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

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

In February 2022, Touro College was granted university status by the Board of Regents of the State of New York.

Touro University celebrated its 50th anniversary at a dinner on December 4, 2022.

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, SLP), the Fast Track Program with the Touro College of Pharmacy, the accelerated Nursing (BSN) Pathway Program, 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.

Faculty members have earned recognition for outstanding achievements, including Dr. John Loike, Professor of Biology, who has published widely in the fields of bioethics and genetics; 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; 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. 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-Sharfman, Vice President and Officer, the Federal Reserve Bank of New York; Samuel Lowenthal, CPA, Partner, DeLoitte; Mindi Lowy, CPA, Partner, PwC; Joel Krasnow, JD, Partner, Milbank; Kalman Yeger, Member, New York City Council; and Simcha Felder, CPA, member of the New York State Senate.

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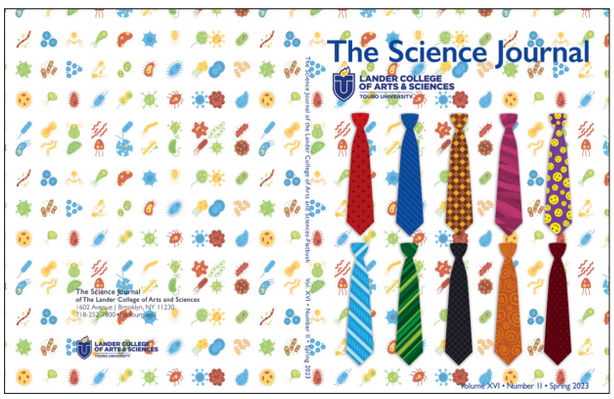

The Cover Picture: The cover illustration, created by Professor Antony O'Hara of the Digital Multimedia Design Program, pertains to the paper "Can Physicians Transfer Bacteria onto Patients through their Neckties?" by Alexandra Pinkhas.

What is the Optimal Timing of Embryo Biopsy for Diagnosing Genetic Abnormalities?

Nekhama Riznyk

Nekhama Riznyk will graduate June 2023 with a Bachelor of Science degree in Biology.

Abstract

Introduction and Purpose: This paper examines current trends in assisted reproductive technology, specifically the use of preimplantation genetic diagnosis (PGD to help in the preimplantation detection of genetic abnormalities such as monogenetic diseases and aneuploidy. PGD can be carried out in different stages, and the timing of the procedure has become one of the major issues in implementation. Three major stages of biopsy have been applied when carrying out PGD: Polar body biopsy (pre-conception), cleavage stage biopsy (3 days after conception), and blastocyst stage biopsy (6 days after conception). The purpose of the paper was to explore these stages and come to a conclusion on the best one in regard to the safety of embryos and the effectiveness of diagnosing genetic abnormalities.

Introduction

Molecular medicine has evolved immensely in the last few years. One of the most important goals of molecular medicine is to provide definitive cures for most, if not all, genetic disorders. Genetic disorders account for a significant number of pediatric admissions and childhood deaths. The progression in molecular therapy and gene-replacement therapy has been slow despite considerable strides in molecular biology and the Human Genome Project (Stern, 2014). Currently, the main approach for managing severe genetic disorders is through prevention mechanisms. Prevention mechanisms have come a long way and have conferred important competencies towards the detection and prevention of genetic disorders in humans. Historically, the ability to recognize genetic abnormalities in embryos or fetuses before they was born were a huge challenge. Years ago, it was impossible to know in early pregnancy the gender of the baby or whether there were any abnormalities. This lack of this information did not allow any attempt to intercede. Before the invention of technology to detect genetic abnormalities in the embryonic or fetal stages, parents would only know genetic abnormalities in their newborns after birth. Advances in medical science, molecular biology, and technology have provided better-timed detection mechanisms with the use of embryo genetic testing (Stern, 2014).

In the early years, the detection of abnormal fetal development improved with with sonography, amniocentesis, and even more recently with genetic testing of an embryo. Human assisted reproduction has entered a completely new era thanks to recent advances in both knowledge and understanding of embryogenesis and preimplantation genetic screening. Biopsied embryos, usually at the blastocyst and morula stages, are checked by DNA microarray or next generation sequencing before cryopreservation, and subsequently, euploid embryos are warmed for transfer into humans (Stern, 2014). One of the key issues in genetic testing has been the timing of biopsies. The embryonal biopsy may often lead to developmental abnormalities in the developing fetus. The earlier the biopsy, the higher the chances of a greater degree of development are affected. It is, therefore, critical to determine the optimal timing for embryonal biopsy to ensure the collection of maximum genetic information while minimizing the risk of developmental damage. Such operations have not been commonly performed in clinics, and their efficacy is uncertain. This review attempts to determine the safest time to sample embryos for genetic defects without producing abnormalities.

Methods

A number of databases including, the PubMed, Touro Online Library, ProQuest, and EBSCO Information Services were searched for scholarly works using key phrases "preimplantation genetic testing," "genetic testing stages," and "optimal timing for embryo biopsy," among others. The search yielded many scholarly articles, with those that comprehensively covered the biopsy stages and included tangible evidence on their effectiveness and challenges being considered for the current study. From this review, a comprehensive overview of the embryo biopsy stages and a conclusion on one has been generated..

Preimplantation Genetic Diagnosis and its Importance

In many in vitro fertilization (IVF) facilities, preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) are standard procedures. IVF is a form of assisted reproduction in which a man's sperm and a woman's eggs are mixed in a laboratory dish outside of the body (Stern, 2014). One or more fertilized eggs (embryos) may be implanted and develop in the woman's uterus. PGD and PGS are examples of assisted reproductive technology (ART), which play a key role in the detection of genetic abnormalities. PGD is an example of preimplantation genetic testing (PGT), which involves the extraction and analysis of DNA from embryos or oocytes in an effort to assess the presence of genetic abnormalities or for human leukocyte antigen typing (Wang, et al. 2018). PGD, which is also known as preimplantation genetic testing (PGT) for monogenic/ single gene defects (PGT-M), has become one of the most important ARTs in the world.

PGD is an important technology for the IVF process, especially for patients who have genetic or chromosomal

problems. PGD is different from other methods of prenatal diagnoses, such as amniocentesis, because it is performed on embryos in an IVF lab as opposed to an intrauterine pregnancy. PGD serves as a detection procedure for genetic abnormalities as opposed to being a therapeutic technology. PGD does not bring changes to the DNA or genetic-related structures and, as such, does not have a therapeutic value (Stem, 2014). As primarily a diagnostic procedure, PGD helps in the identification of single gene disorders, which the couple in question might have a risk for. PGD also helps identify chromosomal abnormalities in embryos which can lead to implantation failure, miscarriages, or children being born with developmental challenges or disabilities. The information obtained from PGD is then used to inform and help physicians and the potential parent(s) decide whether or not to transfer the embryo to the uterus. PGD helps in increased success in pregnancies and reduces the chances of single gene disorders in children (Stern, 2014).

The first successful biopsy of a human embryo for PGD was accomplished in 3-day-old embryos with 6–8 cells (Parikh, et al. 2018). According to Wang et al. (2018), the first pregnancies which had applied PGD were reported in 1990 for two couples who were considered to be at risk of transmitting X-linked intellectual disability and adrenoleukodystrophy to their offspring. PGD evolved considerably, and in 1992, the first birth after screening for cystic fibrosis was reported. Since then, PGD has become instrumental in assessing for many genetic conditions in oocytes and embryos, including sickle cell disease, hemophilia A, Duchenne muscular dystrophy, and Down syndrome, Tay-Sachs disease, Edwards syndrome, spinal muscular atrophy, and inherited ocular cancer and eye disease (Wang, et al. 2018).

Timing of Biopsy

The timing of a biopsy for PGD has been one of the most consistent challenges. Since PGD requires the extraction of DNA, the risk of considerable damage is always a possibility. When choosing the timing of the biopsy, therefore, several factors need to be considered. Scientists have to consider if the timing of the biopsy allows for the accurate diagnosis of the genetic conditions or chromosomal errors for which the polar bodies or embryos are being screened. For instance, a biopsy carried too early might risk missing critical errors with a significant impact on the development of the embryo and the resulting offspring. The timing of a biopsy also has to take into consideration if the errors identified at a given period are an accurate and consistent representation of a corresponding abnormality. Embryos may, for instance, be biopsied at a time when they can self-repair, and errors may not be a true reflection of genetic abnormalities. Based on this factor, the timing may influence the discarding of normal embryos. When choosing the optimal timing for carrying out a biopsy, consideration must also be taken of the safety of the specimen. For instance, the timing must be done such that damage to the embryo is minimized and its integrity preserved. Lastly, the timing must be done such that enough time is allowed for timely embryo selection (Scott, et al. 2013a).

Biopsy Stages and Procedures

Theoretically, the biopsy can be performed at all preimplantation stages, but only three have been suggested: on unfertilized and fertilized oocytes (for polar bodies, known as PBs), on day three cleavage-stage embryos (for blastomeres), and on blastocysts (for trophectoderm cells). The opening of the zona pellucida and the removal of the cells are always part of the biopsy procedure. For the breaching of the zona pellucida, extrusion or aspiration for the removal of PBs and blastomeres, and herniation of the trophectoderm cells, there are various techniques, including mechanical, chemical, physical and laser technology.

Polar Body Biopsy

A polar body biopsy involves taking a polar body, which is a small haploid cell that forms simultaneously with an egg cell during oogenesis but does not have the ability to be fertilized. Polar body biopsy is a unique method when compared to other techniques discussed in this paper due to its timing. This method is classified as a pre-conception diagnosis since it involves the removal and analysis of the first and second polar bodies following meiosis. Polar body biopsy is, therefore, used for the indirect diagnosis of possible genetic abnormalities in the corresponding oocyte. Shortly before ovulation, the diploid oocyte in a female's ovary is reduced in half in the first meiotic division. Here, one set of the chromosomes remains in the oocyte while the second set is segregated in the first polar body. The oocyte at this stage is composed of one set of paired chromatids. After fertilization, the second meiotic division occurs where the set of chromatids are separated, with one set of the chromatids being left in the oocyte and the second extruded in the second polar body. The first and second polar bodies do not play any roles in the development of the embryo. Polar bodies, however, provide important information regarding the genetic constitution of the oocyte and have been shown to be useful in predicting the genetic constitution of the embryo and the presence of genetic abnormalities

inherited from the mother (Montag, et al., 2009).

The introduction of laser-assisted technology for breaching the zona pellicuda has been one of the most useful developments in polar body and embryo biopsy. When used, this technique increases the effectiveness of the breaching procedures and lowers the time required to complete the exercise. After breaching the zona pellicuda, polar bodies are removed and screened for genetic abnormalities. Polar body biopsy is usually indicated for human leukocyte antigen matching and the detection of monogenetic abnormalities of maternal origin. Mostly, polar body biopsy is used to check for errors in the number and structure of chromosomes. When looking for monogenetic conditions, polymerase chain reaction is the procedure used in conjunction with the use of specific primers (Montag, et al. 2009).

Polar body biopsies are extremely safe in preimplantation diagnosis due to their timing (Macas, et al. 2008). The primary benefit of using polar bodies in PGD is that they are not required for successful fertilization or normal embryonic development, ensuring that the embryo is not harmed (Scott, et al. 2013). Even though polar body biopsy assesses genetic constitution at the pre-conception stage, it is still a useful technique in the detection of monogenetic conditions and chromosomal aberrations. According to Fragouli et al. (2010), the detection capacity of polar body biopsy is based on the understanding that the gain or loss of a chromosome in the polar body is usually accompanied by the same happening in the corresponding oocyte. As such, genetic screening on a polar body provides a safe way to analyze for aneuploidy in the corresponding oocyte.

One of the drawbacks of PB biopsy is that it only provides information about the maternal contribution to the embryo. As a result, only cases of maternally inherited autosomal dominant and X-linked disorders that are exclusively maternally transmitted can be diagnosed, while autosomal recessive disorders can only be diagnosed in part. In some countries such as Austria, polar body biopsy is the main available form of PGD since carrying out a biopsy on embryos is considered unethical and illegal (Montag et al, 2009).

Another disadvantage of using polar body biopsy in the detection of monogenetic conditions and aneuploidy is the increased likelihood of diagnostic errors, which can occur due to genetic material degradation or recombination events that result in heterozygous first polar bodies. The predictive value for aneuploidy for polar body biopsy has remained low and makes the use of the procedure less helpful when compared to the embryo stages. According to Montag et al. (2009), the analysis of chromosomes 13, 16, 18, 21, and 22 using polar body biopsy would only identify about 50 percent of all numerical chromosomal abnormalities leading to miscarriages in the first trimester. It was established that the per chromosome predictive sensitivity for blastocyst chromosomal complement using polar body biopsy was 61.7 percent. This figure is relatively low when compared to blastocysts analysis which yielded a predictive sensitivity value of 86.4 percent. Polar body biopsy also tends to be disadvantaged in regard to its error prediction sensitivity due to the dynamics of meiosis. For instance, 91.7 percent of the errors in the first meiotic division arose from premature sister chromatid pre-division, and more than half were corrected in the second meiotic division through a balancing chromosome segregation event (Capalbo, et al. 2013).

In a study, it was established that when polar body biopsy was used in high-risk women with repeated implantation failure, the rate of aneuploidy in the fetus was 65.5 percent compared to 45.2 percent in blastocyst examination. Additionally, the implantation and pregnancy rates in women involved in the study were 11.5 percent and 21.4 percent, respectively, when using polar body biopsy. These rates were low compared to 58.3 percent and 69.2 percent, respectively, when using blastocyst analysis (Fragouli, et al. 2012). Due to these challenges, polar body biopsy tends to give false positives and false negatives in PGD. As such, it is not a very reliable procedure in detecting aneuploidy and monogenetic conditions.

Cleavage Stage Biopsy

Cleavage stage biopsy is a preimplantation technique used on 3-day-old embryos and with at least 6 blastomeres. A cleavage-stage biopsy is the most commonly used approach in PGD, the process accounted for about 90 percent of all PGD cycles up to 2012. Biopsy at the cleavage stage is founded on the principle that during this stage, the blastomeres are equivalent and totipotent such that when a single blastomere is removed, it will provide an adequate and fully representative sample for genetic analysis. In addition, a biopsy at this stage will only compromise at most an eighth of the embryo and, as such, preserve developmental integrity. Here, only a single blastomere is required. When carrying out this procedure, it is important to identify one blastomere with a single and clearly visible interphase nucleus (Thornhill, 2012).

Cleavage stage biopsy resembles chorionic villus sampling (CVS) and amniocentesis since the aim of all three processes is to collect sufficient tissue from the embryo to facilitate effective diagnosis. CVS is carried out in two critical micromanipulation steps: the penetration of a part of the zona pellicuda surrounding the embryo and

the removal of at least one cell. There are a number of zona pellicuda penetration methods including mechanical methods, chemical methods, and laser-assisted techniques. These techniques are described briefly alongside their impact on embryo safety.

Mechanical penetration of the zona pellicuda is achieved through partial or complete penetration. Partial zone dissection involves the use of a fine needle to go through the membrane on two separate points. Then, a slit is made between the two points of penetration while avoiding damage to the embryo. Cells can be extracted by using narrow-diameter micropipettes or through controlled pressure on the zona to push the cells out. The best approach to get cells out is through making two slits on the zona to create a flap which can then be flipped open to allow for more flexibility in regard to the size of the opening. Mechanical methods of zona penetration have been shown to pose risks such as lysis of the blastomeres and pushing out of blastomeres from the embryo.

The most common chemical method of penetrating the zona pellicuda in cleavage-stage biopsy is the use of zona drilling using acidified Tyrode's solution, which has a pH between 2.2 and 2.4. This acidic solution penetrates the zona by dissolving the zona glycoproteins. An advantage of this technique is that it only affects a small, localized area of the zona, usually between 20 and 30 µm. This localized effect is achieved by using a micropipette with an inner diameter of between 5 and 10 µm and placing it in direct contact with the zona and gently expelling acidified solution (Thornhill, 2012). This approach is useful in that it creates an opening with a desirable diameter for the extraction of cells. However, the process can be harmful when it results in damage to blastomeres. The process may expose blastomeres to harmful acidic solutions and affect the developmental trajectory of the embryo.

The last procedure used in cleavage stage biopsy is non-contact laser. Laser ablation is the most common procedure used in the penetration of the zona pellicuda in PGD. The preferred approach is the use of near-infrared solid compact diode 1.48 µm laser (Thornhill, 2012). Lasers are extremely precise and have been shown to produce consistent and reproducible results when used appropriately. The use of lasers is also advantageous in that it results in less contamination and saves more time when compared to both mechanical and chemical approaches. The benefits, limitations, and factors critical to the success of each of the three methods of penetration are summarized in Table 1 below:

Cleavage stage biopsy is a usually done on the third morning after insemination. The exact timing of the procedure tends to vary based on the procedures carried out in different laboratories and may be determined by patient-specific or cohort-specific factors. Biopsy is sometimes carried out on day 2 but has been shown to be problematic due to retardation in the cleavage rate and collection of blastomere samples of small size. As such, day 3 embryos are considered the best for cleavage stage biopsy.

Cleavage stage biopsy has its advantages and disadvantages in regard to the safety of the embryos and the predictive value for genetic abnormalities. The efficacy of successful cleavage stage biopsy was reported at 98 percent by an ESHRE PGD Consortium report covering over 150,000 embryos in clinical PGD cycles. Data on the success rates of pregnancies is largely unavailable, although a recent comprehensive analysis placed the pregnancy success rates after cleavage stage biopsy at 30 percent following a transfer of embryos (Thornhill, 2012). In a study by Mastenbroek et al. (2007), it was established from 408 women (206 in the PGS group and 202 in the control group that had IVF but did not undergo PGS) that the pregnancy success rate was 25 percent in the PGS group and 37 percent in the control group. In addition, live-birth rates were also lower in the PGS group at 24 percent compared to 35 percent in the control group. These numbers point to a challenge in the pregnancy success rates when using cleavage stage, which is the most predominant pre-implantation genetic screening and

Zona Penetration Method	Benefits	Limitations	Factors Critical to Success
Mechanical	• Least invasive to embrio(safer) • Improveved survival after freeze-thaw? • Inexpensive	• Difficult to learn • Operator Dependent • Time-consuming	• Operator skill essential • Appropriate microtools needed
Chemical (Acidified Tyrode's solution)	• Relatively inexpensive • Widespread clinical experience	• Operator Dependent • Difficult to limit apurture size • Effect on cryptopreservation? • Double tool optimal	• Acidified Tyrode's pH 2.2-2.4 • Sensitive control of acid • Rinse acid from embryos
Laser (1.48 µm noncontact diode)	• Rapid and reproducible • Simple to use • Documentation/measurement software	• Capital cost (30-60,000 US dollars) • Not all system prtable • Invisible thermal damage/stress	• Laser alignment and calibration • Pulse duration and number • Distance between laser and zona

Table 1: A summary of the benefits, limitations, and factors critical to the success of mechanical, chemical, and laser methods of penetrating the zona pellicuda. (Source: Thornhill, 2012).

testing procedure used in hospitals currently.

Chromosomal mosaicism is another key challenge for cleavage-stage biopsy. Chromosomal mosaicism is generally understood as the occurrence of at least two distinct cell lines in a person. Chromosomal mosaicism occurs in up to 80 percent of the embryos which undergo cleavage stage biopsy (Thornhill, 2012). Mosaicism is the leading factor in the occurrence of high rates of false positives and negatives in PGD following cleavage stage biopsy and has numerous implications on the safety of embryos.

Blastocyst Stage Biopsy

Blastocyst stage biopsy is carried out after cells progress form the blastomere to the blastocyst stage. Here, cells are more pronounced and easier to identify when compared to blastomeres. When PGD is done on the embryo, it is an invasive procedure. As an invasive procedure, it is important that the impact of biopsy is lowered to the lowest possible point. Blastocyst biopsy is founded on the principle of reducing the impact of the biopsy procedure by reducing the possible amount of the embryo's biomass lost through the process. In cleavage stage biopsy, approximately 12 to 25 percent of the embryo's mass is lost (McArthur, 2014). Such a huge loss of biomass can have detrimental effects on the embryo, especially the ability to implant and develop into a viable fetus. Blastocysts are characterized by more biomass and having more than 100 cells. When biopsies are carried out on blastocysts, only a small portion of the biomass is removed, and the inner cell mass is left intact.

Blastocyst stage biopsy is preceded by embryo hatching, usually on day 3, 5, or 6 following fertilization. When hatched on day 3, the cells can be cultured for 2 more days to allow for the expansion of the blastocoel and extrusion of the trophectoderm cells (McArthur, 2014; McArthur, et al., 2008). Breaching of the zona pellicuda occurs on either day 5 or day 6 following fertilization. Breaching can be done using mechanical, chemical, or laser approaches. The laser method is the most preferred. Once removed, cells can undergo PGD analysis while the embryo is returned to a culture (McArthur, 2014).

Studies over the last decade have shown that trophectoderm biopsy carried out on day 5 or 6 following fertilization is the most effective approach to achieving high rates of embryo safety and effectiveness of diagnosis. Day 5 or 6 biopsy of the blastocyst facilitates better implantation, higher pregnancy success rates, and higher rates of live births. Blastocyst biopsy is effective because it avoids some of the pitfalls of polar body and cleavage stage biopsy such as mosaicism and increases the rates of success. Despite the benefits of blastocyst stage biopsy, this stage of genetic screening is limited to a few hours to a day before embryo transfer. Furthermore, cells acquired from the trophectoderm via mechanical or laser resections are not always suitable for Fluorescence in situ hybridization (FISH) because of the difficulty of isolating and fixing their nuclei (McArthur, et al. 2008).

Ethical Issues

Although PGD has created ethical concerns, it may lessen the need for abortion during pregnancy. The approach can be used to determine the embryo's sex before birth, and so could be used to pick embryos of one sex over the other in the context of "family balancing." Other "social selection" choices that introduce socio-economic issues may be feasible in the future. Some embryos that are unharmed are placed in a woman's uterus, while excess ones and those that are impacted are rejected or donated to science. PGD has the ability to screen for attributes like intelligence and beauty that aren't medically necessary, as well as negative traits like impairments. This has been seen as a counterintuitive and contentious suggestion by the medical establishment. The possibility of a "designed baby" is linked to the PGD technology, raising concerns that more genetic screening will lead to a modern-day eugenics' movement. On the other hand, a procreative beneficence principle is presented, which is a hypothetical moral requirement for parents who have the ability to choose their children to favor those who are predicted to have the best life. One argument in favor of this approach is that certain features (like empathy, memory, and so on) are "all-purpose methods" in the sense that they help the child realize whatever life goals he or she may have. According to Veit (2018), there is no essential moral difference between "creating" and "choosing" a life, making eugenics a natural outcome of embracing the procreative beneficence principle.

Conclusion

Polar Body versus Cleavage Stage Versus Blastocyst Stage Biopsy: Which is the Best?

Three stages were analyzed: polar body biopsy where PGD is done pre-conception; cleavage stage biopsy, which is done 3 days after fertilization; and blastocyst stage biopsy, which is done 6 days after fertilization. Of the three stages, blastocyst stage biopsy was the best one in terms of safety and effectiveness in diagnosis. Using the blastocyst stage biopsy in PGD provides+ more genetic material for sampling, was safer for embryos, increased chances of implantation, and raised the success rates of pregnancies when compared to the other two.

The major difference between these techniques is the

	Embryos transferred *n*	Transfer procedures *n*	Pregnancy per retrieval	Implantation per embryo	Miscarriages	Live birth or ongoing pregnancy	Multiple at confinement		
							Single	Twin	Triple
Day 3 biopsy + day 5-6 transfered *n* = 91 retrievals	1 2 3 All av. 1.5	38 28 3 69 (75.8%)	11 12 1 24/91 (26.4%)	11 15 1 27/103 (26.2%)	4 1 0 5/91 (20.1%)	7 10 1 18/91 (19.8%)	7 7 1 15	 2 3 Multiples (16.7%)	 1
Day 5-6 biopsy + day 5-6 transfered *n*=177 retrievals	1 2 3 All av. 1.1	105 8 0 113 (63.8%)	54 4 58/177 (32.8%)	54 5 59/121 (48.8%)	8 1 9/58 (15.5%)	46 3 49/177 (27.7%)	46 2 48	 1 1 Multiple (2%)	

Table 2: A comparison of the clinical outcomes of day 3 (cleavage stage) and day 6 (blastocyst stage) biopsies. (Source: McArthur, et al. 2012).

time in the reproductive cycle where each is executed. Polar body biopsy is carried out on polar bodies pre-conception; cleavage stage biopsy is carried out 3 days following conception, and blastocyst stage biopsy is carried out six days after conception. As such, the difference in each of the stages boils down to timing. The difference in timing confers unique advantages and drawbacks to each procedure as discussed in the above section. Important features of each stage include the safety of the embryos after biopsy and effectiveness in accurately providing genetic diagnosis so as to ensure the safety of the consequential fetus and baby.

Polar body biopsy has its advantage when it comes to ensuring the safety of embryos. Polar body biopsy does not affect the development of the embryo since polar bodies are not involved in any further development of the embryo. Polar body biopsy is also effective in assessing aneuploidy in embryos. When it comes to the effectiveness of diagnosing genetic abnormalities, polar body biopsy is the least reliable method when compared to cleavage stage and blastocyst stage biopsies.

Most of the debate on the timing of biopsy for PGD has been between day 3 (cleavage stage) and day 6 (blastocyst stage). The table above compares the clinical outcomes of day 3 (cleavage stage) and day 6 (blastocyst stage) biopsies.

From the table above, it is clear that blastocyst stage biopsy is superior to cleavage stage biopsy in regard to effectiveness and safety. When used, blastocyst stage biopsy showed superior numbers in pregnancy per retrieval (32.8 percent compared to 26.4 percent for cleavage stage), implantation per embryo (48.8 percent compared to 26.2 percent for cleavage stage), lower miscarriage numbers (15.5 percent compared to 20.1 percent for cleavage stage), and more live birth or ongoing pregnancy numbers (27.7 percent compared to 19.8 percent for cleavage stage) (McArthur, et al. 2012). While biopsies of 8-cell blastomeres are currently the most common in IVF laboratories all over the world, biopsying the blastocyst trophectoderm on days 5 or 6 is the more preferable technique to cleavage-stage blastomeres on day 3. Blastocyst biopsies yield more genetic material than cleavage-stage embryo biopsies. By using fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and comparative genomic hybridization (CGH) technologies, a more significant number of cells accelerates genetic analysis, offers more accurate results, and aids in the detection of genetic and chromosomal abnormalities. Since blastocyst stage biopsy is done on day five post-fertilization, a trophectoderm biopsy is performed on embryos that have completed the initial cell differentiation phases (compaction and cavitation) during mammalian preimplantation development. As a result, these embryos have the best chance of implantation. It has also been established that the rate of aneuploidy in blastocysts is substantially lower than in cleavage stage embryos. Finally, compared to blastocysts, a biopsy done on cleavage-stage embryos is more harmful.

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Is Growth Hormone the Fountain of Youth?

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Abstract

Finding the cure for aging has been a sought-after quest for as long as the world has existed. Growth hormone has been shown as a possible treatment to negate the phenotypic effects of aging. Growth hormone is released from the adenohypophysis in response to sleep, exercise and stress. This in turn stimulates insulin-like growth factor-1 (IGF-1) secretion from the liver. Growth hormone circulation decreases in volume during aging. Studies on growth hormone therapy have indicated youth-like benefits, such as the reversal of sarcopenia, improved cognitive function, and boosted immunity. However, creating an imbalance of growth hormone and insulin-like factor-1 also has its detriments. IGF-1 has been linked to cancer and diabetes. The purpose of this study was to determine if there is sufficient evidence of growth hormone's anti-aging effects to consider it as an effective age-reversal therapy. Based on the health risks, insufficient degree of positive results, and the benefits of low insulin-like growth factor-1 for evading cancer, growth hormone is likely not the anti-aging drug being sought.

Introduction

Aging is by far the most considerable risk factor for chronic diseases and their related deaths worldwide. Commonly recurring conditions like cardiovascular disease and cancer, are known to occur at a much higher rate among the aging. In fact, beginning at midlife, the incidence of mortality from chronic disease doubles nearly every 7-8 years (Rae et al., 2010). This presents an important dilemma for a world population that is getting increasingly older, with a higher population density of those aged 60 or over every year. Without any intervention, this could cause a major problem for hospitals and healthcare infrastructure as they attempt to keep up with the higher demand for medical care. Some of the negative impacts of aging are the result of cellular and molecular damage inflicted by the dysfunction of human body systems and other environmental factors over time. This can lead to conditions such as organ hypertrophy, sarcopenia, and a compromised immune system. Much research has been done on this subject, with anti-aging interventions performed on both animals and humans, yielding some promising results (Weindruch & Walford, 1988). Reversing aging could positively impact both longevity and health span, giving the elderly more "youthfulness" and a higher quality of life in their later years. In specific, Growth Hormone (GH) treatments have been identified as a possible solution to the aging crisis. With aging, GH pulsation decreases in both frequency and quantity and becomes almost nonexistent in some. Human Recombinant Growth Hormone (hRGH) therapy has been shown to increase the body's muscle to adipose ratio in younger subjects with growth hormone deficiency (Elbornsson et al., 2013) and could possibly counteract the effects of sarcopenia. It is hypothesized that GH would also cause organ growth, including the thymus which is very important for immunity-providing T cells. However, side effects of GH treatment must also be considered, with the increased ability of cancerous growths to proliferate. Another negative effect of GH therapy to examine is diabetes mellitus. This can occur as the result of over targeting on the GH/IGF1 axis, leading to dampened insulin sensitivity. Overall, it must be determined if GH is a worthy treatment for aging prevention, at what dosage is it most affective, and if it could be combined with other drugs known to combat its side effects.

Methods

Databases including Google Scholar, PubMed, EBSCO, and ProQuest were used to search for peer-reviewed articles and journals with relevant scholarly information. Articles were reviewed and analyzed for both accurate contemporary content as well as any conflict of interest. Keywords used for searching included "Growth Hormone", "Aging", "GH-IGF-1 axis", and "Somatopause."

Discussion

What is Growth Hormone?

Human growth hormone (hGH), or somatotropin, is the most abundant hormone in the adenohypophysis, accounting for up to 10% of the total weight of the pituitary gland (Devesa & Devesa, 2020). This small proteohormone is originally stimulated by growth hormone releasing hormone (GHRH) from the hypothalamus in the brain. Stress, exercise, and sleep stimulate the release of GHRH to the adenohypophysis and the resulting GH secretion by somatotrophs into the bloodstream. As its name suggests, growth hormone was initially discovered for its growth inducing properties on longitudinal bones in children. However, the effects of GH were quickly recognized to be much greater than just longitudinal growth. One of the earliest pioneers of GH treatment wrote, "Pituitary growth hormone is distinctive in causing growth of almost all tissues…" (Raben, 1962). We now know that growth hormone increases the growth of most cells by increasing amino acid uptake, and that it also regulates metabolism through lipolysis in fat tissue (Tresguerres et al., 2022). Therefore, GH plays a large role not just in children and adolescents, but also in adults that have "ceased" growing.

GH circulation levels reach their maximum soon after birth and remain high throughout the early stages of life. After reaching the full growth potential of adulthood,

there is a slow decline in GH levels caused by less hypothalamic GHRH production. Measuring GH levels in the body has long been a problem for researchers, because of its nature of pulsatory secretion. The highest GH levels are always found during sleep and fluctuate greatly throughout the day. As a result, new tests to indirectly detect GH have been developed, with the most common way being the measurement of plasma IGF-1 (Insulin-like growth factor-1). This method was proven by measuring IGF-1 levels in the aging to see that they closely resembled the downward curve of GH levels (Rudman et al., 1990). IGF-1 levels directly correspond to those of growth hormone since IGF-1 acts as a GH mediator and regulator in the GH/IGF-1 axis.

The GH/IGF-1 Axis

Once GH is secreted from the pars distalis, it travels through the bloodstream to target tissues. These include the muscle, adipose tissue, and liver. The adipose tissue then decreases as it undergoes lipolysis. On the other hand, an increase of protein and amino acid uptake results in growth of muscle tissue. When GH reaches the liver, the liver secretes IGF-1 which acts as a mediator to affect both chondrocytes and the body's organs. GH affects nearly all cells in the body, either directly or through IGF-1 secretion. However, since IGF-1 closely resembles insulin's chemical structure, this results in competition for insulin binding sites and a dampened insulin sensitivity, which can lead to hyperglycemia (LeRoith, 2007). The regulation of GH occurs on both the hypothalamic and pituitary levels. Somatostatin is released by the hypothalamus and inhibits both growth hormone and GHRH activity. IGF-1 acts to increase somatostatin and inhibits GH both directly and indirectly, through GHRH inhibition. So, in essence, GH regulates itself. Sleep, exercise, and trauma act as stimulants for GH. Ghrelin, sometimes called the hunger hormone, which is produced in the gastrointestinal tract, also acts as a stimulant for GH. This is the well-known GH/IGF-1 axis for regulating growth hormone activity, especially in the longitudinal growth which occurs in children.

Growth Hormone Deficiency (GHD) in Children and Adults

In 1985, the FDA approved the use of hRGH (human recombinant growth hormone) as a treatment for children suffering from GHD. Growth hormone deficiency is characterized by a dysfunction of the hypothalamus or pituitary gland which results in an inadequate amount of circulated GH. The causes of this dysfunction can be either congenital or etiological, with its effects seen in children as well as adults. GHD in children is sometimes referred to as pituitary dwarfism and is easily identified by a lack of growth and short stature. GH treatment in children has shown overwhelming success rates in dose-dependent increase of growth and stature (Chatelain et al., 1994). GH has become somewhat standardized for children with small-stature as a result of GHD. In adults however, GHD diagnosis becomes much more complicated. Adult patients may present with obesity and a lack of lean muscle, but this is not always apparent. Hypoglycemia often accompanies GHD since it causes dysregulation of the GH/IGF-1 axis, although there can be many other causes of low blood sugar levels. Additionally, measuring GH levels requires constant and persistent testing using GH/IGF-1 assays to obtain accurate results (Melmed, 2019). Diagnosis can often only be made after putting together the entire picture. GH treatment for adults with GHD was FDA-approved in 1996 and patients immediately showed major improvements in body composition and bone health, as well as a self-reported increase in quality of life in both male and female cohorts (Attanasio et al., 1997).

Is GH Treatment Safe?

The effects of GH therapy have been monitored over the past 30 years, in thousands of patients, to determine that it is generally safe for children and adults. The risk of developing cancer both for children and for adults is not affected by GH treatment. Cancer recurrence also seems to be unaffected in children, while more data is still necessary to determine the risk of recurrence in adults. However, increased IGF-1 levels have been linked to several types of neoplasms including prostate and colon cancer. Additionally, it is likely that the anabolic effects of GH and IGF-1 would aid cancerous growth once a malignant growth is already present (Jenkins et al., 2006). The incidence of type 2 diabetes mellitus has also been shown to be unchanged after GH treatment, even though insulin sensitivity is a common concern when increasing the GH/IGF-1 axis. Moreover, it can be hypothesized that risk of cardiovascular disease actually decreases, since levels of LDL cholesterol are decreased when compared to GHD. Growth Hormone has been known to cause peripheral edema, arthralgias, and carpal tunnel syndrome in older patients (Hersch & Merriam, 2008). There are several other rare cases of illness, including intracranial hypertension, scoliosis, and sleep apnea, although direct relation to GH therapy has not been confirmed (Allen et al., 2016).

Acromegaly and Excess GH Secretion

On the other hand, immoderation of GH secretion, which is generally caused by a pituitary adenoma, also yields negative results. Gigantism results when excess growth

hormone is secreted before the long bones have fused. When left untreated, patients commonly develop diabetes and arthritis. Acromegaly, which is the consequence of increased GH production in adults, carries a greater risk for hypertension and cardiomyopathy. These health risks result in morbidity rates of up to three times that of their age group (Adelman et al., 2013). Although, since the cause of this disorder is hypersecretion due to adenoma, there can be many other hormones that will also be present in excess which can also contribute to adverse effects.

GH/IGF-1 Axis in Centenarians

Centenarians are perhaps the closest model you can find for a healthy and delayed aging process. The ability of these long-lived people to thrive in old age can usually be attributed to their success in avoiding and outlasting major illness, like cancer, cardiovascular disease, and diabetes mellitus. There are several factors that affect healthy aging, with genetics playing a large role in avoiding disease entirely. In fact, several aging associated diseases, including Alzheimer's and cardiovascular disease, are practically nonexistent in extremely old individuals (Zhang et al., 2020). Therefore, studying the long-lived and their offspring can likely yield insight for anti-aging techniques.

There have been several studies documenting the GH/IGF-1 axis in long-lived individuals, but the results appear controversial. Many studies found an incidence of decreased levels of plasma IGF-1 among centenarians (Bonafe et al., 2003;Van der Spoel et al., 2015). On the other hand, one study showed that low IGF-1 levels in centenarians predicted impendent mortality (Arai et al., 2008). Some even reported no change in the GH/IGF-1 axis of centenarians when compared to those over the age of 65 (Paolisso et al., 1997). There are several factors that could influence the effectiveness of these studies. It is hard to determine the role of GH/IGF-1 in aging based on this data, especially when taking into account the normal age-related decline in GH secretion. Additionally, other important longevity factors include socioeconomic situations and exceptional genetics when it comes to predisposition for certain illnesses. Accordingly, it is likely that more research is necessary to accurately determine a pattern of GH levels in centenarians. Studying the offspring of these individuals could be a better option for obtaining enough data, since it can be difficult to find and test centenarians due to a small sample size, as well as their physical limitations of old age.

GH Therapy as An Anti-Aging Drug

The idea that growth hormone could be used in an aging prevention capacity is based on established knowledge of growth hormone's functional properties in metabolism and growth. It is well documented that GH secretion declines with age starting from as early as 30 years old. After that point GH secretion decreases about 15% more every decade of life (Garcia, et al., 2000). With this progressive loss of growth hormone circulation, it is hypothesized that this deficiency plays a large role in several aging processes. Firstly, changes in body composition in the aging favor a higher ratio of adipose tissue and less lean body mass. Additionally, hypertrophic processes are reflected in many organs such as the liver, spleen, and bone. GH replacement could be used to prevent and reverse this aging decline, much like we see its effectiveness regarding the adipose to muscle ratio in GHD patients.

In a landmark study, GH replacement therapy was introduced to 21 men over the age of 60. After a 6-month treatment period, the results showed major improvements, increasing muscle mass by 8.8 percent and skin density by 7.1 percent, and decreasing the mass of adipose tissue by 14.4 percent. Bone density also showed a marginal increase. This data indicated that GH is indeed responsible at least partially for the aging phenotype. GH therapy could be a breakthrough in the treatment of aging decline, as well as common age-related conditions like sarcopenia (Rudman et al., 1990). Additionally, the fact that there were practically no side effects in this cohort indicated that GH could be used safely in aging patients if given at low/replacement doses.

This study though, was only a small representation of GH success. The sample size was 21 patients, and the treatment period was only 6 months. More research would be required to identify long-term anti-aging improvements and any adverse effects. One such concern was that although GH therapy could reverse the effects of aging by inducing growth of internal organs, maybe it would also aid cancerous growths. Although, research in children and adult GHD has shown no such risk, there is not much data on older adults where the incidence rates of cancer are already heightened. Additionally, the side effects found in the test group included hypertension and hyperglycemia. Over longer periods of treatment, it is likely that insulin sensitivity could decrease and lead to diabetes mellitus. Other adverse effects reported from GH administration at therapeutic levels (although the muscle to adipose ratio increased greatly) included peripheral edema, carpal tunnel syndrome, and arthralgia, which occurred in up to 46% of patients in one study (Blackman et al., 2002). In replacement therapy though, these symptoms were not seen, other than a low incidence of joint pain. It is important to consider that when looking through the lens of health span rather than longevity, maybe such a

treatment would be worth the risk. The ultimate goal is to delay aging, in the sense of a longer feeling of youthfulness, rather than merely extending life after aging. However, because of the great possibility of serious illness, additional research would be necessary before declaring the unearthing of the fountain of youth.

A better way to measure effectiveness of GH could be by using epigenetic clocks in place of chronological age. Epigenetic aging clocks are a type of biological clock which measures DNA methylation levels. DNA methylation is a type of DNA modification that increases with age and plays a role in proper gene expression and function. Since this methylation is a dynamic process, it can accurately predict biological age in regard to many health risks (Field et al., 2018).

A study was conducted showing the impact of GH on thymic involution and immunity from an epigenetic aging perspective. The shrinking of the thymus is a common effect and a key factor in many aspects of aging. The reduction of the functional mass of the thymus, the thymic fat free fraction (TFFF), results in decreased t-cell production and a compromised immunity from major illnesses like cancer and pneumonia. Lack of t- cells can also result in inflammation and can lead to pain and atherosclerosis. Growth hormone replacement therapy combined with DHEA (dehydroepiandrosterone) and metformin, was administered to 9 patients in 2 separate trials. The results, as seen by MRI imaging, showed rejuvenation of the thymic fat free fraction of the thymus, indicating greater immunity t-cell production. The purpose of using DHEA and metformin with the GH was to counter the possible effects of hyperinsulinemia on thymic growth. Hyperinsulinemia is often caused by insulin resistance which could be caused in this case by elevated IGF-1 levels. Immunophenotyping confirmed a higher lymphocyte-monocyte ratio and an increase in recent thymic emigrant t cells, which is associated with better outcomes in 8 different cancers. Most importantly, the epigenetic age of both cohorts increased by a mean of 2.5 years when tested using 4 of the most accurate epigenetic clocks, DNAm, Pheno, Hannum, and GrimAge. (Fahy, 2019). This discovery indicates that GH treatment confers an important immunity boost, which could potentially offset the increased risk of cancer caused by its growth properties. Additionally, the use of a combination of treatments to counter side effects could possibly be useful when trying to combat negative effects of growth hormone treatment including diabetes mellitus. Overall, a reduction in biological age is a strong indicator of GH efficiency in the reversal of aging processes.

Another less studied effect of GH therapy is regarding memory function. The association between GH therapy and memory gain has already been established in studies of GHD children and adults. Researchers have determined that memory will predictably decline in growth hormone deficient patients when compared to a similar control group. When treated with GH however, these patients experienced cognitive improvements in relation to their previous state when deficient in growth hormone (Maruff & Falleti, 2006). The precise mechanism for this link between GH/IGF-1 and memory is not currently known, but we do know that GH does have the ability to cross the blood-brain barrier. It has been speculated that GH could affect the dopamine concentration in the hippocampus, thereby increasing memory and cognition (Arwert et al., 2005). However, we must consider that this improvement in cognitive behavior was mostly seen in younger adults with child onset GHD. Another possible explanation for this phenomenon is the development and maturation of the human brain in early adulthood. More research in older patients must be conducted in order to determine if GH indeed boosts cognitive functioning.

Although GH therapy promotes many age-reversal effects, one study found low GH/IGF-1 levels in the elderly to be a conservation process extending longevity. In a group of 184 subjects above the age of 90 (93 with low IGF-1 levels and 91 that exhibited high IGF-1 levels when compared to the median), it was found that females in the low IGF-1 group survived two times as long as those with high IGF-1 levels. Interestingly, male subjects with low IGF-1 tested the same as those above the median. When comparing both males and females who had a history of malignancy, the survival rate increased to nearly 2.5 times longer for both males and females with low IGF-1 than that of the control group (DeVito et al., 2022). This suggests that GH and IGF-1 decline in aging may in fact be the body's way of self-preserving especially when there is a history of malignancy. One mechanism for this phenomenon could be the increased activity of IGF-1 binding proteins leading to an imbalance in protein homeostasis. For the aged, such an imbalance to homeostasis could mean an inefficient response of the cells to stress, in addition to major health implications on cellular maintenance, causing a decrease in longevity.

GH Treatment and Moderation in Animal Studies

For years it has been known that mice that have a pituitary deficiency of GH have a much longer lifespan than their ordinary siblings (Brown-Borg et al., 1996). Ames dwarf mice, for example, which are deficient in GH, thyroid stimulating hormone, and prolactin, lived a median of 50 percent longer for both males and females. However, when these mice were treated with recombinant GH immediately

after birth, their median survival decreased by 22 percent when compared to a control group, proving the association between growth hormone modulation and longevity (Sun et al., 2017). Interestingly, in the same study normal mice that were injected with GH did not exhibit any change of lifespan compared to untreated controls. A possible explanation for this is that small doses of GH are more tolerable in the early stages of life, given that GH levels are already at maximum.

To better study GH modulation in mice, two laboratories genetically modified mice so that one group had a disruption in the growth hormone receptor (GHR) gene and the other had a modification in the GHRH gene. In both cases there was a major extension of life in both genders (Sun et al., 2017). One of the purposes of this experiment was to better show that GH was responsible for lengthening life, even when all other hormones such as TSH and prolactin are functioning normally. Additionally, this showed that it is possible to create a genetic GH/IGF-1 modulation system without any previous history of GHD. If this could be replicated in humans, it could possibly lead to extended longevity, although the appropriate time for such a change to low GH/IGF-1 would likely have to take place during adulthood to prevent child GHD issues.

GHR Disruption and Laron Syndrome

It is of worthy mention that the longest-lived mouse, winner of the Methuselah prize for longevity in laboratory mice, was a genetically modified growth hormone receptor knockout (GHRKO) mouse that survived almost 5 years. This was an important discovery since the GHRKO gene correlates greatly with patients who have Laron Syndrome (LS). In cases of LS, patients are also found to have a low serum IGF-1 while exhibiting high levels of GH (Duran-Ortiz, 2021). IGF-1 is linked to cancer, with effects on cell proliferation, angiogenesis, apoptosis, and metastasis. Increased IGF-1 has even been thought to cause resistance to chemotherapy treatments (Jenkins et al., 2006). People with LS often live to normal life expectancy or longer and are even immune to certain types of cancer. In one study of LS with 222 patients, not one of them developed cancer while there was a cancer prevalence of 8-24 percent among relatives (Shevah & Laron, 2007). This supports the idea that low IGF-1 levels are associated with longevity in humans. Indeed IGF-1 blockers are currently under investigation as a possible chemotherapeutic agent (Zhang & Douglas, 2004).

Conclusion

Growth Hormone therapy in the aging has shown many of the positive effects that we have seen in GHD children and adults. However, it seems that excess GH/IGF-1 can also act as a detriment to longevity. Cancer proliferation seems more likely with high levels of IGF-1 and the development of diabetes mellitus from acquired insulin insensitivity remains a concern. Furthermore, animal studies have shown that low GH and IGF-1 levels are strong indicators for longevity. To declare GH as an anti-aging drug would require more research into these health risks. The results of GH therapy have not indicated that they are worth the risk of adverse effects. Perhaps testing other doses of GH could reverse aging more, or using a combination of medications could limit negative results. At this point it is impossible to determine if growth hormone is the fountain of youth, or merely the body's way of protecting itself from age-related diseases.

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Sanfilippo Syndrome: Symptoms, Therapies and the Search for a Cure

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Abstract

Sanfilippo syndrome, also named Mucopolysaccharidosis III (MPS) is an uncommon disorder that develops due to abnormalities in the nervous system and brain. Aspects of this condition include losing motor and mental function beginning at just a few months old. Four different proteins are involved in the breakdown of Heparin Sulfate (HS) in the extracellular matrix."When one of these proteins is missing or inadequate, Mucopolysaccharidosis III (MPS), can result ("Sanfilippo Syndrome", 2019) The specific protein that is insufficient will determine which of the four corresponding forms of Sanfilippo syndrome is present."All variations of Sanfilippo Syndrome are easily identified by a variety of somatic symptoms accompanying the progressive deterioration of the Central Nervous System (CNS), all of which will be described further in this research paper. The goal of this study is to investigate the progress made to find a remedy for this devastating disorder, as well as to discuss the symptoms and pathology of the condition. Although there is currently no cure for Sanfilippo Syndrome, several researchers have observed that there may be some hope in symptom management and therapy."Particularly, treatment may include Substrate Reduction Therapy (SRT), Enzyme Replacement Therapy (ERT), and gene therapy." As of late, the biggest obstacle that scientists must circumvent while searching for a cure is the blood-brain barrier, which can block treatments from passing between the Central Nervous System and the blood. In the research paper below we will more deeply examine the various characteristics of the disease and current treatment options.

Introduction

"Sanfilippo syndrome develops due to inflammation in the nervous system and brain cells, which inevitably results in neurodegeneration. This unusual condition consists of various indicative symptoms, such as weak muscle control, loss of cognitive skills, and premature death. Fortunately, various studies have demonstrated that a patient's condition can improve through symptom management and therapy. However, for now, therapies can only provide for a slight improvement from the symptoms of the disorder, and regretfully, there is no cure. Nonetheless, it is important to note that experts have made encouraging progress despite the complexity of the disorder.

Sanfilippo syndrome is a rare neural disease belonging to the group of Lysosomal dysfunctions. "This condition arises when one of the four enzymes responsible for degrading Heparan Sulfate (HS) is missing or damaged. "The enzyme deficiency causes a build-up of partly decomposed HS in the lysosomes of the cell in the organs and tissues. HS, a sugar-based macromolecule, is mostly found in the extracellular matrix, where it aids in cell communication and strengthens glial cells. The buildup of HS impairs CNS function because it causes astrocytes and microglia to produce proteins that indicate inflammation. Consequently, the levels of toxins in the blood rise, intracellular communication is damaged, and extensive inflammation in the brain causes neurodegeneration. (Valstar et al., 2008). Following this basic interpretation of the pathology of Sanfilippo Syndrome, it is important to ask, what progress has been made in finding a cure. In order to evaluate that we must also ask, what does the diagnostic and treatment plan look like, and how effective is it?

Materials and Methods

"The research for this paper is based on academic research papers, scientific journals, and medical databases such as PubMed and ProQuest. Touro University's online library has been essential in providing material and sources for this article. All sources have been vetted for reliability, correctness, and only the latest research has been used.

Discussion

"The process of diagnosing Sanfilippo syndrome is long and complicated. Various symptoms such as difficulties in communication and interpersonal skills resemble symptoms of autism spectrum disorder (ASD). "To eliminate a misdiagnosis of ASD, doctors will first order an autism diagnostic observation plan. A urine sample is then obtained from the patient and tested for elevated levels of glycosaminoglycan (GAG), which would indicate an excess amount of Heparin Sulfate in the body. While urine findings can confirm that the illness is Sanfilippo syndrome, a more thorough examination of enzymatic activity is required to determine which of the four variants is present. The clear presence of facial features that are indicative of the disorder might influence the diagnosis as well ("Sanfilippo Syndrome," 2019).

The scenario below depicts a typical diagnosis of Sanfilippo syndrome in a young child. After suffering hyperactivity and other behavioral issues since the age of three, a seven-year-old child was brought to a neurologist for assessment. His medical history showed normal birth and that he had previously reached all age-appropriate milestones in both walking and talking. However, he later developed restlessness and other abnormalities in his psychomotor development. He was originally misdiagnosed with ADHD, but when no change was found after drug therapy, the doctors sought a different explanation. A physical assessment showed somewhat coarse facial features and a low nasal bridge. Despite the fact that initial lab results of urine, blood, thyroid, and plasma showed

no abnormalities, the findings of the physical examination together with the boy's telltale behavioral issues led the doctors to prescribe additional testing to confirm their suspicion of MPS III/ Sanfilippo syndrome. Another round of testing showed a complete lack of activity of one for the four indicative enzymes, supporting the diagnosis of Sanfilippo syndrome. The patient would now go through a lengthy process of therapy and support in effort to slow degeneration as much as possible and increase quality of life (Kartal, 2016).

The symptoms of patients with Sanfilippo syndrome are broken into three phases as described in the Journal of Inherited Metabolic Disease. The first stage, from ages 1-2, begins after a typically unremarkable pregnancy, and consists of delayed movement and speech. Telltale physical features might become noticeable, including a frontal bossing forehead, rough facial features, and prominent eyebrows. The next couple of years are part of phase two, which include extreme behavioral and mental problems, also called childhood dementia. Additionally, during the second phase, Sanfilippo patients will typically experience insomnia, hyperactivity, restlessness, aggressive tendencies, and permanent loss of all communication skills. They also suffer from various uncomfortable somatic symptoms such as extreme hair growth, enlarged liver, stiff joints, and loose stool. When all vital functions like swallowing and other areas of muscle control are lost, the patient has reached the terminal stage. Life expectancy is generally around 20 years (Delgadillo et al. 2011). Table 1, based on a collection of data by Muschol, et al., represents a full list of neurological and somatic symptoms indicative of Sanfilippo Syndrome:

Although unfortunately there is no cure, scientists are constantly researching therapies that can hopefully improve or stabilize the effects of Sanfilippo syndrome. Enzyme Replacement Therapy has been FDA approved for limited use to help people cope with this disorder. However, its efficacy has been insufficient so far, as it does not influence the CNS and can only aid with the somatic symptoms. The reasoning is straightforward: it is unable to pass the blood-brain barrier without invasive and complex medical procedures. While it is necessary for the patient to undergo an unpleasant procedure to administer the ERT via an implant, testing on dogs has revealed that the therapy can indeed reduce the appearance of HS substrates, based on the dose of ERT given. Most importantly, after injecting the highest dose of ERT, the presence of HS substrates was lessened to near normal (Pearse and Iacovino, 2020).

One of the most encouraging treatment prospects for this disease is gene therapy. When conducting trials on

Table 1: Clinical indications of Sanfilippo Syndrome (Muschol, et al. 2022)

Cerebral and Nervous:	Possible Expression
Mental	Insufficient language and speaking skills
	General retardation of neural development
	Continuous deterioration of life skills and cognitive capability
Behavioral	Violent/harmful activity
	Agitated and overactive behavior
	Hyperorality
	Stubbornness and outbursts
	Fearlessness
	Noncompliant behavior
	ADHD
	Inability to sit still
	hypersensitive to touch/heat/cold
	Autistic mannerisms
	insomnia
Mechanical	insufficient fine and gross motor skills
	Abnormal walking pattern
	Muscle tightness
Misc	Spasms and convulsions
Somatic:	
Physical form	Crude physical characteristics of the face
	Thick and roughened skin and hair
	Excessive hair growth
	Prominent forehead
	Larger than normal head circumference
Stomach/Intestines	Diarrhea or frequent loose bowels
	Constipation
	Abdominal pain
	Hernias
	Enlarged spleen or liver
ENT	Partial deafness
	Regular ear infections
	Fluid in the middle ear
	Constant stuffy nose
	Need for tonsils/adenoid removal at a young age
Eyes	Loss of vison due to retinal disease
Cardiology	Irregular heartbeat
	Weak heart
	Heart-valve disease
Skeletal/muscular	Pigeon-toeing
	Walking on tiptoes
	Stiff joints
	Bone-tissue death in parts of the femur
	Curved spine
Respirational	Chronic rapid breathing in newborns
	Pneumonia
	Abnormal breathing while sleeping

mice it was found that after just one injection of a viral vector carrying a recombinant gene, the resulting enzyme activity was restored to standard levels. The way this works is as follows: The virus containing the healthy gene sequence is delivered intravenously and taken up by nearby cells. The goal is that the cells should learn the viral genetic code, which will allow it to fulfil missing functions despite the faulty enzyme. The viral recombinant genes will then be released it into the bloodstream, where they will continue to present the healthy gene to the rest of the body. When experimental gene therapies were trialed on mice, it clearly increased their survival rate by rectifying the HS lysosomal storage issues and thereby lessening neurodegeneration. However, one important shortcoming to note is that gene therapy has only been effective in treating young patients so far. Nevertheless, this new avenue of treatment is now being evaluated as a possible cure and may yet offer substantial improvements treating this ailment (Pineda, 2013).

Substrate Reduction Therapy aims to reduce the severity of Sanfilippo syndrome using a different technique. Instead of creating new enzymes to degrade HS, doctors release substrates into the body that can diminish its production to begin with. One type of molecule that can achieve this is Genistein, which can alter the production of HS by completing the coding during protein synthesis. When mice were test treated with Genistein, researchers found that they not only created less HS, but they also had enhanced lysosomal storage and less inflammation was evident in their brains. However, researchers are not satisfied with the minimally reduced level of HS that SRT yields. Data from clinical trials showed that even a high dosage of Genistein yielded just a minute decrease in HS. Still, research is advancing, aiming to continue testing higher and higher of doses of Genistein, in hopes they will be able to lower it enough to lessen symptoms of Sanfillipo (Beneto et al., 2020)

As of yet, the only real way to aid in tending to the care of Sanfillipo patients is palliative support. Medical resources such as antibiotics, surgery, and other drugs are vital the prevention and cure of frequent infections, hernias, and diarrhea. Antiepileptic medications have been proven essential to control seizures, and overr-the-counter drugs like melatonin are effective in in soothing behavioral issues such as insomnia and aggression. (Lavery et al., 2017)

Results

Understandably, the prognosis of Sanfilippo Syndrome is terribly painful for patients to hear, and heartbreaking for loved ones to watch. Therefore, researchers are devoting a significant amount of time and effort searching for a way to increase the comfort and improve life expectancy for such people. Amazingly, scientists have identified of all the genetic factors that contribute to MPS III. However, treatments are still in the experimental stage, and finding cure has not been straightforward at all. One such limitation is that while studies are frequently conducted on animals, the results cannot always be accepted as reality for humans. This is because at the end of the day there are many profound distinctions in the biological makeup of rodents and people. Although scientists know that these trials may be totally irrelevant, they continue to experiment on mice and other animals in the hope that they will discover something that will be instrumental in curing Sanfilippo syndrome (Beneto et al., 2020).

Doctors who are optimistic that a cure can be found are continually assessing trial results, testing new therapies, and evaluating possible remedies. Additionally, there are many public resources on the internet that provide information about the disease and offer opportunities to join clinical trials. More than thirty clinical trials in SRT, ERT, and gene therapy, have transpired throughout the past 20 years. Specifically, The Journal of Inherited Metabolic Disease presents detailed results on a new Substrate Reduction Therapy trial that took place in Europe. Nineteen patients ranging from 2 to 19 years old were each given a 5-milligram dose of a genistein extract three times per day for a year. Every twelve weeks, professionals calculated the examined the effects of the drug by collecting bloodwork, surveys, and DNA samples. Patients felt no adverse effects of the drug after trialing the drug for a year. Although the patients did not report improvement on their cognition or behavior according to the disability surveys, labs confirmed that those who underwent genistein therapy had minimal accumulation of Heparin Sulfate, and less inflammation. Additionally, doctors observed a decrease in the frequency of infections, and improvement in some other somatic symptoms (Delgadillo et al. 2011). These results were duplicated in a study from 2013 with an identical number of patients and a significantly higher dose of genistein (160 milligrams). Once again, the results exhibited no harmful side effects, and the rate of mental degeneration did not attenuate. However, the quantity of stored Heparin Sulfate was diminished, and behavior improved (Pearse & Iacovino, 2020).

It is critical to monitor the success of these experimental therapy in order to maximize the life expectancy of people with Sanfilippo syndrome. As of now, each continual trial of SRT, ERT, and gene therapy brought the same mixed results. On one hand, they were unsuccessful in stopping or undoing the deterioration in the brain. On

the other hand, they were effective in somewhat lessoning the somatic symptoms of the disorder, such as irregular behavior and insomnia. With the ongoing advancements in science and technology, patients hope to steadily increase their ability to manage Sanfillipo symptoms using drugs and other treatments ("Sanfilippo Syndrome," 2019).

The biggest hurdle that scientists need to circumvent in their search for a cure is the blood-brain barrier. In its function of preventing large molecules from diffusing from the blood to the brain, it inadvertently prevents macromolecules in medication from diffusing as well. Therefore, many times Sanfillipo cures have improved somatic symptoms but have not slowed the neurodegeneration. It is possible to deliver medicine to the brain by injecting it directly into the cerebrospinal fluid or cerebral tissue. The first experimental treatment to successfully overcome this obstacle was approved by the FDA in 2019. Seelos Therapeutics created a drug called Trehalose, which is small enough to diffuse through the blood-brain barrier. Hopefully, further testing will show that Trehalose can reduce toxic buildup in the CNS (Press Release, 2020). The results of these trials are encouraging the idea that a cure might soon be found. It is hopeful that with the continual success and data collection and research, there will be a breakthrough in the near future.

Sanfillipo Syndrome is a severely debilitating disorder that causes behavioral issues, degeneration of mental and motor skills, and usually results in death within 20 years. Scientists have given their utmost to finding a cure for this heartbreaking condition, and in doing so, they have pushed the limits of medicine farther than ever before. They have made some progress on treatments such as Substrate Reduction Therapy, Enzyme Replacement Therapy, and gene therapy. Although these therapies have not improved cognitive decline, Sanfilippo Patients are thankful to use these therapies to help regulate the somatic symptoms. In the meantime, research is still constantly developing, and now that researchers have identified their biggest obstacle as the blood-brain barrier, it is hopeful that they will discover a way to bypass it.

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Improving the Prediction and Management of Intrauterine Growth Restriction

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Abstract

Intrauterine growth restriction (IUGR) is a potentially fatal and often missed obstetric complication. The fetus is deprived of vital blood, oxygen, and nutrients due to faulty maternofetal circulation, leading to a severe lack of fetal growth. Since current prenatal testing is highly ineffective at diagnosing the condition, many babies with IUGR are negatively impacted before, during, and after birth. This paper assesses alternative and innovative detection and management methods of IUGR. Current routine prenatal care includes simple fundal height measurements to screen for IUGR. This analysis finds that other testing may increase the rate of detection of the disease. Maternal serum analytes, uterine artery dopplers, and fetal heart rate analysis all provide relatively small rates of detection. However, due to their non-invasive nature, they offer the option of taking a multipronged testing approach to increase the chance of an IUGR pregnancy being properly diagnosed and managed. Possible management includes aspirin and early delivery; however, this analysis does not find a consistently positive effect for either of these options, despite the pathology of the disease suggesting that these approaches would improve outcomes. More research is needed to find optimal diagnostic testing and treatment for intrauterine growth restriction.

Introduction

Intrauterine Growth Restriction (IUGR) is a condition in which there is abnormal or impaired growth of the fetus inside the uterus that is due to a pathological cause. The growth of the fetus in the womb is a significant predictor for the outcome of the pregnancy. Low-weight fetuses are at a greater risk of intrauterine injury and death. An astounding 50% of stillbirths have been found to fit the criteria for IUGR. For pregnancies that result in liveborn infants, the detection of fetal growth restriction is abysmal, with as many as 85% of IUGR cases going undetected until delivery (Albu et al., 2014).

Ideal fetal growth is allowed by placental ability to handle injury caused by outside stimuli. In IUGR, the placenta shows abnormal maternal spiral arterioles, dysregulated villous vasculogenesis, and profuse fibrin deposition. Regardless of the cause for these abnormalities, the villi of the trophoblasts show severe injury to the epithelium and stress to the endoplasmic reticulum, leading to a lack of regulation. The escalating stages of placental dysfunction that develop through the pregnancy, as the IUGR diagnosis surfaces, eventually leads to a reduction of blood flow and limitation of nutrient transfer to the fetus (Scifres and Nelson, 2009).

Currently, the main method of detection of IUGR includes fundal height measurements at prenatal visits, with abnormal findings indicating the need for biometric testing, which uses specific formulas to estimate fetal weight based on general fetal growth curves. Primary management of IUGR is limited to monitoring fetal well-being at specific intervals, and, when fetal risks are greater than neonatal risks, intervening by administering steroids and delivering the fetus at a hospital that is equipped to deal with a seriously ill neonate (Militello et al., 2009).

New research suggests other methods of predicting IUGR, potentially improving outcomes of pregnancies afflicted with fetal growth restriction. This paper will analyze the different approaches and explore their effectiveness in improving disease management.

Materials and Methods

Research on detection methods for intrauterine growth restriction was obtained from peer-reviewed scientific articles and academic journals. These were accessed via Touro University Library as well as Google Scholar, using various databases including PubMed, ProQuest, and Ebsco. The main keywords and phrases used to obtain research data for the paper include "IUGR pathology," "IUGR testing," "IUGR treatment," and "IUGR neonatal outcomes."

Placental Pathology

The most common cause of IUGR is dysfunctional maternal-fetal circulation (Vandenbosche and Kirchner, 1998). The development of the placenta to support and nourish the fetus throughout gestation is a complex process involving the formation of an entirely new uteroplacental circulation. A fetus's growth can be restricted due to faulty development of the uteroplacental circulation. The fetus is deprived of vital nutrients and oxygen, causing its failure to reach its growth potential (Albu et al., 2014).

Placental exchange is controlled by a complicated interaction between placental growth, rates of blood flow through the placenta, expression of transporter proteins, and metabolic needs of placental tissue. This interaction is regulated by hormones from the mother, fetus, and the placenta. When operating properly, this system allows for an adequate flow of blood, oxygen, and nutrients to the fetus. Humans have hemochorial placentas, with a limited cellular barrier separating the mother's blood from the blood of the fetus. The deep trophoblast invasion poses risks for developing placental complications during pregnancy, such as IUGR (Burton and Fowden, 2015).

A study of 69 placentas, with 45 of them from pregnancies complicated by IUGR (birth weight <10th percentile), and 24 of them from uncomplicated pregnancies with fetuses

who were average for gestational age (AGA), showed certain abnormalities in the placentas of IUGR fetuses. There was increased decidual vasculopathy, infarct, increased syncytial knots, villous fibrosis, and more extensive deposition of perivillous fibrin. Additionally, the placentas of IUGR-complicated pregnancies were considerably lower weights than the placentas from uncomplicated pregnancies. These findings suggest the main disease processes that are associated with placental pathology of intrauterine growth restriction. Included in those findings are chronic uteroplacental insufficiency and abnormal uteroplacental vasculature, coagulation pathologies of the fetoplacental, intervillous, and uteroplacental vasculature, as well as chronic inflammatory lesions. Ultimately, the fetus is deprived of adequate blood flow, causing the low birth weight that is characteristic of IUGR (Park et al., 2002).

Fundal Height Measurement

Current screening and surveillance protocols for intrauterine growth restriction in low-risk pregnancies include obtaining a thorough medical and obstetric history and measuring the fundal height at each prenatal visit after 24 weeks. The threshold determined by the American College of Obstetricians and Gynecologists as an indication of IUGR is a discrepancy of at least 3 between gestational age in weeks and measurement of fundal height in centimeters (ACOG practice bulletin).

The reliability of this measurement as a predictive tool is questionable, with research showing low effectiveness. A large study evaluated the efficacy of fundal height measurements as a predictive tool. There are two methods of using fundal height to predict IUGR. The first is looking at a single value of fundal height, however, this method is very inaccurate. In this study, they found that it has low sensitivity, ranging from 0 to 52% from weeks 22 to 35. The other option is to observe the measurements over a period of time during the pregnancy. This method also had low predictive value, detecting only 56% of IUGR pregnancies, even when looking at values through the full gestational period. The results also indicated an unacceptably high rate of false positives (Rosenberg et al., 1982). Therefore, this examination that is routinely performed at prenatal visits can easily miss a large percentage of IUGR cases, increasing the risk of neonatal morbidity and mortality.

Serum Markers

Maternal serum analytes have been examined to assess their role in the detection of IUGR. Two important analytes which have been studied in relation to IUGR and other pregnancy complications are free beta human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) (Ong et al., 2000). Beta hCG has an important role in placental development by stimulating vasculogenesis and angiogenesis. These provide the placenta with sufficient blood flow from the mother and adequate nutrition for the embryo while the uterine endometrium is invaded (Gridelet et al., 2020). PAPP-A, a protein secreted by syncytial trophoblasts, enters circulation following implantation of the blastocyst. The protein releases insulin-like growth factor (IGF) by cleaving IGF binding protein. The released IGF participates in cell differentiation, multiplication, and trophoblast invasion. Therefore, it is important for the development of the placenta. Additionally, IGF also controls the absorption of amino acids and glucose, thus directly impacting the growth of the fetus (Shah et al., 2020).

Due to the important roles that β-hCG and PAPP-A play in placental and fetal development, it would be reasonable to suggest that a decrease in their levels may predict intrauterine growth restriction, even at an early stage. A screening study performed at antenatal clinics measured levels of both maternal serum analytes between 10 and 14 weeks gestation. The levels were then compared, based on the ultimate outcomes of the pregnancies. Approximately 20% of the pregnancies that resulted in complications including IUGR, had levels of PAPP-A that were below the 10th percentile of the reference range. About 15% of complicated pregnancies, including those which developed IUGR, showed □-hCG levels below the 10th percentile of the reference range (Ong et al., 2000).

Of note, a much larger study consisting of 4390 pregnancies showed conflicting results to the previous study. Between weeks 11 and 13 of these pregnancies, the mothers' PAPP-A and □-hCG markers were checked. In the 172 pregnancies which ultimately developed fetal growth restriction, their PAPP-A levels were significantly lower. However, their □-hCG levels were not significantly lower, making the results of the previous study questionable, particularly due to its small sample size (Spencer et al., 2005). Others also concluded that abnormal levels of □-hCG are not as reliable as PAPP-A in predicting adverse pregnancy outcomes, including IUGR (Gaccioli et al., 2018).

Other markers have been studied due to their roles in placentation, with varying results. Angiogenic factors are key elements in the uterus's vasculature remodeling during pregnancy. Certain placental products with proangiogenic and antiangiogenic properties are secreted and regulated to ensure ideal placentation and to allow for ideal development and growth of the fetus (Gaccioli et al., 2018). Placental growth factor (PlGF), an important proangiogenic protein that supports fetoplacental circulation, is secreted by the placenta throughout the entire

pregnancy. PlGF is detectable in maternal serum, where it affects the well-being of the endothelium. An increased risk of developing IUGR has been observed in pregnancies with low levels of this protein in the first trimester. (Romero et al., 2008, and Karagiannis et al., 2011). For antiangiogenic factors, there has not been consistent findings regarding their predictiveness of IUGR. When maternal serum levels of soluble endoglin (sENG) were high in the first trimester, it seemed to indicate an association with IUGR. However, for another antiangiogenic factor, soluble fms-like tyrosine kinase-1 (sFLT1), the opposite was true. Low levels of sFLT1 indicated a greater risk of developing FGR (Smith et al., 2007). Proangiogenic factors seem more promising than antiangiogenic factors in showing a consistent relationship with the presence of IUGR.

Ultrasound Markers

An alternate method of non-invasively surveying the development of the placenta and assessing blood flow to the fetus involves utilizing ultrasound technology. Maternal-fetal blood flow interactions are assessed by using uterine artery doppler (UAD). A study involving a large number of participants was conducted to assess the ability of UAD to predict the onset of IUGR. Included in the study were 3,010 singleton pregnancies. Of these pregnancies, 565 of them delivered SGA (small for gestational age) neonates (<10th percentile). They looked at resistance indices and the prevalence of bilateral notching in abnormal versus normal pregnancies during weeks 11 to 14. Both of these measurements can indicate resistance to blood flow which is an important factor in IUGR. In women whose pregnancies resulted in SGA neonates, their resistance indices were found to be considerably higher than what was seen in healthy pregnancies (median uterine artery RI, 0.74 vs. 0.70). Additionally, there was a higher incidence of bilateral notching in the abnormal pregnancies. The study discovered that the mean uterine artery resistance index decreased as gestational age at the time of delivery increased, with a statistical significance of P = 0.01. This study shows very promising results for using early ultrasound to predict the development of IUGR (Melchiorre et al., 2009).

Another study took a different approach to the utilization of uterine artery dopplers to predict the development of IUGR and other adverse outcomes. Rather than look at ultrasound findings from a single period, they examined trends in the dopplers and assessed their association with pregnancy outcomes. A cohort of 870 pregnancies underwent ultrasound evaluation during the first trimester (11-14 weeks), which was then followed in the second trimester (19-22 weeks) by repeat evaluation. The trend seen within the two intervals was a significant linear decrease of mean uterine artery pulsatility indices. A decrease in the prevalence of bilateral notching was only seen during weeks 11-13 of the first trimester. With continued follow-up, it was found that 37 (4.25%) of the pregnancies subsequently developed IUGR. When retroactively comparing these pregnancies UAD's with pregnancies that had normal outcomes, it was found that the complicated pregnancies had considerably higher average pulsatility indices and a greater occurrence of bilateral notching in both intervals studied. Further comparison showed that in complicated pregnancies more than in uncomplicated pregnancies, the bilateral notching persisted (30% vs. 8%), abnormal first-trimester pulsatility indices shifted to normal in the second trimester (14% vs. 4%), and normal first-trimester pulsatility indices became abnormal in the second trimester (13% vs. 4%). The study was able to find an association between these UAD findings and the level of risk for the development of adverse outcomes. Pregnancies with persistent notching and/or shifting pulsatility indices from the first to the second trimester were associated with an intermediate risk of developing hypertensive disorders including IUGR. Pregnancies that showed unrelenting abnormally high pulsatility indices had the greatest risk for IUGR and other negative pregnancy outcomes (Gomez et al., 2006).

Both studies on the role of ultrasonography in the prediction of IUGR show comparable results. UAD's were significantly different in complicated pregnancies when compared with uncomplicated pregnancies. These abnormalities were already apparent in early pregnancy, during the first trimester. However, with low predictabilities, the test does not recognize a high percentage of subsequent IUGR cases. Thus, the usage of UAD allows for a non-invasive but not consistently reliable prediction method for the development of IUGR.

Fetal Heart Rate Analysis

Fetal heart rate is an easily measured predictor of fetal well-being. Starting from 28 weeks of gestation, a healthy fetus goes through periods of active sleep and quiet sleep, with changes in heart rate reflecting the fetus's state of sleep. During active sleep, there is an increase in fetal movement and a high heart rate variability. During the quiet sleep period, there is little fetal movement and low heart rate variability. An indicator of fetal well-being is a period of active sleep. A study was performed to determine whether fetal heart rate can be used as an early predictor of IUGR. Because of the impact that restricted blood flow has on a fetus, they presumed that the cardiovascular system, or more specifically the heart

rate, would be affected, thereby providing a marker for the condition. They utilized cardiotocography (cCTG) to measure fetal heart rate, and they used the Dawes/Redman automated system of interpretation. Included in the research was a group of 1,630 IUGR pregnancies and a group of 1,630 same-sex and gestational age healthy pregnancies. Both groups showed increased periods of activity at more advanced gestational ages. However, they made an important finding specific to the IUGR group. Before 35 weeks gestation, the IUGR fetuses showed a significantly lower percentage of active sleep when compared to the healthy fetuses. They showed a delayed or compromised sleep state organization, indicating a lack of maturation for their gestational ages. Based on their results, it seems that the quality of this risk marker is better earlier in gestation (24-34 weeks), since the gap narrowed after that point. Of note, the AUC was only 0.76. However, the study does provide evidence of the effectiveness of fetal heart rate analysis in the detection of IUGR (Stroux et al., 2017).

IUGR Management

Once IUGR is detected and further testing determines the extent of placental dysfunction and stress on the fetus, treatment can be initiated. One treatment that has been researched repeatedly is the use of aspirin to prevent or treat IUGR. Aspirin has been found to affect placental vasculature. It works by inhibiting the production of thromboxane A2 without impacting prostacyclin levels. This results in vasodilation as well as a reduction in platelet aggregation. Since the placental pathology in IUGR includes increased syncytial knots at the terminal villi which impedes blood flow to the fetus, aspirin can reduce the damage by increasing perfusion to the placenta (Ali et al., 2018).

A randomized controlled trial in Egypt was conducted to determine the impact of aspirin on fetal weight in cases of idiopathic asymmetrical IUGR with abnormal UAD indices. One group of women with this complication was given 75 milligrams of Aspirin daily for a period of 4 weeks. The second group diagnosed with IUGR and abnormal dopplers received no intervention. Results showed significant improvement in the group taking aspirin, specifically in regard to fetal weight. The resistance index and pulsatility index decreased in the aspirin group, indicating an improvement of blood flow to the fetus. In terms of neonatal outcomes, the group that received aspirin had higher APGAR scores, although it is worth noting that the number of admissions to the NICU was similar in both groups (Ali et al., 2018). In contrast to this study, a clinical trial conducted on 90 pregnant women showed contradicting results. These women were at an increased risk of developing IUGR due to previous histories of pre-eclampsia. Half of the women were given 80 milligrams of aspirin daily and the other half were given a placebo. The results of the study indicated that there was no statistically significant difference in the pregnancy outcomes, with an IUGR rate of 27.9% in the aspirin group, and 25.6% in the control group (Abdi et al., 2020). Currently, the American College of Obstetricians and Gynecologists believes that there is not enough evidence to support the use of aspirin in the treatment of IUGR. Of note, the first study that found aspirin beneficial for the prevention and treatment of IUGR had a large overlap between the standard deviation error bars, suggesting the possibility that the results are not truly statistically significant. The results showed an increase in fetal weight in the aspirin group (1745±201 gm) when compared to the control group (1534±36gm). However, despite a p-value <0.05, there is a significant overlap in these results, making the results less likely to represent an actual clinical improvement in cases of IUGR.

ACOG also does not recommend bed rest as a means to treat IUGR (ACOG Practice bulletin). This is in agreement with a study of 107 women with suspected fetal growth restriction who were split into a bed rest group and an ambulatory group. There was no evidence of improvement in the growth restriction or neonatal outcomes in the bed-rest group (Gulmezoglu & Hofmeyr, 2000).

Ultimately, the management of IUGR comes down to proper delivery timing to optimize neonatal outcomes. When the risk of a compromised uterine environment outweighs the risk of prematurity, delivery is indicated (ACOG Practice Bulletin). The Growth Restriction Intervention Trial assessed delivery timing for IUGR fetuses at gestational ages of less than 34 weeks. The study population included women carrying fetuses who had restricted growth, and their physicians were unsure whether early delivery would improve outcomes. They were divided randomly into 2 groups; the early delivery group delivered their babies within 48 hours of diagnosis, and the expectant management group underwent continued fetal monitoring until delivery couldn't be delayed anymore. There were equal rates of corticosteroid administration for fetal lung development in both groups. Both groups showed similar rates of death perinatally, as well as equal cognitive, behavior, motor, and language abilities seen in follow-up at 6 to 12 years. Preterm delivery did not significantly improve short and long-term outcomes in cases of IUGR (Walker et al., 2011). Another similar study was performed, but this study population included

women who were past 36 weeks gestation. The results were the same, with no benefit seen in early delivery when compared to the expectant management group (Boers et al., 2010).

Despite these findings, two suggestions are made by ACOG in cases of diagnosed IUGR: 1) in cases of isolated FGR, the fetus should be delivered at 38 0/7–39 6/7 weeks and 2) in cases of FGR complicated by other risk factors including abnormal dopplers and maternal risk factors, delivery should occur at 34 0/7–37 6/7 weeks (ACOG Practice Bulletin). Although the studies on delivery timing did not show significant improvement in outcomes with early delivery, the risk of stillbirth in IUGR fetuses, especially those with abnormal dopplers, is high. Therefore, the risk to the growth-restricted fetus in utero is considered higher than the risk of late prematurity.

If a growth-restricted fetus is at severe risk of negative outcomes prior to 34 weeks gestation, indicating the need for preterm delivery, certain steps can be taken to provide the best chance of a positive outcome. Firstly, the delivery should take place in a center that has a NICU, and a maternal-fetal medicine specialist should be consulted to ensure proper planning. Additionally, if IUGR has been diagnosed and subsequent fetal monitoring indicated the need for early delivery, corticosteroids should be given to the mother prior to delivery since it aids in fetal lung maturation, thereby improving neonatal outcomes (Roberts & Dalziel, 2006). If IUGR is not detected until the fetus is in distress, there may not be sufficient time to administer the corticosteroids, leading to an increased risk of neonatal respiratory distress.

Conclusion

The detection of intrauterine growth restriction at a point in pregnancy when proper management can still be initiated is low. Data suggests that aside from current methods, including fundal height measurements, there are other approaches which can improve the detection rate. Implementing a multipronged approach, including analyzing maternal serum analytes, observing uterine artery dopplers, and assessing fetal heart rate trends, can potentially improve the rate of early diagnosis of IUGR by a significant amount. Each method alone did not surpass current detection rates from fundal height measurements. However, by combining the three approaches along with current practices, more cases of IUGR can be detected, thus avoiding neonatal morbidity and mortality caused by late or missed diagnoses. None of the testing discussed in the paper poses any risk to the mother or fetus since they are non-invasive tests. Therefore, it may be a reasonable option to add testing during prenatal care to better predict cases of IUGR. If IUGR is detected, some research has shown that the administration of aspirin can improve outcomes. This would be expected based on the vascular pathology of IUGR and the mechanism of action of aspirin. However, due to conflicting data from reliable sources, more research is required to determine aspirin's effect on uteroplacental circulation. Finally, results of various studies have not shown expected improvement in outcomes with early delivery, yet ACOG continues to recommend the delivery of a high-risk IUGR fetus prematurely. It is reasonable to suggest that this is based on the assumption that the risk of prolonged impaired blood flow is too high, outweighing the relatively mild risks of late prematurity. However, more research must be conducted to ascertain the effectiveness of early detection methods and management protocols.

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Is Nanotechnology an Effective Treatment for Diabetic Wounds?

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Abstract

Diabetes Mellitus is increasingly impacting millions of adults worldwide. Multiple complications are associated with the disease, including non-healing chronic wounds. Treatment of diabetic wounds and foot ulcers is a complex challenge, as standard treatment options are ineffective. This review focuses on the use of nanotechnology as a treatment option. Nanomaterials include inorganic and organic nanoparticles as well as nanofilms and fibers. Analysis of the benefits of these nanoparticles will be discussed which include their antimicrobial, anti-inflammatory, antioxidant, and wound healing capabilities. A critical overview of the possible benefits will be assessed from in vitro and in vivo studies. Overall, this class of treatment options seems promising, but more research is necessary to determine long efficacy.

Introduction

As of 2021, an estimated 537 million adults have diabetes worldwide, and the numbers continue to rapidly increase (IDF Diabetes Atlas, 2021). Diabetes includes Type 1, Type 2, and Gestational diabetes. In Type 1 diabetes the body doesn't produce enough insulin and is often diagnosed at a young age. It accounts for 5- 10% of diabetes cases. Type 2 diabetes involves the dysregulation of insulin. While genetics plays the only role in type 1 diabetes lifestyle choices add to genetic factors in adults with Type 2 diabetes.

Diabetes is an illness that affects various organ systems as well as arterial and vascular functions. Diseases such as diabetic retinopathy, diabetic neuropathy, and kidney failure are all complications of diabetes mellitus (Thiruvoipati & et al., 2015). An important issue for diabetic patients is painful wounds that have trouble healing. These diabetic wounds often develop at the feet, being referred to as "diabetic foot ulcers" but can also develop in other parts of the body. Quite often, these wounds necessitate lower extremity amputations in patients. Standard treatment methods including bandages, topical creams, and medications don't work well against these chronic wounds. Researchers have been working to develop new methodologies to reduce the difficulties that are associated with chronic wounds. The application of nanomaterials as a treatment option shows promise in the future of diabetic wound treatment. This review will focus on metal-based nanoparticles, lipid-based nanoparticles, and biopolymer-based nanomaterials as some of the forms of nanoparticle therapies in treating diabetic wounds. It will attempt to assess whether nanoparticles are effective in the treatment of diabetic wounds, by analyzing the benefits and potential risks of this treatment choice.

Methods

Evidence-based research was gathered from scholarly journals accessed via databases including ProQuest, EBSCOhost, and Science Direct, with access granted via Touro college. All relevant research publications were gathered and analyzed for information on nanoparticles for the treatment of diabetic wounds.

Chronic Wounds

The wound healing process consists of several steps, hemostasis, inflammation, proliferation, and remodeling (Grubbs & Manna, 2022). Diabetic wounds may have issues at any of these stages which will then prolong the healing process. Individuals diagnosed with diabetes are at risk for developing peripheral artery disease (PAD). PAD is the blockage of the vessels that carry blood to the lower extremities which results in decreased blood flow. One cause for the lack of proper healing is insufficient vascular flow, which is critical for healing (Okonkwo & DiPietro, 2017).

Additionally, the immune system involved in wound healing appears to malfunction in diabetic patients. Macrophages and monocytes are important cells of the innate immune system that play a role in wound healing. Normal wounds have shown macrophages switch from a proinflammatory phenotype (M1) to a pro-repair phenotype (M2) during the healing process. This ensures a proper transition to allow for the regrowth of tissue. However, it is believed that in diabetic wounds this process is altered and the M1 macrophages are extended causing a prolonged inflammation stage and preventing the wound from continuing to heal. In a study performed on mice with diabetic-induced wounds researchers observed the pro-inflammatory phenotype of macrophage activity even at day 10 post-injury. The healthy group showed a transition to the healing phenotype of macrophages on day 5 post-injury. As a result of this, the diabetic mice had increased inflammatory cytokines, diminished levels of growth factor, and decreased angiogenesis resulting in impaired wound healing (Mirza & Koh, 2011).

MicroRNA 21 (miRNA 21) is the microRNA that plays an important role in the transition of the macrophages from the inflammatory phenotype to the reparative phenotype. Levels of miRNA 21 are reduced in diabetic wounds and therefore prevent the normal conversion in the phenotype of the macrophages. The reduced levels also impact the migration of fibroblasts and keratinocytes to the wound. Fibroblasts and keratinocytes are cells that execute regrowth and re-epithelialization in wounds and respond to increased levels of miRNA 21. Based on this, the decreased levels of miRNA at the wound play a role

in the increased inflammatory stage and decreased healing (MacLeod & et al., 2016).

Hyperglycemia causes decreased vascular flow and damage to endothelial cells via the production of reactive oxygen species (ROS). Abnormally high levels of ROS cause oxidative stress on cells and lead to cell death (Burgess & et al., 2021).

When wounds do not heal properly it can result in an increased risk of infections and biofilm formation. Additionally, there is evidence that diabetic skin has higher rates of bacterial colonization (Jagadeesh & et al., 2017). Overall, there are multiple factors associated with reduced wound healing in diabetic patients.

Forms of Treatment

There are many forms of treatment that attempt to improve the healing of diabetic wounds. For something to be considered an effective treatment method, it should include certain properties; the ability to absorb exudate, allow for effective water vapor transmission and contain antibacterial and anti-inflammatory properties. Additionally, having a drug loading capability, elasticity and strength are also beneficial. The treatment method should also provide a moist environment for wound healing. However, most wound treatment options do not contain all these properties, and diabetic wounds that are treated with conventional methods often produce scarring (Shalaby & et al., 2022). It is important to protect diabetic wounds from any microbe, as any infection will further complicate the healing process. Simple dressings can provide protection to the wound while it attempts to heal (Whittam & et al., 2016). Other treatment options include topical medications, gene therapy, and oxygenation therapy. Larval therapy has also been tested for aiding the healing process of chronic wounds. The larvae consume dead tissue from diabetic wounds and their secretions have been shown to contain antibacterial peptides which improve wound healing (Romeyke, 2021).

Nanotechnology

Nanotechnology is a recent introduction to treating diabetic wounds. Nanoparticles range from 1 to 100 nm in size, giving them the benefit of having a large surface area to volume ratio. The micro size of the particles allows for them to have unique properties when interacting with compounds (Maneesha & et al., 2016). Numerous nanoparticles are being tested which provide unique improvements to healing. The main classifications for nanoparticles are carbon-based, metal-based, semiconductor, ceramic-based, lipid-based, and polymeric (Ibrahim & et al., 2019). This review will focus mainly on metal and lipid-based nanoparticles. Metal-based nanoparticles such as gold and silver nanoparticles show anti-bacterial and anti-inflammatory properties (Pangli & et al., 2021). Zinc oxide nanoparticles are studied for drug delivery as well as for biocompatibility (Huang & et al., 2017). Lipid nanoparticles have been analyzed for use as a drug delivery system. Biological polymers have also been tested for incorporation into nano-formulations that can be applied for wound healing. Nanoparticles can be applied to the wound either topically or injected, and formulated into films, fibers, and gels.

Synthesis of Nanoparticles

The process of synthesizing nanoparticles differs for organic and inorganic particles. Metal nanoparticles are inorganic and can be produced via physical and chemical methods. Chemical synthesis involves the use of a reducing agent, and the type of agent used affects the character of the nanoparticles formed (Ghazali & et al., 2014). There are harmful effects that synthesizing metal nanoparticles has on the environment and their toxicity is an issue. Therefore, there has been much research for finding alternate safe methodologies to form metal nanoparticles rather than using chemical reducing agents.

Bio-mediated methods of synthesizing metal nanoparticles involve using biomaterials as the reducing agents which reduces the toxicity and damage to the environment. Zinc nanoparticles have been produced using extracts of Dovyalis caffra fruit as a bio-mediated reducing agent (Adeyemi & et al., 2019). Another biological method for synthesizing gold nanoparticles is using Magnolia kobus and Diopyros kaki leaf extracts. The rate of reaction was higher, compared to using a chemical-reducing agent. They also showed that the temperature of the reaction and the concentration of the plant extract affected the size and shape of the particle. This gave a way to control the desired size and shape of the nanoparticles being synthesized (Song & et al., 2009). An interesting plant extract that has also been used for gold nanoparticle synthesis is that of Chamaecostus cuspidatus. This plant possesses interesting qualities in the treatment of diabetes, and in India, it is referred to as "the insulin plant". The gold nanoparticles that were synthesized showed anti-diabetic effects and did not show toxicity when tested in vivo (Ponnanikajamideen & et al., 2019). Plant-based reducing agents seem optimal as compared to microbe-derived reducing agents since they require less purification and have fewer harmful side effects. There are hundreds of other bio-mediated ways that have been discovered to synthesize metal nanoparticles, including the use of bacteria, fungi, algae, and plant extracts as reducing agents.

Metal Nanoparticles

Silver Nanoparticles (AgNP)

Silver nanoparticles have been widely researched and shown to have antimicrobial and anti-inflammatory properties. Silver had a long history of being used in wound treatment but with the introduction of antibiotics, it became less popular. Recently, there has been renewed interest in using silver nanoparticles for their antibacterial properties, because of an increase in antibiotic resistance. Silver nanoparticles can be used alone or in conjunction with antibiotic treatment.

One method for observing the antibacterial effect that silver nanoparticles have was done using AgNP synthesized from cyanobacteria. These silver nanoparticles were tested for anti-MRSA capability in diabetic wounds. At low concentrations, the nanoparticles showed promising action against MRSA infection. Additionally, when using a combination of AgNP combined with 0.5% chloramphenicol, a strong antibiotic, there was practically 100% anti-MRSA activity. This was significant; indicating that combinations of AgNP with antibiotics produced significantly better antibacterial capabilities than antibiotics alone (Younis & et al., 2022).

Another example of AgNP's antibacterial properties is its effects on gram-negative bacteria. These silver nanoparticles were synthesized with sodium citrate to stabilize them. Gram-negative bacteria are often antibiotic resistant and therefore make controlling and treating these strains of bacteria quite challenging. Hospitals are frequently challenged by the spread of these bacteria. P. aeruginosa is a gram-negative strain of bacteria that causes many nosocomial infections, contributes to biofilm production, and is resistant to many forms of antibiotic treatment. Three strains of P. aeruginosa were shown to have significant susceptibility to the treatment of AgNP at 5.0 micrograms/ml concentration. Even more impressive were the results of silver nanoparticles on hospital strains of these bacteria which had shown resistance toward 11 types of antibiotic treatment. When these hospital strains were treated with 5.0 micrograms/ml concentration of AgNP there was 99.9% bacterial death, after 12 hours. Addressing the toxicity factor, these particles showed low toxicity at 5 micrograms/ml when tested. However, for extra safety, the concentration levels should be even lower when using them as a coating for medical devices, and the release of the silver ions should be slow (Salomoni & et al., 2017). This experiment shows the success of silver nanoparticles even on difficult-to-treat hospital-associated strains of resistant bacteria.

A study observing the wound healing potential of silver nanoparticles involved the bio-mediated synthesis of AgNP using Carica papaya extract. Using plant-based synthesis helps lower the risks of toxicity of the nanoparticles. The AgNP was tested in vivo on rats with streptozotocin-induced diabetes on excision wounds. The group that was treated with topical application of the nanoparticle solution showed the best wound healing. The other groups included treatment with povidone-iodine, an extract of Carica papaya leaves, and a control group that did not have as good of an outcome as the nanoparticles. After 14 days these biosynthesized silver nanoparticles successfully closed the wound by 100%, whereas other forms of treatment only partially reduced the wound area (Chandnani & et al., 2022).

Another method tested AgNP on human dermal fibroblasts that were taken from live donors and tested in vitro. The particles produced a decrease in wound inflammation by lowering the expression of the levels of Interleukin IL-6 levels. This decrease was observed when using concentrations of AgNP at 0.25 and 2.5 micrograms/ml. These interleukins are important in the inflammatory response of the immune system against invading pathogens. An issue with overexpression of these interleukins can cause chronic inflammation problems (Ambrozova & et al., 2017). As discussed previously, the decreased healing of diabetic wounds stems from increased and prolonged inflammation.

Gold Nanoparticles (AuNP)

Gold Nanoparticles (AuNP) have also been evaluated for their antimicrobial, and anti-inflammatory properties. One study used AuNPs that were synthesized from Acalypha Indica plant extract. The AuNPs were embedded within the cotton fabric and tested via in-vitro and in vivo uses. The gold-nanoparticle-coated cotton was tested on strains of E. coli and St. epidermidis and after 24 hours the zone of inhibition (ZOI) was analyzed. Zone of inhibition tests the effect of antibacterial treatment on the growth of the bacterial colonies. The AuNP-coated cotton showed antibacterial properties, creating zones of inhibition against E. Coli (26mm diameter) and S. Epidermidis (31 mm diameter). These were the highest ZOIs when compared with other control forms of antibacterial treatment such as Acalypha indica extract-coated cotton (Boomi & et al., 2020).

The research also showed the antioxidant properties of the extract of gold nanoparticles via an in-vitro study. Free radicals cause damage to cells, and it is important to find methods that use antioxidants to protect against the damage of free radicals. A DPPH solution which is a compound that has stable free radicals was used to analyze the antioxidant properties of gold nanoparticles. As The

concentration of AuNp extract increased; the antioxidant abilities also increased. When tested, he extract containing the gold nanoparticles had an Ic50 value of 16.25 µg/mL. For DPPH an extract that produces a value of IC50 that is between 10-50 mg/ml is considered to have strong free radical scavenging abilities. The gold nanoparticles showed highly significant antioxidant capabilities. The wound-healing properties of these AuNP were tested in vivo on mice containing diabetic-like wounds. When compared with a control group the mice whose wounds were treated topically with AuNP extract, exhibited much better healing. This treatment seemed to improve blood vessel formation and collagen matrix remodeling, both of which allowed for faster wound healing and skin regeneration. Additionally, the nanoparticles shortened the inflammation stage of the wounds, which helped with the healing (Boomi & et al., 2020). An extended inflammatory stage is a critical issue of diabetic wounds as discussed earlier. Treatment that is successful in reducing the stage of inflammation in these wounds is promising.

Another study using nanoparticles made from leaf extract of Physalis peruviana produced similar results in the treatment of diabetic wounds. This study also tested the in-vitro aspect of gold nanoparticles against different strains of gram-positive and gram-negative bacteria, as well as a strain of gram-positive fungus. As the concentration of AuNPs increased so did their zones of inhibition against all strains that were tested. The zones of inhibition were the best detected against the gram-positive fungus (C. albicans) and S. aureus and B. subtilis gram-negative (E. Coli). The zones of inhibition for P. aeruginosa were slightly less, but the gold nanoparticles showed antimicrobial properties. When tested against E. coli the gold nanoparticles were as effective as many forms of antibiotics tested. The wound healing potential was tested on rabbits who had diabetic-like wounds. The test compared the results of treatment with standard cotton versus gold nanoparticle-embedded cotton. The rabbits treated with the cotton containing the nanoparticles portrayed faster-wound healing at the 6-day mark compared to the control group. After 14 days the wounds treated with gold nanoparticles were fully healed without signs of infection thus portraying the wound-healing advantage of the AuNPs (Stephen & et al., 2022).

Zinc Oxide Nanoparticles

Zinc oxide nanoparticles are a group of inorganic nanoparticles with promising antibacterial properties. Zinc oxide has been approved as "safe for use" in biological applications and is cheaper compared to other types of inorganic nanoparticles. In one study zinc oxide nanoparticles were tested against different strains of gram-positive, gram-negative, and biofilms in-vitro. The researchers found that using a 500nm particle size of ZnO-NP was more effective as compared to smaller sizes. In gram-negative bacteria such as E. Coli and P. aeruginosa, there was 50% bacterial death when treated with 500 µg/ml of zinc oxide nanoparticles. At 1000mg/ml with 24-hour incubation, no colonies survived. For gram-positive bacteria, S. aureus 92% were killed when treatment comprised 500 µg/ml of ZnO-Np, and 98% with 1000 µg/ml of ZnO-NP. After 24 hours of incubation with the nanoparticles, there were no colonies. Significantly, ZnO-NPs were effective against MRSA, drug-resistant S. aureus. After treatment of 750 µg/ml and 6 hours of incubation, there were few surviving colonies. The zinc-oxide nanoparticles also showed success against biofilms when tested on established biofilms of P. aeruginosa and S. aureus. With increasing dosage strength of ZNO-NP, there was diminished biofilm formation and survival. Interestingly it was shown using scanning electron microscopy, that the ZnO-NP fights bacteria by attacking the bacterial cell membrane, as seen by S. aureus where after a minimal dose of ZnO-NP the membrane was completely disarranged (Rashmirekha & et al., 2014).

A second part of the study tested the in vivo antibacterial properties of zinc oxide nanoparticles in the treatment of S. aureus infections in mice. After treatment with ZnO-NP, the bacterial burden was significantly reduced compared to the control. The rats treated with the Zinc oxide nanoparticles exhibited reduced inflammation and improved skin composition in the infected wounds (Rashmirekha & et al., 2014).

Titanium Dioxide Nanoparticles (TiO2 NP)

Titanium Dioxide Nanoparticles have also been tested for their diabetic wound healing capabilities. This class of metallic oxide- nanoparticles is stable and exhibits minimal toxicity. Green synthesis of TiO2 NPs is a safer method for producing these particles. In one study TiO2 NPs were made from Ocimum sanctum, a plant extract, and embedded into a chitosan gel-based drug delivery system. This provided a uniform dispersion of the particles when applied topically to a wound. This experiment was performed in vivo on rats with Streptozotocin-induced diabetes and excision wounds. The rats were split into groups with a control group, a treatment of just chitosan gel, a group treated with Tio2 nanoparticles in chitosan gel, and a group that received a treatment of silver sulfadiazine, a topical antibiotic. The control group exhibited inflammation in the wound while the other three groups didn't show signs of infection or pus formation. The group

that was treated with the zinc oxide nanoparticle gel as well as the ones treated with the silver sulfadiazine showed the best wound healing after 7, 14, and 21 days. The least inflammation was in the groups treated with TiO2 NPs gel, which is likely due to the anti-inflammatory properties of chitosan combined with the titanium oxide. The measurement of the wound contraction of the diabetic wounds clearly shows the success of the Titanium dioxide nanoparticles with chitosan gel. Overall, this experiment was successful in demonstrating the possibility of using Titanium oxide nanoparticles for improved healing (Ahmad & et al. , 2022).

Lipid Nanoparticles

Lipid-based nano formulations can be classified into two main groups, liposomes and lipid nanoparticles. They function similarly but are structurally different. Liposomes consist of a lipid bilayer with an aqueous center, while lipid nanoparticles don't have that aqueous middle. The preferred method of synthesizing solid lipid nanoparticles is via high-pressure homogenization which reduces the size of the sample to a small particle (Kumbhar & et al., 2022). Lipid nano formulations possess unique characteristics that make them an ideal drug delivery system for diabetic wounds. Firstly, their small size and lipid makeup allow them to deliver drugs in a regulated manner and advance the interaction between the drug and the wound site. Additionally, the lipid-based particles share similar composition to bodily lipids and allow for successful tolerability as a drug delivery system and making them biodegradable (Dilara & et al., 2017).

Lipid nanoparticles are amongst the most widely researched organic nanoparticles and have many useful features. Lipid nanoparticles as drug delivery systems improve wound healing of diabetic wounds for multiple reasons. These particles were shown to have better wound penetration compared to other drug delivery systems. Additionally, they also can carry and deliver a wide variety of drug molecules and increase the half-life of the drugs (Matei & et al., 2021). One study incorporated lipid nanoparticles as a carrier for siRNA to diabetic wounds. A critical issue in the healing of diabetic wounds is extensive inflammation. Overproduction of the TNF alpha cytokine by the macrophages promotes fibroblasts in the wound to produce the MCP-1 chemokine which then signals an increase of macrophages and monocytes to the wound increasing inflammation in an inflammatory loop. siRNA therapy blocks the overproduction of TNF alpha thereby directly improving the healing process. This study showed the success of using lipid nanoparticles as the vehicle of transport for siRNA therapy. Wound cultures were prepared to mimic characteristics of a real diabetic wound, including a macrophage- fibroblast culture that exhibited the cytokine properties of a real wound. The lipid nanoparticles delivery of siRNA sequence was successful in reducing both the TNF alpha cytokine as well as the Mcp-1 chemokine thereby successfully reducing two inflammatory factors of diabetic wounds (Kasiewicz & al., 2016).

Another study researched the use of tissue scaffolds together with the drug simvastatin combined in a lipid-based carrier. The data showed these lipid particles were successful in encapsulating the simvastatin. Additionally, the results showed that it was a stable delivery in combination with the tissue scaffolds treatment. The success of the study in vitro diabetic wound models proved the feasibility of lipid-based drug delivery systems (Dilara & et al., 2017).

Additionally, solid lipid nanoparticles (SNL) were tested as a drug delivery vesicle for ATRA (all-trans retinoic acid) in an in-vivo study. ATRA has been shown to have some wound-healing properties however it can also be harmful to wounds if applied for more than a short time period. Chitosan is a polysaccharide compound that has low toxicity, biocompatibility and can control the release of drugs. The study attempted to evaluate chitosan film for drug administration method but because of the low solubility of ATRA (hydrophobic) in chitosan (which is hydrophilic), the researchers sought other delivery systems for incorporating the ATRA. One key benefit of solid lipid nanoparticles is that they can bind both hydrophilic and hydrophobic drugs. The ATRA was incorporated into solid lipid nanoparticles delivered to the chitosan film. This encapsulation prevents the overdosage of the drug to the wound while incorporating chitosan. The SNL-ATRA chitosan treatment helped the healing process by increasing the collagen deposition in the wounds. It also appeared to reduce the infiltration of neutrophils into the wounds, which helped healing, as diabetic wounds often have increased infiltration of neutrophils which cause inflammation and a reduced rate of healing. There was no sign of skin inflammation from the ATRA which was critical in proving that the SLN were successful in delivering the ATRA in a way that was regulated and sustained. This is another example where the use of lipid nanotechnology as a drug delivery system helped healing of diabetic wounds (Valquíria & et al., 2020).

Polymeric Nanoparticles

An additional class of organic nanomaterials include Biopolymeric nanoparticles, which as a group include proteins and polysaccharides, and may consist of chitosan, collagen, silk fibroin to name a few. The benefit of using

biopolymeric nanoparticles is that they are biodegradable and biocompatible (Sundar & et al., 2010) . They have been used successfully as a drug delivery system, and as a carrier for other treatments.

In one study, cellulose nanocrystal films were tested, since nanocellulose is a biopolymer that can be extracted from a variety bacteria and plants. It was used as an antimicrobial wound dressing film using cellulose nanocrystals as a drug delivery system for curcumin. Nanocellulose is an excellent material for wound dressings due to its impressive compressive properties and excellent ability to absorb exudate. Curcumin increases levels of growth factor β1, a protein involved in cell growth and proliferation. This compound also protects skin cells from oxidative damage. The film containing curcumin improved wound healing in a diabetic rat model after 7 days of topical application. The film was 99.9% effective at inhibiting bacterial growth at the wound, which is important for wounds where bacteria infect and impair healing. Compared with the control group the treated wounds also showed fewer inflammatory cells. Cellulose film was an effective system for curcumin and was successful in treating method for diabetic wounds (Woei & et al., 2018).

An in vivo study testing the use of silk fibroin/chitosan scaffolds containing adipose-derived stem cells (ADSC) to treat diabetic wounds. Rats with diabetic-induced wounds were split into a control group, a silk fibroin/chitosan scaffold grafts group, and a group that was given the ADSC-containing grafts. The group treated with the ADSC scaffold grafts showed the highest levels of wound size contraction. This was likely due to the properties of the ADSC which increased cell infiltration, epithelialization, and angiogenesis, all playing an important role in improved wound healing (Wu & et al., 2018).

Nanomaterials in various forms can be integrated into a variety of methodologies for the possible treatment of diabetic wounds, implemented for treatment in many different forms. Biopolymer films are effective as they are flexible and often contain wound-healing agents. They are also biodegradable and usually transparent allowing for monitoring of the wound healing without constant opening of the film. Nanofiber-based dressings have a high surface-to-area ratio and are therefore effective in drug delivery. These dressings also have a diameter size that is similar to that of the extracellular matrix, which aids in cell proliferation, adhesion to the skin, and wound healing. Hydrogels consist of networks of linked polymers and high moisture content. They are effective at providing a moist environment for the wound, absorbing exudate, and preventing infections. Using biopolymer-based hydrogels for drug delivery seems to lower the toxicity issues with many drugs. Their composition allows for them to be malleable and adhesive making them an ideal choice for a wound dressing Biopolymer-based foams and bandages are flexible and have high absorbency for wounds with exudate. They can also deliver drugs and other therapeutic agents (Alven & et al., 2022).

Harmful Effects of Nanoparticles

Recently studies have shown that nanoparticle treatment is a safe and effective way to treat chronic wounds; however, there is still concern over the safety of using nanomaterials. Before implementing a new class of treatment options, it is vital to ensure the safety of the treatment. It is also important that the possible risks are clearly understood by the user. The challenge with evaluating the safety of nanoparticles lies in the newness of this treatment. It is hard to make a definite conclusion about safety when there hasn't been enough time to fully evaluate all the lasting effects. A big concern of nanoparticle therapy is the concern of toxicity. The issue of toxicity arises mostly regarding metal-based nanoparticles. Silver nanoparticles as explained earlier contain many important anti-microbial and anti-inflammatory properties. They have also been shown to have associated toxicity. Research testing their toxicity is limited to in vitro studies as well as short-term in vivo studies on animals. Therefore, it is challenging to understand the ramifications that these silver nanoparticles would have on complex wounds over an extended period of time. Studies have found that the silver nanoparticles disassociate with silver ions and the ions cause the toxicity (Edwards-Jones, 2022). However, despite issues with toxicity, there have been attempts to minimize the toxicity of silver nanoparticles by using stabilizing agents during the synthesis of the particles. Additionally, when testing silver nanoparticles in vitro it appeared that the biologically synthesized silver nanoparticles caused less cytotoxicity compared with chemically synthesized ones. It also appears that the toxicity causes an increase in the production of reactive oxygen species ROS (Desai & et al., 2022).

An in vivo study tested the toxicity factor of green synthesized gold nanoparticles. Researchers took mice and injected them with treatments of the biologically synthesized nanoparticles and orally injected them daily for 10 days. After 10 days, the mice did not exhibit any symptoms of toxicity. Furthermore, when the kidneys, liver, and lungs of the mice were tested they did not show increased toxicity compared with the control group of mice. These results agreed with others showing that biologically synthesized nanoparticles were not toxic to cells (Kumar & et al., 2017).

Titanium oxide nanoparticles must be carefully regulated as extensive application can cause cytotoxicity of the dermis and aging of the skin. This is caused by oxidative stress from increased free radicals and the diminished collagen (Pormohammad & et al., 2021).

Solutions for the issue of toxicity may include using biological mediated factors for the synthesis. Also, lowering the concentration of the particles can reduce the risk. It appears that research into the toxicity risk of using nanoparticles for practical treatment is still in the early stages. The information on the long-term negative effects of the usage of the compounds investigated in this review will require further elucidation before a better understanding of the various short-term and long-term health risks is better understood.

Conclusion

In conclusion, nanotechnology seems promising in the future of diabetic wound treatment. The diversity of nanomaterials ranges from inorganic to organic and can be incorporated into many different forms of treatment. Based on in vitro and in vivo animal studies, these particles exhibited anti-microbial, anti-inflammatory, and wound-healing capacities. The concern for the toxicity of these nanoparticles needs to be further researched. Using biological mediated reducing agents, as well as, lowering the concertation of nanoparticles in treatment may help reduce risks of toxicity. Extensive research should be implemented before this can be used as a widespread treatment option.

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How Can We Eliminate Peanut Allergies?

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Abstract

For a long time, people with peanut allergies have had to carefully read labels, carry around emergency epinephrine injections, and live in fear of accidentally consuming an otherwise benign food that could potentially kill them. Thankfully, researchers have been working on ways to alleviate the harmful effects of these allergies and perhaps even find a cure. In the Learning Early About Peanut allergy (LEAP) study performed in 2015, researchers examined whether the early introduction of peanut consumption could be used to prevent peanut allergies. According to the study, peanuts can be introduced orally to high-risk infants who are sensitized, as well as to non-sensitized infants in the early stages of development (Du Toit et al., 2015). Current research has increased our understanding of peanut allergies and has led to the development of treatments and preventions for this condition. Additionally, the mechanism of peanut allergies and how it differs from other food-borne allergies has been studied in detail, which has helped to revolutionize our understanding of this condition. This knowledge is being used to develop new ways to prevent and possibly cure peanut allergies.

Introduction

In the United States, 32 million people suffer from allergies related to food (Gupta et al., 2019). Approximately 9 million of them suffer from allergies to peanuts (Wang, 2021). Peanuts are found in many foods people regularly consume, in many instances, only trace amounts are present. This can be due to using peanut oil or even producing benign foods in a factory that also produces peanut-containing foods. Studies show that an estimated 0.85% of all emergency room visits in the United States are related to allergic reactions, underscoring the high morbidity and possibly mortality (Carrillo-Martin et al., 2020).

Not all people who have peanut allergies will have identical reactions. Some will only have a mild allergic reaction with pruritic hives, while in the most severe cases, the consumption of the allergen can result in anaphylactic shock, which can lead to death if not treated promptly. It is estimated that between 0.7%-2% of cases of anaphylactic shock result in death (Bock, 2021).

The focus of this paper is to explain the pathophysiology of peanut allergies as well as discuss possible preventions and treatments for this allergy.

Methods

Data was found using Touro University's online library, specifically ProQuest and PubMed databases. Supplemental information was obtained using UpToDate. The main keywords used were peanut allergy, Ara h 2 and 6, Bamba, and LEAP.

Discussion

Pathophysiology of Allergic Reactions

An allergic reaction falls into one of the four categories of Gell and Coombs' classical division of immune responses. The type of hypersensitivity is classified based on the mechanism used. Hypersensitivity type I is known as immunoglobulin E (IgE)-mediated. The mechanism involves the release of IgE antibodies against soluble antigens and is also known as an "immediate reaction." When the host is exposed to an antigen, two levels of response occur, sensitization and effect. During sensitization, the host is exposed asymptomatically to the antigen. Following reintroduction, anaphylactic or atopic responses are produced. There is a possibility that a mild reaction can occur upon first exposure to an antigen. However, most reactions will not happen unless it is exposed to an antigen a second time. Hypersensitivity type II engages IgG and IgM antibodies, activating the complement system and cell lysis. A type III hypersensitivity is also called an Immune Complex Reaction. It occurs when IgG, IgM, and sometimes IgA antibodies are involved. These antibodies bind with the antigen and form a large complex that can trigger a more significant immune response and form large complexes that can cause tissue damage, especially in areas of narrower vasculature. T-cell-mediated reactions are involved in type IV hypersensitivity, also called delayed hypersensitivity. T-cells or macrophages are activated by cytokine release, causing tissue damage (Warrington et al., 2011). The prevalence of type I allergies is 48%, followed by type IV allergies at 18%, type III allergies at 10%, and type II allergies at 6% (Żukiewicz-Sobczak et al., 2013).

It is the focus of this article to examine type I hypersensitivity as it relates specifically to peanuts.

Peanut Allergy Pathophysiology

Peanut allergies are classified as type I reactions or immediate hypersensitivity reactions. An individual with a peanut allergy experiences a range of symptoms when they consume peanuts or come into contact with peanut protein. Whenever a peanut allergy sufferer consumes peanuts or encounters peanut protein, the immune system perceives peanut proteins as harmful invaders and mounts an immune response. This immune response is mediated by mast cells, which are found throughout the body, particularly in tissues that have direct contact with the outside environment, including the skin, respiratory tract, and digestive tract. Histamine and leukotrienes are released by mast cells in the presence of peanut allergens, which cause inflammation and allergic symptoms (Al-Muhsen et al., 2003).

Sensitization and Effect

Allergic reactions involve a two-step process: sensitization and effect. During the sensitization phase of Type I hypersensitivity reactions, allergens (or antigens) are presented to T-cells which is a type of white blood cell whose job is to help recognize and eliminate pathogen by presenting the pathogen to an antigen-presenting cells (APCs). T-cells then stimulate B-cells, another type of white blood cell, to produce IgE antibodies, which bind to the Fc receptors on mast cells and basophils. When the free antigen causes the crosslinking of these bound IgE antibodies on mast cells and basophils, it leads to the degranulation of the cells and the release of histamine, proteolytic enzymes, and other mediators such as prostaglandin, cytokines, leukotrienes, platelet-activating factors, macrophage inflammatory proteins, and tryptase. This results in increased vascular permeability, peripheral vasodilation, and smooth muscle contraction, which can cause symptoms such as increased mucous secretions, bronchospasm, abdominal cramping, rhinitis, and potentially hypovolemia or hypoxia. Pulmonary angioedema or general angioedema can also occur due to fluid shifting into interstitial space. Individuals may experience pruritus and local responses, such as is the case with asthma, or an individual can experience a whole-body response which can include diffuse hives and anaphylaxis (Abbas et al., 2022).

Peanuts contain several proteins that cause allergic reactions, including Arachis hypogaea 1 (Ara h 1), Ara h 2, Ara h 3, and Ara h 6. These proteins are found in the seed coats of peanuts. They are resistant to digestion, which allows them to provoke an immune response when they encounter the immune system. The proteins belong to the 2S albumin protein family, a group of proteins found in many plants. Plants use these proteins to combat pests and diseases. The immune systems of individuals with peanut allergies mistake these proteins for harmful invaders and mount an immune response when detected. Studies have shown that Ara h 2 and Ara h 6 are the most allergenic proteins in peanuts, meaning that they are most likely to trigger an allergic reaction. Ara h 6 is very similar to Ara h 2 in terms of sequence identity, secondary and tertiary structure, and allergenic activity. Ara h 2 and Ara h 6 have about 59% sequence identity and a similar overall structure. While Ara h 2 is well-known as a strong allergen, it has recently been discovered that Ara h 6 is also highly allergenic. Based on their similarities in both physical and immunological properties, it is likely that Ara h 2 and Ara h 6 should be considered as closely related allergens. Other proteins in peanuts, such as Ara h 1 and Ara h 3, are also allergens, but they are not as potent as Ara h 2 and Ara h 6. Therefore, treatments for peanut allergies often focus on these two proteins (Mueller et al., 2014).

In severe cases, an allergic reaction can trigger anaphylaxis. This life-threatening allergic reaction can cause symptoms such as diffuse angioedema, a drastic drop in blood pressure, and respiratory distress due to airway constriction. Anaphylaxis can be treated with medications such as epinephrine, but it requires immediate medical attention and can be fatal if not treated promptly. One of the most common causes of food-induced anaphylaxis is peanuts. The average peanut contains 200 mg of protein. For most people with peanut allergies, symptoms begin after consuming less than 1 peanut, and for highly allergic individuals, even trace amounts can cause symptoms. Based on a study designed to determine the minimum dose of peanut protein that will elicit a reaction in highly sensitized individuals, subjective symptoms have been reported even with doses as low as 100 µg, while objective signs have been observed at 2 mg (Al-Muhsen et al., 2003).

Initial Exposure Phenomenon

Approximately 70% of children with peanut allergies reported symptoms at their first known exposure. Considering that symptomatic IgE-mediated allergic reactions occur following initial exposure to an allergen, a prior unnoticed exposure is likely to have occurred. Sensitization may occur because of fetal exposure to allergens in breast milk that may arise after the mother consumes peanuts (Al-Muhsen et al., 2003).

Studies show that peanut allergens can be found in breast milk after consumption. One such study was done in 2014 that showed that the Ara h 6 protein from peanuts was evident in human breast milk just 10 minutes after being fed 30 g of roasted peanuts (Bernard et al., 2014).

Diagnosis of Peanut Pllergies through Clinical Testing

Peanut allergies can be detected by IgE testing to individual peanut protein components in the suspected individual's blood rather than the entire peanut protein. Compared to crude peanut-specific IgE, IgE against Ara h 2 has shown to have better discrimination than that against other peanut-specific IgE. The diagnostic uncertainty will, however, persist in 5% of cases even when using component-resolved diagnostics, so food challenges will be necessary to clarify the diagnosis. In addition, peanut allergy component testing has only a small diagnostic advantage over skin prick tests (SPT) (Hemmings et al., 2020).

While skin prick tests and high levels of peanut specific IgE can indicate sensitization to peanuts, an oral food challenge (OFC) test is often necessary to confirm a diagnosis of peanut allergy. However, OFC tests can be risky and time-consuming, and may not always be performed.

In addition to its use for seasonal allergies, a conjunctival provocation test can also be used to diagnose peanut allergies in children. During the test, an allergen is applied to the conjunctiva, the membrane that covers the white of the eye. It is then observed if there are any allergic reactions (Lindvik et al., 2017).

Outgrowing Peanut Allergies

It was previously believed that peanut allergy is unlikely to be outgrown, but recent studies have shown that this may not always be the case. One study found that around 21.5% of patients with peanut allergies were able to outgrow their allergies (Skolnick et al., 2001). In another study, children with intermediate levels of peanut-specific IgE had a 55% chance of outgrowing their allergy, while those with lower peanut-specific IgE levels had a 63% chance, and those with an undetectable peanut-specific IgE had a 73% chance of successfully passing an oral challenge with peanut (Fleischer et al., 2003). The data suggests that patients with a peanut specific IgE level of 5 or less may have at least a 50% chance of outgrowing their allergy. However, it is crucial to note that peanut allergy can sometimes reappear after a negative skin test or challenge, especially if peanuts are not regularly consumed (Al-Ahmed et al., 2008).

Studies Aimed at Preventing Peanut Allergies

A clinical trial was conducted in 2013 to evaluate the safety and effectiveness of a candidate vaccine called EMP-123 for the treatment of peanut allergies. EMP-123 is a mix of recombinant hypoallergenic variants of the peanut allergens Ara h 1, 2, and 3, encapsulated in inactivated E. coli cells and administered rectally as an adjuvant. Inactivated E. coli can serve as a carrier for vaccine components, expressing specific antigens from the target pathogen. However, the trial was unsuccessful as 5 out of the 10 enrolled patients experienced adverse reactions and had to drop out. It is possible that the adverse reactions experienced by the patients were due to incomplete removal of the IgE-binding epitopes from the allergens (Wood et al., 2013).

In 2015, researchers conducted the Learning Early About Peanut Allergy (LEAP) study. It was discovered that Jewish children in the United Kingdom were 10 times more likely to develop a peanut allergy than Israeli children of similar ancestry. A study of infants in the United Kingdom found that peanut-based foods are usually not consumed during the first year of life. In Israel, peanut-based foods are usually introduced when infants are approximately 7 months old. The goal of the study was to determine whether introducing peanuts to infants at high risk of peanut allergy early could prevent peanut allergy development. A total of 640 infants aged 4 to 11 months were enrolled in the study. There was a high risk that these infants would develop a peanut allergy due to their severe eczema, egg allergy, or both. Two groups of infants were assigned randomly: one group received peanuts as snacks at least three times a week, and the other group avoided peanuts altogether. By using a food challenge test, the study assessed whether children developed peanut allergies at age 5. Peanut allergy rates were significantly lower in the intervention group (1.9%) than in the control group (13.7%). This is evidence that the early introduction of peanuts to infants at high risk of peanut allergy can effectively prevent the occurrence of peanut allergies later in life (Du Toit et al., 2015).

Follow-up research (LEAP-On) also showed that the consumption group maintained peanut allergy reductions 12 months after allergen avoidance (Palladino, Breiteneder, 2018).

Interestingly, Bamba, a popular Israeli snack containing approximately 50% peanut protein, was chosen as the preferred peanut snack in the LEAP study. Among the children aged 4 to 16 who consumed peanuts in the LEAP trial, 70% ate Bamba as their primary peanut food during the first 12 months (Hindley et al., 2018).

The LEAP study's use of Bamba to reduce the prevalence of peanut allergy has raised questions about its allergen composition and the levels of specific peanut allergens that may be associated with tolerance to peanuts. A study was done to measure the levels of the major allergens Ara h 1, Ara h 2, and Ara h 6 in Bamba and compare them to those in other peanut-containing foods, as well as estimate the doses of these allergens that may be tolerable in individuals who regularly consume Bamba. The allergen levels in Bamba were measured by an enzyme-linked immunoassay. Bamba's allergen levels were measured at Ara h 1, 2388 µg; Ara h 2, 1988 µg; and Ara h 6, 2341 µg. Thus, the weekly doses of allergens consumed by children in the LEAP study were calculated to be 83 mg Ara h 1, 120 mg Ara h 2, and 127 mg Ara h 6 (total 330 mg/wk). As opposed to other peanut products, Bamba has relatively similar levels of the three major allergens. Previous research has shown that peanut butter usually contains 2-4 times more Ara h 1 than Ara h 2 or Ara h 6, while peanut flour can have up to 20 times more Ara h 2 and Ara h 6 than Ara h 1. Peanut flour used in clinical trials of oral immunotherapy (IOT) also tends to have 2-10 times more Ara h 2 than Ara h 1. According to these findings, consuming around 330 mg of specific peanut allergens per week may help to prevent peanut allergy and induce tolerance to peanuts. (Hindley et al., 2018).

An additional clinical trial, published in 2016, included 40 children with suspected or known peanut allergies aged 9 to 36 months who were treated with early intervention OIT (E-OIT) to evaluate its safety and effectiveness. The children were randomly assigned to receive E-OIT at doses of 300 or 3000 mg/day in a double-blinded fashion. The primary endpoint was sustained unresponsiveness at 4 weeks after stopping E-OIT, was assessed by a double-blinded, placebo-controlled food challenge. The outcomes were compared with those of 154 matched standard-care controls. The results showed that 78% of the children in the intent-to-treat analysis and 91% in the per-protocol analysis achieved unresponsiveness over a median of 29 months. Peanut-specific IgE levels significantly declined in the E-OIT-treated children, who were 19 times more likely to successfully consume dietary peanuts than the matched standard-care controls. Allergic side effects during E-OIT were common but mild to moderate in severity. These findings suggest that E-OIT has an acceptable safety profile and is highly effective in rapidly suppressing allergic immune responses and achieving safe dietary reintroduction of peanuts in children (Vickery et al., 2017).

A phase II clinical trial was conducted at 8 centers in the US to evaluate the safety and efficacy of AR101 (Palforzia), a novel oral biologic drug for the treatment of peanut allergies. The study enrolled 55 peanut-sensitized individuals between the ages of 4 and 26 years, all of whom had symptoms triggered by less than 143 mg/day of peanut protein as assessed by a double-blind, placebo-controlled food challenge. The patients were given daily doses of Palforzia or placebo, with the dosage gradually increased from 0.5 mg to 300 mg/day. The results showed that Palforzia generally improved the patient's symptoms and tolerated 18-fold higher amounts of peanut protein after treatment (Bird et al., 2018).

Treatments

Though many OITs are being researched and developed, Palforzia is the only current FDA-approved treatment to help expose children and teenagers to peanut proteins in controlled amounts in a medical facility. Treatment with Palforzia is typically initiated in a healthcare setting, where the patient can be monitored for signs of allergic reactions. Once the maintenance dose is reached, treatment can be continued at home (Erlich, 2022).

Palforzia is not intended to cure peanut allergies rather it is meant to lessen the severity of future reactions. Thus, patients receiving Palforzia are strongly advised to always carry injectable epinephrine just in case they experience a severe allergic reaction (Heo, 2021).

In the hope of curing peanut allergies in adults, a clinical trial was performed to test the use of a monoclonal antibody injection used to target the cytokine interleukin-33 (IL-33) in peanut-allergic individuals (Chinthrajah et al., 2019). IL-33 is a protein involved in the immune system and is thought to play a role in the development of allergic reactions. In peanut-allergic individuals, IL-33 has been found to be elevated in the blood and the skin. IL-33 may stimulate the production of other cytokines, and immune cells that contribute to the allergic reaction. Blocking IL-33 has been shown to reduce the severity of allergic reactions in animal models (Ding et al., 2018).

The trial included a small group of 20 participants and found that a single dose of the antibody, called Etokimab, significantly improved desensitization to peanuts as determined by standardized food challenges and SPTs. There was also a trend towards reducing atopy-related events (such as asthma, eczema, food allergy, and allergic rhinitis) in the group receiving Etokimab compared to the placebo group. The results showed that the group receiving treatment with Etokimab showed a 73% and 57% increase in the amount of peanut protein they could tolerate on days 15 and 45, respectively, while the placebo group saw no improvement on either day. Additionally, the group receiving the placebo reported more instances of atopy-related conditions such as asthma, eczema, food allergy, and allergic rhinitis compared to the group receiving the treatment (60% vs. 7%, respectively). These results suggest that IL-33 blockade may effectively inhibit allergic responses throughout the human body. Further studies are needed to understand the mechanisms behind these effects and to determine the potential of anti-IL-33 therapy for the treatment of peanut allergies (Chinthrajah et al., 2019).

Quality of Life

In peanut-allergic patients, increased stress and anxiety levels can negatively impact their quality of life (QoL) due to the burden of avoiding peanuts strictly and fearing anaphylaxis (Heo, 2021).

In a study to determine QoL in children with severe peanut allergies, researchers studied 20 children with severe peanut allergies, as well as a group of 20 children diagnosed with insulin-dependent diabetes mellitus. A comparison between the two groups was chosen because both conditions need to be monitored daily and can potentially result in adverse effects if they are not managed properly. The study evaluated the children using two disease-specific QoL questionnaires while also having them photograph their facial experiences over a 24-hour period. Results showed that the QoL of children with peanut allergies was worse than that of those with

insulin-dependent diabetes mellitus, with peanut-allergic children experiencing more fear of adverse events, anxiety about eating and feeling more restricted by their allergies in physical activities. However, they also reported feeling safer when carrying epinephrine kits and were not anxious about eating at familiar restaurants. The difference between the two groups appears to be related to higher anxiety levels in the peanut allergy group, particularly in situations outside of the home or school. While this anxiety may help promote adherence to strict allergen avoidance measures, it can also lead to mental health problems if the restrictions are unrealistic or unfounded. Children with peanut allergy must constantly be aware of allergen hazards in their environment and carry an injection to save their life in case of accidental exposure (Avery et al., 2003).

Conclusion

Unfortunately for those living with peanut allergies, there is currently no approved cure for adults suffering from peanut allergies. Until recently, it has been one of the few allergies that people would not grow out of over time. However, there are now several OITs, including one that is FDA-approved, that help wean children and teenagers who have peanut allergies so that they no longer have a severe immune response to the allergen. Further research is currently underway aiming to cure peanut allergies once and for all. Until then, it is important to continue practicing diligence, such as carrying an epinephrine injector and closely adhering to FDA reporting on trace amounts of peanuts in food products.

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Alcohol Consumption and its Effects on the Liver

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Abstract

Thousands of people die each year in the United States from alcohol related deaths. Why does the metabolism of alcohol cause severe damage to the body? Ethanol enters the body in the form of wine, and other alcoholic beverages and travels through the digestive system and then to the liver where it is metabolized. There are a series of steps involved in the breakdown of ethanol, and toxic byproducts are formed such as reactive oxygen species (ROS). These oxygen radicals can lead to damaged deoxyribonucleic acid (DNA) and to the formation of adducts. Chronic alcohol consumption can lead to liver inflammation and liver failure. Researchers are continuing to study and understand the specific mechanisms of liver damage as well as possible interceptions and cures.

Introduction

There are approximately one-hundred-forty-thousand alcohol related deaths in the United States each year, which is more than three-hundred-eighty deaths per day. The deaths are primarily a result of heavy episodic drinking (HED) that led to liver disease, heart disease, and cancer (Centers for disease Control and Protection, 2022). Alcohol consumption is a serious risk factor for many diseases because of its toxic effects on the body.

The Centers for Disease Control and Prevention (CDC) established guidelines for what is considered excessive alcohol consumption. Binge drinking is defined as consuming four or more drinks on one occasion for a woman and five or more drinks for a man. A woman drinking eight or more drinks a week and a man drinking fifteen or more is considered heavy drinking (Centers for Disease Control and Prevention, 2022).

There are some observed trends with alcohol consumption among gender, race, and age. In the United States, over 55% of adults aged 26 and older consume alcohol each month, and one in every four adults in this age bracket engage in HED (Witkiewitz, Litten, & Leggio, 2019). Men are more likely to engage in HED, possibly due to peer pressure or to achieve the feeling of masculinity. There is a correlation between white American males overconsuming alcohol more than other races (Wade, 2020). Alcohol abuse affects both men and women, but men are more likely to develop alcohol related diseases and die from them. Men are four times more likely than women to develop cirrhosis and liver cancer (Yerby, 2022).

Long term consumption of alcohol correlates to the development of many diseases. Alcohol consumption has been shown to have a detrimental impact on the development of infectious diseases such as tuberculosis, human immunodeficiency virus (HIV), and pneumonia since alcohol negatively affects the immune system. It is not fully understood how, but there is a correlation between heavy alcohol consumption and the development of cancer. Some say that the acetaldehyde, a product of alcohol metabolism, is itself carcinogenic. Additional diseases that alcohol consumption is related to include: diabetes, cardiovascular diseases, neuropsychiatric disorders (like epilepsy), and liver and pancreatic diseases (Rehm, 2011).

Alcohol is one of the leading causes of death in humans, and a leading risk factor in many diseases. What is it about alcohol that makes it so toxic to the human body, specifically to the liver, and is there any way to intercept the process from causing severe liver damage and eventual death?

Methods

The research acquired for this paper was compiled from the databases of Touro College Online Libraries and was supplemented by additional websites. Keywords used to aid in finding relevant articles include: alcohol, liver, acetaldehyde, and reactive oxygen species.

Discussion

Alcohol has negative effects on the entire body; the liver suffers the most severely since it is the primary location of alcohol metabolism (Cioarca-Nedelcu, Et. Al. 2021). This paper analyzes common alcohol metabolic pathways and the harm of the byproducts formed.

Pathway Through the Body

"The effects of alcohol on various tissues depend on its concentration in the blood over time (Zakhari, 2006)." The rate that alcohol is absorbed, metabolized, distributed, and excreted determines the blood alcohol concentration (BAC). Environmental factors such as how fast one drinks, or what they drink, as well as genetic factors can contribute to one's BAC levels. Rate of alcohol elimination varies among individuals and things like age, time of day, and chronic drinking can all affect the rate of alcohol elimination from the body (Zakhari, 2006).

Alcohol enters the body through the oral cavity and a very small amount is directly absorbed into mucosal tissue and then into the blood stream. The majority of the alcohol then travels down the esophagus to the stomach. Once in the stomach, the mucosal layer of the stomach absorbs a small amount of the alcohol and sends it directly into the blood stream and then to the liver. This is why there is an almost immediate alcohol rush response soon after drinking.

The vast majority of alcohol will be absorbed into the blood stream from the intestines. From the stomach, alcohol travels through the pyloric sphincter to the small

intestines. If alcohol is consumed on an empty stomach, the sphincter will be fully open, allowing the alcohol to enter the intestines all at once and ultimately hit the bloodstream faster. However, if there is food in the stomach, the sphincter will open only slightly, so the alcohol will steadily reach the bloodstream (Cottle, 2021).

The alcohol encounters the enzyme alcohol dehydrogenase which converts ethanol to acetaldehyde - a more toxic substance than the ethanol itself. Another enzyme converts acetaldehyde to acetate which is less toxic. However, if one consumes too much alcohol that overwhelms the liver, some of the ethanol will escape into the blood stream without first being converted to something less toxic (Cottle, 2021).

From the liver, the blood goes to the heart, and the heart sends the blood to the lungs. Some of the ethanol actually leaves the body simply by breathing, but the percentage that does not travels back into the heart. The heart pumps and sends the rest of the ethanol first to the brain and then to the rest of the body. Ethanol affects the majority of the body aside from bone and fatty tissue. Ethanol is water soluble and fat tissue is lipids so they will not mix, which is why women who have more fatty tissue and less blood volume than men are affected more by alcohol consumption (Cottle, 2021).

Ethanol triggers the sympathetic nervous system – fight or flight, causing rapid heart rate and sweating (Cottle, 2021). In the brain, ethanol will affect the function of neurotransmitters. Ethanol will increase the function of inhibitory neurotransmitters like GABA, glycine, and adenosine, causing people to feel sedated. At the same time, ethanol inhibits the function of excitatory neurotransmitters like glutamate, also contributing to the alcohol induced feeling of sedation (Valenzuela , 1997). Additionally, the hypothalamic-pituitary axis will recognize the ethanol and, in an effort to maintain homeostasis, it will trigger the adrenal glands to release cortisol, epinephrine, and norepinephrine causing the feelings of stress and adrenaline. The pituitary will also produce less antidiuretic hormone (ADH). Typically, ADH affects the amount of blood flowing through the vessels in the kidney and thereby controls the amount of urine produced. With limited ADH, the vessels in the kidney will dilate and thus allow more urine production, which is why people have frequent urination when they consume alcohol. This results in dehydration since the body has lost so much fluid (Cottle, 2021).

Alcohol Metabolism

One chemical mechanism alone will not account for alcohol induced damage in a person. Rather, a series of processes will occur when one consumes alcohol, and combined, they will lead to critical damage (Wu & Cederbaum, 2003).

Alcohol is metabolized in the liver through a series of steps known as oxidative metabolism. The major oxidative pathway for metabolism of alcohol in the liver involves an enzyme called alcohol dehydrogenase. When alcohol enters the liver, it encounters the alcohol dehydrogenase enzyme in the cytosol of hepatocytes. This enzyme converts alcohol into acetaldehyde, a toxic and highly reactive molecule (Tuma & Casey, 2003).

Then, the enzyme aldehyde dehydrogenase converts acetaldehyde into acetate. The oxidation of alcohol reduces nicotinamide adenine dinucleotide (NAD+) by two electrons to form a nicotinamide adenine dinucleotide (NADH) molecule-the starting material for the cellular respiratory chain. "As a result, alcohol oxidation generates a highly reduced cytosolic environment in liver cells," leaving the liver vulnerable to the reactive byproducts of alcohol metabolism (Zakhari, 2006). During the respiratory chain, oxygen radicals, such as hydroxyl, peroxide and superoxide, are produced as intermediates. These oxygen radicals are, for the most part, converted to water before they can damage any cells. With increased alcohol intake there is a greater volume of NADH and possibility for oxygen radical formation (Cederbaum & Wu, 2003).

The enzyme cytochrome P450 2E1, located mainly in the endoplasmic reticulum of hepatocytes in the liver, can also be used to convert alcohol into acetaldehyde in an oxidative process. However, this chemical mechanism also produces highly reactive oxygen species (ROS) as a byproduct (Tuma & Casey, 2003).

Another oxidative pathway in metabolizing alcohol involves the enzyme catalase, located in the peroxisomes of cells. Catalase oxidizes ethanol in the presence of hydrogen peroxide. Researchers have found increased catalase activity and hydrogen peroxide production in the liver of rats who were given alcohol over time. This is a minor pathway in metabolizing alcohol (Zakhari, 2006).

Reactive Oxygen Species

A significant process leading to alcohol induced damage was found to be the production of reactive oxygen species (ROS). "Both acute and chronic alcohol exposure can increase production of ROS and enhance peroxidation of lipids, protein, and deoxyribonucleic acid (DNA) (Wu & Cederbaum, 2003)."

"Repeated formation of these reactive oxygen species, especially in heavy drinkers, surpasses the liver's capacity for fighting oxidative stress by consuming all resources of the local enzymatic and non-enzymatic antioxidant molecules (Cioarca-Nedelcu Et. Al. 2021)." Excessive

production of ROS or limited activity of antioxidants can lead to oxidative stress and eventually result in cell death (Tuma & Casey, 2003).

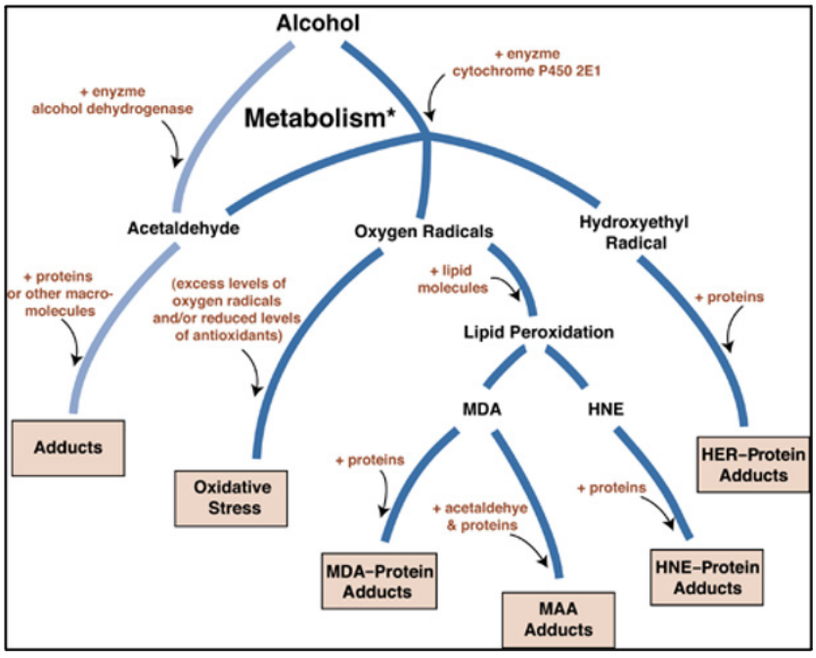
The oxygen radicals are toxic because they can interact with polyunsaturated fat molecules in cell membranes, in a process called lipid peroxidation." The result of ROS interacting with phospholipids is the formation of electrophiles that are reactive aldehyde derivates: malondialdehyde (MDA), 4-hydroxy-2,3-alkenal, and 4-hydroxy-2-nonenal (HNE), similar to acetaldehyde (Tuma & Casey, 2003). Di Luzio, in 1966, was the first to discover the peroxidation of lipids after alcohol consumption, and this was confirmed by other studies (Galicia-Moreno & Gutiérrez-Reyes, 2014). "In addition to damaging cells by destroying membranes, lipid peroxidation can result in the formation of reactive products that themselves can react with and damage proteins and DNA (Wu & Cederbaum, 2003). These lipid peroxide derived compounds can induce a strong inflammatory response in the liver, also known as alcoholic hepatitis (Cioarca-Nedelcu, Atanasiu, & Stoian, 2021).

The oxidative modification of proteins can alter their function and structure eventually leading to proteolysis. First, proteins will be slightly modified, but they can still function. Next, proteins will slightly unfold, exposing the hydrophobic regions of proteins. If the damaged protein has not yet been degraded, "it forms an aggregate with other proteins, lipids, and sugars (Galicia-Moreno & Gutiérrez-Reyes, 2014)."

Acetaldehyde along with the lipid peroxide derivatives MDA and HNE can interact with proteins and other complex molecules forming adducts as a product. The aldehydes typically interact with the lysine amino acid and most commonly affect the following proteins: hemoglobin, albumin, tubulin, lipoproteins, collagen, cytochrome, and ketosteroid reductase. Research has found that these adducts that are formed from the metabolism of alcohol are a leading cause of alcohol induced liver disease (Tuma & Casey, 2003). In other words, the ROS oxidation of lipids yields products that interact with proteins and can cause changes to the protein's three-dimensional structure and cross linking of proteins (Wu & Cederbaum, 2003).

Research has been done to confirm that adducts are indeed formed in-vitro as a result of alcohol consumption. The first study done detected antibodies for aldehyde adducts, like acetaldehyde, MDA, and HNE, in the blood of chronic alcohol consumers. The adducts formed were seen as foreign, and therefore, elicited an immune response and production of antibodies. Further studies proved adduct formation in the liver, specifically in the primary liver cells-hepatocytes. Acetaldehyde adducts were primarily found in the perivenous regions of the liver, an area around the vein carrying blood out of a lobule in the liver. MDA and HNE adducts were found in damaged parts of the livers of chronic alcohol consumers (Tuma & Casey, 2003).

Research has found a link between adduct formation resulting from alcohol consumption and liver dysfunction and disease. Liver damage has been seen in the same area where acetaldehyde adducts accumulate, indicating a correlation. Additionally, acetaldehyde adducts were found in areas of inflammation and fibrosis (scar tissue formation) in advanced liver disease. MDA adducts were found in the same places as acetaldehyde adducts. Aldehydes were found to interact specifically with the lysine amino acid, and evidence was found showing dysfunction in lysine reliant enzymes like calmodulin and a cytoskeleton protein: tubulin. Research has found that modification of even five percent of a person's tubulin molecules by acetaldehyde can impair one's cytoskeleton function. This explains the observed protein transport pathway and protein secretion dysfunction in the liver of alcoholics. The altered tubulin can also lead to disorganized hepatocytes which can



Tuma & Casey, 2003

lead to more severe liver damage (Tuma & Casey, 2003).

Research has found the extracellular matrix (ECM) in the liver to also be affected from adducts. Disorder in the ECM production can result in scar formation in the liver, hepatic fibrosis. Accumulation of irregular ECM is associated with the stage of alcohol induced liver disease prior to cirrhosis. Another study found that alcohol consumers with aldehyde adducts trigger a specific immune response producing antibodies that can harm the liver. This hypothesis was supported by an experiment where a guinea pig with aldehyde adducts was given alcohol, resulting in hepatitis (Tuma & Casey, 2003).

ROS, specifically the hydroxyl radical, reacts with the carbon atoms in the nitrogenous bases of DNA, and they abstract hydrogen from thymine and 2-deoxyribose. Guanine has the greatest tendency to become damaged from oxidation, and if it is not repaired, it can become mutagenic since it will pair with adenine instead of cytosine. There is a hypothesis that the ethanol induced morphological and functional abnormalities in the hepatocytes are due to the loss of integrity in DNA pairing, leading to inaccurate protein synthesis. Studies were done with rats where they were given alcohol over time, and modified DNA was seen in the rats' mitochondria (Galicia-Moreno & Gutiérrez-Reyes, 2014).

Alcohol Intake Causing Liver Disease

Since ROS are naturally produced, the body has mechanisms, including the use of an antioxidant Glutathione (GSH), to rid the body of them. However, with long term alcohol consumption, these mechanisms become impaired, so ROS can accumulate and lead to liver damage. (Wu & Cederbaum, 2003).

Liver Diseases

Researchers have found that many liver diseases resulting from alcohol are due to the products of alcohol breakdown in the liver (Tuma & Casey, 2003).

The liver reacts to alcohol causing fatty liver, inflammation, and the breakdown of liver cells. Hepatic steatosis, also known as a fatty liver, is when a minimum of five percent of the liver weight is made up of intrahepatic triacylglycerols. Hepatic steatosis is the primary response when the liver is overly exposed to alcohol. It is considered a reversable process once a person stops drinking alcohol (Cioarca-Nedelcu, Atanasiu, & Stoian, 2021).

Long time alcohol consumers are prone to develop Alcoholic Steatohepatitis, a chronic liver disease described as inflammation of a fatty liver. Kupffer cells, macrophages located in the sinusoids of the liver, defend the liver when it is exposed to pathogens from the portal venous tract (a vein carrying blood from the GI tract, gallbladder, and spleen to the liver). With chronic alcohol intake, Kupffer cells can switch from anti-inflammatory to a pro inflammatory state. Activated Kupffer cells generate reactive oxygen radicals and nitrogen species that trigger the release of several cytokines like TNF alpha, chemokine, and interleukins, which ultimately will attract leukocytes to the liver causing inflammation (Cioarca-Nedelcu, Atanasiu, & Stoian, 2021).

"The main trigger of Kupffer cells' immune response in subjects with chronic ethanol consumption is an endotoxin known as lipopolysaccharide (LPS)," an endotoxin found in the gut. Alcohol has the ability to increase intestinal permeability and destroy tight interepithelial junctions between intestinal cells, allowing pathogenic bacteria and endotoxins to leak into the portal system. The process of bacteria translocating from the gut to the liver is known as endotoxemia. LPS reaches the liver, the Kupffer cells respond by generating reactive oxygen and nitrogen species, and ultimately inducing liver inflammation and damage. "In conclusion, there is a strong connection between high endotoxemia and the progression of alcoholic liver disease from liver steatosis to alcoholic hepatitis (Cioarca-Nedelcu, Atanasiu, & Stoian, 2021)."

Cirrhosis is a progressive disease of the liver where hepatocytes are replaced with scar tissue. There are four stages of this disease beginning with hepatitis: inflammation of the liver. Abdominal discomfort is usually felt at this stage, and if the inflammation is treated at this point, it can inhibit the progression to stage two. Next, there is scarring as result of the inflammation, obstructing the blood flow to the liver. At this point if treated, the liver may be able to recover or slow the progression of liver disease. The third stage is cirrhosis where liver tissue that was destroyed over time is replaced by scar tissue. This scarring is permanent damage to the liver, making the liver hard and lumpy.

The scar tissue will eventually block the blood flow through the portal vein into the liver, causing the blood to travel to the spleen resulting in additional problems. The final stage is liver failure. At this stage, if no immediate medical intervention is done, like a transplant, a person will die. "Cirrhosis of the liver caused by years of alcohol abuse or being overweight can be avoided by making changes in the early stages of the disease… Once your liver is severely damaged and scarred, there is no way to repair the damage (Kumar, 2002)."

A primary function of the liver is to filter out toxins from the blood. When the liver is replaced by scar tissue due to alcohol induced damage, it struggles to filter toxins, resulting in the accumulation of toxins in the blood

continuing onto the heart, lungs and then the brain. Some of the blood from the portal system will not even enter the liver and rather bypass the liver in a phenomenon known as portal–systemic shunting and continue onto the brain. The toxins, such as ammonia and manganese, can build up in the brain and lead to a disorder known as hepatic encephalopathy. Researchers have confirmed this when they found ammonia and manganese in the brains of those with severe liver disease. Symptoms of hepatic encephalopathy include cognitive dysfunction and problems with motor function such as tremors and poor coordination. Eventually, patients can end up in a coma called a hepatic coma since it was the dysfunction of the liver which led to the toxic accumulation. Lactulose, a sugar molecule that is not absorbed by the body, draws ammonia into the intestines and eliminates it with the undigestible sugar. This is the primary treatment for chronic hepatic encephalopathy (Butterworth, 2004).

The liver, the second largest organ in the body, has the capability to regenerate. Regeneration is different than scarring in that it is actually reproducing liver tissue as opposed to scar tissue. The liver has this feature because it is in charge of metabolizing toxins in the body (Cottle, 2021). With chronic alcohol consumption and development of cirrhosis, the liver can no longer regenerate, and the liver fails to function. Most doctors will require patients needing a liver transplant due to alcohol abuse to be sober for at least six months. Many people are not candidates for a liver transplant because they have medical problems such as cancer, hypertension, or substance abuse disorder (Feng, Roayaie, & , 2023).

Possible Treatments

There are numerous treatments for alcohol use disorder including the use of drugs and treatment programs like Alcoholic Anonymous. Acamprosate, a drug that targets the glutamate system in the body, works to inhibit alcohol dependency. Another drug, Naltrexone, reduces alcohol cravings. Naltrexone does have a risk of causing hepatotoxicity if more than the approved amount is taken. Therefore, patients with active liver disease, and acute hepatitis or liver failure should not use Naltrexone. The first FDA approved drug in 1951 was Disulfiram. Disulfiram works by inhibiting aldehyde dehydrogenase, the enzyme that converts acetaldehyde into acetate. With the enzyme disabled, if alcohol is consumed it will cause acetaldehyde to accumulate, resulting in unpleasant symptoms like tachycardia, headaches, nausea, and vomiting (Witkiewitz, Litten, & Leggio, 2019).

For treating the excessive production of ROS: "Numerous investigations have found that administering antioxidants, agents that reduce the levels of free iron, or agents that replenish GSH levels can prevent or ameliorate the toxic actions of alcohol (Wu & Cederbaum, 2003)."

Where Research is at Now

"Innovative approaches to better identify the mechanisms through which adducts cause liver injury remain challenging goals. The development of new therapeutic interventions for patients with alcoholic liver disease aimed at modifying or preventing adduct formation also poses a challenge for investigators. Both of these endeavors represent fertile areas for future research and should provide valuable information concerning alcohol's toxic effects on the liver and the treatment of alcoholic liver disease (Tuma & Casey, 2003)."

"Nowadays, multiple studies targeting alcohol-induced liver disease have shown that administration of agents that eradicate superoxide anions, such as a superoxide dismutase and agents that restore reduced glutathione, such as N-acetyl cysteine, can successfully counteract oxidative stress. Moreover, through their anti-inflammatory activity, corticosteroids can decrease the local release of cytokines and the local collagen production, especially in alcoholic hepatitis. Last but not least, silymarin, which is a strong antioxidant, is a strong cell membrane stabilizer and an antifibrotic agent (Cioarca-Nedelcu, Atanasiu, & Stoian, 2021)."

Conclusion

Alcohol has short term effects on the body, affecting the lining of the digestive tract, the neurotransmitter function, and cognitive function. Over time, alcohol consumption can have more detrimental effects on the body. The liver suffers the most severely, first with inflammation, then steatosis, and finally with cirrhosis. The breakdown of alcohol yields byproducts called reactive oxygen species that are toxic to lipids, proteins, and DNA. ROS interacting with parts of the body causes damage and formation of adducts. Adducts were found in the liver of long-term alcohol consumers. There are some medications to help the effects, but if consumption of alcohol is not stopped, the only option at times is a liver transplant. Research is focused on trying to inhibit the ROS from causing damage.

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Can Physicians Transfer Bacteria onto Patients through their Neckties?

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Abstract

Hospital-acquired infections are exacerbated by the presence of transmission agents such as bacteria on neckties. Physicians can put patients at risk of infections if bacteria contaminate their ties during their normal hospital routines. In the hospital setting, some of the most common bacteria include Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Enterococcus, Streptococcus pneumoniae, Methicillin-resistant Staphylococcus aureus (MRSA) and Clostridioides difficile. Studies have shown that neckties can act as transmission agents, especially when they come into contact with patients. Additionally, the type of material used to make a necktie determines the viability of bacteria. Wearing neckties increases risk for immunocompromised patients which can lead to prolonged hospital stay and increased mortality. Given that the issue contributes to poor health outcomes and increased healthcare costs, new policies must be implemented. Researchers have suggested a complete ban on physician neckties or impregnation of fabrics in ties with metals such as copper and silver which can aid in the reduction of nosocomial infections.

Introduction

Most people are aware of the dangers posed by hospital environments but rarely consider doctors' attire as the source. White coats, wristwatches, neckties, stethoscopes and other medical instruments are potential vehicles for germ transfer from doctor to patient (Mehta et al., 2014). Neckties are an interesting piece of clothing as they are considered a mark of professionalism. Mostly worn by male doctors, they add to the professional appeal. However, neckties do not serve any functional role and could contribute to nosocomial infections. In hospitals, doctors caring for patients and doing rounds come into contact with various pathogens. While hand washing removes most pathogens that could be transmitted through touch, neckties may pose a greater health risk. There is no evidence that neckties add to the patient-doctor relationship, and they likely contribute to bacterial transmission in the hospital setting. Traditionally hospitals have relied on disinfection of surfaces and objects to curb the spread of illnesses. However, these measures might not be enough given that doctors walk around with germs swinging from their necks. An understanding of hospital microbiology is critical to solving the issue of hospital acquired infections.

In healthcare settings bacteria are prevalent, and measures must be taken to reduce the chances of hospital-acquired infections. Bacteria are living single-celled organisms found everywhere in the environment. While some microscopic organisms can be beneficial or harmless, others can cause infections leading to hospitalizations and even death. Even worse, when bacteria become antibiotic-resistant, infections are very difficult to treat. Susceptible patients, such as those with compromised immunity, are at increased risk of infection even from minor exposure. Some common bacterial infections can cause diphtheria, pneumonia, sepsis, and urinary tract infections, bloodstream and gastrointestinal infection. The bacteria associated with do these illnesses include Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Enterococcus, Streptococcus pneumoniae, Methicillin-resistant Staphylococcus Aureus and Clostridioides difficile (Mehta et al., 2014). Hospital-acquired infections contribute to increased costs of treatment and longer hospital stays. Fomites should be removed from the hospital setting to ensure that bacteria do not spread.

The fabric used in a necktie can provide a rich environment for bacteria to thrive. If neckties go unwashed, exposing patients to them will likely contribute to the spread of infection. Depending on the colony forming units formed on neckties, transmission of bacteria from physician to patient is possible. Therefore, researchers have proposed that physicians get rid of their neckties due to the possibility of transferring bacteria.

According to the Center for Disease Control and Prevention (CDC), about 5% of patients acquire infections during healthcare visits. Infections come with a high price tag, costing about 30 to 40 billion dollars, and reducing infections could bring huge savings, as complications associated with care are to blame for the excess costs (Haque et al., 2018). The costs are incurred because of longer hospital stays and the possibility of antibiotic-resistant bacteria. To reduce the chance of acquiring opportunistic infections, immunocompromised patients might be isolated. In addition, sterile gloves should be worn, especially when doctors touch areas that might be infested with bacteria. Gowning, masks, and eye protections ensure that body fluids, secretions, and splashes do not contaminate the physician during care, especially when respiratory diseases occur. Additionally, shoe coverings and head coverings might be required in some cases. One measure is isolation, which involves physically separating the patient from others. Infections are serious and life-threatening, and hospitals have guidelines for dealing with them.

Healthcare workers also ensure that patient care items are handled properly, and one-time-use items are disposed of properly. Airborne precautions must also be considered, and respiratory protection protects from bacterial droplets in the air. Healthcare professionals must play a role in reducing nosocomial infections which results in reduced quality of care. They must identify all possible avenues through which infections can be spread, including

beds, rails, textiles, and physician attire (Mehta et al. 2014). Hospital staff cleans and disinfects items, making them safe for use. Physicians also engage in practices that ensure that germs are not transmitted to unsuspecting patients, such as washing hands, gowning, and wearing masks and gloves.

Even though doctors wash their hands regularly, neckties are mobile fomites that professionals rarely consider even though they frequently come into contact with human secretions and germs. Also, as they are considered clean, neckties are rarely washed making them mobile germ carriers. Exposure to these fomites can introduce disease-causing organisms that increase the likelihood of mortality, especially for immunocompromised patients. Therefore, minimizing exposure to neckties is critical to the reduction of nosocomial infections.

Neckties are considered a professional piece of attire. However, questions have started to emerge. In the medical field, the connection between the transmission of germs and neckties is still controversial, and their usefulness in medical practice is questionable. Given that new medical outfits are being embraced, for instance scrubs are now commonly worn, which can still afford doctors the benefit of being identified as professional. If the item contributes to an increase in hospital-acquired infections, then a call to ban them should be enforced. However, if they are not potential hosts for pathogens, then doctors should be able to wear ties without objection.

Method

The research employed a literature review of ten preselected articles covering the topic. These articles were obtained from recognized databases such as PubMed, Science Direct, Embase, and Cochrane Library. The search terms physician, neckties, and bacteria were included in the initial search which yielded more than fifty articles. In addition, terms such as textiles and bacterial survival were added to the search. A review of the abstracts identified the best articles for this study. The process led to the selection of ten relevant articles that would be used to determine whether physicians can transmit bacteria to patients.

Discussion

The bacterial content and count differ depending on the practices employed by physicians. McGovern et al. (2010) employed a literature review of various studies to make these conclusions. For instance, an examination of pathogens present in neckties was conducted in a study by the UK Department of Health. The study was to determine if wearing neckties had any beneficial function to patient care. In one of the studies, neckties from 40 doctors in a Scottish hospital were sampled to determine the bacterial content. Staphylococcus aureus was isolated from eight doctor's neckties. In yet another study, it was established that two out of the five doctors working in intensive care units had coagulase-negative staphylococci (CoNS). The results were constant with the hypothesis that neckties were rarely laundered and colonized by pathogens. McGovern et al. (2010) also discussed another study conducted in London that indicated physician neckties had higher bacterial count than their shirt pockets. While shirts were frequently laundered, neckties were considered less dirty as they never came into contact with the body. These notions led to less frequent laundering of neckties, even among physicians. The same research also compared bow ties and neckties that gynecologists and obstetricians wore. The results indicated that there was no difference, and the ties had similar levels of bacterial infestation. The bacterial levels were collected in the study on the third day of wearing the ties.

Antibiotic-resistant bacteria were prevalent in physician neckties. Methicillin-resistant Staphylococcus aureus (MRSA) is hard to treat as it has become resistant to most antibiotics. The bacteria were isolated from most neckties during some of the studies. In another study involving 95 male physicians, only 20% objected to not wearing a tie (McGovern et al., 2010). Skin flora found on the sampled ties suggested that laundering was ineffective in eliminating all bacteria (McGovern et al., 2010). Ties were handled regularly, and pathogenic organisms colonized one in five. The conclusion is that ties can be reservoirs for healthcare-associated infections and should be eliminated from healthcare practices.

The spread of nosocomial infections, common in intensive care units, can be addressed by determining potential vectors. According to Dixon (2000), heavy growths of coagulase-negative staphylococcus were identified in two of the five ties tested in their study. During the research, the ends of five neckties, from male doctors working in the intensive care unit, were blotted and then placed on blood agar plates. The plates were incubated for a forty-eight hour period, and then the number of bacterial colony-forming units were counted. Heavy growth in two neckties was noted, with more than three colony-forming units observed after forty-eight hours. Given the bacterial count, results indicated that neckties are a potential source of infection.

Fifty doctors were enrolled in a study, half of whom were surgeons. Of the group, sixteen never cleaned their ties, while twenty did not account for when they had last cleaned their ties. Another fourteen estimated that the last time their ties had been cleaned was seventy-three days earlier (Lopez et al. 2009). The data is concerning,

especially because they reported wearing their shirts for less than two days after a wash. The colony-forming units on ties ranged from 13 to 950 compared to shirts with 2 to 51 colony-forming units. The most abundant bacteria, Staphylococcus aureus, was isolated in thirteen of the ties. The colony-forming units for this bacterium were zero to eighty-six colony-forming units.

The goal to decrease hospital-acquired infections also led to a study to investigate the effect of shirt sleeves and ties on the transmission of bacteria from doctors to patients. The controlled investigation was well designed, as ties and sleeves were inoculated with Micrococcus leteus using a swab, and cultures were obtained immediately after inoculation. Cultures were obtained from the simulated patients' cheeks, right hand, and abdomen area. Before the stimulated examination, the patients had no micrococcus growth. The physicians wore the inoculated clothing as they examined mannequins dressed in hospital gowns. The simulated patients were laid on hospital beds, simulating the exact patient/doctor interactions. The ties were unsecured, allowing them to come into contact with patients. Cultures were collected before doctors' standard history and physical examination during visits. After the simulated encounters, the clothes were again cultured to determine their bacterial contamination levels. The tie was also deliberately touched on the mannequin, and the site was cultured. After the encounters, results indicated more than 300 colony-forming units on each article of clothing. Cultures taken from cheeks, abdomen, and hand sites also showed at least a five unit growth in colony-forming units. Patients were contaminated after encounters. Four of the five patients had bacterial colonization when physicians wore a long-sleeved shirt with a tie. Source (Weber et al., 2012).

Two of the five patients had bacterial colonization when the physician wore a short sleeve shirt. There was higher colonization when the physician wears long-sleeved shirts and ties (Weber et al., 2012). Bacteria are often transmitted from the physician to the patient, a factor that increases the likelihood of nosocomial infections.

Another study investigated patients' attitudes regarding physicians attire. The researchers conducted 160 surveys, in which patients were asked about their preference for medical attire to see whether there is a link to hospital infections. Most patients preferred doctors in scrubs or professional outfits, including a long-sleeved shirt and a necktie. One of the proposed medical attires was a bare below-the-elbow outfit, which got the lowest votes in all categories. Younger people did not show any preference and would accept it if physicians wore any of the uniforms. On the other hand, older people preferred the long sleeve and tie look. Notably, scrubs were considered the most hygienic of the three attire choices while those with a bare below-the-elbow outfit were considered the least hygienic. Scrubs also scored high when it came to physicians being identifiable. Source (Bond et al., 2010).

Given that over 200,000 patients contract nosocomial infections, while 8,000 die, healthcare-acquired infections must be curbed. The cost of healthcare is estimated to be upwards of 45 billion dollars. A systematic review noted that healthcare workers, patients, and visitors are responsible for the spread of infection in hospital settings. The study reviewed articles from 1966 to 2017 and only considered neckties or a comparison with other vectors such as stethoscopes and identification badges to be the reason for the spread. The studies revealed that neckties were likely contaminated with S. aureus, methicillin-resistant S. aureus, and S. citrus (Pace-Asciak et al., 2018). Heavy growth of pathogenic bacteria was detected on most neckties, a factor that could compromise the health of immune-competent patients. The same studies also noted that most patients preferred that doctors be dressed in professional attire that aids in identifying them. Various researchers investigated the influence of the type of fabric on the spread of bacteria. They showed data on the survival of bacteria on textiles. The evaluation was critical as it advanced understanding the role of specific fabrics as sources of transmission. The materials investigated in the study were cotton, synthetic, and mixed fibers. In cotton, widely used in neckties, bacterial survival rates ranged from less than an hour to more than ninety days. Enterococcus spp. survives ninety days and is most commonly associated with urinary tract infections in hospitalized patients. E. coli, associated with stomach pains, vomiting, and bloody diarrhea, lives up to forty-five days on cotton fabric. Other bacteria, such as V. cholerae, Salmonella spp., and C. jejuni, are viable for eight, five, and three hours respectively. With an increase in humidity, the survival rates for some bacteria, such as S. aureus and S. pyogenes, increased. Synthetic fibers, such as polyester, had very high bacteria survival rates compared to cotton. Bacteria survival rate ranged from seven to one hundred and six days. When the bacterial count was low, the number of survival days were lower. As in cotton, humidity increased the survival rate for E. coli, S. prognes, and S. aureus. In mixed fibers, bacteria were able to survive for up to ninety days. The greatest survivability was for Enterococcus spp., E. coli, and S aureus, which reported forty-nine, forty-five, and forty-one days, respectively (Kampf, 2020). The results presented note that most nosocomial pathogens are equipped to survive on surfaces such as textiles and fabrics.

Koca et al. (2012) also conducted a study reviewing the survival rates of nosocomial pathogens on textiles and fabrics. Fabrics are common in hospitals and most likely have a place in the chain of infection. Koca et al. (2012) isolated the bacteria used in the test from patients at a hospital in Turkey and Israel. Test fabrics included wool, cotton, polyester, and silk. Bacteria were then introduced to the materials, and survival rates were determined. The materials were placed at room temperature, and survival time was counted. Colony-forming units with loads of 106 to 108 per 100cm2 were likely to contaminate patients and any items in the environment. Figure 2 summarizes the results collected during the three-month study.

Some bacteria had higher survival rates on the materials. This study confirms that textiles can transmit bacteria to surfaces and patients. Therefore, the choice of material does influence whether a necktie will spread infections from physician to patient.

Figure 2

The survival of bacterial and fungal isolates on various fabrics

Microorganism	Length of survival (no. of days) of individual isolates on			
	Cotton-Polyester	Cotton	Wool	Silk
E. faecium	51	49	49	49
S. aureus	37	37	41	37
E. coli	37	45	45	45
P. aeruginosa	23	13	33	33
A. baumannii	19	19	7	19
S. maltophilia	7	7	7	7
C. albicans	6	6	12	12
C. tropicalis	9	3	>30	24
C. krusei	6	3	>30	21
C. glabrata	>30	>30	>30	>30
C. parapsilosis	>30	>30	>30	>30
G. candidum	6	21	12	6
A. fumigatus	>30	>30	>30	27
C. neoformans	>30	>30	>30	>30

Figure 3:

The bacteria binding ratios to fibers and cloths

Bacteria	Average binding ratios of the 5 strains				
	Acrylic % (SE)	Cotton % (SE)	Nylon % (SE)	Polyester % (SE)	Wool % (SE)
Staphylococcus aureus					
Methicillin-sensitive	87.6 (3.6)	2.0 (2.0)	0.9 (1.8)	96.2 (2.7)	63.2 (23.6)
Methicillin-resistant	86.9 (5.5)	1.0 (1.7)	0.7 (1.4)	87.6 (19.1)	49.5 (29.0)
Pseudomonas aeruginosa	95.4 (4.5)	8.1 (12.0)	14.9 (9.0)	99.9 (0.2)	84.7 (13.2)
SE, Standard error.					

The type of material used in making hospital attires, such as neckties, is critical. As noted, materials like wool, polyester, and acrylic had high binding properties for some species of bacteria. On the other hand, nylon and cotton had lower binding properties making them preferable for neckties. The evidence presented should guide the physicians' decision-making during the purchase of neckties. Lower binding properties mean neckties are less likely to carry and transmit bacteria to patients even when exposed to bacteria. Under the appropriate moisture and temperature conditions, some textiles support microbial growth. They become an important source of bacteria that can contaminate patients and personnel. Physicians always contaminate their work attire, including neckties, when performing activities on patients with bacteria in wounds or urine (Borkow & Gabbay, 2008). As such, prevention is critical, and several strategies have been proposed. Biocidal materials are especially critical in the hospital setting and should have antimicrobial properties. They should also be effective against antibiotic-resistant bacteria such as Methicillin-resistant S aureus. Impregnation using copper oxide is a proposed strategy, as it reduces the transmission properties of different textiles. The oxidization of copper atoms is meant to weaken bacteria as they pull electrons from the atoms making up the cell wall. Eventually, the cell wall breaks, leading to the death of the bacteria. The method has been widely applied, and hospitals embrace copper surfaces in what is known as contact killing. Other heavy metals, such as gold and silver, have extra killing power as they disrupt cell membranes. These metals are more expensive, making copper the most preferable because of its abundance. Its use might reduce the number of hospital-acquired infections, which puts immune-compromised individuals at risk.

A comparison of bacteria-binding properties on different materials was also conducted. In a laboratory setting, researchers used different types of bacteria and materials purchased from the market. Strains of bacteria were introduced into the fabrics, and colony-forming units were then determined after the initial introduction. The results indicated that acrylic and polyester fibers had the highest binding ratios of about 87 and 96 percent, respectively. Figure 3 summarizes the results collected from the five strains of bacteria.

Pseudomonas aeruginosa, mostly associated with infections of the blood and lungs after surgery, had the highest binding properties in acrylic, polyester, and wool. Methicillin-resistant S. aureus had the highest binding in wool, polyester, and acrylic, indicating that items such as

ties made with those material could be easily contaminated. Cotton and nylon had the smallest binding ratios, indicating that they might be preferable for attire worn while attending patients. Source (Takashima et al., 2004).

Discussion

Physician attire should be functional and not endanger patients, but this is not always the case. Patients' attitudes influence how physicians choose to dress. For instance, one study showed that patients preferred formal wear and reported higher satisfaction levels. While scrubs can protect regular clothing from stains and patients from infection, it is not always the attire choice. Doctors choose to project the professional and conservative image associated with a medical practice and don the white coat, shirt, and tie, which has become the physician uniform. For over 100 years, the necktie has been an icon for professionalism. The white coat is symbolic of purity, and in combination with a button-down shirt and necktie is standard formal attire for doctors (Pace-Asciak et al., 2018). Physicians also touch their ties, ensuring that bacteria will be transferred to doctors. However, the concern is that doctor attire can contribute to hospital-acquired infections, with neckties especially concerning because they dangle precariously over places where bacteria lurk.

Nosocomial infections are a problem in most healthcare settings and are especially concerning for immunocompromised individuals. In these settings, the presence of bacteria can result in illnesses contracted in the hospital, which includes blood infections, surgical site infections, respiratory tract infections, and gastrointestinal issues. Nosocomial infections contribute to increased rates of mortality. Extended hospital stays, due to infections, increase healthcare costs that could have been avoided. In addition, some infections are antibiotic-resistant and very hard to treat. Gram-positive bacteria, E. coli, Klebsiella, Acinetobacter, and Methicillin-resistant S aureus, pass on genetic material that allows future bacteria to become drug resistant. Concern about the spread of the bacteria warrants the elimination of objects that might be considered fomites and have no function in a hospital setting. Given that getting rid of neckties does not undermine most patients' confidence in their physicians, it is prudent to eliminate them.

Evidence from research indicates that n the hospital setting, coughs, sneezes, touching with hands, food particles, and patients contribute to bacterial infestation. Most articles analyzed reveal that neckties are rarely laundered as they are considered clean even when they are contaminated. Some physicians could not remember the last time they had washed their neckties, while those that could estimate mentioned seventy-three days. Wearing a necktie for two months is cause for alarm. The colony-forming bacterial units on the ties were higher than those on shirts which represents a greater risk of potential infection (Lopez et al., 2009). In addition, ties come into contact with patients during routine checkups, a factor that could contribute to increased infections among patients. Even with rigorous sanitation protocols in hospital settings, failure to take care of the doctor's attire might contribute to hospital-acquired infections.

Having determined that ties do have bacterial infestations, it was critical to determine whether the germs can be transmitted to patients. The study by Weber et al. (2012) simulated physician-patient interaction and determined that microorganisms can be transmitted from physician to patient. Even though hand washing has been instrumental in decreasing the incidence of bacterial transmission, ties do contribute to the process. Hanging objects such as lanyards and stethoscopes can also be colonized. When patients touch the objects, bacteria are introduced, thus increasing the likelihood of infection. In the Weber et al. (2012) study, deliberate contamination was accomplished by touching the tie to the dummy which resulted in a significant increase in colony-forming units. While conducting examinations, clinical activities often involve touching and leaning over the patient, increasing the probability of bacterial transfer.

Survival of bacteria at room temperature also affects bacterial transfer. Polyester proved to be the most dangerous material to be used in a hospital setting; some species of bacteria survived for two hundred and six days. On cotton, bacterial survival was as long as 90 days. With elevated humidity levels, bacterial survival rate increased. Contaminated textiles are a transmission source. Changing the material to copper may be more expensive, but it is most preferable because of its abundance. Its use might reduce the number of hospital-acquired infections.

Putting a halt to nosocomial infections involves tough measures in disinfection and eliminating fomites, especially ones not needed for service delivery. The hospital setting had different fomites which attribute to the spread of bacteria from physicians to patients. Neckties are especially concerning as they seem to be unnecessary objects that increase the chances of infection. Doctors who rarely wash their ties pose a great risk to patients who are already immune compromised. The call to ban neckties is to protect vulnerable individuals exposed to hospital-acquired infections. Given that patients do not have a problem with physicians losing their neckties, enforcing this measure and protecting those visiting hospitals from infections is prudent.

Future Research

The research covered most issues related to bacterial contamination from physician neckties. These include reasons for the difficulties in changing the dress code worn by doctors for centuries. Other issues found were how neckties contributed to the spread of bacteria in the hospital setting. In addition, there was an exploration of how different textiles used in making ties contribute to bacteria survival rates. Other factors, such as bacterial binding, were also explored in the research.

Since the study is directed toward nosocomial infections by bacterial contamination, future research should also cover viruses and fungi. Given the recent pandemic, the Covid-19 virus became a reason for increased mortality, especially among patients with comorbidities. Chances of physician-to-patient virus transfer are high, given that it can stay on surfaces for a long time. An investigation into neckties' role in spreading viral and fungal infections would serve as an important point for the debate on eliminating neckties in the hospital setting. Further research may include other common objects such as pens, lanyards, stethoscopes, and blood pressure machine cuffs used from patient to patient. Their ability to spread bacteria might contribute to increased hospital infections.

In addition, methods of preventing bacterial contamination should be explored. In this study, the impregnation of textiles with metals such as copper and silver was somewhat effective in eliminating some bacteria. Further research can establish ways that can be used to reduce bacterial infestation, especially antibiotic-resistant.

Conclusion

The patient-physician relationship dynamic is based on trust and the Hippocratic Oath. Patients trust that physicians will deliver the appropriate care and not harm them during healthcare visits. Neckties are crucial for physicians to represent themselves professionally. However, they do not add to physicians' trust levels. Their ability to carry disease-causing pathogens makes them likely to hinder patients' trust in their doctors. Nosocomial infections can be costly for healthcare and lead to increased morbidities and mortalities. Therefore, physicians should avoid neckties that contribute to increased infections because of the pressure to reduce hospital-acquired infections. While other vectors of pathogen delivery can be disinfected through cleaning and antiseptics, neckties pose a challenge. Physicians move with the neckties from patient to patient, making it likely that bacteria will be acquired. In addition, the materials used have properties that make neckties possible bacterial breeding grounds.

The survival rate of bacteria also varied with the type of material and the species of bacteria. Bacteria that can survive up to three months can infect more patients. If physicians are to wear neckties, selecting materials that do not allow the survival of bacteria is the best choice. These include materials such as cotton and nylon that had the lowest bacterial binding ratios of all the materials. The introduction of silver and copper oxide nanoparticles into different materials is meant to reduce the antimicrobial load on health worker uniforms. The procedure is effective on some strains of bacteria and can be used on neckties to make them bacterial-resistant. Cotton and polyester neckties can be coated with cationic copper that endows them with potent broad-spectrum antibacterial properties. The process makes it safer for doctors to have neckties in the workplace as it is an innovative weapon in fighting against hospital-acquired infections. The downside is that such neckties might be more expensive and ineffective when bacteria become resistant. Factors that make it possible for bacteria to thrive, such as high moisture content and lower temperatures, should also be checked. Physicians should avoid situations that result in increased colonization of bacteria.

The remaining alternative is the ban on wearing neckties. It has been proven beyond reasonable doubt that neckties can harbor and aid in transmitting bacteria between physician and patient. These healthcare workers become mobile spreaders of infection and can affect even the most vulnerable patients. The call to ban neckties is well-informed and should be implemented for the safety of patients. Physicians should also not dwell on the traditional professional look, as safety comes first in healthcare settings.

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Singer's Voice Quality - Genetic or Environmental Influences?

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Abstract

This paper explores the singing voice in terms of the vocal mechanism's key anatomical features and the physiology of the vocal function. It focuses on phonation and resonation and examines the anatomy and physiology of the vocal tract and the respiratory system. The aim is to determine whether the singer's voice is only the product of genetics or due to environmental influences. It conducts a procedure to evaluate how singers use their voices to modify breathing, pitch, volume, and timbre through resonance shaping. It also investigates the hereditary transmission of a good singing voice, on chromosome 12q, between relatives, and whether vocal similarities between family members are due to shared DNA. This paper explores dietary and external environmental influences that affect the voice. The results suggest that the extent to which genetics plays a role in determining an individual's voice needs to be better established.

Introduction

The quality of a singer's voice is a source of fascination, captivating audiences across all genres. Although some people believe that the quality of a singer's voice is solely a result of their genetic attributes, a closer examination of the issue reveals that the quality of a singer's voice is also a product of their environment and experiences. The vocal range, pitch, and resonance of a person's voice are all distinctive features (Marrie, 2022). Singing a song and giving a speech requires a wide range of physiological, neurological, aerodynamic, enviromental and psychological factors, thus raising the question of how much genes dictate a person's voice and how much the influences of the environment (Proctor, et. al. 2010). A voice's pitch, volume, and timbre are as crucial to people's perception as the words spoken. Skillful singers can control the sound they produce by changing the shape of their vocal tracts and the way their vocal folds vibrate. Voice quality may or may not be influenced by one's genes, and a person's voice box, or timbre, may be influenced by their genetic makeup regarding how their vocal cords and laryngeal muscles are formed (Erickson-DiRenzo & Thibeault, 2016). Heat, humidity, altitude, and diet plays a vital role in a singer's voice quality. This research aims to determine whether the quality of a singer's voice is solely the product of genetics or due to environmental influences.

Methods

Sources for this review were located by using various search engines such as Proquest, Pubmed and EBSCO, some of which were obtained through the Touro University Library system.

Discussion

The human voice is a result of an intricate bio-mechanism that integrates several specialized body systems into a single, coordinated whole for singing and speaking (Marrie, 2022). The larynx has many important physiological roles besides phonation, including protecting the airway (during swallowing), regulating breathing pressure (during respiration), and pressure-valving (during speech production, in childbirth, defecation, and weightlifting). The vocal folds vibrate in reaction to changes in air pressure triggered by the respiratory system, resulting in spoken sounds (Van Lierde et al., 2005). A person's voice is an example of an acoustic instrument; the individual's breath powers it, and its various moving parts serve as oscillators. The larynx, also known as the voice box, is the anatomical pathway that is located in the middle of the neck, between the 4th and 6th cervical vertbrae. It comprises three regions. (Brenner 2022)

- Supraglottis - the region situated between the lower boundary of the hyoid bone, epiglottis, and the vestibular folds, sometimes referred to as the false vocal cords.
- Glottis - the opening located between the vocal folds and the arytenoid cartilages on either side of the larynx, and the structures that border it.
- Subglottis - the space below the vocal cords extending up to the trachea.

Pharyngealization and Voice

To further define the properties of modal voice's articulatory characteristics that determine the physiological reference points it shares or differentiates from other auditory categories: pharyngealized voice, raised larynx voice, lowered larynx voice, and faucalized voice. Furthermore, to investigate the modal voice's articulatory relationship to the laryngeal voice's attributes, comparisons are made with a modal voice to these other categories (Proctor, 2010). Perceptual and auditory studies have shown that the pharyngealized and elevated larynx voices are similar in articulatory function but distinct in pitch. Pitch differences distinguish the articulatory similarities between a faucalized voice and a lowered laryngeal voice (Marrie, 2022). It is necessary to interpret the phonetic literature to comprehend the rationale behind the correlation between pharyngealization and laryngeal elevation and the correlation between pharyngeal postures and phonation style (Proctor et al., 2010).

Is Voice Quality Affected by Genetics?

Very little work has been done to determine the extent to which genetics play a role in developing an individual's voice (Erickson-DiRenzo & Thibeault, 2016). Despite this,

it is believed that shared laryngeal anatomy in the DNA of related individuals contributes to the resemblances in the vocal characteristics of members of the same family. (Van Lierde et al., 2005). Because relatives often share a childhood, it can be challenging to tell whether a stuttering problem stems from genetics or the Pharynx (Erickson-DiRenzo & Thibeault, 2016). Nonetheless, sex is one genetic factor that affects the voice. Vocal fold tissue in males is more robust than in females (Van Lierde et al., 2005). In other words, even before puberty, their voices are typically lower in pitch than women's. The vocal cords are a pair of elastic bands stretched across the larynx (the voice box) which cause vibrations when air is drawn from the lungs. The average fundamental frequency of a human male is about 125 hertz (Hz), while that of a female is about 210 hertz (Hz), and that of a child is about 300 hertz (Hz). When a person's fundamental frequency increases, their voice becomes higher in pitch.

Vocal talent may or may not be influenced by one's genes (Van Lierde et al., 2005). On the one hand, a person's voice box, or timbre, may be influenced by their genetic makeup regarding how their vocal cords and laryngeal muscles are formed (Lennon, 2019). On the other hand, those in the same family who share a talent for singing may have a similar vocal tone. A professional singer can attest that their voice is confused with their sister's. However, as a person develops and acquires personal singing preferences over time, the environment can significantly impact the quality of their voice (Watts et al., 2008). One's ability to consistently practice singing and learn tips from professional voice teachers and performers will determine the extent to which one is talented (Kayes, 2015).

How is Gene AVPR1A Related to Music?

Music ability has traditionally been viewed as an innate talent; a sensitive, knowledgeable, and skillful capacity to create, recognize, and perform music. Even so, much of the evidence on its genetic background is still lacking. The current study was conducted to address this issue. To evaluate musicality, participants were assessed with the Individualized Music Therapy Assessment Profile (IMTAP), a valid measure developed in Brazil. Results indicated that one particular gene – AVPR1A – was nominally linked to musicality outcomes when examining its microsatellite RS1 segment; replicating prior findings that showed associations between AVPR1A and engagement in musical activities. (Mariath, Luiza Monteavaro, et al.)

How Environmental Factors Affect a Singer's Vocal Quality

Environmental factors such as temperature, humidity, and altitude can have a significant impact on a singer's vocal quality. It has been documented that low humidity can lead to a vocal fatigue, while high humidity can make it difficult to produce clear, articulate notes. Temperature also plays a role in vocal quality, as it is believed that warmer temperatures can lead to increased vocal fatigue and make it harder to control the voice. Altitude can also have an impact, as the lower air pressure can make it more difficult to maintain a steady vocal sound, especially if the singer is singing high notes (Lam, 2013).

In addition, the vocal quality of a singer can be impacted by a range of dietary factors in the environment. Recent research has shown that several dietary components in the environment, such as vitamins, minerals, proteins, and carbohydrates, can have a direct effect on the vocal quality of a singer. For example, a study conducted (Zhang et al. 2018) found that the intake of vitamin B12, calcium, and magnesium had a positive effect on vocal quality and can help improve the range, volume, and clarity of a singer's voice. Furthermore, a study (Gao et al. 2020) found that the intake of carbohydrates and proteins had a positive effect on vocal quality, while the intake of fat had a negative effect. These findings suggest that dietary factors in the environment can have a direct impact on a singer's vocal quality.

Benefits of Vitamin B12, Calcium and Magnesium

Singers can benefit from a healthy lifestyle, including having adequate amounts of vitamins, minerals, and other essential nutrients in their diet. Vitamin B12, calcium, and magnesium are three important nutrients that can help singers maintain vocal health. Vitamin B12 helps maintain healthy blood cells, calcium helps build strong bones and keep them healthy, and magnesium helps maintain muscle and nerve function. All three of these nutrients are important for singers as they help support vocal health, allowing singers to reach their full potential. Furthermore, a study published in the Journal of Voice found that individuals with adequate levels of Vitamin B12, calcium, and magnesium had better vocal control and endurance than those with lower levels. (Erkan, et. Al. 2020)) This highlights the importance of these three nutrients for singers.

Discussion

Experiment subjects in this study all came from the same family of professional singers and shared certain defining traits of a high-quality singing voice. Comparatively, the control group consisted of professional singers who did not come from a musical family. Five controls were utilized in each age range (teens to adults) and both sexes (adult male, adult female, teen female, teen male). Furthermore,

12 speech-language pathology master's degree candidates from nearby universities were recruited to serve as auditory-perceptual judges. Jurors were expected to have normal hearing, have taken a graduate-level course on voice disorders, and be familiar with the many auditory-perceptual classifications of voice quality.

A cardioid microphone was placed on each participant's head, a few centimeters from the center of their mouths, to record their speech production. It maintained a consistent distance between the lips of the speaker and the microphone throughout the recording process. The recordings were transferred to a computer running the Computerized Speech Laboratory, a commercially accessible hardware/software package for analyzing vocals. One of the authors used custom-made software to do acoustic analysis on the various voice samples and to collect auditory-perceptual assessments of voice quality type, severity, and pitch for the recorded voice stimuli.

The recordings were made with the participants seated quietly. After a full breath in, participants were instructed to sustain the vowel /a/ for about 4 seconds at a volume and pitch that they found most natural. All the acoustic analyses that followed the perceptual listening task that the judges completed were conducted with vowel prolongations as the experimental stimuli. One of the writers created specialized software to measure eight acoustic variables related to phonatory biomechanics and voice quality. Both studies made use of the software and demonstrated its usefulness.

Results and Observations

Vocal comparisons revealed similarities between pharyngealized and raised laryngeal voices. Particularly within the articulatory structure, which involves a striking engagement of the aryepiglottic tongue-root retraction and vocal cord elevation. These engagements appear to be effects of a sphincter mechanism. This setting becomes more apparent in the presence of the [a] vowel. In contrast, the larynx is open, the aryepiglottic folds are stretched, and the larynx is lowered in a faucalized voice, also known as a "lowered larynx voice." A pharyngealized voice and a voice with a dropped larynx appear at low pitches, while a voice with a raised larynx and a faucalized larynx only emerge at higher frequencies. Four voice quality options were explored, each correlated with the pharyngeal fricative, approximant, trills, and stop limitations. Pharyngealized and elevated laryngeal voices provide the most similar patterns, as they both include activation of the aryepiglottic sphincter during consonant articulation (Proctor, 2010). The tongue retracts even further as the aryepiglottic sphincter weakens in a depressed larynx voice or a faucalized voice. But each pharyngeal passage can be made separately from these considerations. Comments about the tongue's base location are worthless unless the sphincter is also mentioned. It was thought that shaded products were unlikely to exist. These subjective evaluations are close approximations of the articulatory similarities between a pharyngealized voice, and a voice produced by a raised larynx and between a depressed voice and a voice produced by a faucalization. In these cardinal postures, sphincteric closure is typically achieved through laryngeal elevation and root tongue retraction, particularly in [a].

A person's Pharynx is the primary source of resonance. It rises into the nose, mouth and then curves around the larynx like a muscular sleeve. (Woodson, 2010). The nasopharynx is crucial to producing vowels and consonants and swallowing mechanics. To have orally delivered consonants and all non-nasalized vowels and generate the intra-oral pressure required to form the plosive and fricative consonants, the soft palate (velum) must be raised. The nasopharynx is exposed when the soft palate is lowered, allowing resonance coupling between the nasal and oral pharyngeal regions. One will need to lower the velum to make nasal consonants or vowels that sound coming from a nose. The soft palate can be pushed up, tightened, or released. The levator palatini muscles are responsible for raising the soft palate, which increases the arch at the back of the oral cavity and, in turn, the volume of the air that may vibrate there. Thus, to increase the size of the opening in the pharynx, the tensor palatini muscles will stretch the palate in a horizontal direction (Woodson, 2010).

The oropharynx, which ranges from the soft palate to the laryngeal inlet, provides the most expansive resonant region in the pharynx (Woodson, 2010). The jaw and soft palate are all part of this area, and they work together or separately to alter the quality of the resonation (Watts et al., 2008). The base of the tongue is also located here, and subtle changes in tongue position can profoundly affect the size of the oral pharyngeal cavity (Woodson, 2010). There are many different muscles in the tongue, intrinsic (found in the tongue body in four pairs) and extrinsic (found on the tongue's surface).

Four sets of muscles make up the intrinsic muscle group of the tongue—superior fibers in the transverse and vertical axes and inferior longitudinal fibers. Tongue length and width can be adjusted by the tongue's intrinsic muscle fibers, which intertwine with the extrinsic muscle fibers to create the tongue's various contours (vertical threads). The superior longitudinal fibers allow the tongue's tip and sides to be curled into a convex shape.

Formants are defined as "the frequencies that define the vocal tract shape and contribute to the production of speech sounds" (Byrne, 1992). They are determined through acoustic analysis and provide insight into how speech sounds are formed. Formants are particularly important in determining vowel sounds, as they are responsible for the distinct qualities of the vowels. Formants can also be used to identify particular words in a sentence or phrase, since the formants of each word can be used to differentiate it from other words in the same sentence. Formants can be used to identify speakers, as the formants of an individual's voice are unique and can be used to differentiate them from other speakers. In summary, formants are frequencies that define the vocal tract shape and can be used to differentiate vowels, words, and individuals.

The tongue is crucial to the resonance of the mouth and throat:

- The shape and placement of the tongue substantially affect the sensitivity of the second formant. The second formant, for instance, is raised when the tongue is high and fronted (as in the palatal 'y' sound), whereas it is depressed when the tongue is "backed" close to the velar area, as in the bilabial 't' sound.
- When the tip of the tongue is protruded or retracted, the third formant is raised or lowered.
- Pulling back on the tongue's body reduces the oral-pharyngeal resonating gap, raising the initial formant. The raised initial formant and the lowered higher formants are characteristics of a "throaty voice quality," which is supposed to be caused by this movement (Watts et al., 2008).

The oropharynx's opening is located at the base of the jaw. The most powerful muscles in the skull are the masticatory muscles, which move the mandible (lower jaw) (Woodson, 2010). These include the masseter, temporalis, and pterygoid muscles. As opposed to being mandibular depressors, these muscles raise the jaw. The temporomandibular joint, which connects the jaw to the skull, is an incredibly complicated structure that allows for a wide range of motion, including up and down, forward, and backward. As their origins are at the movable hyoid, the supra-hyoid muscles (the anterior belly of the digastric, the geniohyoid, and the mylohyoid) can lower the jaw, but doing so may threaten the hyoid's stability. The need to let gravity takes over when dropping the mouth to sing, along with the need to do other laryngeal movements, is emphasized. According to singing, the jaw's function in word articulation is secondary to its role in resonance. Since the fundamental frequency of high soprano singing may be as high as or higher than the first formant, lowering the mandible is particularly effective in increasing the resonating airspace in the mouth. Consonant articulation is the lips and tongue's primary functional role in speech and singing (Kayes, 2015). Vocalists and public speakers can alter the formant frequencies of their voices by lengthening or shortening the vocal tract through lip expansion and protrusion.

The long muscles of the Pharynx

Suspending and elevating the larynx posteriorly are the salpingopharyngeus and the palatopharyngeus, which are joined by the stylopharyngeus and attach to the larger horn of the thyroid cartilage (Woodson, 2010). These muscle fibers are very elongated. The larynx will be raised or lowered due to their actions, changing the vertical resonant airspace.

Laryngopharynx or Hypopharynx

The aryepiglottic and epiglottic folds provide entry to the larynx, which is in the lower pharynx. From the laryngeal intake to the cricoid cartilage, this area is referred to as the hypopharynx or epilarynx (Woodson, 2010). The free superior epiglottic border, the aryepiglottic folds, and the inter arytenoid folds define its anterior, lateral, and posterior limits, respectively. Different acoustic results, including the singer's formant and the resulting "twang" or "belting" vocal sounds, have been linked to this region's selective narrowing or broadening. Using modeled vocal tracts, showed that the epilarynx's narrowing can operate as an "attractor" for all formant frequencies, boosting the volume of resonances at those frequencies.

Control of Pitch and Range in Singing

Vocal fold vibrations "slice" the air stream into short bursts of airflow, which are the primary component of voiced sound. In a normal situation, this requires the lungs to have a higher air pressure than the surrounding environment and for the glottis to be closed or partially closed. This occlusion of the airway by the glottis excites the vibrating air molecules in the vocal tract and creates the glottal wave, the essential building block of spoken sound (Van Lierde et al., 2005). Vocal fold health is shown in a periodic cyclical motion, a hallmark of harmonic motion. When the glottis closes or partially opens, it creates what is known as a glottal wave, and each period has a closed and open phase (Van Lierde et al., 2005). The fundamental frequency [F0] is a quantitative representation of pitch that is directly related to the number of cycles per second of the vocal folds. Because of the vocal folds' mechanical qualities and their connection to other structures and muscle groups, the frequency of vocal fold vibration can be changed in several ways.

The Effect of Temperature on the Vocal Cords

Temperature has a significant impact on a singer's vocal quality. Changes in temperature can affect the singer's vocal cords and cause them to become strained, as demonstrated by a study conducted by Corbin et al. (2015). The study found that when a singer's vocal cords were exposed to a temperature of over 40°C, the vocal cords became tense and strained. This increased tension leads to a decrease in the singer's vocal quality, as the cords become less supple. Furthermore, the study showed that when the temperature was decreased, the singer was able to produce a better vocal quality. A study found that both hot and cold temperatures had a negative effect on the vocal quality of professional opera singers (Kedzierski et al. 2016). The study found that, as the temperature increased, the singers' vocal ranges became more limited, and their vocal quality decreased. Conversely, lower temperatures resulted in a stronger and more powerful vocal quality.

The Effect of Humidity on the Vocal Cords

Humidity can have a significant impact on the vocal quality of a singer. Vocal quality decreases with increasing humidity. The study also showed that singers experienced increased difficulty in controlling vocal intonation, pitch accuracy and breath control when humidity was increased. The researchers concluded that humidity has a negative effect on vocal quality and that singers should take extra care to protect their vocal folds from high humidity (Fujimoto, et al., 2018). This can be done by keeping the vocal folds hydrated and by avoiding prolonged exposure to high humidity.

The Effect of Altitude on the Vocal Cords

Altitude has been shown to have a significant effect on vocal production in singers. According to a study, singers who perform in high-altitude locations often have trouble in vocal production due to the thin air. The thin air caused by the high altitude affects the singer's breathing and can lead to a decrease in vocal quality and range, as well as a decrease in vocal stamina. This can be attributed to the fact that the air is less dense in higher altitudes, resulting in decreased airflow and less resonant sound. (Clark and Brown 1991) The study also suggested that singers should be aware of their vocal needs when performing in high-altitude locations and take steps to ensure their vocal production is not affected by the thin air. This includes making sure to take breaks if needed, drinking plenty of fluids, and using vocal warm-ups to help maintain vocal quality and range.

Dietary Choices affecting vocal quality

Diet is an important factor in maintaining vocal quality for singers. Proper nutrition helps to maintain a healthy singing voice, as well as avoiding foods and drinks that can weaken the vocal cords and impair singing performance. In recent studies, it has been found that dietary choices can have a profound impact on the vocal quality of a person. One study found that even minor changes to one's diet can have far-reaching consequences for the overall quality of their voice. The study concluded that a diet rich in fruits, vegetables, and fiber can have a positive effect on vocal performance, while a diet that is low in fiber and high in processed foods can have a negative effect. This study serves as evidence that dietary choices can have a significant effect on vocal performance and should be taken into consideration when choosing a diet. (Bennet. 2020) In addition to following a healthy diet, singers should also avoid foods and drinks that can weaken the vocal cords or impair singing performance. Foods and drinks that contain caffeine, alcohol, and refined sugar should be avoided, as these can cause dehydration and weaken the vocal cords. Additionally, singers should avoid dairy products, as these can cause mucus buildup in the throat and impair singing performance.

Conclusion

The quality of a Singer's Voice is a product of musical genetics combined with the singer's environmental factors- they work simultaneously. Three interconnected systems—respiratory, phonatory, and resonant—control the production of vocal sounds while singing. Vocalizations occur when the vocal folds vibrate in response to the air pressure created by the respiratory system. Singing requires a change in breathing rhythm from normal, quiet breathing. Some singers stop breathing entirely to sing more expressively or alter their rate, rhythm, and volume. Phonation allows one to change the quality of one's voice by adjusting the tension of vibrations produced by the vocal folds. By adjusting the length and tension of their vibrating vocal folds, singers gain access to a broad spectrum of fundamental frequencies. The vocal tract, a component of the respiratory and digestive systems, serves as a resonating area that singers may manipulate to produce vowels and other voice qualities. All these features have been argued to serve practical purposes when singing. Due to the complex nature of the voice production's mechanism, various singers can produce identical sounds by employing a variety of vocal tract configurations, each of which is entirely valid. Sex hormones have varying effects on the voice throughout the life cycles of both sexes, but they are more noticeable during puberty. Overall, an adult

male's larynx is about 40% larger than an adult female. As a result of these distinctions, it is recommended that the female voice not be considered a mere diminutive of the male vocal tract. It has been proposed that the female adult provides a more trustworthy normative model of voice than the male. Meanwhile, research into the role of genetics in our vocal abilities was scant (Lennon, 2019).

Environmental factors such as temperature, humidity, and altitude can have a significant impact on a singer's vocal quality. It is important for singers to be aware of the environmental conditions they are performing in, as these factors can have a negative effect on their vocal performance. Especially, altitude can have a significant effect on vocal production in singers. Therefore, singers should be aware of the potential effects of altitude on their vocal production and take measures to ensure their vocal quality is not affected. (Clark, G. D., and R. L. Brown) Moreover, humidity has a significant effect on the vocal quality of singers. Singers should take extra care to protect their vocal folds by keeping them hydrated and avoiding prolonged exposure to high humidity. (Fujimoto, et al., 2018) Therefore, singers should take care to ensure that the temperature of the environment in which they are performing is conducive to their vocal quality. This can be achieved using air conditioning, heating, or insulation, depending on the environment. (Kedzierski et al. 2016)

In addition, environmental dietary factors, as well as certain environmental elements, can have a major influence on a singer's vocal quality. Overall, diet plays an important role in maintaining vocal quality for singers. Eating a balanced diet with adequate amounts of vitamins, minerals, and other essential nutrients, as well as avoiding foods and drinks that can weaken the vocal cords or impair singing performance, can help to maintain a healthy singing voice. It is important for singers to maintain a healthy diet, as well as protect themselves from environmental pollutants and excessive noise levels, to achieve optimal vocal performance.

Despite this, it is believed that shared musical genes like AVPR1A are a big contributing factor for a singer's voice. Furthermore, laryngeal anatomy DNA, like every other physical attribute, accounts for at least some vocal similarities across family members. However, because relatives often share an upbringing, it can be challenging to determine if an effect is genetic or has environmental roots (Lennon, 2019). Despite this, one genetic difference affects the voice: sex. Males often have more prominent vocal folds than females. The result is that even in early adulthood, men tend to have deeper voices than women (Lennon, 2019)

Voice is produced by the vibration of vocal cords, which are stretched along the larynx (the voice box) and move when air is drawn from the lungs. Their fundamental frequency is proportional to their length, size, and tension and is typically around 125 Hz in males, 210 Hz in women, and 300 Hz in children. The fundamental frequency is directly related to pitching; a higher frequency means a higher voice.

In conclusion, the quality of a Singer's Voice is a combination of both genetic and environmental influences. Vocal production is a complex process involving the respiratory system, phonatory system, and resonant system. The voice can be manipulated to produce a variety of sounds and qualities. In addition, sex hormones also play a role in the development of the voice, with males typically having larger larynges than females. Thus, both genetic and environmental factors contribute to the quality of a singer's voice. Therefore, singers should be aware of the factors that influence their voice and strive to improve both their technical and artistic skills to reach their fullest potential.

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Intraoral Appliance Therapy - A Better Alternative for Apnea than CPAP?

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Abstract

Obstructive sleep apnea (OSA) is a condition where there is a physiologic hindrance of airflow to the lungs, triggering the brain to interfere with the sleep cycle and awaken an individual to provide sufficient oxygen flow. For years, the continuous positive airway pressure (CPAP) machine has been the gold standard of care for patients suffering from OSA. Nonetheless, the machine has its flaws of being bulky, noisy, and other side effects, causing a low adherence rate and thus a lower relief rate of OSA symptoms. Accordingly, there have been many researchers seeking a more effective way to treat OSA, such as intraoral devices that manipulate the jaw and tongue placement to prevent pharyngeal airway collapse. The purpose of this paper is to compare the efficacy of continuous positive airway pressure (CPAP) and intraoral devices in the treatment of obstructive sleep apnea (OSA) based on the available evidence from original studies. To diagnose OSA, the studies used the Epworth sleepiness scale (ESS), the apnea hypopnea index (AHI), polysomnography (PSG), and the respiratory disturbance index (RDI). All studies found that, when adhered to properly, CPAP is more efficient in reducing OSA symptoms such as excessive daytime sleepiness, nighttime arousals, and hypertension, especially in more severe cases. Nonetheless, these studies also found CPAP to have lower adherence rates when compared to intraoral devices, influencing the effectiveness of the treatment. Furthermore, it was found that the margin for treatment relief between CPAP and intraoral devices decreased with a decline in OSA severity. This can be explained due to the fact that less severe cases do not require as rigorous treatment to control by symptoms. Accordingly, treatment planning needs to be individualized according to severity and expected adherence of each patient. In patients with mild-to-moderate OSA, intraoral devices seem to provide adequate relief of symptoms, while boasting a much higher adherence rate. Furthermore, in patients with severe sleep apnea, CPAP treatment continues to prove superior results. However, in patients who do not use the machine properly, intraoral devices may be considered.

Introduction

Almost nothing is as precious as a good night's sleep. It is essential in promoting optimal health and well-being. For adults aged 18–60 years, at least seven hours of sleep each night is recommended. However, in 2014, the CDC declared a sleep disorder epidemic within the United States based on a study that found that more than a third of American adults are not getting enough sleep on a regular basis, oftentimes due to sleeping disorders (Liu, et. al., 2014). These include insomnia, parasomnias like sleep walking, sleep related bruxism, snoring, obstructive sleep apnea (OSA), central sleep apnea, and several others. This is concerning, as aside from the immense economic impact, which is estimated at a loss of 411 billion dollars or 2.28% U.S. GDP, sleeping less than seven hours per night is associated with increased risk for diabetes, stroke, obesity, hypertension, coronary heart disease, and frequent mental distress (Hafner, et. al., 2017). In addition, insufficient sleep has been proven to impair cognitive performance, which can increase the likelihood of motor vehicle accidents, industrial accidents, medical errors, and loss of work productivity (Basner, et. al., 2017). Thankfully, there are several effective treatments, specifically for obstructive sleep apnea and snoring, that offer promising solutions to the sleep epidemic. They include behavioral modification (e.g., weight loss and alteration in sleep posture) or interventions such as maxillomandibular osteotomy, continuous positive airway pressure therapy (CPAP), intraoral appliances (IOA) like maxillary oral appliances, and mandibular advancement splints (MAS), also called mandibular advancement devices (MAD). This paper will specifically focus on CPAP and intraoral device treatments with the purpose of determining if intraoral device therapies provide superior alternative treatments when compared to CPAP machines in the treatment of OSA?

Methods

This document was written by researching peer reviewed scholarly articles and medical journals to assess the efficacies of CPAP and intraoral appliances. Online scholarly databases were searched for relevant articles, including Google Scholar and PubMed. While most of the material found is available to the public, many of the articles required special access, which was provided by Touro College.

Discussion

Diagnosing Sleep Disorders

There are several different tests that are employed in diagnosing sleep disorders. The present reference or "gold" standard is the polysomnogram (PSG). PSG is defined as the continuous monitoring and simultaneous recording of physiologic activities like eye movements during sleep, using a combination of continuous electroencephalogram (EEG) and electrocardiography. EEG records electrical cortical activity and calculates relative differences in electrical fields across brain regions to analyze sleep states, specifically rapid eye movement (REM) sleep, using body position sensors (Markun, et. al., 2020). The data collected and integrated in this method is termed the "sleep study."

Regarding sleep apnea specifically, the apnea-hypopnea index (AHI) is the primary measurement for diagnosis (Thornton, et. al., 2012). This is an average that reflects the total number of apneas and hypopneas that occur during

a single hour of sleep. The respiratory disturbance index (RDI) is a numeric index which helps to define the degree of apnea (Abeyratne, et. al., 2010). RDI is calculated as the number of apnea, hypopnea, and respiratory-effort related arousals per hour of sleep. A respiratory-effort related arousal is defined as a breathing abnormality detected by the EEG during a sleep study that does not fit the requirements for apnea or hypopnea and is instead considered an "arousal" event that is connected to a respiratory effort (Kushida, et. al., 2005). The diagnosis severity of an apnea patient for both the AHI and RDI is as follows:

- Normal Sleep: AHI/RDI < 5 events/hour
- Mild apnea: AHI/RDI between 5-15 events/hour
- Moderate apnea: AHI/RDI between 15-30 events/hour
- Severe apnea: AHI/RDI > 30 events/hour

Other common diagnostic tools used in detecting symptoms of OSA are the Epworth Sleepiness Scale (ESS) and Multiple Sleep Latency Test (MSLT), the former being the most common measure of subjective daytime sleepiness and the latter in detecting objective daytime sleepiness. The ESS is a validated eight-question survey asking patients to rate the perceived likelihood of falling asleep in various situations. The test is scaled from a score of 0 to 24, with 0 indicating no daytime sleepiness, 24 indicating the most severe sleepiness, and a score of 11 or greater the standard for determining significant sleepiness. For the MSLT, patients are instructed to try to fall asleep in a dark quiet room four or five times at two-hour intervals. The MSLT score is the average number of minutes required to fall asleep, which is measured by electroencephalography. Normal adults score between 10 and 20 minutes with anything below indicating sleepiness and below five indicating pathologic drowsiness.

Sleep Apnea

Two distinct respiratory conditions comprise sleep apnea-hypopnea syndrome: central (originating in the central nervous system) and obstructive apnea (involving collapse of the anatomical structures of the upper airway). In some instances, however, the conditions can be mixed (co-existing central and obstructive clinical symptoms). This paper will solely focus on the obstructive component of sleep apnea.

Obstructive sleep apnea (OSA) is a common disorder in the general middle-aged population, affecting approximately 2% of women and 4% of men (Young, et. al., 1993). Although nonobese individuals may suffer from OSA, obesity is a primary epidemiologic risk factor. In fact, increases in body mass index, neck circumference, and central accumulation of adipose tissue are effective predictors of disease (Young, et. al., 2004). According to American Academy of Sleep Medicine, OSA is defined as a respiratory condition that, despite continued breathing efforts, sees a reduction or cessation of airflow. When this happens during sleep, muscles around the pharynx such as the platoglossus and palatpharyngeus muscles relax, causing soft tissue in the back of the throat to collapse and obstruct the upper airway (Remmers, et. al., 1978). The human pharynx is uniquely susceptible to collapse due to the presence of a floating hyoid bone, a longer airway, and a less direct route for inspired air to travel. All these factors increase the pharynx's sensitivity to alterations in anatomically imposed mechanical loads (Patil, et. al., 2007).

As a result of an obstruction, there may be partial decreases (hypopneas) and/or total pauses (apneas) in breathing. Hypopnea is usually defined as a 25% to 50% reduction in oronasal airflow combined either with a reduction in oxyhemoglobin saturation or an arousal from sleep. Snoring, heart rate abnormalities, and paradoxical breathing are specific diagnostic criteria for this population group. Apneas, on the other hand, are defined as cessations in breathing lasting at least 10 seconds while asleep (Shiroh, et. al., 2009). Most apnea gaps last between 10 and 30 seconds but in severe cases can last for a minute or longer. This may lead to a sudden decline in blood oxygen saturation, with oxygen levels dropping by as much as 40% or more in extreme circumstances. The body will then alert the brain of the oxygen shortage, resulting in a short awakening from sleep before briefly restoring breathing. In a single night, this sequence can repeat itself hundreds of times, resulting in a disturbed sleep pattern that frequently causes excessive daytime sleepiness and hypoxemia. Most OSA sufferers snore loudly and regularly, stopping only when their airway is restricted or closed.

Treatments

Considering the detriment OSA poses on health and daily function, it is essential to seek out remedies. There are several ways to treat mild to severe OSA, but this review will primarily focus on CPAP and intraoral appliance therapies in terms of their effectiveness in treating OSA.

Continuous Positive Airway Pressure

Continuous Positive Airway Pressure (CPAP) machine is a medical device used to treat sleep apnea. The CPAP machine delivers a constant flow of air pressure through a mask that is worn over the nose or mouth, which helps to keep the airway open and prevent pauses in breathing. The CPAP machine consists of three main parts: a motor,

a mask, and a hose. The motor generates the airflow and is usually located in a small unit that can fit on a bedside table. The hose connects the motor to the mask. The mask can be either a full-face mask that covers both the nose and mouth or a nasal mask that covers only the nose. The pressure of the airflow delivered by the CPAP machine can be adjusted to suit the patient's needs. The doctor or sleep specialist will typically prescribe a specific pressure level based on the severity of the patient's sleep apnea (Chen, et. al., 2012). The most common side effects experienced when using CPAP include discomfort, dry mouth or nose, skin irritation, claustrophobia, gastrointestinal problems, mask leaks, and conjunctivitis.

CPAP has been proven by countless studies to drastically improve the symptoms of OSA such as snoring, daytime sleepiness, decreased blood oxygen saturation, AHI and hypertension. Accordingly, CPAP has been considered the "gold standard" of treatment for all severities of OSA. For instance, Patel and colleagues performed a meta-analysis on a diverse population, including twelve trials totaling 706 patients, and concluded that CPAP reduced the ESS score by an average of 2.9 points more than the placebo in patients with OSA. The treatment proved more effective for moderate to severe cases than those with mild OSA. This suggests that although the ESS score only slightly decreased, it is significant since the sample size was very large. Supporting these findings, another study on the long-term effects of CPAP on blood pressure in OSA patients concluded that CPAP does improve daytime sleepiness as well as significantly controls hypertension in patients with OSA. The 36 patients who received CPAP treatment scored on average over three points lower on ESS (a six-point decrease from baseline) than the control group. Also, hypertension control was improved in 69.4% of CPAP users compared to only 43.2% of control subjects over a 36-month range (Huang, et. al., 2015). While hypertension was controlled significantly, the patients in this study had much lower BMI's than most other studies. Obesity is an independent risk factor which may contribute to worsening of blood prssure control and is not affected by CPAP.

While it does seem that CPAP is very effective in improving daytime sleepiness, another analysis suggests otherwise. A study conducted on the effect of CPAP in normalizing daytime sleepiness and quality of life in patients with moderate to severe OSA, found that CPAP did not normalize daytime sleepiness responses despite seemingly adequate use for a substantial proportion of the 174 patients. In total, 40% of patients in the three-month-long trial had an abnormal ESS score at its conclusion. Of the patients who used CPAP for more than seven hours per night, 80.6% had a normal ESS score after treatment (Antic, et. al., 2011). This study reported a generally low overall nightly CPAP usage in 45% of patients, which can explain the failure of CPAP treatment to normalize ESS. However, this may not be the explanation, since even the 19% of patients with abnormal ESS pretreatment values in the subgroup that used CPAP for more than seven hours per night failed to have normal ESS scores after treatment.

This issue of non-adherence to CPAP continues to plague the treatment. A study conducted on CPAP adherence of patients with OSA found that of 903 subjects referred for a sleep study and CPAP treatment, only 248 continued to follow up for treatment after one month. They claimed to be adherent (using the Kribbs et al definition of adherence as ≥ 4 hours per night for at least 70% of the days which has been used in many studies). Within this population, their subjective adherence was 85.1%, and their objective adherence was 64.5%. While both groups reported many side effects, the objectively non-adherent group complained of adverse effects more frequently. There was around a 10% difference between the groups for patients bothered by machine noise, air leakage, dry mouth and nose, morning headache and sleep discomfort (Selepci, et. al., 2013). It seems that even within the seemingly most "compliant" group, as displayed by the fact they attended follow ups, 45% still did not adhere with their device use. This can most probably be explained by the higher percentage of side effects reported by the later sub-group. Nevertheless, another justification for the low ratio of patient follow-up may be due to the low social and economic status of the patients, making extended treatment unaffordable.

Intraoral Appliances

A mandibular device, also known as a mandibular advancement device (MAD), is a type of oral appliance used to treat OSA. The device is custom fit to the patient's mouth by a dentist or sleep specialist. It typically consists of two separate dental trays that sit over the upper and lower teeth, connected by metal hinges. The lower tray is designed to hold the lower jaw in a slightly forward position, which helps to prevent the tongue and soft tissues at the back of the throat from collapsing and obstructing the airway during sleep. Side effects are typically mild and temporary, rarely needing intervention, and include dry mouth, hypersalivation, jaw pain, and sensitive teeth upon awakening (Fritsch, et. al, 2001).

A randomized control trial was conducted by Gotsopoulos et. al. with the aim of evaluating the effect of a MAS on both objective and subjective daytime sleepiness and a range of other symptoms in OSA. The MAS device

featured a basic design with separate upper and lower acrylic appliances anchored onto the dental arches that cover the occlusal surfaces of all teeth. A screw system enabled incremental advancement of the jaw. In contrast, the control device consisted of the upper appliance alone, which had no protrusive effect on the mandible. Patients were informed that the aim of the study was to examine the efficacy of oral appliance therapy for OSA by comparing two appliances. All patients were above the age of 20 and had evidence of OSA on polysomnography (RDI $\geqslant$ 10/hour), while suffering from at least two of the following symptoms: daytime sleepiness, snoring, witnessed apneas, or fragmented sleep. The 73 patients who participated for the duration of the study period consisted of 59 men and 14 women and, as a group, were middle-aged and overweight. After undergoing a PSG, OSA severity subgroups revealed a predominance of moderate (with 41 patients (56%)) and severe (21 patients (29%)) OSA. Eleven of the original group only showed a mild OSA and were not included in the results. There were 38 patients (52%) considered subjectively sleepy, scoring greater than 8 on the Epworth Sleepiness Scale, a reliable and validated self-administered questionnaire (Rosenthal, Dolan, 2008). After randomization, group one consisted of 30 males and 6 females with an average BMI of 28.4[Equation]5.2 and group two consisted of 29 males and 8 females with an average BMI of 29.6[Equation]4.1.

At the conclusion of the study, the MAS devices proved to improve various sleep metrics. The most significant change was in the RDI which decreased by 15 ± 4 from 27 ± 2 disturbances per hour at baseline to just 12 ± 2 disturbances per hour when using an MAS device. The control group only showed a slight reduction to 25 ± 2 disturbances per hour. This amounts to a 52% reduction in mean RDI when using the MAS device. Furthermore, the MAS resulted in a substantial reduction in objective snoring frequency (207 ± 20 vs. 366 ± 21) and in both average and maximum snoring intensity. However, there was no significant difference in mean sleep efficiency or mean total sleep time. Objective daytime sleepiness improved appreciably during active treatment. The MSLT indicated that 35 patients (48%) demonstrated a normal MSL score with active treatment, compared to only 25 patients (34%) with the control treatment (a normal MSL defined as between 10 and 20 minutes to fall asleep). There was also a small improvement in subjective daytime sleepiness with the MAS when compared to the control device (7 ± 1 versus 9 ± 1 mean ESS score). Active treatment produced a normal ESS score in 60 patients (82%), compared with 45 patients (62%) on the control treatment. Interestingly, the control device still showed a significant reduction in subjective daytime sleepiness from the baseline (9 ± 1 versus 11 ± 1) (Gotsopoulos, et. al., 2002).

Although these results do demonstrate significant objective and subjective evidence of symptom improvement in patients with mild to severe OSA with MAS therapy, patients still did not reach the threshold of a normal RDI score. In addition, 52% of patients still scored abnormally on the MSLT. Both these factors can possibly be attributed to the short treatment of 4 weeks, as longer treatment may yield better results. Also, the two-point decrease in the ESS score may not be clinically significant, since it is only a slight decrease in a small sample size and, as with any subjective report, the main limitation of the ESS is that it is open to response bias. However, given that the placebo used was very convincing in appearance to the active treatment and still there was a 20% discrepancy in normal ESS scoring between the two groups, the slight decrease in ESS score may be relevant.

In a study aimed at testing the efficacy of CPAP versus oral appliance therapy in mild to moderate OSA patients, 114 patients were included over a three-month period. The placebo group was given pills. The subjects were middle aged (47.0 ± 0.9 years), predominantly male (80%) and overweight, with mild to moderate OSA (AHI, 5–30 per hour). CPAP adherence was objectively measured by an inbuilt meter and showed CPAP pump usage to be on average 4.2 ± 0.3 nights per week and for an average of 3.6 ± 0.3 hours per night. The MAS adherence was measured subjectively for 49 of the 85 subjects who completed MAS treatment via subject diary and reported an average of 5.3 ± 0.3 nights per week of usage for 5.5 ± 0.3 hours per night over the entire treatment period. 38 of 88 (43%) subjects treated with CPAP received adequate treatment, while 37 of 49 (76%) subjects treated with MAS (for whom there was usage data) received adequate treatment. Of the patients who started treatment with mild OSA, 28% preferred CPAP and 41% preferred MAS, as they found it easier to use (Barnes, et. al., 2004). These results demonstrate that while both treatments were more effective than the placebo in improving quality of life and subjective (but not objective) sleepiness, neither treatment proved better than the other since both reported a 9.2 score on ESS (baseline was 10.7). In addition, although usage of the MAS device was reported to be significantly higher than CPAP, CPAP was superior to the MAS in treating obstructive sleep breathing events with the CPAP scoring a 4.8 AHI and MAS 14 (Baseline was 21.3). This can be easily explained by the fact that the MAS device usage was subjectively reported leading to bias. Also, although subjects reported that CPAP was the most difficult treatment to use, they felt that it was the most effective.

A much more recent study however, found that there is no clinically relevant difference between MAD and CPAP in the treatment of mild/moderate OSA. Of the 57 patients who completed the six-month trial, only those assigned to treatments saw drastic improvements in AHI and RDI, with CPAP displaying a decrease of 19.5 and 13.5 respectively and the MAD displaying a decrease of 16.3 and 13 points respectively. In addition, snoring had decreased more frequently in the MAD group and had disappeared more frequently in the CPAP population. Regarding usage of appliances, the MAD group utilized their appliance 90.6% of the nights and the CPAP group used theirs 82.9% of nights (Arab, et. al., 2010). This indicates that patients were more likely to adhere to the MAD usage than the CPAP machine. Furthermore, although there was a small sample size in this study, the longer treatment time in this trial was a tremendous strength. In addition, the previous trials titrated (the process of determining the proper air pressure for CPAP and protrusion for MADs, by gradual manipulation) the CPAP objectively while the MADs were titrated by their dentist creating the possibility of bias in the evaluation of improvement. This trial, on the other hand, titrated both CPAP and the MAD as objectively, as PSG recordings were made for each MAD patient, thus reducing biases.

Conclusion

These studies do suggest that although intraoral devices are effective treatments of OSA, especially for mild to moderate severities, they are slightly less effective than CPAP in decreasing AHI, RDI, MSLT and ESS scores as well as in controlling hypertension. Nonetheless, clinical outcomes for CPAP and intraoral devices are very similar for mild to moderate severities. Moreover, the major difference between devices is with adherence and side effects. Patients find it very difficult to use CPAP due to factors such as discomfort and machine noise during use. Conversely, patients find the intraoral devices more comfortable and easier to use. Side effects for the intraoral devices were usually mild and temporary, while with CPAP they persisted with use. Accordingly, treatment planning needs to be individualized according to severity and expected adherence of each patient. Since intraoral devices are easier to adhere to and are only marginally less effective than CPAP for mild-to-moderate OSA, they are a superior solution over CPAP. For severe cases of OSA, CPAP is still the preferred course of treatment since it is demonstrably more successful at reducing symptoms than intraoral devices. However, in patients who do not use the machine properly intraoral devices may be considered.

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Is Intermittent Fasting a Better Option than Continuous Calorie Restriction?

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Abstract

Intermittent fasting has become an increasingly popular diet for weight loss in the United States in the past ten years. Proponents of intermittent fasting claim that restricting calories via a fasting period yields more significant weight loss and better improvements in biomarkers for longevity than restricting calories continuously. The goal of this paper is to determine whether intermittent fasting leads to weight loss and is more beneficial than continuous calorie restriction. The paper first details the physiological processes which can occur during fasting that are theorized to render fasting advantageous. It then reviews the limited available research that experimentally compares intermittent fasting with continuous calorie restriction. Based on the review, it concludes that intermittent fasting is just as effective as continuous calorie restriction in causing weight loss and other health improvements. Intermittent fasting also does not lead to any short-term detrimental effects. There is not enough substantial evidence to assume other than these conclusions. The work also details some risks associated with intermittent fasting. More, higher-quality studies are necessary to confirm the findings of this review.

Introduction

Plutarch, an ancient Greek writer once wrote, "Instead of using medicine, better fast today". Fasting, the abstinence from all or some kinds of food, has been practiced for spiritual reasons for centuries. It remains a part of almost every major religion today. Fasting has long been theorized to induce an improvement in health, slowing of aging, and the delayed onset of disease. Paracelsus, one of three fathers of modern Western medicine wrote, "Fasting is the greatest remedy—the physician within" (Vasim et al., 2022). Recently, fasting has become more common as a method for weight loss. Maintaining a healthy body weight is essential to living a long, healthy life because being obese or even overweight increases a person's risk of heart disease, stroke, type 2 diabetes, certain types of cancer, and mental illness (Centers for Disease Control and Prevention, 2022). Unfortunately, obesity and overweight in the United States are as prevalent as it has ever been. According to 2017–2018 data from the National Health and Nutrition Examination Survey (NHANES), almost one-third of American adults (30.7%) are overweight and more than 2 in 5 adults (42.4%) are obese (U.S. Department of Health and Human Services). This creates the need for effective, safe, and maintainable diet and weight loss programs. One effective and safe technique to help people lose body fat is a continuous daily calorie restriction. Current recommendations for the treatment of overweight and obesity is a daily consumption of only 70-80% of daily weight maintenance requirements. Over a 6-month period, this approach normally yields a modest weight loss of 5-10% (Das et al. 2017). However, many people find it too difficult to constantly count calories. Because of this problem, a new fad called Intermittent Fasting has become increasingly popular. Intermittent fasting refers to a diet regimen in which a person has consistent periods of extremely limited caloric intake followed by an eating window. It can involve a fast on 2 nonconsecutive days each week, a 24-hour fast on alternate days, or a daily fast for 16 hours. The advantage of intermittent fasting is that the program does not restrict one's calorie intake during the eating window (Cienfuegos, et al. 2020). The goal is to yield a net calorie deficit in a more sustainable way. Proponents of intermittent fasting allege that the program is not only more effective than a continuous calorie restriction program in facilitating weight loss, but that it also improves biomarkers related to longevity (Anton et al., 2018). Is intermittent fasting more advantageous than a moderate continuous calorie restriction? This paper will discuss if there is sufficient scientific evidence to determine whether intermittent fasting is more effective than continuous calorie restriction in helping achieve a healthy weight, and whether fasting provides other health benefits beyond energy deficit and weight loss.

Methods

Data was collected using ProQuest, Google Scholar, and PubMed databases through Touro College's online library. Among the key phrases used were "intermittent fasting," "calorie restriction," "time restriction eating," and "weight loss."

Discussion

It is well known that restricted calorie intake is an effective way to lose body fat (Das, et al. 2017). This means reducing the intake of average daily calories, while still getting the nutrition that the body needs. Beyond weight loss, restricted calorie intake also has been linked to slower aging, longer lifespans, and better health biomarkers in animals (Masaro, 2000). For example, caloric restriction has been shown in animals to slow cancer formationl, reduce the likelihood of stroke, and increase the brain's ability to resist neuron deterioration and dysfunction in models of Alzheimer's and Parkinson's (Bruce-Keller et al., 1999; Mukherjee et al., 2002).

Research has suggested that dietary restriction facilitates health benefits for humans as well. For example, in a long-term study with normal and slightly overweight adults, Das, et al. (2017) found that a 25% calorie restriction led to a loss of fat mass and better overall markers

for heart and metabolic health. These indicators included LDL, cholesterol, insulin sensitivity and blood pressure. Intermittent fasting is a diet technique designed to allow one to restrict their average calorie intake more easily than one that continuously restricts calories.

There are some issues involving the effectiveness of intermittent fasting for weight loss that must be investigated. One issue is that fasting may cause the participant to engage in less physical activity and calorie expenditure ,as one feels weak while fasting. Also, periods of fasting may cause the body to hold on to fat in preparation for continued malnutrition. Furthermore, intermittent fasting may not lead to an average calorie intake deficit at all. When one can only eat at certain times, they may compensate for this energy deficit by eating more during the eating window. Additionally, people also tend to indulge more with the knowledge that they will be restrained soon. Critics of intermittent fasting use these arguments to claim that the diet may lead to an overall calorie surplus and weight gain (Templeman et al., 2021).

This discussion will first detail the potential theories behind the belief that intermittent fasting is more advantageous than continuous calorie restriction. It will then examine if an intermittent fasting regimen facilitates a net calorie restriction and weight loss in people. Then, the claim that the fasting period component of intermittent fasting offers an advantage will be reviewed, with a focus on weight loss and cardiovascular and metabolic disease risk factors. When reviewing this last claim, any evidence being cited must have produced results that are independent of calorie restriction. This way, it can be determined that the fasting period alone is responsible for the observed differences. Any studies that fail to make this distinction will not be discussed.

The Theories Behind the Advantage of Intermittent Fasting

There are two components of intermittent fasting - a net calorie deficit and a fasting period. Advocates of intermittent fasting insist that intermittent fasting naturally will lead to a decrease in net calorie intake, as there is a period in which one cannot consume food (Vasim et al., 2022). There are different theories to explain why the second component, the fasting period, which does not exist in continuous calorie restriction, may be more effective in causing weight loss and other health biomarkers beyond calorie deficit.

Intermittent fasting may more effectively flip the "metabolic switch" than continuous calorie restriction (Anton et al., 2018). This switch is when the body uses stored fat for energy instead of glucose. The idea behind this is that by restricting food for extended periods of time, glucose levels in the blood drop, forcing the body to use fat stores more quickly and efficiently for energy (Puchalska & Crawford, 2017). This shift from the use of glucose from glycogenolysis to the use of fatty acids and fatty acid-derived ketones is what leads to weight loss. After long periods of food deprivation, the body stops lipid synthesis and fat storage. Instead, the body utilizes fat in the form of free fatty acids and fatty-acid-derived ketones. The metabolic switch happens during fasting because glycogen stores in hepatocytes are low and subsequent adipose tissue lipolysis generates fatty acids and glycerol (Cahil, 2006). The time it takes for the switch to happen depends on liver glycogen content and on the amount of energy used while fasting. Yet, the metabolic switch usually happens between 12 to 36 hours after a fast starts. First, fats (triacylglycerol and diacylglycerol) are broken down into free fatty acids. After they are released into the blood, the free fatty acids are then transferred into the liver's hepatocytes where they are metabolized to produce ketones (Gano et al., 2014). At the same time, other cell types, including astrocytes in the brain, may also start producing ketones. The ketones are taken into cells where they are processed to make acetyl coenzyme A, which then enters the Krebs cycle to produce ATP. By way of these physiological processes, ketones are the energy source for muscle and brain cells during fasting (Cahil, 2006). This means that during fasting, the major energy source the body utilizes is ketones instead of glucose. Because research indicates that ketones are the preferred fuel for both the body and brain (Puchalska & Crawford, 2017; Volek et al., 2015), this can mean that fasting can be beneficial to the body.

Another theory states that autophagy is greatly enhanced during fasting. Autophagy is defined as "a process by which a cell breaks down and destroys old, damaged, or abnormal proteins and other substances in its cytoplasm" (Koutouroushis & Sarkar, 2021). Fasting, by providing a short nutrient deprivation, forces cells to focus on maintenance and to be more efficient with less. This causes cells to become more adept at cleaning themselves and recycling components. The effects of this upkeep bolster mental and physical performance in the fed state as well. Some researchers, such as Kanasaki et al. (2019), suggest that it affects the hormones that control hunger, such as ghrelin, insulin, and glucagon. In a study involving fruit flies, Ulgherait et al. (2021) found that maintenance of a 20-hour daily fasting diet for 30 days, without net calorie restriction, resulted in greater autophagy and consistent, significant lifespan extension in fruit flies.

Finally, studies show that intermittent fasting tends to

improve the gut microbiome which is linked to the improvement of many age-related diseases (Zarrinpar et al., 2014). A healthy gut microbiome helps with digestion, destroys harmful bacteria, and helps control one's immune system (How Your Gut Microbiome Impacts Your Health, 2022). These show potential benefits which include reduced inflammation, higher microbial diversity, and the making of favorable microbial compounds in the form of fatty acids (Liu et al., 2020). These compounds affect the expression of the circadian rhythm genes in the liver. In this way, intermittent fasting is believed to restore the body's normal circadian clock. This has been shown in studies involving mice (Zarrinpar et al., 2014). This reset of the body's internal clock is theorized to allow the body to effectively manage metabolism, sleep, and behavior. With the set schedule that intermittent fasting provides, the dieter is able to take advantage of this reset internal clock by eating consistently when the body is ready for it.

Intermittent fasting has also been shown to decrease measures of assumed obesogenic microflora while increasing the amounts of assumed obesity-protective microflora (Zarrinpar et al., 2014). Ozkul et al. (2020) found that nine people had higher levels of beneficial gut bacteria, including Akkermansia, Faecalibacterium , and Roseburia, after Ramadan. Ramadan is the Muslim holy month in which individuals fast from dawn to sunset. However, this study was limited in sample size and was unable to determine the impact of weight loss and diet on these changes, thus leaving the effects of potential contributing factors unclear.

The extent of the occurrence of the mechanisms mentioned here may vary based on duration of the fast. However, due to limited research available, all types of intermittent fasting will be grouped together to review if fasting has been shown to yield any beneficial results.

Intermittent Fasting For Weight Loss

Many studies have shown that an intermittent fasting diet regimen is effective in decreasing calorie intake causing weight loss. One study found that a 36-hour fast did not lead to compensation of energy the following day in 24 lean adults (Johnstone et al., 2002). Although it was only one fast period, it shows evidence against the claim that fasting leads to subsequent overeating. However, the fact that these were lean adults might have influenced these results. People who are overweight are more likely to overindulge during the eating window.

In another study, researchers set out to determine whether intermittent fasting successfully leads to calorie deficit without calorie counting, and whether it is a worthwhile option for weight loss. The study explored the effects of eight-hour time-restricted feeding on body weight and metabolic disease risk factors in obese adults. For over 12 weeks, 23 obese subjects participated in a daily 8-hour time-restricted feeding intervention. The subjects ate whatever they wanted during their eating window. Controls (n=23) were told not to change their eating habits and to maintain their weight. The intermittent fasting group had a decrease in body weight by ~3% and energy intake by 341 kc/d compared to controls. This study shows that intermittent fasting produces a mild caloric restriction and weight loss without calorie counting (Gabel et al., 2018).

Similarly, others also concluded that intermittent fasting is an effective diet approach for the reduction of HbA1c and is similar to continuous calorie restriction in its ability to help one lose weight. Researchers compared the effect of intermittent fasting with continuous calorie restriction on glycemic control and weight loss in overweight and obese patients with type 2 diabetes. For 12 months, 97 participants with type 2 diabetes either utilized an intermittent fasting diet for 2 nonconsecutive days per week (n=51) or a 30% continuous calorie restriction diet (n=46). Both groups experienced ~5-6 kg in weight loss. Similarly, Cienfuegos et al. (2020) also demonstrated that 35 obese participants on a 4 and 6-hour per day intermittent fasting regimen, yielded a ~550 kcal/d energy restriction and weight loss (~3%) after 8 weeks (Carter et al., 2018).

Those who are opposed to intermittent fasting for weight loss point to a recent study that found that intermittent fasting offers no benefits whatsoever in people. Lowe et al. (2020) set out to determine the effect of 8-hour daily intermittent fasting on weight loss and metabolic risk markers. In this 12-week randomized clinical trial, 116 adults were randomized into two groups. One group was told to eat their daily calorie requirements in three structured meals per day. The intermittent fasting group was instructed to eat ad libitum from 12:00 pm until 8:00 pm and refrain from eating from 8:00 pm until 12:00 pm the next day. There was significant weight loss in the intermittent fasting group; however, there was no difference in weight loss between the two groups. Not only that, but there were also no meaningful observed differences in fat mass, fasting insulin, blood sugar, HbA1C, or blood lipids between the groups. Time-restricted eating did not even result in less energy intake. Moreover, people in the intermittent fasting group also lost on average about 3.5 lbs. of lean mass. Normally, only about 20-30% of total weight loss is lean mass but here, the proportion of lean mass loss was 65%. There are potentially other factors that caused this loss of lean

mass. It is possible the participants did not eat enough protein or engage in enough physical activity. The shift in activity can lead to dwindling lean mass. More research is required to account for these factors.

The researchers conclude that fasting does not lead to an overall calorie deficit and is not more effective in causing weight loss than eating regular meals throughout the day. They claim that the weight loss of the three-meal group indicates that short-term weight loss can result from just participation in a weight loss study alone. However, this conclusion is flawed because perhaps eating three structured meals each day on a set schedule utilizes the body's circadian rhythm to more efficiently metabolize fat as well. Although this study had a relatively large sample size with a wide range of BMI among participants including both men and women, it does conflict with many studies that do show that intermittent fasting does indeed lead to a calorie deficit and weight loss. An earlier, more advantageous eating window, would perhaps lead to compatible results as people tend to eat more at night. This study should be repeated to account for any errors.

Intermittent Calorie Restriction vs Continuous Caloric Restriction

Contrary to widespread belief, most research has found that a fasting period, although not detrimental, does not cause more weight loss than continuous calorie restriction. However, there is a lot of conflicting research about fasting and its ability to offer metabolic benefits beyond weight loss.

In one important study, Anson et al., (2003) showed that a fasting period leads to health benefits similar to continuous calorie restriction, even without overall energy intake deficit or weight loss. C57BL/6 mice were placed in one of three groups: fed ad libitum, alternate-day intermittent fasting, and daily calorie restriction. Throughout the twenty-week trial, mice in the intermittent fasting group ate almost the same amount of food as the mice fed ad libitum. This is because, on feeding days, they ate about twice as much as the fed ad libitum mice ate. However, no application about energy compensation can be made to other animals because researchers point out that this is a specific characteristic of the strain of mice they used and does not occur in other mice. The mice in the daily calorie restriction ate 60% of the energy eaten by the ad-libitum-fed animals and had a 49% lower body weight. In contrast, the mice that intermittently fasted had only slightly lower body weights than those in the ad libitum-fed group. Yet, the intermittent fasting mice did have improvements in glucoregulatory health indicators. In the mice fed ad libitum, the fasting serum concentrations of glucose and insulin averaged 150 mg/dl and 3,400 pg/ml, respectively. In contrast, in the mice of both the daily restriction and intermittent fasting groups, the concentrations of glucose and insulin decreased drastically to 100 mg/dl and 700–1,100 pg/ml, respectively. Increased insulin sensitivity is a key beneficial physiological change that happens in mammals when their calories are restricted. Decreased fasting plasma levels of glucose and insulin reflect this change.

When reviewing this study, however, it is important to note that throughout the twenty weeks, there was some overall energy restriction in the intermittent fasting group. This energy restriction, which may have led to the group's slight weight loss, may have contributed to the favorable results. However, the negligible difference in energy consumption, although it perhaps contributed, could not have been solely responsible for the very favorable insulin resistance data. This animal study indicates that although the fasting period itself does not cause weight loss, it can still facilitate the beneficial effects of caloric restriction in regard to glucoregulatory health.

Harvie et al. (2010) also found that intermittent fasting is better for cardiovascular and glucoregulatory health in humans. Over a 6-month period, two groups of obese premenopausal women were instructed to, every week, consume only 75% of the weekly calories required for weight maintenance. However, the two groups accomplished this with two different eating schedules. One group (n=47) did so by consuming only 75% of their daily calorie needs each day. The other group (n= 42) did so with an intermittent energy diet by consuming only 25% of their daily energy needs on two nonconsecutive days while eating the rest of their allowed weekly calories on the other days. Importantly, intermittent energy did not lead to overeating on the other five days and weight loss was similar between the groups. In the intermittent fasting group, weight reduced from a mean of 81.5 kg to 75 kg compared to a decline from 84.4 kg to 78.7 kg in the continuous calorie restriction group. The two groups had similar drops in body fat, hip, bust, and thigh circumference, and composition of weight loss. Both groups experienced mild reductions in fasting serum insulin and improvements in insulin sensitivity. However, these reductions were greater in the intermittent fasting group. Correspondingly, there was a moderate rise in adiponectin levels in the intermittent fasting group, but not in the continuous calorie restriction group. Adiponectin is a hormone that helps with insulin sensitivity and inflammation. Adiponectin is important because its anti-inflammatory properties protect the vascular system, heart, lungs, and colon (Adiponectin: What It Is, Function & Levels). Also, slow-acting AOPP (Advanced Oxidation Protein Product), which is a protein product that promotes oxidative stress, the imbalance of

free radicals and antioxidants, (Advanced Oxidation Protein Product - an overview), decreased in the intermittent fasting group and had a slight increase in the continuous energy restriction group. Both diets yielded similar reductions in total and LDL cholesterol, triglycerides, and blood pressure. This study demonstrates that in humans, a fasting period may be more effective in increasing insulin sensitivity and adiponectin while reducing oxidative stress and inflammation. However, a few things should be taken into account when reviewing this study. The study only included women. Also, energy intake records were based on self-reporting, which means that they were not entirely accurate. On the other hand, in contrast to traditional intermittent fasting, subjects were allowed to eat 25% of average daily calories on "fasting" days. It is possible that completely fasting on those days would result in even more drastic health improvements than observed in this study.

Gabel et al. (2019) found similar results. The first 6 months of the year-long trial consisted of weight loss followed by 6 months of weight maintenance. Subjects with insulin resistance were grouped into an intermittent fasting group and a continuous calorie restriction group. Both groups achieved a 25% calorie restriction per day during the first 6 months. The intermittent fasting group (n=11) participants consumed 25% of their daily calorie requirements on fast days, and 125% on alternating feast days. Continuous calorie restriction participants (n=17) consumed 75% of their energy needs every day. During the next 6 months, participants were instructed to consume an additional 25% every day to reach the energy intake required for weight maintenance. Controls (n=15) did not change their eating habits throughout the twelve months. At the end of the year, reductions in weight were similar (~6-8 kg) between the intermittent fasting group and the continuous calorie restriction group. However, the intermittent fasting group had a bigger reduction in fasting insulin by month 12 (−52%) than the continuous calorie restriction group (-12%). Intermittent fasting also yielded a greater decrease in HOMA-IR (a marker of insulin resistance) by month 12 (−53%) when compared to continuous calorie restriction (-10%). However, there were no significant differences detected between the two groups regarding other metabolic disease risk factors, including blood pressure, plasma lipids, and inflammatory mediators. This study is very limited by its sample size.

Sutton et al. (2018) also found that intermittent fasting can improve glucoregulatory health and blood pressure. In this 5-week randomized trial, eight overweight, prediabetic men were split into two groups. One group adopted an intermittent fast schedule with a 6-hr daily eating period while the other group had a 12-hr daily eating window. The two groups followed diets that were designed to maintain their weight and participants were required to eat only the food provided. All meals were monitored by study staff to account for potential differences in food intake or meal frequency. At the end of a 7-week washout period, intermittent fasting did not alter glucose levels; however, it did lower fasting insulin by 3.4 – 1.6 mU/L. and insulin resistance by 36 – 10 U/mg. The intermittent fasting also lowered blood pressure but had no effect on arterial stiffness or cholesterol. Researchers say the effect of the lowered blood pressure was similar in effectiveness to anti-hypertensive medications such as angiotensin-converting enzyme (ACE) inhibitors. Intermittent fasting also reduced oxidative stress but did not affect inflammatory markers. The study also found that intermittent fasting reduced hunger in the evenings. This study suggests that intermittent fasting, even when there is no calorie deficit or weight loss, can improve insulin levels, blood pressure, insulin sensitivity, and oxidative stress levels. This study was specifically well done because it did not rely on self-reporting for calorie intake. However, this trial was extremely small and must be repeated in a larger trial that includes women.

There are many stronger studies, however, that find that a fasting period offers no health benefits.

In the study conducted by Carter et al. (2018) detailed above, weight loss and HbA1c levels between the continuous and intermittent fasting restriction groups were similar. Trepanowski et al. (2017) also found that intermittent fasting is not more advantageous than daily calorie restriction in aiding with weight loss and health biomarkers. Over a one year period, 46 obese participants spent 6 months on a weight loss regimen followed by 6 months on a weight maintenance regimen. For all participants, the weight loss regimen involved a 25% overall average calorie restriction, while the weight maintenance regimen involved no overall calorie restriction. However, the 46 participants who completed the study were in one of two groups. The alternate-day fasting group (n=21) was instructed to consume an average 75% of their daily calorie requirements using alternate-day fast (25% on fast days, 125% on eating days). The other group (n=25) used a daily 25% calorie restriction. During the 6 months of weight maintenance participants in both groups were to consume 100% of their requirements by adding 25% daily from the previous six months. An additional 23 participants made up the control group. Based on the data, both experimental groups experienced similar physical activity and consumed equal amounts of energy throughout the year. However, weight loss was similar between the intermittent fasting group and the daily calorie restriction group at months six (~-6.8%) and twelve

(-~5-6%). Weight regain from months 6 to 12 was also not substantially different between the alternate-day fasting group and the daily calorie restriction group. At months 6 and 12, there were no statistically significant differences between the intermittent fasting group and the daily calorie restriction group for blood pressure, heart rate, total cholesterol levels, triglyceride levels, high-sensitivity C-reactive protein, homocysteine levels, fasting insulin, fasting plasma glucose, fat mass, lean mass, or visceral fat mass. The results of this randomized clinical trial revealed that intermittent fasting did not lead to greater adherence, weight loss, or weight maintenance than continuous calorie restriction. It also did not show any improvement in indicators of cardiovascular or metabolic disease risk factors compared with continuous calorie restriction. This study is especially critical because of its sample size and because it compares intermittent fasting and continuous calorie restriction in the context of consuming the same amount of calories. It also backs the claim that intermittent fasting does not cause lean mass loss or less physical activity.

However, this study has a slight limitation. Researchers point out that the intermittent fasting group ate more than prescribed on fast days, and less than prescribed on feast days. This means the "fast days" were not actual fast days. Failing to adhere to restrictions on fast days, in essence, transforms an intermittent fasting diet into a continuous calorie restriction diet. It, therefore, becomes more difficult to draw any conclusion from this study about a subject who will actually adhere to an intermittent fasting diet by consuming very little on fast days.

However, another well-done recent study, Liu et al., (2022), found similar results. 139 Chinese overweight and obese adults were randomly assigned to either restrict calories by 75% using a daily intermittent fast, eating from 8am-4pm, (n=70) or a daily-calorie-restriction regimen (n=69) for 6 months. This time frame was chosen to coincide with the Chinese participant's biggest meal. This weight loss program was followed by 6 months of weight maintenance. By the end of 12 months, physical activity and average caloric deficit were similar in the two groups, although these were based on self-reporting. However, weight loss (6-8kg), fat mass and lean mass loss, and reductions in waist circumference were similar between the groups. There was also no difference in fasting glucose, lipids, and insulin levels along with blood pressure. Additionally, side effects including fatigue, dizziness and headaches were similar between groups.

These results back the last study and show that although fasting does not affect physical activity or lead to higher calorie intake, it does not facilitate weight loss beyond calorie deficit. This study also demonstrates that an intermittent fasting regimen does not lead to a greater loss of lean mass or weakness if followed with a proper diet. The strengths of this study, including sample size and duration of the diet, show that intermittent fasting is essentially the same when considering all potential effects of dieting.

Although most studies show that intermittent fasting is as effective as continuous calorie restriction, there are two that suggest otherwise.

Templeman et al. (2021) concluded that that a calorie deficit achieved while fasting can be less effective than continuous calorie restriction for weight loss. For 3 weeks, 6 lean, healthy adults were equally divided into three groups of 12. The first two groups consumed a net 75% of the required calorie requirements. Group 1, the continuous calorie restriction group, did so by consuming 75% of their usual daily calorie intake every day. Group 2 fasted on alternate days and consumed 150% of their usual calorie intake on other days. The third group, Group 3, did not undergo any overall calorie restriction and only utilized a fasting period. Although they fasted on alternate days, they ate 200% of their normal calorie intake on eating days. This design allowed researchers to determine if fasting itself without a change in energy intake contributes to weight loss. At the end of the study, those in Group 1, lost an average of 1.91 kilograms of body mass almost entirely due to fat loss while those in Group 2 lost an average of 1.60 kg with only half of the reduction due to loss of body fat. Group 3 lost an average of 0.52 kg with a minimal loss of body fat. All metabolic health levels such as blood sugar levels, cholesterol, and blood pressure or fat tissue gene expression remained constant among the study participants. This study is especially important in determining whether the fasting period itself can aid in weight loss. On one hand, Group 1 lost 1 kg more body fat than Group 2. This would suggest that consuming the same calorie amounts while fasting leads to less fat mass loss. However, the third group that only used intermittent fasting as a means of weight loss, managed to lose a minimal amount of fat mass over the three weeks. This indicates that the fasting period doesn't negatively affect fat mass loss. Researchers suggest that the difference between Group 1 and Group 2 is partly due to a reduction in physical activity in Group 2. They also determined that intermittent fasting does not lead to beneficial cardiovascular or metabolic effects. This is an invalid conclusion because the weight loss also did not either improve the metabolic and cardiovascular health of the participants. The researchers themselves say that there were no improvements because the participants were lean at the start of the trial.

Although this study was brilliantly designed, it was

extremely short in duration and small in sample size. This study involved only lean healthy participants, which may be the reason fasting did not result in significant weight loss. The body of a lean person may not switch to ketosis as readily as the body of an overweight or obese one. It is likely because of this reason that this study conflicts with the others. However, this study, demonstrates that fasting is not more effective in causing weight loss than continuous calorie restriction in lean individuals. The weight loss from Group 3 is too insignificant to support that fasting itself is a means of weight loss. Additionally, this weight loss can be attributed to participating in a weight loss study. This trial should be repeated with a longer experimental period and a larger sample size.

Another study (Catenacci et al., 2016) suggests that utilizing intermittent fasting may be less beneficial than continuous calorie restriction when it comes to weight loss, but more beneficial when it comes to weight regain. Twenty-six obese adults completed a diet of either a zero-calorie alternate-day fast or continuous calorie restriction for 8 weeks. The continuous calorie restriction group was given a regimen, with meals, designed to create a 14% deficit from estimated daily energy requirements. The intermittent fasting group was not allowed to eat on fast days but was allowed to consume as much as they wanted on the other days. Results were reviewed at the end of the 8-week intervention and after 24 weeks of follow-up. Importantly, over the 8 weeks, self-reported average daily energy deficits were considerably higher in the intermittent fasting compared to the continuous calorie restriction, 47 and 28%, respectively. However, absolute weight loss was similar between the groups. To explain the lack of correlation between energy deficit and weight loss in the intermittent fasting group, researchers suppose that the groups underreported food intake. Additionally, fasting may have led to less physical activity and energy expenditure than in the continuous energy restriction group. There were no differences in weight regain between the 8-week intervention and the 24-week follow-up; however, the intermittent fasting group gained more lean mass and less fat mass than the continuous calorie restriction group. These results suggest that intermittent fasting may have generated metabolic or hormonal changes that resulted in a favorable pattern of weight regain. This study also demonstrates that intermittent fasting is effective for weight loss.

At first glance, the study supports intermittent fasting over continuous calorie restriction. However, an application can only be made to compare an alternate-day fast vs. a continuous calorie restriction diet regimen initially aimed to facilitate just a 14% daily calorie deficit. In fact, something the researchers fail to point out is that the continuous restriction group doubled the original 14% goal of energy deficit throughout the eight weeks. This is significant and indicates that a more ambitious 25% daily restriction goal can be more advantageous than intermittent fasting. However, the continuous energy restriction group in this study was not burdened with making healthy food choices, choosing appropriate portion sizes, and most importantly ,counting calories. These factors may have led to more favorable results than those which would occur in a real-life situation. This study should be repeated with a bigger sample size and with controls to account for these factors. For the purposes of this work, this study shows that an intermittent fasting regimen is safe and leads to a major overall energy deficit. It conflicts with other studies by suggesting that intermittent fasting may lead to less weight loss than would be expected based on reported energy deficits.

Intermittent Fasting and the Brain

Although there are many studies indicating that intermittent fasting can lead to improved brain health and resistance to neurological disorders in animals (de Cabo & Mattson, 2019), most of them do not control for calorie restriction to isolate the effects of fasting. However, in one study, Baik et al., (2020) determined that intermittent fasting increased markers for neurogenesis in the hippocampus of groups of mice that intermittently fasted for three months. The hippocampus is responsible for learning and memory. Mice that were grouped into intermittent fasting groups were deprived of food every other day for either 12 or 16 hours. The other group had no calorie restriction and ate ad libitum. The fasting mice showed higher amounts of specific protein markers than the ad libitum mice did. This indicates that the mice that fasted were creating new neurons at a faster rate and more efficiently. Researchers claim that the data indicated that there was no significant difference in the overall energy intake between groups. This means that the markers for neurogenesis were detected due to fasting alone. However, the researchers fail to include this data, which is unfortunate considering the significance of this claim. More animal studies like this must be carried out to further explore these findings.

Intermittent Fasting- Mood, Sleep and Alertness

Roky et al. (2000) explored how Ramadan intermittent fasting affected daytime alertness, mood and oral temperature in ten healthy subjects. Ramadan is the month in which Muslims do not drink and eat daily between sunrise and sunset. Researchers found that daytime oral

temperature, alertness, and mood decreased during fasting. However, it is fair to assume that this negative consequence was not a result of the fasting itself but rather a consequence of poor sleep habits during Ramadan. Eating and drinking only at night is disruptive to sleep patterns and the maintenance of a healthy circadian rhythm. Therefore, an intermittent fasting regimen that encourages healthy sleep habits, like an eating window between 12 AM and 8 PM, may not result in such negative effects. One review (McStay et al., 2021) looking at the influence of intermittent fasting on sleep found no overall negative effects. More research that accounts for sleep deprivation is required before any conclusions about alertness and mood can be made.

Risks of Intermittent Fasting

While there may be benefits to intermittent fasting, one must consider some possible negative ramifications. One of the topmost risks associated with intermittent fasting is dehydration. This still holds true even when one consumes non-caloric beverages during their fasting periods. Intermittent fasting can also put someone in a state of ketoacidosis which presents substantial health risks and can lead to hypoglycemia. Hypoglycemic symptoms include anxiety, irritability, low energy, and sleep disturbances. Furthermore, intermittent fasting may play a role in the development of AFRID (Avoidant/Restrictive Food Intake Disorder), especially if one starts intermittent fasting as a child or adolescent. In such cases, individuals will show a lack of interest in food consumption (Harding, 2021). Anybody with underlying health conditions should therefore consult with a doctor before starting an intermittent fast regimen.

Conclusion and Further Research

Based on the currently available research, intermittent fasting is on par with continuous calorie restriction in causing weight loss. Although it is not superior to continuous calorie restriction in causing weight loss, it is a safe and effective approach when done with proper diet and physical activity. Yet, there are conflicting studies on whether fasting offers additional benefits beyond weight loss. Most of the studies that suggest advantages in decreased blood pressure, insulin resistance, glucose levels, and stress levels are of low quality. These involve either a small sample size, self-reporting of food totals, or insufficient duration of the study. The stronger studies reviewed here found that although the fasting period does not hurt, it does not help either. They indicate that both regimens yield similar improvements in glucoregulatory, cardiovascular, and inflammatory biomarkers as well as changes in body composition. Therefore, one who feels that intermittent fasting is the easiest weight loss option should go ahead with it, as long as he makes sure to get proper nutrition and hydration. He should also first consult his doctor to make sure that he is aware of all the risks involved and that he is healthy enough to fast on a consistent basis.

Being that the current literature on human intermittent fasting is limited, more well-done research is required to fully validate the findings of this review. Additionally, a lot of questions remain. None of the current literature explores the long-term effects of fasting in humans, and there is a lot more to learn about the effects of different types of intermittent fasting. We also do not confidently know the effect that age and baseline health and weight have on the effects of fasting.

Long-term studies done in the future should be designed like the Templeman et al. (2021) conducted. They should involve sample sizes of at least 100 men and women from different backgrounds and different ages. To ensure accuracy, all calorie intake and physical activity should be recorded by researchers and not the participants themselves. It should also be repeated with participants with and without various cardiovascular and metabolic diseases who are lean, overweight, and obese. This may be very difficult to pull off practically, but these adjustments can go a long way to truly determine the effects of intermittent fasting.

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Rheumatoid Arthritis: Traditional Drugs vs. Alternative Medicine

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Abstract

Rheumatoid arthritis is a systemic autoimmune disease which is inflammatory and chronic. This disease affects the joint linings of those with this condition, which causes painful swelling. Complications of this disease can cause problems in the patients' eyes, skin, heart, lungs, and other organs. Being that there is no cure for Rheumatoid Arthritis, different treatment approaches are aimed to reduce the current pain and to slow further damage. In this paper, we will present a summary of past and present treatments to address the best possible treatment options for Rheumatoid arthritis. We will place an added emphasis on the use and effectiveness of alternative medicine and treatments used to treat this disease.

Introduction

Rheumatoid Arthritis is a progressive autoimmune disease which causes inflammation in the linings of the joints. Reasons behind the onset of rheumatoid arthritis include but are not limited to: genetics, environmental conditions (alcohol, smoking, birthweight), as well as hormones (Nazir, 2021). This disease initially affects small joints, and gradually progresses to large joints (Jacqueline Bullock, 2018). Rheumatoid arthritis can affect both sides of the body, which sets it apart from other forms of arthritis. The disease can start at one part of the body, such as the eyes, and eventually affect many parts and systems. The estimated global prevalence of rheumatoid arthritis is 0.2-1% (Won, 2018). The risk of developing rheumatoid arthritis is five times higher in women than men (Panel, 2004). Common symptoms include joint stiffness, swelling, tenderness, and nodules under the skin. Cases of remission are also highly evident with this disease. Treatments for Rheumatoid arthritis generally include immunosuppressive drugs, anti-inflammatory drugs, and steroids which can slow Rheumatoid arthritis progression and save the joints from permanent damage. These anti-inflammatories decrease the swelling and tenderness, which provides temporary pain relief for those with the disease. While these medications have proven to be effective, alternative treatments, such as acupuncture and CBD will also be explored. While acupuncture treatments have been around for 3000 years, CBD was only first isolated from marijuana in 1940, and its structure was reported in 1963 (Crocq, 2020). These two alternative medications/treatments have been shown to stimulate certain chemicals that reduce swelling and inhibit immune responses and allergic reactions.

Methods

To analyze the best current treatment options for Rheumatoid Arthritis, this research paper is composed of systematically searched papers and articles from MEDLINE, PubMed, the American college of Rheumatology, as well as additional health review sources which are peer reviewed, screened using multiple information sources, and have proven clinical trials of treatments for Rheumatoid arthritis.

Traditional Medications

Methotrexate

Methotrexate inhibits the synthesis of DNA, RNA and proteins by binding to dihydrofolate reductase (Lopez-Olivo, 2014). In simple words, methotrexate is an immunosuppressive drug which helps reduce inflammation that causes stiff and swollen joints in patients with rheumatoid arthritis. Methotrexate is not a pain killer, rather it attacks and inhibits inflammation which is the common pain factor in rheumatoid arthritis. Being that it attacks the source of the problem, methotrexate can reduce destruction of the joints which is caused by inflammation. On the flip side, we need to look at the possible side effects from this drug in order to determine if the benefits outweigh the negatives. Side effects from methotrexate can be diverse and affect many organs. Pericardial serositis is a rare complication. (Albrecht, 2010). Stomatitis is very prevalent during methotrexate treatment, with percentages as high as 37% found in a controlled study (Albrecht, 2010). Headaches, dizziness, and fatigue have all been reported. Nausea, vomiting, and malaise are the most frequent adverse side effects of methotrexate. According to the American Society of Health-System Pharmacists, Methotrexate should only be taken to treat cancer or other conditions that are very severe and which cannot be treated with other medications or treatments (MedlinePlus, 2022). This is due to the life-threatening side effects that it can cause. These side-effects include a decrease in the number of blood cells and severe liver damage. For people with a history of liver disease, Methotrexate is generally ruled out altogether. The fact that this medication is the first treatment option for rheumatoid arthritis necessitates additional research into other treatment options including alternative medicine. This is especially true since Rheumatoid Arthritis isn't deadly on its own, but rather complications and inflammation can shorten a patient's lifespan. Some of these complications include infection, cardiovascular disease, and respiratory disease. (Naz, 2007). Cardiovascular disease is so common with patients with rheumatoid arthritis that, according to the Arthritis Foundation, more than 50% of early deaths in people with rheumatoid arthritis occur due to cardiovascular disease, and patients with rheumatoid arthritis have a 50% to 70%

higher risk for cardiovascular disease than the general public (Foundation, 2022).

Hydroxychloroquine

Hydroxychloroquine is an anti-rheumatic drug that can decrease the pain and swelling of rheumatoid arthritis. This medication has been used to successfully treat all forms of arthritis for over 75 years. What is interesting about this drug is that it's not clear why it is effective at treating arthritis and other autoimmune diseases. Research suggests that it interrupts the inflammatory response by regulating and interfering with the communication of cells in the immune system (Puravath, 2022). Although it is an effective drug, it is usually reserved as an option for people whose rheumatoid arthritis has not responded to other options. This may be in part due to the severe side effects associated with this drug. Hydroxychloroquine can affect the heart's rhythm and lead to abnormal heartbeats, otherwise known as arrhythmias. This is in part due to the drug causing a QT-prolongation, in which there is a delay of ventricular repolarization (Ulrich, 2022). This electrical pattern is linked to an increased risk of deadly heart rhythms. In addition, taking hydroxychloroquine for a long period of time can harm the retina, causing serious and usually irreversible vision loss. This condition, known as hydroxychloroquine toxicity, can occur in roughly 1% of patients who take a cumulative dose of 1,000 g of hydroxychloroquine for 5 to 7 years of normal use (Phillips, 2014). These two mentioned side-effects are only some of the more serious side effects for patients taking hydroxychloroquine. The most common side-effects are: stomach pain, skin rash, nausea, vomiting, loss of appetite, headaches, muscle weakness, cramps, spasm. According to a study of 166 participants, at least one adverse event was experienced by 37.9% of percent of patients taking hydroxychloroquine (Sharanappa, 2020).

Leflunomide

This drug relieves symptoms of rheumatoid arthritis by inhibiting the body from producing too many of the immune cells responsible for the inflammation and swelling caused by the disease. As per the American College of Rheumatology, leflunomide is effective in treating rheumatoid arthritis as it can block the formation of DNA, which is critical for replicating cells, such as those in the immune system. It also suppresses the immune system to reduce inflammation that causes swelling and pain experienced by rheumatoid arthritis patients (Sha, 2022). There are some concerning side-effects of leflunomide which we need to address. First off, leflunomide is not a feasible option for those who are pregnant or are planning to become pregnant. This is due to the harm that it can cause to the fetus. In addition, you must avoid pregnancy during the two years following leflunomide treatment (MedlinePlus, 2015). It is troubling for a drug to have negative effects on the development of a fetus even two years after ceasing treatment. Additionally, leflunomide can cause liver damage that can be life-threatening. Other side-effects include but are not limited to: stomach pain, mouth ulcers, headaches, dizziness, dry skin, higher blood pressure.

Prednisone

This medication is a corticosteroid that has been proven to relieve and slow down inflammation and swelling through suppressing the body's immune system. This result is obtained by stopping the production of certain pro-cytokines. This weakening of the immune system blocks the chemicals that ordinarily cause inflammation as part of the body's immune response (Carter, 2022). Although prednisone is generally the first-choice corticosteroid when dealing with rheumatoid arthritis, there are many associated risks. Since prednisone weakens the immune system, you are more likely to get infections while taking it. For this reason, patients taking prednisone are warned to avoid being near people who are sick or have recently been sick. Patients are also advised not to receive live vaccines due to the chance of infection. With regards to the side-effects of prednisone, there are the common ones which include headaches, insomnia, mood changes, acne, fatigue, weak muscles, heartburn. However, there are more serious side-effects which are reported while taking this drug. These side-effects include seizures, irregular heartbeat, shortness of breath, cardiovascular disease, and impaired wound healing. The differentiation of a patient experiencing light side-effects versus serious side-effects is generally a result of whether the patient takes prednisone for a short period versus a long period of time, as well as lower doses versus higher doses. Patients taking prednisone for short periods or generally only experience minor side-effects such as insomnia and mood changes, while those taking prednisone for longer periods of time experience weight gain, osteoporosis, cataracts, and hypertension (Golden, 2022).

Sulfasalazine

This drug treats rheumatoid arthritis by killing harmful bacteria and acting to reduce the process driving inflammation. Sulfasalazine belongs to a group of medicines called aminosalicylates. The drug acts on the inflamed lining of the gut. More specifically, it acts locally in the colon to reduce inflammation, as well as throughout the body by inhibiting the formation of prostaglandins,

thereby controlling pain and inflammation (Cheifetz, 2022). This drug has a major advantage over leflunomide as it can be prescribed to take throughout all stages of pregnancy and is compatible with breastfeeding in healthy, full-term infants (National Rheumatoid Arthritis Society, 2020). Although this drug seems to have a lower degree of toxicity than others that are used to treat rheumatoid arthritis, there are still common and even serious side-effects associated with it. The most common side-effects are: headaches, nausea, and abdominal discomfort (Rheumatology, 2022). What is interesting to note is that serious side effects such as stomach ulcers, are less prevalent while taking sulfasalazine than while taking non-steroidal anti-inflammatory drugs such as ibuprofen. However, this drug can potentially cause more serious adverse reactions such as leukopenia, rash, abnormal liver function tests, and dyspnea. The upside is that these reactions reverse after treatment with the drug is stopped (Scott, 1988). One of the most serious but rare side-effects of the drug is sulfasalazine induced lung toxicity. But even this rare side-effect does not appear to be worrisome, as the majority of patients with suspected sulfasalazine-induced lung disease improve within weeks of drug withdrawal, and even the need for corticosteroids is debatable (Parry, 2002).

Alternative Treatments/Management

Alternative medicine has gained much popularity as of late. So much so, that a recent study by the National Center for Complementary and Alternative Medicine reported that 38 percent of U.S adults and about 12 percent of children are using some form of alternative medicine (Bas III, 2009). Additionally, an increasing number of medical colleges have started offering courses in alternative medicine. The advantages of using alternative treatment methods in general and for this disease specifically are numerous. To begin, by using alternative medicines and treatments, you can avoid the side-effects which are associated with the traditional medicine options. These potential serious side-effects include liver damage, bone marrow dysfunction, life-threatening infections, hyperglycemia, interstitial lung disease, and hypertension. Alternative treatment options also put a strong focus on prevention, whereas traditional medicine only intervenes once a disease is already present. While the benefits of traditional medicine may outweigh the negatives for patients with severe cases of rheumatoid arthritis, nonpharmacological treatments should be considered as complementary and alternative therapy options for patients whose rheumatoid arthritis does not cause excessive pain, but rather only minor to moderate discomfort throughout the day.

Acupuncture

Used as a natural healing therapy, acupuncture has been practiced in China for more than 3000 years. It has gained legitimacy and acclaim through acupuncture research which was initiated in the eighteenth century and developed rapidly since then. A major breakthrough for acupuncture validation occurred in the United States 1996, when the US FDA redefined the conception of acupuncture and moxibustion and admitted them as therapeutic methods (Xing, 2013). Acupuncture treatments in the United States exploded in 1997, when the National Institutes of Health consensus panel concluded that acupuncture was an effective treatment for postoperative dental pain (National Institutes of Health, 2009). More than 10 million acupuncture treatments are administered annually in the United States alone (NYU Langone Medical Center, 2014). Acupuncture works by triggering specific points on the skin with needles. Since each acupuncture needle produces a tiny injury at the insertion site, it signals to let the body know that it needs to respond. These acupuncture points stimulate and affect the activity of multiple sensory neurons, which in turn increase blood flow to certain parts of the body. Acupuncture has also been thought to restore the normal flow of energy (known as the qi) in the body. Most Traditionally and most commonly, needle penetration is the means of opening qi (Van Hal, 2022). Although the actual mechanism by which acupuncture affects rheumatoid arthritis is controversial, the anti-inflammatory effect has been the most proposed mechanism that has been mentioned. The thought is that acupuncture provides nonanalgesic effects via suppression of inflammatory response, improvement of blood flow, and relaxation of muscle tone (Chou, 2018). Other proposed mechanisms include regulating plasma adrenocorticotropic hormone, serum cortisol levels, activity of synovial nuclear factor kappa B, and the release of endorphins (Hui, 2010). The common side-effects of acupuncture include soreness and minor bleeding at the insertion site. In a survey of 34,000 treatments by traditional acupuncturists, Dr. MacPherson et al found no serious adverse events and only 43 minor ones (McPherson, 2001). In another survey, Dr. Melchart et al found 7.1% minor adverse events and 5 serious ones among 97,733 acupuncture patients (Melchart, 2004). There is a very slight risk of infection associated with acupuncture treatment. As per an evaluation of the frequency and severity of adverse events reported for acupuncture, moxibustion, and cupping between 2000-2011, which included 117 acupuncture reports, there was only 239 reported cases of infections associated with acupuncture treatment (Xu, 2013). These studies indicate that serious adverse events are rare and that acupuncture is generally a safe intervention.

Diet Changes

It has been suggested that you can reduce inflammation by cutting out certain foods from your diet. These foods include processed foods, salty foods, red meat, refined grains such as white bread, pasta, and white rice. Other foods include snack foods such as cookies, chips, crackers, pastries and fried foods. Drinking sodas and other sweetened drinks may also increase inflammation. Obesity also has a strong effect on rheumatoid arthritis. According to a review of 13 studies involving 13,562 rheumatoid arthritis patients and 400,609 total participants, there was a 13% increase in rheumatoid arthritis risk for every 5 kg/m2 in patients with rheumatoid arthritis (Skocynska, 2018). Interestingly, the rheumatoid arthritis incidence in Southern Europe is 0.3%-0.7%, compared with Northern Europe and North America, where the rheumatoid arthritis incidence is 0.5%-1.1%. Seemingly, the Mediterranean diet plays a strong role in this result. The Mediterranean diet consists of fish, olive oil, and cooked vegetables. Most importantly, fish consumption has been associated with the effect of omega-3 long chain polyunsaturated fatty acids against poly-inflammatory disease (Alamanos, 2005). Not only can life-long consumption of these foods decrease the chance of rheumatoid arthritis, it can also decrease the severity as well. In addition to the fatty acids, research has shown that probiotics, vitamin D, and antioxidants all have positive effects on limiting disease activity (Vadell, 2020). These dietary interventions seem to indicate that there are several foods and supplements that have the potential to reduce disease activity by lowering inflammation levels. These dietary changes are also being recommended after increasing evidence that altered microbiota in the gut of rheumatoid arthritis patients is what is responsible for disease progression (Khanna, 2017). Another interesting diet worth noting is the "Seven days fasting diet." This diet entails fasting for 7-10 days with partial nutrient intake of herbal teas, parsley, vegetable broth, garlic, juice extracts from carrots, beets and celery. The 7-10 days of fasting is then followed by one year of a vegan diet. Remarkably, studies on this diet observed a strong decrease in the number of swollen joints, tender joints, and overall pain. Specific pain reduction, such as a decrease in the duration of morning stiffness, stronger grip strength, erythrocyte sedimentation rate, white blood cell count, and C-reactive protein were also noted (Kjeldsen-Kragh, 1991).

Exercise/Physical Activity

As per the CDC, it is recommended that adults should perform at least 30 minutes of moderate physical activity five days a week for a total of 150 minutes (Prevention, 2022). Regular physical activity provides many health benefits for the general population and especially for patients with chronic diseases. Improvements in cardiovascular health have always been associated with regular exercise. This is even more important for patients with rheumatoid arthritis, as patients with rheumatoid arthritis are almost twice as likely to develop cardiovascular diseases than the general population (Blum, 2019). This cannot be ignored since cardiovascular related diseases are the main cause of reduced life expectancy in patients with rheumatoid arthritis. Unfortunately, it is estimated that more than 80% of rheumatoid patients are physically inactive in some countries (Soka, 2008). This high rate of physical inactivity plays a strong part in regards to the patient's overall health and especially the progression of rheumatoid arthritis. Regular exercise has been shown to increase muscle mass, improve muscle strength, physical function and joint mobility (Cooney, 2011). Another reason while regular exercise is crucial in the treatment of rheumatoid arthritis is due to two thirds of patients suffering from cachexia, which is significant muscle wasting. This causes muscle weakness and fatigue. Through regular exercise, a rheumatoid arthritis patient can limit the effects of cachexia and improve their overall muscle strength. In addition, high intensity resistance exercise has been shown to reverse cachexia and to restore muscle mass, which improves physical function (Lemmey, 2009).

Cannabidol (CBD)

CBD products are easily available over the counter and are marketed as wellness products. CBD is a phytocannabinoid that can reduce inflammation and pain. CBD can decrease inflammation through its effect on cytokines. Cytokines are secreted by immune cells upon stimulation. Their role is to balance the initiation and resolution of inflammation. CBD dysregulates cytokine production by immune cells and disrupts the immune response, in turn reducing inflammation caused by an autoimmune disease such as rheumatoid arthritis. Clinical studies have also confirmed that CBD reduces the levels of pro-inflammatory cytokine and inhibits T cell proliferation (Atalay, 2019). Although evidence of CBD treatment for rheumatic diseases remains preclinical, it has shown a lot of promise. As per a study that was conducted to evaluate patients' perceived efficacy of CBD, 83% of participants reported improvements in pain after CBD use. In addition, 66% of participants reported greater physical function and sleep quality. These remarkable statistics provide a lot of promise for future CBD research. With regards to possible side-effects of CBD, the common ones include dry mouth, lightheadedness, low blood pressure

and drowsiness. More serious side-effects of CBD include dose-related liver damage. This generally occurs when CBD is used in conjunction with other medications such as leflunomide (Meissner H, 2022). All in all, CBD seems like a generally safe and effective intervention for rheumatoid arthritis.

Discussion and Conclusions

It is important to understand that we have much more data and clinical evidence for traditional medications prescribed for rheumatoid arthritis than for alternative treatments. Non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and conventional disease-modifying antirheumatic drugs (DMARDs) have been the go-to medications over the past century. Especially with the advancement of modern medicine, we can develop powerful medications that can control inflammation caused by autoimmune diseases such as rheumatoid arthritis. We cannot undermine the proven results of these drugs. However, a stronger emphasis must be placed on the current and future research on alternative medications and treatments. More awareness needs to be placed on lifestyle changes such as diet changes, which can dramatically decrease the prevalence of inflammatory diseases. When a patient first sees a doctor for an inflammatory condition such as rheumatoid arthritis, the first response should be to investigate the patient's past and current diet as well as the patient's exercise habits or the lack of. Especially for patients with only mild symptoms in stage 1 of rheumatoid arthritis, alternative treatments such as acupuncture and CBD can be tried as a first resort to curb inflammation levels. It is important to note that stage 1 is early-stage rheumatoid arthritis, in which there is inflammation inside the joint but no damage to the bone. In this stage, it is likely that the disease will go into remission if diagnosed and treated properly (Lovering C, 2022). With effective treatment, the progression of the disease can be slowed down even if the disease doesn't go into remission. For severe and debilitating cases, the use of powerful drugs may be warranted to prevent excessive pain and bone damage. To conclude, it is important to look at both the traditional and alternative path before prescribing short-term and long-term treatment plans for rheumatoid arthritis.

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