APPROVAL

We certify that we have read the thesis submitted by Layla Herfi titled "**DIAGNOSTIC USE OF GREMLIN GENE EXPRESSION IN ULCERATIVE COLITIS**" and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Educational Sciences.

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DIAGNOSTIC USE OF GREMLIN GENE EXPRESSION IN

MASTER THESIS

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NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MOLECULAR MEDICINE

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M.Sc. THESIS

Layla HERFI

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Name and Surname of the Student

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Layla Herfi

Abstract

Diagnostic Use of Gremlin Gene Expression in Ulcerative Colitis

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Background - Ulcerative colitis (UC) is a persistent chronic inflammation of the colon and rectum. Recent studies suggest that the gremlin gene, *GREM1*, may influence UC growth and progression. Genetic research has identified many susceptibility genes that contribute to the onset and progression of UC. The "*GREM*" gene produces the "Gremlin" protein, which blocks BMP signaling. Inflammatory diseases and cancer are linked to BMP pathway dysregulation.

Aim- The objective of this study was to examine the diagnostic utility of GREM1 gene expression in UC. If a significant association is established between the expression of GREM1 gene and UC, additional research will be conducted to assess its viability for routine diagnostic purposes.

Materials and Methods - The cases with histopathological diagnosis of UC, non-specific colitis, and normal mucosa were identified at a private university hospital between 2021-23. The process of RNA isolation was conducted to extract total RNA from the biopsy samples, followed by cDNA synthesis to convert the RNA into complementary DNA (cDNA). The PCR conditions for specific gene amplification were optimized using Gradient PCR. The expression levels of the target genes were quantitatively measured using quantitative real-time PCR (RT-qPCR).

Results – A total of 50 cases were included. The correlation analysis between the total values of *GREM1* and the Housekeeping gene yielded a negative correlation coefficient and a p-value exceeding 0.0001, indicating a lack of statistical significance. This finding suggests that the expression of *GREM1* may not be beneficial in the diagnosis of UC.

Keywords: Ulcerative colitis, Gremlin gene, Biomarker, Gene expression, BMP.

SUMMARY

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that has a global prevalence and affects a substantial number of individuals. This condition exerts a considerable influence on the overall well-being and quality of life of affected patients. The timely and precise identification of a medical condition is of utmost importance in order to enhance the treatment and the patient's results. The gene known as *GREM1*, commonly referred to as the gremlin gene, has recently garnered attention as a potential biomarker in the context of UC. This gene is of interest due to its involvement in the regulation of the bone morphogenetic protein (BMP) pathway, which is known to play a crucial role in both inflammation and tissue homeostasis. Nevertheless, the extent to which *GREM1* can be used as a diagnostic tool in the context of UC has not been thoroughly investigated. The objective of this study was to examine the association of UC and the expression levels of *GREM1*. The research encompassed the process of extracting RNA from biopsy samples, subsequently synthesizing complementary DNA (cDNA), and conducting real-time quantitative polymerase chain reaction (RT-qPCR) analysis to measure the expression of *GREM1*. The findings indicated a lack of statistical significance in the association of *GREM1* expression and UC, implying that additional factors may exert a more substantial influence on the pathogenesis of the disease. Notwithstanding these constraints, the present study elucidates the potential of *GREM1* as a diagnostic biomarker in the context of UC. To confirm these findings, more research is needed with larger sample sizes and a wider range of patient populations. This will help researchers learn more about how *GREM1* might affect how diseases progress and how well treatments work. Also, a thorough look at how *GREM1* and other genes involved in the pathways that lead to UC interact with each other may lead to a deeper understanding of how this disease works. The research findings have broader implications beyond the realm of diagnosis, as they provide opportunities for the development of targeted therapies and personalized medicine strategies for individuals diagnosed with UC.

By gaining a deeper comprehension of the molecular mechanisms associated with *GREM1*, healthcare professionals can devise more efficacious therapeutic approaches aimed at managing inflammation, mitigating the severity of diseases, and augmenting the overall well-being of patients. In conclusion, this thesis makes a valuable contribution to the continuous endeavors aimed at enhancing the administration of UC and promoting the field of precision medicine in the realm of gastroenterology.

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List of Abbreviations:

5-ASA- 5-aminosalicylic acid

APCs- Antigen-presenting cells

BMP - Bone morphogenetic protein

Buffer PKD – Proteinase K digestion

Buffer RPE – Second RNA wash buffer with ethanol

CAC – Colitis associated colorectal-cancer

CDI – Clostridioides difficile

cDNA - Complementary DNA

CMV - Cytomegalovirus

COPD – Chronic obstructive pulmonary disease

CpG - Cytosine and guanine

CST – Cell signaling technology

DNAse - Deoxy ribonuclease

dNTPs - Deoxy ribonucleotide triphosphates

EDTA – Ethylene-diamine-tetraacetic acid

EMR – Endoscopic mucosal reaction

EMT - epithelial-mesenchymal transition

ESCC - human esophageal squamous cell carcinoma

ESD – Endoscopic mucosal dissection

EUS- Endoscopic ultrasound

FAO – Fatty Acid Oxidation

FGF4- Fibroblast growth factor

Fig. - Figure

Fwd primer - Forward Primer

FC - Fulminant Colitis

GO - Gene Ontology

GREM1 - Gremlin 1

GWA – Genome-wide association

HLA – Human leukocyte antigen

IBD – Inflammatory bowel disease

IL - Interleukin

IPAA - Ileal Pouch-Anal Anastomosis

lncRNAs - long non-coding RNAs

JAK – Janus kinase

Mad-CAM1 - Mucosal Addressin Cellular Adhesion Molecule-1

MAPK – Mitogen activated protein kinase

miRNAs - microRNAs

mL – Milliliter

MM – Master mix

mRNA - Messenger RNA

ncRNAs - Non-coding RNAs

NSAIDs – Nonsteroidal anti-inflammatory medicines

PCR - Polymerase Chain Reaction

PRDC – Protein related to Dan and Cerberus

p-Value- P

QOL - Quality of Life

RA – Rheumatoid arthritis

Rev primer - Reverse Primer

RNA - Ribonucleic Acid

RNase – Ribonuclease

Rev primer - Reverse Primer

RT - Reverse transcriptase

RT-qPCR - Real-Time Quantitative Polymerase Chain Reaction

SHH – Sonic hedgehog signaling

SMAD - Sma and Mad-Related Protein

TAE – Tris-acetate EDTA

TBE – Tris-borate EDTA

Th1 - T-helper 1

Th17 - T-helper 17

Th2 - T-helper 2

TNF - Tumor Necrosis Factor

TGF - Transforming Growth Factor

UC - Ulcerative Colitis

USAG1 – Uterine sensitization-associated-gene 1

UV – Ultraviolet

VEGFR2 – Vascular endothelial growth factor receptor 2

WGS- Whole genome sequencing

WNT – Wingless-related integration site

µl - Microliter

µM – Micromole

CHAPTER I

Introduction

Statement of the Problem

Ulcerative colitis (UC) is a persistent inflammatory condition of the colon and rectum, classified as a chronic inflammatory bowel disease. The present diagnostic techniques for UC, such as endoscopy and histopathology, are restricted by factors such as invasiveness, cost, and variability in interpretation. Consequently, an imperative exists for a dependable and non-intrusive diagnostic methodology that can precisely detect and track UC. The expression patterns of the Gremlin gene have exhibited promising indications as a biomarker for UC. Nevertheless, complete clarification of the diagnostic efficacy of gremlin gene expression in UC is yet to be achieved.

The problem statement underscores the inadequacies of current diagnostic techniques for UC and recognizes the prospective utility of the gremlin gene as a diagnostic biomarker. The statement underscores the necessity for additional inquiry into the diagnostic utility of gremlin gene expression in the context of UC. The problem statement may be utilized as an initial step in developing research objectives, constructing an investigation methodology, and assessing the diagnostic effectiveness of the gremlin gene in the diagnosis of UC.

Purpose of the study

The aim of the investigation concerning the diagnostic utility of *GREM1* expression in UC is to assess and appraise the feasibility of the *GREM1* as a biomarker for the purposes of diagnosing and monitoring UC.

We have evaluated the variance in expression levels of the *GREM1* between individuals diagnosed with UC and those who are in a healthy state of well-being or diagnosed with nonspecific colitis.

Research Questions / Hypotheses

The expression levels of the Gremlin gene have the potential to function as a diagnostic indicator for UC. This could facilitate precise discrimination and identification of individuals with UC from those who are healthy or have other gastrointestinal disorders.

Does the severity and progression of UC correlate with the level of the Gremlin gene's (*GREM1*) expression?

Significance of the study

Enhanced Diagnostic Precision: If the investigation confirms the diagnostic efficacy of gremlin gene expression in UC, it has the potential to enhance the precision of UC diagnosis. This phenomenon has the potential to facilitate prompt identification and intervention, thereby facilitating timely and suitable disease management.

The conventional diagnostic techniques for UC, including endoscopy and histopathology, are associated with invasiveness, discomfort, and high expenses. In the instance where research illustrates the effectiveness of gremlin gene expression as a non-invasive diagnostic indicator, it has the potential to provide an alternative approach that is more amenable to patients and involves less invasiveness.

The investigation of the correlation between the expression of the gremlin gene and the severity of disease or clinical outcomes can potentially impact personalized medicine and treatment approaches for individuals with UC. The identification of patients who are at an elevated risk of disease progression could potentially facilitate the implementation of more intensive treatment strategies to improve clinical outcomes.

The investigation's results regarding the association between the expression of the gremlin gene and the severity of the disease may have significant implications for the surveillance of disease activity in individuals with UC. The analysis of gremlin gene expression has the potential to function as a biomarker for evaluating disease progression or treatment response, thereby enabling more precise and efficient disease management. Therapeutic targeting may be a viable option if the study establishes a substantial correlation between the expression of the gremlin gene and UC. This could potentially lead to the development of targeted therapies that focus on modulating the gremlin pathway.

Limitations

The generalizability of the findings may be impacted by the limited sample size of the study. The limited number of participants in the study may not be sufficient to accurately reflect the heterogeneous population of individuals with UC, thereby constraining the generalizability of the findings.

Selection bias may occur in a study if it is based on a particular subset of individuals with UC or if certain populations, such as those with concurrent medical conditions or atypical disease presentations, are excluded. The potential impact of this could be on the ability to generalize the findings to the wider UC population.

The disease known as UC is characterized by heterogeneity, as evidenced by the presence of variations in clinical presentation, disease severity, and treatment response. The potential heterogeneity of UC patients may not have been adequately considered in the study, which could restrict the generalizability of the results to various subpopulations.

The impact of confounding factors, including but not limited to medication utilization, disease activity, coexisting medical conditions, and lifestyle factors, may not be sufficiently regulated or adjusted for in the research methodology. The aforementioned factors have the potential to exert an influence on the expression of gremlin genes, thereby giving rise to biased or deceptive associations.

The analysis of gremlin gene expression may present technical challenges, including potential discrepancies in sample collection, RNA isolation, and gene expression analysis methodologies. Procedural inconsistencies have the potential to introduce variability, which can have a negative impact on the reliability and reproducibility of the outcomes.

External validation of the study's findings may be necessary in separate cohorts to ascertain the dependability and applicability of the outcomes. The absence of validation in diverse populations or research contexts may constrain the robustness of the study's findings.

Definition of Terms

Ulcerative colitis - It is classified as a chronic inflammatory bowel disease (IBD) characterized by aberrant immune system responses that result in inflammation and ulceration of the mucosal lining of the colon.

GREM gene - The gene *GREM1*, also known as Gremlin 1, belongs to the DAN family of BMP antagonists and encodes a protein. *GREM1* has been found to be associated with various diseases, such as polyposis syndrome, hereditary mixed polyposis syndrome, and hereditary mixed polyposis syndrome. The signaling by BMP and angiogenesis (CST) pathways are associated with the subject matter. The gene in question has been associated with cytokine activity and BMP binding based on its Gene Ontology (GO) annotations. *GREM2* is a significant paralog of the gene.

Bone morphogenetic pathway -The bone morphogenetic proteins (BMPs) are a cohort of signaling molecules that pertain to the Transforming Growth Factor-β (TGF-β) superfamily of proteins. Bone morphogenetic proteins (BMPs) were originally identified for their osteogenic properties but have since been recognized as pivotal players in various physiological systems.

CHAPTER II

Literature review

Ulcerative colitis (UC) is a persistent inflammatory condition of the gastrointestinal tract that primarily impacts the colon and rectum. This disease manifests itself through various symptoms, including abdominal pain, diarrhea, and rectal bleeding. The study of UC holds great importance due to its widespread occurrence and profound implications for a large population worldwide, leading to significant implications for their overall well-being. The importance of a timely and precise diagnosis cannot be overstated, as it is compounded by the difficulties associated with differentiating it from various other gastrointestinal disorders. Comprehending the progression of disease is of utmost importance, as UC encompasses phases of remission and flare-ups, resulting in persistent inflammation and damage to the colon. The ongoing development of therapeutic interventions plays a pivotal role in effectively addressing symptoms and improving the overall quality of life for patients. In addition, the examination of genetics, environmental influences, and personalized medicine holds promise for yielding significant insights into the fundamental mechanisms of diseases and advancing the creation of more targeted therapeutic interventions. Recognizing the significance of UC in healthcare systems is imperative for optimizing resource allocation.

The early 20th century marked the discovery of bacterial associations with intestinal illness, including Bacillus coli (1909), streptococci (1911), and B. coli communis (1913). This period also saw increased attention given to the bacterial causes of UC. Despite failing to meet Koch's prerequisites, the potential involvement of bacteria had a significant impact on the management of UC for a prolonged period. Hurst administered a polyvalent anti-dysenteric serum via intravenous injection, while Leusden administered an autologous fecal bacteria vaccination. Later, sulfonamides and antibiotics were extensively employed. Matthew Baillie's publication titled "Morbid Anatomy of Some of the Most Vital Parts of the Human Body" suggests that UC was a probable cause of patient mortality during the latter part of the 18th century. In 1859, Samuel Wilks, a London-based medical practitioner, authored the initial "impactful" account of "ulcerative colitis," citing the case of a 42-year-old woman who succumbed to prolonged bouts of fever and diarrhea.

The clinical presentations comprise the rectum, which is the distal part of the colon, being extensively affected as the principal location of inflammation, and a well-defined confluent inflammation pattern that ceases abruptly with a clear demarcation and transitions into the normal mucosa of the colon. There is evidence to suggest that smoking may have a protective effect, and it has been observed that UC often presents itself following smoking cessation. During the post-mortem examination, an observation was made of transmural ulcerative inflammation in the colon and terminal ileum. At first, the medical diagnosis assigned to this condition was "simple ulcerative colitis," but subsequent analysis revealed that it was indeed Crohn's disease (Porter, Kalla, & Ho, 2020). Wilks and Moxon's 1875 case report documented an early instance of UC, wherein a young woman's entire colon was afflicted with inflammation and ulceration, ultimately leading to her demise due to severe bloody diarrhea (Kirsner, 2001).

The initial pathological observations of UC indicated widespread mucosal and submucosal engagement, which commenced in the rectum and rectosigmoid and advanced towards the proximal end to affect the complete colon. This condition was characterized by diffuse inflammation of the mucous membrane, accompanied by the presence of lymphoid cells, eosinophils, and plasma cells, chronic inflammatory cells, vascular congestion, depletion of goblet cells, and the formation of crypt abscesses. The phenomenon of inflammatory necrosis affecting arteries, veins, or both, resulting in vascular occlusions and subsequent colon infarction, was first documented by S. Warren and S. Sommers in 1953 in a cohort of patients diagnosed with UC. Buie and Bargen identified vascular "thrombotic phenomena" as the underlying pathological mechanism for UC in 1933. UC is a chronic ailment that impacts the gastrointestinal tract. This condition is characterized by abnormal inflammation of the inner surfaces of the rectum and colon, which collectively constitute a significant portion of the large intestine. Inflammation and ulceration of the large intestine are common occurrences. While the onset of this condition can occur at any point in an individual's life, it frequently presents itself for the first time during the period spanning from 15 to 30 years of age. Throughout an individual's lifespan, inflammation often experiences periodic exacerbations, leading to the recurrence of various indications and symptoms.

In a prospective study carried out in the United Kingdom, it was observed that second-generation South Asian immigrants displayed a greater prevalence of UC when compared to the European population. The rates of UC were found to be 17.2 and 7 per 100,000 population per year for second-generation South Asian immigrants and the European population, respectively. The reported range of incidence and prevalence of UC spans from 1.2 to 20.3 (Aslam, et al., 2022).

The progress in medical therapy has facilitated the efficacious treatment of UC, thereby diminishing the necessity of surgical intervention. It has been reported that a significant proportion of patients diagnosed with UC, approximately 30%, will necessitate surgical intervention at some point in their lifetime. Moreover, within the first year of diagnosis, approximately 10% of individuals will require surgical treatment. People who have been diagnosed with UC often have their first surgery be a restorative proctocolectomy with ileal pouch-anal anastomosis (IPAA). The existing body of research has predominantly indicated an improvement in the health-related quality of life (QOL) for individuals who have been diagnosed with severe UC (Aslam, et al.).

The etiology of IBD is believed to stem from an inequilibrium between the Th1 and Th2 immune cell subsets. Nevertheless, current research has established that the Th17 subset and its related cytokines also play a crucial role as inflammatory mediators, operating autonomously from the anti-TNF mechanism.

Additionally, research has shown that *IL-23* upregulates Th17-driven inflammation while *IL-12* primarily elicits a Th1 response in the human body. The aforementioned data has instigated extensive investigation into *IL-12* and *IL-23*, leading to the development of Ustekinumab, an antibody that selectively targets the commonly shared p40 sub-unit of anti-*IL12* and 23. The primary objective of the UNIFI study was to evaluate the effectiveness of ustekinumab in individuals diagnosed with moderate to severely active UC, following its successful application in treating moderate to severely active Crohn's disease, as documented in previous trials (Gajendran, et al., 2019).

Gremlin 1, a glycoprotein that is widely preserved, belongs to the Cerberus and Dan subfamilies of BMP antagonists that are secreted. It exhibits a more favorable interaction with BMP2, BMP4, and BMP7. The protein *GREM1* plays a crucial role as an antagonist of BMP in ensuring proper limb outgrowth and patterning during the developmental stages of vertebrates.

GREM1 expression is a crucial requirement for cellular proliferation and branching morphogenesis during the formation of the lung and the organogenesis of the kidney. Aberrant expression in adulthood has been associated with orofacial clefting, osteoarthritis, spontaneous bone fractures, liver fibrosis, lung fibrosis, and renal fibrosis. The impact of *GREM1* on BMP seems to be independent of its proangiogenic and proinflammatory properties, as per the available evidence (Ren, Laria, & Smid, 2019).

As per previous research, Gremlin has the potential to elicit a proinflammatory response in endothelial cells, leading to an upregulation of proinflammatory molecules that are implicated in leukocyte extravasation. The discovery suggests that the gremlin protein could potentially serve as a proinflammatory agent within cellular systems. The current study showed that the process of epithelial-mesenchymal transition (EMT) in human esophageal squamous cell carcinoma (ESCC) was helped by the presence of *GREM1*, a protein that is released by mesenchymal stromal cells. The observed effect was found to be partially mediated via the transforming growth factor (TGF)/BMP signaling pathway. The TGF family comprises three mammalian members, namely TGF-1, TGF-2, and TGF-3, which have been observed to exert an impact on diverse physiological processes. The activation of the TGF-signaling pathway is typically facilitated by transcription factors belonging to the SMAD family (Qu, Liu, & Wang, 2020).

Epidemiology And Aetiology of Ulcerative Colitis

With an annual incidence of around 10 per 100,000 individuals, UC affects around 300 persons per 100,000 people in affluent nations, including the United States. Since incidence and prevalence rates for UC are about same in men and women, unlike most autoimmune illnesses, it does not appear to favor one sex over the other. Early adulthood is the most frequent age for UC to manifest. But it may happen to anybody, at any age. Approximately 35% of new instances of UC in 2018 were in adults aged 17 to 40, according to a recent study of the disease's epidemiology throughout time in Great Britainopens in a new tab or window, with roughly 25% of new cases occurring in both teenagers and those aged 40 and older. The same statistics showed that these rates had significantly changed since 2000, with incidence declining by nearly one-third in older people while doubling in those between the ages of 10 and 16 (Gever, 2022).

Socioeconomic Status

The impact of socioeconomic status on individuals with UC can vary significantly and have far-reaching implications for the disease. In these circumstances, there are several pivotal themes to contemplate concerning the socioeconomic determinants that underlie UC.

The accessibility of healthcare resources, including specialists, diagnostic tests, and drugs, may be influenced by an individual's socioeconomic status. Individuals who belong to a lower socioeconomic stratum may encounter challenges in accessing healthcare services that are timely and sufficient, thereby potentially impacting the management and treatment of UC (Ruiz-Casas, Evans, & Limdi, 2021).

One of the challenges in accessing UC pharmaceuticals, treatments, and healthcare services is the issue of affordability, which can pose a significant barrier for individuals with lower socioeconomic status. The expenses associated with treatment, which include but are not limited to prescription medications, frequent medical appointments, and hospitalizations, have the potential to create financial limitations and limit compliance with prescribed treatment plans (Patel, et al., 2018).

The employment and work productivity of an individual can be significantly impacted by UC, leading to decreased work productivity and a potential work disability. Individuals with UC may experience a financial burden, particularly if they encounter limited availability of sick leave, disability benefits, or employment flexibility (Patel, et al.).

The socioeconomic factors associated with UC may have a significant influence on an individual's overall quality of life. The presence of financial impediments, inadequate resources, and challenges in managing the ailment can collectively lead to escalated stress levels and compromised physical well-being. The correlation between healthy aging and socioeconomic status is significant, as older adults with higher wealth tend to exhibit a higher probability of good health (McMaughan, Oloruntoba, & Smith, 2020).

Education and skills may vary depending on the level of socioeconomic status, which can affect an individual's understanding and familiarity with UC. Individuals belonging to a lower socioeconomic stratum may encounter restricted access to information pertaining to illnesses, preventive measures, and treatment options. This phenomenon may impact an individual's ability to make informed decisions pertaining to their healthcare (Zajacova & Lawrence, 2018).

It is critical to understand that socioeconomic issues might have an impact on UC administration and results. Efforts to increase healthcare accessibility, cost, patient education, and support systems can all contribute to reducing the socioeconomic inequalities associated with UC. Healthcare professionals, patient advocacy organizations, and public health programs all play an important role in increasing awareness, improving access to care, and promoting equitable healthcare for people with UC from all socioeconomic backgrounds.

Pathophysiology of Ulcerative Colitis

It is crucial to have a thorough understanding of the cellular components that make up the colon in order to investigate the molecular mechanisms underlying UC. The innermost layer of the colon, known as the mucosa, is comprised of a single-layered columnar epithelium that is characterized by a delicate brush border.

The previously mentioned epithelium plays a crucial role in the maintenance of gut homeostasis through its functions as a physical and biochemical barrier, a central point for coordinating immune defense, and a mechanism for facilitating interactions between bacteria and immune cells. It also refers to invasions characterized by the presence of 'crypts of Lieberkühn'. The crypts harbor intestinal stem cells that play a crucial role in the effective regeneration of the intestinal epithelium. The differentiation of these stem cells occurs through the involvement of transitory proliferative cells, and as they progress through the transition zone, they undergo additional differentiation.

Ultimately, the differentiated intestinal epithelial cells are shed into the lumen. Intestinal epithelial stem cells have the potential to differentiate into a variety of cell types, including but not limited to enterocytes, paneth cells, goblet cells, and neuroendocrine cells. The gut is primarily composed of absorptive cells, with the notable distinction of crypt cells that are predominantly secretory in nature (Kaur & Goggolidou, 2020).

UC pathophysiology is attributed to various factors. The gut microbiota displays a reduction in diversity and a change in metabolic equilibrium, as evidenced by a decrease in short-chain fatty acids. The hallmark characteristic of UC is a diminished synthesis of mucin 2, a type of colon mucin, within the mucus layer. The diminution of the mucus layer and the modifications in the microbiota result in a disruption of the epithelial barrier, thereby facilitating the ingress of the microbiota. The disruption of the intestinal epithelium caused by apoptotic foci and altered expression of tight junction proteins leads to increased permeability of the barrier, allowing for greater passage of microbes.

This event activates macrophages and antigen-presenting cells (APCs), ultimately resulting in the expression of chemokines that attract neutrophils (Kobayashi, Siegmund, & Le Berre, 2020). There are two distinct mechanisms that can affect leukocyte recruitment. The pathogenesis of UC involves an upregulation of *CXCL8*, which serves as a chemoattractant for leukocytes originating from the systemic circulation towards the mucosal layer. The mucosal blood vessel endothelium displays an increase in mucosal addressin cellular adhesion molecule-1 (Mad-CAM1), assisting in the attachment of leukocytes and their movement out of the blood vessels and into the mucosal tissue (Lynch & Hsu., 2022). The etiology, level of inflammation, and illness phenotype are significantly impacted by the enteric microbiota. The equilibrium between the host's mucosal immunology and intestinal microbiota seems to be in a state of homeostasis, potentially playing a role in the development of UC. Consequently, non-pathogenic microorganisms elicit an atypical response (Lynch & Hsu.).

Types of Ulcerative Colitis

Ulcerative colitis, referred to as UC, is categorized as an inflammatory bowel disease (IBD) characterized by chronic inflammation of the colon and rectum. There are multiple types or classifications of UC, which are determined by various criteria.

1-Ulcerative Proctitis a particular form of UC that mainly affects the rectal area. Given that the inflammation is confined to the rectal area, this represents the least severe and most restricted form of UC. Ulcerative proctitis is characterized by inflammation that is confined to the rectum, which represents the distal portion of the large intestine. The inflammatory response is localized to a specific site.

Clinical manifestations of ulcerative proctitis comprise rectal bleeding, commonly observed as hematochezia or blood on sanitation tissue, and urinary urgency. Additional symptoms encompass discomfort in the rectal area, inflammation, and the passage of mucus.

Ulcerative proctitis is typically regarded as a milder manifestation of UC; however, in some instances, it may progress to involve more extensive portions of the colon, leading to distinct forms of UC such as proctosigmoiditis or left-sided colitis. In order to identify the progression of an illness, it is imperative to engage in consistent monitoring and adhere to a regimen of follow-up appointments with a healthcare professional. The therapeutic approach for ulcerative proctitis aims to mitigate inflammation, alleviate symptoms, and attain remission. Topical rectal therapies such as mesalamine suppositories or enemas are commonly employed as the primary mode of treatment to specifically target inflammation in the rectal region. Under specific conditions, healthcare professionals may suggest the administration of oral medications, such as aminosalicylates or corticosteroids. (Burakoff & Karagozian, 2007).

The topic of interest is the management of diseases. Apart from medical interventions, recommendations for lifestyle modifications and dietary adjustments may be suggested to ameliorate symptoms and enhance the overall well-being of the gastrointestinal tract. Frequent consultations with a gastroenterologist are imperative to oversee the progression of the disease, modify treatment modalities as necessary, and manage any complications (Leiper & Moris, 2007).

2- Protosigmoiditis a form of UC is characterized by rectus and sigmoid colon inflammation. When compared to ulcerative proctitis, the manifestation of UC in the sigmoid colon is deemed to be a more severe variant of the ailment owing to the spread of inflammation beyond the rectum and into the inferior segment of the colon. Proctosigmoiditis is distinguished by the following characteristics:

The rectum and sigmoid colon exhibit inflammation, which is a hallmark of protosigmoiditis. The anatomical term "sigmoid colon" denotes the curved portion of the large intestine located immediately superior to the rectum, which assumes an S-shaped configuration. The response to inflammation is localized to the sigmoid colon.

Proctosigmoiditis is characterized by clinical manifestations such as hematochezia, abdominal cramps or discomfort, and an increased frequency of defecation. Rectal bleeding, the passing of mucous, and a sensation of incomplete evacuation of stool may also be experienced by certain individuals.

Disease Progression: It is regarded as a minor form of UC since the inflammation is only present in the rectum and sigmoid colon. Under specific conditions, the inflammatory process may extend to a larger area of the colon.

The objective of proctosigmoiditis therapy is to mitigate inflammation, alleviate symptoms, and achieve remission. Aminosalicylates, such as mesalamine, are frequently prescribed pharmaceuticals for the purpose of mitigating inflammation and maintaining remission. In cases of elevated severity, corticosteroids or immunosuppressive medications may be employed.

The management of diseases may involve recommendations for lifestyle and dietary modifications in addition to pharmacological interventions, with the aim of regulating symptoms and promoting optimal gastrointestinal well-being. To assess the level of inflammation and adjust treatment as necessary, routine disease monitoring by colonoscopy or other imaging methods may be necessary (Christophi & et al., 2016).

3- Left-sided colitis is a subtype of UC characterized by inflammation extending from the rectum to the splenic flexure of the colon. The current state differs from proctosigmoiditis in that there is a greater degree of colonic involvement, which causes UC to manifest more severely as a result of inflammation. The hereditary inclination of individuals with left-sided UC in contrast to those with a more widespread condition remains undetermined.

The following are the principal characteristics of colitis that impact the left colonic region. In left-sided colitis, the rectum, sigmoid colon, and descending colon—all of which are on the left side of the colon—are all marked by inflammation. The inflammatory process frequently advances towards the left upper quadrant of the abdominal region.

Common symptoms associated with left-sided colitis comprise rectal bleeding, abdominal pain or cramps that are confined to the left side, a feeling of incomplete evacuation of the bowel, and unintentional weight loss. Certain individuals may encounter symptoms of fatigue and fever.

The course of UC is recognized to exhibit variability contingent on the site of the inflammatory process. Particularly, it has been found that proctosigmoiditis is a milder type of ailment than left-sided colitis. Under certain conditions, an inflammatory response may expand to encompass both the transverse and right colon, resulting in the manifestation of pancolitis.

The principal aim of managing left-sided colitis is to mitigate inflammation, effectively handle symptoms, and achieve a state of remission. Pharmaceutical interventions, including aminosalicylates (e.g., mesalamine), corticosteroids, immunosuppressants, or biologic medicines, may be recommended depending on the severity of symptoms and the extent of inflammation.

The management of left-sided colitis involves medication, lifestyle modifications, and dietary adjustments. Additionally, it may entail consistent monitoring through colonoscopy or other imaging methods to gauge the degree of inflammation, assess the efficacy of treatment, and identify any potential complications. In cases where pharmacological interventions prove ineffective in mitigating symptoms or where complications such as profuse hemorrhaging or the development of a toxic megacolon arise, surgical measures may become necessary (Haghighi & Lashner, 2004).

4-Pancolitis, also known as universal colitis in scholarly literature, is a variant of UC characterized by significant inflammation throughout the colon. The sigmoid colon, ascending colon, descending colon, and rectum are all affected by this form of UC. These further characteristics are noteworthy features of pancolitis:

Pancolitis is a pathological state characterized by inflammation that spans the entirety of the colon, including the area from the rectum to the cecum, which represents the anatomical juncture between the colon and the small intestine. The anatomical structures that comprise the sigmoid colon, ascending colon, descending colon, transverse colon, and rectum are all included.

The clinical manifestations of this condition encompass hematochezia, abdominal distress or spasms, increased frequency of defecation, reduction in body mass, fatigue, and pyrexia. The symptoms associated with this type of UC are often more severe in comparison to other forms due to the prevalence of inflammation throughout the body.

UC is a condition that presents with varying degrees of severity, with pancolitis being the most severe manifestation. This form of UC is characterized by inflammation that affects the entire colon. In severe cases, pancolitis may be linked to adverse outcomes such as toxic megacolon, colon perforation, or an elevated susceptibility to colon cancer. It is imperative to engage in consistent monitoring and seek counsel from a gastroenterologist to evaluate the progression of the illness.

The utilization of colonoscopy is considered an established method for characterizing UC, as it enables a direct evaluation of the ongoing mucosal inflammation. In certain cases, the assessment of disease activity may necessitate the utilization of a colonoscopy. The procedure is characterized by a high cost, invasiveness, and the need for patient preparation, resulting in prolonged waiting times and discomfort. Additionally, there exists a small yet noteworthy probability of complications. Hence, it is plausible to employ a serum marker, namely endocan, or a stool marker, namely fecal calprotectin, for the dual purpose of monitoring patients and determining the appropriate course of treatment for the ailment (Albayrak & Sebin, 2023).

5-Fulminant Colitis (FC) is a highly severe form of UC that has the potential to result in death. The condition is characterized by a pervasive inflammatory response and a rapid onset of clinical manifestations. Fulminant colitis necessitates prompt medical intervention and is often associated with hospitalization.

The following are key attributes of fulminant colitis:

Severity of Symptoms: Fulminant colitis is typified by pronounced symptoms that exert a significant influence on an individual's well-being and daily activities. Possible symptoms include notable abdominal discomfort, persistent and abundant bloody diarrhea, dehydration, elevated body temperature, rapid heart rate, a considerable reduction in body weight, and indications of systemic inflammation.

The occurrence of fulminant colitis may lead to the development of toxic megacolon, a life-threatening condition that is distinguished by an extensively enlarged colon. Additional hazards comprise colon perforation, sepsis (systemic infection), and an elevated susceptibility to colon cancer.

Prompt medical intervention is necessary for patients with fulminant colitis owing to the gravity of their symptoms and potential ramifications. Frequent observation, administration of intravenous fluids and electrolytes, provision of nutritional support, and pharmacological intervention aimed at reducing inflammation and alleviating symptoms are typical rationales for hospital admission.

The therapeutic interventions available for fulminant colitis encompass the reduction of inflammation, prevention of complications, and stabilization of the patient's condition. The initial treatment strategy for reducing inflammation expeditiously frequently involves the administration of corticosteroids via intravenous route. In cases where corticosteroids fail to provide substantial relief, alternative interventions such as biologic therapy, immunomodulators, or surgical procedures may be considered.

In cases of fulminant colitis, a surgical procedure known as total colectomy involves the complete removal of the colon, which may be followed by the creation of either an ileostomy or a pouch operation, also referred to as restorative proctocolectomy (Rubin & et al., 2021).

The etiology of severe and fulminant colitis remains elusive in a significant number of cases. Studies have demonstrated that colitis can be aggravated by the presence of Clostridioides difficile (CDI) and cytomegalovirus (CMV) infection. Severe and fulminant colitis may be instigated by various factors, such as polypharmacy, discontinuation of smoking, noncompliance with maintenance therapy, and pseudo-medical resistance, particularly mesalamine intolerance (Wang & et al., 2019).

Involvement Pattern of Ulcerative Colitis

Ulcerative colitis (UC) is not an infectious ailment and therefore lacks the ability to propagate from one individual to another through infection. The multifactorial etiology of UC is postulated to encompass environmental, genetic, and immunological factors. The precise etiology of UC remains incompletely elucidated; however, it is postulated to arise from an aberrant immune response within the gastrointestinal tract.

UC is characterized by the potential to spread throughout the colon and impact multiple segments. This phenomenon is primarily due to the inflammation that originates within the individual's body as opposed to any external factors. UC usually exhibits a progressive spread, originating in the rectum and potentially extending to the proximal segments of the colon.

The subsequent variables could potentially facilitate the dissemination or progression of UC: The term "immunological dysregulation" pertains to an aberration in the standard operation of the immune system. UC is postulated to originate from an aberrant immune response within the gastrointestinal tract, wherein the immune system erroneously targets the mucosal lining of the rectum and colon. The persistent immunological response has the potential to lead to the spread of inflammation all over the colon (Lee, Kwon, & Cho, 2018).

The intensity of inflammation and its distribution pattern in the colon may be influenced by genetic factors. The development and progression of UC may be subject to the influence of various environmental factors, including but not limited to nutrition, stress, smoking, and specific medications.

It is imperative to bear in mind that while these variables may exacerbate symptoms or trigger flare-ups, they do not directly precipitate the transmission of UC (Vedamurthy & Ananthakrishnan, 2019).

Genetics

The susceptibility and pathogenesis of UC are significantly influenced by genetic factors. Genetic studies have identified multiple genes that are linked to UC. These genes participate in diverse biological processes such as immune regulation, inflammation, epithelial barrier function, and microbial interactions. The intracellular pattern recognition receptor *NOD2/CARD15* has been linked to detecting bacteria, and genetic mutations in this gene have been found to make people more likely to get UC. The association between susceptibility to UC and genetic factors of notable significance has been established. Notably, the *IL23R* receptor, which controls the immune response, and the cytokine *IL10*, which reduces inflammation, have been found to be important genetic factors in this case.

The *NOD2/CARD15* gene encodes the *NOD2* protein, which functions as an intracellular pattern recognition receptor that is responsible for detecting bacterial components. The presence of mutations in the *NOD2* gene has been correlated with an elevated susceptibility to the onset of UC. The *NOD2* molecule is of paramount importance in the regulation of the immune response to bacterial pathogens. Its malfunction can result in an atypical immune response and subsequent inflammation in the gastrointestinal tract.

Interleukin-10, commonly abbreviated as *IL10*, is a type of cytokine that possesses anti-inflammatory properties and plays a crucial role in regulating immune responses. The linkage between UC susceptibility and genetic variations in the *IL10* gene underscores the significance of adequate immune regulation in the pathogenesis of UC. The cytokine *IL-10* functions as an anti-inflammatory agent and plays a crucial role in preserving immune homeostasis within the gastrointestinal tract. Disruptions in immune regulation leading to the development of UC can be attributed to defects in the production or function of *IL10*.

Tumor necrosis factor (TNF) is a cytokine that is known to exert a pivotal role in immune responses through its ability to stimulate and enhance inflammatory processes. It has been shown that polymorphisms in the *TNF* gene make people more likely to get UC and make the condition worse when it does happen. This observation implies that dysregulation TNF signaling might be implicated in the development and progression of UC.

TNF plays a crucial role in the facilitation of inflammation and tissue injury in individuals with UC. The potential contribution of anomalies in TNF production or signaling pathways to the persistent inflammation characteristic of this disease is noteworthy.

Epigenetics Modifications

The term "epigenetics" was initially employed to denote the intricate interplay between the genome and the environment that underlies the processes of development and differentiation in advanced organisms. Currently, the expression is employed to denote inheritable modifications that do not arise from variations in the nucleotide sequence of DNA. Modifications of this nature have the potential to impact the regulation of gene expression, leading to changes in cellular activity and conduct. Epigenetic modifications encompass DNA methylation, histone modifications, and gene regulation mediated by non-coding RNA (Handy, Castro, & Loscalzo, 2011).

The process of DNA methylation entails the insertion of a methyl group into a DNA molecule, usually at cytosine residues situated in a CpG dinucleotide context, as a biochemical mechanism.

DNA methylation could repress gene expression by preventing the binding of transcription factors and other regulatory proteins to the DNA. The regulation of genes is a crucial mechanism that holds significant importance in the context of normal development as well as various ailments, including cancer and neurological disorders (Phillips, 2008).

DNA is packaged and organized into a small structure called chromatin with the help of proteins called histones. Histone proteins have the ability to undergo various post-translational modifications, such as acetylation, methylation, phosphorylation, and ubiquitination, among others. The alterations possess the capability to influence gene expression by modifying the structure of chromatin. Histone acetylation is frequently associated with gene expression that is active, while histone methylation can have either activating or repressive consequences based on the region and context (Millán-Zambrano, Burton, Bannister, & Schneider, 2022).

Non-coding RNAs (ncRNAs) represent a class of RNA molecules that possess significant regulatory capabilities over gene expression despite their lack of protein-coding capacity. Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have been recognized for their significant impact on the regulation of gene expression. miRNAs have the capacity to interact with mRNAs and modulate their translation into proteins through repression or promotion. (lncRNAs) exhibit various mechanisms by which they can engage with chromatin and regulate the modulation of gene expression (Non-coding RNAs (ncRNAs): a guide, 2023)**,** (Nature Methods, 2022)**.**

Epigenetic changes are reversible and can be triggered by a variety of environmental variables such as food, stress, and pesticide exposure. They are essential in proper development, cellular differentiation, and disease processes. The association between epigenetic dysregulation and a range of medical conditions, such as cancer, neurological disorders, cardiovascular ailments, and autoimmune diseases, has been established.

Presentation

Ulcerative colitis (UC) is linked to a range of symptoms that exhibit variations in both severity and duration. The UC presentation may encompass instances of diarrhea. The prevailing manifestation of UC is characterized by the persistence of repeated and prolonged episodes of hematochezia. The frequency and urgency of bowel movements may demonstrate variability, as may the consistency of fecal matter, which may range from loose to liquid.

The majority of individuals diagnosed with UC commonly experience gastrointestinal pain and cramping. Generally, this sensation of unease is restricted to the inferior left quadrant of the abdominal region. The intensity of discomfort may vary across a spectrum from mild to severe (Menard, 2021).

The occurrence of blood in the feces, known as hematochezia, is a frequently observed symptom of UC. Hematochezia, or the presence of blood in the stool, may appear as a light red or maroon color that is either intermingled with the fecal matter or as distinct streaks (Sissons, 2021).

The sensation of urgency to defecate is often present even when the fecal matter is of minimal volume and is characterized by a consistent and sudden urge to evacuate the bowels.

The elevated state of urgency may potentially disrupt routine activities and impede the ability to rest.

Tenesmus is a prolonged sensation of incomplete bowel emptying or the desire to evacuate feces even when the rectum is vacant. It can induce pain and a persistent need to stretch.

Insomnia: an overall sense of exhaustion can result from chronic inflammation and the body's reaction to UC.

Weight Loss: Those with UC might lose bodyweight in more severe instances or during flare-ups owing to decreased appetite, malabsorption, or higher metabolic needs.

Fever: Fever can develop during acute flare-ups or in the presence of complications such as infection or inflammation-related systemic symptoms.

Extra-intestinal Symptoms: UC can also affect other regions of the body, resulting in extra-intestinal symptoms. Joint discomfort (arthritis), skin rashes, eye inflammation, liver diseases, and mouth ulcers are examples of these.

The way UC manifests itself varies from person to person, and the intensity and mix of symptoms might alter gradually (Levine & Burakoff, 2011).

Stages of Ulcerative Colitis

Mild Stage

At this stage, the condition is distinguished by mild inflammation and limited symptoms. Possible symptoms may include mild diarrhea, which may be accompanied by blood or mucus, mild stomach discomfort, and a generally satisfactory health status. Patients in this phase often exhibit a positive response to therapeutic interventions and may attain remission expeditiously.

Moderate Stage

Moderate UC is characterized by elevated levels of inflammation and increased sensations of discomfort. The incidence and severity of diarrhea, along with gastrointestinal discomfort and muscular contractions, may increase. At this stage, individuals may experience weight loss, fatigue, and a diminished quality of life. In order to alleviate symptoms and attain a state of remission, it may be necessary to utilize therapeutic interventions such as anti-inflammatory pharmaceutical agents.

Severe Stage

The identification of severe UC can be attributed to the presence of severe symptoms and extensive inflammation. The incidence of diarrhea increases and is often accompanied by hematochezia. Common symptoms include abdominal discomfort, cramping, and urinary urgency. Individuals in this phase may exhibit a significant reduction in body weight, extreme fatigue, a low red blood cell count, and impaired daily functioning. In order to achieve remission, it may be necessary to administer more potent medications, such as corticosteroids or biologic therapies.

Fulminant Stage

The stage of UC known as fulminant colitis is regarded as the most severe and has the potential to result in death. It induces a state of extensive inflammation throughout the colon, leading to severe symptoms and potentially fatal outcomes. Possible symptoms of the condition may include severe diarrhea accompanied by frequent and bloody stools, chronic stomach discomfort, fever, dehydration, and indications of systemic inflammation.

In rare cases, hospitalization, intravenous medications, and intensive monitoring may be required for the management of fulminant colitis. In cases where medical intervention proves insufficient or complications arise, surgical intervention may be necessary to excise the affected colon (Pabla & Schwartz, 2020).

Treatment

The goal of UC treatment is to reduce symptoms, induce and sustain remission, and enhance patients' lifestyles and quality of life. Treatment strategies are often tailored to an individual's age, overall health, and drug reaction, as well as the intensity and breadth of inflammation.

Medications

Aminosalicylates (5-ASA) are commonly employed as the initial therapeutic option for mild to moderate UC due to their anti-inflammatory properties. Mesalamine and sulfasalazine are examples of such medications. (Cheifetz & Cullen, 2022)

Corticosteroids, namely prednisone or budesonide, may be prescribed for brief durations to mitigate inflammation during episodes of exacerbation. Nonetheless, owing to potential adverse consequences, extended utilization of the medication is generally not recommended. (Caramori, Mumby, Girbino, Chung, & Adcock, 2019).

Immunomodulators: In situations of moderate to severe UC, pharmaceuticals such as azathioprine, mercaptopurine, or methotrexate may be administered to regulate the immune system and decrease inflammation.

Biologic drugs, such as anti-TNF medications (e.g., infliximab, adalimumab) or other targeted therapy (e.g., vedolizumab, ustekinumab), may be utilized for those with moderate to severe UC who are intolerant to existing treatments or frequently experience flare-ups.

When traditional treatments are not successful, Janus kinase (JAK) inhibitors, such as tofacitinib, may be utilized to manage mild to severe UC. The mechanism of action of these pharmaceutical agents involves the inhibition of the JAK pathway, which is recognized to play a role in the modulation of the immune response. (Li, Zheng, & Chen, 2017).

Symptom Management:

Anti-diarrheal Medications: To treat diarrhea symptoms, over-the-counter or prescription anti-diarrheal medications such as loperamide may be used.

Pain Reducers: Nonsteroidal anti-inflammatory medicines (NSAIDs) should be avoided since they have the potential to exacerbate UC symptoms. For pain management, acetaminophen may be prescribed instead.

Supplements for iron: Iron supplements may be administered to replenish iron levels in situations of anemia caused by continuous bleeding.

Lifestyle Modifications:

Changes in Diet: While individual triggers vary, some people with UC find that avoiding foods, including as spicy or high-fiber meals, can help control symptoms. Keeping a food diary and working with a trained nutritionist might help you discover your own trigger foods.

Stress Prevention: Although stress does not cause UC, it can aggravate symptoms. Exercise, meditation, and therapy can all be excellent stress-reduction approaches.

Surgical Intervention:

Surgical intervention may be advantageous for individuals afflicted with severe UC who exhibit a poor response to treatment or experience complications such as toxic megacolon or significant bleeding. An ileal pouch-anal anastomosis (IPAA) or a permanent ileostomy may be established after the excision of the colon and rectum, commonly known as a proctocolectomy, depending on the unique clinical circumstances. Consistent monitoring, modifications to medication, and alterations to lifestyle can facilitate the management of symptoms, the attainment of remission, and the enhancement of long-term outcomes.

Endoscopic Treatment

Endoscopic procedures are highly conservative surgical interventions that employ an endoscope, a pliable tube equipped with a light source and a camera at its distal end. The management of UC involves the utilization of techniques that enable direct visualization and treatment of the inflamed segments of the colon and rectum.

Endoscopic Mucosal Resection (EMR): EMR is a treatment that eliminates aberrant or precancerous tissue from the colon or rectum lining. It entails lifting and cutting away the targeted tissue with an endoscope. In situations of dysplasia or early-stage malignancy linked with UC, EMR can be useful.

Endoscopic Submucosal Dissection (ESD):(ESD) is an upgraded form of EMR which enables for the removal of bigger lesions or tumors from the colon or rectum. It entails removing the lesion from the digestive tract's underlying layers. Endoscopists with advanced training frequently do ESD.

Balloon Dilation: In individuals experiencing UC, inflammation of the colon or rectum can create strictures or narrowing of the path. Balloon dilation is inserting an endoscope with a balloon connected into the constricted region. After that, the balloon is inflated to expand the route and remove the impediment.

Endoscopic Ultrasound (EUS): The technique known as endoscopic ultrasound (EUS) combines the use of endoscopy and ultrasound imaging. This technique facilitates the examination of the various layers comprising the intestinal wall as well as the adjacent anatomical structures. EUS has the potential to assist in the assessment of inflammation severity in UC and provide valuable guidance for therapeutic interventions.

Endoscopic Therapy for Bleeding: UC can occasionally produce bleeding in the colon or rectum. To reduce bleeding and facilitate regeneration, endoscopic procedures such as thermal coagulation, argon plasma coagulation, or the use of hemostatic clips can be utilized.

Topical Therapies: Endoscopic approaches may also include the direct application of drugs to the afflicted parts of the colon and rectum. Corticosteroids, immunomodulators, and other anti-inflammatory medications may be used to decrease inflammation and speed up healing.

Surgical Treatment

In certain circumstances, surgical therapy for UC may be considered if medicinal medication fails to control symptoms, problems emerge, or the illness is severe. The predominant surgical intervention for UC is the procedure of total proctocolectomy with IPAA. The procedure involves the complete removal of both the colon and rectum, followed by the construction of a pouch utilizing the terminal portion of the small intestine, known as the ileum. The bag has an attachment to the anus, allowing for the elimination of fecal matter. This therapeutic approach preserves anal function while circumventing the need for a permanent ileostomy or stoma.

Another surgical treatment is Ileostomy: When an IPAA is not feasible or the patient is not a good candidate, an ileostomy is an alternate surgical option. This entails making a stoma on the abdominal wall and bringing a tiny opening of the ileum to the surface. The stoma is fitted with a pouch, and waste is extracted in an external bag.

Colectomy with End-Ileostomy: A colectomy with end-ileostomy may be performed if the rectum is unable to survive due to serious medical conditions or complications. The colon and rectum are removed, and the ileum is then attached to the surface of the abdomen in order to create an ileostomy.

After a comprehensive assessment and discussion with a gastroenterologist and a colorectal surgeon, surgical therapy for UC is usually recommended. The decision whether to have surgery is influenced by a variety of criteria, including illness severity, medical care response, quality of life, complications, and patient desire. It's crucial to understand that UC surgery is not a cure, although it can give long-term remission and relief from symptoms in many patients.

Ulcerative Colitis and Gremlin Genes

The aggravated familial risk and the significant phenotypic concordance in monozygotic twins with CD have long been indicative of a genetic foundation for IBD, with an incidence of 5–30% in families of affected individuals and a 50–75% phenotypic concordance. Those who have been diagnosed with Crohn's disease (CD) exhibit a higher likelihood of possessing relatives who have a history of inflammatory bowel disease (IBD) in comparison to those who have been recognized with UC (Rubin, Shaker, & Levin, 2012).

Gremlin proteins, which are both encoded by the genes *GREM1* and *GREM2*, are secreted proteins that have an impact on a variety of physiological processes, including cell division, proliferation, and tissue development. It has been demonstrated that they can interact with and modifying signaling pathways, including the BMP pathway (Luo, Chang, & Yi, 2021).

The present statement asserts that Gremlin-1 (*GREM1*) is a member of the cystine knot superfamily and functions as an antagonist of bone morphogenetic protein (BMP). The regulation of BMPs plays a crucial role in embryonic development, organogenesis, and tissue differentiation (Park, et al., 2020).

The discovery of 260 susceptibility loci (including frequent and uncommon genetic variants) associated with IBD has been made possible by genetic research, including genome-wide association studies (GWA), whole genome sequencing (WGS), and fine mapping investigations (Liu, et al., 2015). The genes that are commonly observed, such as *IL23-R, IL-12, JAK2, CARD9, TNFSF18*, and *IL-10*, could encode both innate and adaptive immunity processes, cytokine signaling, and immunological sensing. Seventy percent of these genes are also detected in autoimmune disorders such as ankylosing spondylitis and psoriasis. The genomic region of chromosome 6 that encodes for human leukocyte antigen (HLA) has been associated with the most prominent genetic indicators within loci that are specific to UC (Goyette, et al., 2015). UC susceptibility is slightly but significantly elevated due to genetic factors. A considerable number of individuals exhibit no discernible genetic predisposition upon evaluation through a polygenic risk score that comprehensively considers all susceptibility loci (lee & Cleynen, 2019).

Structure And Types of Gremlin Gene

Gremlin-1 is a BMP antagonist that belongs to the DAN family and operates by directly interacting with the growth factor to primarily disrupt the action of BMP-2 and -4 (Church, et al., 2015). It has crucial roles in the growth of the kidney, lung, and bones (Costello, Cahill, Martin, Gaine, & McLoughlin, 2009). The expression of Gremlin-1 is observed in the basal region of intestinal crypts, where it plays a crucial role in preserving the stem cell reservoir. This is achieved by counteracting the BMP activity that originates from the mesenchymal cells. (Kosinski, et al., 2007). The upregulation of Gremlin-1 has been demonstrated in the stromal cells of tumors, facilitating the establishment of a conducive microenvironment for the proliferation of cancerous cells (Davis, et al., 2014).

In terms of embryogenesis and tissue development, Gremlin 2 (*GREM2*) is a potent antagonist BMPs. The variable expressivity of *GREM2* mutations has been observed to occur even among individuals within the same family. The mode of inheritance for this trait is autosomal dominant, characterized by incomplete penetrance (Kantaputra, Kaewgahya, & Hatsadaloi, 2015). The genes *GREM1* and *GREM2*, commonly referred to as Gremlin genes, have been identified as significant contributors to the inflammatory response, specifically in relation to select chronic inflammatory conditions. Empirical evidence indicates that the expression of Gremlin-1 is elevated in the pulmonary system of individuals diagnosed with chronic obstructive pulmonary disease (COPD) and asthma. The research findings indicate that Gremlin-1 has the potential to induce inflammation in the conditions through the suppression of BMP signaling. This may result in an irregularity in the control of immune responses and the emergence of persistent inflammation.

Likewise, the upregulation of Gremlin-1 expression is observed in the synovial tissues of individuals diagnosed with rheumatoid arthritis (RA). Within this context, it has been demonstrated that Gremlin-1 serves to stimulate the generation of cytokines that are pro-inflammatory in nature while also bolstering the viability and reproduction of inflammatory cells. Such actions may serve to facilitate the onset and advancement of rheumatoid arthritis (Nygaard & Firestein, 2020).

The precise function of Gremlin-2 in the context of inflammation remains relatively understudied. However, existing research indicates that it may exert a modulatory influence on immune responses. A study demonstrated that Gremlin-2 exhibited upregulation in the livers of mice suffering from acute liver injury. The study further suggested that Gremlin-2 may have the potential to enhance the survival of liver cells and impede inflammation (Sanders et al., 2017).

The subject of discussion is *GREM1*, also known as Gremlin 1.

The genomic locus of *GREM1* is situated on chromosome 15q13.3 (Jaeger et al., 2012). The molecular weight of Gremlin-1 is 20.7 kilodaltons; however, its electrophoretic mobility is observed to be around 24–25 kilodaltons, which is attributed to the process of glycosylation (O'Reilly, 2021).

The subject of discussion is *GREM2*, also known as Gremlin 2.

The genomic locus of *GREM2* has been identified on chromosome 1p36.12.

In terms of gene structure, it can be observed that the *GREM2* gene is smaller than *GREM1* and occupies a genomic region of approximately 13 kilobases (kb) on the chromosome (O'Reilly).

Function of Gremlin Proteins

The ongoing inquiry pertains to the molecular functionality of *GREM1*. The gene under consideration is purported to hold importance in the mechanisms of both carcinogenesis and metanephric kidney organogenesis. The protein in question functions as an antagonist of BMP and is essential for the initial development and arrangement of limbs. Consequently, it plays a pivotal role in sustaining the feedback loop between FGF4 and SHH. The ongoing inquiry pertains to the molecular functionality of *GREM1*. The gene under consideration is purported to hold importance in the mechanisms of both carcinogenesis and metanephric kidney organogenesis. This protein, which functions as a BMP antagonist and is necessary for the first outgrowth and patterning of limbs, plays a crucial role in maintaining the FGF4-SHH feedback mechanism.

The dose-dependent modulation of BMP4 signaling is observed to result in downregulation. The antagonist of BMP2 has been observed to inhibit the differentiation of osteoblasts mediated by BMP2 in vitro. Functions as a monocyte chemotaxis inhibitor.

Overexpression of a certain factor has been observed to impede the proliferation or survival of non-transformed cells while leaving transformed cells unaffected (O88273 · GREM2_MOUSE, n.d.).

The protein denoted as Gremlin 2 (*GREM2*), alternatively referred to as Protein Related to Dan and Cerberus (PRDC), is categorized as a constituent of the DAN family of BMP antagonists. These closely related paralogs, which include Gremlin 1, Dan, Dante (or Coco), Cerberus-like 1, Uterine Sensitization-Associated Gene-1 (*USAG-1*), and Sclerostin, are included in this category (Pearce, Penny, & Rossant, 1999). The discovery of *GREM2* dates back roughly fifteen years; however, its biological function and the mechanism through which it inhibits BMP have not been fully elucidated. The mesenchymal expression of *GREM2* has been detected during the developmental stages of the spinal cord and lung. Its potential role has been proposed in the development of various structures such as follicles, neurons, and bones (Ideno, Takanabe, Shimada, & Imaizumi, 2009). The results of in vitro experiments indicate that *GREM2* exhibits inhibitory effects on Bmp2 and Bmp4, but not on Tgfβ or Activin. Although some members of the DAN family, such as Dante and *GREM1*, have been linked to various medical conditions such as pulmonary arterial hypertension, chronic kidney disease, and cancer, the specific role of *GREM2* in disease pathogenesis has yet to be extensively investigated (Cahill & et al., 2012). Alternative terms for the *GREM2* gene *PRDC, DAND3, STHAG9*, and *CKTSF1B2.*

CHAPTER III

Methodology

Research design

The study employed a retrospective cohort design, which is an observational study design that retrospectively examines past data to investigate the association between exposure to specific risk factors or interventions and the incidence of an outcome. The study design involves the identification of a cohort of individuals who have been subjected to a particular factor of interest, such as a specific treatment or exposure, alongside a control group of individuals who have not been exposed to the same factor.

Participants

Patients at the Near University Hospital who have been diagnosed with inflammatory bowel diseases.

Data collection tools / Biopsy

The retrieval of patient records was conducted through the hospital information system, specifically Nucleus version 9.37.77, for the period spanning from 2021 to 2023. The search was performed using specific keywords including "colon," "rectum," "inflammation," "ascending colon," and "descending colon." The cases that met the criteria for a diagnosis of inflammatory bowel disease were chosen. The control group consisted of cases exhibiting nonspecific colitis and colonic changes within normal limits. The slides and formalin-fixed, paraffin-embedded blocks of the selected cases were obtained from the archives of the pathology laboratory located at the Near East University Hospital. A pathologist reviewed all cases microscopically and selected one representative block from each case. Exclusion criteria were applied to cases lacking a tissue block or showing indeterminate histology.

Materials

Chemical Reagents

Elution buffer, Ethanol, Ethidium bromide, Proteinase K, Xylene for deparaffinization,

Kits

cDNA synthesis kit (Hibrigen, Turkey), RNA isolation Kit (Qiagen, Germany)

Oligonucleotide

The primer pairs utilized for *GREM1* were acquired from Turkey (Macrogen, Europe).

RNA Isolation Kit Materials

Buffer PKD, Proteinase K, DNase booster buffer, Dnase I stock solution, Buffer RBC, Buffer RPE, Rnase-free water.

Software System

Nucleus version 9.37.77 for patient's record, The GelCapture software packages have been used for the purpose of visualizing and scrutinizing the gel images as well as preserving the imaging data. The data has undergone statistical analysis through the utilization of the Statistical Package for the Social Sciences (SPSS).

Methods

Biopsy Samples

The obtained samples from the pathology lab at the Near East University Hospital archive were cut into 4μM and placed in an Eppendorf tube.

RNA isolation

The RNA extraction was performed using the QIAGEN kit. Since the extraction is made on biopsy samples, the deparaffinization step is necessary to get rid of the paraffin wax from the tissues.

A 1000 μL of Xylene reagent is set to heat up at 75°C in a warm bath. The xylene reagent is then added to the 4μM tissue sample in an Eppendorf. After washing the xylene, 1000 μL of ethanol is added twice for 10 minutes and then vortexed vigorously for 10 seconds to assure that there is no paraffin wax found. The tissue is then left to dry.

The samples are then incubated for 3 minutes at 56°C and left to cool down. 150 µL of buffer PKD is added and vortexed, and afterwards the centrifuge is used for 1 min at 11,000g. Following on, 10 µL of proteinase K is added to digest the proteins and is mixed gently by the pipette. The samples will first be incubated at 56°C for 15 minutes, then at 80°C for another 15 minutes.

Before moving onto the next step, the lower colorless phase should be transferred into a 2 ml microcentrifuge tube. An ice tray is then used for 3 minutes of incubation before centrifuging for 18 minutes at 17,000 g. The supernatant will be transferred into an unsupplied microcentrifuge tube. Dnase booster buffer (16 L) and Dnase I stock solution are both added and mixed, then vortexed to gather the liquids from the sides of the tube. 15 minutes of incubation at room temperature followed. An addition of 320 µL of buffer RBC to adjust the binding condition, 720 µL of ethanol (stored at -80°C) is added and mixed well with the sample, 700 L of the sample is transferred into an Rneasy MinElute spin column inside a 2 ml closed tube, 15 s of centrifugation is performed for 15 s at the speed of 8000 g, the flow through is then discarded, and this step is performed twice. An addition of 500 µL buffer RPE (44 µL of ethanol is added to the buffer RPE) is added to the Rneasy MinElute spin column and is then centrifuged for 15 s at a speed of 8000 g. Another 500 µL of buffer RPE is added and centrifuged for 2 minutes at a speed of 8000g. The Rneasy MinElute spin column is placed onto a new 2 ml collection tube, the lid remains open, and it is centrifuged at full speed (17,000 g) for 5 minutes. Finally, the Rneasy MinElute spin column is placed in a new 1.5-ml collection tube, 20 µL of Rnase-free water is added directly into the spin column, and it is centrifuged for 1 min at the maximum speed of 17,000g for RNA elution.

The RNA samples are being stored at -20°C.

cDNA Synthesis

The cDNA synthesis was performed utilizing the HIBRIGEN cDNA synthesis kit.

Table1 displays the three components included in the kit.

Table 1– *Standard protocol for the synthesis of cDNA, this protocol was used for 50 RNA samples.*

Component	Volume x1
Reaction Buffer	4 µL
Enzyme Mix	1 µL
Total RNA	5 µL
Nuclease-free dH20	10 µL
Total Volume	20 µL

The cDNA samples are set into the PCR device **(Table 2)**

Table 2 *The cDNA synthesis lasted for an hour and 10 minutes.*

STEP	Temperature	Time
CDNA synthesis	42	60 minutes
Inactivation of kit	80	10 minutes

Primer Optimization for Gradient PCR

Prior to commencing the experiment, it is necessary to activate the primer kit (Thermo, Hibrigen) intended for use with the *GREM1* gene. Activation involves the addition of 250µL of distilled water to both the forward and reverse primers. Subsequently, the solution was diluted through the incorporation of 10µL of primer stock and 90µL of distilled water (ampoule water). (**Table 3 and Table 4).**

Table 3 *First primer optimization, in this trial the result was a faint band.*

Protocol	**1x**
MM- Master Mix	12.5
Forward F	1.25
Reverse R	1.25
H2O	9
cDna	2

Table 4 *PCR condition cycle is set for 40x cycle*

Stage	**Temperature**	**Time**
Initial Denaturation	95	3 minutes
Denaturation	95	0.80 seconds
Annealing	57-62	0.20 seconds
Extension	72	0.45 seconds
Termination	72	3 minutes

The second optimization of the primer was conducted as a result of the occurrence of primer dimers in the initial optimization. In this second optimization, an additional 1 µL of cDNA was added. **(Table 5 and Table 6).**

Table 5 *The second optimization of the primer.*

Protocol	**1x**
MM	12.5
F	1.25
R	1.25
H20	9
cDNA	3

Table 6 *Annealing time was decreased from 0.20 to 0.10, 40x cycles*

Stage	**Temperature**	**Time**
Initial Denaturation	95	3 minutes
Denaturation	95	0.80 seconds
Annealing	56-61	0.10 seconds
Extension	72	0.45 seconds
Termination	72	3 minutes

Table 7 shows the protocol for the third trial of primer optimization where glycerol was used, **Table 8** shows the PCR cycle where the time of all stages were increased.

Table 7 *Third primer optimization*

Protocol	1x
MM	12.5
F	1.25
R	1.25
H20	9
cDNA	2
Glycerol	1

Table 8 *Denaturation time was increased*

Stage	**Temperature**	**Time**
Initial Denaturation	95	5 minutes
Denaturation	95	0.30 seconds
Annealing	56-61	0.20 seconds
Extension	72	0.45 seconds
Termination	72	3 minutes

Table 9 shows the protocol for the third trial of primer optimization where H2O amount was reduced, **Table 10** shows the PCR cycle where the time of all stages were decreased.

Table 9 *Fourth primer optimization*

Protocol	**1x**
MM	12.5
F	1
R	1
H20	8
cDNA	2
Glycerol	1

Table 10 *Fourth primer PCR Cycle*

Stage	**Temperature**	**Time**
Initial Denaturation	95	2 minutes
Denaturation	95	0.30 seconds
Annealing	57-62	0.40 seconds
Extension	72	0.45 seconds
Termination	72	5 minutes

Table 11 presents the fifth trial for the primer optimization

Table 11 *Fifth primer optimization*

Protocol	**1x**	16x
MM	12.5	200
F	1	16
R	1	16
H20	8	128
Glycerol	1	16
cDNA	2	

Table 12 describes the forward and reverse primer pairs for the *GREM1* gene

Table 12 *states the primer pair for the GREM1 gene.*

Oligo name	**Base Sequence 5'-3'**
M- Forward	5'GGGAGCCCTGCATGTGAC3'
M- Reverse	5'GGCTGTAGTTCAGGGCAGTT3'

Gel-Electrophoresis

The technique of gel electrophoresis is extensively employed for the purpose of segregating and scrutinizing molecules of DNA, RNA, or protein based on their respective magnitude and electrical charge. The process of gel electrophoresis involves a series of fundamental steps.

Prepare the gel according to the established protocol. To create an agarose gel, 6g of agarose powder was dissolved in a buffer solution of TAE x 2 = 200 ml and heated until the agarose was fully dissolved. The process involves pouring the liquid agarose into a designated gel tray and subsequently inserting a comb to establish wells for the purpose of sample loading. The gel should be permitted to solidify prior to proceeding. It is worth noting that TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA) buffers are frequently employed as standard running buffers for DNA electrophoresis.

The TAE used was diluted by the addition of 20ml + 980 ml H2O.
For the preparation of samples, it is recommended to combine DNA, RNA, or protein samples 10 ml with an appropriate loading buffer that incorporates a tracking dye "Blue Leather Dye 3 ml". The utilization of the loading buffer serves the purpose of facilitating sample visualization during electrophoresis while also furnishing density for the loading of the sample into the wells. Sample Loading: With precision, use a micropipette to meticulously load the previously prepared samples into the designated wells of the gel. It is advisable to exercise caution in order to prevent sample leakage by refraining from overfilling the wells.
Perform gel electrophoresis by inserting the gel tray into an electrophoresis chamber that has been filled with the appropriate running buffer. Attach the electrodes to the power source, taking care to properly orient the positive and negative terminals. To conduct the analysis of molecules, it is necessary to subject the gel to an electric current at an appropriate voltage and duration, which may vary depending on the type and size of the molecules under investigation. A visual representation of the outcomes is recommended. Upon completion of the electrophoresis run, the gel should be meticulously extracted from the electrophoresis chamber. Utilize an appropriate dye, such as ethidium bromide, to stain the gel for DNA.

It is recommended to gently agitate the gel within the staining solution to ensure optimal staining. To visualize and destain the bands, it is recommended to eliminate the excess dye by utilizing a destaining solution. Subsequently, the separated bands can be visualized through the utilization of ultraviolet (UV) light or other appropriate visualization techniques. Record the outcomes by acquiring a visual representation of the gel.

RT-qPCR

This method is widely employed in the field of molecular biology and is utilized for the purpose of assessing and quantifying the quantity of RNA present within a given sample. The procedure encompasses two primary stages: reverse transcription (RT) and quantitative polymerase chain reaction (qPCR). During the initial stage, known as reverse transcription, the process involves the conversion of RNA into complementary DNA (cDNA) through the utilization of an enzyme known as reverse transcriptase. The utilization of this technique enables the quantification of gene expression levels through the conversion of RNA molecules into complementary DNA (cDNA), which exhibits enhanced stability and is better suited for polymerase chain reaction (PCR) amplification. During the second stage, known as quantitative polymerase chain reaction (qPCR), the complementary DNA (cDNA) is subjected to amplification through the utilization of specific primers and a DNA polymerase enzyme. For polymerase chain reaction (PCR) monitoring in real time, fluorescent dyes or probes that have a strong affinity for the amplified DNA are used. As the polymerase chain reaction (PCR) proceeds, the fluorescence signal exhibits a direct correlation with the quantity of amplified DNA. Quantitative data on gene expression levels can be obtained by determining the initial amount of RNA in the sample through the comparison of fluorescence signals to a standard curve or reference genes. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is a technique that is often used to study gene expression, find biomarkers of disease, and confirm the results of high-throughput sequencing projects. The technique provides a high degree of sensitivity, specificity, and precision in quantifying gene expression levels, rendering it a valuable instrument in diverse domains such as biomedical research, diagnostics, and drug discovery.

Table 13 shows the first trial for RT-qPCR

Table 13 *Protocol for RT-qPCR first trial*

Component	**1x**	**51x**
SYBR	10	510
Forward	1	51
Reverse	1	51
H2O	8	407
Glycerol	1	51
cDNA	2	

Table 14 notice the quantity of cDNA was increased by 1 µL for accurate results.

Table 14 *Second trial for the RT-qPCR*

Component	**1x**	**51x**
SYBR	10	510
Forward	1	51
Reverse	1	51
H2O	8	407
Glycerol	1	51
cDNA	3	

Table 15 shows the mix for preparing the ACTB gene (housekeeping gene) for 50 samples.

Table 15 *Housekeeping gene ACTB gene*

Components ACTB	**1x**	**51x**
SYBR	10	510
Forward	1	51
Reverse	1	51
H20	6	306

CHAPTER IV

RESULTS

Patients and Pathological Information

By typing relevant keywords on the hospital information system, we identified 308 cases of colon biopsies taken between 2021-2023. All cases were reviewed microscopically by a pathologist and one representative block was selected from each case. 200 cases were excluded because of insufficient tissue size, 58 cases were excluded because of indeterminate histologic diagnosis. A total of 50 cases were included. 33 of the cases were male (66%) and 17 (34%) were female. The mean age of the patients was 61 (range 14-89). Thirty-two (64%) cases were diagnosed with UC and 18 (36%) were in the control group. The latter included 8 biopsies from healthy individuals and 10 from nonspecific colitis.

RNA Synthesis and Ratio Measurement

The process of RNA synthesis and ratio measurement was conducted on a total of 50 samples utilizing the cDNA synthesis kit. The ratio of RNA was determined using the nanodrop technique, and the resulting data is presented in the accompanying, **Table 16**

Table 16 *RNA Ratio Results*

Biopsy Number	RNA Ratio	UC/ CONTROL (CC)
999	1.88	CC
252	2.12	CC
353	1.98	CC
907	1.81	CC
227	1.95	CC
339	1.92	CC
210	1.60	CC
908	1.96	CC
774	1.76	CC
64	1.98	CC

Table 16 *continued*

214	1.62	CC
42	2.00	CC
1980	2.13	CC
1844	1.97	CC
1	1.81	CC
312- 1A	2.05	CC
127	1.88	CC
1328	1.64	CC
1169	1.94	UC
1634	1.96	UC
321	1.96	UC
1776	1.70	UC
116	1.65	UC
132	1.88	UC
163	2.18	UC
202	1.66	UC
256	2.00	UC
204	2.07	UC
289	1.98	UC
374	1.85	UC
453	1.51	UC
1196	1.79	UC
163	2.18	UC
2006	2.00	UC
2014	1.98	UC
1969	1.66	UC
389	1.52	UC
2057	2.01	UC
370	2.00	UC
667	1.93	UC
520	1.90	UC
529	2.30	UC

Table 16 *continued*

1477	1.81	UC
81	1.69	UC
2057	1.87	UC
471	1.70	UC
1303	1.80	UC
228	2.00	UC
68	1.70	UC
1344	1.90	UC

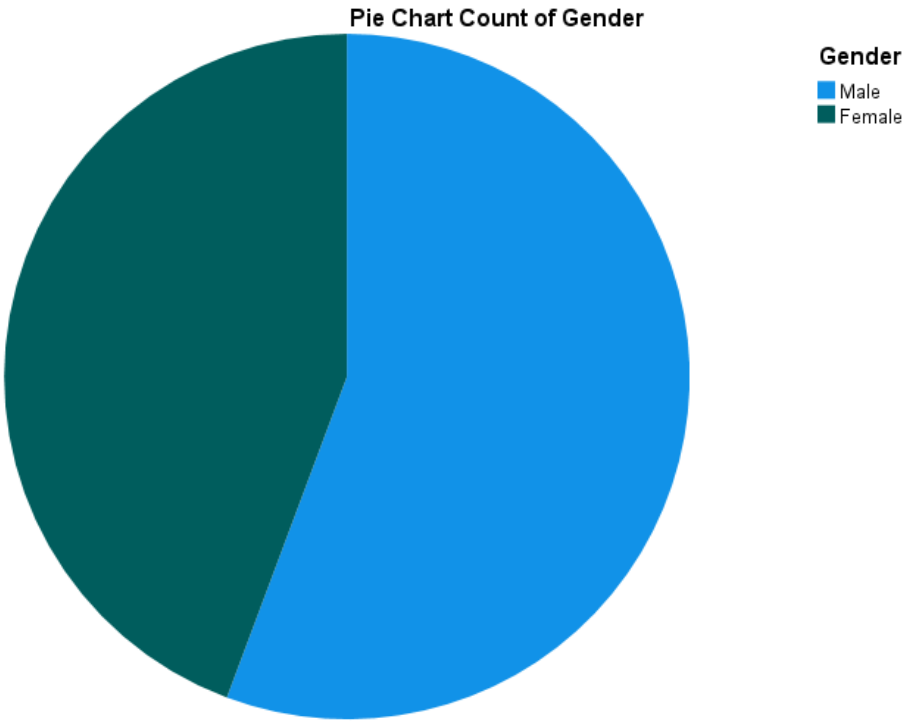
Data Overview

The study's sample consists of 50 participants out of 307 samples, the total number of samples have 171 male participants (55.5%) and 136 females (44.5%), ages between 14 years to 91 years with an average of 54 years. (**Table 17 and Figure 1).**

Table ***17*** *Gender of total participants*

Gender			
		Frequency	Percent
	Male	171	55.5
	Female	136	44.5
	Total	307	100.0

Figure 1 *Pie chart count of Gender for Samples*



The sample included patients who had been diagnosed with UC as well as a control group comprising individuals who did not have the condition. The incidence of UC is more pronounced in the geriatric demographic in comparison to younger cohorts. UC has the capacity to present itself during any phase of an individual's lifespan. The occurrence of inflammation exhibits spatial variability across patients, manifesting in diverse anatomical regions. **(Table 18 and 19).**

Table *18* *Age of total samples*

Age of Total Samples

Mean	Number of Samples	Minimum	Maximum
56.45	307	14	91

Table *19* The study's sample size overview.

Total No. of Biopsy Samples	Age	Gender	Control Cases No.	UC Cases No.
50	14-89	F/M	18	32

As shown in **Tables 20 and 21 and Figure 2**, out of the 307 samples 50 were chosen to be participants in this study, aging between 14 years and 89 years with an average of 61 years. 33 were male (66%) and 17 were female (34%).

Table 20 *Gender of Participants*.

	Frequency	Percent
Male	33	66.0
Female	17	34.0
Total	50	100.0

Figure 2 *Pie Chart Count of Gender of Participants*

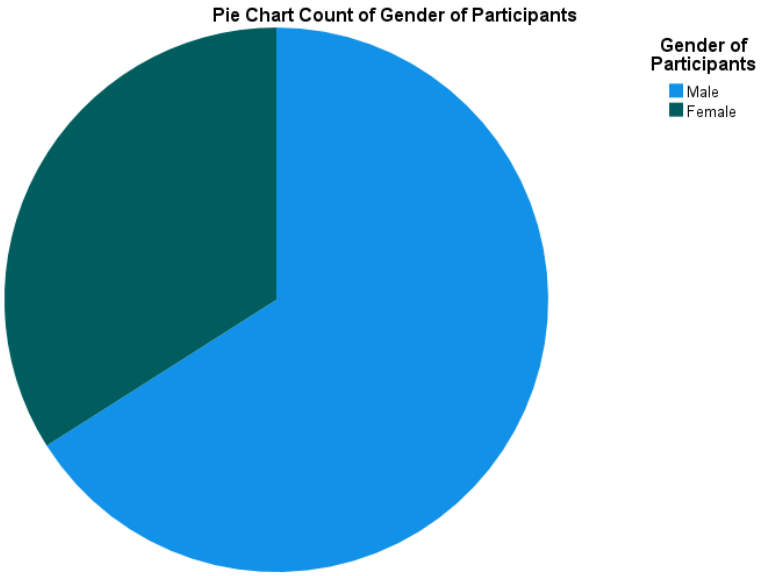


Table *21* *Age of Participants*

Mean (Avg.)	Number of Participants	Minimum	Maximum
61	50	14	89

Statistical Analysis

The statistical analysis of the Housekeeping Gene and the Gremlin-1 for the two groups (Controlled cases and UC) for all the 50 samples is presented in **Table 22**.

Table 22 *CT Average Values*

Statistics

		Ct Control cases	Ct UC	Housekeeping Control Case	Housekeeping UC
N	Valid	18	32	18	32
	Missing	32	18	32	18
Mean		27.1717	27.8722	33.9150	32.9156
Median		27.5850	27.9150	33.8750	32.8500
Std. Deviation		2.29419	1.50028	1.29447	1.40887

When we analyzed the 50 samples, we found out that 18 cases are control cases with a mean of 27.17±2.29.

And out of 50 samples 32 are UC cases with a mean of 27.87±1.5.

For the Housekeeping gene 18 out of 50 are for control cases with a mean of 33.91±1.29 and 32 for UC cases with a mean of 32.91±1.4.

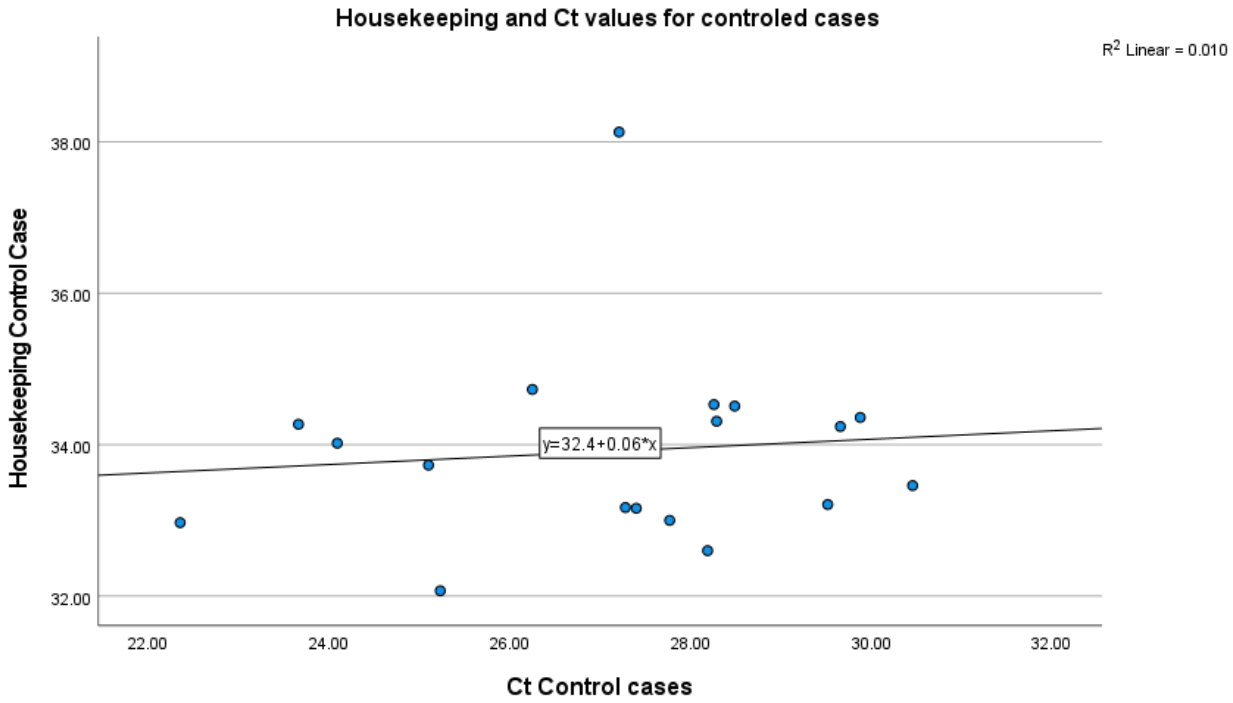
The gene correlation analysis of Gremlin-1 for the two groups (Control Cases and Housekeeping gene for Control Cases) for all 18 samples is presented in the **Table 23,** and shown graphically in **Figure 3**. A p-Value of ≤ 0.05 is significant.

Although the there is a positive correlation between Control Case and Housekeeping gene, Control Case p-Value > 0.0001, that means there is not a significant correlation.

Table 23 *Correlation between Control Case and Housekeeping Gene*

			Ct Control cases	Housekeeping Control Case
Spearman's rho	Ct Control cases	Correlation Coefficient	1.000	.187
		Sig. (2-tailed)	.	.458
		N	18	18
	Housekeeping Control Case	Correlation Coefficient	.187	1.000
		Sig. (2-tailed)	.458	.
		N	18	18

Figure 3 *CT Control Cases*



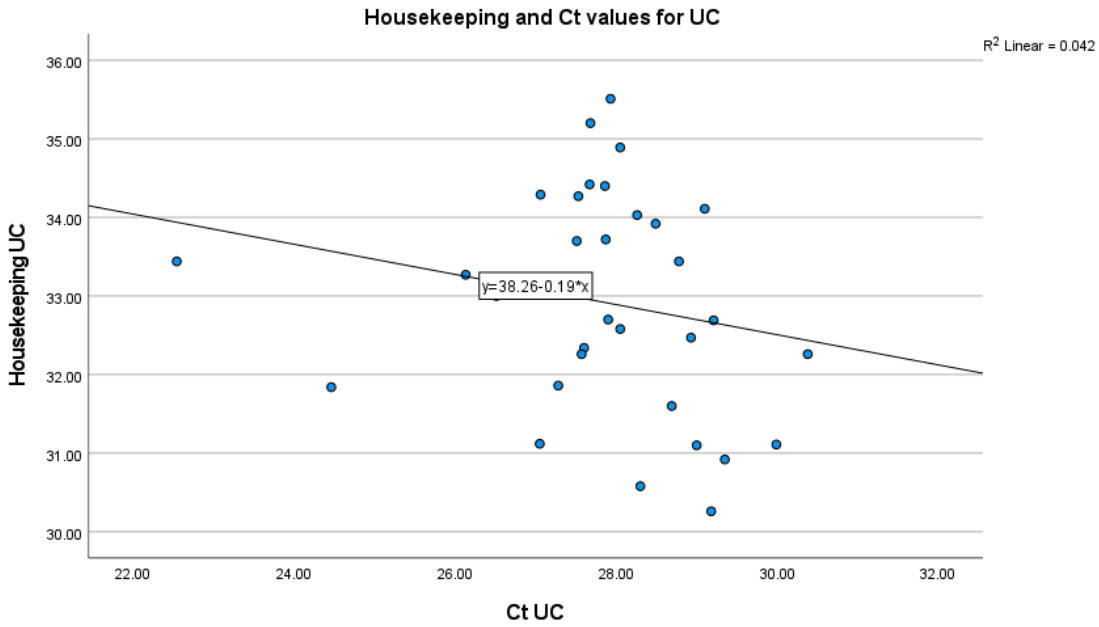
In **Table 24** we can see the correlation analysis between the two groups (UC cases and housekeeping gene for UC), and their graphical presentation in **Figure 4**.
for all 32 samples. While the p-Value > 0.05 shows no significant correlation between *GREM1* and housekeeping gene for UC, the correlation coefficient shows a negative correlation between the two.

Table 24 *Correlation between GREM1 and Housekeeping gene.*

Correlations

			Ct UC	Housekeeping UC
Spearman's rho	Ct UC	Correlation Coefficient	1.000	-.282
		Sig. (2-tailed)	.	.118
		N	32	32
	Housekeeping UC	Correlation Coefficient	-.282	1.000
		Sig. (2-tailed)	.118	.
		N	32	32

Figure 4 *Housekeeping and Ct Values For UC*

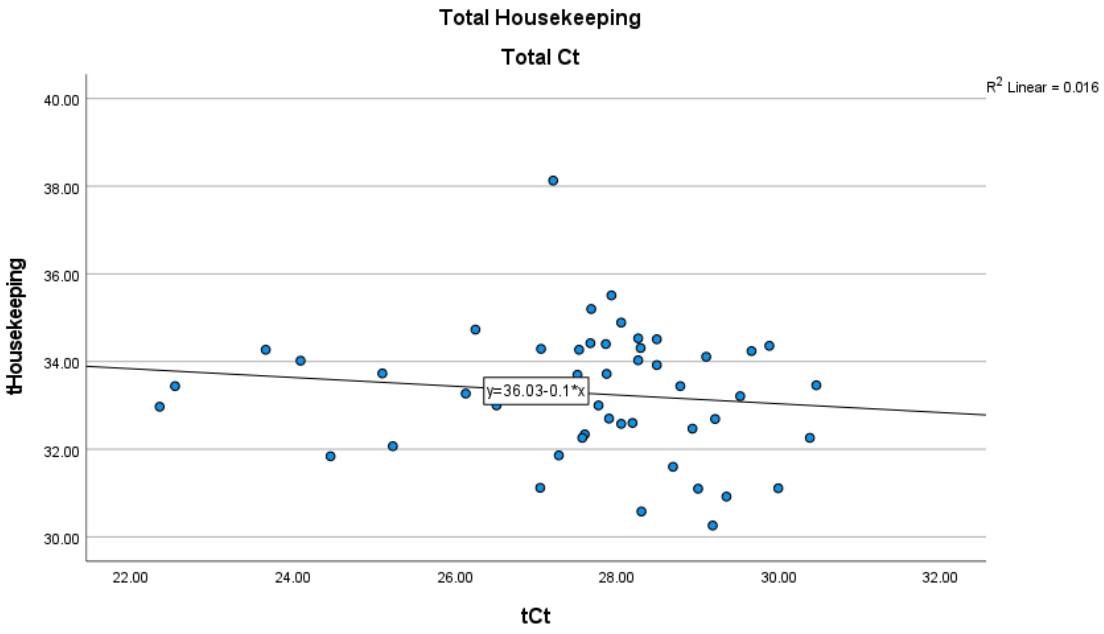


The analysis of the correlation between the total values of *GREM1* and the Housekeeping gene reveals a negative correlation coefficient and a p-value lower than 0.05, indicating a lack of statistical significance. **(Table 25 and Figure 5).**

Table 25 *Correlation between all 50 GREM1 samples and the housekeeping gene.*

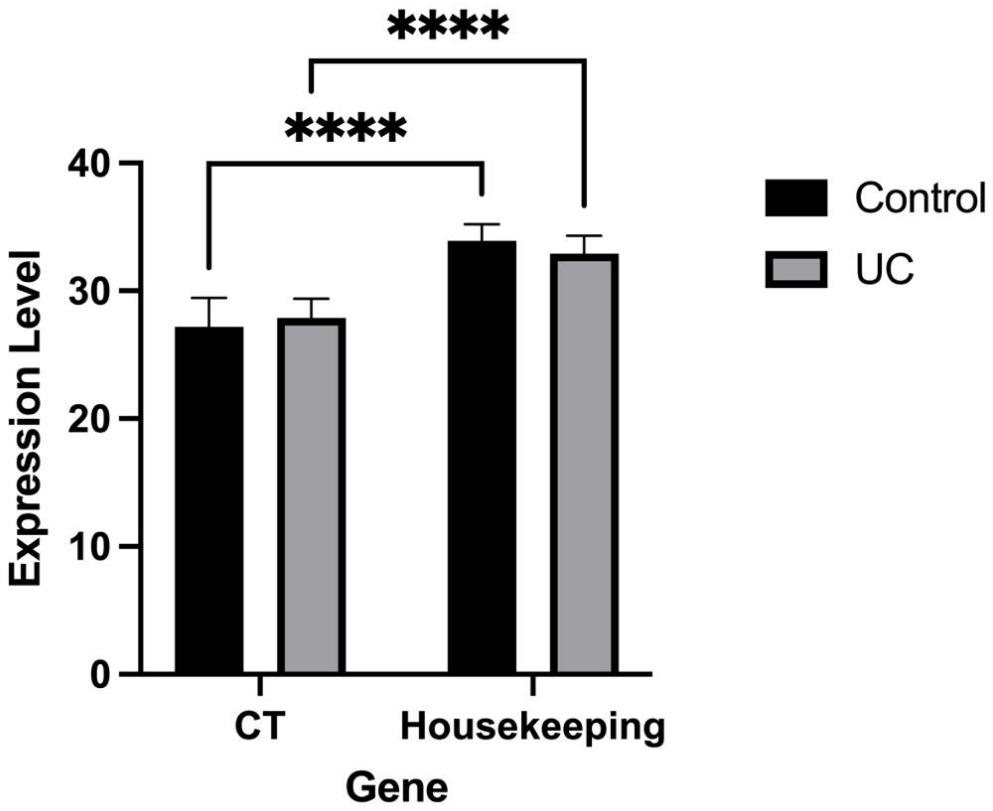
Correlations				
			housekeeping	Ct
Spearman's rho	Housekeeping	Correlation Coefficient	1.000	-.140
		Sig. (2-tailed)	.	.333
		N	50	50
	Ct	Correlation Coefficient	-.140	1.000
		Sig. (2-tailed)	.333	.
		N	50	50

Figure 5 *Total Housekeeping Gene Ct Value*



The gene expression profiles for the control cases and UC are presented in **Figure 6** showing that there is no significant correlation.

Figure 6 *gene expression profile*



Results Interpretation

Although the present results indicate a limited association between *GREM1* and the Housekeeping gene, it should not be inferred that *GREM1* lacks biological significance in the context of UC. Additional research is necessary to thoroughly investigate the correlation between *GREM1* expression and UC. This entails conducting studies with larger sample sizes, encompassing diverse populations, and employing alternative normalization strategies.

The findings highlight the intricate nature of gene expression analysis and emphasize the significance of meticulous interpretation. Although the correlation analysis in this study did not yield statistically significant results, the overall findings and additional analyses conducted have the potential to provide valuable insights to the field of UC research. Subsequent investigations may expand upon the present findings and investigate additional variables that could potentially impact the expression of *GREM1* in the context of UC.

CHAPTER V

DISCUSSION

Ulcerative colitis (UC) is a complex chronic inflammatory disease of the colon that requires the evaluation of clinical, colonoscopic, pathological, and laboratory findings for a definitive diagnosis. In histopathological examination, one of the most important findings used for distinguishing UC from other inflammatory diseases is the presence of crypt distortion. The loss of the regular shape and arrangement of the crypts, which are tubular structures on the inner surface of the large intestine, indicates damage to the intestinal cells from ongoing inflammation. In a study that looked at the gene expression patterns of human colon tops and basal crypts, it was found that intestinal cryptal myofibroblasts and smooth muscle cells at the colon crypt expressed a BMP antagonist called *GREM1*. This finding suggests that *GREM1* expression may play a role in the development of crypt distortion in patients with UC. In addition, the BMP pathway is known to have a crucial role in both inflammation and tissue homeostasis. These studies paved the way for us to investigate the diagnostic utility of *GREM1* expression in UC. When we looked at *GREM1* expression in biopsy samples from people with UC and people who were healthy or had nonspecific colitis as controls, we did not find a big difference between the two groups. Our study suggested that *GREM1* expression may not be used for distinguishing UC from other conditions affecting the colon. This finding contradicts the previous hypothesis that *GREM1* expression may be associated with crypt distortion in UC. Our study suggested that the role of *GREM1* and the BMP pathway in UC pathogenesis may be more complex than previously thought and may vary depending on the stage and severity of the disease. Further studies are needed to elucidate the molecular mechanisms underlying crypt distortion and inflammation in UC. The *GREM1* gene is of considerable significance owing to its regulatory function in a range of physiological processes, with a particular emphasis on its participation in the development of bone and cartilage. Nevertheless, recent studies have revealed the wider ramifications of this gene, encompassing its influence on inflammatory and pathological disorders. Within the realm of UC, the *GREM1* gene has attracted considerable interest due to its potential involvement in regulating inflammatory reactions and promoting tissue regeneration within the gastrointestinal tract.

In the case of colorectal cancer (CRC), the gene *GREM1* has been found to have higher levels of expression in people who have CRC. This implies the potential role of the gene in facilitating the development of tumors or functioning as a diagnostic marker. In addition, finding out that *GREM1* is linked to inflammatory conditions other than UC could give important information about common molecular pathways and possible therapeutic targets.

A recent study revealed a significant association between *GREM1* expression and angiogenesis in UC carried out by (SUN, et al., 2020). In this study, the researchers evaluated the expression of *GREM1* and its association with angiogenesis in individuals who had been diagnosed with UC. This study indicated a considerable increase in the expression of *GREM1* within the inflamed colonic mucosa. This led the researchers to hypothesize that *GREM1* may have a role in the angiogenic mechanisms that are linked with UC. In a similar way, the study by (Gundersen, et al., 2019) showed that the expression of Gremlin was much higher in the inflamed mucosal tissue of people with UC than in the non-inflamed mucosa. The research provides evidence that *GREM1* may play a role in the development of UC, even though the association between *GREM1* and the severity of illnesses was not specifically investigated in this study (Merino, et al., 1999). However, in our research, we did not find a significant expression of *GREM1* in patients with UC compared with patients with non-specific colitis or healthy individuals.

The study "Stromal Cells in the Pathogenesis of Inflammatory Bowel Disease" looks at how important it is for myofibroblasts to control BMP signaling and the release of Wnt ligands in the intestinal crypts. The results of the study show that BMP antagonists, such as Gremlin and Noggin, are present in the bottom part of the colon crypt, where myofibroblasts are found. The antagonists effectively hinder the signaling of BMP within the specified region while concurrently allowing its occurrence in the upper crypt regions. The observed variation in BMP signaling within the crypt indicates the presence of diversity within the myofibroblast population.

In addition, it has been shown that the Wnt ligands made by Gli1-positive fibroblasts near the bases of intestinal crypts are very important for maintaining the integrity of the epithelium and helping stem cells renew themselves. Another study found that fibroblasts that express CD90, a subgroup of fibroblasts that express Gli1, are actively involved in making BMP antagonists and Wnt ligands, which helps organoids grow. It is significant to note that the fibroblasts that express CD90 can be classified into two distinct populations depending on whether SMA is present or absent. This observation implies that fibroblasts play a role in preserving epithelial homeostasis and barrier function, which are often compromised in cases of IBD. The processes mentioned above play a critical role in the maintenance of intestinal homeostasis and the understanding of IBD pathogenesis (Barnhoorn, et al., 2020). The article "Recent developments on BMPs and their antagonists in inflammatory bowel diseases" looks at how BMPs and BMP antagonists contribute to the pathophysiology of IBD and colitis-associated CAC. The research shows how important BMP signaling is for keeping the gut in balance and how it could be used as a therapeutic target or biomarker for IBD and CAC. BMPs and antagonists, such as BMP4, BMP6, BMP7, GREM1, SOST, Nog, and FST/FSTL1, have been looked at to see what role they play in IBD. The authors engage in a comprehensive examination of their influence on inflammatory mechanisms, tissue regeneration, and the regulation of stem cells within the framework of IBD. The review article also acknowledges that there are numerous aspects of BMP signaling in IBD that are still not well understood and have received limited research attention. This includes the combined effects of multiple BMPs and their antagonists. Also, more research needs to be done to see if other molecules like BMP could be used as new biomarkers in people with IBD. The article concludes by recommending further investigation into BMP signaling to develop innovative therapeutic interventions for the treatment of IBD. This recommendation is especially important because anti-BMP6 reagents, exogenous BMP7, and FST have all shown they can reduce pro-inflammatory cytokines in cases of colitis.

The review says that the article, on the other hand, covers a wider range of research. It looks at multiple BMPs and antagonists in the context of how inflammatory bowel disease happens and how they might be used as therapeutic targets. This article emphasizes the need for more research into BMP signaling and other relevant molecules as possible biomarkers in people with IBD, (Xie, et al., 2023).

"Targeting Gremlin 1 Prevents Intestinal Fibrosis Progression by Inhibiting the Fatty Acid Oxidation of Fibroblast Cells," another article, talks about a study that looked at the role of *GREM1* and how it affects the growth and activation of fibroblast cells. The study revealed a notable elevation in *GREM1* levels within fibrotic colon tissues obtained from both human and murine subjects. Experiments that looked at how *GREM1* worked showed that it helped intestinal fibroblast cells grow by increasing fatty acid oxidation (FAO) by turning on VEGFR2 and the MAPK signaling pathways that came next (Yang, et al., 2021).

In the study "Fibroblast-derived Gremlin1 localization to epithelial cells at the base of the intestinal crypt," the different ways *GREM1* mRNA and protein are expressed in the colon were examined. According to the research, fibroblasts in the muscularis mucosa express *GREM1* before releasing it from this layer. The Gremlin protein is secreted and exhibits a distinct pattern of localization within the colonic crypt. There is a gradient effect in this location, with the highest levels of the protein found at the bottom of the crypt, close to the muscularis mucosa. As one moves towards the luminal surface, the distribution of the protein becomes more diffuse. The results of the study show that there is a paracrine signaling loop between fibroblasts and epithelial cells.

This loop may help keep the stem cell niche in the colonic crypt. The *GREM1* protein, which acts as an antagonist to BMP signaling, facilitates this process. Considering our research into how the expression of the *GREM1* gene can be used to diagnose UC, both studies look at how *GREM1* is involved in intestinal processes. The mentioned research looks at the different ways that *GREM1* mRNA and protein are expressed in the colon and tries to figure out what role it might play in keeping the stem cell niche alive. Both studies make important contributions to our understanding of *GREM1* in the gastrointestinal tract, though they focus on different parts of *GREM1's* biology and how it affects health and disease (Dutton, et al., 2019).

There are certain limitations of our study. First, the small sample size may restrict generalizability and not adequately represent UC's diversified population. If a study includes only a fraction of UC patients or excludes populations with concurrent medical illnesses or atypical disease presentations, selection bias may develop. This may hinder generalization to the UC population. Clinical presentation, illness severity, and treatment response vary in UC.

The study may have overlooked UC patients' heterogeneity, which could limit its applicability to different subpopulations. Medication use, disease activity, comorbid medical disorders, and lifestyle factors may not be adequately controlled or corrected in the research approach. These factors may affect *GREM1* gene expression, leading to skewed or false relationships. Sample collection, RNA isolation, and gene expression analysis may vary for *GREM1* expression analysis. Variability from procedural irregularities can reduce outcome reliability and reproducibility. For reliability and application, the study's findings may need external validation in other cohorts. No validation in varied groups or research environments may limit the study's conclusions.

To sum up, the main goal of our study was to see if *GREM1* gene expression could be used to diagnose UC. For this purpose, we extracted RNA from biopsy samples and performed RT-qPCR analysis. The negative correlation coefficient supported the study's findings that there was no statistically significant relationship between the expression of *GREM1* and UC.

Potential future directions for our study may involve investigating additional biomarkers or genetic factors that could potentially be linked to UC. Furthermore, it is worth exploring alternative gene expression patterns or signaling pathways that may offer valuable insights into the pathogenesis of UC. When it comes to improving our methods, adding different types of samples, or making the RNA extraction process more efficient could make our results more accurate and easier to reproduce. By delving into alternative advanced methodologies or utilizing omics-based strategies, such as transcriptomics or proteomics, researchers may gain a more comprehensive understanding of the molecular alterations linked to UC.

In addition, it would be advantageous to broaden the scope of our research in order to examine the potential diagnostic and prognostic significance of *GREM1* in other gastrointestinal disorders. In summary, our study provides a significant contribution to the comprehension of UC, and subsequent research endeavors can utilize these findings to further enhance the understanding and treatment of this intricate ailment.

CHAPTER VI

Conclusion and Recommendations

In summary, the objective of our study was to examine the diagnostic utility of gremlin gene (*GREM1*) expression in individuals with ulcerative colitis (UC). The results of our study indicate that there is no statistically significant correlation between the total values of *GREM1* and the Housekeeping gene. This suggests that there is no association between *GREM1* expression and disease severity in the cohort of UC patients that we examined.

The findings of this study indicate that *GREM1* may not be a dependable biomarker for evaluating the severity of disease or forecasting the progression of UC. Nevertheless, it is crucial to acknowledge the constraints inherent in our research, such as the restricted number of participants and the possibility of uncontrolled variables that may have influenced our findings. The intricate nature and diversity of UC as a pathological condition may play a role in the variations observed in the expression of Gremlins among distinct groups of patients.

Notwithstanding the constraints, our investigation makes a valuable contribution to the comprehension of the diagnostic capacity of *GREM1* expression in UC. This sentence shows how important it is to keep looking for and studying new biomarkers and molecular pathways that could help people with UC be found, have their outcomes predicted, and have their treatments tailored to them.

Our study contributes to the existing body of literature regarding the involvement of gremlins in UC. However, additional research is necessary to gain a more comprehensive understanding of the specific mechanisms through which *GREM1* contributes to the development and progression of this disease. Furthermore, it is imperative to conduct larger-scale studies that encompass a wide range of patient populations and implement rigorous control measures to account for confounding variables. These measures are essential in order to authenticate our findings and establish more definitive evidence.

In the context of how UC is diagnosed, our research shows how important it is to investigate other biomarkers and molecular pathways that could help find people with this condition, predict how they will do, and make sure their treatments are just right for them.

Although our study did not find diagnostic utility in *GREM1*, it is important to recognize that this does not diminish the importance of exploring other potential biomarkers and pathways that could provide valuable insights into the management of UC.

In conclusion, our research enhances our comprehension of the diagnostic capacity of *GREM1* gene expression in UC. This statement underscores the importance of ongoing research and a comprehensive approach in order to gain a thorough understanding of the intricate characteristics of UC and identify dependable diagnostic indicators that can enhance patient management and outcomes. Future investigations can aim to expand upon the knowledge generated from this study in order to offer more comprehensive insights into the pathogenesis and management of UC. This, in turn, has the potential to enhance patient care and treatment strategies.

Recommendations

Recommendations According to Findings

The study's findings provide a basis for suggesting several recommendations for future research and clinical practice. Given that the correlation between the expression of the Gremlin gene (*GREM1*) and the severity and progression of UC has not been statistically significant so far, it is important to do more research with a larger and more diverse group of people to learn more about the link between the expression of the Gremlin gene *GREM1* and the severity and progression of UC. Augmenting the sample size has the potential to augment the statistical power and enhance the reliability of the findings. Furthermore, in order to gain a deeper comprehension of the involvement of *GREM1* in the pathogenesis of UC, it is imperative to conduct mechanistic studies. By looking at how *GREM1* interacts with other genes and signaling pathways, scientists can learn a lot about how it is controlled and find possible therapeutic targets. This could help the development of targeted therapeutic approaches that focus on changing the way *GREM1* is expressed for better control of UC. In addition, the investigation of gene-environment interactions is essential for gaining a comprehensive understanding of the intricate characteristics of UC. The integration of multi-omics data, which includes genomic, transcriptomic, and proteomic data, could provide a full understanding of the molecular causes of UC, and make it easier to find new biomarkers and therapeutic targets. Even though this study did not find a strong link between the two, it's still worth thinking about how *GREM1* expression could be used as a non-invasive biomarker to diagnose UC. Additional validation studies conducted in diverse patient populations and various clinical settings have the potential to provide further insights into the diagnostic efficacy of this approach. The integration of *GREM1* expression with other well-established biomarkers has the potential to enhance the precision of diagnosing and monitoring UC. In conclusion, it is imperative for future investigations to prioritize the comprehension of *GREM1*'s involvement in disease prognosis and the response to treatment. The study of *GREM1* expression as a possible predictor of how a disease will turn out and how well a treatment will work could help improve personalized medicine and patient care.

In conclusion, this study did not find statistically significant evidence of expression of *GREM1* between patients with and without UC, but it does add to what is known about how *GREM1* might be involved in UC. By engaging in additional research in the domains of extensive-scale studies, mechanistic inquiries, gene-environment interactions, and clinical validation, we can enhance our comprehension of UC and facilitate the development of more precise and individualized strategies for its treatment.

Recommendations for Further Research

This thesis makes several suggestions for future research that aim to improve understanding of the diagnostic value of Gremlin gene expression in UC and its possible clinical applications. The confirmation of the diagnostic utility of Gremlin gene expression necessitates the conduct of validation studies in independent and larger cohorts of patients with UC. It is imperative to incorporate diverse populations in these studies in order to enhance the generalizability of the findings. Furthermore, the utilization of longitudinal studies to monitor the temporal alterations in Gremlin gene expression among individuals with UC can yield valuable insights regarding its viability as a biomarker for disease surveillance and prognostication. The examination of the dynamics of Gremlin gene expression during periods of remission and relapse holds potential for enhancing our comprehension of its involvement in the progression of the disease. Furthermore, an investigation into the molecular mechanisms that govern the regulation of Gremlin gene expression in UC can provide valuable insights into its role in the development and progression of the disease. The exploration of the interplay between the Gremlin gene and various genes or signaling pathways has the potential to reveal previously unidentified targets for therapeutic intervention. Furthermore, conducting a comparative analysis of the diagnostic efficacy of Gremlin gene expression in relation to other well-established biomarkers for UC can provide valuable insights into its potential utility in clinical settings.

The integration of Gremlin gene expression with other biomarkers has the potential to enhance the precision of UC diagnosis. Moreover, the performance of functional investigations aimed at comprehending the biological ramifications of modified Gremlin gene expression in UC can yield significant knowledge regarding its involvement in the pathogenesis of the condition. Utilizing cell lines or animal models, in vitro and in vivo experiments can achieve this goal. Also, looking into the therapeutic implications of targeting the Gremlin gene in UC could lead to the discovery of new ways to treat the disease. The evaluation of the impact of manipulating the expression of the gremlin gene on disease activity and inflammation is of utmost importance in the advancement of targeted therapeutic approaches. Also, studying the relationship between Gremlin gene expression and different environmental factors, such as diet, lifestyle, and gut microbiota, can help us understand the pathogenesis of UC more fully and come up with personalized ways to treat it. Lastly, the combination of data from different omics methods, such as genomics, transcriptomics, proteomics, and metabolomics, can give a full picture of the molecular composition of UC. The utilization of this integrated approach has the potential to uncover previously unidentified biomarkers and therapeutic targets. By incorporating these suggestions into future research endeavors, it is possible to enhance our comprehension of the involvement of the Gremlin gene in UC, thereby potentially facilitating more precise diagnostic methods, enhanced disease management, and the development of personalized treatment approaches.

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Appendices

Appendix A

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :27.04.2023
Meeting Number :2023/113
Project number :1716

The project entitled "Diagnostic use of gremlin gene expression in ulcerative colitis" (Project no: NEU/2023/113-1716) has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	*Decision*	*Meeting Attendance*
	Approved (✓) / Rejected (X)	*Attended (✓) / Not attended(X)*
Prof. Dr. Tamer Yılmaz	✓	✓
Prof. Dr. Şahan Saygı	✓	✓
Prof. Dr. İlker Etikan	✓	✓
Doç. Dr. Mehtap Tınazlı	✓	✓
Doç. Dr. Nilüfer Galip Çelik	*X*	*X*
Doç. Dr. Dilek Sarpkaya Güder	✓	✓
Doç. Dr. Gulifeiya Abuduxike	✓	✓
Doç. Dr. Burçin Şanlıdağ	✓	✓

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Appendix B

Turnitin Similarity Report

DIAGNOSTIC USE OF GREMLIN GENE EXPRESSION IN ULCERATIVE COLITIS

ORIGINALITY REPORT

12%	10%	8%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.science.gov Internet Source	1%
2	"Yamada' s Textbook of Gastroenterology", Wiley, 2015 Publication	1%
3	docs.neu.edu.tr Internet Source	<1%
4	worldwidescience.org Internet Source	<1%
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18	Zhuo Xie, Gaoshi Zhou, Mudan Zhang, Jing Han, Ying Wang, Xiaoling Li, Qirui Wu, Manying Li, Shenghong Zhang. "Recent developments on BMPs and their antagonists in inflammatory bowel diseases", Cell Death Discovery, 2023 Publication	<1%

19	Reyhaneh Abgoon, Printha Wijesinghe, Cathie Garnis, Desmond A. Nunez. "The Expression Levels of MicroRNAs Differentially Expressed in Sudden Sensorineural Hearing Loss Patients' Serum Are Unchanged for up to 12 Months after Hearing Loss Onset", International Journal of Molecular Sciences, 2023 Publication	<1 %
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42	Zhengzhe Feng, Xiaoxi Zhang, Li Li, Chuanchuan Wang, Mingtao Feng, Kaijun Zhao, Rui Zhao, Jianmin Liu, Yibin Fang. "Tumor-associated macrophage-derived exosomal microRNA-155-5p stimulates intracranial aneurysm formation and macrophage infiltration", Clinical Science, 2019 Publication	<1 %
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CV

LAYLA HERFI

Scientist

PROFILE

I am a passionate, geneticist with 5 years of academic and practical experience in the fields of medical genetics, Pathology and Medical Laboratory. As a motivated recent graduate, I am keen on gaining experience in these fields.

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SKILLS

- Excellent time management abilities
- Strong leadership and communication techniques
- Great teamwork skills
- Good organization capabilities
- Reliable and personable

LANGUAGES

Arabic
English
French Beginner
Turkish Beginner

EDUCATION

NEAR EAST UNIVERSITY (Lefkoşa, Northern Cyprus)
2022 - 2023
Master degree in Molecular Medicine with **high honor**

NEAR EAST UNIVERSITY (Lefkoşa, Northern Cyprus)
2017 - 2021
Bachelor degree in Molecular Biology and Genetics with **CGPA 3.85** and **high honor**

ACADEMIC EXPERIENCE

Medical Genetics Lab
02.2023 – 06.2023
RNA extraction, Gene analysis and PCR.

Cytogenetics Lab
02.2023 – 06.2023
cDNA synthesis and real-time PCR.

Pathology Lab
02.2023 – 06.2023
Biopsy dissection and Biopsy collection.

COVID Lab
02.2021 – 07.2021
PCR analysis.

Urinalysis Lab
10.2020 – 01.2021
Urine and faces analysis.

Biochemistry Lab
10.2020 – 01.2021
HIV, Blood Levels, Blood Glucose Levels, Vitamins Level and Ferritin.