risk of having neonatal lupus erythematosus (Sammaritano, 2016). These autoantibodies have been linked to the development of congenital complete heart block and noncardiac neonatal lupus erythematosus expressions such as: transaminitis, reversible thrombocytopenia, and photosensitive rash (Brito-Zero'n, et. al. 2014).

Congenital complete heart block is the most serious condition linked to anti-Ro/SS-A and anti-La/SS-B antibodies and occurs in approximately two percent of babies born to mothers with these antibodies. If the mother previously had a child with congenital heart block, then the risk for the second child having it increases to eighteen percent; if the mother previously had two children with congenital heart block then the risk increases to fifty percent (Brucato, et. al. 2001). In more than eighty percent of children with congenital heart block the mother had anti-Ro or anti-La antibodies. Typically, congenital heart block develops between sixteen and twenty four weeks of pregnancy, and it can be recognized by low fetal heart rate which is less than sixty beats per minute. Anti-Ro and anti-La antibodies attack the myocardium and fetal atrioventricular node. This causes immune mediated inflammation and fibrosis in tissues that are affected, resulting in various levels of heart block or cardiomyopathy (Llanos, et. al. 2012). The risk of death for babies affected is around ten-twenty percent and most of those who survive require a permanent pacemaker.

Various treatments for congenital heart block have been tried. Because of their capability to diffuse across the placenta, fluorinated steroids are used for cases that involve myocarditis, hydrops, or incomplete heart block due to its potential to reverse the affects (Friedman, et. al. 2009). Exposure to hydroxychloroquine throughout pregnancy might lower the chances of development of congenital heart block (Izmirly, et. al. 2012).

Treatments/Management of SLE Pregnancy

Firstly, patients with lupus who are considering pregnancy should be closely followed by their rheumatologist and obstetrician. Lupus should be quiescent, and the patient should be on medications that are low risk for approximately 6 months before conception. Recent studies show that antimalarial medications are beneficial for the

mother and baby and have few side effects, and therefore should be taken throughout pregnancy. In one study of one hundred and eighteen lupus pregnancies, poor pregnancy outcomes were dramatically reduced in the women who were taking hydroxychloroquine. "Preterm delivery rates were 15.8% in that group versus 44.2% in untreated patients, and rates of IUGR were 10.5% versus 28.6%" (Leroux, et. al. 2015). In a different study, women who stopped taking hydroxychloroquine suffered remarkably more lupus activity than women who continued hydroxychloroquine (Clowse, et. al. 2006). The use of hydroxychloroquine throughout pregnancy in patients with lupus minimizes the number of flares and hypertensive disorders. Hydroxychloroquine is safe to use during pregnancy, and there have been no "reported malformations, growth restriction and ocular, auditory, or neurological toxicity in exposed fetus" (Ruiz-Irastorza, Khamashta, 2011). Hydroxychloroquine is secreted in breast milk; however, there were no reports of negative effects in children who were breastfed.

In the case of disease reactivation during pregnancy, corticosteroids are usually used. Since fluorinated corticosteroids diffuse across the placenta, they should not be taken during pregnancy. On the other hand, non-fluorinated corticosteroids, (prednisone, prednisolone, methylprednisolone, hydrocortisone) are broken down by placental 11 beta-hydroxysteroid dehydrogenase, and only ten percent of drug dosage crosses the placenta. That said, non-fluorinated corticosteroids are connected to various complications such as diabetes, preeclampsia, and hypertension; therefore, low doses are recommended (prednisone<7.5 mg/day) (Ruiz-Irastorza, et. al. 2012). A dose of above ten mg/day of prednisone has been linked to a higher chance of developing dyslipidemia, arterial hypertension, maternal hyperglycemia, and fluid retention. Non-fluorinated corticosteroids are only slightly passed into breast milk and is permitted during breastfeeding. However, if the dose is high then women should wait four hours after taking the corticosteroid to breastfeed.

Most immunosuppressive medications are stopped during pregnancy and breastfeeding, except azathioprine in doses up to two and a half mg/kg/day, cyclosporin, and tacrolimus. Although cyclosporin has been deemed safe to use during pregnancy, it has been linked to an increased chance of preeclampsia, hypertension, and gestational diabetes (Ateka-Barrutia, Khamashta, 2013). Mycophenolate mofetil, methotrexate, and cyclophosphamide are not safe to use during pregnancy and should be switched to safer drugs.

NSAIDs are overall safe to use throughout pregnancy if they are limited to short term usage. However, long term use of NSAIDs have been linked to cardiac and renal failure, fluid overload, and hypertension in the mother, and renal disorders and oligohydramnios in the fetus. The use of these medications should only take place at the end of pregnancy, after thirty weeks of gestation (Ostensen, et. al. 2006).

Antiplatelets and anticoagulants are also used to treat lupus. The use of low-dose aspirin (75-100 mg/day) and dipyridamole is safe to use during pregnancy. Aspirin can be used even throughout labor or epidural anesthesia to decrease the chance of hemorrhagic issues. Since heparins do not diffuse across the placenta, they are safe to use throughout pregnancy and breastfeeding. However, warfarin is damaging to the fetus during organogenesis (the first six-ten weeks of pregnancy) and therefore should not be taken during this timeframe (Ostensen, et. al. 2006). Patients taking these medications should be switched to heparin when pregnancy is confirmed.

Conclusion

Systemic Lupus Erythematosus is a disease with varying severity in the population, but one that can have devastating effects on mother and baby. Advances in the understanding of the disease as well as treatment and prevention of flare ups have allowed women with lupus to have successful and healthy pregnancies with favorable outcomes. It is imperative however, that women with lupus seek guidance from their rheumatologist and obstetrician and focus on prenatal care for the best possible outcome for mother and baby.

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Do People with Hashimoto's Disease need a Thyroidectomy?

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Abstract

Hashimoto's Thyroiditis (HT) is one of the most common autoimmune diseases (Hiromatsu et al. 2013 p.13). It primarily affects the thyroid gland. The thyroid gland influences growth and regulates the body's metabolism by manipulating hormonal levels. Hashimoto's thyroiditis affects thyroid function through mechanisms that cause the hormone levels to become dysregulated. The standard therapy for Hashimoto's thyroiditis is hormone replacement. This approach helps most patients by regulating their thyroid hormones, though there are some individuals who fail treatment. Untreatable patients are plagued with weight gain, sleep disturbances, and other symptoms. Certain factors in thyroid disease may cause various secondary complications including psychiatric manifestations. Research into these patients has shown that the removal of the thyroid gland seems to improve their clinical condition. Therefore, in the situation when significant symptoms remain after what appears to be adequate medicinal treatment, the possibility of surgery should be entertained. This review discusses the pathological underpinnings of Hashimoto's disease and reviews some of the published literature from the past ten years in relation to the treatment of HT.

Introduction

The thyroid gland is a butterfly-shaped endocrine gland found in the lower front portion of the neck. The thyroid primarily controls metabolic function. This includes hormone production, growth, and bodily maturation. Thermoregulation, as well as brain maturation and other cognitive functions, are also controlled by the thyroid (Drake 2018). Thyroid dysfunction can be due to hyperthyroidism, the overproduction of thyroid hormone, or hypothyroidism, which is the underproduction of hormones. The most common symptoms of hyperthyroidism are weight loss and palpitations, while the most common symptoms of hypothyroidism are weight gain and lethargy (Guyton, Hall 2006 Pp. 938-940).

According to the American Thyroid Association (ATA), there are approximately twenty-million people in the United States who suffer from thyroid disease. Of this twenty-million, about fourteen million of them suffer from an autoimmune condition known as Hashimoto's thyroiditis (American Thyroid Association, General Information). An autoimmune disease develops when the body's immune system begins attacking its own organs or particular proteins. Autoimmune diseases are caused by autoantibodies, which respond to normal organs as foreign invaders. Hashimoto's Thyroiditis occurs when the body attacks either the thyroid cells or their product thyroxine.

Hashimoto's Thyroiditis can develop in patients with either a genetic or environmental susceptibility (Pyzik et al. 2015). Hiromatsu et al. studied some of the current concepts of genetic susceptibility to Hashimoto's Thyroiditis. They noted that, specifically, the concordance rate for HT in identical twins (monozygotic) is 55%, while in non-identical twins (dizygotic) it is 0%. Moreover, they also observed that there is a higher rate of concordance of thyroid autoantibodies not associated with Hashimoto's Thyroiditis in monozygotic twins than in dizygotic twins. In these siblings, 80% of monozygotic twins had autoantibodies versus 40% in nonidentical twins (Hiromatsu et al. 2013). This study concluded that genetics is a significant factor in HT.

Several genetic loci have also been identified as associated with Hashimoto's thyroiditis, most notably multiple human leukocyte antigen-DR (HLA-DR) isotopes. HLA-DR is a lymphocyte surface receptor. When genetically altered it affects what is presented to the T-cells which elicits an immunologic response. This response can influence the selectivity and binding of peptides, which appears to predispose individuals to the development of HT (Hiromatsu et al. 2013). Other immune modulators released from the lymphocytes, such as interferon-gamma can also instigate the development of HT.

Environmental triggers that affect individuals susceptible to Hashimoto's thyroiditis include infections, dietary factors, and pregnancy. Although iodine deficiency can cause thyroid diseases, excess iodine can induce autoimmunity by increasing the body's sensitivity to abnormal thyroglobulin. Thyroglobulin is the protein produced by the thyroid and is the precursor to the thyroid hormones triiodothyronine (T3) and thyroxine (T4). In the presence of excess iodine, thyroglobulin can be abnormally iodinated. This can lead to the production of autoantibodies as well as to the toxicity of the thyroid cells and hypothyroidism (Hiromatsu et al. 2013).

With all this in mind, the two main autoantibodies linked to Hashimoto's Thyroiditis are anti-thyroglobulin and antithyroid peroxidase. An anti-thyroglobulin antibody binds to thyroglobulin which limits availability and subsequent release of T3 and T4 thyroid hormones. The thyroglobulin directly affects the functional aspect of the thyroid and its reduction, or absence contributes to a patient's hypothyroid symptoms. Antithyroid peroxidase, on the other hand, targets thyroid follicular cells or thyrocytes. The way it does this is by tagging the thyroid cells for destruction which can in turn lead to hypothyroidism. HT is an insidious disease that can take years before becoming clinically significant (Guyon, Hall 2006, Pp. 940-943).

Many patients eventually require treatment with thyroid hormone replacement. This therapy involves being prescribed a bio-identical thyroid hormone medication,

such as Levothyroxine also known as Synthroid or Armor Thyroid. For most patients, the treatment works well, and the symptoms are resolved. There are circumstances though where hormone replacement fails and in cases like these clinical research has shown that the surgical excision of the thyroid or thyroidectomy should be considered. This potentially works because it reduces the antibody load and the antibody's potential effects on other targets (Goldvog et al. 2019, p. 462). The main objective of this paper is to review the possible solutions to treatment-resistant Hashimoto's thyroiditis disease.

Methods

Data was collected through Touro College's online library using several scientific scholarly databases such as ProQuest and PubMed databases. The key-phrases used were Hashimoto's Disease, Hashimoto's thyroiditis, thyroidectomy, and autoimmune thyroiditis. Only peer-reviewed journals and English language articles were analyzed.

Discussion

Autoimmune thyroid disease was first described in 1912 by the Japanese surgeon Hakaru Hashimoto (Pyzik et al. 2015). He identified a cohort of patients with lymphoid infiltration of the thyroid with associated destruction of the thyroid glands which he called "struma lymphomatosa" (Hiromatsu et al. 2013). The concept of autoimmune diseases was not recognized until the 1950s and even currently many of these diseases are not fully understood. Hypothyroidism is diagnosed by a thyroid function blood test that shows elevated thyroid stimulating hormone (TSH) which reflects a reduction in the thyroid's function (Drake 2018). Unlike many other diseases, patients who suffer from hypothyroidism do not exhibit symptoms over a set timeline. Some patients develop their symptoms over days to months, while for others, symptoms evolve over years to decades (Lee 2020). In other words, hypothyroidism can exist in many states which can range from a subclinical harmless state to a significant clinical presentation (Chaker et al. 2017). Table 1 shows a flow chart on the process of diagnosing the disease.

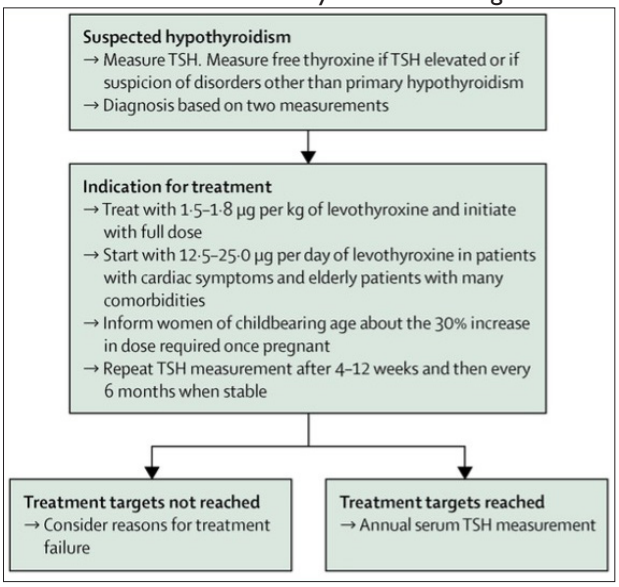

Table1: Diagnostic scheme (Chaker et al. 2017)

There is controversy as to when medical professionals should start treating patients with clinical hypothyroidism. According to the American Association of Clinical Endocrinology (AACE) guidelines, there are a few opinions as to when treatment commences. Treatment generally relies on the Thyroid Stimulating Hormone (TSH) level. TSH responds to feedback inhibition of the pituitary gland, the organ which produces TSH. As the levels of thyroid hormone increase, the TSH decreases, and as the levels of thyroid hormone decrease the TSH increases. This is an example of a negative feedback loop (Guyon, Hall 2006, p. 939). The American Thyroid Association's guidelines suggest that when the TSH levels approach 10 mIU/L patients are clinically hypothyroid and need treatment. Others claim that there are therapeutic reasons to start treating at lower levels. Regardless, treatment is always dependent on the clinical symptoms being addressed (Garber et al. 2012, p. 1112).

The most common symptoms of hypothyroidism in adults are weight gain, fatigue, hot/cold intolerance, and memory loss (brain fog). Table 2 lists the signs and symptoms related to hypothyroidism. The symptoms for the diagnosis of hypothyroidism are reflective of the hormonal insufficiency in different organs (Carle et al. 2014, p. 593). Symptoms vary by age and health status. An increase in the severity of symptoms may predict a harsher course of hypothyroidism. Clinical symptoms were originally used to diagnose hypothyroidism. A variety of outdated scoring systems such as the Billewicz score system and the Zulewski score system were utilized. With the advent of a sensitive blood test for TSH, these systems have been phased out and the fluctuation in TSH levels is what is used to guide the treatment of the disease.

Thyroid function has also been proven to play a crucial role in the cognitive development of youth and many other facets regulating nervous system activity. Neuropsychiatric symptoms refer to a gamut of emotional and cognitive complications that are directly related to alterations in the brain (Dickerman, Barnhill 2012). Due to the close association between thyroid and the nervous system function, disturbances in the body's

TABLE. Signs and Symptoms of Hypothyroidism

Signs	Symptoms
Eyebrow and hair thinning (C)	Fatigue
Skin coarsening (C)	Weight gain
Tendon relaxation phase slowing (C)	Cold intolerance
Facial/periorbital edema (O)	Mental slowing
Macroglossia (O)	Muscle weakness
Bradycardia (O)	Reduced exercise capacity
Pericardial effusion (R)	Constipation
Pleural effusion (R)	Xeroderma
Rhabdomyolysis (R)	Depression
	Menstrual irregularities

C = common; O = occasional; R = rare.

metabolic state can be related to a range of neurological signs and symptoms. These signs include headaches, mood, and cognitive disorders, ophthalmoplegia (eye movement disorder), tremors, and muscle weakness. Hypothyroidism can cause psychiatric symptoms such as depression, anxiety, memory deficits, and even psychosis. According to medical guidelines, testing for thyroid disease is a case-by-case decision, but patients who present with psychiatric issues should all be screened for thyroid dysfunction (Dickerman, Barnhill 2012, p.130).

In the neuropsychiatric realm, a sudden worsening in a patient's hypothyroidism may result in myxoedema coma, a life-threatening condition that is also associated with a rapid decline in mental health. The degree to which subclinical hypothyroidism and mild hypothyroidism impacts moods and cognitive functions, as well as whether these symptoms respond to treatment, remains controversial (Stasiolek 2015). A case study reported a 61-year-old woman who first presented to the emergency room complaining of severe chronic daily headaches. According to the patient, the headaches started 9 months prior and were described as, "bi-lateral with pressing-type quality, without associated symptoms such as nausea, photophobia, and did not worsen with exercise,". The patient also presented with depressive symptoms which were evaluated by a psychiatrist. The woman was subsequently diagnosed as having depressive syndrome. She was prescribed antidepressants but the medication failed to correct the problem. The patient later had a full medical evaluation which included blood work. The blood work revealed an increased anti-TPO antibody titer. This patient was diagnosed with encephalopathy related to autoimmune thyroid disease, which is also known as Hashimoto's encephalopathy (Correia et al. 2019).

Another physical ailment associated with hypothyroidism is reduced cardiac output. This is a result of the relaxation of vascular smooth muscle tissue. This relaxation occurs because the thyroid hormones which control the pacemaker-related genes reduce the heart rate and cardiac output which subsequently increases arterial stiffness leading to hypertension. (Udovcic et al. 2017, p. 55). Because of this process, a person's heart rate will elevate in the presence of excess thyroid hormone (hyperthyroidism) or reduce if the person has less thyroid hormone present (hypothyroidism). The change from normal levels to abnormal levels produces a domino effect of increased arterial stiffness, which then causes increased systemic vascular resistance. A variety of medicinal conditions can arise due to these changes which include atrial fibrillation and heart failure (Udovcic et al. 2017).

Thyroid dysfunction can also be associated with changes in body temperature and body weight. The thyroid-stimulating hormone regulates the basal metabolism, thermogenesis, and lipid glucose metabolism (Sanyal, Raychaudhuri 2016). "According to the National Health and Nutrition Examination Survey, obesity affected 32.2% of adults in 2003–2004." (Biondi 2010, p. 3614). A study performed in India at the Medwin Hospital's department of Endocrinology and Obesity Clinic found that thyroid dysfunction was realized more in individuals that were obese. All the patients included in the study underwent a physical examination which included, "Height, weight, waist circumference, presence of goiter, acanthosis nigricans or peripheral stigmata of dyslipidemia and blood pressure measurement," (Verma et al. 2008). Their study consisted of a total of 1075 patients. The study was divided into two subgroups. One group was made up of 625 patients who were enrolled in the hypothyroidism clinic, and the other group was 450 patients who were enrolled in the obesity clinic. Of the patients from the hypothyroidism clinic, 44% of them found that their body mass index (BMI) was greater than 25 kg/m2 with the typical BMI for a healthy person being anywhere between 18.5 and 24.9 kg/m2. Of the patients from the obesity clinic, only 33% had hypothyroidism (Verma et al. 2008). They concluded that most people who suffer from hypothyroidism have the likelihood of being obese.

Thyroid glands are also prone to develop nodules known as goiters. A goiter refers to an abnormal enlargement within the thyroid gland. It is important to stress that the majority of people that have goiters do not have any thyroid gland dysfunction. People with Hashimoto's

thyroiditis, however, seem to have more nodules than the average person. This leads to patients with Hashimoto thyroiditis having a slightly higher risk of developing thyroid cancer (Fish 2019 p.334). Some researchers say that the higher rate for papillary thyroid carcinoma is due to the presence of an autoimmune-response, or possibly anti-tumor immune response to the immune mediators (Graceffa et al. 2019, p.5).

As mentioned previously in this paper, the primary treatment for Hashimoto's disease consists of hormone replacement. Hormone replacement aims to balance the patient's biochemical system. While taking out the thyroid might prevent an antibody response, if a patient does not have a significant quality of life issue, doctors would not suggest having a total thyroidectomy (Promberger et al. 2014, p. 979). There are cases in which a patient is still affected by the symptoms of hypothyroidism, even after taking the correct dosage of medication and correcting the abnormal TSH level. In that instance, doctors might suggest that the patient undergo a total thyroidectomy (Pollock et al. 2001, p.894).

There are some standard indications as to when it is appropriate to have a thyroidectomy beyond having thyroid cancer. When a patient starts to exhibit compressive symptoms such as discomfort while swallowing, the feeling of strangulation, or tightness in the neck, it is appropriate to surgically remove the thyroid gland (Pradeep et al. 2011). Greater than 63% of patients with HT identified with the symptom of compression (McManus et al. 2011, p. 336). Another symptom that hormone replacement cannot cure is painful Hashimoto thyroiditis. Painful HT is also known as acute exacerbation of HT. This condition is not very common and in most instances is treated adequately with painkillers. Having a total thyroidectomy is thought to be the best treatment for this condition especially when painkillers do not provide relief (Peng et al. 2020, p.12).

In certain instances, patients with Hashimoto's disease may not necessarily experience a resolution of their complaints with medication. A study of 426 women who were all planning on having their thyroids removed for various reasons including HT. Before surgery, they all answered the SF-36 questionnaire, which is a set of generic, and easily administered quality-of-life measurements. The results established that patients who had high anti-TPO levels had lower quality of life measures even though they were taking the correct dosage of medication based on their TSH levels (Ott et al. 2011, p.165).

In the Annals of Internal Medicine, Guldvog et al., who performed the first randomized controlled trial, demonstrated that the removal of the thyroid gland in patients with histologically verified Hashimoto's disease can improve their quality of life by normalizing their anti-TPO antibody titer levels. This study was performed on patients between the ages of 18 to 79 years. They all had anti-TPO titers above 1000 IU/ml (normally less than 35 IU/ml). In total there were 150 test subjects all of whom had persistent Hashimoto-related symptoms. Before the data was collected and the surgery was performed, all the patients were monitored for thyroid function until they achieved euthyroid status or normal thyroid function. Participants were either assigned to undergo total thyroidectomy with continued hormone replacement or to only receive hormone replacement therapy. After 18 months, the anti-TPO levels of the surgical group had fallen from a mean of 2232 IU/ml to 152 IU/ml, and the general health score of the surgical group improved from 38 to 64 points. The chronic fatigue frequency in the surgical group decreased from 82% to 35% of the individuals. The overall score was improved compared to the original SF-36 questionnaire. The control group showed no significant changes. The researchers hypothesized that the improvement in symptoms could be related to the normalization of serum anti-TPO antibody titers. (Goldvog et al. 2019).

Dr. Trevor Angell interpreted and reviewed the study done by Guldvog et al. and concluded that the results were compelling. Guldvog et al. indicates that patients with Hashimoto's thyroiditis might have persistent symptoms due to more than just the thyroid dysfunction. They found that a thyroidectomy may modulate the immune response by removing the antigen and thereby reducing the inflammation and the inflammatory mediators. With the removal, the symptoms caused by HT would subsequently be alleviated (Angell 2019, p.180).

Nevertheless, the initial plan of treatment for patients who have the symptoms of hypothyroidism should be supplemental hormone replacement. In the case when patients do not respond positively to hormone replacement, a total thyroidectomy has proven to be effective as well. For a patient that chooses to have surgery, a risk analysis of surgical complications must be evaluated. One study predicted that if more people start removing their thyroids then complications will increase 12-fold. This rate, according to the literature, would change from 3.05% to 36.6% (Memeh et al. 2020).

Conclusion

In conclusion, not every person who has Hashimoto's thyroiditis should have thyroid surgery. According to the research reviewed, having a thyroidectomy might only be recommended to patients who have Hashimoto's Thyroiditis and who are experiencing neuropsychiatric symptoms after their TSH levels are stabilized. There

needs to be more research evaluating and specifying the benefits of having a thyroidectomy. Larger test groups need to be observed, and the follow-up period should be extended. Though they are not frequent, complications of total thyroidectomy are real, and the risk-benefit ratio must be assessed on a case by case basis.

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