An-Najah National University

Faculty of Graduate Studies

THE RELATIONSHIP BETWEEN SYMPTOMS SEVERITY, MENTAL HEALTH, LIFESTYLE FACTORS, AND GUT MICROBIOME AMONG IRRITABLE BOWEL SYNDROME PATIENTS, A CROSS-SECTIONAL STUDY, WEST BANK, PALESTINE

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Dedication

With great reverence and unshakable admiration, I present my master's thesis as a dedication to Gaza's tenacious spirit, an inspiration of resilience in the face of suffering. This work is devoted to the fearless people who dare to dream and fight persistently for a better future.

May its findings serve as a monument to the Gazan people's resilience and tenacity, inspiring hope and sparking progress.

My family and my fiancé, whose support and affection have been the foundation of my path. Your belief in me has inspired my commitment to achieving this goal.

To my doctors and instructors, whose enthusiasm for knowledge fueled mine. Your mentoring has transformed not only my academic career, but also my future goals.

For my friends, who supported me through my struggles and celebrated each victory. Your companionship made the experience unforgettable, and I dedicate this degree to our adventures together.

Finally, to myself the sacrifices, challenges, and unwavering pursuit of aspirations

This degree celebrates resilience, progress, and the infinite possibilities inside.

Yasmeen

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All thanks to the Dr . ... an external supervisor, and all thanks and appreciation to the doctor... an internal examiner.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

THE RELATIONSHIP BETWEEN SYMPTOMS SEVERITY, MENTAL HEALTH, LIFESTYLE FACTORS, AND GUT MICROBIOME AMONG IRRITABLE BOWEL SYNDROME PATIENTS, A CROSS-SECTIONAL STUDY, WEST BANK, PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Signature:

Date: **24/3/2025**

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Abstract

Background: Irritable bowel syndrome (IBS) is a common functional disorder primarily characterized by chronic abdominal pain. IBS is a global issue that impacts the quality of life for patients. Its pathophysiology involves an interaction of psychosocial and environmental factors. Additionally, dysbiosis of gut microbiota and the gut-brain axis affect patients' mental health and influence IBS symptoms. Therefore, the study's objective is to determine the relationship between the symptoms of IBS patients and their mental health, lifestyle, certain types of diet, and gut microbiota diversity in comparison to healthy controls.

Methods: In this cross-sectional study, we aimed to find the relationships among IBS patients' mental health, physical activity, MEDAS adherence, and gut microbiota composition, comparing both healthy controls and the patients themselves according to symptom severity. A total of 237 IBS patients and 30 healthy control participants participated in this study. All participants completed a questionnaire to evaluate the factor that was aimed to study. For gut microbiota analysis, stool samples were collected for DNA extraction using semi-quantitative PCR to quantify selected bacterial species. Statistical analyses were performed to evaluate the relationship between all factors.

Results: Microbiota diversity analysis using alpha and beta diversity showed significant variance between healthy controls and IBS patients, evaluated using

Bray-Curtis dissimilarity with a value of 0.88. Mental health distress, physical activity, sleep problems, and smoking had significant relationships among them, while no significant relationship was detected between BMI and MEDAS diet adherence. When using the Shannon index among patients with varying severity of symptoms, different microbiota diversity was observed, showing significant relationships with smoking, sleep problems, and mental health distress. However, no variations were shown between BMI, and MEDAS diet adherence.

Conclusion: This study demonstrates differences in gut microbiota diversity between IBS patients and healthy controls, and among patient groups with varying symptom severity. There is a significant relationship involving mental health distress, smoking, and sleep problems for both groups. These findings emphasize the importance of managing IBS patients based on their lifestyle and mental health status alongside medications.

Keywords: IBS, MEDAS, gut-brain axis, microbiota, symptom severity.

Chapter One

Introduction

1.1 Background

Irritable bowel syndrome (IBS) is one of the most common disorders of gut-brain interaction (DGBI) in the worldwide[3]. It is a chronic functional gastrointestinal disorder described by abdominal pain associated with irregular defecation or a change in bowel habits[4]. There are four types of bowel pattern subtypes in IBS: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), mixed IBS (IBS-M), and alternating IBS (IBS-A) [5]. Individuals who don’t suffer from symptoms of either diarrhea or constipation are classified as having unsubtyped IBS (IBS-U) [5].

Medical professionals use the Rome IV criteria for diagnosing functional gastrointestinal disorders like IBS [6]. These criteria are based on patients with symptoms, including relief with defecation, which is linked with a change in stool frequency, and the stool's appearance as estimated in the Bristol stool chart [7]. Also, they may suffer from bloating, cramps, flatulence, fecal urgency, sensation of incomplete evacuation, and relief of pain or discomfort after defecation [8]. These symptoms can vary depending on age and sex [9].

IBS prevalence can vary from one country to another, however, in the overall population it is estimated that IBS disorder affects around 7% to 15% of the population. Also, the prevalence of IBS tends to be in women more than in men, with nearly twice the incidence when compared with men [6]. Around 2.8% to 25.7% of the prevalence of IBS was in Asian children [10]. This broad range indicates that IBS is one of the serious health concerns in Asian countries [10].

A recent study found that the onset of IBS in younger age groups was linked with urogenital symptoms and infections, whereas in older age, it was associated with psychosocial health and musculoskeletal problems [11]. Other factors associated with IBS in older age include life stress, and recurrent healthcare utilization like primary care providers, gastroenterologists, and emergency departments. The nature and intensity of pain in older individuals have some differentiation, while some suffer from intermittent discomfort, others have constant and severe pain, and sleep disorders like insomnia or disrupted sleep patterns. These sleep disturbances may happen due to abdominal pain, fecal urgency, or because of anxiety [12].

Also, many of research documented that IBS can have an important effect on a person's mental health [13]. One-third of IBS patients have several psychiatric symptoms and emotional disorders, like depression and anxiety, these psychological effects can lead to reduced patients' physical activity. Emotional distress may decrease energy and motivation, that leads a negative effect on individuals to begin and continue their exercise routines [13].

For 12 years a cohort study was done on 5574 participants who have IBS [14]. The research emphasizes that to decrease the effect of low-risk sleep behaviors and increasing the higher healthy sleep score, lead to decreased severity of symptoms in IBS patients (Hazard Ratio [HR] 0.81, 95% Confidence Interval [CI] 0.79–0.83). Moreover, this research shows that from 29.4% to 32.4% of this decrease in these risks may be because of the enhanced mental health of these patients. Also, this study shows that the higher levels of psychological distress (HR 1.16, 95% CI 1.14–1.17) and neuroticism (HR 1.11, 95% CI 1.10–1.12) were linked to developing IBS symptoms positively. Healthy sleep was linked with a percentage of 8.3% to 9.7% to decrease psychiatric symptoms and therefore reduce the incidence of IBS [14]. Additionally, participants who have the lowest scores of healthy sleep and the highest scores in mental health, have a higher genetic readiness for IBS, and so the highest risk of developing IBS disorder [14].

The Mediterranean diet, one of the diets that have a lot of health advantages, also been shown to be a good chance for managing IBS disorder. In this dietary pattern vegetables and fresh fruits are prime concerns, as well as whole grains, proteins, and healthy fats, from nuts and olive oil [15]. This dietary pattern provides the body with high fiber, which leads to regulated bowel movements and relieves some of IBS symptoms, particularly constipation. Additionally, the Mediterranean diet encourages the intake of different types of food, as well as some fermented foods, such as yogurt, which can help with gut health and alleviate discomfort related to IBS [16]. Moreover, the diet helps to reduce red meat and processed foods and may help decrease the intake of triggers which can worsen some of IBS symptoms [17]. Although the response to dietary changes may alter from one individual to another, using a Mediterranean diet can be one of the strategies for those who seek nutritional approaches to eliminate their IBS symptoms and enjoy a large range of delicious and nutritious diets [12].

In a study done on IBS patients, it was shown that a higher consumption of fruits, vegetables, sugar, and butter was linked to enhanced severity of IBS symptoms [18]. Through multivariate analysis, several foods in the Mediterranean diet were associated with reduced IBS symptoms. Additionally, greater adherence to a modified Mediterranean diet correlated with a lower presence of potentially harmful bacteria like *Faecalibacterium*, *Streptococcus*, and *Escherichia coli*, as well as higher levels of some beneficial bacteria like *Akkermansia* and *Bifidobacterium* [19].

A diet that restricts fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) is nowadays recommended to manage the symptoms of IBS [20]. FODMAPs are a group of short-chain carbohydrates that aren't effectively absorbed from our bodies, resulting in increased content of water and improved movement of intestines [21]. In the colon, these carbohydrates are fermented rapidly, leading to the production of gases, and enhancing bloating and distention in the intestines [22].

Studies have shown that in individuals who follow a low FODMAP diet, the levels and the abundance of *Bifidobacterium* in their gut will decrease [21]. Additionally, there is a depletion in the overall population of bacteria in the gut of individuals with a low FODMAP diet [23]. Probiotics and prebiotics have considerable attention as therapeutic options for re-establishing a beneficial and healthy gut microbiome[24]. Probiotics are live microorganisms, and taking it in adequate amounts, can reflect positively on individual health [25]. These beneficial bacteria, such as *Akkermansia*, *Lactobacillus*, and *Bifidobacterium* species, can help to enhance the diversity of gut microbiota and improve digestive health [12], and relieve symptoms like abdominal pain, bloating, and irregular bowel movements [26].

The human intestine includes a huge number of microbes, which together make a complex ecosystem that is responsible for normal physiological processes [27]. In healthy individuals, the function of the gut microbiota is to absorb nutrients from the diet, support immune responses, and maintain homeostasis in the gut [28]. When bacteria make fermentation to the dietary fibers and resistant starch, Short-chain fatty acids (SCFAs) are produced in the colon [29]. Many of research has demonstrated that

SCFAs have an important role in the regulation of neuro-immunoendocrine pathways [28]. In the gut, the production of SCFAs per day is dependent on many factors, like the amount of fiber in the diet, the composition of microbiota, and transit time [30].

The importance of the gut microbiota in depression and anxiety is evidenced by studies indicating that there are lower levels of SCFAs in non-human models of depression, and in fecal IBS concentrations when compared to healthy controls [31]. Moreover, recent research has explained that the influence of the microbiota not only on the gastrointestinal (GI) tract but also plays an important role in the bidirectional communication between the GI tract and the central nervous system (CNS) [32]. There are several pathways to how the gut microbiota may influence brain function. Microorganisms may involve their function in CNS processes bidirectionally through the vagus nerve, immune system modulation, the hypothalamic-pituitary-adrenal (HPA) axis, and the metabolism of tryptophan [33]. Additionally, the microbiota can synthesize neurotransmitters and produce metabolites, including SCFAs, which possess neuroactive properties [32]. Acetate, propionate, and butyrate are the primary SCFAs produced in the colon via bacterial fermentation of dietary fibers and resistant starch [30]. In addition to their well-known role in energy supply, trophic factors, and the regulation of T regulatory cell colonies, there is lot of evidence that SCFAs do important physiological effects on numerous organs, particularly the brain [34]. There is other evidence showing that there is a connection between gut microbiota and IBS and patients' lifestyles[35]. Similar to smoking, which can alter the gut microbiota and influence mucosa-associated microbiota (MAM) by increasing *Firmicutes* and decreasing *Bacteroidetes* in smokers' gut [23]. Nicotine slows the emptying of gastric so postprandial distress symptoms can occur -bloating and nausea-, also smoking slows the time of mouth-cecum transit. Nicotine decreases the secretion of pancreatic juice, which may lead to diarrhea. Diarrhea can also happen in smoker IBS patients because smoke leads to delayed small intestinal transit, which leads to increased pathogenic bacteria , and encourage diarrhea [36].

To summarize, IBS has many challenges, like the high prevalence, absence of biological markers and organic lesions, the drugs that are ineffective and not suitable for all patients. Also, regardless of the severity of the symptoms they are uncomfortable to all of the patients. Moreover, there is many factors contribute to trigger these symptoms, for example: the patient's lifestyle, mental health, dietary pattern, BMI, and their interaction with gut microbiome. Therefore, there's a need for a research which links all these challenges together, to have a better understanding of this disorder and development of more efficient treatments.

1.2 Function of microbiota

1.2.1 Gut microbiota and polysaccharides fermentation

Microorganisms in the human gastrointestinal tract play an important function in human health and disease [37]. There are lots of essential functions done by the microbiome in the GI tract, These include fermentation of the indigestible food components to become absorbable metabolites [37]. This starts when we eat products that contain polysaccharides that can't be completely digested in the digestive system, like cellulose, hemicellulose, gums, pectin, lignin, β-glucan and mucilage. The benefit of these polysaccharides dependent on their ability to fermented, also the physiochemical properties [34].

In the human bowel, two major phyla dominate the microbiome kingdom, which includes the *Bacteroidetes* – the Gram-negative bacteria- and *Firmicutes* (like *Butyricicoccus*, *Lactobacillus*, *Streptococcus*, and *Faecalibacterium*) which are the Gram-positive one [38].

Bacteroidetes can degrade polysaccharides more than *Firmicutes* do, it can degrade only some selected polysaccharides, while *Bacteroidetes* can metabolize a broad range of them [39]. Living conditions and our daily diets influence the proportion of Firmicutes and Bacteroidetes in our intestines, so we have big variations between humans [40]. These GI bacteria are used to break polysaccharides by the carbohydrate-active enzymes (CAZymes) [41]. For example, (137.1 CAZymes) are the enzyme that codes by The *Bacteroidetes* per genome on average, while (39.6 CAZymes) are in *Firmicutes* [41]. When polysaccharides transfer to the cell surface of the bacteria, hydrolysis happens, which means this type of bacteria has signal sequences to export the polysaccharide lyases and glycoside hydrolases to the cell surface[39]. In *Bacteroidetes* they have near to 81% of glycoside hydrolases and polysaccharide lyases which have signal sequences, while *Firmicutes* have only 19% of the signal sequences [42]. Moreover, *Bacteroides* can metabolite carbohydrates in several pathways and encode various degradative enzymes like glycoside hydrolases, carbohydrate esterases, and polysaccharide lyases, which grants them the power to metabolize carbohydrates [40].

The mechanism of polysaccharide breakdown in these types of bacteria includes three major systems: ABC-transport system, Sus-like transport system, and cellulosome-like scaffolded enzyme system [1] as illustrated in figure 1.1.

The Sus-like transport system was named after the starch utilization system (Sus) discovered [43]. Genetic clusters called the polysaccharide utilization loci (PUL) encode the enzymes in the Sus-like transport system[44]. They are important for encoding primary proteins for the capture, breakdown, and import of specific polysaccharides [45]. Nearly, about 18% of the gut *Bacteroidetes* contain a (PUL) genome, which show their ability to metabolite carbohydrate. By using, SusE, SusD, and SusF lipoproteins to turn on TonB-dependent transporter SusC to transport maltooligosaccharides, which are then released into the cell by SusG [43]. These polysaccharides by α-glucosidase and neopullulanase in the periplasm break down into glucose and maltose. After that, they move out to the cytoplasm [46].

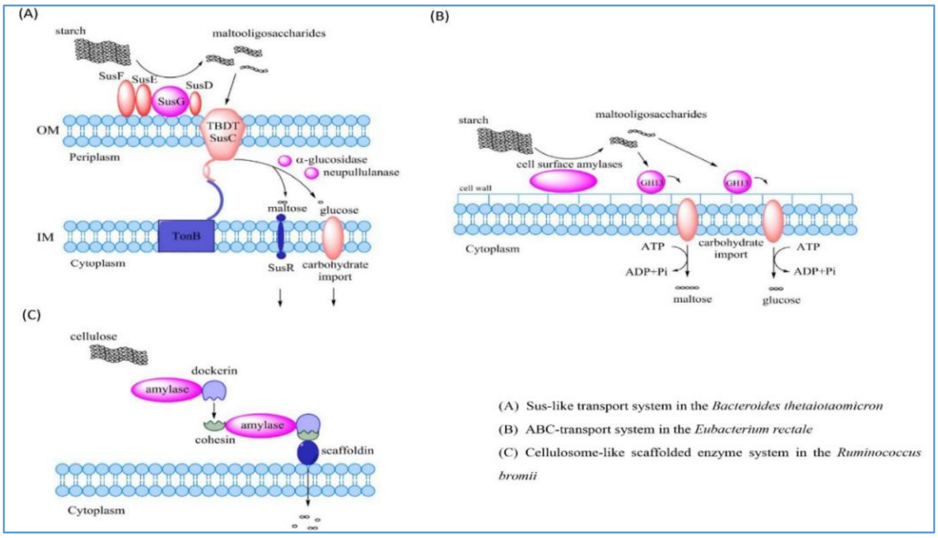
Cell surface amylases are used by *Firmicutes'* ABC transport system to break down long-chain starch into short-chain maltooligosaccharides [44].

Maltooligosaccharides with a length between three to seven maltose or glucose can be transported to the cytoplasm using two separate ABC transport solute-binding proteins [47].

Cellulosome-like scaffolded enzyme systems work on resistant starch and cellulose. Multi-enzyme complexes called cellulosomes, bring substrates and enzymes together on the surface of cells using dockerin-cohesion protein to break down celluloses, hemicelluloses, and cellulose-related polysaccharides into monosaccharides [1].

Figure 1.1

Mechanisms used by intestinal bacteria in the degradation of polysaccharides [1]



Humans rely on gut bacteria to break down their complex nutrients and metabolites, like SCFAs, important food ingredients in nature [39]. SCFAs, including propionate, acetate, and butyrate, are mainly produced by microbial fermentation in the intestines during the breakdown of undigested food fibers, peptides, and proteins [48].

Propionate and butyrate are more prevalent in the distal intestines than in the proximal intestines, whereas acetate is only released in the distal intestines [49]. Additionally, pentanoate, formate, and branched-chain fatty acids (BCFAs) are found at lower levels in the intestines of rodents and humans. These BCFAs are derived from the fermentation of branched amino acids [49].

Specific intestinal microbes from food mediate the production of SCFAs [6]. Various microbial groups can produce acetate, while propionate and butyrate are predominantly generated by specific pathways and bacterial genera [6].

Most SCFAs need specialized transporters for their uptake, with the majority requiring active transport delivered by two receptors: the monocarboxylate transporter 1 (MCT-1) and the sodium-coupled monocarboxylate transporter 1 (SMCT-1). These transporters are highly expressed in the gastrointestinal tract [40]

SCFAs can be transported from the gut into the lacteal lymphatic system and/or by hepatic portal circulation [50]. While acetate is detectable in peripheral blood, propionate, and butyrate are metabolized by hepatocytes and have concentrations in the systemic circulation [49]. Butyrate, in particular, has beneficial effects but is challenging to deliver into the colon due to its short half-life and low compliance rate with rectal administration [51]. However, targeting and delivering butyrate-producing intestinal bacteria may help solve this problem and improve overall health [51].

1.2.2 Gut microbiota and immune system

There is a strong connection between our gut microbiota and the immune system, which maintains homeostasis in the intestinal and avoids inflammation [52]. If there is an impairment in this connection, the pathogenic bacteria will increase, which leads to the destruction of the epithelial barrier and increases the infections [53].

After food is consumed, the gallbladder is stimulated, leading to the flow of primary liver-derived bile acids (BAs) to the duodenum, where they help emulsify dietary fat [52]. While most of these BAs are reabsorbed in the ileum and transported by the enterohepatic circulation back to the liver, some of them are transformed into secondary BAs by microbiota [53].

Both primary and secondary BAs interact antagonistically or agonistically with a family of nuclear (FXR) and G-protein-coupled receptors (GPRs) known as BAs-activated receptors (BARs), thereby modifying cellular signaling and immune response [54]. Recent studies have shown that secondary BAs, such as 3β-hydroxydeoxycholic acid (isoDCA), interact with the farnesoid X receptor on dendritic cells (DCs) to enhance the differentiation of regulatory T cells, offering potential for novel therapeutics [2].

BCFAs are produced from the fermentation of certain branched amino acids like leucine, valine, isoleucine, and pentanoate can enhance glycolysis and acetyl-CoA levels, and also regulate IL10 [55]. They act as agonists in eukaryotic G-protein-coupled receptors (GPRs), which are involved in various signaling pathways [56]. When propionate and acetate bind to GPR43 and GPR41 on colon cells, they activate ERK/MAPK and p38, leading to an inflammatory response [57]. Additionally, enteroendocrine cells sense SCFAs through GPR43 and GPR41, leading to the induction of certain hormones [2].

Lumen's guts are rich in SCFAs which help in reducing their inflammation, protect them from any invasion, and look after the integrity of the barrier by GPCRs or by using sodium-dependent monocarboxylate transporter-1 (SLC5A8), which entery the butyrate and move it into the colonic epithelial cells from the lumen, leading to stable hypoxia-inducible factors (HIFs), and active histone acetyltransferase, to inhibit histone deacetylases (HDACs) [55]. Which are enzymes responsible for changing the modulation of the nucleosome by cleaving from histones and several non-histone proteins its acetyl groups from acetyl-lysine, that way the gene expression is regulated [1].

Aside from it is as HDAC-inhibitory, SCFAs are capable of enhancing the activation of the mammalian target of rapamycin (mTOR), which is a master regulator of energy homeostasis and cell growth [55]. Which leads to enhanced glycolysis and increased acetyl-CoA [33]. When there is an excess of acetyl-CoA, they enters the tricarboxylic acid cycle (TCA) and are converted into citrate [1]. When we use ATP-citrate lyase (ACLY), -which is a pharmacologic inhibition -, it is an enzyme involved in converting TCA-derived citrate to become acetyl-CoA and reducing the production of IFN-γ in acetate-treated CD8+ memory T cells [58]. Acetyl-CoA in a nuclear serves as a substrate in converting acetyltransferases and facilitates conjugate acetyl groups to histones, which regulate the expression of gene and then the production of some cytokines like IFN-γ and Interleukin-10 (IL-10) [57].

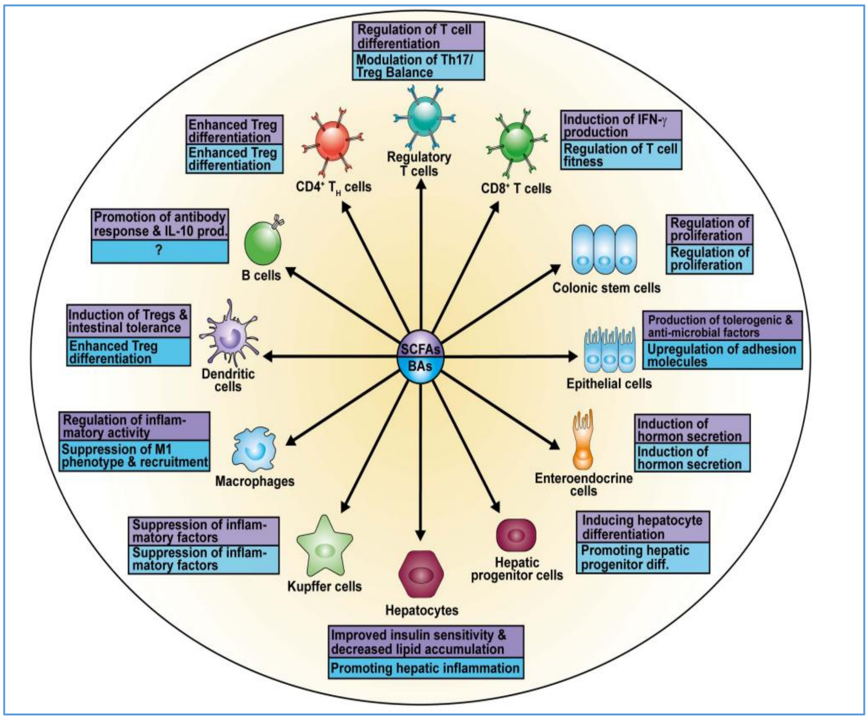
Another function of SCFAs on host immune cells is to work as anti-inflammatory, by active DCs and macrophages to express tumor necrosis factor-α (TNF-α) or regulate some cytokines expression like Interleukin-12 (IL-12), and Interleukin-6 (IL-6)[58].A recent study show the effect of SCFAs on epithelial cells. In intestinal epithelium SCFAs stimulate retinoic acid (RA) production, which is vitamin A derivate converted through aldehyde dehydrogenases, and their function is to associate with signaling and extension of peripheral T-regulatory (Tregs) in response to an immunosuppressive response [33].SCFAs can mediate immunosuppression by inducing IL-10 in various immune cells or by suppress inflammatory macrophages in the lamina propria lead to hyporesponsiveness in commensal bacteria [59]. Moreover, when butyrate treats epithelial cells, production of Interleukin-18 (IL-18)- by GPR109a-mediated mechanism enhances, which contribute to protects from colorectal carcinogenesis and help in intestinal homeostasis [60].

According to the tissue or cell SCFAs are located, they do their functions. For example, in intestinal epithelium, they maintain it is physiology by controlling the cellular turnover [61]. It also regulates the activation of immune cells and it is differentiation, which includes macrophages, T-lymphocytes, DCs, and neutrophils[59]. Another function of SCFAs is to protect cells from pathogen infection by changing the acidity of pathogens' intracellular [34].

In particular, butyrate stimulates Treg cell differentiation, Butyrate also adjusts anti-inflammatory differentiation, like forkhead box protein P3 (Foxp3), which has the function is to suppressing the inflammatory responses [60]. Butyrate controls the production of the cytokine T-helper cells and encourages the integrity of the intestinal epithelial barrier, which helps decrease exposure between luminal microbes and the mucosal immune system and avoid aberrantly response [60]. After GPR109a binds on macrophages and DCs, butyrate encourages the expression of IL-10 and limits the production of IL-6, increasing the development of Treg cells, while preventing the expansion of Th17 cells [62]. As a result, GPR109a strong anti-inflammatory pathways increase apoptosis and protect the colon from cancer by avoiding inflammation-induced colon cancer [57]. On the other hand, GPR109a can work as a tumor promoter, like in keratinocytes, when receptors express upregulated epidermoid carcinoma [59] all summarized on diagram 2. *Bifidobacterium* species produce high quantity of acetate [62]. They in neutrophils reducing NF-kB and play it is role as anti-inflammatory [62].

Figure 1.2

How SCFAs and BAs affected cell types[2]



1.3 Gut-brain axis and microbiota

There is bidirectional communication between the brain and gut, endocrine, neural, and inflammatory mechanisms also participate in this compunction [34].

During the prenatal period of each human, and the first three years of life, the Gut-brain microbiome is programmed, however, it can be modified throughout life by diet, prebiotics, probiotics, stress, and medications [63].

Mode of delivery (vaginal delivery or Cesarean section (C-section)) highly influences the infant microbiota [34]. Through vaginal delivery, the neonate gets much of the maternal vaginal microbiome, especially *Prevotella* species and *Lactobacillus,* as vagina's mother enriches these species, also neonate may get some of the fecal microbes [63].

While in C-section, neonate microbes are rich in *Staphylococcus* [64]. In a study included 2 million children, who were delivered by C-section, it was found that C-section increased the risk of having inflammatory bowel disease, asthma, deficiencies in the immune system, and many chronic diseases [65].

If an infant is a formula-fed or breastfed, the gut microbiota will be different accordingly [66]. Breastfeeding leads to better neurodevelopment and helps improve the development of the gut microbial communities [64]. Human milk oligosaccharides, which are too large to be digested and absorbed in the infant's small intestine, play a crucial role in motivating the development of the gut microbiome [65]. Formula-fed infants tend to have a more complex *Bifidobacterium* profile with more diverse communities resembling adult microbiome [66].

During lifetime, an individual's diet have an impact on their health and microbial communities [64]. Many studies have shown that temporary changes in diet can affect the gut microbiome and gene expression patterns, while evidence suggests that long-term changes may not have the same effect [64]. Additionally, there is an evidence to suggest that changes in the gut microbiome due to diet can be linked to alterations in the structure of the brain [15].

Microglia, which make up about 10% to 15% of all glial cells in the brain, are considered the macrophages of the central nervous system (CNS) and are the most numerous innate immune cells in the CNS [64]. They are an example of the interaction between the CNS and the microbiota [15]. These cells play a role in CNS development in the early stages and, when antigens are present, they carry out phagocytosis and modulate inflammation throughout human life [66]. Microglia also have a homeostatic function by continuously surveying the CNS environment and directly communicating with neurons, blood vessels, and astrocytes [33]. Microbial-derived SCFAs play a role in promoting the maturation and proper function of microglia [15].

Another example showing the interaction between CNS and microbiota is the astrocyte [67]. Which play a role in homeostasis, clearance of neurotransmitters, storage of glycogen, maintain the blood-brain barrier (BBB), support signaling of neurons, and have a function on neuroinflammation [33]. By activating aryl hydrocarbon receptors (AhRs) from Microbial metabolites, inflammation is reduced by regulating type I interferon signaling in the astrocytes [68].

Moreover, microbiota and its metabolites contribute to modifying brain processes and behaviors, including responsiveness to stress, pain modulation, and emotional behavior [69].

The gut-brain axis signaling involves two dynamic barriers: The first is the intestinal barrier, which contains tight junctions that interconnect the epithelial cells to form basal monolayers [67]. It also includes a dynamic mucus layer that excretes IgA and some antimicrobial peptides to recognize specific microbial products and activate the defense of antimicrobials and inflammation in the intestine [70]. Another function is to maintain homeostatic conditions, allowing for a constant environment in the gut lumen via immune cells in the gut and mucosa-associated lymphoid tissue from micro-fold cells [71]. Also dynamic barrier maintains a glycoprotein-rich biofilm, which microbes can degenerate when there is low dietary fiber, causing rising pathogen susceptibility [72].

The second barrier is the BBB, which contains cerebral endothelial cells interconnected via tight junctions [73]. The BBB acts as a diffusion barrier between the cerebrospinal fluid of the central nervous system and the circulatory system [72]. The gut microbiota can modulate the expression of proteins in tight junctions, affecting the permeability of this barrier [72]. There is evidence that SCFAs can influence the maintenance and development of the BBB through epigenetic modifications [74].

In cases of IBS, the pathophysiology involves changes in gut microbiota composition, a decline in the integrity of the intestinal mucosa, and low-grade inflammation [70]. These factors can trigger fluctuations in the activity of the ENS, which can affect the brain [74]. In the chronic IBS brain, efferent signals can be perceived as painful or unpleasant, leading to chronic pain or discomfort [74].

Some evidence shows that there is an increased density of gray matter in the hypothalamus, while a decrease in the prefrontal cortex in the IBS brain [72]. Another study done on patients with IBS show different brain responses in the pain matrix and retardation mode network [33]. Different studies find differences in IBS brain network alterations in different genders [75]. IBS patients illustrate an increase in the thickness of cortical in the pre-and post-central gyrus, while a decrease in the left subgenual anterior cingulate cortex (sgACC) and bilateral insula thickness when compared to healthy female controls [75].

There is an evidence that by modulating gut microbial, subcortical and cortical regions may be disrupted [70]. As well as alteration of the posterior cingulate gyrus on left dorsal part, make differences in glycine, glutamate, cysteine, and histidine alteration of the frontal gyrus to the right putamen in the right superior, change in uracil, thymidine, tryptophan, succinate and 2- deoxyuridine [76]. This interaction can be modified in IBS by perverse signaling of tryptophan, which is significant as a substrate in the synthesis of serotonin [72].

1.4 Problem statement

A recent review in 2024, showed that the global prevalence of IBS is estimated to range from 12.7% to 14.5% [33]. Due to the absence of identifiable biological markers or organic lesions, the diagnosis of IBS is typically based on clinical findings, and gastroenterologists heavily rely on these findings to confirm a diagnosis [12]. Some specialists use probiotics to treat patients with IBS [24]. However, while the efficacy of probiotics showed general improvement in IBS symptoms, others have reported worsening of certain symptoms, such as abdominal pain and bloating [77]. It is now evident that microbial factors play crucial roles in the pathophysiology of IBS [63].

Additionally, other factors such as age, gender, lifestyle, and diet have been identified as contributors to the development of IBS [9]. However, to date, there has been no comprehensive study that establishes an association between these factors, particularly in the Palestinian context. Furthermore, the impact of these factors on IBS may vary among individuals and from one country to another, making it essential to conduct a comprehensive study that gathers data from different regions to gain a more holistic understanding of IBS.

A well-designed study examining the interaction of gut microbotia, age, gender, lifestyle, and dietary habits in developing and managing IBS in Palestine. By use of a healthy sample as control. This study could provide important information about specific risk factors, which help to get more efficient treatments.

1.5 Study significance

The goal of this research is to explore in more depth the factors that lead to develop and trigger the IBS symptoms, especially in Palestinian IBS patients. By identifying these factors and understanding their impact.so that help healthcare providers to develop management strategies for their patients, through dietary modifications, adjustments to their lifestyle, and some interventions to enhance healthier gut microbiota, by comparing their gut microbiota and lifestyle with healthy control.

Additionally, the findings from this study are to participate in broader scientific knowledge about IBS disorder and may help healthcare professionals worldwide to develop their personalized approaches to manage and relieve symptoms.

1.6 Study objectives

1.6.1 Main aims

To determine the relationship between symptoms severity, IBS mental health, and lifestyle factors among IBS patients.

1.6.2 Specific aims

– To determine the association between selected Gut microbiota species diversity and lifestyle factors among Palestinian IBS patients visiting GI outpatient clinics and private clinics, as compared to healthy people.
– To determine the association between selected Gut microbiota species diversity with mental health status, using the General Health Questionnaire (GHQ) among

Palestinian IBS patients visiting patients' clinics and private clinics, as compared to healthy people.

– To determine mental health status using GHQ and the factors associated with IBS symptom severity using Birmingham IBS questionnaire among Palestinian IBS patients visiting GI outpatient clinics and private clinics.

1.7 Research questions and hypothesis

1.7.1 Research questions

1. Is there a relationship between selected Gut microbiota species diversity and lifestyle factors among Palestinian IBS patients visiting GI outpatient clinics and private clinics?
2. How does mental health among Palestinian IBS patients visiting GI outpatient clinics and private clinics influence selected Gut microbiota species diversity ?
3. What is the relationship between IBS symptom severity and lifestyle factors and mental health status among Palestinian IBS patients visiting GI outpatient clinics and private clinics?

1.7.2 Hypothesis

1.7.2.1 Alternative non-directional hypothesis

1. There is a relationship between selected Gut microbiota species diversity and lifestyle factors among Palestinian IBS patients visiting GI outpatient clinics and private clinics.
2. There is a relationship between selected Gut microbiota species diversity and mental health among Palestinian IBS patients visiting GI outpatient and private clinics.
3. There is a relationship between IBS symptom severity and lifestyle factors and mental health status among Palestinian IBS patients visiting GI outpatient clinics and private clinics.

1.7.2.2 Null hypothesis

1. There is no relationship between selected Gut microbiota species diversity and lifestyle factors among Palestinian IBS patients visiting GI outpatient clinics and private clinics.

2. There is no relationship between selected Gut microbiota species diversity and mental health among Plastinian IBS patients visiting GI outpatient clinics and private clinics.
3. There is no relationship between IBS symptom severity and life lifestyle factors and mental health status among Plastinian IBS patients visiting GI outpatient clinics and private clinics.

1.8 Literature review

In 1951, the first clinical trial on IBS patients aimed to recognize emotions life style as a stimulus to colonic function. It divided these triggers into diet, allergic reactivity, physical activity, and infection [78]. The study suggested that IBS represents a bodily change linked with emotional alternation [78]. In 1999 they succeeded in developing the Rome criteria for IBS based on clinical symptoms like the appearance of abdominal pain associated with pain and alternation in bowel habit, rather than examining a biological marker to diagnose this disorder [79]. The prevalence of this disorder was near 10–15% of the general population, and up to 50% of them, their symptoms worsened over time [79]. They define that symptoms vary from one patient to another according to the patients' predominant bowel habits into constipation-predominant IBS, diarrhea-predominant IBS, and IBS with alternating bowel movement [80]. The prevalence of these symptoms can vary according to the region in which the study was conducted. In the United States, for example, the prevalence of IBS in 2001 was 11.6%, about 3.6% of them suffer from constipation, while 1.8% have diarrhea.

These patients cost the U.S. annually about 2.4 million for only visiting the physician, and also about 2.2 million for their drug prescriptions [80]. One of the studies which was done on 877 IBS patients to assess their quality of life and compare them with healthy control, found that IBS patients have impaired in their quality of life because of the pain, fatigue, less in energy and their ability to do their physical activity [81]. Then the studies focus on gut -brain axis, as it plays a critical role in alternating gut function in health and disease [82]. This axis is bidirectional, as signal from the brain to the gut confirm the digestive procedure based on the overall state of the body (relaxation or stress, wakefulness or sleep), while the gut signal to the brain controls reflex action and impact on mood [82]. One of the studies which was done to clarify how gut brain axis contributes in IBS pathophysiology, was done on 14 IBS patients and 14 healthy control to assess the serotonin level on both –as this hormone plays a critical role in maintain gut motility , secretion , and influence mood [83].

They found that there is a depletion in serotonin in IBS patients when compared with healthy ones, which affects their mood [83]. Another study was done to study corticotropin-releasing hormone (CRH), which also plays a role in mediating stress. The study was done on 10 healthy control and 10 IBS patients and found that this hormone enhances the motility of the gut, sensation in visceral, and bad mood following gut activation, without affecting the hypothalamus–pituitary–adrenal axis in IBS patients [84]. Studies also show that chronic stress plays a critical role in improving IBS disorder and enhancing its symptoms. This chronic stress can amplify the response of neuroendocrine and pain modification, which over a long time leads to changes in bowel function, and impaired hemostasis [85].

One of the studies that demonstrate that, was done on 770 IBS patients, which all fill a questionnaire and assess their mental health. The outcome of them was feeling hopeless, nervous, and anxious. Also, they tire easily and suffer from low energy and sleep difficulty [85]. These studies show scientists that IBS patients can suffer from sleep apnea and insomnia. A study was done to show how psychological factors like sleep can impact the severity of IBS symptoms. They illustrate that IBS patients with sleep problems had an increase in their abdominal pain and bloating [86]. Moreover, they found that also smoking and physical activity play a role in IBS disorder. Until 2007, scientists showed that cigarette smoke contained nicotine and toxic components which can increase the permeability of the gut, and that leads to making the intestine more sensitive, and increasing stress. They also found that smoking disrupts the neurotransmitter system, which responds to regulating the function of the gut, which enhances abdominal pain and bloating. A study was done in 2007, there were 720 participants, and 221 didn't smoke [87].

A serum sample was collected to assess their immunoglobulin. Smoking participants had low levels of IgG. Their IgG serum level was 995 mg/dl, while for non-smokers was 1110 mg/dl, with a (p-value < 0·001), which leads to a decrease in IL-6 [87]. Another study included 104 men and 97 women, to assess how smoking can affect gastric secretion. They found that the basal and stimulated gastric acid were higher in smoker one compared to the healthy situation [88]. The gastric acid secretion was associated with the duration of participants' smoking habit, and the number of cigarette smoke. In this study show that in some country like in Korea, the prevalence of IBS patients smoker less than healthy one do, while in U.S., Iran, and Turkey there was no relationship between IBS and smoking [88]. Studies between 2008 and 2011 show that physical activity can relieve symptoms of IBS, in particular bloating, constipation, and abdominal pain. Regular physical activity can help decrease stress and regulate the sympathetic nervous system, reducing patients' hypersensitivity [89]. About 56 patients participated in a study to know which type of IBS (IBS-C, IBS-D, and IBS-M), physical activity can eliminate its symptoms. All of them underwent regular physical activity for 12 weeks and then found that there was no significant difference between them, but IBS-C patients got notable improvement in their symptoms [90].

A randomized control trial was done on 102 patients, in two groups, one underwent regular physical activity, while the other group kept their usual life. They found that physical activity can help to reduce the severity of symptoms, and it can be a treatment in case of mild symptoms, with some dietary management [91]. Lots of studies emphasize their studies on how diet can modify IBS symptoms, and which foods trigger their gut. About 46 patients were fill several questioners to estimate their IBS symptoms using Birmingham IBS questioner, quality of life using IBS Quality of life questioner, and dietary habit using the MoBa Food Frequency questioner [92].

After that, they have guidance about their diet for about 4 months and refill the same questioners. They found that the score of Birmingham IBS questioner was decrease, which mean their severity decrease, with a p-value= .001, also the score of IBS Quality of life questioner was increase, with a p-value=.003 [92]. In these 4 month, there was no difference in the uptake of their calories, but there was notable decrease in the consumption of some fruits and vegetables, which is FODMAPs diet [92].

A randomized control trail was done to compare between two groups of IBS patients with different measure of fermentable carbohydrate, one with 9 gram while the other 50 gram for four day, and they showed decrease in IBS symptoms in the first group [93]. Another randomize trail was done on IBS patients, and undergo to a diet with low fermentable carbohydrate, and that lead to decrease in their symptoms when compared with normal Australian diet, and have more comfort with their stool consistency, especially IBS-D patients, which also have change in their stool frequency [93]. A review was conducted, shows that limiting the intake of fermentable carbohydrates harms prebiotic supplementation in patients suffering from intestinal disease.

This restriction may reduce *Bifidobacteria* abundance and may lead to dysbiosis [93]. While most of studies have the opposite result of this study and found that diversity and abundance of gut microbiota are not affected by these restrictions, unfortunately, the data in this area are too limited [94]. Another type of diet was shown the ability to reduce the severity of IBS symptoms, which is the MEDAS diet. Scientists suggested that the MEDAS diet contains anti-inflammatory properties because this diet is rich in monounsaturated fats like olive oil, and omega-3 fatty acids in seafood which can help to reduce gases, and inflammation of the gut [95].

They also suggest that this diet balances fiber, particularly the soluble fiber of fruits, vegetables, and whole grains, which helps to improve stool consistency. Using Rome III criteria, 1134 IBS patients participated in this study to investigate how adherence to the MEDAS diet was linked with IBS disorder [16]. All of them fill Short Mediterranean Diet Questionnaire. They subgroup them into categories according to age: 17-24 years, 25-35 years,35-49 years, 50-64 years, and above 65 years. Patients aged 17-24 and 25-35 got the low adherence diet. Their score was .45±.20 with a $p < .05$.

Which means the adherence leaned toward enhancement because of increased patient awareness, as alternation in their lifestyle and dietary habits overtime [16]. Another study was done to determine the relationship between MEDAS diet adherence and gut microbiota composition. About 120 IBS patients participated in this study, and their adherence to the MEDAS diet was assessed using MedDietScore, then the patients were divided into groups according to their adherence level [96]. While gut microbiota composition was evaluated using quantitative PCR. Gas chromatography was used to assess SCFAs in the sample stool [96].

They found that a low abundance of *Escherichia* coli was seen in patients with high adherence to the MEDAS diet with a p -p-value =.022, while a high ratio of *Bifidobacteria* to *E. coli*, with a p-value= .025, and increased level of *candida albicans* with a p-value=.039. Also, they have a larger molar ratio of acetate [96]. In this study, they also found that junk food decreases the abundance of *Lactobacillus* and bacteria producing butyrate [96]. Lots of studies was done to assess the effect of IBS disorder on gut microbotia depend on gut-brain axis [97]. In normal situation, the two major phyla are *Bacteroidetes* like (*Bacteroides*), and Firmicuts like (*Bifidobacterium*, *Butyricicoccus, Faecalibacterium*, and *Roseburia*) play a key role in health of gut [97]. Studies show that Firmicuts create SCFAs, which affect the response of brain in stress and pain. While *Bacteroidetes* fragmented carbohydrate and play a role in modulating the mood [98]. One study was suggested that tryptophan and the 5-HT system play a crucial role in the brain-gut-microbiome axis, with various delicate and essential functions [99].

Most attention has been focused on treatment strategies targeting direct manipulation of the 5-HT system. However, these treatments are only partially effective for some patients with stress-related disorders of the brain-gut axis due to the diverse roles of 5-HT and the heterogeneity of these disorders [99]. The proper functioning of the brain-gut-microbiome axis, which affects an individual's quality of life, relies on appropriate 5-HT signaling throughout life to support the development, function, and maintenance of this axis. Additionally, various preclinical experimental strategies have shown that both tryptophan availability and 5-HT signaling are significantly influenced by the composition of the gut microbiota [99]. In a different randomized, controlled trial involving patients with IBS and diverse bowel habits, the intake of *Bifidobacterium infantis* more than 8 weeks was linked to symptomatic improvement as well as the normalization of the interleukin 10: interleukin 12 ratio in plasma [100]. In a review, which included 24 studies comparing gut microbiota between IBS patients and healthy control, was shown that in most studies *Enterobacteriaceae* increased in IBS when compared with control patients, and its alternation the gut environment by enhancing the gut motility [101].

Also, *Lactobacillus* was increased, and it is a response to enhanced bloating and abdominal pain[101]. *Bacteroides* was also in most studies was an increase, particularly in IBS-D and that may be due to some type of *Bacteroides* like *Bacteroides fragilis* producing a toxin that can impair gut motility and enhance diarrhea. While *Bifidobacterium* was decreased, which is a response in eliminating depression in a most study of this review [101]. One study was done on 2020, investigate the mental health of IBS and their gut microbotia [102]. They show that alpha diversity was low in IBS patients, associated with increase of mental health distress, and have a high diversity of

Bacteroides and low abundance of *Lachnospiraceae*, when compare with control one. Also they show that there is differences in beta diversity between IBS patients with depression and IBS patients with good mental health [102]. Other study was done to link gut microbiota and sleep disturbance in IBS, they show that *Faecalibacterium*, *Akkermansia* associated with good sleep quality and duration [103]. A review was done in 2024 to investigate complex factor can enhance IBS together. They show the relationship between gut microbiota, mental health, and physical activity in IBS patients.

They demonstrate that *Lactobacillus* increase in regular physical activity, so lactose can break down, and maintain the acidity of the gut, and enhance the diversity of gut microbiota. Also *Akkermansia* was increased with regular physical activity, which lead to reduce the inflammation of gut and maintain mental health [65]. In 2025, scientists use *Bifidobacterium*, *Lactobacillus* strain, and other bacteria strains as probiotic, in addition to some dietary modification. But till now these treatment not affective for all patients, and they emphasize that treating these patients should done based on the combination of all factor that may influence their disorder, like lifestyle, their education, and their mental health, in addition to their severity of symptoms [104].

Chapter Two

Methodology

2.1 Study design

The current cross-sectional study was conducted to include IBS patients and healthy individuals in An-Najah National University Hospital outpatients and private clinics, in Nablus, Palestine.

The collected data included: socio-demographic: age. Gender, marital status, working status, and economic status. Medical history: chronic diseases. Lifestyle: self-reported smoking practices, physical activity, mental health, and sleeping patterns. Nutrition-related data: weight, height. Dietary pattern. IBS-related data: date of diagnosis, and Birmingham IBS questionnaire. Questionnaire regarding. The General Health Questionnaire (GHQ-12), International Physical Activity Questionnaire (IPAQ), and Mediterranean Diet Adherence Screener (MEDAS).

2.2 Study population and setting

A study of the human population was categorized into two groups.

– IBS adult patients who seek medical care at the outpatient departments of An-Najah National University Hospital and private clinics in Nablus, Palestine.
– The control group is healthy individuals who don't have any complications making them excluded from the research, and they are 30 individuals and are of the same age and gender as the patients.

2.2.1 Inclusion criteria

For IBS patients: all IBS patients in An-Najah National University Hospital outpatients a private clinic. Aged 18 years and above.

For control: any healthy individual in An-Najah National University Hospital outpatient and private clinic his age and gender is the same for IBS patients.

2.2.2 Exclusion criteria

While Patients will be excluded if they have any of these following conditions:

A. Patients diagnosed with other chronic GI disorders that may affect the gut microbiome i.e. IBD, celiac patients.

B. Patients have therapeutic interventions which manipulate gut microbiota like antibiotics, probiotics, prebiotics, and fecal microbial transplantation.

C. Patients with recent GI major surgery (less than six month)

2.3 Study time frame

After receiving approval from the Institutional Review Board (IRB) of An-Najah National University in 5 October 2023 (Appendix C), sampling was conducted from December 2023 to May 2024 at An-Najah National University Hospital in Nablus, Palestine. This choice is informed by the hospital's academic standing, encompassing an endoscopy unit and a GI department, which are anticipated to facilitate the accomplishment of the research objectives significantly.

Also, data analysis, reviewing of literature, and writing the study were continued until December 2024.

2.4 Study variable

2.4.1 Dependent variables

IBS quality of life

2.4.2 Independent variables

1. Gut microbiome.
2. Lifestyle: smoking, sleep, and physical activity.
3. Mental health.
4. Nutritional status assessment: anthropometry, dietary pattern.
5. Weight status BMI.
6. Background variable: Sociodemographic.

2.5 Study sample

Sample size was calculated using open epi software for sample size calculation, considering. The IBS severity symptoms is the primary outcome .The alpha level was considered 5%, power 80. Results from previous study showed that the mean of IBS severity was 24 and the standard deviation was 5 [105]. The estimated effect size was considered 10% from the mean. The required sample size is 198 participants, considering 15% drop out, the required sample size is 230 Sampling frame: Convenience sampling will be employed for participant selection, wherein any patients visiting the hospital's outpatient departments and private clinics will be invited to take part in the study. The approach chosen for this purpose is systematic convenience sampling, owing to its cost-effectiveness, efficiency, and straightforward implementation. As such, this sampling technique is deemed favorable for the study's requirements. For the gut microbiome study, a sub-sample (30 patients) was selected from the total participants, also (30 healthy individuals) were selected as the same gender and age for control [106].

2.6 Measurement tools and study instruments

2.6.1 IBS Symptoms

Birmingham IBS questionnaire was used to verify the IBS patients. We used the gastrointestinal symptom rating scale [117] for the symptoms. The Birmingham IBS symptom score uses a self-completed questionnaire that includes 14 questions. These questions evaluate the frequency of IBS-related symptoms and use the Rome II criteria for functional intestinal disorders. Each of these questions follows a standardized response scale, with a 6-point Likert scale. The scale starts from 0, which means the person never suffers from this symptom to 5, which means the patient suffers from the symptom all the time – as shown in Table 3.3 chapter 3 -. This scoring system gives a quantitative measure of the frequency and severity of IBS symptoms reported by the persons [107]. The validity of the Birmingham IBS symptom score was assessed by comparing the scores with the scores of IBS-specific quality of life (QOL). The analysis gives a negative association between each symptom dimension and the validated IBS-QoL dimensions. That means if the symptoms increased, the quality of life decreased. Spearman's correlation coefficients were calculated to measure the power of these associations [108]. This tool is widely accepted, universally reported, has content validity by experts, and is reliable with Cronbach's alpha of .773.

2.6.2 Gut Microbiota

From approximately 60 patients, stool samples from the first defecation of the day were collected in clean, dry screw-top containers and transferred to the laboratory within 2 hours. The samples were then frozen at -70 °C until further analysis. Fecal DNA was extracted from a stool sample using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's instructions. First, nearly 200 mg of stool was placed in a tube containing buffer and centrifuged. After that, the tube was incubated and then vortexed vigorously. Following this, the supernatant was collected in a new tube containing buffer and Proteinase K, and the mixture was vortexed and incubated before adding ethanol. The next step was to load the lysate onto a QIAamp spin column, wash it with buffer, and elute the DNA. The final step in the DNA extraction process involved centrifuging the sample and transferring the DNA into a new tube.

To amplify the 16S ribosomal DNA (rDNA), a set of primers targeting the major microbiota groups was used. All polymerase chain reaction (PCR) reactions were performed in 30 μl reaction volumes using semi-quantitative PCR[109] . The reaction mixture consisted of 15 μl PCR Master Mix, 0.2 μM of forward and reverse primers, and approximately 10 ng of template DNA. The thermal cycling protocol included an initial denaturation step at 98°C for 1 minute, followed by 30 cycles of denaturation for 10 seconds at 98°C, annealing for 30 seconds at 50°C, and elongation for 60 seconds at 72°C. A final extension step was carried out for 5 minutes at 72°C. Following PCR amplification, the mixture of PCR products was subjected to purification using a DNA purification kit.

To measure the diversity of these bacteria, there is two main methods to assess the diversity in the gut, to have a clear indication about the health of individuals' gut, have well understand about if there is any dysbiosis which contribute to disease, and provide insight into the richness and compositional between the sample measured. One of them is beta diversity, which used to evaluate the difference in community composition between samples. Bray-Curtis dissimilarity was used in this study, because of its sensitivity to abundance data for selected gut microbiota species between 30 stool samples from healthy controls and 30 stool samples. The equation used is:

Bcij=2Cij /Si +Sj -I and j mean healthy control and IBS patients respectively -Si and Sj mean the abundance of all species in group I and j Cij the sum lesser abundance for each species found in both healthy control and IBS patients [102].

The second method which use to assess within sample microbial diversity was alpha diversity, which was used to assess the diversity within the IBS subgroups. In our study, we used Shannon index diversity as accounts both the richness and evenness. Thirty stool sample patients were divided into three groups according to the severity of their symptoms (ten samples were from patients with mild symptoms, twelve from patients with moderate symptoms, and the rest from those with severe symptoms) using the Birmingham IBS questionnaire. To assess the differences in their diversity, the Shannon index equation used as the follow:

$$H = -\sum_{i-1}^{s} Pi * lnpi, \text{ then } E_H = H/InS \qquad 1$$

-H mean diversity index, S mean species count-Pi mean proportion of S made up of its species [110].

2.6.3 Dietary pattern

A 14-point Mediterranean Diet Adherence Screener (MEDAS) questionnaire is used to approximate adherence to the Mediterranean diet [111]. The MEDAS questionnaire consists of 12 questions referring to the frequency of food consumption and the rest is regarding particular food intake habits that are characteristic of the Spanish Mediterranean diet. Each question got either 0 or 1, individuals with a score from 0 to 5 got a low adherence, while those from 6 to 9 got a moderate adherence. individual above that got a high adherence score- as shown in Appendix B - reflecting the degree of adherence to the Mediterranean diet as evaluated in the study context [111].

2.6.4 Mental health

In the 1970s Goldberg, GHQ was developed by Goldberg in the 1970s to assess an individual's mental health status. This questionnaire is designed to inquire if the respondent has recently experienced specific symptoms or behaviors related to their mental well-being. Each item on the GHQ is evaluated using a four-point scale, with response options ranging from "less than usual" to "much more than usual" [112]. For instance, when using the GHQ-12, the total score can be 36 or 12, depending on the chosen scoring method. The two most commonly employed scoring methods are the bi-modal style (0-0-1-1) and the Likert scoring style (0-1-2-3) - as shown in Appendix B- . The GHQ-12 is favored for its brevity, simplicity, and ease of completion [112].

2.6.5 Physical Activity

The International Physical Activity Questionnaire (IPAQ) is a tool used to assess an individual's level of physical activity. It employs a scoring system that categorizes respondents into three levels of physical activity: high, moderate, or low [13] – as shown in Appendix B -. High Level of Physical Activity: Those scoring High on the IPAQ engage in physical activity equivalent to approximately one hour of moderate to vigorous intensity activity per day. This includes either: Vigorous-intensity activity on at least 3 days a week, resulting in a minimum total physical activity of at least 1500 MET (Metabolic Equivalent of Task) minutes per week, or Engaging in physical activities such as walking, moderate intensity, or vigorous intensity exercises on 7 or more days a week, achieving a minimum total physical activity of at least 3000 MET minutes per week.

Moderate Level of Physical Activity: Those scoring Moderate on the IPAQ are involved in physical activity roughly equivalent to at least half an hour of moderate intensity activity on most days [13]. This includes one of the following: engaging in vigorous intensity activity or walking for at least 30 minutes on 3 or more days per week. Participating in moderate intensity activity or walking for at least 30 minutes on 5 or more days per week. Combining walking, moderate intensity, or vigorous intensity activities for at least 30 minutes on 5 or more days a week, achieving a minimum total physical activity of at least 600 MET minutes per week [13]. Low Level of Physical Activity: Scoring Low on the IPAQ indicates that the individual does not meet the criteria for either moderate or high levels of physical activity. This suggests that the individual is not engaging in a sufficient amount of physical activity based on the IPAQ's criteria [13].

2.7 Statistical Analysis

Data expressed as tables and graphs, Categorical data presented as percentages and continues data as mean ± standard deviation or median (interquartile range) depending on normality of data, between the study arms will be tested by the appropriate significance tests for primary and secondary endpoints. T-test for numerical and chi-square for categorical or nonparametric for non-normal distribution. One-Way ANOVA test is also used to determine if there are any statistically significant variations between the means of three or more independent groups. IBM SPSS statistic software version 25 will be used for data entry and testing. The significance level will be for one-sided p-value and set at $p<0.05$.

2.8 Ethical consideration

For participation in the study, all participants received oral and written informed consent (appendix B). The study carried out with the approval of An-Najah National University's Institutional Review Board (appendix A).

Chapter Three

Results

3.1 Participants' Characteristics

a. Socio-demographic characteristics

IBS patients were invited to participate in the study through an online survey, and only patients diagnosed by specialists were included. The total number of participants reached 237, with a certain number 58 male and the rest female. Thirty of these patients, agreed to provide a stool sample. To serve as controls, 30 individuals who didn't suffer from IBS or any other study-excluding condition were included. The number of female participants in the control group was 26, while the number of male participants was 4.

The study finally involved 267 subjects: 237 (88.7%) IBS patients and 30 (11.2%) controls. The mean age was 37.1±13.6 years, ranging from 18 to 65. The mean age for IBS patients was 37.1±13.4 years and for control, it was 36.7±14.8 years, $p>0.05$ using an independent sample t-test. As shown in Table 3.1 the majority of the subjects were female in IBS they were 189, also in control they were 26. There were 161 married individuals, while 76 were not married. (66.2%) of IBS patients live inside the city, while the remaining (33.8%) live outside the city. In the healthy group, all participants live inside the city.

One hundred thirty-eight IBS patients live with their husbands, 90 of the IBS patients live with their families, and only 9 live with neither a husband nor their families. Among healthy individuals, 19 live with their husbands, 14 live with their families, and only one lives with neither IBS nor their family. (35.9%) of IBS patients have a school education, while those who have higher education in healthy subjects were (76.7%). 106 of the IBS patients work, while 131 do not work. 20 healthy people do not work, while the remaining 10 do work. Most of the patients (67 of the total) have a monthly income between 1500-3000 ILS.

Table 3.1

Participants' characteristics according to groups [presented as number (%)]

Parameter	IBS patient (n=237) (%)		Control (n=30) (%)		Total (n=267)		P value
gender							
female	189	(79.7)	26	(86.7)	215	(80.5)	0.264
male	48	(20.3)	4	(13.3)	52	(19.5)	
Marital Status							
Married	161	(67.9)	18	(60)	179	(67)	0.250
Not Married	76	(32.1)	12	(40)	88	(33)	
Residence area							
Inside the city	157	66.2)(	30	(100)	187	(70)	NA
Outside the city	80	(33.8)	0	(0)	80	(30)	
Living condition							
With the husband	138	(58.2)	15	(50)	153	(57.3)	0.253
Other than that	99	(41.8)	15	(50)	114	(42.7)	
Educational level							
School Education	85	(35.9)	7	(23.3)	92	(34.5)	.122
Higher Education	152	(64.1)	23	(76.7)	175	(65.5)	
Working condition							
yes	106	(44.7)	10	(33.3)	116	(43.4)	0.161
no	131	(55.3)	20	(66.7)	151	(56.5)	
Monthly income							
Less than 1500 ILS	37	(22)	5	(26.3)	42	(22.5)	.837
Between 1500-3000 ILS	67	(39.9)	8	(42.1)	75	(40.1)	
More than3000 ILS	64	(38.1)	6	(31.6)	70	(37.4)	

* p<0.05, ** p<0.01, using Chi-square test, NA: not applicable

b. Participants lifestyle and physical characteristics

As demonstrated in Table 3.2, most of the patients are smokers with a percentage of (59.9%), while (34.6%) are not a smoker. Only (5.5%) of them were a previous smoker. In healthy control (96.7%) are smokers. Only one person is not a smoker. (37.1%) of IBS patients slept less than 6 hours or more than 8 hours daily, while (62.9%) of the patients slept between 6-8 hours daily, went to sleep at 2:27 am ±8:14 at range, and woke up at 7:22 am ±3.01.

Compared with healthy individuals, who sleep at 5:22 am ±6:47 at range and wake up at range 6:53 am ±2:05. (86.7%) of them slept between 6-8 hours, while the rest slept less than 6 or more than 8 hours daily. 106 of the IBS patients didn't sleep during the day in healthy ones, 7 didn't. 89 of the patients sometimes sleep, while only 14 in healthy do. 146 of IBS patients suffer from sleep problems, most of them suffer from Insomnia (62.6%), followed by Sleep apnea (13.2%).

When comparing the problems you suffer from during sleep time in healthy people most of them didn't have any problems (90%). When using the IPAQ score to differentiate participants' level of physical activity, in IBS patients 130 of them had in low score, while 99 in moderate score, and only 8 of them got a high score. In healthy people most of them got moderate scores -25 of them, the rest were in low scores and no one got a high score.

Table 3.2

Participants' lifestyle according to groups [presented as number (%)]

Parameter	IBS patient (n=237) (%)		Control (n=30) (%)		Total (n=267)		P value
Smoking status							
Yes	82	(34.6)	1	(96.7)	83	(31.1)	.000**[1]
No	155	(65.4)	29	(3.3)	184	(68.9)	
Duration of sleep							
Less than 6 hours or more than 8 hours daily	88	(37.1)	4	(13.3)	92	(34.5)	.006**
Between 6-8 hours daily	149	(62.9)	26	(86.7)	175	(65.5)	
sleep during the day							
Yes	42	(17.7)	9	(30)	51	(19.1)	.062
No	106	(44.7)	7	(23.3)	113	(42.3)	
Sometimes	89	(37.6)	14	(46.7)	103	(38.6)	
Suffer from sleep problems							
Yes	146	(61.6)	3	(10)	149	(55.8)	.00**[1]
No	91	(38.4)	27	(90)	118	(44.2)	
Physical activity							
low	130	(96.3)	5	(3.7)	135	(50.6)	.00**[1]
moderate	99	(79.8)	25	(20.2)	124	(46.4)	
high	8	(100)	0	(0)	8	(2.9)	
Mean±sd							
hour of going to sleep	2:27 am	8:14	5:22 am	6:47	2:46 am	8:07	0.065
hour of waking up from sleep	7:22 am	3:01	6:53 am	2:05	7:19 am	2:55	.386

* $p<0.05$, ** $p<0.01$, using Chi-square test,[1] using exact

3.1.2 IBS-related data

a. IBS symptoms

Around 58.9% of IBS patients suffer from chronic disease rather than IBS, and most of them suffer from one or more of these diseases (diabetes mellitus (DM) type 2, hypertension, asthma, rheumatoid arthritis, and migraine headaches). In Table 3.3, IBS patients answered the Birmingham IBS questionnaire. 72 of them reported experiencing pain in their abdomen most of the time, while 50 of them had this pain some of the time, and 38 of the patients had this pain all of the time .72 of the patients suffer from loose, mushy, or watery bowel motions all the time, 45 of them reported experiencing it some of the time. In contrast, 39 don’t suffer from it. (37.1%) didn't have diarrhea in the last four weeks, (26.2%) reported having diarrhea a little of the time, (16%) reported having it sometimes, (23.2%) reported having hard bowel motions most of the time, and 21.1%) reported having it a little. (20.7%) reported having it sometimes (24.1%) of the patients reported needing to strain to pass a stool motion a little of the time, while (20.7%) reported needing to strain most of the time.

A good bit of the time, 56 of the patients suffering from constipation, while 52 of them experienced it a little of the time, and 27 of them had it all of the time. 70 patients reported discomfort in their abdomen after eating most of the time. 54 of the patients suffer from this discomfort all the time, while 42 of the patients suffer from this discomfort sometimes. (22.8%) of the patients this abdominal pain prevented them from sleeping little time, while (19.4%) reported this happened sometimes and none of the time.73 of the IBS patients had little time urgency to go to the bathroom, while 54 sometimes had this urgency. More than half of patients don't suffer from mucoid stool, while only .4% all of time suffer from .

Table 3.3

IBS symptoms severity of the patients [presented as number (%)]

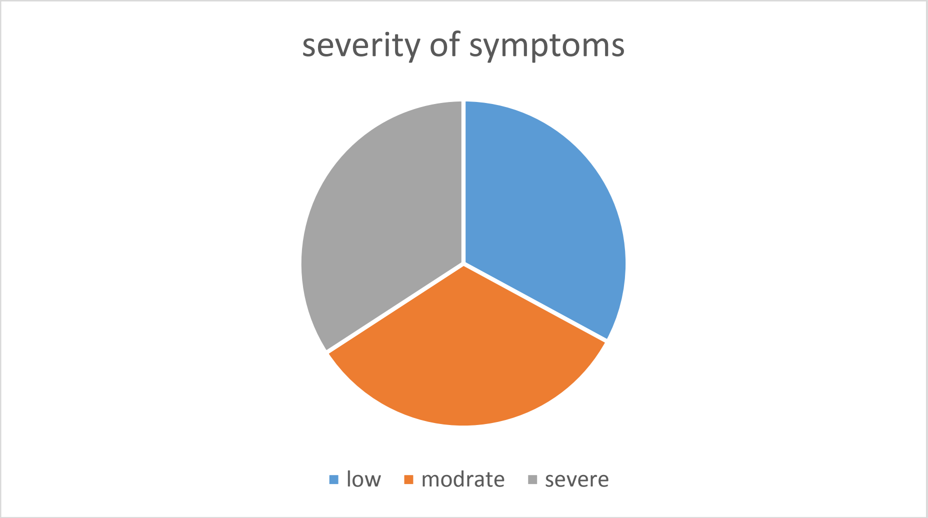
	All of the time (n)	(%)	Most of the time (n)	(%)	A good bit of the Time (n)	(%)	Some of the time (n)	(%)	A little of the time (n)	(%)	None of the time (n)	(%)
During the last 4 weeks, how often have you had discomfort or pain in your abdomen?	38	16	72	30.4	49	20.7	50	21.1	18	7.6	10	4.2
How often have you been troubled with loose, mushy or watery bowel motions during the last 4 weeks?	11	4.6	34	14.3	36	15.2	45	19	72	30.4	39	16.5
How often during the last 4 weeks have you been troubled with diarrhoea?	6	2.5	14	5.9	29	12.2	38	16	62	26.2	88	37.1
During the last 4 weeks how often have you been troubled by hard bowel motion	31	13.1	55	23.2	29	12.2	49	20.7	50	21.1	23	9.7
During the last 4 weeks how often have you felt the need to strain to pass a motion (stool)?	21	8.9	49	20.7	25	10.5	40	16.9	57	24.1	45	19
During the last 4 weeks how often have you been troubled by constipation?	27	11.4	45	19	25	10.5	56	23.6	52	21.9	32	13.5
During the last 4 weeks how often did you experience pain or discomfort in your abdomen after eating?	54	22.8	70	29.5	38	16	42	17.7	23	9.7	10	4.2
How often has you abdominal pain prevented you from sleeping, or woken you during the night during the last 4 weeks?	12	5.1	40	16.9	39	16.5	46	19.4	54	22.8	46	19.4
During the last 4 weeks how often have you leaked or soiled yourself?	15	6.3	26	11	20	8.4	32	13.5	56	23.6	88	37.1
How often during the last 4 weeks have you suffered from a feeling of urgency (feeling that you must immediately rush to the toilet to pass a stool)?	5	2.1	32	13.5	34	14.3	54	22.8	73	30.8	39	16.5
How often have you passed mucus or slime in your stools over the last 4 weeks?	1	.4	22	9.3	19	8	31	13.1	35	14.8	129	54.4

b. IBS severity

After answering the eleven questions of the Birmingham IBS questionnaire, the mean score ranges from 0 to 5. The mean scores are categorized into tertiles (low, moderate, and severe) as follows: scores from 0 to 1.69 indicate low symptom severity, scores from 1.7 to 2.49 indicate moderate symptom severity, and scores from 2.5 to 5 indicate severe symptoms. (34%) of the patients had severe symptoms, while the rest equally divide in moderate and low symptoms with a percentage 32.9% of the each - as shown in figure 3.1.

Figure 3.1

Severity of symptoms in IBS patients



3.1.3 Subjects' nutrition and mental health data between IBS and healthy participants

In Table 3.4, the comparison of mental health between IBS patients and healthy individuals is presented. According to GHO scoring, 150 out of the IBS patients were classified as normal, while the remaining 87 patients experienced mental distress. In the healthy group, 90% were classified as normal, while only 10% experienced mental distress. When considering BMI, 6 IBS patients were underweight, 90 were normal weight, and 78 were overweight, while the remainder were obese. In the healthy group, 12 were normal weight, 10 were overweight, and 10 were obese.

Regarding nutritional instruction, 56.5% of IBS patients received it at some point in their lives, while about 40% of healthy individuals did. When asked about the source of receiving their nutritional instruction, 96 of the patients were from their doctor or healthcare professional, and only 4 were healthy. 118 of the patients used online search to research nutritional information 18 of the healthy used the same method. After using the MEDAS score, 33 individuals had low adherence, 136 had moderate adherence, and 68 had high adherence. Among the healthy individuals, 4 had low adherence, 22 had moderate, and 4 had high adherence.

Table 3.4

Participants' nutrition and mental health according to groups [presented as number (%)]

Parameter	IBS patient (n)	(%)	Control (n)	(%)	Total (n)		P value
Mental health							
normal	150	(63.2)	27	(90)	177	(66.3)	.003**
Mental distress	87	(36.7)	3	(10)	90	(33.7)	
BMI							
Under weight and normal	96	(41.4)	12	(40)	108	(41.2)	.979
Over weight	78	33.6)(	10	(33.3)	88	(33.6)	
obese	58	(25)	8	(26.7)	66	(25.2)	
Received nutritional instruction							
yes	134	(56.5)	12	(40)	146	(54.7)	0.065
no	103	(43.5)	18	(60)	121	(45.3)	
source of receiving nutritional instruction							
nutrition specialist	53	(22.4)	9	(30)	62	(23.2)	.039*
Doctor or healthcare professional	96	(40.5)	5	(16.7)	101	(37.8)	
Other than that	88	(37.1)	16	(53.3)	104	(39)	
source for researching nutritional information							
nutrition specialist	55	(23.2)	8	(26.7)	63	(23.6)	.268
Doctor or healthcare professional	64	(27)	4	(13.3)	68	(25.5)	
Search online or in brochures	118	(49.8)	18	(60)	136	(50.9)	
Mediterranean Diet Adherence Screener							
Low adherence	33	(13.9)	4	(13.3)	37	(13.9)	.175
Moderate adherence	136	(57.4)	22	(73.3)	158	(59)	
High adherence	68	(28.7)	4	(13.3)	72	(27)	

* p<0.05, ** p<0.01, using Chi-square test, NA: not applicable

3.1.4 Factor associated with severity symptoms in IBS patients

a. Association between socio-demographic factor and severity symptoms among IBS patients

In Table 3.5 relationship between the severity of symptoms in IBS patients and their Socio-demographic characteristics is checked. Using the independent-samples t-test to compare the mean in gender. The female mean was 2.1±0.79, while the male mean was 1.9±0.95, with a non-significant p-value of 0.196. The same test was used for marital Status, the mean for married patients was 2.1±.79, whilst for not married was 2.2±0.83, and the p-value was not significant. The mean for those who live inside the city was 2.1±0.85, while the mean was 2.1±.77 for those who live outside the city, with a p-value of 0.666. Patients who live with their husbands get a mean of 2.1±0.82. Compared with live other than that get a mean of 2.2±.77, with no significant value. The p-value for the educational level was .098. Patients who worked got a mean of 1.99±0.84 with no significant value when compared with those who didn't work. A One-Way ANOVA test was used to compare the means for patients' monthly income. The mean for patients who got less than 1500 ILS, was1.9±0.85, and for one who got between 1500-3000 ILS, the mean was 2.1±0.87. while the mean for those who got more than 3000 ILS was 1.9±0.79, with no significant value.

Table 3.5

Socio-demographic characteristics with severity symptoms [presented as mean ± SD]

Parameter	Mean±sd		P value
gender			
Female (n=189)	2.1	.79	.196[1]
male(n=48)	1.9	.95	
Marital Status			
Married(n=161)	2.1	.81	.296[1]
Not Married(n=76)	2.2	.83	
Residence area			
Inside the city(n=157)	2.1	.85	.666[1]
Outside the city(n=80)	2.1	.77	
Living condition			
With the husband(n=138)	2.1	.82	.380[1]
Other than that(n=99)	2.2	.83	
Educational level			
School Education(n=85)	2.2	.81	.098[1]
Higher Education(n=152)	2.0	.83	
Working condition			
work(n=106)	1.99	.84	.068[1]
Not work(n=131)	2.2	.80	
Monthly income			
Less than 1500 ILS(n=37)	1.9	.85	.636[2]
Between 1500-3000 ILS(n=67)	2.1	.87	
More than3000 ILS (n=64)	1.9	.79	

[1] using independent-samples t-test test, [2] using One-Way ANOVA test

b. Association between IBS patient's lifestyle, physical factors and their severity symptoms

In Table 3.6 the relationship between the severity of symptoms in IBS patients and their lifestyle and physical characteristics is explained. A One-Way ANOVA test was used for Smoking status with a non-significant p-value of 0.401. The mean of smoker patients was 2.00± 0.82, while none was 2.07±0.81. The mean of previous smokers was 1.94±1.01. The same test was used for if patients sleep during the day, the mean for those who sleep was 2.29±0.66, while for those who did not sleep was 2.04 ±0.82. those who slept Sometimes during the day got a mean of 2.08±0.87, with no significant p-value. Patients with low physical activity got a mean of 2.1±0.83, whereas those with moderate physical activity had a mean of 2.13±0.80. The mean for patients with high physical activity was 2.3±0.75, and the p-value was not significant. The independent-samples t-test was used to compare the mean for the duration of sleep, with no significant p-value. The mean for those who sleep less than 6 hours or more than 8 hours daily was 2.19±0.77. On the other hand for those who sleep between 6-8 hour daily the mean was 2.05±0.84. The p-value for if the patients suffer from sleep problems was significant. The mean for those who have sleep problem was 2.28±.75, while for those who didn't have any problem was1.82±0.85.

Table 3.6

IBS patient's lifestyle and physical characteristics with severity symptoms [presented as mean ± SD]

Parameter	Mean±sd		P value	
Smoking status				
Yes(n=83)	1.01	.11	.000**[1]	
No(n=184)	1.16	.37		
Duration of sleep				
Less than 6 hours or more than 8 hours daily(n=88)	2.19	.77	.223[1]	
Between 6-8 hours daily(n=149)	2.05	.84		
sleep during the day				
Yes(n=42)	2.29	.66		
No(n=106)	2.04	.82	.247[2]	
Sometimes(n=89)	2.08	.87		
Suffer from sleep problems				
Yes(n=146)	2.28	.75	.000[1**]	
No (n=91)	1.82	.85		
Physical activity				
Low(n=130)	2.1	.83		
Moderate(n=99)	2.13	.80	.627[2]	
High(n=8)	2.3	.75		
hour of going to sleep	11:48 pm	1:35	10:55 pm	2:04
hour of waking up from sleep	6:53 am	2:05	7:25 am	2:23

[1] ** p<0.01 using independent-samples t-test test, [2] using One-Way ANOVA test

c. Association between IBS patients' Nutrition, mental health and their severity symptoms

IBS patient's mental health had a significant p-value by using an independent-samples t-test test, with a mean of 1.91±0.82 for normal one, and 2.43±0.73 for those who have mental distress. While the p-value for BMI was not significant using a One-Way ANOVA test, the mean for patients with under and normal weight was 2.00±0.77. Patients with overweight got a mean of 2.15±0.92, and those who were obese got a mean of 2.18±0.71. The independent-samples t-test was used to compare the mean for if the patients received nutritional instruction with a non-significant p-value. The mean for patients who received nutritional instruction was 2.13±0.77, while those who didn't receive were 2.07±0.88. A One-Way ANOVA test with no significant p-value was done for the source of receiving nutritional instruction, 2.00±0.86 was the mean for those who got it from a nutrition specialist, and the mean for those who got the nutritional instruction from doctor or healthcare professional 2.25±0.74. Patients who got nutritional instruction other than these sources got a mean of 2.01±0.86. The same test used for the source for researching nutritional information, with a p-value of 0.551, the patient who asked nutrition specialists got a mean of 2.05±0.91, those who asked a doctor or healthcare professional got a mean of 2.20±0.83. Searching online or from brochures got a mean of 2.08± 0.78. Low adherence Mediterranean diet mean was 2.30±0.85, while for those with moderate adherence was 2.09±0.81, and for high adherence the was2.05± 0.83 with a non-significant p-value using the same test.

Table 3.7

IBS patient's Nutrition and mental health with severity symptoms [presented as mean ± SD]

Paramete	Mean±sd		P value
Mental health			
Normal(n=150)	1.91	.82	.000[1**]
Mental distress(n=187)	2.43	.73	
BMI			
Underweight and normal(n=96)	2.00	.77	.314[2]
Overweight(n=78)	2.15	.92	
Obese(n=58)	2.18	.71	
Received nutritional instruction			
Yes(n=134)	2.13	.77	.583[1]
No(n=103)	2.07	.88	
source of receiving nutritional instruction			
nutrition specialist(n=53)	2.00	.86	.084[2]
Doctor or healthcare professional(n=96)	2.25	.74	
Other than that(n=88)	2.01	.86	
source for researching nutritional information			
nutrition specialist(n=55)	2.05	.91	.551[2]
Doctor or healthcare professional(n=64)	2.20	.83	
Search online or in brochures(n=118)	2.08	.78	
Mediterranean Diet Adherence Screener			
Low adherence(n=33)	2.30	.85	.352[2]
Moderate adherence(n=136)	2.09	.81	
High adherence(n=86)	2.05	.83	

[1] using independent-samples t-test test, [2] using One-Way ANOVA test

3.1 Gut microbiota-related data

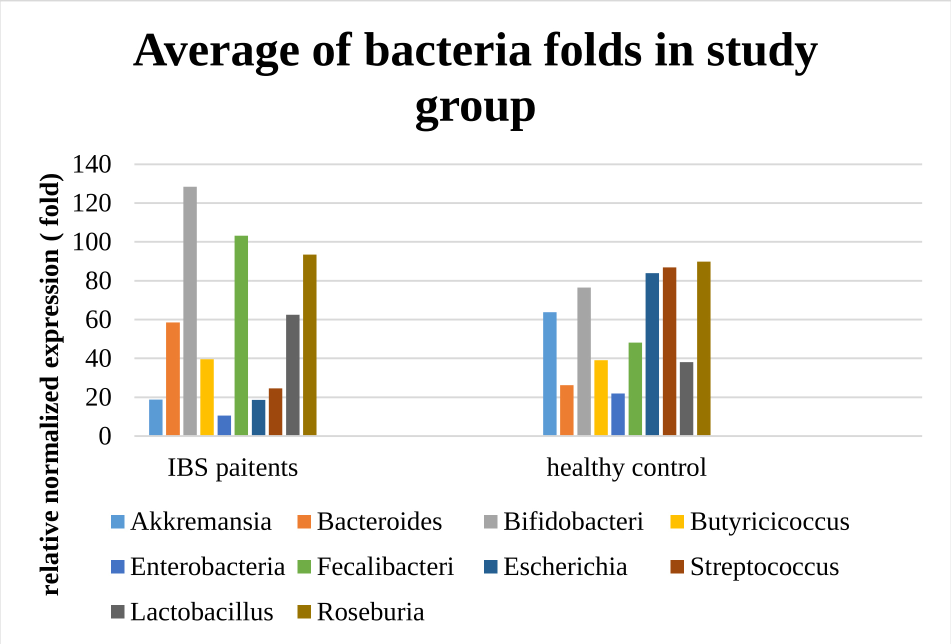
3.2.1 Gut microbiota-related data between healthy and patient participants

3.2.1.1 Average of bacteria fold in healthy and patient participants

All control participants shared their stool samples for this experiment, while only 30 patients shared their stool samples by using semi-quantitative PCR to estimate the quantity of each of these bacteria (*Bifidobacterium*, *Butyricicoccus*, *Enterobacteriaceae*, *Faecalibacterium*, *Akkermansia*, *Bacteroides*, *Escherichia*, *Streptococcus*, and *Roseburia*) for each sample. The average of *Akkermansia* in patients was 18.86 fold, while for control was 63.83 fold. For *Bacteroides* the fold in patients was 58.45, whereas in control was 26.23. In patients, the fold for *Bifidobacterium* was 128.4, while for control, it was 76.56. The average of *Butyricicoccus* in patients was 39.62, while for control, it was 39.1. For *Enterobacteriaceae*, the fold in patients was 10.51, whereas in control was 22.01. For, *Faecalibacterium* the fold in patients was 103.12, whereas in control was 48.2. The average of *Escherichia* in patients was 18.68, while for control was 83.89. In patients, the fold for *Streptococcus* was 24.61, while for control was 86.90. The average of *Lactobacillus* in patients was 62.40, while for control was 38.05. For, *Roseburia* the fold in patients was 93.54, whereas in control was 89.86. As seen in figure 3.2.

Figure 3.2

The average of bacteria folds in the study group



3.2.1.2 Association between selected Gut microbiotia species in IBS patients and healthy participants

For *Akkermansia* bacteria, 13 of IBS patients had significant fold expression, while 17 of them had no significant fold expression. For healthy control 16 of them had significant fold expression, while those who had no significant fold expression was 14, with a p-value of .303. 47.7% of IBS patients had significant *Bacteroides* fold expression, while the rest had no significant fold expression, also in healthy control 23 of them had significant fold expression with no significant p-value. 20 of the healthy controls had significant *Bifidobacterium* fold expression, while 10 of them had no significant fold expression. Of IBS patients 17 of them had significant fold expression.

Who had significant fold expression for *Butyricicoccus* bacteria in IBS patients was 21, while in healthy control was 22, with no significant p-value. For *Faecalibacterium* bacteria in IBS patients, 22 of them had significant fold expression, while 23 in healthy control .The p-value for *Escherichia* bacteria was not significant, with 11 patients had significant fold expression, and 17 healthy control. 22 of healthy control had significant *Streptococcus* fold expression. In contrast, only 8 of them had not significant fold expression. Of *Lactobacillus* bacteria in IBS patients, 19 had significant fold expression, while 21 in healthy control, 11 of patients had no significant fold expression. For *Roseburia* bacteria, 18 of IBS patients had significant fold expression, while 12 had no significant fold expression. For healthy control 19 of them had significant fold expression, while who had not significant fold expression was 11, with a p- value of .500, as demonstrated in table 3.8.

Table 3.8

Association between bacteria count and study group [presented as number (%)

Type of bacteria	IBS patients				Healthy control				P value
	Significant (n)	(%)	Not significant (n)	(%)	Significant (n)	(%)	Not significant (n)	(%)	
Akkremansia	13	44.8	17	54.8	16	55.2	14	45.2	.303
Bacteroides	21	47.7	9	56.3	23	52.2	7	43.8	.771
Bifidobacteria	17	45.9	13	54.1	20	54.1	10	43.5	.596
Butyricicoccus	21	48.8	22	51.2	22	51.2	8	47.1	.500
Enterobacteria	12	40	19	60	18	60	12	40	.196
Faecalibacterium	22	48.9	8	53.3	23	51.1	7	46.7	.500
Escherichia	11	45.8	19	52.8	13	54.2	17	47.2	.792
Streptococcus	19	46.3	11	57.9	22	53.7	8	42.1	.580
Lactobacillus	19	47.5	11	55	21	52.5	9	45	.392
Roseburia	18	48.6	12	52.2	19	51.4	11	47.8	.500

Using Chi-square test

3.2.1.3 Association between selected Gut microbiotia species and physical activity in IBS patients and healthy participants

The p-value of *Akkermansia* was significant, with a value of .019, participants with low physical activity and significant expression of *Akkermansia* was 7, while 16 of them had no significant fold expression. 21 of the participants with moderate physical activity had a significant expression of *Akkermansia*, while 15 did not. Only one participant with high physical activity had a significant expression for the same bacteria. In comparison, the p-value for *Bacteroides* was not significant. Participants who had significant expression of *Bacteroides* with low physical activity was 16, while those who had not significant was 7. Moderate physical activity participants with no significant expression were 9.

Only one participant with high physical activity had a significant expression for the same bacteria.11 of the participants with low physical activity had significant expression of *Bifidobacterium*. At the same time, 12 were not significant.25 participants with moderate physical activity had a significant expression for the same bacteria, with a significant p-value. Participants who had low physical activity and significant expression of *Butyricicoccus* were 17, while those with no significant expression were 6. Participants with moderate physical activity and a significant expression for the same bacteria were 26. There were no participants with high physical activity and significant expression. The p-value for *Enterobacteriaceae* was not significant. Participants who had low physical activity and significant expression of *Enterobacteriaceae* were 10, while those with no significant expression were 13.

Participants with moderate physical activity and a significant expression for the same bacteria were 19, while those with no significant expression were 17. Only one participant with high physical activity showed a significant expression for the same bacteria. The p-value of *Faecalibacterium* was not significant, with a value of .502, participants with low physical activity and significant expression of *Faecalibacterium* was 17, while 6 of them had no significant fold expression. 28 of the participants with moderate physical activity had a significant expression of *Faecalibacterium*, while 8 did not. Only one participant with high physical activity showed a non-significant expression for the same bacteria. Participants who showed low physical activity and significant expression of *Streptococcus* were 17, while those with no significant expression were 6. Participants with moderate physical activity and a significant expression for the same bacteria were 24, while those with no significant expression were 12. No one of the participants with high physical activity showed a significant expression for the same bacteria.13 of the participants with low physical activity had a significant expression of *Lactobacillus*.

At the same time, 10 were not significant. 27 participants with moderate physical activity had a significant expression for the same bacteria, with a non-significant p-value. Also, the p-value for *Roseburia* was not significant. Participants who had significant expression of *Roseburia* with low physical activity was 14, while those who had not significant was 9. Moderate physical activity participants with no significant expression were 13. Only one participant with high physical activity showed a non-significant expression for the same bacteria, all were explained in the table in appendix D.

3.2.1.4 Association between selected Gut microbiotia species and BMI in IBS patients and healthy participants

About 13 of the participants whose weight was low or normal, had a significant expression of *Akkermansia*. At the same time, 10 were not significant.10 participants with overweight had a significant expression for the same bacteria, while 12 of them did not.6 obese participants showed significant expression for *Akkermansia* bacteria, with a non-significant p-value. The p-value for *Bacteroides* was also not significant, with a value of .524, participants who gate low or normal weight and got a significant expression of *Bacteroides* was 17, while 6 of them had no significant fold expression. 16 of the participants with overweight had a significant expression of *Bacteroides*, while 6 did not. 11 of the obese participants had a significant expression for the same bacteria. Participants who gate under or normal weight and significant expression of *Bifidobacterium* were 15, while those with no significant expression were 8.

Participants with normal weight and a significant expression for the same bacteria were 16. Obese participants and significant expression of this bacteria were 11. About 17 of the participants whose weight was low or normal, showed a significant expression of *Butyricicoccus*. At the same time, 6 were not significant.19 participants with overweight had a significant expression for the same bacteria, while 3 of them did not.7 obese participants showed significant expression for *Butyricicoccus* bacteria, with a non-significant p-value. At the same time, the p-value for *Enterobacteriaceae* was also not significant, with a value of .418, participants who gate low or normal weight and got a significant expression of *Enterobacteriaceae* was 10, while 13 of them had no significant fold expression. 14 of the participants with overweight had a significant expression of *Enterobacteriaceae*, while 6 did not. 8 of the obese participants had a significant expression for the same bacteria. For *Escherichia* bacteria, 8 of the participants whose weight was low or normal had a significant expression of *Escherichia*.

With each other, 15 were not significant.13 participants with overweight and had a significant expression for the same bacteria, while 9 of them did not.3 obese participants showed significant expression for *Escherichia* bacteria. The p-value for *Streptococcus* bacteria was also not significant, with a value of .168, participants who gate low or normal weight and got a significant expression of *Streptococcus* was 17, while 6 of them had no significant fold expression. 16 of the participants with overweight had a significant expression of *Streptococcus*, while 6 did not. 8 of the obese participants had a significant expression for the same bacteria.7 of obese participants got significant expression for *Roseburia* bacteria, while 8 of them did not. For whose with overweight, 18 of them showed significant expression for this bacteria, and 5 got not, with a non-significant p-value. As demonstrate in appendix D.

3.2.1.5 Association between selected Gut microbotia species and mental health of participants in IBS patients and healthy participants

For *Akkermansia* bacteria, 8 of the participants with normal mental health had a significant expression of *Akkermansia*. With each other, 22 were not significant. 6 participants with mental distress had a significant expression for the same bacteria, while 9 of them did not, with a non-Significant p-value. About 36 of the participants whose normal mentally, had a significant expression of *Bacteroides*. At the same time, 9 were not significant.8 participants with mental distress had a significant expression for the same bacteria, while 12 of them did not.

The p-value for *Bifidobacterium* was significant, with a value of 0.011, participants who's normal mentally and had a significant expression of *Bifidobacterium* was 32, while 13 of them had no significant fold expression. 5 of the participants who had mental distress, had a significant expression of *Bifidobacterium*, while 10 did not. For *Butyricicoccus* bacteria, 32of the participants with normal mental health had a significant expression *Butyricicoccus*. With each other, 11 were not significant. 9 participants with mental distress had a significant expression for the same bacteria, while 6 of them did not, with a non-Significant p-value. The p-value for *Enterobacteriaceae* was significant, with a value of .036, participants who's mentally normal and had a significant expression of *Enterobacteriaceae* was 26, while 19 of them had no significant fold expression. 4 of the participants who had mental distress, had a significant expression of *Enterobacteriaceae*, while 11 did not. About 35 of the participants whose mentally normal, had a significant expression of *Faecalibacterium*. At the same time, 10 were not significant.10 participants with mental distress had a significant expression for the same bacteria, while 5 of them did not. For *Escherichia* bacteria, 20 of the participants with normal mental health had a significant expression *Escherichia*. With each other, 25 were not significant. 4 participants with mental distress had a significant expression for the same bacteria, while 11 of them did not, with a non-Significant p-value.

The p-value for *Faecalibacterium* was also not significant, with a value of 0.337, participants who's mentally normal and had a significant expression of *Faecalibacterium* was 35, while 10 of them had no significant fold expression. 10 of the participants who had mental distress, had a significant expression of *Faecalibacterium*, while 5 did not. For *Streptococcus* bacteria, 33 of the participants with normal mental health had a significant expression *Streptococcus*.

With each other, 12 were not significant. 8 participants with mental distress had a significant expression for the same bacteria, while 7 of them did not, with a non-Significant p-value. About 30 of the participants whose mentally normal, had a significant expression of *Roseburia*. At the same time, 15 were not significant.7 participants with mental distress had a significant expression for the same bacteria, while 8 of them did not, as demonstrate in appendix D.

3.2.1.6 Association between selected Gut microbotia species and MEDAS in IBS patients and healthy participants

Around 6 of the participants with low adherence to the MEDAS diet, had a significant expression of *Akkermansia*. At the same time, 5 were not significant.16 participants with moderate adherence to the MEDAS diet had a significant expression for the same bacteria, while 20 of them did not.7 participants with high adherence of the MEDAS diet showed significant expression for *Akkermansia* bacteria, with a non-significant p-value. For *Bacteroides* bacteria, 7 of the participants who had low adherence to the MEDAS diet had a significant expression of *Bacteroides*.

With each other, 4 were not significant.27 participants with moderate adherence to the MEDAS diet, had a significant expression for the same bacteria, while 9 of them did not.10 high adherence to the MEDAS diet participants showed significant expression for *Bacteroides* bacteria. The p-value for *Bifidobacterium* was not significant, with a value of 0.299, participants who got low adherence to the MEDAS diet and got a significant expression of *Bifidobacterium* was 6, while 5 of them had no significant fold expression. 22 of the participants with moderate adherence to the MEDAS diet had a significant expression of *Bifidobacterium*, while 14 did not. 9 of the participants with high adherence to the MEDAS diet, had a significant expression for the same bacteria. Around 9 of the participants who got low adherence to the MEDAS diet, had a significant expression of *Butyricicoccus*. At the same time, 2 were not significant. 25 participants with moderate adherence to the MEDAS diet had a significant expression for the same bacteria, while 11 of them did not. 9 participants with high adherence to the MEDAS diet showed significant expression for *Butyricicoccus* bacteria, with a non-significant p-value.

The p-value for *Enterobacteriaceae* also was not significant, with a value of 0.580, participants who had low adherence to the MEDAS diet and got a significant expression of *Enterobacteriaceae* was 5, while 6 of them had no significant fold expression. 19 of the participants with moderate adherence to the MEDAS diet had a significant expression of *Enterobacteriaceae*, while 17 did not. 7 of the participants with high adherence to the MEDAS diet, had a not significant expression for the same bacteria. For *Escherichia* bacteria, 3 of the participants who had low adherence to the MEDAS diet had a significant expression of *Escherichia*.

With each other, 8 were not significant.15 participants with moderate adherence to the MEDAS diet, had a significant expression for the same bacteria, while 21 of them did not. 6 high adherence to the MEDAS diet participants showed significant expression for *Escherichia* bacteria. The p-value for *Lactobacillus* was not significant, with a value of 0.308, participants who had low adherence to the MEDAS diet and got a significant expression of *Lactobacillus* was 6, while 5 of them had no significant fold expression. 25 of the participants with moderate adherence to the MEDAS diet had a significant expression of *Lactobacillus*, while 11 did not. 9 of the participants with high adherence to the MEDAS diet, had a significant expression for the same bacteria. For *Roseburia* bacteria, 6 of the participants who had low adherence to the MEDAS diet had a significant expression of *Roseburia*. With each other, 5 were not significant. 23 participants with moderate adherence to the MEDAS diet, had a significant expression for the same bacteria. As explained in appendix D.

Further analysis was done, to examine each item from the MEDAs questioner with the diversity of the gut microbiota between groups, but no one got a significant relationship.

3.2.2 Gut microbiota related data between the severities of symptoms in IBS patients

3.2.2.1 Association between the severity of symptoms in IBS patients and selected Gut microbioita diversity

Ten of the patients got a stool sample suffering from mild symptoms with an average 27.24±58.71 fold for *Akkermansia* bacteria, while the average of *Bacteroides* in these patients was 10.45±20.62, the fold of *Bifidobacterium* was 56.05±125.96, while for *Butyricicoccus* 75.63±148.62. For Enterobacteri was 1.58±2.44. The fold for *Faecalibacterium* in this bacteria was 13.59±25.46, while for *Escherichia* was 52.77±127.21. The fold of *Streptococcus* was 47.08±126.19, while that of *Lactobacillus* was 32.23±40.83. For *Roseburia* the fold was 4.01±7.85. The fold of each bacteria for each patient is demonstrated in figure 3.2A below.

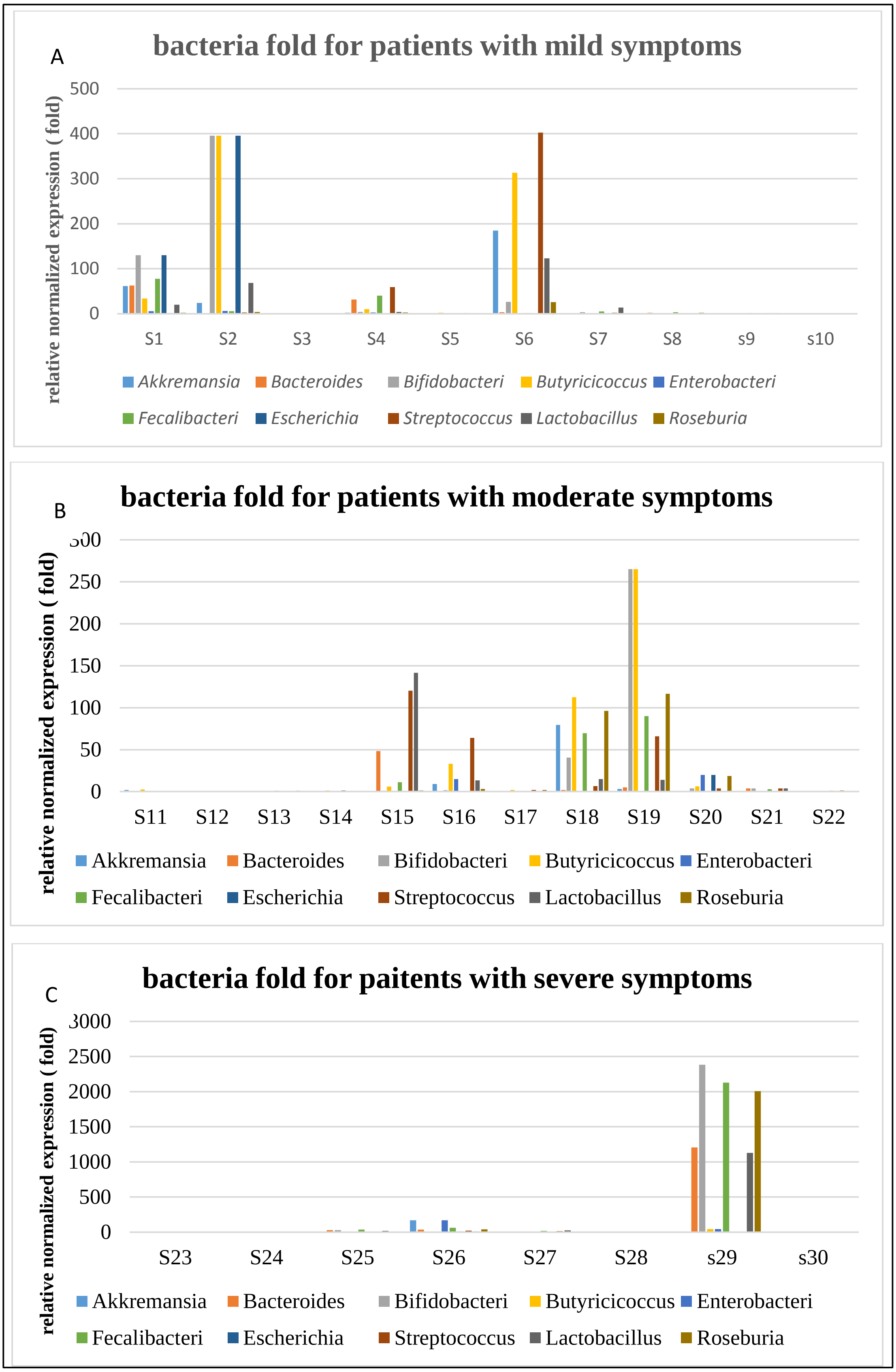
Twelve patients got samples suffering from moderate symptoms, the fold of *Akkermansia* bacteria was 7.88±22.70, while the average of *Bacteroides* in these patients was 5.02±13.70, and the fold of *Bifidobacterium* was 26.37±76.02, while for *Butyricicoccus* 35.81±79.04. For Enterobacteri was 3.03±6.85.The fold for

Faecalibacterium in this bacteria was 14.85±30.82, while for *Escherichia* was 1.94±5.69. The fold of *Streptococcus* was 22.4±39.31, while that of *Lactobacillus* was 15.71±40.10. For *Roseburia* the fold was 19.93±40.94. The fold of each bacteria for each patient is shown in figure 3.2B below.

The rest of the patients who gave a stool sample suffered from severe symptoms the fold of their *Akkermansia* bacteria was 21.47±58.73, while the average of *Bacteroides* in these patients was 159.86±423, and the fold of *Bifidobacterium* was 302.77±840.06, while for *Butyricicoccus* 7.44±14.43. For *Faecalibacterium* was 280.93±747.21. The fold for *Enterobacteriaceae* in this bacteria was 26.94±58.36, while for *Escherichia* was 1.33±3.13. The fold of *Lactobacillus* was 148.17±396.56, while that of *Streptococcus* was 4.32±7.88. For *Roseburia* the fold was 256.69±706.65. The fold of each bacteria for each patient is explained in figure 3.3C below.

Figure 3.3

Bacteria fold for patients with Symptoms



Note: A: Mild Symptoms; B: Moderate Symptoms; C: Severe Symptoms

3.2.2.2 Comparison between selected Gut microbiota species and severity symptoms in IBS patients

For *Akkermansia* bacteria, 4 of IBS patients with mild symptoms had significant fold expression, while 6 of them had not significant fold expression. For patients with moderate symptoms, 5 of them had significant fold expression, while those who did not have significant fold expression were 7. Of patients with severe symptoms, 4 of them had significant fold expression. Also, 4 had no significant fold expression with a p-value of .432. 9 of IBS patients with mild symptoms had significant *Bacteroides* fold expression. In contrast, 6 had no significant fold expression, in patients with moderate symptoms 5 of them had significant fold expression.

In contrast, 7 with moderate symptoms had no significant fold expression, with no significant p-value. 6 of the IBS patients with mild symptoms had significant *Bifidobacterium* fold expression, while 4 of them had no significant fold expression, in IBS patients with moderate symptoms 6 of them had significant fold expression, and 6 of them had no significant fold expression. Who had significant fold expression for *Butyricicoccus* bacteria in IBS patients with mild symptoms was 6, while not significant was 4. For patients with moderate symptoms, 9 of them had significant fold expression, while those who did not have significant fold expression 3. Of patients with severe symptoms, 6 of them had significant fold expression, and also 2 had no significant fold expression with a p-value of .326, which is not significant. For *Faecalibacterium* bacteria in IBS patients with mild symptoms, 8 of them had significant fold expression, while 2 had no significant fold expression. Of patients with moderate symptoms 8 of them had significant fold expression, while who did not have significant fold expression was 4. Of patients with severe symptoms, 6 of them had significant fold expression, and 2 had no significant fold expression with a p-value of .491, which is not significant.

The p-value for *Escherichia* bacteria also was not significant, with 5 patients with mild symptoms having significant fold expression, and 5 patients with mild symptoms had no significant fold expression. Of patients with moderate symptoms, 4 of them had significant fold expression, while who had not have significant fold expression was 8. Of patients with severe symptoms, 2 of them had significant fold expression, and 6 had no significant fold expression 7 of patients with mild symptoms had significant *Streptococcus* fold expression. As demonstrated in table appendix D.

3.2.3 Diversity of selected Gut Microbiota species between the study groups

By measuring beta diversity using Bray-Curtis dissimilarity, the dissimilarity value between IBS patients and healthy control was 0.88, which is high because it's near 1, and that means there are different species between them, and IBS patients have less diverse, which means IBS have dysbiosis.

By measuring the Shannon index to compare the diversity between the three groups, the mild symptoms diversity was 9.67, while for moderate one was 9.45, and the severe one was 7.84.

Chapter Four

Discussion

4.1 Introduction

The goal of our study was to better understand and investigate the link between patients' lifestyles and mental health, particularly in correlation with the severity of symptoms and the diversity of gut microbiota. Additionally, these factors distinguish between the patients and their healthy controls. The study involved two hundred thirty-seven IBS patients and thirty healthy controls who completed a questionnaire evaluating their mental health, physical activity, adherence to the MEDAS diet, smoking habits, and severity of symptoms. The Birmingham IBS questionnaire assesses symptom severity for IBS patients according to the Rome II criteria. Thirty stool samples were collected from each group.

To check that, stool analysis using semi-quantitative PCR was done for different types of bacteria. The diversity of each group was assessed using the Shannon index between groups of patients with different symptoms, and Bray-Curtis dissimilarity between IBS patients and their healthy control. Statistical analysis was done to link participants' lifestyles and their mental health status, with the diversity of gut microbiota. This study shows differences in the gut microbiota diversity between IBS patients and their control, as well as between groups of patients with different symptoms. And significant relationship between mental health, smoking, and sleep problems in the two groups. Moreover, physical activity between IBS patients and their healthy control. At the end, this study and its outcome, emphasize the importance of managing IBS patients based on their lifestyle and mental health status, together with traditional medication which can enhance their microbiota diversity

4.1.1 Severity of IBS symptoms

Our study examined the severity of symptoms using the Rome II criteria. 34% of the patients had severe symptoms, while moderate and low symptoms had 32.9% each. Compared with previous studies that used the same Rome, the percentage of the subgroup was as mild 40%, moderate 35%, and severe 25% [6]. The percentage of the symptoms can vary according to the country of the research, and which rome is used to diagnose IBS patients –rome I/II/III or IV-. In one study, when using Rome III the percentage of mild was 41.1%), while the moderate percentage was (44.2%) and the percentage of severe was (8.8%), while the rest was normal. In the same participant when using Rome IV, the percentage of mild was (11.1%), while the moderate percentage was (41.1%) and the percentage of severe was (46.8%), while the rest was normal[69]. That is because in Rome IV patients report having either constipation or diarrhea rather than mixed stool pattern, and report stool type when it is abnormal, which makes the prevalence of subgroups enormously vary from one Rome to another.

There are two main differences between Rome II, which we used in our experiment, and Rome IV, which is the most commonly used Rome these days. First, in Rome IV, they try to help doctors diagnose and treat better by splitting symptoms that do not always come with pain, like bloating, diarrhea, and constipation. They remove discomfort as a symptom and use it rather than "pain," which makes the subgroup of symptoms more accurate.

4.1.2 Differentiation and similarity between IBS patients and healthy control

4.1.2.1 Differentiation between IBS patients and healthy control

One of the aims that our study was to investigate patients' mental health, and compare it with a healthy control one. According to our result, by using GHQ, (36.7%) of the patients have mental distress, with a significant relationship (P value =.003). Our study got the same result as most studies done, for example in a review done in 2020, including 10 studies done on IBS patients, most of them got the same significant relationship [113]. To explain this result, we can say "gut-brain axis" is the most suitable answer.

Patients with chronic stress and mental health distress, have sleep disturbances, and that was significant in our study. There is a significant relationship between IBS patients and sleep disturbance, with (P value =.000), about (61.6%) of the patients suffer from sleep disturbances. That is because of the gut-brain axis. Also by HPA axis plays a role in these disturbances. GI tract mucosa by it is enterochromaffin cells, produces melatonin, which is a response for initiating sleep and maintaining it. When there is gut dysbiosis and IBS, the level of melatonin can change, leading to sleep disturbance.In our study, not only sleep disturbance was in our study, as well the duration of their sleep and if they suffer from any sleep problems was asked about. 88 of the patients didn’t sleep the hour which any human needs –between 6 to 8 hours -, with a significant relationship (P-value=.006). Moreover, only 91 of the patients didn’t have any sleep problems, with (P-value=.000). To link that with their disorder, deprived of sleep for a long time leads to a raised number of lymphocytes, monocytes, leukocytes, and neutrophils in peripheral blood. Moreover that leads in prolong periods to increase the concentration of IL-6 and tumor necrosis factor-alpha (sTNF-α) receptor 1, which are mediators for the nervous system. One of the study that have like our study outcome, suggest that circadian rhythm disruption lead to enhance intestinal epithelial barrier permeability [114].

In our study, 130 patients out of 237, have poor physical activity, while only 8 of them have high physical activity, and that clearly negatively impacts their disorder, with a (P-value=.0001). Physical activity plays a good role in IBS patients, as increasing of physical activity, gas transit and colonic transit change, and that eliminate the symptoms, especially abdominal bloating. Patients who suffer from constipation can relieve this by doing some physical activity, which was confirmed by a recent study, which demonstrates that about 54% get a good improvement after 12 weeks of doing physical activity [115]. Again the brain axis plays a role, as stress triggers neuroendocrine response, while physical activity can minimize anxiety, depression, and fatigue. Also, it can help to avoid retrogradation of IBS symptoms. Physical activity plays directly on IBS symptoms like constipation and bloating and indirectly on their mental health, which also leads to minimizing their symptoms. Another investigation found in our study was the relationship between IBS and smoking. The smoker IBS patients was 82 participants with a (P-value of .000). A recent study identified smoking as the cause of postprandial distress syndrome in most of the cases with a (P value=.001), smoker-induced diarrhea (P -value = 0.025), urgency (P-value = 0.001) and flatus (P-value = 0.012) when compared with non-smoker [116].

Also in our result, the value of Bray-Curtis was 0.88, which is an indication of dysbiosis. This can affect mental health and increase sleep disturbances by decreasing SCFAs, leading to anxiety, stress, and sleep apnea. Also, dysbiosis can change the synthesis of some neurotransmitters like gamma-aminobutyric acid (GABA), which enhance anxiety and sleep apnea. Moreover, dysbiosis affects systemic inflammation, as shown in previous studies, similar to current study [31]. Another study shows that dysbiosis can alter the HPA axis, leading to increased secretion of cortisol and insomnia[33]. Smoking is also a reason for dysbiosis in our IBS patients, as it is a pro-inflammatory factor, which can enhance inflammation and dysbiosis. Many studies confirm our result, like this result which shows that smoking decreases the abundance of good bacteria when compared with healthy ones [117].

Also, in our results, the abundance of *Akkermansia* decreased in IBS patients (18.86 folds) when compared with healthy people (63.83 folds) and because of smoking [117], which reduced gut permeability, systemic inflammation, increased anxiety sleep apnea, and disrupted the gut-brain axis. One of the reviews done in 2024, supports our result and shows that *Akkermansia* is integrity for the intestinal barrier, and regulates the immune response, which reduces it due to poor lifestyle like smoking increases inflammation and anxiety[19].

4.1.2.2 Similarity between IBS patients and healthy control

Our study presents that there is no variation in the adherence of MEDAS between IBS patients and healthy controls (P-value=.175). Most of the previous studies show inverse results, they have a statistical relationship between IBS and healthy control. One of them these studies, in which all participants used the MEDAS diet as their diet for 8 weeks, and they saw that there is relief in constipation in patients who suffer from, they demonstrated that this type of diet encourages to intake of different types of food, also intake some of fermented foods such as yogurt, which can help in gut health and eliminate discomfort related with this disorder [16]. Moreover, the diet helps to reduce red meat and processed foods and may help decrease the intake of triggers that may cause the symptoms worsen.

This variation in our result is because of variation in the study populations. In our study, all participants are already using the Mediterranean diet despite if there are patients or healthy. Also, this type of diet is suitable for only some types of IBS, like IBS-C, as this type of diet has high fiber, so it can reduce their symptoms. Although MEDAS is a healthy diet, but it have higher FODMAPs quantities, like some type of vegetables (onions and garlic), whole grains, and legumes. As low FODMAPs are the suitable diet to reduce IBS symptoms, IBS patients should decrease the certain type from the MEDAS diet, which has a high quantity of FODMAPs to make it more suitable.

Another thing else, patients vary in the type that are sensitive to, which can interfere with MEDAS components. Intestinal microbiota studies always focus on bacteria in their research, while a recent study investigated the relationship between MEDAS and bacterial and colon fungus, they showed that fungal gut microbiota change by MEDAS diet, which can improve GI disorder [12]. Further investigation about how MEDAS affects both bacteria and fungus is needed, to clarify if this diet is suitable for IBS or not.

Another thing in our study that got different result than most of the previous results get was BMI, in our study there was no significant result (P- value=.979). Previous study, like this study found that (31.9%) of IBS patient participant was obese, with a significant statically relationship when compared with control participants (P-value =.002) suggesting that there is reduce in Bacteroidetes, while in people with high BMI Firmicutes is increase [27].

Also, they show that a diet with high fat increase level of endotoxin in plasma, and primarily enhances of inflammatory responses like TLR4, whose activation it in the prefrontal cortex relates to visceral pain. Moreover, HPA plays another role, when cortisol - which is the final product in this axis- contributes to obesity by influencing appetite regulation through play on hormonal stimulation by increasing ghrelin and inhibiting leptin. High BMI itself is a source of stress for participants. Our result got an inverse result may be due to the type of diet differing from one region to another, although we asked the participants about the MEDAS diet, we didn't make any intervention about their diet. To get more specific information about whether there is a relationship or not, all participants should have the same diet for a specific period, which helps to study BMI as one factor, and that help to concentrate on food quality rather than calories.

4.1.3 Differentiation and similarity between IBS subtype severity symptoms

4.1.3.1Differentiation between IBS subtype severity symptoms

In our finding, there is a significant relationship between the severity of symptoms and smoking. In our research, the mean of those who smoke was 1.01±.11 with a (P-value .000). Most of the Previous studies got the same result , like which were done on 1601 patients, show that about 766 of the patients suffer from severe symptoms,661 of them with moderate symptoms, while the rest with mild symptoms. 80% from the patients who was smoker, suffer from severe symptoms with a (P-value =.000)[118]. As smoking increases the pro-inflammatory cytokines, a rise in a heavy smoke, enhances the inflammatory medium in the gut leading to worse symptoms. Moreover, heavier smokers lead to decreased blood flow, which leads to more discomfort and bloating [75]. Another cross-sectional study which was done on 3363 participants, looked for the relationship with smoking, not only as one factor, but how influenced the patient's lifestyle, in addition to their dietary habits and physical activity, but there was no association. They suggest that look for all the factor, make accurate result rather than when look for smoking alone [119].

In our result another good relationship was between the severity of symptoms and mental health (P-value=.000). The same result was obtained in a study done in 2021 on 1601 patients, they showed that high level of anxiety and depression was linked with more severe symptoms of IBS [118]. Another study was done in Egypt which supports our findings. They found that (88.5%) of the patients have severe anxiety, and (77.9%) of them with severe IBS symptoms [11].

Few studies, like this study, emphasize that although mental health links with IBS directly due to the gut-brain axis, mental health doesn't have a direct relationship with patients' severity of symptoms. They thought patients' awareness of their symptoms (ex. abdominal pain and discomfort) was more related to the severity than mental health itself, which can influence their quality of life and how patients manage their symptoms [49]. Another study that supports this result was done, they confirmed that mental health can vary due to how the patients cope with their symptoms, regardless of the direct physical effect of IBS [3]. Smoking and mental health disturbance can gather to worse the severity of symptoms. Some patients use smoking as a coping technique to reduce poor sleep, stress, and anxiety and it may reduce for a short time, but for a long time, smoking increases inflammation and reduces the immune system which leads to severe symptoms. That means the patients fail to cope with his symptoms, so his anxiety and stress increase more.

Suffering from sleep problems also has a good relationship with the severity of symptoms, with a (P value=.000). One previous study that supports our result, was done on 10 patients with mild severity, 69 was moderate, while 63 with severe symptoms, 32 of them have poor sleep quality [22]. Deep sleep aids the body in repairing tissues, decreasing inflammation, and helping the immune system. Increased sleep problems, lead to increased inflammation and reduce the immune system, making the symptoms more severe [53]. Another study in Jeddah, Saudi Arabia shows that (69%) of patients with severe symptoms had poor sleep, and that is due to a cycle link between psychological and physiological factors. gut-brain axis which alternates the sleep pattern can enhance inflammation and increase the severity [9].

Our study showed using shannon index, that mild symptoms got a high diversity when compared with other groups, then moderate and finally severe symptoms with a value of 9.67, 9.45, and 7.84 respectively. That means our patients who suffer from severe symptoms have less diversity and more dysbiosis, and that can link with our result about have a statically relationship between the severity and mental health, smoking, and sleep problem. As *Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, and *Lactobacillus* got a relationship with the severity of symptoms.

Bacteroides, *Bifidobacterium* and *Lactobacillus* got a bidirectional relationship with mental health. When the amount decrease, their production of SCFAs decrease, and that leads to increased anxiety by impacting the neurotransmitter and function of the brain, which also affects the quality of sleep. For *Enterobacteriaceae*, when their overgrowth, dysbiosis increases. This in our result present more in severe patients symptoms, so systemic inflammation is enhanced, which can also be enhanced by smoking more and lead to affect mood and increased mental distress. Our result was supported by this review which show that the symptoms and anxiety increase when *Bacteroides* decrease and *Enterobacteriaceae* increase [34].

4.1.3.2 Similarity between IBS subtype severity symptoms

Although many of studies show that MEDAS diet can elimante the severity of IBS symptoms like this study ,which suggest that vegetables and fresh fruits are prime concerns in this diet. Also, it provides the body with high of fiber, which leads to regulating bowel movements and relieves some of IBS symptoms, particularly constipation. Moreover, MEDAS encourages the intake of different types of food, and some fermented foods such as yogurt help in gut health and decrease IBS symptoms [15]. Still, our result gives no association between this type of diet and the severity of symptoms in IBS patients, with a (P-value=.352).

One of the studies that got our result was done in 2024, they suggest that some types of food are high in FODMAPs, which is a response for enhancing the symptoms of IBS. They also found that MEDAS lowers the abundance of certain bacteria like *Streptococcus*. Moreover, they found that there is no type of diet suitable for all individuals. Individuals can vary in food which may trigger them [20]. To make the relation clearer, more investigation with follow-up the patients need, to compare the severity before they use the MEDAS diet and how the severity becomes after they use this type of diet.

Despite an association between physical activity and IBS patients in general, there is no statistical relationship between the severity of IBS patients and their physical activity and body mass, with a (P-value=.627), and (P-value=.314) respectively. A few studies look for the relationship between the severity and physical activity. One of study which demonstrates that IBS symptom severity can decrease when patients do moderate-intensity aerobic training for not less than 180 minutes per week, which helps to enhance the immune system and helps to secrete une(GLP-1) which helps to upgrade the gut function [26]. Another study has shown that exercise helps to increase the diversity of gut microbiota, which helps to increase the making of SCFA and decrease inflammation [65].

In our study there is no association because we don't know what type of physical activity the participants do. Some types of physical activity can reduce symptoms (like anxiety and stress) such as walking and yoga more than other types. Also, we don't know the duration of their activity. As we mentioned in the study above, patients should at least train at least 180 minutes. Further investigation of the type and duration of physical activity should be done to make the association more clear. One of the studies that got an association between BMI and severity of IBS symptoms was done in the Netherlands with a (P-value=.049) [118]. While one of the studies that supported our result got a (P-value=.302). They suggest that these symptoms may be caused by psychological factors, such as somatization, which can enhance the symptoms regardless of BMI. They also found that a higher BMI increases the symptoms of IBS-C more than other types [5].

Despite there being a differentiation in diversity between groups with different severity in our result, as mentioned above, there is no association between this diversity and MEDAS and BMI, and there is no certain type of bacteria that also gets an association. One of the new reviews shows no difference in the diversity between groups with different symptoms after 6 weeks of using MEDAS type in their life, they suggest that the duration should be more long and it is not suitable for all patients due to containing some high FODMAPs food [15].

While for physical activity there is differentiation in diversity, also *Bifidobacterium* and *Akkermansia* got a statical relationship with physical activity, but this diversity didn't got a statical relationship with the group. One of the studies which support and discuss our result, show that the increase in this patient fold may be due to his type of food, as *Akkermansia* increases in the gut by certain types of food containing polyphenols, which can be from tea, grapes, and apples. Polyphenols were shown to increase *Akkermansia* by creating its favorite environment in the gut, also it enhance the ratio of Firmicutes/Bacteroidetes [27]. Further investigation with specific types of food and physical activity with long-duration follow-up needs to make the association between dysbiosis, MEDAS, and physical activity clearer.

4.2 Strength points and limitations of the study

4.2.1 Strength points

To our knowledge, this is the first study on Palestinian people to evaluate all of these factors that can influence IBS patients and link them with their microbiota. The sample size for how to complete their questionnaire was large and based on previous studies. Moreover, this questionnaire and the analysis of stool samples were measured using a serial number, which maintained the privacy and confidentiality of the patients. Lastly, the same patients were compared with healthy controls, and between them, that helped prevent potential bias when doing them in a separate study.

4.2.2 Limitations

Rome II criteria is used rather than Rome IV to assess the severity of symptoms, due to limitations in budget. Also, IBS patients are outpatients, and a stool sample is not available every time the patients, the samples deteriorate after a short time if not kept at a suitable temperature, and it was collected during the Gaza war, so it was difficult to get more samples. At last, like the MEDAS diet, it got more accurate investigation when their follow-up.

4.3 Recommendations and Conclusion

4.3.1 Recommendations

It is recommended to conduct further studies with more stool samples.

- It is recommended to reduce stress as soon as possible.
- For some variable like MEDAS longitudinal study can help to get deeper relationship.
- It is recommended for healthcare providers to develop management strategies for their patients, through dietary modifications, adjustments to their lifestyle, and some interventions to enhance healthier gut microbiota.

4.3.2 Conclusion

This study shows differentiation in gut microbiota diversity (*Butyricicoccus, Enterobacteriaceae*, *Faecalibacterium*, *Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Escherichia*, *Streptococcus*, *Lactobacillus*, and *Roseburia*) between IBS patients and healthy controls with a dysbiosis in IBS patients when compared with healthy control using Bray-Curtis dissimilarity. As well as, between groups of patients with different severity of symptoms, with high diversity in patients with mild symptoms, while a high dysbiosis in patients with severe symptoms. Moreover, this study shows that there is a significant relationship between mental health distress, smoking, and sleep problems between IBS and their control, as well as between patients with different severity of symptoms. IBS patients and health control also have a relationship with physical activity. However, the MEDAS diet shows no relationship between any of the groups. As well as BMI. Specialize more for bacteria that have a relationship. There is a differentiation in the abundance of these bacteria between patients with different bacteria and their physical activity (*Akkermansia*, and *Bifidobacterium*). Also in the same group, there is a significant relationship between the mental health of this abundance of bacteria (*Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, and *Lactobacillus*).

These findings emphasize the significance of taking into consideration managing IBS patients based on their lifestyle and mental health status, together with traditional medication which can improve gut microbiota diversity.

List of Abbreviations

Abbreviation	Meaning
ACLY	ATP-citrate lyase
AhRs	aryl hydrocarbon receptors
BARs	BAs-activated receptors
BAs	bile acids
BBB	blood-brain barrier
BCFAs	branched-chain fatty acids
CAZymes	carbohydrate-active enzymes
CNS	central nervous system
C-section	Cesarean section
DCs	dendritic cells
DGBI	disorder of gut-brain interaction
DM	diabetes mellitus
FODMAPs	fermentable oligosaccharides, disaccharides, monosaccharides, and polyols
Foxp3	forkhead box protein P3
FXR	family of nuclear
FXR	family of nuclear
GABA	gamma-aminobutyric acid
GHQ-12	The General Health Questionnaire
GI	gastrointestinal
GPRs	G-protein-coupled receptors
HDACs	histone deacetylases
HIFs	hypoxia-inducible factors
HPA	hypothalamic-pituitary-adrenal
IBS	Irritable bowel syndrome
IBS-A	Alternating irritable bowel syndrome
IBS-C	Irritable bowel syndrome with constipation
IBS-D	Irritable bowel syndrome with diarrhea
IBS-M	mixed Irritable bowel syndrome
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-18	Interleukin-18
IL-6	Interleukin-6
IPAQ	International Physical Activity Questionnaire
isoDCA	3β-hydroxydeoxycholic acid
MAM	mucosa-associated microbiota
MCT-1	monocarboxylate transporter 1
MEDAS	Mediterranean Diet Adherence Screener
mTOR	mammalian target of rapamycin
PCR	polymerase chain reaction
PUL	polysaccharide utilization loci
QOL	quality of life
RA	retinoic acid

rDNA	ribosomal DNA
SCFAs	Short-chain fatty acids
sgACC	subgenual anterior cingulate cortex
SLC5A8	sodium-dependent monocarboxylate transporter-1
SMCT-1	sodium-coupled monocarboxylate transporter 1
sTNF-α	tumor necrosis factor-alpha
Sus	starch utilization system
TCA	tricarboxylic acid cycle
Th	T-helper
TNF-α	tumor necrosis factor-α
Treg	T-regulatory

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Appendices

Appendix A

IRB approval

An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. Oct. 2023/103

IRB Approval Letter

Title of Research:

The relationship of symptoms severity, mental health, lifestyle factors with gut microbiome among Irritable bowel syndrome patients, a cross-sectional study from Palestine

Submitted by:
Yasmeen Mahdi Sa'd Al- Deen

Supervisor:
Manal Badrasawi

Aproved:
25th October. 2023

Your Study " **The relationship of symptoms severity, mental health, lifestyle factors with gut microbiome among Irritable bowel syndrome patients, a cross-sectional study from Palestine**" reviewed by An-Najah National University IRB committee and was approved on 25th October. 2023

Hasan Fitian, MD

IRB Committee Chairman

IRB
Institutional Review Board

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faximile (970) (09) 2342910| E-mail : IRB@najah.edu

Appendix B

Application form for approval to participate in scientific research

The Relationship Between Symptoms Severity, Mental Health, Lifestyle Factors, and Gut Microbiome Among Irritable Bowel Syndrome Patients: A Cross-Sectional Study, West Bank, Palestine

علاقة شدة الأعراض، الصحة النفسية وعوامل نمط الحياة مع البكتيريا المعوية لدى مرضى القولون العصبي

دراسة مقطعية، الضفة الغربية، فلسطين.

DATA SHEET

نموذج طلب موافقة على المشاركة في بحث علمي

بموجب هذا النموذج، يُطلب منك المشاركة في دراسة بحثية لرسالة ماجستير حول علاقة شدة الأعراض، الصحة النفسية وعوامل نمط الحياة مع البكتيريا المعوية لدى مرضى القولون العصبي. وهي دراسة مقطعية من فلسطين.

يجُرى هذا البحث بغية الإجابة على أسئلة مهمة، الأمر الذي قد يساعد على تغيير أو تحسين حياة الأشخاص المصابين بالقولون العصبي. لن تكون عرضة لأي أخطار خلال مشاركتك في هذه الدراسة عدا احتمالية الشعور بالإحراج تجاه بعض المعلومات دقيقة. لا يتطلب منك إلا أن تقوم بالإجابة على هذا الإستبيان، و إحضار عينة براز .

يجوز لك عدم المشاركة في الدراسة ،كما يجوز لك الانسحاب من الدراسة في أي وقت كان ، ولن تطبق عليك أي عقوبة أو تخسر أي مزايا إذا رفضت المشاركة أو إذا قررت التوقف .

نؤكد لك بأن كافة المعلومات التي سيتم جمعها هي لأغراض البحث العلمي فقط وسيتم الحفاظ على السرية التامة وعدم استخدامهذه المعلومات لأغراض أخرى .

قبل موافقتك على المشاركة بالدراسة ،سيقوم الباحث بتلخيص المعلومات المهمة التي ستسمح لك باتخاذ قرار المشاركة أوعدمه .

في حال كان لديك أي أسئلة حول الدراسة يمكنك الاتصال في أي وقت ب:

رقم الهاتف:0597642643

<u>موافقة المشارك:</u>

لقد تم قراءة المعلومات المدرجة أعلاه بتمعن

وأوافق على المشاركة بهذه الدراسة البحثية ☐

أوافق على إحضار عينة براز. ☐

اسم المشارك بالأحرف الكبيرة الواضحة:____

رقم الهاتف:_______________________________

توقيع المشارك: _______________________________

التاريخ:_________________________

فريق البحث:

الطالبة ياسمين مهدي سعد الدين.

الدكتورة منال الحاج حمد.

الدكتور محمد التميمي .

الدكتور قصي عبدو.

Mediterranean Diet Adherence Screener

Components	**Criteria/scoring** التقييم /المعايير		
Sweets حلويات	≥2servings/week حصة طعام/اسبوع≥2	Yes نعم	No لا
Red meat اللحمة الحمراء	<2 servings/week حصة طعام/اسبوع<2	Yes نعم	No لا
Cooked meat لحمة مطبوخة	servings /week ≥1 حصة طعام/اسبوع≥1	Yes نعم	No لا
Eggs بيض	2-4servings /week حصة طعام/اسبوع2-4	Yes نعم	No لا
Legumens بقوليات	≥2servings/week ≥2 حصة طعام./اسبوع	Yes نعم	No لا
White meat لحمة بيضاء	servings /week 2 حصة طعام/اسبوع2	Yes نعم	No لا
Fish/ seefood مأكولات بحرية /سمك	servings /week ≥2 حصة طعام/اسبوع≥2	Yes نعم	No لا
potatoes بطاطا	<3 servings week 3 حصة طعام /اسبوع>	Yes نعم	No لا
Low-fat milk products منتوجات الألبان قليلة الدهون	2servings /day 2حصة يوم/ طعام	Yes نعم	No لا
Nuts and olives مكسرات وزيتون	servings / day 1-2 1_2حصة طعام / يوم	Yes نعم	No لا
Herbs , spices and garnish اعشاب وبهارات ومقبلات	1servings / day≥ 1≥ حصة طعام / يوم	Yes نعم	No لا
Fruits فواكه	3-6 servings /day 6_3 حصة طعام /يوم	Yes نعم	No لا
vegetables خضروات	>2 servings /day يوم /2 حصة طعام>	Yes نعم	No لا
Olive oil زيت زيتون.	3 servings /day ≥ يوم /3≥ حصة طعام	Yes نعم	No لا
Cereals الحبوب	3-6 servings /day 3_6 حصة طعام /يوم	Yes نعم	No لا

General Health Questionnaire

Question Item	0	1	2	3
Have you been able to concentrate? هل تمكنت من التركيز؟	Better than usual أحسن من المعتاد	Same as usual نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كثير أقل من المعتاد
Have you lost sleep from worry هل فقدت النوم من القلق؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كثير أكثر من المعتاد
Have you felt you are playing a useful part in things هل شعرت أنك تلعب دوراً مفيداً في الأشياء؟	More so than usual أكثر من المعتاد	Same as usual نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كثير أقل من المعتاد
Have you felt you are capable of making decisions هل شعرت أنك قادر على اتخاذ القرارات؟	More so than usual أكثر من المعتاد	Same as usual نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كثير أقل من المعتاد
Have you felt constantly under strain هل شعرت أنك تحت الضغط باستمرار؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كثير أكثر من المعتاد
Have you felt you could not overcome difficulties هل شعرت أنه لا يمكنك التغلب على الصعوبات؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كثير أكثر من المعتاد
Have you felt you are able to enjoy day-to-day activities هل شعرت أنك قادر على الاستمتاع بالأنشطة اليومية؟	More so than usual أكثر من المعتاد	Same as usual نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كثير أقل من المعتاد
Have you felt you are able to face problems هل شعرت أنك قادر على مواجهة المشاكل؟	More so than usual أكثر من المعتاد	Same as usual نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كثير أقل من المعتاد
Have you felt unhappy and down هل شعرت بالحزن والإحباط؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كثير أكثر من المعتاد
Have you been losing confidence in yourself هل فقدت الثقة في نفسك؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كثير أكثر من المعتاد

Have you been thinking of yourself as a worthless person هل كنت تفكر في نفسك كأنك شخص لا قيمة له؟	Not at all على الاطلاق	No more than usual ليس أكثر من معتاد	Rather more than usual بل أكثر من معتاد	Much more than usual كتير أكتر من المعتاد
Have you been feeling reasonably happy هل شعرت بسعادة نوعا ما؟	More so than usual أكثر من المعتاد	About same as usual تقريباً نفس المعتاد	Less than usual أقل من المعتاد	Much less than usual كتير أقل من المعتاد

International Physical Activity Questionnaire

During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling? خلال الأيام السبعة الماضية، كم يوماً مارست فيه نشاطاً بدنياً مرتفع الشدة؟	No vigorous physical activities —-> Skip to question 3 لا أقوم بأي نشاط بدني مرتفع انتقل مباشرة إلى السؤال رقم ٣ <-الشدة		___days per week يوم في الأسبوع___
How much time did you usually spend doing vigorous physical activities on one of those days? في المعتاد، كم من الوقت قضيته في ممارسة نشاط بدني مرتفع الشدة في أحد تلك الأيام؟	Don’t know/Not sure لا أدري/أو غير متأكد	___minutes per day دقيقة في اليوم___	___hours per day ساعة في اليوم___
During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a	No moderate physical activities—->Skip to question 5 انتقل إلى السؤال <-لا أقوم بأي نشاط بدني معتدل الشدة رقم ٥		___days per week يوم في الأسبوع___

regular pace, or doubles tennis? Do not include walking. خلال الأيام السبعة الماضية، كم يوماً مارست فيه نشاطاً بدنياً معتدل الشدة؟			
How much time did you usually spend doing moderate physical activities on one of those days? في المعتاد، كم من الوقت قضيته في ممارسة نشاط بدني معتدل الشدة في أحد تلك الأيام؟	Don’t know/Not sure لا أدري/ أو غير متأكد	___minutes per day دقيقة في اليوم	___ hours per day ساعة في اليوم___
During the last 7 days, on how many days did you walk for at least 10 minutes at a time? خلال الأيام السبعة الماضية، كم يوماً مارست فيه المشي لمدة 10 دقائق على الأقل في كل مرة؟	days per week يوم في الأسبوع___	No walking——>Skip to question 7 انتقل مباشرة إلى <—لا أقوم بالمشي إطلاقا السؤال رقم	
How much time did you usually spend walking on one of those days? في المعتاد، كم من الوقت قضيته في ممارسة المشي في أحد تلك الأيام؟	___hours per day ساعة في اليوم___	___ minutes per day دقيقة في اليوم___	Don’t know/Not sure أو غير متأكد/لا أدري
During the last 7 days, how much time did you spend sitting on a weekday? خلال الأيام السبعة الماضية، كم من الوقت قضيته جالساً في أحد هذه الأيام من غير أيام الإجازة الأسبوعية؟	___hours per day ساعة في اليوم___	___ minutes per day دقيقة في اليوم___	Don’t know/Not sure أو غير متأكد/لا أدري

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Approval from Faculty of Graduate Studies

Reload Page

20201974FGSANNUPS
جامعة النجاح الوطنية
كلية الدراسات العليا
مكتب العميد

نموذج تحديد عنوان الأطروحة و المشرف

***** يجب توفر جميع الشروط التالية لتحديد عنوان الأطروحة و المشرف :**
- أن يكون مسار الطالب أطروحة ** الشرط متحقق **
- أن يتم الطالب 12 ساعة . ** الشرط متحقق ** **** عدد الفصول أقل أو يساوي 4 ****
- أن لا يكون الوضع الدراسي للطالب "مفصول من البرنامج". ** الشرط متحقق **
- المعدل التراكمي للطالب أكبر أو يساوي من 2.8 ** الشرط متحقق **

اسم الطالب :	ياسمين مهدي زياد سعد الدين	**رقم التسجيل :**	12154016
اسم البرنامج :	ماجستير الكيمياء الحيوية السريرية	**مسار الدراسة:**	أطروحة
عدد الساعات المعتمدة التي انجزت حتى الان:	25	**المعدل التراكمي:**	3.12
الوضع الدراسي :	يدرس		
عنوان الطالب :	نابلس	**رقم الهاتف المحمول :**	0597642643
البريد الالكتروني :	yasmeen99_s@hotmail.com		
لغة الرسالة :	--------------------		
عنوان الأطروحة باللغة العربية :	علاقة شده الاعراض, الصحه النفسيه و نمط الحياه مع البكتيريا المعوية لدى مرضى القولون العصبي . دراسه مقطعية من فلسطين		
عنوان الأطروحة باللغة الانجليزية:	The relationship of symptoms severity, mental health, lifestyle factors with gut microbiome among IBS patients, a cross-sectional study from Palestine.		
النسخة الإلكترونية من مقترح الاطروحة :	pdf.12154016-1		

رقم المشرف الأول : 2758	**اسم المشرف الأول:** منال محمود حسن بدرساوي
المشرف الثاني :	**يعمل في جامعة النجاح:** نعم **رقم المشرف الثاني:** 3533 **اسم المشرف :** قصي عبد الفتاح اسماعيل عبدة **رتبة المشرف :** استاذ مساعد

ملاحظة المشرف :	اوافق على الاشراف و اقر المقترح			**التاريخ :**	2023-09-26
ملاحظة المنسق :	موافق	**رأي المنسق :**	موافق	**التاريخ :**	2023-10-02
ملاحظة رئيس القسم :	موافق	**رأي رئيس القسم :**	موافق	**التاريخ :**	2023-10-03
ملاحظة مدقق الدراسات :	تم الموافقة من قبل مجلس الكلية	**رأي مدقق الدراسات :**	**موافق / عبء الاشراف :** أدنى من الحد	**التاريخ :**	2023-10-04
ملاحظة عميد الدراسات العليا :	لا مانع	**رأي عميد الدراسات العليا:**	موافق	**التاريخ :**	2023-10-05

قرار مجلس الكلية			
تم تغيير العنوان من قبل مجلس الكلية :	نعم		
عنوان الأطروحة باللغة العربية :	علاقة شدة الأعراض, الصحة النفسية وعوامل نمط الحياة مع البكتيريا المعوية لدى مرضى القولون العصبي . دراسة مقطعية من فلسطين		
عنوان الأطروحة بالغة الانجليزية:	THE RELATIONSHIP OF SYMPTOMS SEVERITY, MENTAL HEALTH, LIFESTYLE FACTORS WITH GUT MICROBIOME AMONG IRRITABLE BOWEL SYNDROME PATIENTS, A CROSS-SECTIONAL STUDY FROM PALESTINE		
رقم المشرف:	2758	**اسم المشرف:**	منال محمود حسن بدرساوي
المشرف الثاني :	**يعمل في جامعة النجاح:** نعم **رقم المشرف المالي:** 3533 **اسم المشرف :** قصي عبد الفتاح اسماعيل عبدة **رتبة المشرف :** استاذ مساعد		
فصل الاعتماد :	الأول	**سنة الاعتماد :**	2023 ** ملاحظة : مثال العام الدراسي 2021-2022 يتم ادخاله على شكل 2021
رقم جلسة الكلية:	434		
تاريخ جلسة الكلية:	01/10/2023		

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Association between physical activity and count of bacteria [presented as number (%)]

Type of bacteria	Low physical activity				Moderate physical activity				High physical activity				P value
	Significant (n) (%)		Not significant (n) (%)		Significant (n) (%)		Not significant (n) (%)		significant (n) (%)		Not significant (n) (%)		
Akkremansia	7	24.1	16	51.6	21	72.4	15	48.4	1	3.4	0	0	.019*[2]
Bacteroides	16	36.4	7	43.8	27	61.4	9	56.3	1	2.3	0	0	.359[2]
Bifidobacteria	11	29.7	12	52.2	25	67.6	11	47.8	1	2.7	0	0	.050*[2]
Butyricicoccus	17	39.5	6	35.3	26	60.5	10	58.8	0	0	1	5.9	.344[2]
Enterobacteria	10	33.3	13	43.3	19	63.3	17	56.7	1	3.3	0	0	.227[2]
Faecalibacterium	17	37.8	6	40	28	62.2	8	53.3	0	0	1	6.7	.502[2]
Escherichia	9	37.5	14	38.9	14	58.3	22	61.1	1	4.2	0	0	.440[2]
Streptococcus	17	41.5	6	31.6	24	58.5	12	63.2	0	0	1	5.3	.216[2]
Lactobacillus	13	32.5	10	50	27	67.5	9	45	0	0	1	5	.268[2]
Roseburia	14	37.8	9	39.1	23	62.2	13	56.5	0	0	1	4.3	.514[2]

[2] using exact

Association between BMI and count of bacteria [presented as number (%)]

Type of bacteria	Under weight and normal				Overweight				Obese				P value
	Significant (n) (%)		Not significant (n) (%)		Significant (n) (%)		Not significant (n) (%)		significant (n) (%)		Not significant (n) (%)		
Akkremansia	13	44.8	10	32.3	10	34.5	12	38.7	6	20.7	9	29	.174[1]
Bacteroides	17	38.6	6	37.5	16	36.4	6	37.5	11	25	4	25	.524[2]
Bifidobacteria	15	40.5	8	34.8	14	37.8	8	34.8	8	21.6	7	30.4	.306[1]
Butyricicoccus	17	39.5	6	35.3	19	44.2	3	17.6	7	16.3	8	47.1	.163[1]
Enterobacteria	10	33.3	13	43.3	14	46.7	8	26.7	6	20	9	30	.418[1]
Faecalibacterium	15	33.3	8	53.3	21	46.7	1	6.7	9	20	6	40	.337[2]
Escherichia	8	33.3	15	41.7	13	54.2	9	25	3	12.5	12	33.3	.491[2]
Streptococcus	17	41.5	6	31.6	16	39	6	31.6	8	19.5	7	36.8	.168[1]
Lactobacillus	13	32.5	10	50	17	42.5	5	25	10	25	5	25	.180[1]
Roseburia	12	32.4	11	47.8	18	48.6	4	17.4	7	18.9	8	34.8	.365[2]

[1]using Chi-square test, [2]* $p<0.05$ using exact

Association between mental health and count of bacteria [presented as number (%)]

Type of bacteria	Normal				Mental distress				P value
	Significant (n) (%)		Not significant (n) (%)		Significant (n) (%)		Not significant (n) (%)		
Akkremansia	23	79.3	22	71	6	20.7	9	29	.328[1]
Bacteroides	36	81.2	9	56.3	8	18.2	7	43.8	.049*[1]
Bifidobacteria	32	86.5	13	56.5	5	13.5	10	43.5	.011*[1]
Butyricicoccus	34	79.1	11	64.7	9	20.9	6	35.3	.202[1]
Enterobacteria	26	86.7	19	63.3	4	13.3	11	36.7	.036*[2]
Faecalibacterium	35	77.8	10	66.7	10	22.2	5	33.3	.337[1]
Escherichia	20	83.3	25	69.4	4	16.7	11	30.6	.181[2]
Streptococcus	33	80.5	12	63.2	8	19.5	7	36.8	.132[1]
Lactobacillus	33	82.5	12	60	7	17.5	8	40	.050*[1]
Roseburia	30	81.1	15	62.5	7	18.9	8	34.8	.142[1]

[1]using Chi-square test, [2]* $p<0.05$ using exact

Association between MEDAS and count of bacteria [presented as number (%)]

Type of bacteria	Low adherence				Moderate adherence				High adherence				P value
	Significant (n) (%)		Not significant (n) (%)		Significant (n) (%)		Not significant (n) (%)		significant (n) (%)		Not significant (n) (%)		
Akkremansia	6	20.7	5	16.1	16	55.2	20	64.5	7	24.1	6	19.4	.575[1]
Bacteroides	7	15.9	4	25	27	61.4	9	56.3	10	22.7	3	18.8	.318[2]
Bifidobacteria	6	16.2	5	21.7	22	59.5	14	60.9	9	24.3	4	17.4	.299[2]
Butyricicoccus	9	20.9	2	11.8	25	58.1	11	64.7	9	20.9	4	23.5	.338[2]
Enterobacteria	5	16.7	6	20	19	63.3	17	56.7	6	20	7	23.3	.580[1]
Faecalibacterium	7	15.6	4	26.7	28	62.2	8	53.3	10	22.2	3	20	.320[2]
Escherichia	3	12.5	8	22.2	15	62.5	21	58.3	6	25	7	19.4	.241[2]
Streptococcus	7	17.1	4	21.1	25	61	11	57.9	9	22	4	21.1	.477[2]
Lactobacillus	6	15	5	25	25	62.5	11	55	9	22.5	4	20	.308[2]

Roseburia	6	16.2	5	21.7	23	62.2	13	56.5	8	21.6	5	21.7	.456[1]

[1]using Chi-square test, [2]* p<0.05 using exact

Type of bacteria	**IBS patients**												**P value**
	mild				**Moderate**				**severe**				
	Significant (n) (%)		**Not significant (n) (%)**		**Significant (n) (%)**		**Not significant (n) (%)**		**Significant (n) (%)**		**Not significant (n) (%)**		
Akkremansia	4	30.8	6	35.3	5	38.5	7	41.2	4	30.8	4	23.5	.432
Bacteroides	9	42.9	1	11.1	5	23.8	7	77.8	7	33.3	1	11.1	.479
Bifidobacteria	6	35.3	4	30.8	6	35.3	6	46.2	5	29.4	3	23.1	.568
Butyricicoccus	6	28.6	4	44.4	9	42.9	3	33.3	6	28.6	2	22.2	.326
Enterobacteria	3	25	7	38.9	3	25	9	50	6	50	2	11.1	.058
Faecalibacterium	8	36.4	2	25	8	36.4	4	50	6	27.3	2	25	.491
Escherichia	5	45.5	5	26.3	4	36.4	8	42.1	2	18.2	6	31.6	.198
Streptococcus	7	36.8	3	27.3	8	42.1	4	36.4	4	21.4	4	36.4	.276
Lactobacillus	8	42.1	2	18.2	5	26.3	7	63.6	6	31.6	2	18.2	.455
Roseburia	7	38.9	3	25	7	38.9	5	41.7	4	22.2	4	33.3	.269

Association between bacteria count and severity symptoms in patients [presented as number (%)]

Using exact

جـــــــــــامعـــــــــــــــــــــة النجاح الوطنية

كليـــــــــــــة الدراســـــــــــــــــات العليـــــــــــــــا

علاقة شدة الأعراض، الصحة النفسية وعوامل نمط الحياة مع البكتيريا المعوية لدى مرضى القولون العصبي دراسة مقطعية، الضفة الغربية، فلسطين

إِعداد

ياسمين مهدي سعد الدين

إشراف

د. منال بدرساوي

د. قصي عبده

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2025

علاقة شدة الأعراض، الصحة النفسية وعوامل نمط الحياة مع البكتيريا المعوية لدى مرضى القولون العصبي دراسة مقطعية، الضفة الغربية، فلسطين

إِعداد

ياسمين مهدي سعد الدين

إشراف

د. منال بدرساوي

د. قصي عبده

الملخص

المقدمة: تعدُّ متلازمة القولون العصبي اضطرابًا يؤثر بشكل كبير على الصحة النفسية للمرضى، مما قد يؤدي بدوره إلى تفاقم أعراض المرض. تهدف هذه الدراسة إلى تحديد العلاقة بين أعراض القولون العصبي والصحة النفسية للمرضى، وعوامل نمط الحياة، وأنواع معينة من الأنظمة الغذائية، وتنوع البكتيريا المعوية، مع مقارنة هذه العوامل بمجموعة من الأفراد الأصحاء.

المنهجية: تم تصميم هذه الدراسة المقطعية لاستكشاف العلاقة بين الصحة النفسية، والنشاط البدني، والالتزام بالنظام الغذائي المتوسطي وتكوين البكتيريا المعوية لدى مرضى القولون العصبي، مع مقارنة النتائج بمجموعة التحكم من الأفراد الأصحاء، وكذلك بين المرضى أنفسهم وفقًا لشدة الأعراض.

شارك في الدراسة ما مجموعه 237 مريضًا يعانون من متلازمة القولون العصبي، بالإضافة إلى 30 شخصًا من الأفراد الأصحاء كمجموعة تحكم. أكمل جميع المشاركين استبيانًا لتقييم العوامل المؤثرة الت يتمت دراستها.

لتحليل البكتيريا المعوية، تم جمع عينات براز من جميع الأفراد في مجموعة التحكم ومن 30 مريضًا بالقولون العصبي تم اختيارهم بناءً على التوافق الديموغرافي. تم استخلاص الحمض النووي من العينات، ثم إجراء PCR شبه كمي لتحديد نسب وجود بعض الأنواع البكتيرية المختارة.

تم إجراء التحليلات الإحصائية لتقييم جميع العوامل التي تمت دراستها معاً.

النتائج: أظهرت تحليلات تنوع البكتيريا المعوية باستخدام مقاييس التنوع ألفا وبيتا فروقًا واضحة بين مرضى القولون العصبي والأفراد الأصحاء. أظهر تحليل بيتا باستخدام مقياس Bray-Curtis dissimilarity اختلافًا كبيرًا بين المجموعتين بقيمة 0.88.

كما تبين أن هناك علاقة ذات دلالة إحصائية بين الضغوط النفسية، والنشاط البدني، واضطرابات النوم، ومدة الأعراض، والتدخين، في حين لم يتم العثور على علاقة ذات دلالة بين مؤشر كتلة الجسم (BMI)والالتزام بنظام (MEDAS) الغذائي.

عند استخدام مؤشر Shannon لتقييم تنوع البكتيريا المعوية بين مرضى القولون العصبي وفقًا لشدة الأعراض، لوحظ وجود اختلافات في التنوع الميكروبي. كما ظهرت علاقة واضحة بين التدخين، واضطرابات النوم، والضغوط النفسية، بينما لم يكن هناك فرق ملحوظ في مؤشر كتلة الجسم، أو النشاط البدني، أو الالتزام بالنظام الغذائي MEDAS.

الاستنتاج: تُظهر هذه الدراسة وجود اختلافات في تنوع البكتيريا المعوية بين مرضى القولون العصبي والأفراد الأصحاء، وكذلك بين مجموعات المرضى بناءً على شدة الأعراض. كما تم إثبات العلاقة القوية بين الضغوط النفسية، والتدخين، واضطرابات النوم مع أعراض القولون العصبي، مما يؤكد أهمية مراعاة العوامل النفسية ونمط الحياة عند معالجة المرضى.

تؤكد هذه النتائج على الحاجة إلى نهج علاجي متكامل يشمل التدخلات في نمط الحياة والصحة النفسية إلى جانب العلاجات الدوائية التقليدية.

الكلمات المفتاحية: متلازمة القولون العصبي (IBS)، نظام MEDAS الغذائي، محور الأمعاء والدماغ، البكتيريا المعوية، شدة الأعراض.