MALTA MEDICAL STUDENTS' JOURNAL

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MMSA

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SCORP Officer '24-'25, Mr Timothy Borg

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SCOME Officer '24-'25, Ms Elisa Psaila

We are proud to present the first-ever edition of the Malta Medical Students' Journal (MMSJ)! For many years, the research efforts of Maltese medical students were spread across three separate journals — Minima Medica, Mind Maps, and Momenta Mea (formerly Materna). This year, we have unified these publications under one umbrella to centralise our efforts and strengthen the journal's recognition both locally and internationally.

This inaugural edition features seven articles, each exploring diverse medical topics. Under the guidance of esteemed academics from the University of Malta and specialised medical professionals, the Malta Medical Students' Journal ensures the highest level of academic credibility and maintains an unwavering commitment to excellence.

We would like to extend our sincere gratitude to Timothy Piasecki, Juliana Zammit, and Manuela Grech, this year's Publication Coordinators, whose dedication and seamless coordination have been pivotal in bringing the MMSJ to life. Their hard work encompassed liaising with authors and tutors, overseeing student reviewing, and organising the MMSA Research Conference.

A heartfelt thank you also goes to our assistants — Micheala Koppens, Lauren Aquilina, and Peter Calleja — for their invaluable support throughout this journey. We are excited to see how the journal continues to grow under the stewardship of the incoming SCOME Officer, Peter Calleja. Additionally, we express our appreciation to MMSA's PRO Keith Calleja and our PR Coordinator Lauren Shaw, whose creative vision and tireless efforts have given this journal a cohesive and professional design that truly reflects the hard work of our contributors.

Lastly, we sincerely thank all the authors for their passion and dedication. Their thoughtful research and commitment to medical knowledge have been the backbone of this publication.

As we celebrate this milestone, we look forward with hope and ambition. The MMSJ stands as a testament to what collaboration, perseverance, and passion can achieve.

May it serve not only as a platform for scientific discourse but also as an inspiration to future generations of medical students to innovate, explore, and lead with purpose. This is only the beginning — and the future is bright.

Timothy Borg | Martine Grech | Elisa Psaila

FOREWORD MESSAGE

Publications Coordinator '24-'25, Ms Manuela Grech
Mind Maps Coordinator '24-'25, Ms Juliana Zammit
Publications Coordinator '24-'25, Mr Tymoteusz Piasecki

In a world overflowing with information, the enduring value of research in medicine remains unmatched. Beyond the easily accessible search results lies a deeper pursuit, one that challenges us to question, explore, and innovate. Research is not just about finding answers; it's about cultivating curiosity, fostering critical thinking, and ultimately, shaping the future of patient care.

The Malta Medical Students' Journal, in its first-ever combined edition uniting the visions of SCORA, SCOME, and SCORP, stands as a testament to the drive, passion, and intellect of our student body. It offers a unique platform where medical students take their first steps into the world of academic research, peer review, and publication — an experience that equips them with indispensable skills for their future as doctors and changemakers.

The articles in this inaugural edition reflect the diversity of medicine and the breadth of student interest: from clinical case studies to public health perspectives, ethical dilemmas, and reflections on gender, human rights, and medical education. Each piece is a window into the inquisitive minds of future healthcare professionals who understand that medicine is not just a practice, but a discipline grounded in lifelong learning and scientific advancement.

This journal would not have come to life without the continued dedication of the SCORP, SCORA, and SCOME teams. We extend our heartfelt gratitude to Timothy Borg, Martine Grech, Elisa Psaila, Lauren Aquilina, Michaela Koppens, and Peter Calleja for their unwavering support and invaluable contributions throughout this journey.

We are also immensely grateful to the peer reviewers who carefully evaluated submissions and offered constructive feedback to our authors. Your commitment to academic excellence truly elevated the standard of this journal.

Finally, we extend our deepest appreciation to the esteemed academics who tutored, mentored, and guided our student authors: Dr. Andrea Cuschieri, Mr. Peter Calleja, Prof. Godfrey Grech, Prof. Pierre Schembri Wismayer, Prof. Marion Zammit Mangion,

and Dr. Lara Ann Saliba. Your encouragement and expertise played a pivotal role in shaping both the content and the confidence of the students involved.

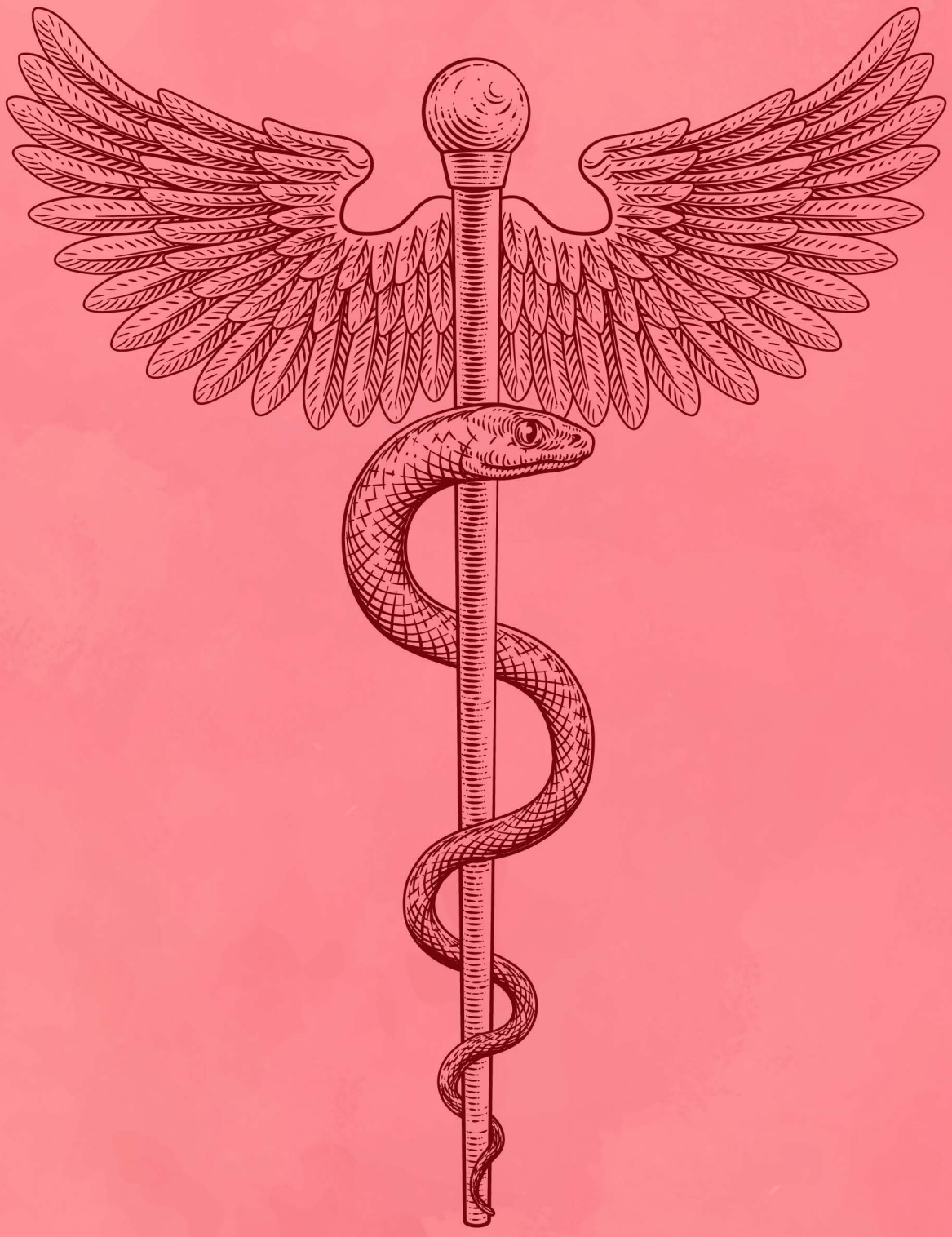
To all the contributors and readers of this journal, thank you for embracing the spirit of inquiry. May this be the first of many editions that continue to inspire a culture of research, collaboration, and academic excellence among Malta's future doctors.

Manuela Grech | Juliana Zammit | Timothy Piasecki

MMSJ Editors 2025-2026

MINICA MEDICA

JOURNALS BY THE MALTA MEDICAL STUDENTS' ASSOCIATION



A Brief Review of the Gut Microbiome: Composition and contribution to Carbohydrate Metabolism

Author: Martina Gauci

Abstract

Many microorganisms form part of the normal, healthy microbiota of the gut for maintaining health and avoiding pathological states. It not only deters the colonisation of pathogens, but also helps in the metabolism of various nutrients. This literature review provides a general overview of the composition of the microbiome in the gut and its role in carbohydrate metabolism.

Keywords: Microbiota of the healthy gut, gut mucosa, bacteria, microbial diversity, carbohydrates, proteins, symbiosis, gene expression, short- chain fatty acids, butyrate, immune system.

Introduction

The microbiome in the gut refers to a vast diversity of microorganisms that colonise the gastrointestinal system of all mammals, together with their collective genome. In the large intestine alone, the colonic contents of bacteria is 1011 – 10 12 colony forming units/ ml (1). Microorganisms in the gut refer to bacteria, viruses, fungi, protozoans and archaea (3–5). In the past few years, there has been great interest in this microbiome as it is associated with many pathological states such as inflammatory bowel disease and diabetes (2,3). Additionally, recent literature indicates that it is important for gut and individual health (2). Contrary to common perceptions, most microorganisms in the gut are not pathogenic, but play important roles such as assisting drug metabolism, contributing to absorption of nutrients and prevention of colonisation of other pathogenic microorganisms (2).

The enterocytes and the non- pathogenic gut microorganisms live in a symbiotic relationship where they both benefit (2, 6). The immune system has evolved to co-exist with the healthy, non-pathogenic microorganisms but still protects the organism from other pathogenic microorganisms (3). The microorganisms benefit from a habitat and a supply of nutrients from the host while the host benefits as the microorganisms contribute to metabolism and immunity. For example, during the fermentation of ingested fibers, bacteria in the gut produce short-chain fatty acids such as butyrate and acetate. The enterocytes make use of the short-chain fatty acids for energy. Meanwhile, these short-chain fatty acids are involved in the modulation of gene expression and cytokine production and are antagonists for the inflammatory response, modulating regulatory T cell production etc. Hence, the relationship between the immune system of the gut and the gut microbiome is important for mucosal homeostasis (7–9, 39).

Composition of the normal gut microbiome

Considerable progress in the study of the gut microorganisms, has been made over the years. The

exact number of all microorganisms or species making up the gut microbiome in humans is not known. However, it has been estimated that the microbiome in the gut is made up of more than 100 trillion cells, 35, 000 of them are bacterial species (8, 10).

In most populations in the West, the microbial diversity on a phylum level appears consistent. However, it is much more complex at the level of genus, species and subspecies. There are many different bacterial species present in the microbiota of an individual which vary from person to person and are thus unique. Indeed, the gut microbiome varies between species as well, complicating the study of the gut microbiome. This is due to many different factors which affect bacterial growth such as age, environment, mode of delivery, nutrition, genotype and others (3, 13, 14).

The composition of the normal gut microbiome varies with age. In infants, the pattern of colonisation of the gut microbiome is disorganised and irregular (14). However, this only remains for a small amount of time. At about age 3, there is a change from the neonatal type to a more stable adult microbiome. During early stages, the gut is colonised by aerobic microorganisms like Streptococci and Enterobacteriaceae. Then, these enable the colonisation of anaerobic microorganisms (2, 13).

The composition of the gut depends on colonisation processes that initiates at birth. The infant acquires microorganisms from the body surfaces of the mother and the environment.

Factors that influence this transfer are diet of the infant, certain medications, hygiene and even mode of delivery (28). During vaginal delivery, the infant acquires microorganisms present in the mother's vagina such as Escherichia coli. In contrast, infants delivered by caesarean section, were found to have more Klebsiella, Enterococcus, Citrobacter and Enterobacter. Apart from impacting the composition of the gut microbiome, this has implications on immunity (13, 14, 15).

Regarding changes in the gut microbiome during childhood and adolescence, few studies have been made and the results vary (29). KOALA Birth Cohort Study reported that the gut microbiome of children between 6 and 9 years is similar to adults (30). Other studies add that while the gut microbiota of healthy children already shows certain adult-like characteristics, it appears to undergo a more extended developmental phase than earlier thought, showcasing its unique paediatric gut microbiota traits (31). For example, Firmicutes and Actinobacteria are found in lesser amount in children than in the adult microbiome. In conclusion, the gut microbiome in children has similar features to the adult gut microbiome and although it is fairly stable, lifestyle and environment may still influence the microbiome (29).

In adulthood, there is a stable relationship between the gut microorganisms and the host, reaching a homeostatic balance able to withstand and overcome threats to the environment of the gut microbiome for example to pathogenic infections (32). Each person has a unique microbiome according to their gender, age and lifestyle. The microbiome could still be affected and changed such as after antibiotics. Thus, the microbiome can undergo change and adapt according to different stimuli (29).

In advanced age, many changes in the body may occur. One change is due to decreasing strength of the immune system. This results in chronic low-grade inflammation which can affect the composition of the gut microbiome (29). This microbiota degeneration is frequently seen to start in people over 70 years (33).

Studies performed by the "Human Microbiome Project and the Metagenome of the Human Intestinal Tract" showed that the human gut microbiome may contain more than 10 million bacterial genes. High and low gene count are two concepts that have emerged from a study involving 169 individuals who were obese and another 123 individuals who were not obese (34). This study showed that both low and high gene counts are involved in diseases and health. Persons with a high gene count have a stronger microbiome in the gut and lower risk of obesity and metabolic disorders. On the other hand, persons with a low gene count have a high risk of having inflammatory bowel disease and other disorders. The reason for this is due to the presence of different bacteria. For example, individuals with a high gene count, have Fecalibacterium species, Butyrivibrio crossotus, Anaerotruncus colihominis among others. Contrastingly, individuals with a low gene count have much more pro-inflammatory bacteria such as Ruminococcus gravis and Bacteroides and others such as Staphylococcus and Dialister (2,11, 12).

Administration of probiotics, prebiotics and symbiotics affect the composition of the microbiome in the gut (15). According to the "World Health Organisation", probiotics refer to microorganisms that when given appropriately, confer advantages to the host's health, such as Streptococcus thermophilus, Lactobacillus planatarum and Bifidobacterium longum (35). Prebiotics are ingredients of food which are not digested such as the polysaccharide inulin that stimulate the activity of bacteria leading to beneficial host effects (16). Synbiotics refer to the use of both probiotics and prebiotics (2). Probiotics can reduce lactose malabsorption and impact immune responses by decreasing the risks for having gastrointestinal infections.

Prebiotics may decrease the risk of colon cancer, increase the efficiency of absorption of minerals and effect hormone levels which act on the gut and regulate food consumption. It is uncertain whether prebiotic effect is brought about as it makes the microbiome in the gut more stable or by inducing population shifts towards microorganisms which are more beneficial (15). The combination of probiotics and prebiotics aids in the growth of useful bacteria which leads to more controlled glucose level and increased sensitivity to insulin (36). Although certain microorganisms play a beneficial roles in the gut, further studies are needed to fully understand their effects on the host (15).

Most bacteria in a healthy gut belong to the phyla Firmicutes and Bacteroidetes. Next, are the Actinobacteria and Verrucomicrobia phyla (3). The distribution of microorganisms in the gut varies spatially and temporally at and beyond the genus level, which lead to different parts of the gut having different micro- habitats (2,15).

The small intestine is dominated by aerobic and facultative anaerobes and is where metabolisation of simple carbohydrates take place. The metabolism of more complex oligosaccharides occurs in the ileum. In contrast, the colon is dominated by strict anaerobes which ferment fibre ingested as part of the diet (36). Figure 1 shows how the gut microbiota varies from the oesophagus to the stomach. As shown in the figure, the dominant genus in the distal oesophagus, duodenum and jejunum is Streptococcus while that in the stomach is Helicobacter. Helicobacter pylori is associated with rich bacterial diversity when it acts as a commensal in the stomach. However, when Helicobacter pylori acts as a pathogen, diversity in the gut has been shown to be reduced. This shows that one microorganism may influence the growth of other microorganisms.The predominant phyla in the large intestine are Bacteroidetes and Firmicutes.Primary pathogens are also present in the colon examples include Escherichia coli,

Salmonella enterica, Vibrio cholera and Campylobacter jejuni.Very few bacteria belonging to the phylum of Proteobacteria are found in the gut. Having no bacteria belonging to this phylum and high amounts of signature genera like Ruminococcus and Bacteroides in the gut is suggestive of a healthy microbiome (2, 37).
The bacterial communities found in the gut are the main community types that have been studied. Very little information is known about viruses, fungi, protozoans and archaea in the gut. However, in recent years there has been more studies and interest about the latter communities as they do play an important role in the gut (13).

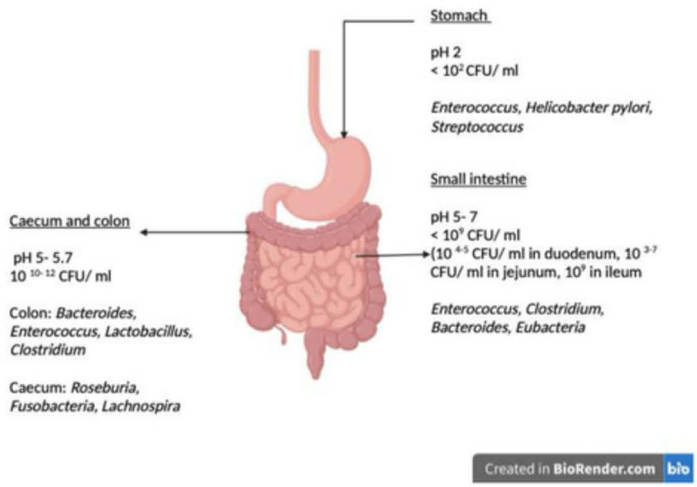


Figure 1: Presence of different bacteria in different sections of the gut. Figure adapted from 'Role of the normal gut microbiota' (2, 3, 6). Created with BioRender.com

It has been thought that the ratio of Firmicutes to Bacteroides can be used to estimate predisposition to diseases. However, the amount of Bacteroides and Firmicutes that are mostly found in the large intestine, vary from person to person. As a result, the use of this ratio is inaccurate and debatable (2). One factor which affects this ratio is diet. For example, diets containing large amounts of fats and carbohydrates lead to changes in the gut microbiome as these diets contain more energy producing substances, thus increasing the dietary energy load. Another example is that prebiotics also affect certain groups of bacteria such as Verrucomicrobia. As a result certain factors affect the arrangement of the gut microbiome that in turn affect its functions such as immunity (1,13).

Apart from a longitudinal difference between the microorganisms found in different parts of the gut, different organisms can be found from the outermost to the innermost layers of the intestine wall (17, 18). This is known as axial differentiation (2). In the mucosal layer, there are more aerobes when compared to the lumen of the intestine (1, 5). For example, Eubacterium rectale is found mostly in crypts while Bacteroides are the most common of the invasive and adhesive populations. The predominant bacteria in the lumen are Ruminococcus, Clostridium, Streptococcus, Bacteroides, Enterobacteriaceae, Lactobacillus, Enterococcus and Bifidobacterium. Contrastingly, those found in the mucus layer are Akkermansia, Lactobacillus, Clostridium and Enterococcus (2).

The microorganisms making up the gut may be classified by enterotypes. The latter are clusters of different bacteria that are stable over gender and geography but react differently to drugs and diet. The three main enterotypes are different in terms of bacteria that make them up and even composition. Bacteroides, Prevotella and Ruminococcus are dominant in enterotypes 1, 2 and 3 respectively (2,19). Enterotype 1 produces energy from carbohydrates and proteins from the diet. This is shown by the presence of high amount of genes that code for specific enzymes such as galactosidases and proteases. Enterotype 2 degrades mucin glycoproteins which are found lining the gut mucosal layer. In fact, Prevotella is one species known to degrade mucin (19).

Enterotype 3 degrades mucin glycoproteins similar to enterotype 2 but also contains membrane transports, especially those of sugars. Thus, enterotype 3 can utilise sugars. This shows that different enterotypes have different ways to produce energy. These bacterial clusters can also produce vitamins (9, 19). Enterotype 1 are associated with the production of riboflavin, ascorbate, biotin and pantothenate while enterotype 2 is associated with the production of thiamine and folate (2, 9, 10). These differences between enterotypes represent different mingling of microbial trophic chain that affects the human host. Interestingly, the enterotypes have functional markers which may be utilised as a diagnostic tool for diseases such as colorectal cancer (14). There are however disadvantages with this mode of classification. In certain diseases, there may not be a significant difference in enterotypes. Moreover, some studies indicate that the distribution of the classes varies between people. Further research is therefore required to determine the extent to which enterotypes are associated with different diseases (1, 16).

Functions of the normal gut microbiome

The relationship between the mucosa of the gut and the microorganisms living in the gut is an example of a symbiotic relationship. The microbiome in the gut plays important roles in many different aspects such as in metabolism and immunity (2, 3). This has been shown from studies where germ-free animals and colonised animals were compared. Animals without a gut microbiome were more at risk of infections, possessed less lymphocytes, thinner mucus walls and demonstrated lower production of digestive enzymes and cytokines among others (1,9). This is consistent with various roles of the gut microbiome. Some of these roles are summarised in Figure 2 below.

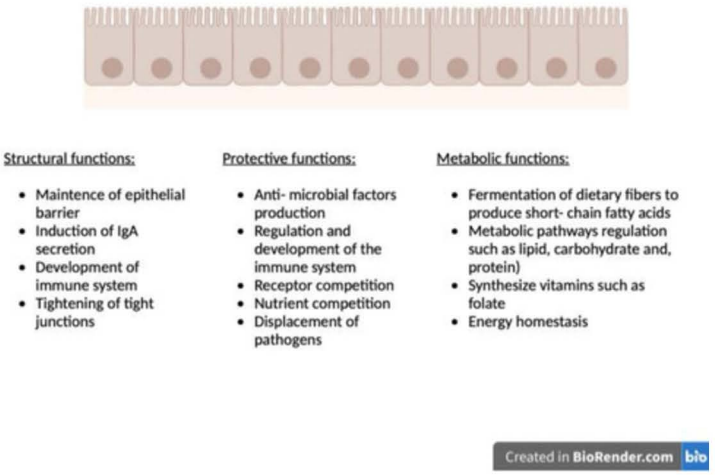


Figure 2: Functions of the bacteria normally found in the gut as part of the natural microbiome. Figure adapted from: "The gut flora as a forgotten organ" (1). Created with BioRender.com

Carbohydrates

The nutrient source of microbiota in the gut is carbohydrates. By fermenting carbohydrates that were not digested proximally, and fermentation of undigested oligosaccharides by microorganisms such as Roseburia, Enterobacteria and Fecalibacterium, short- chain fatty acids (SCFA) are produced (20). Examples of short- chain fatty acids include acetate, butyrate and propionate, where the latter is one of the most important SCFA produced by the gut microbiome due to its various functions (2). Colonocytes use butyrate as an energy source. The liver absorbs and extract acetate and propionate to act as substrates for gluconeogenesis and lipogenesis. (36) Depending on the structure of the fiber and bacterial species, different SCFA are produced (21, 36). For example, Akkermansia mucophilia is one of the intestinal commensals which produce propionate when fiber is ingested (22). Some gut microorganisms that produce butyrate include Faecalibacterium prausnitzii and Eubacterium rectale (7). Most microorganisms that produce butyrate are part of the Clostridium group within the phylum Firmicutes. Acetate co enzyme A transferase pathway as well as butyrate kinase enzymes are used in the production of butyrate by

these organisms (38).

Fiber ingested as part of the diet is important for various metabolic functions such as insulin sensitivity, to maintain a healthy weight and lowers the risk for a wide range of diseases such as colon cancer, inflammatory bowel disease and obesity. In fact, it is hypothesised that the short- chain fatty acids formed, might be helpful to prevent cancer, metabolic syndrome and disorders affecting the bowel (2). Butyrate may play a protective role against colorectal cancer by the production of anti-carcinogenic metabolites, for example shikimic acid. In fact, colorectal cancer patients have lower levels of Roseburia, which is one of butyrate-producing organisms (38). SCFAs may be a protective factor for non-alcoholic fatty liver disease by inhibiting de novo lipogenesis and lipolysis (36). Various diseases such as diabetes and arteriosclerosis have been associated with a reduction in the bacteria producing the SCFA (23). For example, in a study conducted in obese mice, butyrate increased the sensitivity to insulin and energy expenditure (12). Evidence shows that both butyrate and propionate regulate hormones of the gut such as ghrelin and glucagon-like peptide and reduce the risk for obesity (20). Among its many roles, butyrate decreases the risk for inflammation as it communicates with intestinal cells to maintain the intestinal environment for proliferation of anaerobic bacteria instead of facultative anaerobes which have been proven to take a part in inflammation (20, 38). Short-chain fatty acids given during clinical studies were found to be beneficial for treating obesity, Crohn's disease, diarrhoea associated with antibiotic use and ulcerative colitis (2). Another point is that such diseases have also been linked to a lower level of the gut bacteria that produces these beneficial SCFA. For example, the decreased level of Anaerostipes and Anaerobutyricum spp which normally produces butyrate from lactate, is associated with a build-up of lactate. Lactate is acidic and such build up may be associated with metabolic syndrome (22).

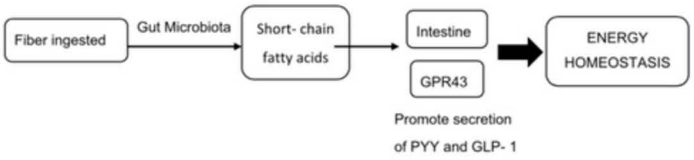


Figure 3: Short-chain fatty acids are produced from fiber ingested in the diet by the microorganisms found in the gut. Among their various roles, short-chain fatty acids bind with GPR43 exerting a positive effect on energy homeostasis. Figure adapted from "The SCFA receptor GPR43 and energy metabolism" (23).

The host might make use of these short-chain fatty acids for example to produce energy. This energy balance in the host is maintained by the short-chain fatty acids ligand-receptor interactions with a receptor which is G- protein coupled receptor 41 (GPR41). GPR41 is one example of a short-chain fatty acid (SCFA) receptor. GPR41 is found in L-cells of the gut which secrete plasma peptide Y (PYY) and glucagon-like peptide (GLP-1) when the receptor is activated by SCFA. Thus, it is also involved in energy homeostasis. Another location where GPR41 is found is in sympathetic ganglia. When propionate binds and activates GPR41 at this site, it results in energy expenditure, release of noradrenaline and an increase in the heart rate. This shows that depending on nutritional status of the body, GPR41 regulates energy homeostasis through sympathetic activity (14).
Furthermore, peptide tyrosine kinase also plays a role. Other functions of these short-chain fatty acids include cell proliferation, thermogenesis, modulation of immunity and modulation of gene expression (2,7,8,12).

Moreover, butyrate does not allow toxic metabolites such as D-lactate to accumulate thus preventing toxic effects from occurring. This is done by enzymes such as glycoside transferases and polysaccharide lyases which are mostly expressed by

the bacterial genus Bacteroides (24). The oxalate produced in the intestine by fermentation of carbohydrates and metabolism by bacteria is harmful as the oxalate can form kidney stones (25). Hence, to prevent this from happening, the oxalate is used by other organisms such as Bifidobacterium, Lactobacillus and Oxalobacter formigenes, thus does not allow oxalate stone to precipitate in the kidneys (2). Furthermore, butyrate regulates the production of regulatory T cells and help the action of macrophages (2, 25).

Gene expression and SCFAs

SCFA which are released by the microorganisms in the gut, regulate gene expression by inhibiting the activity of histone deacetylase (HDAC) which is one of the enzymes needed for histone acetylation during gene expression (26). Both butyrate and propionate inhibit HDAC1 and HDAC3, promoting the production of regulatory T cells in the peripheries (12). This modulation of histone acetylation may be one of the therapeutic strategies used to prevent and treat certain diseases. The anti-inflammatory effect is done by suppressing the activation of nuclear factor kB, upregulating peroxisome proliferator- activated receptor gamma and inhibiting the production of interferon gamma (12). Other functions of butyrate are on the immune system by influencing the expression of cytokines, the migration of immune cells, cell adhesion and also affecting processes taking place in cells such as apoptosis and cell activation (12, 27).

In obese and type 2 diabetic patients, the methylation of free fatty acid receptor 3 (FFAR3) promoter region was significantly less than that in lean individuals (12). This shows a link between body mass index and degree of methylation in FFAR3 promoter region. Subsequently, there may be a connection between epigenetics and the metabolic effect of SCFAs on the host (27).

In conclusion, the SCFA that are produced by fermenting fibres acquired from the diet by anaerobic microorganisms of the gut microbiome, influence metabolism and immune response pathway positively (4). SCFA and target molecules may be used in targeted treatment of certain diseases such as type 2 diabetes, intestinal bowel diseases and obesity (4, 12, 21).

Further studies

A lot of advancement has been made in methods to study the microbiome of the gut. However, the role of other microorganisms apart from bacteria need to be investigated. Furthermore, there needs to be improved experimental models which can represent the microbiome of the gut in humans more accurately. A deeper understanding of the human gut microbiome allows a deeper insight into the interactions between microorganisms and the host. This knowledge will improve healthcare through more personalized and targeted therapies. Additionally, it will also lead to the development of drugs with different targets and more specific modes of action. This could be achieved by longitudinal studies involving people of different characteristics such as age and race and investigate the relationship between health and changes in their gut microbiome. Future studies should be conducted in an integrative manner where structure and function are regarded together (3,11,15). Moreover, more studies are necessary to discover the exact mechanism of receptors such as GPR41 and GPR43 and their regulation in the hope to be used as therapeutic targets for diseases such as obesity (14, 23). Furthermore, clinical trials are needed to determine the role of SCFA on diseases and the mechanisms underlying such interactions (22).

Declarations

Author confirms no conflict of interest.

List of Abbreviations

Abbreviation	Definition
SCFA	Short-chain fatty acids
GPR	G-protein coupled receptor
PYY	Plasma peptide YY
GLP	Glucagon-like peptide
HDAC	Histone deacetylase
FFAR 3	Free fatty acid receptor 3

Author contribution

This is the work of medical student, Martina Gauci.

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Glioblastoma Multiforme Metastasis to the Parotid Gland: A Systematic Review of Case Series and Case Studies.

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Structured Abstract

Introduction

Glioblastoma multiforme (GBM) is a highly aggressive, IDH-wildtype grade IV brain tumor with rapid growth, invasiveness, and poor prognosis despite treatment. Extra-neural glioblastoma (GBM) metastasis to the parotid gland is exceedingly rare and inadequately documented.

Methods

A systematic review following PRISMA2020 guidelines for case-reports or case-series documenting histologically confirmed GBM metastasis to the parotid gland was conducted for studies published until 01/05/2024 (CRD42024517593).

Results

13 studies were included, with general good reporting quality. Average age on GBM diagnosis was 42.73 years (range: 25 – 58 years), with metastasis occurring after 9.77 months (range: 2.5 – 24 months). 8 patients had intracranial GBM metastasis and 9 had additional extra-neural metastasis, 7 being lymph-nodes. Frontal lobe and temporal lobe GBM involvement was noted in five tumours respectively, with two involving the parotid and one the occipital lobe. All parotid metastases were ipsilateral to the site of the original GBM tumour. All patients underwent resection and postoperative radiotherapy for their primary tumours.

Discussion

GBM metastasis to the parotid gland is exceedingly rare and occurred in younger individuals, with all documented cases showing ipsilateral spread which may suggest a pattern of lymphatic spread, though the limited data makes this challenging to confirm definitively. Additional research and case documentation are necessary to better understand the mechanisms and pathways of extra-neural metastasis, particularly to the parotid gland.

Keywords: GBM, Glioblastoma, Parotid Metastasis, Parotid, Case Studies.

Introduction

Glioblastoma multiforme (GBM) is the most aggressive and prevalent primary brain tumour in adults, classified as grade IV IDH-wildtype by the World Health Organisation (1, 2). Characterised by its rapid growth and invasiveness, GBM poses a significant clinical challenge, often leading to poor prognosis despite available treatments, with a median survival of 12-15 months (3, 4). With an incidence of approximately 3.2 cases per 100,000 people worldwide, GBM predominantly affects older adults, with the average age of occurrence being 64 years old (5). It shows an increased incidence and mortality in males, with females having a better prognosis (6). GBM has a complex pathophysiology, exhibiting immune escape, tumour heterogeneity and genetic mutations contributing to its poor prognosis (7). Their highly infiltrative nature due to a number of proposed theories for metastasis, also makes complete surgical resection difficult (3). Despite advances in multimodal therapies, including surgery, radiotherapy and temozolomide-based chemotherapy, there is still a critical need for ongoing research to better understand its biology and develop more therapeutic strategies (8). Extracranial metastasis is notably rare, occurring in less than 2% of patients (9). No specific value of central nervous system metastasis rate of glioblastoma has been found at the time of writing of this paper. However, metastasis to the parotid gland, while even rarer and less documented, presents a unique challenge in both diagnosis and management. Due to the scarcity of cases, the mechanisms of GBM metastasis to the parotid and its clinical implications remain poorly understood. The need for a systematic review becomes apparent as it would establish and analyse the limited available data, enabling healthcare professionals to better recognize and manage this rare phenomenon. Such a review could provide valuable insights into the diagnostic patterns, treatment strategies, and prognostic factors for patients with parotid metastasis from glioblastoma, ultimately contributing to improving patient care and outcomes in these rare cases.

Materials and Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) checklist 2020, and the Cochrane Handbook for Systematic Review of Interventions (10, 11)

Study Identification and Selection

PubMed, MEDLINE, Google Scholar, Science Direct, Scopus and Semantic Scholar databases were searched for relevant articles published up until 01/05/2024 without any language restrictions. Combinations of the following keywords were used to generate the search strategy: Glioblastoma, Parotid, Metastasis.

The inclusion criteria were as follows:

1. The articles were case reports or case series
2. The articles clearly described the development of histologically confirmed GBM and parotid metastasis, according to the latest CNS tumour classification guidelines at the time of the respective publication
3. Articles included cases of GBM metastasis to the parotid gland
4. Articles reported patient characteristics, prognostication and clinical outcomes

Snowballing manual review of the reference lists of retrieved articles was conducted to expand the sample. Gray literature was not searched in this review. Individual cases reported within case series that did not meet the above inclusion criteria were not included in our analysis.

Data Extraction and Analysis

Data from selected articles were extracted using a standardised data collection table. Descriptive statistical analysis coupled with qualitative assessment of results was conducted.

JBI

The Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Case Reports was used to assess the quality and risk of bias in 13 case reports by evaluating key aspects like patient demographics, clinical history, diagnosis, and outcomes. Each report was assessed against eight criteria, with responses marked as "Yes," "No," "Unclear," or "Not Applicable." Reports meeting at least five criteria were included, while those with more than two "Unclear"

Results

Study Characteristics

From the 13 included case reports, the earliest case was published in 1965, and the most recent case report was published in 2022; only 1 study was published after the new World Health Organization 2021 CNS tumour classification (1). Each article was evaluated using the JBI critical appraisal tool to assess for usability in this review. Of the 13 articles, 2 did not discuss the patient's prognosis after parotid metastasis was confirmed, however all the included texts exhibited an overall low risk of bias and high quality of reporting.

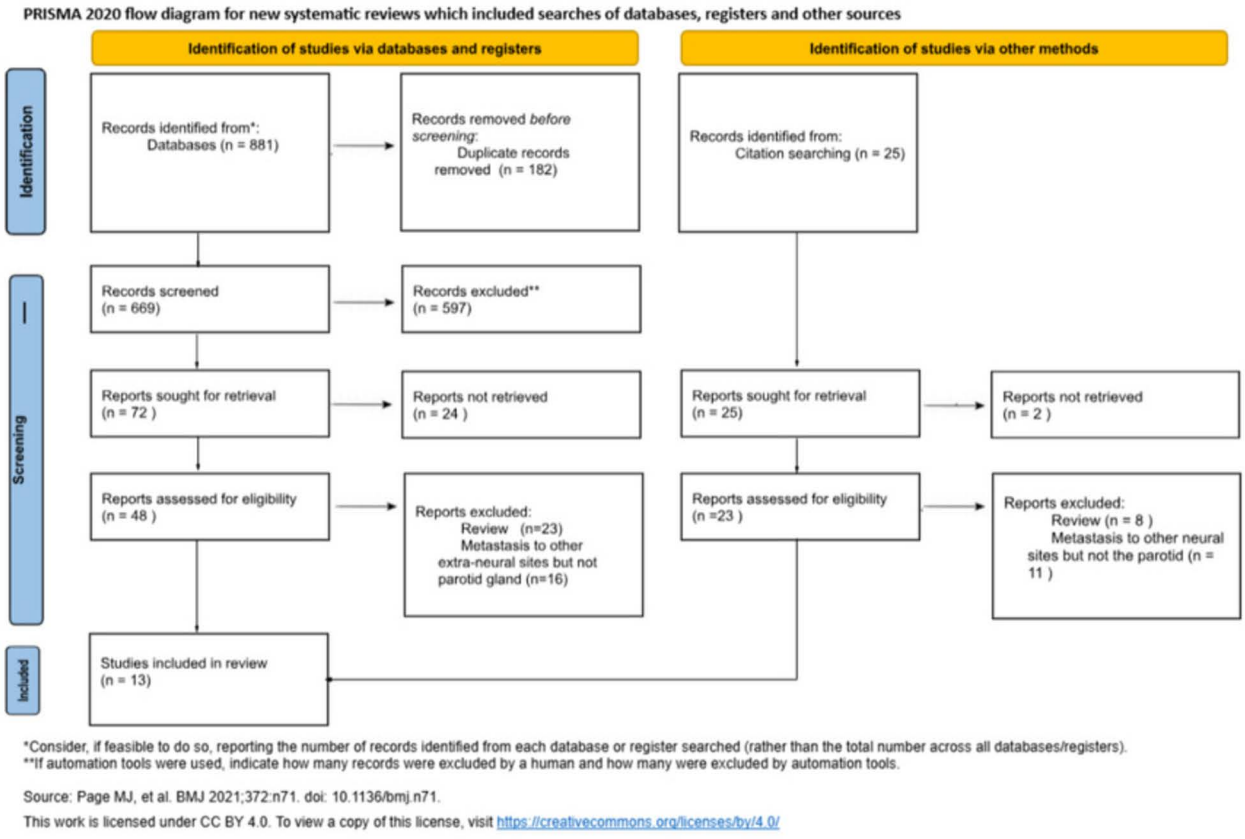

Figure 1: PISMA 2020 Flow Diagram: GBM, Parotid, Metastasis

Figure 2: JBI Appraisal Table

Glioblastoma Patient Characteristics and Management

The ages of the patients range from 24 to 64 at the age of presentation with GBM, with the majority being above the age of 40. Of the patients, 10 (76.92%) were male, and mean age of GBM detection was 46.2 years. 15.4% (n = 2) of patients had recorded concurrent diseases. Both cases had hypertension. 7 patients (53.8%) had GBM on the left hemisphere of the brain, and 8 (61.5%) patients had GBM tumour involvement in the temporal lobe.

All patients with specified data received radiotherapy and surgical resection for their primary GBM and 6 patients were recorded to receive chemotherapy (46.2%) , 5 of which received temozolomide (83.3%). 7 were recorded to have headache as a symptom of their primary tumour (53.8%).

Author, year	Gender	GBM Detection Age	Parotid Detection Age	Concurrent Diseases	GBM Mass Brain Hemisphere	Side of Parotid Mass	GBM Mass Size	Tumour Site	Duration between Primary Tumour and Metastsis	Radiotherapy received for Primary Tumour	Surgical Treatment received for Primary Tumour	Chemotherapy received for Primary Tumour	Radiotherapy received for Metastasis	Surgical Treatment received for Metastasis	Chemotherapy received for Metastsis	Symptoms of primary tumour	Symptoms of metastasis	Concominant metastasis	Prognosis as after dx of metastasis
Jie W, Bai J, Li B. 2018.	M	45	47	none	Right	Right	Not specified	mets: inferior right parotid, right parotid ear area, right deep parotid lobe	2 years	Y	Y: Brain GBM resection	N	N	Y: right total parotidectomy with selective neck dissection	N	Not specified	right parotid lump increasing in size, painful on touch	right deep cervical area and right temporal bone	Not specified
Alhoulaiby S, Abdulrahman A, Alouni G, Mahfoud M, Shihabi Z. 2020	M	53	53	10 year history of medically treated HTN and herpetic encephalitis 4 months earlier	Left	Left	On resection, 5cm in greatest dimension of multiple tissue fragments	original: left temporoparietal mass mets: recurrence in left temporal lobe expanding to left maxilla, mandible, parotid gland, infiltrating surrounding structures, hypoechoic lesions in parotid gland, enlarged left jugular lymph nodes	6 months post-surgery	Y: 60 Gray over 5 sessions/week for 2 months	Y: Craniotomy and resection of brain lesion	Y: Temozolomide 250mg tablets for 6 months, 5 consecutive days per month	N	N	N	Severe headaches, paraplegia (lower extremity)	Swelling in left cheek	cervical lymph nodes	Pt passed away 4 months after mets diagnosis, 9 months after initial surgical intervention
Taha M, Ahmad A, Wharton S, Jellinek D. 2005	M	33	33	none	Left frontal lobe	Left	/	Left frontal lobe primary tumour	7 months	Y: 60 Gy post craniotomy	Y:: 2 craniotomies for debulking	N: Planned but not started	Y	Y: Open biopsy of parotid	Y: PCV Chemo (Procarbazine, CCNU, Vincristine)	Generalised headache, behavioural changes	Painful, tender left-sided facial swelling initially misdiagnosed as infective parotitis	cervical lymph node mets	Pt passed away 3 months after detection of parotid mets
Ozan Baskurt , Yunus Kurtulus , Ahmed Yasin Yavuz and Idris Avci (2022)	M	42	43	none noted	Occurence 1: right temporal lobe Occurence 2: right temporal lobe	right	Occurence 1: 5*4cm Occurence 2: 6*5cm	original: right temporal lobe mets: right preauricular region, superficial lobe of parotid gland to upper part of previous craniotomy border, multiple pathological lymnadenopathies were detected in right submandibular area	between occurence 1 and 2: 7 months between occurence 2 and mets:(6 follow up + 15 months) = 21 months between occurence 1 and mets: 27 months	Y: 60 Gy/30 fractions RT	Y1: subtotal resection of the tumour via temporal craniotomy Y2: total removal of tumour within temporal lobectomy	Y1: Temozolomide at 75mg/m^2 one month after surgery Y2: bevacizumab irinotecan	Y	N	Y	Occ1: headache, syncope, tinnitus Occ2: left hemiparesis with muscle strength 2/5	Swelling in right neck region	right preauricular region. right submandibular area, subcutaneous to scalp of prior craniotomy scar, cortico-subcortical cystic encephalomalacia in right temporal lobe, multiple new distinct lesions and recurrent intracranial tumour growth	Pt declined further treatment and continues his life 39 months after the primary diagnosis of intracranial glioblastoma
Alfredo E. Romero-Rojas • Julio A. Diaz-Perez • Deirdre Amaro • Alfonso Lozano-Castillo • Sandra I. Chinchilla-Olaya (2013)	M	26	26	none noted	Left frontal lobe	Left	Not specified	left frontal lobe primary tumour	6 months	Y	Y	Y: temozolomide (140mg/day for 42 days)	Y	N	Y: temozolomide 300mg/day	severe hedaches and dysaesthesias	progressive growth of a nodule in left cheek and generalised bone pain	cervical lymph nodes (IIB, III and IV)	Total survival from dx of 2 years was documented
Jeroen Swinnen MD, Geert Gelin MD, Sabine Fransis MD, Jan Vandevenne MD PhD, Sofie Van Cauter MD PhD (2019)	F	56	56 (+6 months)	Arterial HTN, morbid obesity	Right temporal lobe	Right	Not specified	Right temporal lobe	6 months	Y: 30 doses of 2 gray on weekdays (Stupp protocol)	Y: resection of the mass and satellite lesions	Oral levetiracetam 500mg 2x daily as seizure prophylaxis as well as oral temozolomide at a ratio of 75mg/m^3 daily. After completion, adjuvant temozolomide regimen changed to 200mg/m^3 intake 5 days/week for 4 weeks repeated in 6 cycles	Not specified	Resection of cervical lymphadenopathy	oral VEGFR 1-3 inhibitor (axitinib) and anti-PD-L1 IgG1 antibody (avelumab) treatment	Headaches, nausea, vomiting, slightly unstable gait	Pain and swelling at right parotid gland	Lung nodules (right lung apex, bilateral millimetric nodules), cervical lymph nodules	Patient passed away 14.5 months after presentation

Author, year	Gender	GBM Detection Age	Parotid Detection Age	Concurrent Diseases	GBM Mass Brain Hemisphere	Side of Parotid Mass	GBM Mass Size	Tumour Site	Duration between Primary Tumour and Metastsis	Radiotherapy received for Primary Tumour	Surgical Treatment received for Primary Tumour	Chemotherapy received for Primary Tumour	Radiotherapy received for Metastasis	Surgical Treatment received for Metastasis	Chemotherapy received for Metastsis	Symptoms of primary tumour	Symptoms of metastasis	Concominant metastasis	Prognosis as after dx of metastasis
Kraft Marcel, Lang Florian, Braunschweig Richard, Janzer Robert Charles. (2008)	M	57 (15 mo before 58)	58	none noted	Right temporal lobe	Right	Not specified	Right temporal lobe	15 months	Y	Y: craniotomy and partial lobectomy	Y: temozolomide	Not specified	N	Y, palliative	Not specified	Pressure sensitive parotid tumour or the right with ipsilateral facial paralysis, House-Brackman n grade IV in a week	Right orbit, thyroid, lung, kidney, breast and colorectal cancer.	Approximately 1.5 months
Ogungbo, Biodun, Perry, Robert Henry, Bozzino, J. M., Mahadeva, Dhirendra. (2005)	F	49	49 (+5 months)	none noted	Left	left	4.5 cm x 3.5 cm	Left occipital lobe	6-8 months	Y: 30 Grey over 6 sessions in 3 weeks	Y: craniotomy and excision of left occipital tumours	Y: CCNU, Procarbazine and metopclopramide.	Palliative oncological treatment not otherwise specified	Palliative oncological treatment not otherwise specified	Palliative oncological treatment not otherwise specified	funny turns', headaches, visual disturbances in the form of recurrent flashing light episodes of 2 weeks duration lasting a few minutes at a time.	unusual swelling on left parotid gland	Lungs	died 16 months after onset of symptoms
U. Kühn1 - H. H. Köhler2 - P. Jecker1 (2003) *Only the 2nd case will be considered as the rest do not have parotid metastasis*	M	58 (minus 6 months)	58	none noted	right	right	Not specified	Right temporal lobe	9 months	Y	Y	not specified	not specified	y: total parotidectomy	not specified	Not specified	increased swelling of right parotid gland for a week	Renewed tumour in right tempora l lobe + liver mets	not specified
CATHERINE C. PARK, MD, CHRISTIAN HARTMANN, MD, REBECCA FOLKERTH, MD, JAY S. LOEFFLER, MD, PATRICK Y. WEN, MD, HOWARD A. FINE, MD, PETER MCL. BLACK, MD, PHD, TIMOTHY SHAFMAN, MD, AND DAVID N. LOUIS, MD (2000) *Only the 4th case will be considered as it is the only one with parotid metastasis*	F	25	25	none noted	right	Not specified	Not provided	Fronto-parietal region, had mets to the scalp	9 months	Y. External beam radiation therapy to 59.4 Gy. Radioactive iodnine seed placement in resection bed	Y. Subtotal resection	Y. Not specified	Not specified	Y. craniotomy for scalp metastases	Not specified	Headaches, nausea, left facial and arm weakness	Progressive symptoms of elevated intractanial pressure from neck mets	Scalp mets, large neck mass	She died 21 monts after diagnosis
Edwin Bolke and Christiane Matuschek (2014) *Only the 2nd case will be considered as it is the only one with parotid metastasis*	M	24	24	none noted	left	left	Not specified	Left temporal lobe, involving greater wing of sphenoid bone, invasion of MCF	Difficult to determine as no data on how long ago the first resection was. However it was ~4 months after presnetation of patient with anxiety and headaches post-resection.	Not specified	Yes: resection	not specified	yes	Y: Parotid and left hemi-neck nodal dissection	Y: temozolomide, paclitaxel, carboplatin, cisplatin, irinotecan and cetuximab	Not specified	anxiety and headaches	dural thickening and extraconal orbital changes, residul GMB outside the CNS with involvement of the sphenoid bone, soft tissues of the masticator space and extraconal orbit with proptosis, tumour in lower neck lymph nodes	Died after 22 months
R. Meqele' , P. Gruß ', K. Bührmonni (1989)	M	66	66	Not specified	left	left	Not specified	left temporal lobe	9 months	started 2.5 weeks post-op, 60 Gy. at 40 Gy to the entire cerebro and 20 Gy to the tumor bed	1. macroscopic removal of entire tumour. 2.macroscopic removal of recurrence, osteoplastic treparation	not specified	not specified	1. Partial parotidectomy 2. Radical parotidectomy and radical neck dissection	not specified	word-finding and memory problems, speech problems and other typical temporal lobe lesion symptoms	left sided periauricular swelling.	Neck mets resembling lymph nodes, spreading to the tonsillar tract	Survived procedures well, died after 3months due to rapid cerebral decompensation
Ali Moghtader (1965)	M	64	64	not specified	left	left	3x4x5 cm	surface of left temporal lobe	3 months between craniotomy and noticing the masses, diagnosis 6 months after craniotomy and diagnosis of mets	total tumour dose of 5070 r with Cobalt 60	satisfactorally excised	not specified	total of 5000 r with a 250 KV machine over 5 weeks	total parotidectomy, with facial nerve sacrifice and radical neck dissection	not specified	not specified	patient noted rapidly growing masses on left side of neck	cervical nodes	died 2 months after paotid mets diagnosis

Figure 3: Data Extraction Table

Parotid Metastasis Patient Characteristics and Management

From all the 13 cases evaluated, 8 (61.5%) patients underwent surgical treatment for the metastasis, 5 (38.5%) patients received radiotherapy, and 6 (46.2%) patients received chemotherapy. Data that was not specified in the cases or left ambiguous e.g. "palliative oncological treatment" that was not otherwise specified, was not included as affirmative for either treatment due to lack of certainty. All 13 cases had concomitant metastases, with 7 cases of metastases to the cervical and/or other lymph nodes not otherwise specified. The duration between the primary tumour and the parotid metastasis ranged from 3 months after their craniotomy to 2 years.
From the cases, 12 out of 13 had ipsilaterally recorded metastasis, and one had the side of metastasis undocumented.

Discussion

Metastasis of GBM to the parotid gland, although rare, highlights the need to understand anatomical and physiological links between the brain's drainage systems and extracranial metastasis. Unlike common extracranial metastases of GBM, such as to the lungs or the pleura (3), parotid metastases are notably ipsilateral, suggesting an undefined route of spreading. While parotid tumours typically present as slow-growing and painless masses, rapid growth, pain, skin tethering/ulceration, cervical lymphadenopathy, or facial nerve palsy raise concern for malignancy, with tail-of-parotid lesions often mimicking cervical lymphadenopathy (13). While hematogenous dissemination often results in diffuse metastases, often bilateral, the parotid's proximity to the brain and shared drainage pathways, may explain this distinct pattern of metastasis.

Lymphatic drainage of the brain, meninges and scalp

Traditionally, the brain was thought to lack lymphatic drainage (14). However, recent studies have shown the presence of dural lymphatic vessels, particularly along the dural sinuses and major blood vessels, which play a role in draining interstitial fluid and cerebrospinal fluid into the cervical lymph nodes (15). When removing the brain from the skull, lymphatic vessels were notably absent in the brain parenchyma and pia mater. Surprisingly, however, a substantial network of lymphatic vessels was visible within the meninges located beneath the skull (15). This has been referred to as the "glymphatic system" (16), defined as a brain-wide clearance pathway that removes interstitial waste and solutes from the CNS by circulating CSF along perivascular spaces. This glymphatic system connects with the sinus-associated lymphatic network to clear solutes along perivascular pathways, suggesting a comprehensive clearance system for waste and immune cell trafficking in the brain (16). In a recent study by Safiye Çavdar et al (2023), the presence of meningeal lymphatic vessels was confirmed along both dorsal and basal dural sinuses in humans, with immunohistochemistry and Western blotting validating their existence (17). Though basal meningeal lymphatic vessels are less common than dorsal ones, their location near cranial exit points could enhance CSF drainage, which may suggest an alternative pathway for CSF transport outside the cranium (17). The scalp's lymphatic vessels follow the venous drainage, with the anterior scalp drains via the parotid nodes, which continue through the deep cervical and submandibular lymph nodes. The posterior scalp (behind the auricle), drains to the occipital and posterior auricular lymph nodes, also known as the mastoid lymph nodes, and then follow to drain into the occipital lymph nodes – which go on to drain the remaining posterior scalp area (18).

The presence of such lymphatic drainage systems can play a more active role in the progression of metastatic cancer than previously understood, even though this theory still needs further research to improve treatment strategies (19). This can be explained through changes in their microenvironment preparing for tumour cell arrival through immunosuppressive signalling and structural remodelling (19). These lymphatic vessels and endothelial cells facilitate the spread of tumour cells, with metastatic nodes often having the primary tumour's genetic composition/diversity (19). In a recent study by Nur et al (2022), a novel route of lymphatic communication between the brain and the parotid lymph nodes (as well as the mandibular lymph node) was revealed, using in vivo hyperspectral fluorescence imaging with Qdot nanoparticles (20). In this study, tracers injected into the left parietal lobe of rats travelled to the right lymph nodes before reaching the left, suggesting an unknown lymphatic pathway potentially involved in brain drainage (20). This further highlights the need for challenging our knowledge of the drainage systems of the brain for the possibility to discover new pathways and systems which could be a passageway for metastatic development.

Venous drainage systems

The brain has a complex venous drainage system, primarily using sinuses. A sinus is a channel / cavity that allows blood or lymph to pass through (21). These sinuses are small venous structures located inside the pia mater (22). These then connect to form cerebral veins, which pass through the subarachnoid space and enter dural sinuses (22). The inferior areas of the frontal and parietal lobes are drained by the cavernous sinus and the superior and inferior petrosal sinuses (23). The temporal lobe is drained by two routes: one using the superficial middle cerebral vein, which passes blood through to the inferior anastomotic vein, which goes on the join the transverse sinus. The other mainly involves the inferior part of the temporal lobe draining into the choroidal vein, which pairs with the thalamostriate vein behind the interventricular foramen to become the internal cerebral vein. This then joins the basal veins to become the great cerebral vein (24). A confluence of sinuses is formed via the superior sagittal sinus and the occipital sinus, which meet over the cerebellum, after which blood follows through to the transverse sinuses (25). The scalp, on the other hand, drains into venous systems, with superficial veins following the arteries, and deep venous veins (18). Small veins originating from the scalp communicate with the dural sinuses using emissary veins, that penetrate the skull (22). The deep scalp is drained by a venous complex in the infratemporal fossa bordered by the medial and lateral pterygoid and temporalis muscle, called the pterygoid plexus (18):. It also encloses the maxillary artery (26), and is drained by the maxillary vein (18). The retromandibular vein, which is the confluence of the maxillary vein and the superficial temporal vein, provides the venous drainage of the parotid gland (27).

Research on venous systems being considered for metastatic potential in the context of neurology currently doesn't give a high yield of literature. In an old study published in 2006 by Edward Tobinick and Charles P. Vega, the cerebrospinal venous system has been described as being a direct vascular route for tumour metastasis (28). Intraglandular lymph nodes which are located in the parotid space are found along the course of the retromandibular vein and in the parotid tail (29). These drain the parotid gland (28). It is also important to note that the parotid tail has been described as a preferential site of parotid metastasis, which might hint towards cancer metastasising via the retromandibular vein (29).

All observed parotid metastases were ipsilateral to the primary tumour, suggesting a direct anatomical

link. For instance, the temporal lobe drains via venous routes (24) and connects to the parotid gland through the pterygoid plexus and retromandibular vein (18, 26, 27). This anatomical continuity raises the possibility that tumour cells exploit such pathways for metastatic spread.

Hypothesised Mechanisms of Metastasis

The primary routes are proposed to explain parotid metastasis: hematogenous spread, venous spread and lymphatic seeding.

Hematogenous and Venous spread

The venous drainage using emissary veins and dural sinuses, along with the brain's proximity to these veins, could serve as a passageway for glioblastoma cells to enter the systemic circulation (30). Metastasis to the parotid gland could therefore potentially occur through direct hematogenous seeding.

Hematogenous spread is a documented phenomenon in brain cancers, with metastatic tumours using this route becoming implanted in the subarachnoid space and then being able to spread to other meningeal surfaces (31). With venous spread, tumour cells disseminate via the bloodstream and are typically entrapped at the grey-white matter junction, where blood vessel narrowing promotes cancel cell arrest (32). This arrest often occurs in the cerebral hemispheres, with the blood brain barrier presenting a major challenge for metastasis and drug delivery due to endothelial cells expressing high levels of efflux transporters like ATP-binding cassette (ABC) proteins that pump drugs and toxins out of the brain (33). Cancer cells that manage to cross the barrier will tend to adopt a perivascular position where they are able to proliferate and form metastases (32). Additionally, CSF drainage occurs via arachnoid granulations that reabsorb fluid into the venous sinuses, providing another potential method for tumour cell dissemination into the CNS through venous routes (32).

Lymphatic seeding

With the lymphatic connections between dura mater and cervical lymphatics and the dural lymphatics that drain into the deep cervical lymph nodes contributing to immune surveillance, these newfound structures have become a suspect in cancer metastasis. In a study by Hu. X et al., (2020), they indicate that meningeal lymphatic vessels (MLVs) play a vital role in brain tumour drainage and lymphatic seeding (32). Dorsal MLVs undergo extensive remodelling when tumours such as gliomas are present, aiding in the transport of immune cells from the brain to the deep cervical lymph nodes (33). This transport is essential for immune response, and disruption of the dorsal MLVs reduces tumour cell drainage to dorsal cervical lymph nodes and weakens the effectiveness of checkpoint therapy (32). This underscores MLVs' potential as a therapeutic target for enhancing immune responses in brain tumours (such as glioblastoma) (33). Historically believed to be an 'immune-privileged' organ, the revealed MLVs and glymphatic pathway challenge this notion by revealing an lymphatic network, including capillary and collecting lymphatics situated along the sigmoid sinus and petrosquamous sinus at the skull base, as well as along the trnsverse sinus and the superior sagittal sinus in the meninges (33). Further research focusing on mapping these routes could provide valuable insights into many aspects of neurology, from cancer treatments as well as treating CNS-associated neuroinflammatory conditions.

Complications and Unresolved Questions

Following the detailed anatomical description and theories of tumour spread, it is important to examine the details that point to a possible undiscovered pathway, possibly lymphatic in nature, in the spread of glioblastoma to the parotid gland. Notably, all cases in the aforementioned case studies reported additional metastases, predominantly to close lymph nodes and lungs.

The parotid metastases varied in treatment approaches. Importantly, regardless of patient survival outcomes, the primary tumour and concurrent metastases were dominant in influencing prognosis.

All observed metastases were ipsilateral to the primary tumour, suggesting a correlation with lymphatic or venous drainage routes – particularly from the temporal lobe, which has drainage patterns through both venous and the aforementioned newly discovered lymphatic systems. This raises the question of whether nearby venous structures, such as the pterygoid plexus, could facilitate metastasis to the parotid given that the maxillary vein transports blood from the pterygoid plexus and then enters the parotid gland (to join the superficial temporal vein and then go on to form the retromandibular vein) (32). Moreover, no direct anatomical structure currently confirms a consistent connection between specific brain regions and the parotid gland, but the consistent ipsilateral occurrence across cases is notable and may imply underlying anatomical pathways yet to be identified or noted as important or relevant.

In the paper by Hu X et al (2020), in which animal models showed that injected brain tracers reach cervical and parotid lymph nodes (which connect to the parotid gland), within minutes – suggesting that localised drainage pathways from the brain might facilitate ipsilateral metastasis. This aligns with the observed patterns of GBM metastasis, where tumour spread occurs on the same side as the primary tumour, supporting a region-specific drainage route that could theoretically transport tumour cells to local lymphatic areas (31). However, there are significant challenges. Differences in brain structures between humans and animals, especially rats (such as the lack of an arachnoid villus in the superior sagittal sinus – with it being in the skull base), limit the direct applicability of these findings to human cases (31). Additionally, the perivascular system's counterflow mechanisms may impede larger particles, like glioblastoma cells, from navigating these routes unless the system is notably compromised (31).

Conclusion

Information on GBM and its methods of metastasis has come a long way in recent years. The brain's newly discovered lymphatic pathways, such as the glymphatic system and meningeal lymphatic vessels (MLVs), have already challenged the traditional idea of the brain being "immune privileged". Ipsilateral parotid metastases in GMB correlate with anatomical drainage routes, such as the pterygoid plexus and retromandibular vein, as well as lymphatic connections to the cervical lymph nodes. These pathways suggest tumour cells could have a tendency to exploit proximity to shared brain-parotid drainage systems, offering a potential mechanism for parotid metastasis.

Despite the aforementioned hypotheses, significant gaps remain. The difference in brain anatomy between animal models and humans limit the applicability of available experimental findings. Additionally, the topic of the glymphatic system in tumour cell clearance and the perivascular system's counterflow mechanisms that may impede metastasis unless disrupted, raises several unanswered questions that require further research to solve and map these pathways conclusively. There are still difficulties in tracking tumour cells in real-time, and a focus on developing methods of tracking tumour cell trafficking could help us understand more about the routes of metastatic dissemination.

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None.

Declarations

Conflict of Interest: N/A

Ethics: N/A

Authors Contribution

Ruby Sciriha Camilleri wrote the manuscript with guidance from Andrea Cuscieri. Ruby Sciriha Camilleri and Nathaniel Gauci carried out the full-text screening and data extraction, Daniele Formosa and Axel Tonna carried out title and abstract screening, Andrea Cuschieri conceptualised the topic, set up the systematic review, draft editing and data analysis. Dr. Christian Zammit gave feedback and guidance.

List of Abbreviations

ABC	ATP-binding cassette
CNS	Central Nervous System
CSF	Cerebro-Spinal Fluid
GBM	Glioblastoma Multiforme
JBI	Joanna-Briggs Institute
MLVs	Meningeal Lymphatic Vessels
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analyses

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Cerebrospinal Fluid Ascites after Ventriculoperitoneal Shunting: A Comprehensive Systematic Review Emphasizing Patient Demographic Attributes

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Abstract

Introduction

An unusual complication of ventriculoperitoneal (VP) shunting is the accumulation of cerebrospinal fluid (CSF) in the peritoneal cavity, resulting in abdominal distention known as CSF ascites. While its impact on the affected individuals' quality of life is significant, knowledge about its demographics and prevalence remains limited. This systematic review aims to systematically assess CSF ascites, prevalence, demographic patterns and contribute to a better understanding of the condition.

Methods

A systematic literature review was conducted in six major databases following the PRISMA 2020 checklist. Articles were included following rigorous screening and pre-defined inclusion criteria and exclusion criteria. A standardised data extraction table was used, and descriptive and inferential statistics were conducted using the Jeffreys's Amazing Statistics Program (JASP). The Joanna-Briggs' Institute (JBI) tool was used to assess the quality of reporting.

Results

79 articles were included, 64 articles documented CSF ascites in children (representing 83 cases), with the remainder describing CSF ascites in adults. An overall high quality and transparent reporting by the included articles was noted. In the children, the median age at VP shunt placement was 12 months. Age at shunt placement didn't differ by gender. The most common conditions were optic pathway tumours. Ascites onset after shunt placement took a median of 10 months (IQR: 40.5 months) and wasn't correlated with age at shunt placement. 92.72% achieved ascites resolution with treatment, with 59.04% receiving conversion to a ventriculoatrial (VA) shunt as treatment. n adult patients, the median age at VP shunt placement was 39.00 years, with no significant gender differences in age distribution. The primary condition was mostly normal pressure hydrocephalus (40.00%, N = 15). Ascites onset after shunt placement took a median of 9 months and did not differ by gender. Age at shunt placement and duration between placement and ascites onset were not correlated. Ascites resolved in 84.62% of patients with the most common treatment being VP to VA shunt conversion.

Discussion and conclusion

CSF ascites, a rare complication of VP shunting across age groups, have diverse causes. This systematic review clarifies its demographics, showing no clear gender or age-related trends in occurrence. The link between primary conditions and demographics echoes disease patterns. Mortality solely from CSF ascites is rare, and treatment success is high. Converting VP to VA shunts stands out as the preferred solution. While data discrepancies limit demographic analysis, this study significantly advances CSF ascites understanding, warranting more research and clinical attention.

Keywords: Ventriculoperitoneal shunts, cerebrospinal fluid ascites, CSF, demographic characteristics, ascites

List of Abbreviations:

Abbreviation	Definition
CI	Confidence interval
CSF	Cerebrospinal fluid
ETV	Endoscopic third ventriculostomy
EVD	External ventricular drain
GBM	Glioblastoma multiforme
IQR	Interquartile range
JASP	Statistical software used for analysis
JBI	Joanne Briggs Institute
NICHD	National Institute of Child Health and Human Development (age categories)
NPH	Normal pressure hydrocephalus
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
TBM	Tuberculous meningitis
VA Shunt	Ventriculoatrial shunt
VCC	Ventriculocholecystic shunt (rare alternative)
VP Shunt	Ventriculoperitoneal shunt
VPS	Ventriculoperitoneal shunt (alternate abbreviation)

Introduction

The prevailing strategy for addressing hydrocephalus prominently revolves around the utilization of ventriculoperitoneal (VP) shunts. However, interventions involving the placement of VP shunts have demonstrated a spectrum of complications, ranging from dysfunction and infection to obstruction and migration (1). Among these complexities, a rare yet significant occurrence emerges, characterized by the emergence of abdominal distention due to the accumulation of cerebrospinal fluid (CSF) within the peritoneal cavity (2). This distinctive clinical manifestation defines CSF ascites, as the ascitic fluid's origin differs from the conventional hepatic, renal, or cardiac etiologies; rather, it stems from an anomalous accumulation of CSF in the peritoneal cavity (3). This accumulation can result in discomfort, abdominal pain, irritability, and challenges in breathing and eating (4–6).

Despite the potential impact of CSF ascites on the quality of life of affected individuals, fundamental insights into the demographic attributes of patients and the scope of known cases remain largely elusive. Yount et al. (1984) were pioneers in examining the literature encompassing case studies detailing CSF ascites, highlighting its occurrence in both pediatric and adult populations. Subsequent efforts by Alexiou et al. (2021) sought to quantify the prevalence of CSF ascites in adults through a systematic review; however, their investigation was confined to a single database and lacked the implementation of quality assessment tools. The following year, Khizar and Zahid (2022) contributed a review encompassing the largest documented series of CSF ascites cases, yet their search lacked systematic rigour and quality evaluation measures. This review endeavours to enhance our current understanding of CSF ascites prevalence, document potential demographic trends associated with the condition, and achieve these objectives through a systematic review using quality assessment.

Methods

This systematic review was generated according to the Systematic Review and Meta-analyses (PRISMA) Checklist 2020 (1), and the Cochrane Handbook for Systematic Reviews of Interventions (2).

Study identification and selection

A systematic search in PubMed, MEDLINE, Google Scholar, Science Direct, Scopus and Semantic Scholar databases were searched for relevant articles published until 20th June 2023, without language restrictions. Combinations of keywords ("ventriculoperitoneal shunt"; "VPS"; "cerebrospinal fluid"; "CSF") and their variations were used to develop a database-specific search strategy in combination with a series of Boolean "AND/OR" operators and asterisk wildcards. Searches of other CSF shunts (such as ventriculoatrial or ventriculopleural) were not conducted since only VP shunts drain CSF fluid into the peritoneum, and by definition, ascites may only occur in the peritoneum. The database outputs were exported as CSV or Bibtext format and uploaded into Rayyan reference managing software (3). Duplicate titles were removed, and an initial screening of titles and abstracts was conducted (by Andrea Cuschieri) to discard irrelevant articles. Full-text screening was then conducted (by Andrea Caruana, James Zerafa, Conor Shaw, Elyssa German, Gillian Attard Montalto, Robert Pisani, Gillian Pace, and Michael Farrugia) with any disputes resolved by Andrea Cuschieri to include or exclude articles.

Inclusion criteria

Included articles must have been 1) cases reports, case series or conference abstracts detailing a case report/ case series; 2) which clearly describe the occurrence of CSF ascites; 3) following the placement of a CSF shunt; 4) in children or adults; 5) which meet at least half of the criteria of the

quality of reporting assessment tool in this systematic review; and 6) reported data per patient.

Exclusion criteria

Articles elucidating instances of ascites in patients with a VPS shunt with a known extra-cranial cause were not included.

Data extraction and analysis

The following data, where possible, were extracted from each study (by Andrea Caruana, James Zerafa, Conor Shaw, Elyssa German, Gillian Attard Montalto, Robert Pisani, Gillian Pace, Michael Farrugia) using a standardised data extraction table: author(s) and year of publication, sex and age on VPS placement, primary underlying condition, surgical treatment for ascites, duration between shunt placement and onset of ascites, and the outcome of ascites. The data was stratified into child adolescent, and adult groups according to age on VPS placement and analysed accordingly. Age on VPS placement in the children and adolescent group was categorised and presented according to the National Institute of Child Health and Human Development (NICHD) age stages (Birth – 27 days; 28 days – 12 mo; 13 mo – 2y; 2 – 5y; 6 – 11 y; 12 – 18y; 19 – 21y) (4). In adults, age on VPS placement was categorised into the following groups: 21 – 24y; 25 – 44y; 44 – 59y; > 60y). In both groups, the duration between shunt placement and the onset of ascites data was also categorised into five distinct groups (< 1 wk; 1 mo - < 6 mo; 6 mo - < 1y; 2 yr - < 5y; > 5y).

The median (range), interquartile range (IQR) and percentages were employed to summarize numerical outcomes for variables categorized as normally and non-normally distributed, respectively. To assess relationships in discrete variables, Pearson's R correlation coefficient and Chi-square testing were utilized. Pearson's R correlation was conducted between age on VPS placement versus duration between shunt placement and onset of ascites data in months.

The test statistic, significant level, population number and confidence intervals were reported, where applicable. Significance across all tests was established at $P < 0.05$. All statistics were conducted using JASP version 0.17.3.

Quality of reporting assessment

Quality of reporting was assessed using the Joanne Briggs Institute (JBI) case report and case series critical appraisal tool. This tool includes eight questions that address the internal validity and risk of bias of case report designs, particularly information and confounding bias in addition to the importance of clear reporting (5). The JBI tool was filled in per the included article (by Andrea Caruana, James Zerafa, Conor Shaw, Elyssa German, Gillian Attard Montalto, Robert Pisani, Gillian Pace, and Michael Farrugia) with disputes resolved by Andrea Cuschieri). Each domain was afforded one of three possible judgements: "reported"; "Not reported" or "Unclear". The overall quality of reporting about paediatric and adult studies was assessed together. Studies which failed to satisfy at least half of the JBI criteria were excluded.

Results

Literature search and quality of reporting assessment.

73 tentative articles were identified, with 132 being duplicates. 236 studies were excluded following title and abstract screening using the predefined inclusion criteria. Thus, 105 reports were sought for retrieval and their full text was assessed in detail for eligibility. Among these, 3 studies could not be retrieved, and 26 studies were excluded following full-text review. Citation-searching of the 77 included studies revealed an additional 3 potential studies, which met our inclusion criteria following an in-depth assessment of their full text, resulting in a total of 79 studies included in this systematic review (6–84) (Figure 1). Of the total 79 included studies, 64 studies described cases of CSF ascites in

children or adolescents, representing 83 patients, while 15 cases pertained to CSF ascites occurring in adults. Quality of reporting assessment using the JBI tool demonstrated an overall high quality and transparent reporting by the included articles. Adverse events (harms) or unanticipated events were perceived to be largely unclearly reported (Figure 2).

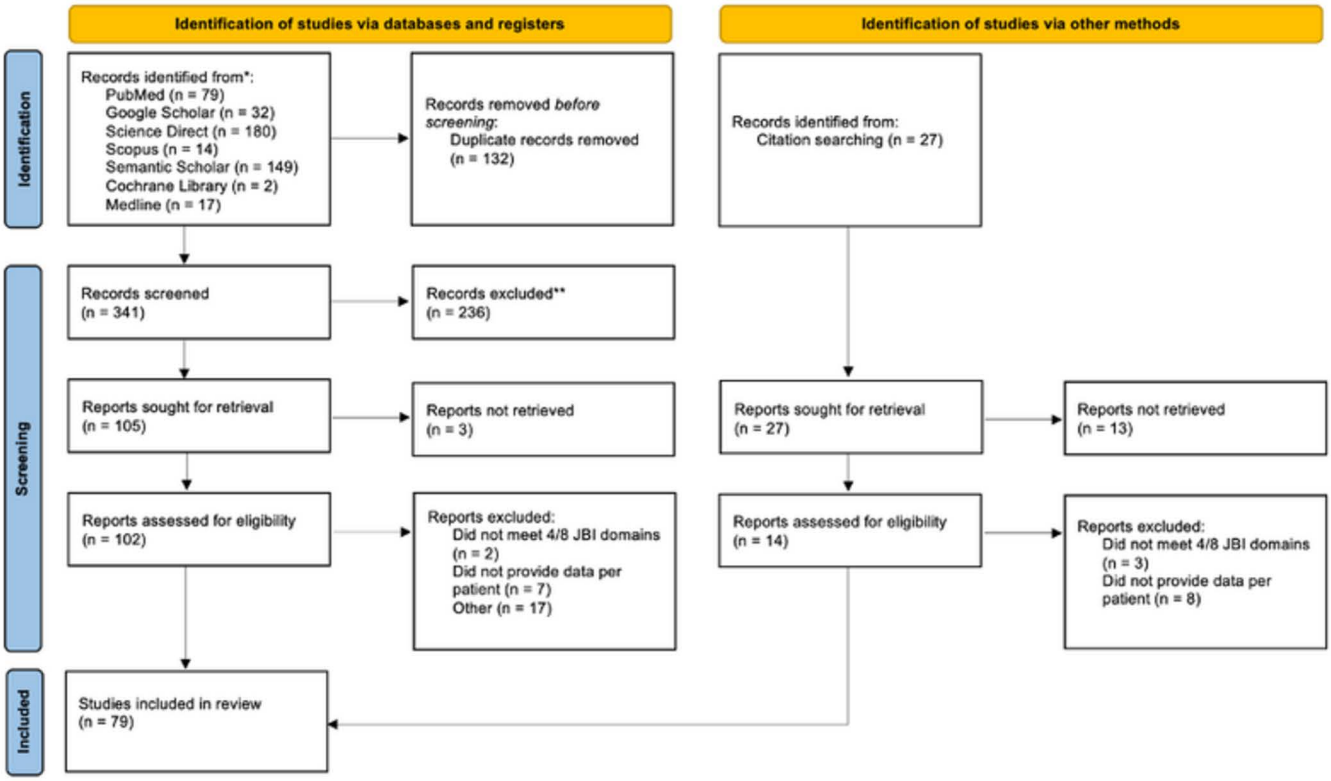

Figure 1: Prisma 2020 flow diagram (1).

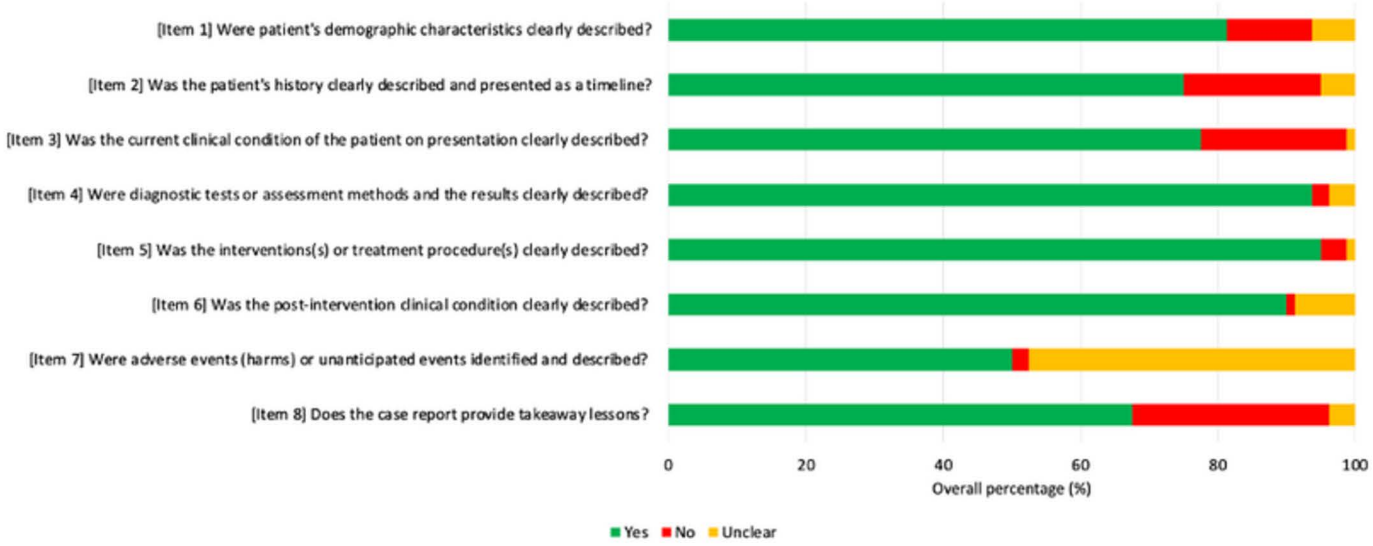

Figure 2: Overall quality assessment appraisal using the JBI tool (5).

CSF ascites in children and adolescents

The median age for VP shunt placement of patients in the children and adolescents group was 12 months [IQR: 41.5 months; N = 69]. The gender distribution of this cohort was almost equal [male = 53.62%, N = 69], and age on VP shunt placement did not differ between males and females [$\chi^2 = 4.499$, p = 0.480, N = 58]. The most common primary underlying condition was optic pathway tumours, followed by hydrocephalus whose cause was not disclosed (Figure 3). The median duration between shunt placement and the onset of ascites was 10 months [IQR: 40.5 months, N = 73), and was not correlated with patients' age on VP shunt placement [r = -0.097, p = 0.431, CI(-0.320, 0.136), N = 73]. 92.72% of patients had complete resolution of the ascites following treatment [N = 83]. Two patients succumbed to the primary underlying condition (17,74), two died due to surgery-related complications (77,79), and the other two remaining patients died due to infection (56), and respiratory distress (62). Conversion of the VP shunt to a ventriculoartrial (VA) shunt was the most common surgical remedy conducted in the children and adolescent cohort [59.04%, N = 83]. Per patient data is presented in Table 3.

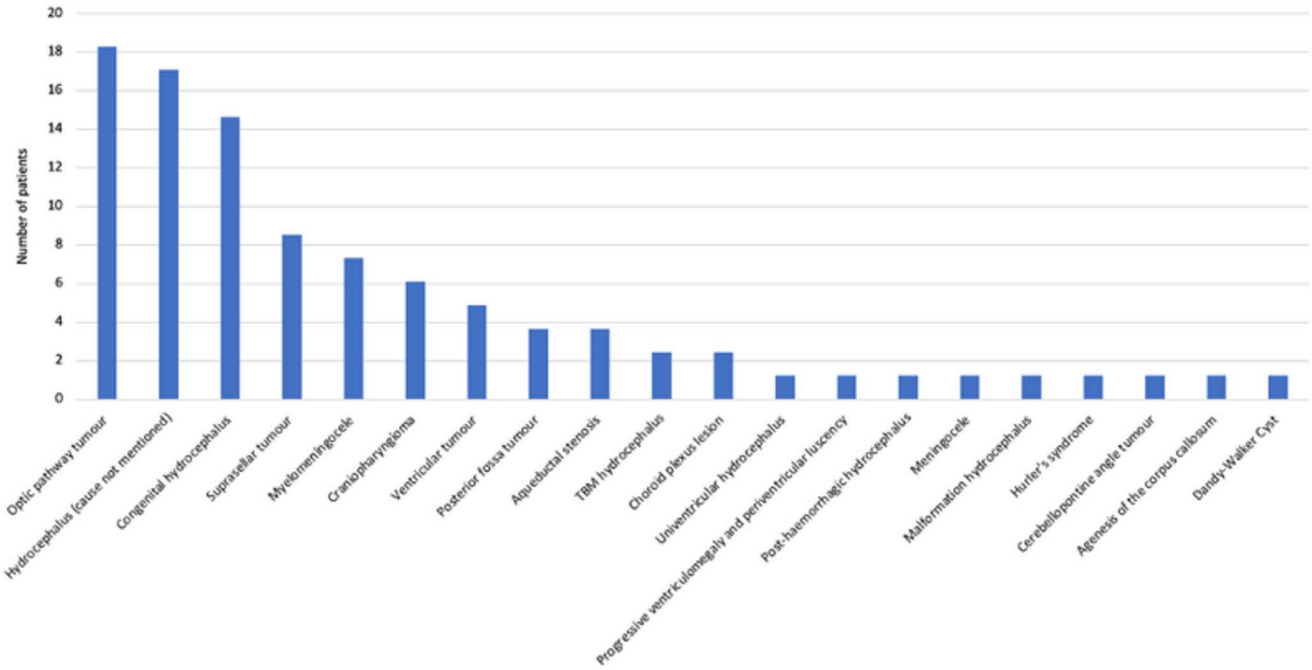

Figure 3: Distribution of the primary underlying condition in the child and adolescent patient cohort.

Table 1: Per patient data of child and adolescent patients who developed CSF ascites

Author, year	Gender	Age on shunt placement	Primary underlying condition	Surgical treatment for ascites	Duration between shunt placement and the onset of ascites	Outcome of ascites
Ray Peckpeck, 1956	F	28 days - 12 mo	Choroid plexus lesion	• Convert to VA • Tumour excision.	< 1wk	Deasurgery-related complications)
Ames, 1967	M	13 mo - 2y	/	• Convert to VA.	/	Resolved
Odeku et al., 1970	/	13 mo - 2y	Hydrocephalus (cause not mentioned)	• Repeated paracentesis.	1 - <6mo	Persistent ascites
Dean & Keller, 1972	M	Birth - 27 days	Aqueduct stenosis	• Repeated paracentesis.	6 mo - <1y	Resolved
Rosenthal et al., 1974	F	13 mo - 2y	Suprasellar tumour	• Convert to VA.	2 - < 5y	Resolved
Parry et al., 1975	F	Birth - 27 days	Aqueduct stenosis	• Laparotomy to evacuate ascites • Convert to VA • VA converted back to VP.	2 - < 5y	Resolved
	F	28 days - 12 mo	Hydrocephalus (Cause not mentioned)	• Shunt revision at the peritoneal end.	> 5y	Resolved
	M	2 - 5y	Dandy-Walker cyst	• Laparascopic drianage • Shunt externalisation and internalisation at the peritoneal end.	2 - < 5y	Resolved

	M	28 days - 12 mo	Hydrocephalus (Cause not mentioned)	• Surgical drainage • Convert to VA.	1 - 4wk	Resolved
Weidmann et al., 1975	F	Birth - 27 days	Congenital hydrocephalus	• Paracentesis • Convert to VA.	1 - 4wk	Resolved
Adeloye & Olumide, 1977	/	13 mo - 2y	Hurler's syndrome	/	/	Death
Mori et al., 1977	/	28 days - 12 mo	Posterior fossa tumour	• Repeated paracentesis.	2- < 5y	Resolved
Lees et al., 1978	/	28 days - 12 mo	Myelomeningocele	• Convert to VA.	1 - < 2y	Resolved
Noh et al., 1979	/	28 days - 12 mo	Posterior fossa tumour	• Convert to VA.	1 - <6mo	Resolved
Ohaegbulam, 1980	/	6 - 11y	Craniopharyngiom a	• Paracentesis.	6 - <1y	Resolved
Adegbite & Khan, 1982	F	6 - 11y	Craniopharyngiom a	• Convert to VA.	1wk - 1mo	Resolved
Agha, 1983	M	6 - 11y	Suprasellar tumour	• Convert to VA.	6mo - <1y	Resolved
Yount et al., 1984	F	12 - 18y	Hydrocephalus (cause not mentioned)	• Paracentesis • Repeatedd taps of shunt reservoir.	2 - < 5y	Resolved
	F	2 - 5y	Hydrocephalus (cause not mentioned)	• Convert to VCC.	6mo - <1y	Resolved
	M	2 - 5y	Optic pathway tumour	• Paracentesis • Low sodium diet.	1 - 4wk	Resolved
	F	28 days - 12 mo	Optic pathway tumour	/	2 - < 5y	Resolved
Goodman & Gourley, 1988	F	28 days - 12 mo	Meningomyelocele	• Convert to VA.	> 5 yr	Resolved
Perez Pena et al., 1990	M	6 - 11y	Malformation hydrocephalus	Convert to VA.	> 5 yr	Resolved
Isla et al., 1991	F	/	Suprasellar tumour	• Convert to VA.	/	Resolved

Tang et al., 1991	F	28 days - 12 mo	Optic pathway glioma	• Convert to VA.	1 - <6mo	Death (due to primary condition)
Suarez et al., 1993	M	/	Craniopharyngioma	• Convert to VA.	/	Resolved
West et al., 1994	/	28 days - 12 mo	Optic pathway tumour	• Convert to VA.	1 - 4wk	Resolved
	/	2 - 5y	Optic pathway tumour	• Convert to VA • Removal of VA shunt • Ventriculostomy • Placement of VP shunt.	2 - < 5yr	Resolved
	/	28 days - 12 mo	Optic pathway tumour	• Convert to VA.	1 - <6mo	Resolved
Britz et al., 1996	M	Birth - 27 days	Congenital hydrocephalus	• Convert to VA.	1 - <6mo	Resolved
Nairn-Ur-Rahman et al., 1996	M	2 - 5y	Hydrocephalus (cause not mentioned)	• Shunt externalisation and internalisation at the peritoneal end.	1 - <6mo	Resolved
Gruber et al., 1997	M	2 - 5y	Optic pathway tumour	• Convert to VA.	6 - <1y	Resolved
Shuper et al., 1997	F	2 - 5y	Optic pathway tumour	• Paracentesis.	1 - < 2y	Death (due to infection)
Yukinaka et al., 1998	F	Birth - 27 days	Congenital hydrocephalus	• Convert to VA.	> 5 y	Resolved
Chidambaram & Balsubramaniam, 2000	F	28 days - 12 mo	Congenital hydrocephalus	• Convert to VA.	2y - < 5y	Resolved
	M	6 - 11y	Optic pathway tumour	• Convert to VA.	1 - <6m	Resolved
	/	6 - 11y	Optic optic pathway tumour	• Convert to VA.	/	Resolved

Gil et al., 2001	/	28 days - 12 mo	Optic pathway tumour	• Convert to VA.	/	Resolved
	/	2 - 5y	Optic optic pathway tumour	• Convert to VA.	/	Resolved
	/	28 days - 12 mo	Optic optic pathway tumour	• Convert to VA.	/	Resolved
Karp et al., 2001	F	13 mo - 2y	Myelomeningocele	• Shunt externalisation at the peritoneal end • Removal of shunt.	> 5y	Resolved
Longstreth et al., 2001	F	28 days - 12 mo	Hydrocephalus (cause of it not mentioned)	• Repeated paracentesis • Laparascopic drianage • Convert to VA.	> 5y	Resolved
Binitie et al., 2002	M	28 days - 12 mo	Progressive Ventriculomegaly and Periventricular Lucency	• Convert to VA.	1 - 4 wk	Death (due to infection)
	F	13 mo - 2y	Congenital Hydrocephalus	• Convert to VA.	1 mo - <6 mo	Resolved
	M	28 days - 12 mo	Congenital Hydrocephalus	• Convert to VA.	< 1 wk	Resolved
Kumar et al., 2003	M	6 - 11y	Hydrocephalus (cause of it not mentioned)	• Paracentesis.	6mo - <1y	Death (due to primary condition)
	M	28 days - 12 mo	Hydrocephalus (cause of it not mentioned)	• Total excision of the third intraventricular tumour.	1 - < 2y	Resolved
	M	28 days - 12 mo	TBM hydrocephalus	• Shunt revision at perionteal end.	2 - < 5y	Resolved
	M	6 - 11y	Suprasellar tumour	• Replace the shunt with a new one.	1 - < 2y	Resolved

Yaqoob et al., 2003	M	12 - 18y	TBM hydrocephalus	• Repeated paracentesis • Shunt externalisation at the peritoneal end • Resection of choroid plexus papilloma.	1 - <6mo	Resolved
Pawar et al., 2003	F	28 days - 12 mo	Ventricular tumour	• Tumour excision.	1 - <6mo	Resolved
	M	13 mo - 2y	Ventricular tumour	• Tumour excision.	1 - <6mo	Death (due to primary condition)
El-Naggar et al., 2005	M	28 days - 12 mo	Post-hemorrhagic hydrocephalus	• Shunt externalisation the at peritoneal end then removed • Convert to VA.	1 - 4wk	Resolved
Dai et al., 2006	F	28 days - 12 mo	Suprasellar tumour	/	/	Resolved
Diluna et al., 2006	M	28 days - 12 mo	Congenital hydrocephalus	• Convert to VA.	2 - < 5y	Resolved
Niggemann et al., 2007	F	13 mo - 2y	Hydrocephalus (cause not mentioned)	• Paracentesis • Convert to VA.	> 5y	Resolved
Smith et al., 2009	M	28 days - 12 mo	Hydrocephalus (cause not mentioned)	• Shunt externalisation at peritoneal • Convert to VA.	1 - <6mo	Resolved
Das et al., 2010	M	6 - 11y	Craniopharyngioma	• Convert to VA.	1 - < 2y	Resolved
Paik et al., 2010	F	28 days - 12 mo	Myelomeningocele	• Convert to VA.	> 5y	Resolved
Al Fawaz & Ahmad, 2011	M	Birth - 27 days	Congenital Hydrocephalus	• Repeated paracentesis	2 - < 5y	Resolved

Duinen et al., 2011	F	12 - 18y	Meningocele	• Convert to VA.	> 5y	Resolved
Mwang'ombe et al., 2012	M	28 days - 12 mo	Aqueductal stenosis	• Repeated paracentesis • Endoscopic third ventriculostomy (ETV).	> 5y	Resolved
Comba et al., 2013	M	28 days - 12 mo	Myelomeningocele	• Convert to VA.	> 5y	Resolved
O'Halloran et al., 2013	F	13 mo - 2y	Suprasellar tumour	• Convert to VA.	2 - < 5y	Resolved
Woodfield & Magdum, 2013	F	28 days - 12 mo	Craniopharyngioma	• Convert to VCC.	/	Resolved
Legault et al., 2014	M	2 - 5y	Posterior fossa tumour	• Repeated paracentesis.	2 - < 5y	Resolved
Tekerek et al., 2014	M	28 days - 12 mo	Congenital hydrocephalus	• Died before treatment.	6mo - <1y	Death (due to respiratory distress)
Upadhyaya et al., 2017	M	13 mo - 2y	Cerebellopontine angle tumour	• Repeated paracentesis • Convert to VA.	6mo - <1y	Resolved
Sachan & Manohar, 2017	M	2 - 5y	Hydrocephalus (cause not mentioned)	• Repeated paracentesis • Shunt externalisation at the peritoneal end • Resection of choroid plexus papilloma.	2y - < 5y	Resolved
Siddiqi et al., 2017	F	Birth - 27 days	Congenital hydrocephalus	• Repeated paracentesis • Convert to VA.	> 5y	Resolved
Hori et al., 2018	M	Birth - 27 days	Congenital hydrocephalus	• Unilateral resection of choroid plexus.	1 - <6mo	Resolved

Mishra et al., 2018	M	2 - 5y	Suprasellar tumour	• Convert to VA.	2 - < 5y	Resolved
Musa et al., 2018	F	2 - 5y	Hydrocephalus (cause not mentioned)	• Repeated paracentesis • Shunt revision at perionteal end • Convert to VA.	1 - 4wk	Resolved
Mohammad et al., 2019	F	Birth - 27 days	Choroid plexus lesion	• Convert to VA.	2 - < 5y	Resolved
Gülşen et al., 2020	M	2 - 5y	Ventricular tumour	• Shunt externalisation the at peritoneal end • Paracentesis • Removal of shunt • EVD placement.	6mo - <1yr	Resolved
Suleiman et al., 2020	F	Birth - 27 days	Agenesis of corpus callosum	• Convert to VA.	> 5y	Resolved
Bekralas et al., 2021	/	28 days - 12 mo	Univentricular Hydrocephalus	• Convert to VA.	>5y	Resolved
Khizar & Zahid, 2022	F	28 days - 12 mo	Myelomeningocoel e	• Paracentesis • Removal of shunt • Endoscopic third ventriculostomy (ETV).	2 - < 5y	Resolved
Mathew et al., 2022	F	6 - 11y	Suprasellar tumour	• Repeated paracentesis • Convert to VA.	1 - <6mo	Death (surgery-related complications)
Mehta et al., 2022	M	28 days - 12 mo	Hydrocephalus (cause not mentioned)	• Convert to VA.	> 5y	Resolved
Eder et al., 2023	F	2 - 5y	Optic pathway tumour	• Convert to VA.	6mo - <1y	Resolved
Gader et al., 2021	M	2 - 5y	Suprasellar tumour	/	< 1wk	Resolved

CSF ascites in adults

The median age of adult patients on VP shunt placement was 39.00 years [IQR: 18 years; N = 11], with the occurrence of ascites being evenly distributed amongst males and females [N = 14]. Age on VP shunt placement did not differ between males and females [χ^2 = 0.972, p = 0.615, N = 11]. Normal pressure hydrocephalus was the most common primary underlying condition [40.00%, N = 15]. The median duration between VP shunt placement and the onset of ascites was 9 months [IQR: 71 months; N = 11] and did not differ between males and females [χ^2 = 2.222, p = 0.528, N = 11]. Age on VP shunt placement and duration between shunt placement were not correlated [r = -0.492, p = 0.124, N = 11, CI(-0.843, 0.153)]. Ascites resolved in 84.62% of included patients (N = 13). One patient succumbed to their primary disease (23), while another died following a pulmonary embolism unrelated to the ascites or primary condition (6). Conversion of VP to VA shunt was the commonest surgical treatment in adult patients (40.00%, N = 15), followed by shunt externalisation at the peritoneal end. Per patient data is presented in Table 2.

Table 2: Per patient data of adult patients who developed CSF ascites.

Author, year	Gender	Age on shunt placement	Primary underlying condition	Surgical treatment for ascites	The duration between shunt placement and the onset of ascites	Outcome of ascites
Cummings et al., 1972	/	45 - 59 yr	Aqueductal stenosis	• Convert to VA	2 yr - < 5 yr	Resolved
Gottfried et al., 2004	M	45 - 59 yr	Post-hemorrhagic hydrocephalus	• Shunt externalisation and internalisation at the peritoneal end	1 mo - <6 mo	Resolved
Montano et al., 2010	F	45 - 59 yr	Large left vestibular schwannoma	• Shunt externalisation at the peritoneal end • Removal of shunt	2 yr - < 5 yr	Resolved
Gómez & Miranda, 2015	M	25 - 44 yr	Normal pressure hydrocephalus	• Convert to VA	> 5 yr	Resolved
Jamal & Abrams, 2016	F	25 - 44 yr	Normal pressure hydrocephalus	• Convert to VA	1 mo - <6 mo	Resolved
Han et al., 2017	M	/	Dandy walker syndrome	• Convert to VA	/	Resolved
Ng et al., 2018	M	25 - 44 yr	Temporal tumour	• Shunt externalisation at the peritoneal end • Peritoneal drain	1 mo - <6 mo	Death (due to pulmonary embolism)
Li et al., 2019	F	/	Loeys-Dietz syndrome	• Convert to VA	/	Resolved

Hironaka et al., 2019	M	25 - 44 yr	intradural extramedullary malignancy melanoma spinal with CSF dissemination	/	6 mo - <1 yr	/
Alexiou et al., 2021	F	> 60 yr	Bilateral Frontal Meningiomas	• Convert to VA	1 mo - <6 mo	Resolved
Azhar et al., 2021	M	/	Normal pressure hydrocephalus	• Shunt ligation	/	Resolved
Ifeyinwa et al., 2021	F	25 - 44 yr	Normal pressure hydrocephalus	• Repeated Paracentesis • Shunt externalisation at the peritoneal end	> 5 yr	Resolved
Low et al., 2021	F	45 - 59 yr	Posterior fossa tumour	• Peritoneal drain	1 mo - <6 mo	Death (due to primary condition)
Wu et al., 2021	M	/	Normal pressure hydrocephalus	• Paracentesis • Shunt ligation	/	/
Shabeeb et al., 2022	F	25 - 44 yr	Normal pressure hydrocephalus	• Repeated paracentesis • Removal of shunt	> 5 yr	Resolved

Discussion

CSF ascites have been traditionally considered a rare complication of VP shunting (15). The cause of CSF ascites is currently unknown, however many aetiologies have been postulated. Such aetiologies include subclinical peritonitis, which obstructs lymphatic drainage (48), heightened CSF protein levels leading to peritoneal malabsorption (9,12,49), and excessive CSF production surpassing absorptive capacity (36). Factors such as multiple shunt revisions (11,40), immunological response to vaccination (40), or shunt degradation (52) can all contribute to peritoneal irritation. Chronic illnesses such as tuberculosis and brain tumours (e.g., optic gliomas and craniopharyngiomas) are associated with elevated CSF protein levels (9,49). The excessive production of choroid plexus papillomas has been linked to ascites (74). To confirm a diagnosis of CSF ascites, it's beneficial to compare the biochemical composition of CSF shunt aspirate with ascitic fluid obtained through paracentesis (13). Spontaneous resolution of CSF ascites occurs when CSF flow is redirected using a VA shunt or endoscopic third ventriculostomy (ETV) (36).

A complete understanding of the demographics of CSF ascites and investigations into possible trends to identify demographic risk factors was not previously available. This systematic review attempted to fill this knowledge gap and has identified 79 known cases of CSF ascites reported in the literature (6–84), suggesting it may be more common than previously thought. Upon closer inspection CSF ascites were documented to occur in more paediatric patients, suggesting it may be rare in adults. Notwithstanding, the number of cases reported in each age group resonates with the demographic characteristics of hydrocephalus, which is eight times more common in children in comparison to adults (85). This is on par with the ratio of the number of paediatric and adult cases

observed in this study. Thus, CSF ascites cannot be classified as a strictly paediatric or adult complication of VP shunting, as it is equally rare in age both groups.

This observation can be further extended to other trends noted in both age groups. In both children and adults, the age upon VP shunt placement and duration between shunt placement and onset ascites were not significantly more prevalent in either gender. Moreover, age upon VP shunt placement was not correlated with the duration between shunt placement and onset of ascites in both groups. Thus, currently, the onset of ascites has no known demographic risk. Yet to date, no case has been reported wherein patients have succumbed to CSF ascites nor of cases that did not resolve following surgical treatment.

The incidence of primary underlying conditions documented in both age groups corresponds to the demographic trends of the respective diseases. Thus, this complication of CSF ascites is curable with appropriate treatment, with documented deaths being attributed to the primary underlying condition, surgical complications or infection. In our systematic review, the conversion of a VP shunt to a VA shunt has been the most commonly documented treatment for curing CSF ascites. Other methods employed were Repeatedd paracentesis and externalisation of the shunt at the peritoneal end.

Strengths and limitations

This article is the first systematic review with quality of reporting assessment to fully document cases of CSF ascites without extra-neural cause reported in the literature. Moreover, stringent inclusion criteria were employed. Nonetheless, the application of such stringent criteria may have led to the inadvertent exclusion of previously reported cases of CSF ascites, as noted in antecedent reviews. Nevertheless, this cautious approach was imperative to uphold the integration of studies marked by robust reporting standards.

The extraction of data encompassing protein levels within both CSF and ascitic fluid was precluded owing to incongruities in the employed reporting conventions and measurement units across the amalgamated studies. Moreover, not all studies encompassed in the analysis afforded a comprehensive portrayal of patient demographic attributes, with a subset failing to accurately delineate parameters such as patient age at the time of VP shunt placement or the onset of ascites. This omission introduces an inherent limitation in the presentation of the demographics associated with CSF ascites within the purview of this study.

Conclusion

CSF ascites, an equally rare complication of VP shunting in both paediatric and adult cohorts, presents a complex array of potential causes, yet the precise physiology governing the generation of ascites is largely undetermined. This systematic review sheds light on the demographic landscape of this condition. Gender, age at shunt placement, and interval to ascites onset exhibited no pronounced patterns, suggesting its random occurrence especially since the trend of primary underlying conditions mirrors disease demographics. Notably, cases of mortality due solely to CSF ascites remain absent, and most cases responded well to treatment. The conversion of VP shunts to VA shunts emerged as the favoured intervention in treating CSF ascites, with appropriate treatment being curative. Discrepancies in data reporting and missing patient attributes added limitations to the demographic analysis. This study, however, constitutes a vital step in advancing our understanding of CSF ascites, its demographics, and its management, prompting further research and clinical consideration.

Author's Contribution

Andrea Cuschieri conducted the idea conceptualisation, systematic literature review search, data analysis, writing the initial daft and editing of the initial draft. Andrea Caruana, James

Zerafa, Conor Shaw, Elyssa German, Gillian Attard Montalto, Robert Pisani, Gillian Pace, and Michael Farrugia carried out article screening, data extraction and writing of the initial draft. Ruby Sciriha Camilleri carried out formatting and editing of the initial draft. Mr. Shawn Agius provided supervision and conducted editing of the initial draft.

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Xenotransplantation Review: Immune Barriers, Tissue Engineering, and Clinical Integration

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Abstract

The imbalance between the high demand for organ transplantation in humans and the insufficient organ supply makes xenotransplantation a promising technology to provide unlimited organs to patients on the waiting list and thus decrease the mortality rate. Xenotransplantation is associated with multiple immunological barriers that can result in xenograft rejection, in addition to the risk of transmitting microorganisms, such as viruses. Scientists continue to develop techniques, including immunosuppressive therapy and genetic modification, to reduce the immunogenicity of xenografts. Decellularisation techniques facilitate the clinical use of porcine heart valves. Additionally, the recellularisation using stem cells offers a new avenue for enhancing graft functionality. This review presents the current understanding of xenotransplantation and the related mechanisms in xenograft infection, discussing the progress in the genetic editing process and the latest clinical trials of pig-to-human xenotransplantation.

Keywords: Xenotransplantation, Immunogenicity, genetically modified pigs, Decellularisation.

Methodology

Literature was sourced from PubMed, EMBASE, and Google Scholar, and supplemented by reputable institutional sources such as NYU Langone Health's News Hub and Press Releases. The search included English-language materials published up to April 2025. Search terms included combinations of "xenotransplantation," "immunogenicity," "genetically modified pigs," "decellularization," and related keywords. Inclusion criteria were original studies, reviews, and clinical reports addressing the physiological, immunological, and technical aspects of xenotransplantation. Non-English articles, abstracts, and studies unrelated to mammalian xenografts were excluded. Selection was based on scientific merit and relevance. This review presents a thematic synthesis rather than a quantitative analysis.

Introduction to Xenotransplantation

Xenotransplantation is a procedure of transferring cells, tissue, or organs between different species. Transferring xenograft between more closely related species (e.g., between primates or rodents) is called concordant xenotransplantation. At the same time, discordant xenografting is performed between

species that are more distantly related, such as between pigs and humans (1). Allotransplantation, transplantation between the same species with different genotypes, is considered an appropriate treatment for patients with end-stage organ failure. The United States' Official information about organ donation and transplantation has shown that, as of January 2019, the number of patients pending transplantation exceeded 113,000 which is a substantial number considering the 36,528 transplantations carried out in 2018 (2).

Moreover, 300,000 patients are waiting to receive a transplant in China, while the number of organs available is only around 16,000 yearly(3)The increasing demand for organ transplantation, coupled with insufficient organ availability, highlights the need for an alternative solution. This makes xenotransplantation a promising technology, as it has the potential to provide an unlimited supply of organs.

The concept of xenotransplantation was first presented in 1667, based on the idea of transferring blood from lambs to humans in a procedure known as transfusion (4). Then, it progressed to the transfer and implantation of animal organs for clinical purposes. For instance, in 1905, an attempt to transplant a rabbit kidney into a human was documented (5)During the 1920s-1990s, trials were conducted on transplanting non-human primate organs (NHP) into humans (6).

NHPs share high level similarity with humans' physiology, anatomy, and immunity in comparison to other species. However, pigs are more preferred as a source of organs due to having fast reproductive maturity of 3-5 months, thus hastening the population's growth rate, which is reflected as unlimited organ's availability. In addition to that, organs obtained from pigs have a sufficient adult size, a high breeding potential, lower maintenance costs, and advanced pig cloning techniques, all of which are advantages that enhance the superiority of using pig organs (7).

Xenotransplantation is associated with numerous barriers, including immune rejection, inflammation, coagulation dysfunction, and cross-species transmission of pathogens. Currently, immune-suppressive therapy and genetic modification have enabled the use of xenogeneic tissue by reducing its immunogenicity (7).

Pathobiology of xenograft

Immune rejection

The severity of the immunological response depends on the form of xenotransplantation, and rejection becomes more dramatic in discordant xenografts, characterised by complete organ damage via vascular thrombosis within minutes to a few hours. In comparison, immune rejection occurs late and is mild in the case of discordant xenografting(1) The immune system is essential for human survival and is composed of a combination of cells, chemicals, and organs that work together as a defence mechanism to attack pathogenic antigens. Immune rejection occurs when the recipient's immune system recognises the transplanted organ as a foreign body, and therefore the organ is damaged and processed. There are three forms of immune rejection (8,9):

1. Hyperacute rejection (HAR) is the most severe type of immune rejection, occurring within minutes to hours, and results in vascular thrombosis, edema, and necrosis.
2. Acute rejection: The patient may develop acute rejection between weeks to several months after transplantation and is identified by many features such as fever, swelling, tenderness at the transplantation site, and the organ becomes impaired.
3. Chronic rejection is a delayed-onset rejection, occurring months to years after graft transplantation, characterised by functional decline of the graft and damage, as well as the presence of tertiary lymphoid organs.

There are similarities in the immunological response

and rejection in allotransplantation and xenotransplantation. Still, the differences rely on the presence of foreign components on the xenograft's surface that initiate an immune reaction with the recipient's system, in addition to the molecular variety in the cross-reactive transmission, such as complements, natural antibodies, macrophages, and natural killer cells.

Inflammation and coagulative dysfunction

Inflammation following xenograft transplantation is characterised by an increase in inflammatory markers, including C-reactive proteins, chemokines, cytokines, and histones. Inflammation induces coagulopathy and enhances the adaptive immune response. Thus, anti-inflammatory approaches will help prolong the survival of the xenograft by preventing systemic inflammation (10). This is achieved by using anti-inflammatory and genetic modification techniques, which involve inserting human inflammatory-regulatory genes into the donor pig's genome. The studies showed that enhancing the expression of heme oxygenase-1(HO-1), an inducible antioxidant enzyme with anti-inflammatory properties (11) on porcine cells, results in effective prevention of the inflammation caused by tumour necrosis factor (alpha TNF-alpha), oxidation and cell apoptosis and corresponds to protecting against coagulation dysfunction resulting from xenotransplantation (12).

In addition to that, TNF alpha induced protein 3 (TNF AIP3) acts as anti-inflammatory protein and in a rodent model shows its ability to inhibit the nuclear factor kappa B (NF-κB) which is a protein transcription factor that regulate the innate immunity and provide resistance against the invading pathogens by creating connection between both the pathogenic and cellular danger signals (13).

Xenografts are susceptible to coagulopathy due to dysfunction in a complex pathway of interactions involving inflammation and innate immunity (14)

Coagulative dysfunction is considered the primary cause of graft failure and is characterised by thrombocyte aggregation, which can lead to thrombosis after transplantation. The process of platelet deposition is triggered by multiple activators, including von Willebrand factor (vWF), collagen, tissue factor (TF), induced thrombin, and adenosine diphosphate (ADP). Additionally, thrombus development can occur due to molecular incompatibility between primates and pigs. Studies have shown that genetic manipulation and pharmacological treatment can contribute beneficially to repressing the coagulopathy that occurs in xenotransplantation (15–18).

Cross-species transmission of porcine endogenous retrovirus

One of the concerns in animal-to-human xenotransplantation is the transmission of zoonotic pathogenic microorganisms, which include bacteria, porcine viruses, fungi, and parasites.

The transmission of bacteria, fungi, and parasites can be eliminated through designated pathogen-free (DPF) breeding, in contrast to porcine endogenous retroviruses (PERVs), which DPF cannot avoid due to their incorporation into the pig genome and subsequent inheritance. PERVs are gamma retroviruses that can cause immunodeficiency and tumours in the hosts. Scientists have not yet reached a consensus on the necessity of inactivating PERV in donor pigs through genetic modification (19). Moreover, research has proved that RNA interference technology can be used to inactivate PERV (20). A clinical trial by Morozov et al., in which pig islets were used as xenotransplants, was achieved without PERV transmission (21), which is a similar finding to the results of another clinical trial, in which pig-to-NHP corneal xenotransplantation was done by Choi et al., as no PERV transmission occurred as well (22).

Although some in vitro experiments have shown PERV transmission from pig to human and from human-to-human cells, the infection was limited to

specific types of cells. These cells had a functional receptor on their surface. Therefore, cells lacking these receptors are protected from infection (23). Additionally, another factor that reduces the likelihood of cell infection is the presence of cellular restriction factors, such as APOBEC3G. This was evidenced by studies which have shown that HEK 293 cells, which do not express APOBEC3G, have a higher probability of being infected by PERV (24). Because immunosuppressants are given when xenograft is transplanted to a patient, in addition to the lack of scientific data about the possibility of PERVs transmission when a solid vascularised procaine organ is transplanted into an immunocompromised recipient, it is imperative to give sufficient attention to the potential ability of PERVs to produce disease, thus preventing the possibility of zoonosis' occurrence.

Eliminating the immunogenicity

Several strategies are employed to mitigate the risks associated with xenotransplantation, including the control of pathogen transmission and the implementation of screening protocols. Additionally, crosslinking techniques assist in preventing immune rejection by controlling the epitopes of the xenograft, in addition to fixing the graft's matrix to suppress its conversion into other tissue types as part of the healing process. Additionally, decellularisation of the tissue before implantation is considered an effective technique for removing xenogeneic cellular components and thereby minimising the immunological response that may occur. Immunosuppressive therapy and genetic medications played a crucial role in the success of xenotransplantation (25).

Immunosuppressive Therapy

Exogenous immunosuppressive agents are considered a treatment to suppress the immune system in the human body. In the context of xenotransplant, these biological or pharmacological agents will help to lower the susceptibility of xenograft rejection by the recipient's immune system. However, using immunosuppressants is associated with side effects, including infection, hypertension, and hyperlipidaemia. There are several exogenous immunosuppressants, including calcineurin inhibitors (cyclosporine A, tacrolimus), steroids, mTOR inhibitors (sirolimus, everolimus), and antimetabolites (mycophenolate mofetil, azathioprine) (26).

Genetic modification of xenograft

Genome editing technologies are concerned with the gain or loss of function by inserting or deleting genes and are used to overcome the xenotransplantation barrier. They work to eliminate Xeno antigen expression, thereby avoiding the recognition of human natural antibodies to those antigens and suppressing the antigen-antibody reaction. Genetic manipulation also involves the insertion of a human transgene that protects the xenografts from the recipient's complement and coagulation pathway (27,28). The current genome editing platforms include: homologous recombination HR, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clustered regulatory interspaced short palindromic repeats (CRISPR) (29).These are synthetic nuclease that edit the genome by inducing double stranded break which is then repaired via two different repair mechanisms: first the homology directed repair in which the homologues DNA sequence is used as a template, the second approach is reconnecting the broken ends at non – homologous DNA sequence (non-homologues end- joining) (30).

The primary Xeno antigen is Galactose-α-1,3-Galactose (Gal), which is synthesised by the enzyme glycoprotein α1,3-galactosyltransferase 1

(GGTA1). However, the antibodies produced in response to the presence of non-Gal antigens trigger NK cell activation without contributing to HAR (31). Accordingly, masking the Gal epitopes prevents HAR and acute rejection to a certain extent. This can be achieved by expressing specific enzymes on the pig cells, including endo-β-galactosidase C (EndoGalC) and H-transferase (HT, also known as α1,2-fucosyltransferase). EndoGalC cleaves the Galβ1-4GlcNAc linkage to detach the Galα1-3Gal disaccharide from Gal epitopes. While H-transferase blocks Gal epitope by transferring fucose from GDP-fucose to the common acceptor substrates by processing the transfer of fucose from GDP-fucose to acceptor substrate and impeding glycosylation, as both H transverse and α1,3-galactocyltransferase have similar substrate specificity(32,33)The expression of the transferase minimises the formation of Gal epitopes by 80-90% (34)Additionally, there is another technique in which the α-galactosidase (GLA) gene is inserted into the pig genome, resulting in the removal of α-D-galactose from Gal epitopes.

Genetic manipulation plays a pivotal role in reducing the reliance on exogenous immunosuppressive agents. The elimination of exoantigen genes significantly reduces humoral immunity. Studies have demonstrated that in humans with humoral immunity against human leukocyte antigen (HLA) class II, the human version of MHC, the SLA class II proteins behave like xenogenic antigens (35). Consequently, cellular immunity is minimised by introducing some genes in the recipient's genome, such as a mutant Human Histocompatibility complex (MHC) II transactivating gene, which downregulates the expression of the swine leukocyte antigen (SLA) class II (36).

Decellularization of Xenogeneic Tissue

What is Decellularization?

The idea of decellularisation had been mentioned since 1975. Decellularisation is the process of extracting the cellular and genetic materials from the extracellular matrix (ECM) scaffold, leaving behind a porous scaffold called the decellularised scaffold/matrix DM (37). The DMs conserve the three-dimensional microarchitecture, organisation, mechanical integrity, and biological composition of the native tissue. The Intact ECM contains structural and functional molecules, including collagen, fibronectin, laminin, glycosaminoglycans, growth factors, and matrix-bound nanovesicles (MBVs), all of which are preserved in the DM. Decellularisation aims to detach the xenogeneic or allogenic antigens, which would elicit an immune response and inflammation upon implantation. Thus, the decellularisation procedure will enable us to obtain xenogeneic decellularised scaffolds and utilise them in various tissue engineering and regenerative medicine strategies, as they will undergo the recellularisation process later. Incomplete cellular removal will provoke an immunological response (38).

Decellularization Methodology

The efficiency of decellularisation is influenced by two main factors: the type of methods used to extract the antigenic materials and the nature of the tissue, which involves the source, thickness, density, and architecture, as well as the lipid content of the native tissue (70). Meeting specific standards is essential for the decellularised tissue to be viable for clinical application. Such standards include having DNA content less than 50 ng/mg, a residual DNA length of less than 200 base pairs, and the absence of nuclear material in tissue stained with 4,6-diamidino-2-phenylindole (DAPI) or haematoxylin and eosin. The decellularisation methods are classified into four main types:

1. Physical methods (e.g., supercritical fluid, freeze-thaw, mechanical shaking, pressure gradient, and electroporation).

2. Chemical methods (Hypotonic and hypertonic solutions, acids and bases, and zwitterionic detergents).
3. Biological (e.g. enzymes: nucleases, collagenase, lipase, trypsin, alpha-galactosidase, and thermolysin).
4. Mixed methods (37,39).

Immunogenicity in decellularised tissue

There are several sources of immunogenicity in decellularised tissue, and incomplete decellularisation is considered the primary source (40). The cellular remnants of the decellularised xenograft typically consist of DNA fragments. The remnants will elicit a Type 1 macrophage response in the recipient, indicating the necessity to adhere to the scaffold's quality standards. Another source of immunogenicity is the presence of PERVs, endogenous retroviruses, which are detected after decellularisation using PCR, fluorescence in situ hybridisation (FISH), and enzyme-linked immunosorbent assays (ELISA) (41,42). Additionally, if the α-Gal epitopes are present on the decellularised xenograft, the corresponding antibodies become activated, and immune rejection occurs. Humans and primates do not express the α-Gal epitopes, which can be overcome through genetic manipulation and enzymatic treatment. These epitope residues are detected using various methods, such as PCR and ELISA, as well as fluorescein isothiocyanate (FITC)-conjugated BSI-B4 lectin (43).

Recellurization with stem cells

Recellularisation of decellularised scaffolds represents a significant advancement in creating functional, transplantable organs. This process, which involves repopulating the ECM scaffold with organ-specific cells to recreate its function and then transplanting it into the human body, holds great promise and inspires further research and development in the field of regenerative medicine (44).

The Native Tissue Derived decellularised ECM (dESM) is identical to the composition and mechanical properties of the original tissue after correcting the decellularisation treatment. This provides a dynamic microenvironment for stem cell behaviour. However, it encounters challenges such as limited donor availability, higher immunogenicity, potential pathogen transmission with decellularised xenografts, and difficulties in scaling up for extensive research. Conversely, Cultured Cell-Derived dECM is produced by cultured cells in vitro and then decellularised, making it suitable for large-scale in vitro studies. It has a lower immune response, allows for the modelling of small tissue regions, and reduces the risk of pathogen transmission by screening ECM-synthesising cells. Despite these advantages, it has poorer mechanical properties and poses difficulties in producing large quantities due to the impossibility of obtaining sufficient primary material (45).
The key components of organ regeneration via recellularisation involve selecting appropriate cell sources, applying optimal seeding methods and providing a physiologically relevant culture environment using bioreactors. This should be completed by reestablishing the parenchyma, vasculature, and support components before the implantation.

The functionality and translatability of each scaffold depend on the types of cells used for recellularisation (45). Various cell types have been studied for seeding the scaffold, including fetal and adult cells, embryonic stem cells, and progenitor cells derived from induced pluripotent stem cells (iPSCs). However, identifying the most suitable cells remains a significant challenge, engaging researchers in the field and calling for further comprehensive, long-term in vivo organ transplantation studies (46).

Mesenchymal stem cells (MSCs) are a significant

focus in the field of regenerative medicine, where extensive research has revealed their remarkable potential in repopulating acellular scaffolds, solidifying their status as a promising functional cell type (47). MSCs, or multipotent stromal cells, possess the capacity for self-renewal and can differentiate into various cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes. They are found in bone marrow, cord blood cells, adipose tissue, dental pulp cells, and amniotic fluid. The MSC tissue is isolated and then undergoes enzymatic digestion to release the cells. The cells are then cultured for three to five days, during which non-adherent cells are removed and adherent cells are maintained and expanded. The primary culture medium for MSCs ideally consists of low-glucose Dulbecco's Modified Eagle Medium (LG-DMEM) supplemented with 1% (w/v) antibiotic/antimycotic and 10% (v/v) fetal bovine serum (FBS) (48). Their interaction with ECM components significantly influences the behaviour and differentiation of seeded MSCs (49). The combination of ECM proteins (e.g collagen types I and IV, laminin), along with growth factors, plays a crucial role in directing MSC activity (50). For instance, collagen II has been shown to encourage chondrogenic differentiation in MSCs (51), while collagen VI favors myogenic differentiation (52). Additionally, the mechanical properties of the scaffold are critical, as more rigid scaffolds tend to enhance osteogenic differentiation, whereas softer ECM materials promote adipogenic differentiation (53).

Moreover, both direct cell-to-cell interactions and indirect stimulation from bioactive molecules produced by neighbouring cells can influence primary cell proliferation and MSC differentiation(54). According to a study (55), MSCs cocultured with astrocytes led to a significant increase in astrocyte VEGF and Tie2 gene expression, as well as an upregulation of Ang1, Tie2, and VEGF protein levels, compared to astrocyte cultures alone. Similarly, when marrow stromal cells were cocultured with microvascular brain endothelial cells (MBECs), there was a marked increase in Tie2, VEGF, and FIK1 gene expression, as well as higher levels of Ang1, Tie2, and VEGF proteins, compared to MBECs cultured alone. Matrix metalloproteinases (MMPs) maintain ECM haemostasis and promote tissue remodelling and degradation. MSCs have been shown to enhance the expression of various MMPs, including MMP1(collagenase-1, which targets collagen I, II and III), MMP3 (stromelysin-1, which targets collagen III, IV, and V), and MMP-13 (collagenase-3, which targets collagen II) (56).

The immunomodulatory effects of MSCs are evident when they are seeded within a decellularised scaffold. These MSCs inhibit leukocyte infiltration, thereby preventing ECM destruction (57). They also facilitate the transformation of macrophages into the M2 phenotype, which is associated with a regenerative tissue response (58). Additionally, MSCs regulate the immune system by suppressing T-cell proliferation, adjusting the Th1/Th2 balance, and managing the functions of regulatory T cells (Tregs) (58), and producing interleukin-10 (IL-10) (59).

MSCs exhibit significant angiogenic properties that enhance the neovascularisation of decellularised scaffolds through several mechanisms. Firstly, after reendothelialisation of the scaffold with endothelial cells, MSCs can be seeded to further support this process (60). MSCs enhance endothelial cell proliferation through the secretion of paracrine factors, thereby ensuring adequate endothelial coverage and preventing thrombosis (61). Moreover, MSCs secrete growth factors that stimulate angiogenesis by promoting the migration of host endothelial progenitor cells and their differentiation into mature endothelial cells, thereby increasing vascular sprouting and supporting graft regeneration (63,64). MSCs activate the Angiopoietin-1 (Ang-1) and Tie2 signalling pathway, where the phosphorylation of Tie2

enhances perivascular cell recruitment, contributing to the stabilisation of the neovasculature. Furthermore, MSCs can migrate to the tunica adventitia and differentiate into pericytes, which provide structural integrity to the developing vasculature and reduce the risk of haemorrhage (62). These combined actions underscore the potential of MSCs in enhancing vascular regeneration and stability in tissue-engineered scaffolds. There are three defined methods to recellularise acellular scaffolds with MSCs: 1) immediate transplantation of undifferentiated MSCs, 2) Tissue culture and transplantation of undifferentiated MSCs, and 3) Tissue culture and transplantation of differentiated MSCs.

The recellularisation efficacy depends on the anatomical, structural, and pathological conditions of the scaffold sources, the method of decellularisation, the surface modification of the scaffolds, and the microenvironment of the cell culture. The limitations of MSCs for recellularisation of acellular scaffolds include challenges with sourcing and isolating the cells, particularly from elderly patients or those with systemic diseases (63). Autologous MSC expansion is time-consuming, and variations in isolation methods can lead to inconsistent quality (64).
The safe dosage for allogeneic MSCs is also unclear due to the potential for the development of alloantibodies (65). Additionally, undifferentiated MSCs may be influenced by surrounding tissues, leading to the formation of unwanted cell types, and even differentiated MSCs require long-term evaluation. Finally, safety concerns include the risk of MSCs increasing tumorigenicity, with links to conditions such as Ewing's sarcoma and gliomas (66,67). Additionally, MSCs can have pro-inflammatory effects and may contribute to the development of fibrosis, which can lead to conditions such as fibrotic lung disease (68). While clinical trials have demonstrated short-term safety, further research is needed to elucidate the long-term risks, particularly about cancer development (69).

Heart valve xenotransplantation

Valvular heart disease (VHD) is the main contributor of global mortality and morbidity. In the US, 2.5% of the total population have VHD, resulting in 25.000 deaths per year (70). In severe cases, the treatment options include valve repair or replacement. A malfunctioning heart valve can be replaced with a mechanical valve prosthesis (MVP) or a bioprosthetic heart valve (BHV) through a procedure called transcatheter aortic valve implantation (TAVI). MHVs have poor hemocompatibility and thus require lifelong, closely regulated anticoagulation therapy, which may result in thrombosis and spontaneous bleeding. Given that xenogeneic bioprosthetic valves lack durability and are susceptible to calcification and degeneration, patients will therefore undergo valve replacement in nearly 10 years (71).

Non-genetically modified pig-derived heart valves first undergo specific processes, including excision, preparation, cultivation, decellularisation, chemical inactivation, and cryopreservation. According to these processes, they may be regulated as a medical device or as a medicinal product. Thus, medical devices such as bio prostheses do not contain viable tissue or cells (72) and are chemically fixed, resulting in a lack of the capability to grow and remodelling (73). An example of these non-living tissues is a glutaraldehyde-fixed bioprosthetic heart valve (GBHV). Moreover, Medicinal product valves, which may contain viable tissue and may not, can be used with or without an acellular matrix. Decellularised porcine heart valves are classified as a medicinal product with the ability to recellularise and remodel, which will reduce the need for follow-up replacement surgeries (74,75). Decellularisation methods cannot achieve a 100%

removal of cellular and nuclear materials, and the most frequent remnant is resilient DNA fragments (76).

Bio prosthetic valves from porcine heart and bovine pericardium undergo decellularisation to eliminate the antigens that provoke an immunological response and inflammation. The most common method used to decellularise the xenogeneic heart valves is detergent decellularisation. The decellularised valves mainly consist of elastic and collagen fibres. GBHVs undergo a chemical fixation procedure using glutaraldehyde that aims to conserve both the structural and mechanical integrity, while also detaching exoantigens. After that, when the GBHV undergoes structural valve deterioration (SVD), the valve becomes thicker and more calcified (76). This process results in a narrowed valve opening, in addition to the deterioration of the valve leaflets. Thus, SVD is correlated with stenosis, regurgitation, and hemodynamic dysfunction. Additionally, calcification is associated with the immune response, primarily due to the presence of pig antigens. The calcification process also occurs in cases of inflammatory conditions, such as tuberculosis (TB).

Patients with GBHVs do not need long-term anticoagulation treatment in comparison to patients with MHVs. Meanwhile, the GBHVs have limited durability, especially in young adults and children (74). Therefore, these patients will undergo repeat valve replacement operations. Currently, the replacement options do not allow the somatic growth of the valve after implantation, resulting in multiple reoperations in paediatric patients within months to a few years, with a higher risk of death than the initial surgery (77).

For this reason, when valve repair in young adult patients is unsuccessful, they are typically offered mechanical heart valves (MHVs). More than 250,000 heart valves are replaced every year worldwide. Approximately 55% of these valves are replaced with mechanical heart valves (MHVs), while 45% are replaced with bioprosthetic heart valves (GBHV) (78). Hence, genetically modified pig-derived valves have been shown to have prolonged survival rates in young adult patients, children, and patients with contraindicated anticoagulant therapy.

Clinical Pig to human kidney xenotransplant

In the US, there are more than 80000 patients with end-stage renal disease (ESRD) waiting to receive a kidney transplant. This highlights the shortage of kidneys and the significant demand for organ supply, making kidney xenotransplantation a promising alternative to allotransplantation. Xenotransplantation provides an unlimited kidney supply. Hence, patients with ESRD will not need to go through chronic dialysis, besides stopping the detrimental effects of brain death on the organs (79). It is essential to control the immunological barriers to fully leverage the potential advantages of kidney xenotransplantation. Thus, genetically engineered pigs would provide an extra organ source with low immunogenicity.

Xenograft rejection due to an antigen–antibody reaction could be inhibited by using genetically engineered pigs as a source of organs. At the same time, immunosuppressive treatment is necessary to control T-cell-dependent adaptive immunity. However, conventional immunosuppressive therapy, approved by the Food and Drug Administration, failed to inhibit the adaptive immunity against pig cells. In contrast, blocking the CD40:CD154 co-stimulation pathway is effective in preventing the T cell response, as evidenced by Buhler et al.

Transplanting genetically unmodified pig kidneys in

NHPs resulted in kidney failure within minutes. However, using genetic engineering techniques to introduce a gene for a single human complement protein into the genome of life-supported pig kidneys extended the survival of the kidneys up to 90 days (80,81). Additionally, by combining the elimination of Gal epitope expression with the induction of one or more complement-regulatory proteins, the survival of the kidneys and the recipient increases to 6 months with normal renal function.

The Emory group conducted pre-transplant antibody screening in monkeys, which revealed that prolonged kidney transplant survival is enhanced by a combination of low antibody levels and anti-CD154 mAb co-stimulation blockade (82). Additionally, long-term survival of pig kidneys is promoted by specific gene editing, as demonstrated by a group from the University of Pittsburgh (83).The longest reported life-supporting pig kidney xenograft survival is 405 days, as reported by Kim et al., which highlights the need to prevent a CD4+ T cell response (84).

In September 2021, researchers at the NYU Langone Transplantation Institute, led by Montgomery et al. (85), conducted the first 'thymokidney' transplants from GGTA1-knockout pigs into two brain-dead human recipients. The procedure involved administering high-dose methylprednisolone daily and intravenous mycophenolate mofetil twice daily. Both xenografts maintained proper function for 54 hours without indications of hyperacute or AMR. However, three days post-study, the deceased subjects experienced multiple organ failure, disseminated intravascular coagulation (DIC), shock, academia, and pancytopenia. This research provides crucial evidence that eliminating genes responsible for synthesising carbohydrate xenoantigens (specifically GGTA1, β4GALNT2, and CMAH) is adequate to prevent hyperacute rejection through this mechanism in humans. The investigation utilised a human preclinical model to assess the efficacy of porcine genetic modifications in avoiding HAR, to determine if porcine cells and pathogens could be detected in the recipient's blood, and to evaluate the safety of performing porcine renal xenotransplantation for future clinical trials.

Porrett et al. (86) transplanted two kidneys from a 10-gene-edited pig into a deceased human recipient and administered an immunosuppressive regimen. Although no hyperacute rejection was observed, the xenograft function appeared unsatisfactory during the 74-h observation period.

Subsequently, Locke et al. (86) reported a case using the same gene-edited pig, with the addition of an anti-C5 monoclonal antibody (mAb) to the immunosuppressive regimen. They found that the xenograft function remained normal for 7 days. However, owing to limited observation time, the outcome remains unknown.

Previous studies have observed kidney xenotransplantation for 2 to 7 days, which has been sufficient to demonstrate hyperacute rejection in pig-to-human models. In the (87) study, the period was extended to 12 days, where two brain-dead patients received single kidney xenotransplants from genetically modified minipigs without CD154-CD40 pathway-blocking antibodies or thymic tissue. Renal function initially resumed, but acute rejection and PCMV reactivation occurred by day 12. Further research is needed to assess whether these treatments can effectively control acute xenograft rejection and significantly extend graft survival. DeVries, C. (2023) conducted a 61-day study on a single-gene knockout pig kidney with thymic tissue transplanted into a 58-year-old deceased male (88). All previous clinical pig-to-human kidney xenotransplants were performed in brain-dead recipients. Massachusetts General Hospital successfully transplanted the first genetically edited pig kidney into a 62-year-old male with type 2 diabetes and hypertension on March 16, 2024.(89) The patient had previously received a

human donor kidney in 2018, which failed after five years, leading to ESRD, and he resumed dialysis in 2023. Monoclonal antibody drugs were used, resulting in good renal function and patient recovery. The long-term viability of the transplanted kidney remains uncertain, but preclinical research suggests it could last more than two years.

In April 2024, Lisa Pisano became the first person to undergo a combined left ventricular assisted device and gene-edited pig kidney transplant. Unfortunately, she passed away on July 7 while under hospice care, after struggling to discontinue in-patient medications that were required to maintain sufficient blood pressure to support the xenografted kidney (90).

In November 2024, NYU Langone Health transplanted a gene-edited pig kidney into Towana Looney, a 54-year-old woman with ESRD, under the FDA's expanded access protocol. The kidney functioned for 130 days, the longest duration a gene-edited pig kidney has worked in a living human. After a period of stable function, Ms. Looney experienced a reduction in renal performance due to acute rejection. This episode followed a reduction in her immunosuppressive therapy, which had been lowered to treat an infection unrelated to the pig kidney. Ms. Looney and her doctors jointly decided that the safest course of action would be to remove the kidney and return to dialysis, rather than increasing immunosuppression. Importantly, there was no evidence of PERVS transmission during the trial (91).

Clinical Pig to human Heart xenotransplant

The University of Maryland team conducted the first human heart transplantation utilising a gene-edited pig heart. The recipient was a 57-year-old male with end-stage heart disease, exacerbated by multiple comorbidities. These comorbidities included pre-transplant adrenal insufficiency, episodes of gastrointestinal haemorrhage, bloodstream infections, and chemotherapy-induced leukopenia. Before the procedure, the patient was bedridden, experiencing cardiac muscle atrophy and intractable ventricular arrhythmias. He required venovenous extracorporeal membrane oxygenation (ECMO) support for 46 days and exhibited significant immobility in the pre-transplant period, which substantially compromised his physical resilience.The transplanted heart demonstrated adequate function for more than 40 days. However, the patient's condition subsequently deteriorated due to increasing levels of anti-pig antibodies in the serum, ultimately resulting in mortality at 60 days post-transplantation. Critical observations have highlighted the importance of meticulous patient selection, as an individual's compromised state limits their ability to recover. Intravenous immunoglobulin (IVIg) was administered to mitigate organ rejection but may have inadvertently contributed to the elevation of anti-pig antibodies, thus complicating the immune response. Furthermore, increased levels of porcine cytomegalovirus (CMV) have been detected, potentially affecting cardiac function. This illustrates the complexities of xenotransplantation and underscores the need for refined procedures to enhance patient outcomes (92).

Although Maryland porcine cardiac transplantation resulted in unfavourable outcomes, it provided valuable insights. If the procedure was performed on a brain-dead donor and observed for three days, it might have appeared successful. Still, it would have failed to reveal crucial information about the risks in ill patients, the use of IVIg, porcine CMV transmission, and challenges in interpreting biopsies. Notably, the Maryland patient survived considerably longer than the first human heart transplant recipient in 1967, who lived for only 18

days. This comparison emphasises the importance of persisting with clinical porcine cardiac transplantation research until it becomes successful (92).

The Maryland medical team performed a second porcine-to-human cardiac transplantation. The recipient, Lawrence Faucette, 58, had end-stage heart disease and was ineligible for a conventional human heart transplant. The surgical team used a genetically modified porcine heart with alterations to 10 genes. Despite efforts to mitigate IVIG and PCMV/PRV infection complications, the patient survived for approximately six weeks post-transplantation. The medical community anticipates a comprehensive report of this case to better understand xenogeneic immune responses and associated complications (93). In a recent study, Moazami et al. (2023) conducted cardiac xenotransplantation from genetically modified porcine donors into brain-dead human recipients. The porcine donors had 10 genetic modifications. Cardiac function was monitored 66 hours post-transplantation. Despite a gradual decline in function due to size incompatibility, there were no signs of cellular or antibody-mediated rejection, nor were there any zoonotic infections (94). Table 1(85–89,91–93,95,96).

Ethical Considerations

Xenotransplantation raises important ethical concerns involving animal welfare, patient safety, and cross-species disease transmission. The use of animals, particularly pigs, for organ harvesting must balance scientific progress with humane treatment and adherence to ethical standards. Informed consent is critical, especially in early clinical trials where long-term outcomes and risks are uncertain. Public health considerations, such as the potential for zoonotic infections (e.g., PERV transmission), also necessitate stringent regulatory oversight (97). This review is based on previously published literature; no new human or animal studies were conducted by the authors, and no ethical approval was required.

Conclusions

Current efforts in clinical porcine xenotransplantation should prioritise kidney transplantation, given its practicality and ethical acceptability. The use of porcine hearts as a temporary bridge for paediatric patients awaiting allotransplantation also holds promise, as it offers a life-saving option during the often-prolonged wait for size-matched donor hearts. Significant progress has been made, yet a deeper understanding of immune rejection mechanisms in genetically engineered (GE) porcine organs transplanted into non-human primates (NHPs) remains essential. These insights will guide the development of next-generation GE porcine models capable of achieving consistent long-term survival (over 9 months) in NHPs with clinically approved immunosuppressive regimens. Achieving this benchmark is critical to building confidence and paving the way for broader clinical application of porcine organ xenotransplantation (94).

	Institution trial date	Organ Donor / Genetic Modifications	Recipient sate	Immunosuppression regime	Survival time	Outcome	Limitation
Kidney	NYU Langone Transplant Institute The 1st on September 25, 2021, and the 2nd on November 22, 2021 (85)	Genetically engineered α1,3-GTKO pig kidney with donor-specific thymic tissue	Brain-dead human subject	daily high-dose methylprednisolone and twice-daily intravenous mycophenolate mofetil.	The kidney functioned for 54 hours,	The kidney functioned for 54 hours. Produced urine and creatinine, macroscopic rejection observed	Short follow-up period, use of suboptimal GTKO pig rather than triple-knockout
	University of Alabama at Birmingham Heersink School of Medicine (86)	Two kidneys from a genetically engineered pig. 10 GE pigs: +hDAF, +hCD46 (complement inhibitors), +hTBM, +hEPCR (anticoagulants), +hCD47, +hHO1 (immunomodulators), -3 pig carbohydrate antigens, - pig growth hormone receptor	57-year-old brain-dead male decedent	daily methylprednisolone taper, anti-thymocyte globulin for a total of 6 mg/kg, and anti-CD20. Maintenance immunosuppression included mycophenolate mofetil, tacrolimus, and prednisone.	74 hours (study termination)	No hyperacute rejection; maintained vascular integrity; urine production; no viral transmission or chimerism; creatinine clearance did not recover.	Short duration; altered physiology of brain death
	University of Alabama at Birmingham Heersink School of Medicine (95)	10 gene modifications, including4 gene knockdowns and knock outs (GTKO, CMAH, B4GALNT2, GHR) and 6 human transgene insertions (CD46, CD55, CD47, THBD, PROCR, HMOX1)	Brain-dead male in his 50s and had AKI superimposed on a history of CKD (stage 2) and HT.	The decedent received a complement inhibitor (anti-C5; eculizumab) 24 hours before xenotransplantation, followed by a solumedrol taper, antithymocyte globulin (6 mg/kg total), and rituximab. Maintenance immunosuppression included tacrolimus, mycophenolate mofetil, and prednisone.	Xenograft function remained normal for 7 days	Produced urine with a concurrent decrease in urine volume Serum creatinine decreased within the first 24 hours, normalised at 48 hours, and remained within normal limits throughout the study duration. Creatinine clearance improved. Normal histology without evidence of thrombotic microangiopathy.	Limited observation time
	NYU Langone Transplant Institute, July 14, 2023 (88)	GalSafe pig kidney with donor-specific thymic tissue. Single-gene knockout pig kidney	The organ was removed on September 13, 2023, from a 58-year-old man who had been on a ventilator, with his family's consent, after being declared dead by neurologic criteria before the xenotransplant.		61 days	mild rejection process that required intensifying immunosuppression medication to reverse it completely.	
	Hainan Medical University, China 2024 (87)	Two pig kidneys Triple-gene knockouts (GGTA1, β4GalNT2, CMAH) and human gene transfers (hCD55 or hCD55/hTBM	Brain-dead	Conventional immunosuppression without CD40-CD154 blockade	12 days	Normal renal function was quickly restored after transplantation Acute rejection by day 12, leading to graft dysfunction Case 1: Signs of both T cell-mediated rejection (TCMR) and antibody-mediated rejection (AMR) Case 2: Primarily exhibited antibody-mediated rejection (AMR and an increase in anti-donor xenoantibodies, particularly anti-A antibodies. Evidence of porcine cytomegalovirus (PCMV/PRV) reactivation in both cases.	Limited observation time, brain-dead model Lack of recent genetically optimised immunosuppressive treatments.

Table 1: Summary of Clinical Pig-to-Human Xenotransplantation (85-89, 91-93, 95, 96)

	Massachusetts General Hospital, March 16, 2024, (89)	Genetically modified kidney pig uses CRISPR-Cas9 technology to make 69 precise edits to the pig's DNA	A 62-year-old male with type 2 diabetes and hypertension. He previously received a kidney from a human donor in 2018, which failed after five years, leading to ESRD, and he resumed dialysis in 2023.	monoclonal antibody drugs	still unknown	Good renal function, patient recovering The longer-term results of the transplant are not yet precise.	
	NYU Langone Transplant Institute, November 25, 2024 (91)	10 GE pigs	Towana Looney, 53- year-old female. She donated a kidney to her mother (1999) and developed kidney failure years later due to hypertensive complications during pregnancy. She is on dialysis since 2016	FDA-authorized, specific regimen: Ultomiris (ravulizumab-cwvz) Empaveli (pegcetacoplan)	130 days-Kidney removed on April 4,2025	Pig kidney functioned for 130 days, then removed; patient returned to dialysis	Single patient case; compassionate use—not part of a formal clinical trial yet
Heart	University of Maryland School of Medicine January 7, 2022, (92)	10 GE pigs	A 57-year-old man with end-stage heart disease and pre-transplant adrenal insufficiency had gastrointestinal bleeding, bacteraemia, and drug-induced leukopenia. He was bed-bound with cardiac cachexia and refractory ventricular ectopy, required 46 days of venovenous ECMO, and was non-ambulatory, reducing his physiological resilience.	(based on blockade of the CD40/CD154 co-stimulation pathway), The patient had received intravenous human immunoglobulin (IVIg),	2 months Died on March 9, 2022,	The pig heart functioned well for >40 days Then serum anti-pig antibodies increased mcfDNA testing indicated an increase in pig cytomegalovirus (CMV),	The patient was in a severely debilitated state before the transplant, which complicates the interpretation of the outcomes. The role of IVIg in the patient's outcome is not fully understood.
	NYU Langone Transplant Institute. June 19, 2022, and July 9, 2022 (96).	Hearts were procured with 10 genetic modifications, including four porcine gene "knockouts" and six human transgenes ("knock-ins") in pigs.	deceased humans			No signs of early rejection in either organ The hearts functioned normally with standard post-transplant medications and without the need for additional mechanical support. No presence of (pCMV) was detected in either case. Strict protocols to prevent and monitor transmission of PERV)	
	University of Maryland School of Medicine September 2023 (93).		58-year-old man with end-stage heart disease.		6 weeks	show signs of rejection in recent days.	

Table 1: Summary of Clinical Pig-to-Human Xenotransplantation (85-89, 91-93, 95, 96)

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The Role Of The Gut Microbiome In Obesity and Diabetes

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Abstract

The gut microbiome plays a crucial role in recirculating and recycling hormones, which is often dysfunctional due to chronic inflammation. Endocrine disorders, such as obesity and diabetes, are prevalent and can be linked to the gut microbiome. Maintenance and reestablishing the gut microbiome can lead to better conditions for those affected by these disorders. By studying the cause and effect of these disorders, it is possible to determine if there is a correlation between the gut microbiome and certain endocrine disorders, potentially leading to complementary treatment, less reliance on drugs, prevention, and reduced susceptibility.

One complementary treatment that could benefit from the gut microbiome is the use of probiotics and their strains of microbiota. Patients who understand their gut microbiome better and understand the causes of inflammation or diseased microbiota could take their health into their own hands. Education on these effects can help patients achieve more control over their lives negatively impacted by the disorders in question.

Introduction

The metabolic repertoire affects the gut's microbiota as the gut microbiome (GM) is responsible for the recirculation of hormones as in recycling and producing them (1). It is therefore not surprising that in endocrine disorders the GM is dysfunctional due to for example chronic inflammation. Endocrine disorders (ED) are vast and numerous yet we focused on some of the most prevalent within our country, those being obesity and diabetes (6)(7).

Throughout this paper we focused on the disorders' linkage with the GM and how maintenance and reestablishing the GM can lead to better conditions for people affected by these disorders. Through studying the cause and effect we can deduce if there exists a correlation between the GM and certain ED (2), perhaps leading to treatment strategies for ED by means of the GM, less reliance on drugs, prevention and possibly reduced susceptibility. One

Considerable progress in the study of the gut microorganisms, has been made over the years. The such complementary treatment worth discussing is the use of probiotics and what strains of microbiota are primarily included, questioning if the use of these strains is any help in the ED we shall investigate (3). Prebiotics (refer to figure 1) are mostly non digestible fibres that stimulate GM bacterial growth like Lactobacilli and Bifidobacteria. Some types of prebiotics include breast milk, soybeans and oats (56), and they have been shown to increase the longevity of probiotics with positive health implications such as increasing short-chain fatty acids (SCFAs), and reducing dysbiosis (58). Synbiotics are when probiotics and prebiotics are combined together, thus helping bowel issues, inflammation and GM modulation (56)(58). Synbiotics have greater resulting effects compared to prebiotics or probiotics alone (58).

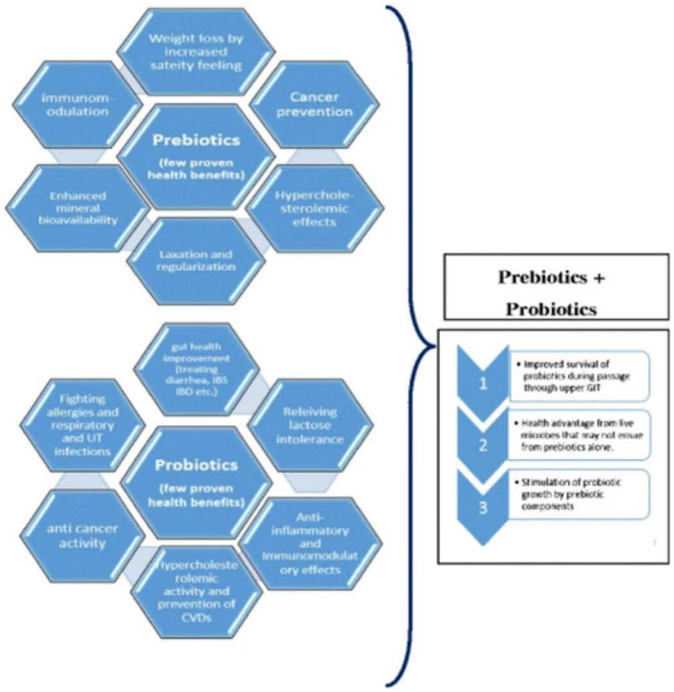

Figure 1: Prebiotics and probiotics (56)

If patients were to understand their GM better and conclude what is causing the inflammation or diseased microbiota, which could be leading to the worsening of their ED, then that education could help patients take their health into their own hands (4). Apart from this, by not only considering the effects of the drugs used regularly in the treatment of the diseases to be discussed (being Metformin, traditional Chinese medicine, acarbose), but through providing a wider knowledge to the patients on these effects, patients may be able to achieve more control later over their lives which are currently negatively impacted by the disorders in question (4).

The host's nutrient metabolism, the metabolism of xenobiotics and drugs, the preservation of the gut mucosal barrier's structural integrity, immunomodulation, and pathogen defence are all facilitated by a healthy gut microbiota. The typical gut microbiota is shaped by a number of variables, an example being the host's diet (5). In physiological conditions, the enterocytes and gut bacteria live together in a symbiotic relationship and are not pathogenic. The primary functions of gut commensals include metabolism of nutrients, of natural and synthetic medications, intestinal barrier function, and preventing colonisation by foreign and potentially pathogenic microorganisms (5). Apart from the impacts listed above, gut microbiota can also have neuroendocrine effects and play a role in appetite control, obesity, and metabolic regulation (5).

Discussion

The leading endocrinological disorder; Obesity

In recent studies it was found that around 70% of the Maltese population were either obese or overweight (7), these numbers show a staggering issue within the maltese population, and obesity is not by the average layman understood to be an endocrine disorder. The World Health Organization defines obesity as having a body mass index (BMI) greater than 30, however the definition varies from country to country (9). Around one third of the world population is either overweight or obese. Moreover, this runs risks of developing complications such as diabetes type 2 (8), cardiovascular disease (10), and inflammation (9). Obesity is a multifactorial condition dependent on genetics, society, economical status, environmental factors and psychosocial ones (8)(9). It has been found to affect the endocrine system by impacting the hypothalamic-pituitary axis, vitamin D homeostasis, sex steroids and thyroid hormones (11). This is because when excess adipocyte deposition is present, increased inflammation, cytokine production and most importantly adipokine production ensues (13), thus causing issues within endocrine regulation and causing insulin resistance as often seen in obesity (13). An unhealthy GM in obesity is commonly seen, as the

bacteria used to degrade polysaccharides, short chain fatty acids and lipopolysaccharides (LPS) will not be diverse enough to efficiently do their role (9).

The GM diversity has been found to decrease significantly in obese patients versus lean patients; this can be linked to the patients' changed metabolism and physiology as a result of being obese (14). It can be noted how essential a thriving, diverse GM in an individual is to maintain systemically good health through studies on the bacteria and their metabolites. An example of this would be the SCFAs released by the GM as part of the fermentation process which activate receptors that are involved in the release of control molecules like Peptide YY (PYY) (13). The latter is known to provide a decrease in appetite in order to prevent over-consumption of food (19). Therefore, damage to the integrity and diversity of the microbiota in question may bring about endocrinological dysregulation in obese patients, such as decreased PYY and hence further increased appetite (19). This demonstrates the importance of the symbiotic relationship between the patient and the GM needing to be respected by the patient themselves, since in the case of an unhealthy diet, commonly consumed by obese patients, it was found that increased levels of LPS (13) were found due to the gram-negative bacteria present in significant numbers within the GM (13). LPS are noted to pose a significant increase in the progression of metabolic disease, and worsening of obesity (13). Furthermore, there seems to be a link between the GM and leptin sensitivity as studies testing this correlation suggest that GM modulation via prebiotic use has been noted to increase leptin sensitivity (20). Hence, it can be understood why leptin resistance is one of the hallmark effects of obesity due partly to this endocrine disorder involving dysbiosis of the gut as one of its many complications (20) (refer to figure 2).

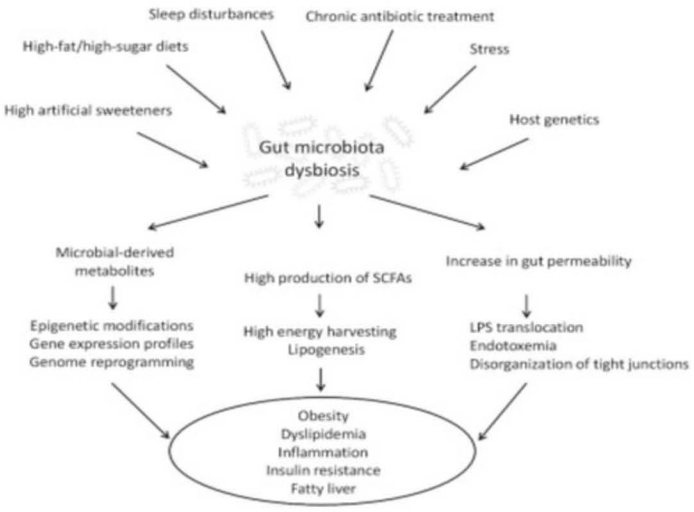

Figure 2 : GM factors that in dysbiosis can lead to or worsen obesity (16)

Many studies have noted a strong link between insulin resistance and an altered GM. For instance, it has been found frequently that Toll-like Receptor 4 can be activated by LPS, which then upregulates inflammatory pathways that promote insulin resistance (21). Another study found that children with obesity and higher HOMA-IR levels, a test to approximate insulin resistance which if high indicates increased resistance (refer to figure 3), were noted to exhibit lower diversity of a number of microbial species in the gut (22). Dysbiosis was also noted to be linked to decreased amino acid and SCFA biosynthetic pathways which are essential in insulin sensitivity and anti-inflammatory effects (22). In the disrupted GM, it is noted through mechanistic studies that increased peptidoglycan biosynthesis occurs and is associated with increased insulin resistance due to its pro-inflammatory roles (22).

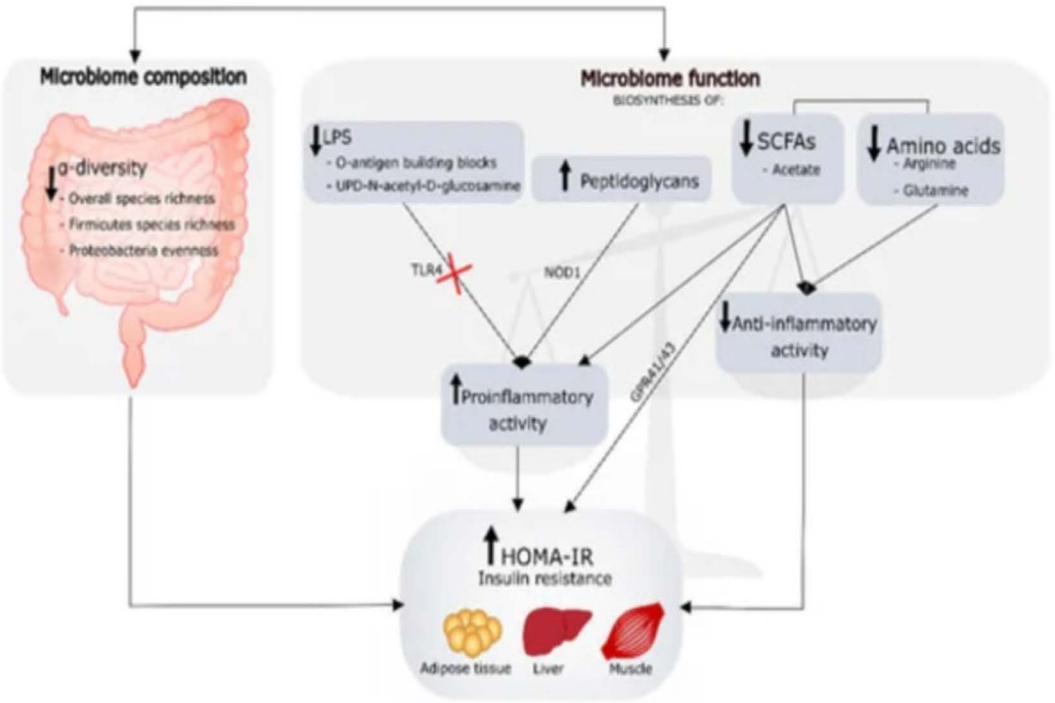

Figure 3: Effects of altered GM all contributing to increased insulin resistance (22)

The dysbiosis in obese patients is a significant factor in increased lipid synthesis and storage (23). With regards to lipid synthesis, bile acid concentration which is essential in repressing hepatic lipogenesis is decreased due to the gut's disrupted microbial diversity and hence contributes to enhanced lipid synthesis (23). In the context of lipid storage, it is related to the metabolic endotoxemia as a result of increased levels of LPS due to the GM dysfunction as mentioned earlier in this section of the review (13, 23). Firstly, the pro-inflammatory cytokines this molecule induces to be produced involve IL-6 and TNF-α, that are responsible for the phosphorylation and hence the inactivation of the insulin receptor, causing the obese patient's body to develop further insulin resistance (23). Secondly, LPS toxicity is noted to promote increased adipocyte size due to its ability to stimulate production of molecules, such as the significant contributor to mesenteric fat deposition called CD14, and Activin A that is important for adipocyte precursor formation, allowing further lipid storage (23, 24).

Dysbiosis is a major factor in increased lipogenesis and adipocyte hypertrophy, which can lead to endocrinological issues. Adipocyte dysfunction in obesity ensues via mitochondrial damage due to hypertrophy of these cells (25). This brings about the release of many inflammatory cytokines that destroy normal adipocyte functioning. Furthermore, increased leptin secretion occurs and if chronic will lead to leptin resistance (25). A plummet in the levels of adiponectin will also occur which in normal, physiological levels would provide insulin sensitisation and protection against inflammation, and hence its benefits are annulled in patients suffering from obesity (25,26).

Since there seems to be a correlation with well-maintained GM and reducing obesity and its associated risks, it is worth mentioning that

adequate exercise (15) and dietary changes that avoid foods that are inflammatory or damaging to the colon and GM would potentially help obese patients lose weight and have reduced endocrine dysregulation (18). The improved metabolic function due to the rich GM being restored, through careful maintenance of diet can improve an obese patient's energy homeostasis focusing on a protein and fat diet over a carbohydrate rich one (16). Faecal transplant can be an alternative method of improving GM richness as used normally for Clostridium difficile bacteria. However, in mice it has been found that when performing faecal transplant on lean patients' GM it may replenish good bacteria within the gut (14)(17). In one study, participants within the combined diet and faecal transplant group experienced significant weight loss as early as day 20, whereas those in the diet-only group saw results much later, after day 90 (35). Reducing dysbiosis remains unclear as to whether it can reduce obesity besides these studies done on mice which have shown ameliorated symptoms of metabolic dysfunction (14)(16).

Traditional Chinese Medicine (TCM) has been found to have anti-obesity effects by regulation of energy metabolism, absorption, feeding behaviour, fat accumulation and elevating chronic mild inflammatory response (38). It was found that ginsenosides improve the composition of GM, increasing SCFAs and receptor proteins too (38). Propolis ethanol extract also regulates GM, glucose tolerance and lipid distribution in high fat diet rats (39). Berberine and curcumin were found to improve intestinal barrier function, increase the number of intestinal probiotics, upregulate the expression of the innate immunity genes Pla2g2a and PYY, and decrease food intake in obese mice (40). Other studies (41) discovered that grape seed can reduce appetite and improve weight loss by regulating GM. Many other TCM herbs have come to assist the GM in its metabolic and endocrinological role in obesity. These can lead to increasing variety of GM, reducing triglyceride and cholesterol level, increased SCFAs, and reducing dysbiosis (42). It must be noted that most of these studies were conducted on high fat diet mice.

Different types of dietary fibre influence gut microbial diversity, composition, and metabolism. Fibre fermentation increases GM diversity and enhances SCFA production to improve insulin sensitivity, reduce inflammation and boost satiety. Therefore, by inadvertently improving GM, dietary fibre will also help alleviate the inflammatory and endocrinological dysfunction caused by obesity (43) (44).

Diabetes Mellitus

This disease accounts for about 90% of individuals with any type of diabetes. Unfortunately, obesity and T2DM have a very high prevalence in the Maltese islands, through a study conducted on a sample representative of the adult Maltese population, which was found to be present in 1 in 10 inhabitants or 10.31% of the sampled group (27). A significant part of this percentage, approximately 40% of the sample with T2DM, were newly diagnosed with the disease while the rest were already known cases (27). This relatively large number of new diagnoses during the sample collection is concerning since it shows the lack of health information that could be provided to the patients in order to decrease T2DM risk, and the increased costs in treating the established disease. The projected healthcare expenditure for T2DM treatment in 2045 is estimated to be €244,136,040, however in 2017 the annual expenses had already reached nearly half of that value (27).

Endocrine disruption, such as that brought about by obesity which increases insulin resistance, can

eventually lead to T2DM by deregulation of beta cells of the pancreas, impairing insulin output, signalling and increasing beta cell apoptosis (28). Especially in obese where the high levels of adipose decrease leptin and adiponectin release, and increase insulin resistance. The low-grade chronic inflammation due to obesity also increases TNF-α thereby reducing insulin sensitivity by blocking GLUT4 insulin sensitive channels (28).

The correlation between the intestinal microbiota and host hormones can influence insulin metabolism, as endocrine dysfunction can lead to T2DM but the GM, if healthy , can modify blood glucose and stimulate beta cells. It is possible that compartmentalization of host microbe response is important in the effects of incretins by means of SCFAs which is a metabolite of the GM to metabolize dietary fibre (29). SCFAs act as signalling molecules by binding to FFAR2 (free fatty acid receptor 2) and FFAR3 (free fatty acid receptor 3) receptors on intestinal L cells, triggering the release of PYY and glucagon-like peptide-1 (GLP-1) (29). These hormones help slow gastric emptying, suppress appetite, enhance insulin secretion, and reduce glucagon levels. SCFAs promote intestinal gluconeogenesis in two ways: Butyrate activates gluconeogenesis-related genes through a cAMP-dependent mechanism and propionate serves as a key substrate for gluconeogenesis, further boosting glucose production in the gut (30)(refer to figure 4).

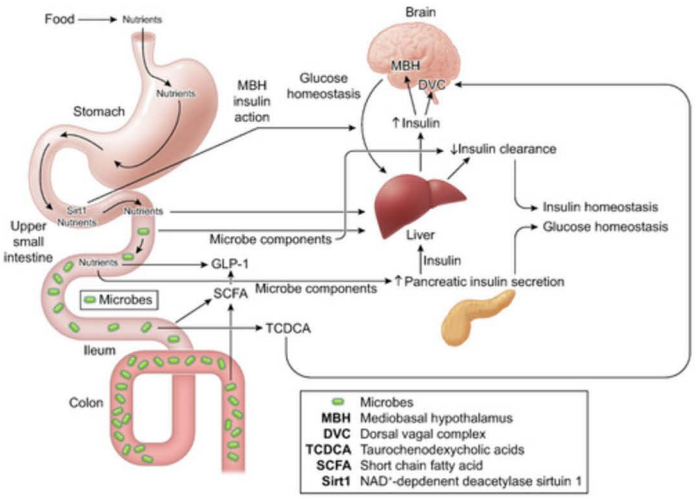

Figure 4 : The GM affecting the metabolic pathways of insulin (29)

T2DM is associated with gut microbiota dysbiosis, particularly an imbalance in Firmicutes and Bacteroidetes populations (31). Findings involve decreased Clostridium spp which is linked to diminished insulin sensitivity, and Akkermansia muciniphila, a metabolically beneficial bacterium, is also reduced in T2DM while a rise in Betaproteobacteria is positively correlated with blood glucose levels (31). Decline in the diversity of the gut microbiome is not strictly correlated with T2DM only, but also with T1DM which is an autoimmune disease, where the gut microbiota plays a role in triggering immune responses. Dysbiosis is observed in T1DM patients, leading to increased gut permeability, allowing bacterial components to enter circulation and activation of immune pathways such as Toll-like receptors (TLRs) promoting inflammation (31, 55). Reduced levels of beneficial bacteria like Faecalibacterium prausnitzii, which helps control inflammation, is also associated with the latter disease (31).

There are many ways and means by which certain changes in the intestinal microbiota encourage and sustain the pathophysiological process of the development of T2DM. These changes can be

exacerbated because of the established disease which worsens its complications and prognosis (30). Firstly, abnormal bile acid metabolism in patients with GM damage have been noted to be at increased risk of T2DM due to decreased insulin secretion and increased glucagon release (30, 57). In normal conditions, primary bile acids are converted into their secondary form through a number of reactions, the most important being 7α-dehydroxylation which converts the primary bile into deoxycholic acid and lithocholic acid which are the two major secondary forms (30). The two latter molecules play a crucial role in enhancing insulin sensitivity by indirectly promoting the release of a subtype of Fibroblast growth factor which acts as a ligand that works in favour of glucose tolerance (60). Known species of bacteria that carry the responsibility of this process are Clostridium and Eubacterium (59). Some studies show that the issue with a diseased gut flora is that there is not a reduction of secondary bile salt secretion, but a rise in its rate which triggers a process involving enterochromaffin cells which, on cue to the abundance of secondary bile acids, release 5-hydroxytryptamine (57). This molecule results in reduced insulin release and promotes glucagon secretion (30).

In addition to abnormal bile acid metabolism, through means of an uncontrolled diet a significantly increased production of branched-chain amino acids (BCAAs) can occur via the GM, which is found to be directly proportional in insulin resistance and thus increased risk of T2DM (30). Specifically, Prevotella copri and Bacteroides vulgatus are most known to be involved in synthesis of BCAAs, which are essential AAs like leucine and valine (30, 61). There is found to be some correlation, via the mTOR signalling pathway, between raised BCAAs and mTOR in its phosphorylated state (62). The latter is able to disrupt insulin receptor signalling, hence explaining how increased BCAAs contribute to the likelihood of T2DM development (62). One study assessed the level of functional gene for BCAA synthase present in a small population of non-diabetic individuals against one of individuals with T2DM (30, 45). Results were in line with BCAAs' connection to T2DM risk, since the disease-bearing patients had raised levels of the functional gene observed in the study (45)(refer to figure 5).

Lastly, gut flora are capable of metabolising histidine and as a by-product of this process, a molecule termed imidazole propionate is formed (46). If accumulated, this molecule plays a role in insulin resistance by a pathway similar to the one involving BCAAs, called the mTORC1 pathway (47). This causes serine present on insulin receptor substrates to be phosphorylated at abnormal levels, which thus acts as the main step to the development of insulin resistance. Harmful bacteria such as Clostridium baumannii are found to be connected to raised amounts of imidazole propionate, while the contrary is noted for anti-inflammatory bacteria (47).

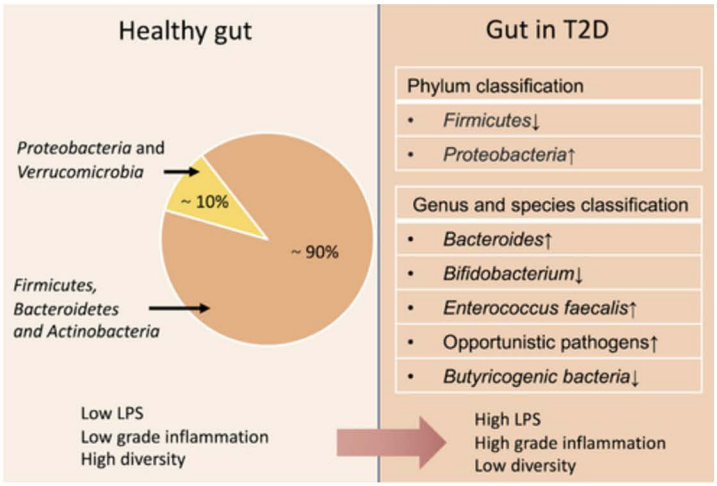

Figure 5: Changes within the GM between a healthy gut and that of a T2DM patient (32)

Probiotics may help in modulating gut microbiota and improving metabolic health in T2DM. Specific probiotic strains such as Lactobacillus,

Firstly, they are able to improve insulin resistance via the action of multi-strain probiotics that contain Lactobacillus species such as L. acidophilus, and action of Bacillus coagulans on members of the interleukin family (33). Particularly IL1β and IL6 are both found to decrease on administration of the aforementioned probiotics (33, 63). Following the decrease of these molecules, insulin resistance has been observed to diminish (33). Secondly, other probiotics such as Bifidobacterium partly play a role in the alleviation of inflammation due to its suppressive effect on other pro-inflammatory cytokines apart from interleukins, such as TNF-α (33). Reduction in inflammation occurs due to activation of AMPK pathway among many others, for which the probiotic supplementation is responsible, which consequently improves patients' blood glucose and HbA1c levels along with improved glucose tolerance and insulin sensitivity (33) (refer to figure 6).

As a combined effect, the wide range of probiotics available currently in the market can aid individuals suffering from T2DM to better control their blood glucose levels and more importantly to reduce dependence on their medications used to increase insulin sensitivity (34), thereby reducing associated side-effects of the medication such as diarrhoea due to metformin (34). This symptom can occur with probiotics but only temporarily, and probiotic administration only carries a small chance of adverse effects in immunosuppressed or compromised patients in which an infection may rarely ensue (34).

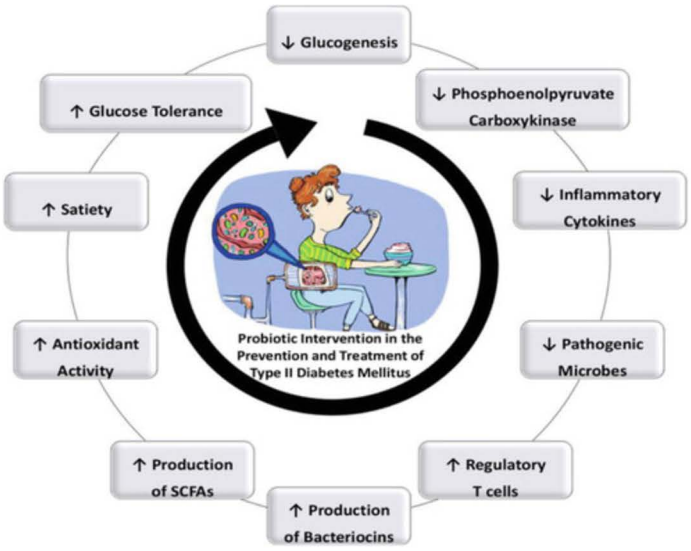

Figure 6: Various beneficial metabolic, inflammatory and immunological changes associated with probiotic intervention in T2DM (36)

In addition to probiotic supplementation, studies have shown the benefits of adapting to certain diet regimens which bring about a healthier GM, with this process being accelerated by incorporation of faecal microbiota transplantation (FMT) (35). A study performed on a group of patients with T2DM demonstrated that a diet rich in probiotics, prebiotics and wholegrain caused a significant increase in both Bifidobacterium and Lactobacillus species in the gut (35). This process occurred at a much faster rate in the group of patients who were offered a FMT. Therefore, the combination of the diet and FMT group effectively rendered a greater and quicker controlled fasting blood glucose and HbA1c level in the patients (35). Apart from the increase in metabolically beneficial bacteria, a plummet in sulfate-reducing groups such as Desulfovibrio and Bilophila was noted. These are species associated with metabolic disorder and inflammation, so their reduction further aided gut health and metabolic balance (35).

Some studies have found that acarbose treatment

for T2DM, in which it helps to avoid postprandial spikes in blood glucose levels, also has a positive effect on the GM composition (37). It has been noted that from acarbose treatment a rise in Lactobacillus and Bifidobacterium can be appreciated, providing better insulin sensitivity and suppressing inflammation (37, 64). Due to acarbose being an α-glucosidase inhibitor, its mechanism of action reduces carbohydrate absorption in the small intestine, effectively allowing higher rates of fermentation by the GM. Since acarbose treatment also correlates with increased Eubacterium rectale which produces SCFAs particularly butyrate, the raised fermentation rates cause a significant rise in SCFA production and these molecules, as discussed previously, are associated with improved metabolic function in T2DM patients (37).

Chinese medicine has been used to treat diabetic symptoms for years and has been found to in various means improve the GM as we discussed in section 1 (38-42). Shanyao has been widely used in diabetes (48). Shanyao was shown to maybe improve glycolipid metabolism and fasting insulin level, and has shown potential benefit compared to hypoglycaemic agents and lifestyle changes only (48). Aside from this, since many TCM methods modulate the GM they can indirectly affect glucose metabolism (42)(49), and this results in improved glucose control and insulin sensitivity (49). It was found that increased TCM treatment could increase the proportion of Firmicutes, Bacteroidetes and Lactobacillales populations within the gut(50)(51). HbA1c was found to be significantly reduced in a TCM treatment group versus control group, suggesting TCM has a hypoglycaemic effect compared to that of only the first line drugs administered in T2DM (49)(50)(51).

Exercise has been correlated with improvements of glucose homeostasis and insulin sensitivity, and GM variety has been exhibited to enhance capabilities of metabolism of SCFA and BCAAs (refer to figure 7)(52). Athletes with healthy GM were found to have increased biosynthesis, for instance of carbohydrates (53). Therefore, since dysbiosis of GM plays a role in insulin resistance and T2D (52), it stands to reason that if one exercises and maintains a healthy GM it will improve diabetes symptoms and risk. The exact mechanism of how one affects the other is not fully delineated, likely driven thanks to microbial butyrate and exercise induced intestinal barrier functions (54).

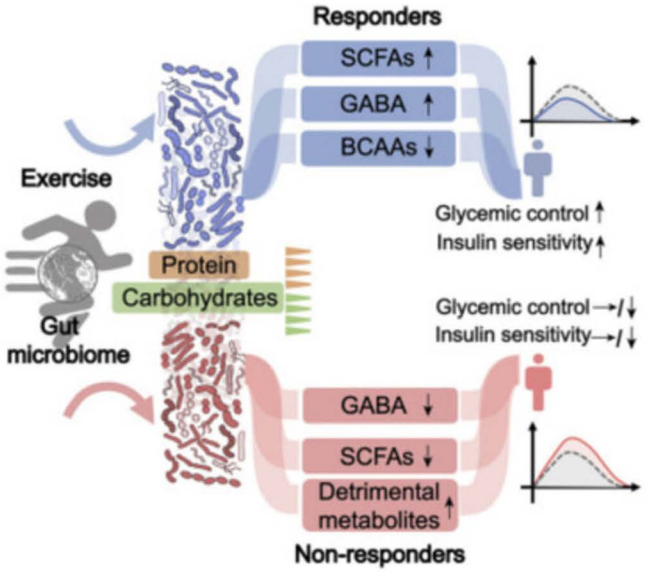

Figure 7: Detailing the input of exercise on GM and indirectly treating T2DM (52)

Conclusion

Through various studies and research done throughout it becomes clear that there exists a significant input of the GM and the endocrine system regulation. The gut microbiome modulates endocrine function through mechanisms such as regulating glucose metabolism, impacting insulin sensitivity, and influencing hormonal signalling via the gut-brain axis. Additionally, it affects systemic

inflammation and metabolic homeostasis, which are central to many endocrine disorders. Therapeutic strategies targeting the gut microbiome, including probiotics, prebiotics, dietary interventions, and faecal microbiota transplantation, hold promise for improving endocrine health and mitigating the progression of these conditions. However, despite its highly promising implications much has yet to be discovered, but possibly less severe progression of endocrine dysfunction along with improved quality of life in these patients is a foreseeable event. Given this possibility, targeting the gut microbiome offers researchers and clinicians novel opportunities to improve overall health and well-being.

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Declarations

We hereby declare no conflicts of interest or attempts to discredit other authors or misinform our readers.

Authors Contributions

Luke Camilleri and Peter Calleja in equal parts co-authors to this article

List Of Abbreviations

GM	Gut Microbiome
ED	Endocrine Disorders
LPS	Lipopolysaccharide
T2DM	Type 2 Diabetes Mellitus
SCFA	Short Chain Fatty Acid
IL	Interleukin
TNF	Tumor necrotic factor
T1DM	Type 1 Diabetes Mellitus
AMPK	Adenosine monophosphate activated protein kinase
FMT	faecal microbiota transplantation
TCM	Traditional Chinese Medicine
AA	Amino Acid
BCAAs	Branched Chain Amino Acids
PYY	Peptide YY

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MIND MAPS

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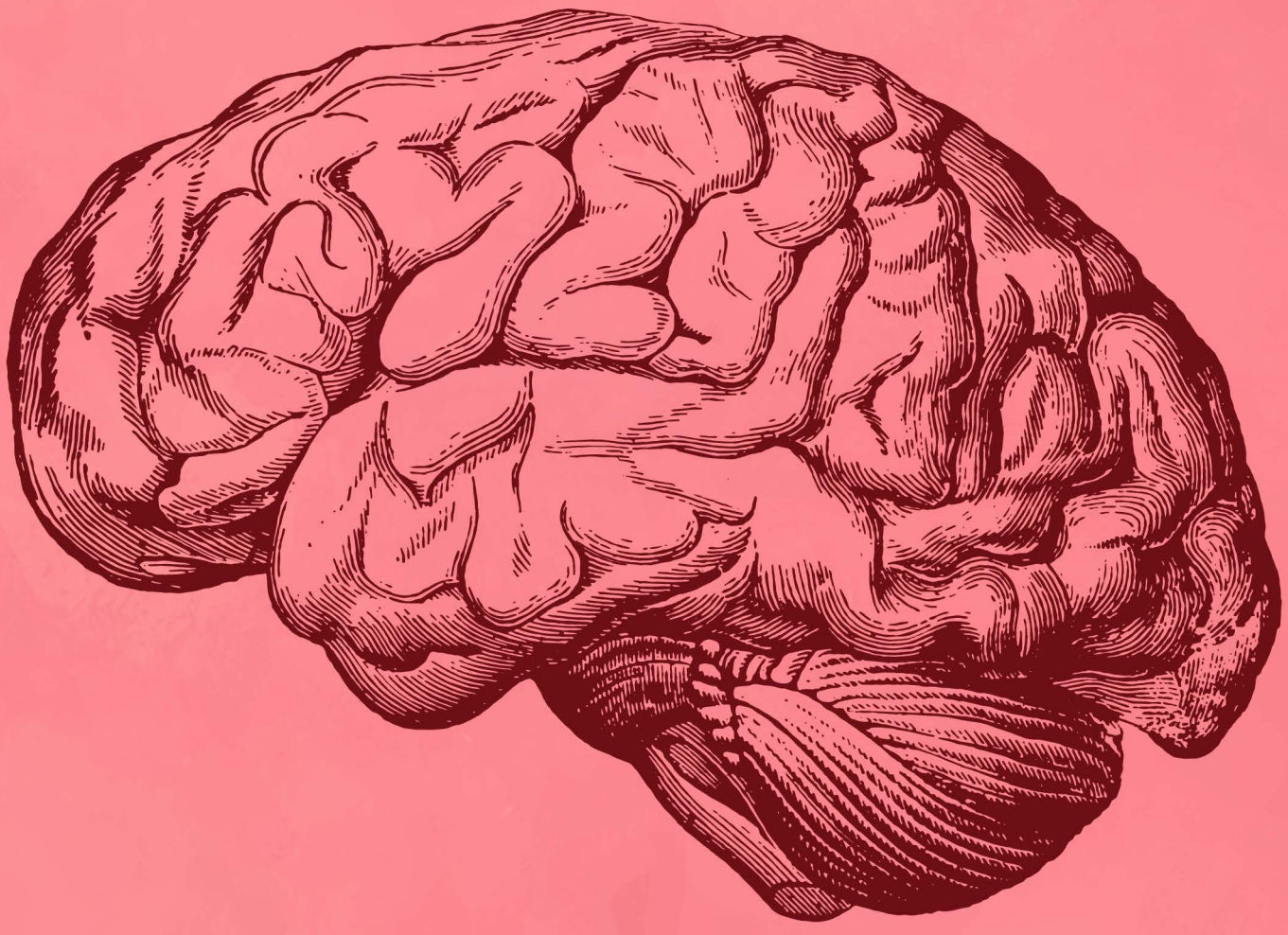

Addressing physician burnout Mental Health Strategies

Author: Kimberley Abdilla

Abstract

Burnout is a state of total exhaustion which is commonly experienced by physicians due to the intense work environment in which they find themselves working in. The causes and effects of burnout will be tackled in this career-related piece, addressing symptoms in which stress and exhaustion can manifest themselves. The symptoms are also taken from a neurobiological perspective, observing how burnout affects different parts of the brain, hence resulting in both physical and emotional distress. The causes of burnout are not only related to the medical aspect of healthcare, but it also involves the legal area of a physician's profession, which may include organisation of health records for instance. The effects on both doctors and patients will be tackled, including issues which the healthcare organisation faces on a daily basis due to the high burnout levels constantly being experienced by physicians. This piece compares the overall performance between a burnt out and a not burnt out doctor, highlighting the importance of being in a mentally fit state when working in such a profession. The latter is crucial so as to ensure that patients' trust is enhanced, and medical errors are prevented. Finally, strategies which aid in preventing and ultimately improving physician burnout will be discussed, so that they may be implemented within healthcare organisations.

Keywords: burnout, physicians, causes, effects, symptoms, strategies

Introduction

Firstly, burnout must be measured in order to fully comprehend the extent to which physicians are experiencing it (1). The next step is to identify what is causing these high levels of stress which depends on both the doctor's professional and personal lives. Furthermore, the strong negative impact on patients and physicians themselves continues to highlight the importance of tackling this issue with utmost priority (2). The only means of improvement when it comes to fostering a positive work environment in healthcare involves compiling a number of strategies to aid in addressing physician burnout. These strategies are requisite to ensure that the physicians and those around them, including patients, do not become negatively affected by this burnout as numerous grave consequences may arise if it is not handled appropriately (2).

Discussion

Measuring physician burnout

The standard way of measuring physician burnout is via the Maslach Burnout Inventory (MBI) which includes a Human Services Survey applicable for healthcare professionals. The instrument, which was released in 1981, consists of a questionnaire assessing the frequency of symptoms occurring over the past year. It does so by analysing three critical aspects of burnout: emotional exhaustion, depersonalisation, and personal accomplishment. This gives an idea of how many physicians are

experiencing this form of exhaustion so that the necessary strategies are compiled to prevent or treat this accordingly (3).

What gives rise to physician burnout?

Primarily, the concept of practicing clinical medicine within itself is one of the main causes of burnout as physicians are constantly dealing with ill, scared, and dying patients (4). Physicians must also keep up with the rapid expansion of medical knowledge and deal with increased administrative burdens related to electronic health records and regulatory requirements. Moreover, lack of leadership skills from immediate supervisors, for instance having an absent or tough boss, can make the work more demotivating. This falls hand-in-hand with the concept of facing scrutiny from administrators on sub-optimal metrics such as patient satisfaction scores and percentage of charts closed within 24 hours (5). This fails to recognise the nature of their work making them feel indifferent towards the crucial work they are carrying out. Furthermore, the struggle to find a work-life balance further contributes to feeling burnt out. Even simple, everyday needs such as sleep, exercise and nutrition are being pushed to the back burner by clinicians in order to keep up with all of the work on their proverbial plates (3). This results in accumulation of stress, fatigue, and clouded judgment when making crucial decisions at the workplace.

What are the symptoms of burnout?

The crucial aspect when it comes to identifying burnout is to notice the symptoms associated with it (refer to Figure 1). Firstly, burnout is a gradual process which occurs over time. Symptoms range from exhaustion and fatigue all the way to experiencing thoughts inflicting self-harm. It is important that as a physician, one does not only look for signs of stress within themself but try to search for them within their colleagues. They might be less productive, absent from work, excessively tired, or appear irritable (6).

Another associated symptom with burnout includes depersonalisation. This is an impaired and distorted perception of oneself, of others, and one's environment and it manifests itself as an affective-symptomatic lack of empathy. A physician may manifest signs such as making unprofessional comments towards co-workers and the inability to express empathy or grief towards patients and their families. Depersonalisation can make a person feel numb, demotivated and isolated (7).

Furthermore, another disorder which arises from burnout is major depressive disorder. It affects how you feel, think and behave and can lead to a variety of emotional and physical problems (5). This manifests itself as anger outbursts, an overload of sadness, emptiness and hopelessness, together with a lack of efficacy. The latter may involve losing interest in your job as a physician and experiencing pessimistic thoughts about how a physician's job serves no purpose. This will surely affect the overall results of patient care since there will be a strain on the doctor-patient relationship if the physician is struggling to communicate effectively with the convalescent individual (5).

Uncontrollable stress has deleterious effects on the prefrontal cortex which is the brain region that governs higher cognition and controls thought, action, and emotion. Moreover, this region of the brain is essential for guiding appropriate social behaviours, including placing patients' interests above one's own. The prefrontal cortex can also act as a 'mental cheerleader' (8), motivating higher goals and regulating emotional circuits, including those that mediate mood and anxiety. During extreme levels of stress, high levels of noradrenaline and dopamine are released in the brain, thus weakening prefrontal cortex function (5). These catecholamines initiate intracellular signalling cascades which rapidly open potassium channels near the prefrontal cortex synapses. This process

can result in diminished working memory and poor decision making, resulting in detrimental effects when working as a physician. Burnout can cause the prefrontal cortex synaptic connections to atrophy. Fortunately, however, these connections can regenerate during sustained periods of nonstress, hence this is why burnout should be addressed and handled accordingly (5).

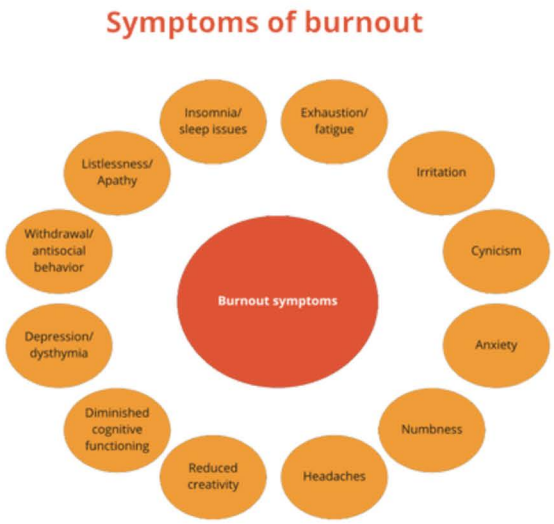

Figure 1: Diagrammatic representation of the symptoms experienced as a result of burnout (9).

Effects of physician burnout on both doctors and their patients

Physician burnout is costly for all. Besides impacting the doctor's themselves, patients are also at risk of being affected. According to a Medscape survey, the highest rates of burnout were concentrated in emergency medicine, internal medicine, and obstetrics-gynaecology (10). Moreover, burnout seems to leave a stronger impact on female doctors, mainly due to family caregiving responsibilities. Physicians who form part of the older population are less likely to suffer from burnout, mostly due to shorter working hours. The main impact is that a greater number of physicians are more likely to leave the profession, resulting in high turnover rates within the healthcare system (11). Furthermore, over time, it is becoming more evident that physicians suffering from burnout are more than twice as likely to be involved with patient safety incidents than physicians who are not suffering from burnout. Patients may perceive a lack of interest or concern for their well-being. It is well known that patients are less likely to trust and follow the advice of a physician who appears disengaged or indifferent about their healthcare (7). In addition, as a symptom of burnout, doctors may experience a reduced sense of personal accomplishment, which in turn may decrease motivational levels and enthusiasm for learning. This further puts patients at risk and lowers their satisfaction levels when they are not treated by confident and competent physicians. Communication within a doctor-patient relationship is a key element. However, burnout can impair a physician's ability to communicate clearly and effectively. This may lead to patients feeling uncertain about their treatment plans, misdiagnosis, and a lack of shared decision-making, hence breaking a patient's trust (2).

The figure below exhibits survey results obtained during an anonymous survey about healthcare well-being, burnout, and patient safety conducted in May 2021 amid the COVID-19 pandemic. This portrays precisely the difference between a mentally motivated doctor and an encumbered one (refer to Figure 2).

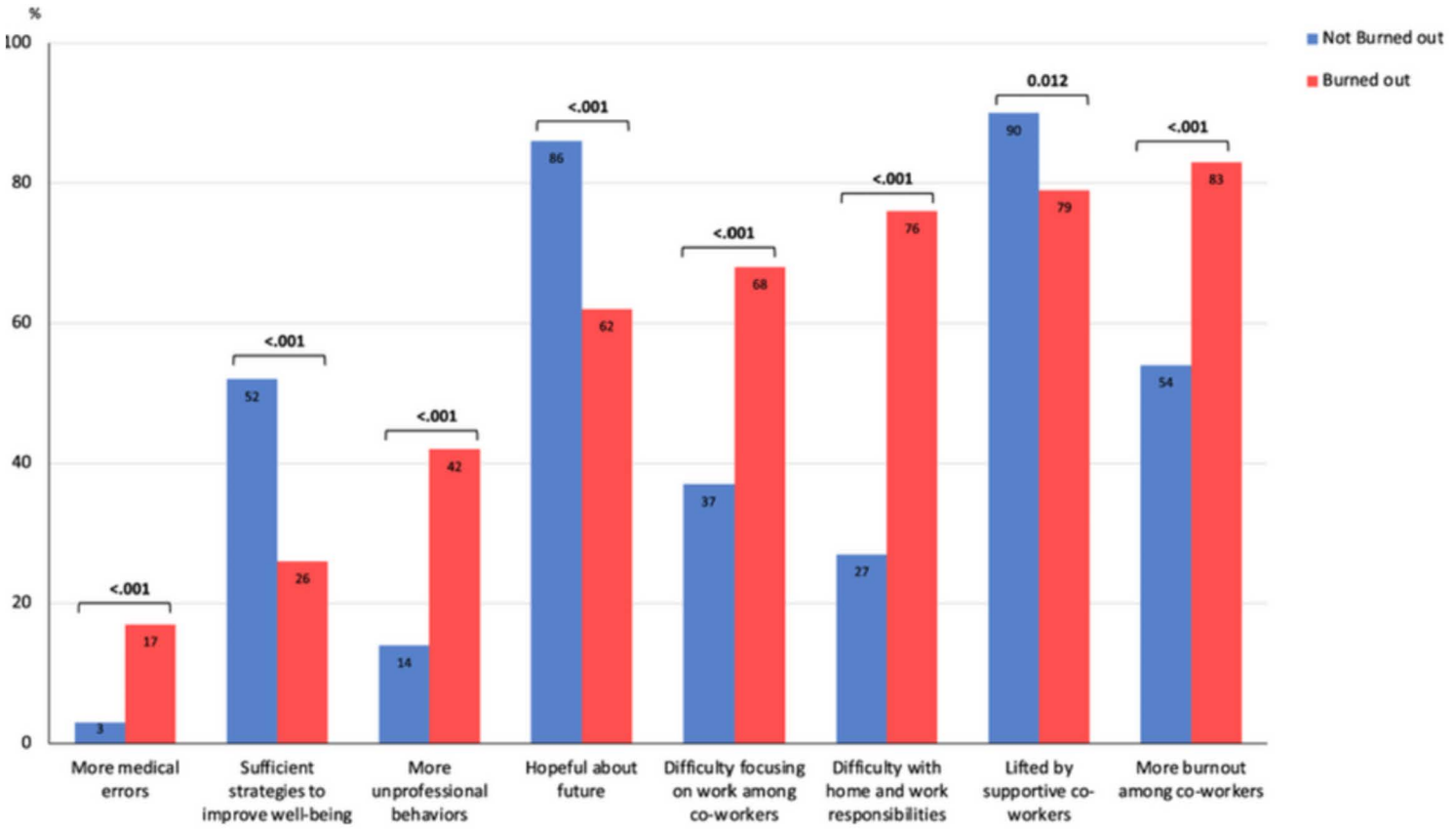

Figure 2: Diagrammatic representation of overall performance of burnt out and not burnt out physicians (12).

Strategies to prevent and improve physician burnout

Healthcare systems must work to create a healthier work environment for their employees as this is a crucial aspect when it comes to supporting physician well-being. One of the most important aspects when working as a physician is to have a strong work-life balance. The system may do this by enabling flexible scheduling and adequate time off. This ensures that doctors are recharged and mentally resilient enough to face the day-to-day challenges on the job. Providing mental health support is another strategy which should be utilised. This may be done by offering counselling sessions and stress management programs (2). The COVID-19 pandemic left a strong impact on healthcare systems, not just in a negative way however. This global rife ended up shedding a light on what physicians truly require when it comes to feeling less stressed at their place of work. For instance, Mount Sinai Health System introduced a 'huddles' program which included psychological first aid, managing moral distress, and addressed common mental health challenges such as anxiety and depression (13). Furthermore, implementing strategies such as offering childcare services together with maternity and paternity leave may help prevent burnout caused due to external factors in the physicians' personal lives. The healthcare organisation should render an environment in which shared accomplishments are celebrated, team building exercises are carried out often, together with organising social events and resident retreats. This is because the most important aspect at the workplace is that physicians feel at ease working with each other, so that they can even aid one another when it comes to dealing with stressful situations on the job (14).

Conclusion

If left unaddressed, physician burnout may lead to emotional exhaustion which may result in a variety of mental health conditions. This compromises patient care by reducing physician engagement and communication, whilst increasing the likelihood of medical errors. Hence, various strategies should be implemented by healthcare organisations to mitigate the high levels of burnout constantly experienced by doctors. It is crucial to foster a culture that values physician well-being so as to ensure the longevity of its workforce whilst offering the best possible patient care. By definition, occupational burnout is precipitated by characteristics of the work environment not by individual problems as such, highlighting the idea that identifying it within the organisation is the first step when it comes to addressing it (14).

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Declarations

Conflict of interest: N.A.

Author's Contribution

I am the primary author of this piece.

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The Use of Imaging in Endometriosis

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Abstract

Endometriosis is a complex and often painful condition characterised by the presence of endometrial tissue outside the uterus. This may lead to inflammation, scarring and symptoms such as pelvic pain and infertility. It may manifest itself as superficial or deep disease as well as ovarian disease with the presence of endometriomas.

This study came to fruition in order to emphasise the importance of imaging with respect to the diagnosis of endometriosis. Whereas previously a surgical approach was the gold standard, imaging has now become the benchmark for diagnosis. The aim of this paper is to highlight the importance of imaging in endometriosis by reviewing literature on various databases such as PubMed and combining the most salient and relevant points. The goal is to underscore the previous points even further.

This literature review revealed that techniques such as ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) are utilised to visualise endometrial lesions, cysts and associated pelvic anatomy variations. Ultrasound, in particular transvaginal, is often the first-line imaging tool primarily because of its accessibility and non-invasiveness. MRI however is considered the gold standard for evaluating deep infiltrating endometriosis and is able to provide a detailed image of soft tissue structures. It is therefore the mainstay investigation prior to management planning and surgical intervention.

In conclusion, the use of imaging does not simply aid with management planning however, is able to assist in monitoring disease progression and response to treatment

Keywords: Endometriosis, imaging, MRI, ultrasound

Introduction

Endometriosis is a gynecological disorder characterised by the presence and growth of tissue which when viewed under a microscope, is similar to endometrial tissue, outside of the uterus. The prime age of diagnosis is between the ages of 30 and 45 however lesions may occur in 1-20% of all women, most often asymptomatically. These lesions can be found throughout the pelvis, most notably in the uterosacral ligaments and surrounding the ovaries where it may form endometriomas or "chocolate cysts". It may also occasionally affect abdominal wound scars, the vagina, bladder, rectum and sometimes even the lungs.

Endometriosis, like normal endometrium within the uterus, responds to oestrogen. This explains why it is known to regress during the menopause as well as pregnancy, and is seen to have fluctuating symptomatology during the menstrual cycle. In severe cases, endometriosis has been seen to cause what is known as a "frozen pelvis" secondary to

progressive fibrosis and the formation of adhesions.

Clinically, a patient may present in a number of various ways ranging from cyclical chronic pelvic pain to dysmenorrhea prior to the onset of menstruation, deep dyspareunia, infertility as well as dyschezia. More acute cases may be characterised by sudden onset abdominal pain secondary to rupture or torsion of an endometrioma. Vaginal examination may reveal thickening in the adnexa, an immobile or retroverted uterus, or may also be completely normal!

Whereby the previous gold standard to diagnosis was via laparoscopy, imaging has slowly taken over mainly because it is less invasive and patients are therefore more likely to comply, however also because of its sensitivity with respect to diagnosing endometriotic lesions.

Literature Review

Moradi et al wrote that there is an average delay of 7-12 years in the diagnosis of endometriosis (1). This delay in diagnosis results in delays of interventions that relieve pain and discomfort associated with this condition, which in some women can be so severe causing them to harm themselves. For this reason, it is imperative to invest in women's health since delays in diagnosis result in significant psychological burden (2).

NICE guidelines recommends ultrasound, which is usually trans-vaginal or trans-abdominal as the primary imaging modality, however, transvaginal ultrasound is preferred. Other guidelines such as ESHRE also include MRI as being one of the primary imaging modalities (3). Patients tend to prefer ultrasound due to its quick and easy process, as well as being relatively cheap, however the use and accuracy of results depends greatly on the operator's skill and experience, as well as the severity of the disease.

Ultrasound

Ovarian endometriomas are arguably the most common forms of endometriosis found on ultrasound. It is generally described as unilocular or multilocular cystic lesions with a ground-glass appearance measuring [X] x [Y] x [Z] with no internal vascularity noted on Doppler imaging. Internal vascularity would prompt investigations for more sinister causes for the ovarian mass. Additional findings may include finding free fluid in the pouch of Douglas as well as tenderness on transvaginal probe pressure correlating with the lesion location. The ultrasound operator may also find endometrial glands within the myometrium, meaning that there is co-existing adenomyosis. Adenomyosis and endometriosis are closely related however may be distinguished since endometriosis is found outside the uterus while adenomyosis is found within the myometrium. To assess the extremity of ovarian endometriosis, the operator may elicit the "sliding sign" using the application of pressure to the trans-vaginal probe to measure the degree of mobility of the pelvic structures. A positive sliding sign is normal; meaning that the anterior rectum glides freely over the posterior uterus. A negative sliding sign is a bad prognostic indicator since it denotes the presence of deep infiltrating endometriosis, meaning that there is involvement of uterosacral ligaments, bladder, bowel, vagina and any part of the peritoneum.

MRI

With MRI, soft tissue can be seen in much greater detail, than with ultrasound, thus it is good to distinguish lesions when the diagnosis is in doubt. However, patients may be reluctant since there is a need to be in a closed space for a long period of time. It is important to mention that even though high-resolution pictures are obtained through MRI, a diagnosis of endometriosis cannot be ruled out if there are no findings on imaging, and further investigations are required when there is a high clinical index of suspicion. MRI uses T1 and T2

weighted images, therefore it is important to distinguish that endometriosis is hyperintense on T1 images and hypointense on T2 images. There will also be shading signs showing layering of blood within the lesion. MRI is useful when a diagnosis of endometriosis is made on ultrasound, but the degree of disease needs to be further quantified. An example of advanced disease seen on MRI is distorted pelvic anatomy due to adhesions and fibrosis associated with deep infiltrating endometriosis.

Despite the many signs and clues seen on ultrasound and MRI which help us in our diagnosis, smaller and more superficial lesions are usually missed (4), thus, this is where laparoscopy starts to play an important role in the imaging-negative patient with a high index of suspicion. Unfortunately, a clinical exam is of little use in most cases of endometriosis since only about half of the larger lesions are thought to be palpable. One main disadvantage of imaging is that in some cases, it is unable to differentiate between ovarian cancer and ovarian endometriosis. In addition, both conditions constitute a rise in Ca125 which does not help in differentiating the two diagnoses. This is especially an issue in older postmenopausal women, who might have had endometriosis for years but never sought help, or got a proper diagnosis, and thus further warrants surgical exploration via laparoscopy (5).

Koninckx et al. describe the inaccuracy of imaging via calculations alleging that a test with 99% sensitivity and specificity still results in 50% false positive results. They also mention the possible integration of artificial intelligence in the future to strengthen the reliability of imaging techniques and slowly start moving away from surgical techniques. When there is uncertainty, surgical options can be particularly damaging especially to the younger patient. This is because endometriosis tends to be a recurring condition, thus adhesion formation from surgery may cause recurrence of endometrial lesions, possibly even in the adhesion or surgical scar! It also poses a threat to the young female's fertility and affects family planning (5). For this reason, many doctors and patients take the collective decision to commence medical therapy without a definite diagnosis, thus contributing to the delay in diagnosis as explained earlier by Moradi et al.

PET-CT

Positron emission tomography- computed tomography (PET-CT) has a wide range of use in oncology, but it is being applied outside of the oncological field in various novel studies overseas to assess its ability to aid in other possibly benign diagnoses, such as that of endometriosis. Since endometriosis may mimic ovarian cancer, it can be applied to identify endometrial lesions and endometriomas (6). It is a promising diagnostic technique since PET involves injection of radiolabeled tracers intravenously to highlight parts of the body that take up that specific tracer, for example, in areas that are more metabolically active. There are many tracers that can be used for this purpose. Initially, studies used F-fluorodeoxyglucose as the radiolabeled substance- which showed promising results (7), however studies are shifting their focus towards the assessment of F-flouroestradiol and F-flourofuranylnorprogesterone as well as the integration of PET-CT with other imaging modalities to improve early-stage diagnoses (8).

MRE

Magnetic resonance elastography (MRE) is used to add an extra dimension to the image formed to determine the tissue's character (mechanical and physical properties such as stiffness and viscosity). It allows the surgeon to know the lesion's characteristics beforehand to be better prepared about what is most likely to be found during surgery, since the lesion's characteristics and

composition influence decisions for removal (8). MRE also overcomes MRI's limitation of not being able to identify the difference between ovarian cancer and ovarian endometriomas in some cases. The downsides of this technique are that it is very expensive, requires investigation for a long time like MRI, and it is not yet available in Malta.

Conclusion

Patients suffering from this chronic, relapsing condition experience reduced quality of life, time off work and overall increased suffering. It is our duty as medical professionals to enhance their quality of life, make sure that our patients feel heard, listen to their concerns and follow them up on any issues they might have. With the advancements in modern medicine, as well as the increase in awareness of the condition, more and more women are being diagnosed and treated accordingly. As physicians, we must always take pelvic and menstrual pain seriously, as they may be dismissed as normal, therefore instead of fixing the problem, we would be contributing to the worsening of the problem. In addition, we must also be aware to find and treat the problem, with the least invasive procedures possible. A management plan in line with the lifestyle and wants of the patient should be devised keeping the patient informed, included, and at the center of all decisions taken.

Declarations

None to declare.

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