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EVALUATION OF THE EFFECT OF FECAL CALPROTECTIN, IL6 VITAMIN B12, FERRITIN, AND SOME MINERALS IN PATIENTS WITH ULCERATIVE COLITIS WITH AND WITHOUT BIOLOGICAL TREATMENT

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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NUHA HAMZA KHEDHIR KHEDHIR

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By Nuha Hamza Khedhir KHEDHIR

June 2023

We certify that we have read this dissertation and that, in our opinion, it is fully adequate in scope and quality as a thesis for the degree of Doctor of Philosophy.

Advisor: Assoc. Prof. Dr. Şevki ADEM
Co-Advisor: Asst. Prof. Dr. Summer Saad ABDULHUSSAIN

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Ebru AKKEMİK
Food Engineering
Siirt University

Member : Asst. Prof. Dr. Ali Rıza TÜFEKÇİ
Chemistry
Çankırı Karatekin University

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Çankırı Karatekin University

Member : Prof. Dr. Volkan EYÜPOĞLU
Chemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
KırıkkaleUniversity

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. Hamit ALYAR
Director of Graduate School

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Nuha Hamza Khedhir KHEDHIR

ABSTRACT

EVALUATION OF THE EFFECT OF FECAL CALPROTECTIN, VITAMIN B12, FERRITIN, AND SOME MINERALS IN PATIENTS WITH ULCERATIVE COLITIS WITH AND WITHOUT BIOLOGICAL TREATMENT

Nuha Hamza Khedhir KHEDHIR

Doctor of Philosophy in Chemistry

Advisor: Assoc. Prof. Dr. Şevki ADEM

Co-Advisor: Asst. Prof. Dr. Summer Saad ABDULHUSSAIN

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Fecal calprotectin can be used to predict histologic remission in patients with UC, although the cut-off level differs across research, depending on the test used to quantify this biomarker and depending on the definition of histologic remission. To understand the role of fecal calprotectin with some immunological, biochemical, and hematological variables in the diagnosis and control of biological therapy for patients with ulcerative colitis and its association with the rest of the study variables, a study of the relationship with the results of the Fecal Calprotectin test for ulcerative colitis patients who take biological therapy and patients who do not take biological therapy, and their relationship with healthy subjects, and an indication of the extent to which parameters are affected by these microbes. Samples of 150 patients (males and females) 50 for each group were collected from patients being referred to the gastroenterology clinic of Azadi Teaching Hospital and private Kirkuk clinic for upper and lower GIT endoscopy in Kirkuk City from October 2021 to June 2022 with clinical symptoms of bloody diarrhea with rectal urgency, rectal bleeding, frequent stools, and mucus discharge from the rectum, tenesmus, and lower abdominal pain and a clinical trial (randomLy) study were done. Interviews were done using a questionnaire that looked for the causal factors of ulcerative colitis. The results of this study show a strong relationship between the fecal calprotectin test with some immunological, biochemical, and hematological variables in patients with biological therapy group and without the biological therapy group compared to the control group where there is a significant difference between the

group without biological therapy and control group in the fecal calprotectin (FC), ferritin, IL-6, Hs-CRP, PCT, K, WBC, platelets, vitamin B12, Na, and Cl, while there were significant differences between the patient with biological therapy group and control group in vitamin D and Folate while there were no significant differences for both groups with a control group in Hb. The results of the study were as follows: High accuracy and precision can be achieved when predicting the development of ulcerative colitis using the level of fecal calprotectin (FC). According to the results of our study, it can be concluded that biological therapy has an important role in UC treatment where there were significantly positive changes in immunological, biochemical, and hematological variables in patients taking the biological therapy group compared with patients without the biological therapy group.

2023, 115 pages

Keywords: Inflammatory bowel disease, Ulcerative colitis, Fecal calprotectin, Vitamin B12, HsC-RP

ÖZET

ÜLSERATİF KOLİTLİ HASTALARDA BİYOLOJİK TEDAVİLİ VE TEDAVİSİZ FECAL CALPROTECTIN, VITAMIN B12, FERRITIN VE BAZI MİNERALLERİN ETKİSİNİN DEĞERLENDİRİLMESİ

Nuha Hamza Khedhir KHEDHIR
Kimya, Doktora
Tez Danışmanı: Doç. Dr. Şevki ADEM
Eş Danışman: Dr. Öğr. Üyesi Summer Saad ABDULHUSSAIN
Haziran 2023

Fekal kalprotektin, UC'li hastalarda histolojik remisyonu tahmin etmek için kullanılabilir, ancak bu biyobelirteç miktarını ölçmek için kullanılan teste ve histolojik remisyonun tanımına bağlı olarak kesme seviyesi araştırmalar arasında farklılık gösterir. Ülseratif kolitli hastalarda biyolojik tedavinin tanı ve kontrolünde bazı immünolojik, biyokimyasal ve hematolojik değişkenlerle fekal kalprotektinin rolünü ve bunun çalışma değişkenlerinin geri kalanıyla ilişkisini anlamak için, Biyolojik tedavi alan ülseratif kolit hastaları ve biyolojik tedavi almayan hastalar için fekal Calprotectin testi ve bunların sağlıklı bireylerle ilişkileri ve bu mikroplardan hangi parametrelerin ne ölçüde etkilendiğinin bir göstergesi. Ekim 2021'den Haziran 2022'ye kadar Kerkük Şehrindeki Azadi Eğitim Hastanesi gastroenteroloji kliniğine ve özel Kerkük kliniğine üst ve alt GİS endoskopisi için sevk edilen hastalardan her grup için 50'şer olmak üzere 150 hastadan (erkek ve kadın) örnekler toplandı. rektal aciliyet ile kanlı ishal, rektal kanama, sık dışkı ve rektumdan mukus akıntısı, tenesmus ve alt karın ağrısı ve bir klinik çalışma (rastgele) yapıldı. Görüşmeler, ülseratif kolitin nedensel faktörlerini araştıran bir anket kullanılarak yapıldı. Bu çalışmanın sonuçları, biyolojik tedavi grubu olan ve olmayan hastalarda fekal kalprotektin testi ile bazı immünolojik, biyokimyasal ve hematolojik değişkenler arasında kontrol grubuna göre güçlü bir ilişki olduğunu ve biyolojik tedavi grubu arasında anlamLı bir fark olduğunu göstermektedir. biyolojik tedavi ve kontrol grubunda fekal kalprotektin (FC), ferritin, IL-6, Hs-CRP, PCT, K, WBC, trombosit, vitamin B12, Na ve Cl ile hasta arasında anlamLı fark bulunurken, biyolojik tedavi alan

hastalar arasında D vitamini ve Folatta grup ve kontrol grubu arasında anlamLı fark bulunmazken, kontrol grubu ile Hb arasında her iki grup arasında anlamLı fark yoktu. Çalışmanın sonuçları şöyleydi: Fekal kalprotektin (FC) seviyesi kullanılarak ülseratif kolit gelişimini tahmin ederken yüksek doğruluk ve kesinlik elde edilebilir. Çalışmamızın sonuçlarına göre, biyolojik tedavi grubu alan hastalarda biyolojik tedavi grubu almayan hastalara göre immünolojik, biyokimyasal ve hematolojik değişkenlerde anlamLı derecede olumLu değişikliklerin olduğu UC tedavisinde biyolojik tedavinin önemLi bir rolü olduğu sonucuna varılabilir.

2023, 115 Sayfa

Anahtar Kelimeler: İnflamatuar barsak hastalığı, Ülseratif kolit, Fekal kalpprotectin, Vitamin B12, hsCRP

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Nuha Hamza Khedhir KHEDHIR

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CONTENTS

LIST OF SYMBOLS

Cm	Centimeter
^{O}C	Degree celsius
g	Gram
KDa	Kilo daltun
L	Liter
µg	Microgram
µL	Microliter
mg	Milligram
mL	Milliliter
Pg	Picogram
rpm	Revolution per minute

LIST OF ABBREVIATIONS

ASUC	Acute severe ulcerative colitis
BRMs	Biological response modifiers
BSA	Bovine serum albumin
CP	Calprotectin
Cl	Chloride
CLL	Chronic lymphocytic leukemia
CD4	Cluster of differentiation
CSF	Colony-stimulating factors
CBC	Complete blood count
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ECL	Electrochemiluminescence
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzy-linked ımmunosorbent assay
ESR	Erythrocyte Sedimentation Rate
EDTA	Ethylenediaminetetraacetic acid
ESPEN	European Society for Clinical Nutrition
ECF	Extracellular fluid
FC	Fecal Calprotectin
GI	Gastrointestinal
GFR	Glomerular filtration rate
G-CSF	Granulocyte colony-stimulating factors
GM-CSF	Granulocyte-macrophage colony-stimulating factors
HCT	Haematocrit
H. pylori	Helicobacter pylori
HGB	Hemoglobin
Hs-CRP	High-Sensitivity C-reactive protein
HER	Human epidermal growth factor receptor
IBD	Inflammatory bowel disease
IL	Interleukin
ICF	Intracellular fluid
IVCS	Intravenous corticosteroids
IBS	Irritable bowel syndrome
JAK/STAT	Janus Kinase/Signal transducers and activators of transcription
kDa	Kilo daltun
LES	Lower esophageal sphincter
MCHC	Mean corpuscular hemoglobin concentration
Ras/MAPK	Mitogen-activated protein kinase
MRP	Myeloid-related protein

P-ANCA	Perinuclear anti-neutrophil cytoplasmic antibodies
PBS	Phosphate buffered saline
PI3K	Phosphoinositide-3-kinase
PMN	Polymorphonuclear leukocytes
PMN	Polymorphonuclear leukocytes
K+	Potassium
PCT	Procalcitonin
PKB/Akt	Protein kinase B
PCFT	Proton-Coupled folate transport
ROC	Receiver operating characteristic
RAGE	Receptor for advanced glycation end products
RDW	Red cell distribution width
SMC	Sensotronic Memorized Calibration
Na+	Sodium
STfR	Soluble transferrin Receptor
TMB	Tetramethylbenzidine
TGF	Transforming growth factor
TBS	Tris- Buffered saline
TB	Tuberculosis
TNF	Tumour necrosis factor
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor
VEGF	Vascular endothelial growth factor

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1. INTRODUCTION

An example of an inflammatory bowel disease is ulcerative colitis (UC). Neutrophils have been thought to be crucial in mucosal injury in UC, albeit their genesis and pathophysiology are yet unknown. One distinctive morphologic aspect of UC is the presence of a significant neutrophil infiltration in damaged mucosa. Proteases and reactive oxygen species are two harmful substances produced and released by activated neutrophils that can harm tissue (Kayo *et al.* 2006).

Ingestion, propulsion, mechanical or physical digestion, chemical digestion, absorption, and feces are the six steps in the digestion process. Ingestion, the first of these procedures, is the process by which food enters the alimentary canal through the mouth. Saliva, which contains enzymes that start breaking down the food's carbohydrates as well as some fat digestion via lingual lipase, gets mixed with the food as it is chewed there. Chewing enhances the food's surface area and enables the creation of a bolus that is the right size (Tapia *et al.* 2023).

The involvement of activated platelets in the polymorphonuclear leukocytes (PMN)-mediated mucosal damage in inflammatory bowel disease (IBD) is still unknown, although the quantity of active platelets increases in the peripheral blood of patients with IBD. The secondary activation of PMN caused by platelet activation in UC may be to blame for the rise in UC-related PMN-mediated tissue damage (Suzuki *et al.* 2001).

A 36 kDa protein belonging to the S100 family is called calprotectin. It has direct antimicrobial actions, plays a part in the innate immune response, and is mostly generated by neutrophils. The content of calprotectin in different body fluids varies according to the level of inflammation present, and it is approximately six times higher in feces than in plasma. Fecal calprotectin measurement is a helpful proxy for gastrointestinal inflammation. It is a useful tool for prioritizing endoscopy since it has a strong negative predictive value for ruling out inflammatory bowel disease (IBD) in undiagnosed, symptomatic individuals and a high sensitivity for detecting the condition.

Fecal calprotectin can be a helpful management tool in individuals with known IBD, showing signs of relapse or mucosal repair to allow therapy to be increased or decreased (Khaki-Khatibi *et al.* 2020, Ayling and Kok 2018).

Commercial calprotectin assays vary significantly in performance as determined by external quality testing, and there is currently no standardized reference material available. Results may be impacted by several variables, such as age, medication, and daily variation. Hence, laboratories should be aware of their own assay's properties and any potential influencing factors (Ikhtaire *et al.* 2016, Ayling and Kok 2018).

1.1 Aim of Study

- Evaluate the role of fecal calprotectin with some immunological, biochemical, and hematological variables in the diagnosis and control of biological therapy for patients with ulcerative colitis and its association with the rest of the study variables.
- Study of the relationship between the Fecal Calprotectin test and some immunological, biochemical, and hematological variables in the diagnosis for patients who take biological therapy and patients who do not take biological therapy, and their relationship with healthy subjects, and an indication of the extent to which parameters are affected by these microbes.

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2. LITERATURE REVIEW

2.1 General Information

As in vitro culture systems, human organoids where stem cells spontaneously self-organize into three-dimensional structures resembling human organs have drawn a lot of attention. Access to the digestive tract in humans is typically restricted, making it difficult to test rapidly advancing theories about diagnosis and treatment. Thus, digestive organoids can be employed as a different model to research the pathobiology of patients and their unique therapeutic responses, providing a cutting-edge method for drug discovery, toxicity, and precision medicine (Funata *et al.* 2021, Jung *et al.* 2018). The gastrointestinal (GI) tract, as well as the liver, pancreas, and gallbladder, make up the digestive system. The GI tract is made up of several interconnected hollow organs that extend from your mouth to your anus. The mouth, esophagus, stomach, small intestine, large intestine, and anus are the organs that make up the GI tract, in the order that they are connected. The digestive system is specially designed to carry out its function of converting food into the nutrients and energy an organism requires to exist. Because the body needs nutrients from the food and liquids it consumes to stay healthy and operate correctly, digestion is crucial. Carbohydrates, proteins, lipids, vitamins, minerals, and water are all examples of nutrients. To use nutrients for vital purposes including energy, cell growth, and repair, the digestive system breaks down and absorbs nutrients from the food and liquids that are consumed (Funata *et al.* 2021, Dedhia *et al.* 2016).

2.2 Digestive System

The digestive system in humans is utilized to carry out the digestion process. The digestive tract, or the group of structures and organs through which food and liquids pass as they are transformed into forms that may be absorbed into the bloodstream, makes up the majority of the human digestive system. The system also includes the organs that supply the juices required for digestion as well as the structures via which

wastes are eliminated. Abdominal organs, their structures, and the activities of the human digestive system (Sato *et al.* 2011).

The tongue and pharyngeal muscles push food out of the mouth and into the esophagus. The movement of food through the digestive tract is an example of propulsion, which is the last voluntary act before feces. Both the deliberate process of swallowing and the uncontrollable process of peristalsis are included in it. To move food forward, smooth muscles of the alimentary wall contract and relax in a series of waves. This process is known as peristalsis. The blending of food and digestive secretions is another function of these waves. Due to the strength of peristalsis, even while standing on your head, food and drinks that you swallow enter your stomach (Shahsavari and Parkman 2022).

Food is moved through the digestive tract via peristalsis, which involves waves of muscle contraction and relaxation that alternate. This picture illustrates how food moves peristaltically. The food bolus is located near the top of the esophagus in the left image, and arrows pointing downhill indicate the direction in which the peristaltic wave is moving. The food bolus and wave movement are closer to the center of the esophagus in the center image than they are to the bottom end of the esophagus in the right image (McQuilken 2021, Kozu *et al.* 2014).

Both mechanical and chemical activities take place during digestion. The process of mechanical digestion is entirely physical and has no impact on the food's chemical composition. Instead, it reduces the size of the food to enhance its mobility and surface area. It entails mastication, or chewing, along with tongue motions that facilitate breaking food into smaller pieces and blending it with saliva. Mechanical digestion happens after the meal exits the mouth as well, despite the common misconception that it only happens during the earliest stages of digestion. Chyme, an acidic "soup," is produced when the mechanical churning of food in the stomach further disintegrates it and exposes more of its surface to digestive fluids. Segmentation, which mostly affects the small intestine, is made up of localized contractions of the circular muscle found in the alimentary canal's muscular layer. These contractions separate little portions of the intestine, allowing their contents to move back and forth while being continuously

divided, broken up, and mixed. Segmentation combines food with digestive fluids and speeds up the absorption by causing food to move back and forth in the intestinal lumen (Kong and Singh 2010, Bognon-Küss 2022).

Digestive secretions break down complex food molecules into their chemical building components during chemical digestion, which begins in the mouth (for example, proteins into separate amino acids). Although the contents of these secretions vary, they often include water, different enzymes, acids, and salts. The small intestine is where the procedure is finished (Nadia *et al.* 2021).

Even after food has been digested, the body cannot benefit from its nutrients until they are absorbed into the bloodstream. This happens as a result of the absorption process, which largely occurs in the small intestine. There, the mucosa's epithelial cells absorb the majority of nutrients from the alimentary canal's lumen into the bloodstream. Lipids are taken up by lacteals and delivered to the bloodstream through lymphatic veins. Later, we'll talk about these processes' specifics. Undigested elements are expelled from the body as feces during defecation, which is the last phase of digestion. Sometimes the digestion process is controlled by a single organ. For instance, just the mouth and anus are used for ingesting and excretion respectively. However, as food passes through the alimentary canal, the majority of digestion processes entail the cooperation of numerous organs and take place gradually (Wang*et al.* 2022).

Ingestion, propulsion, mechanical digestion, chemical digestion, absorption, and feces are the digestive processes. The picture depicts how food moves through the body's primary organs after leaving the mouth. Near the organs where they occur, corresponding textboxes list several processes including propulsion, chemical and mechanical digesting, and absorption.In the mouth, some chemical digestion takes place. Alcohol and aspirin are two examples of substances that can be absorbed in the mouth and stomach (Figure 2.1) (Millynn 2022).

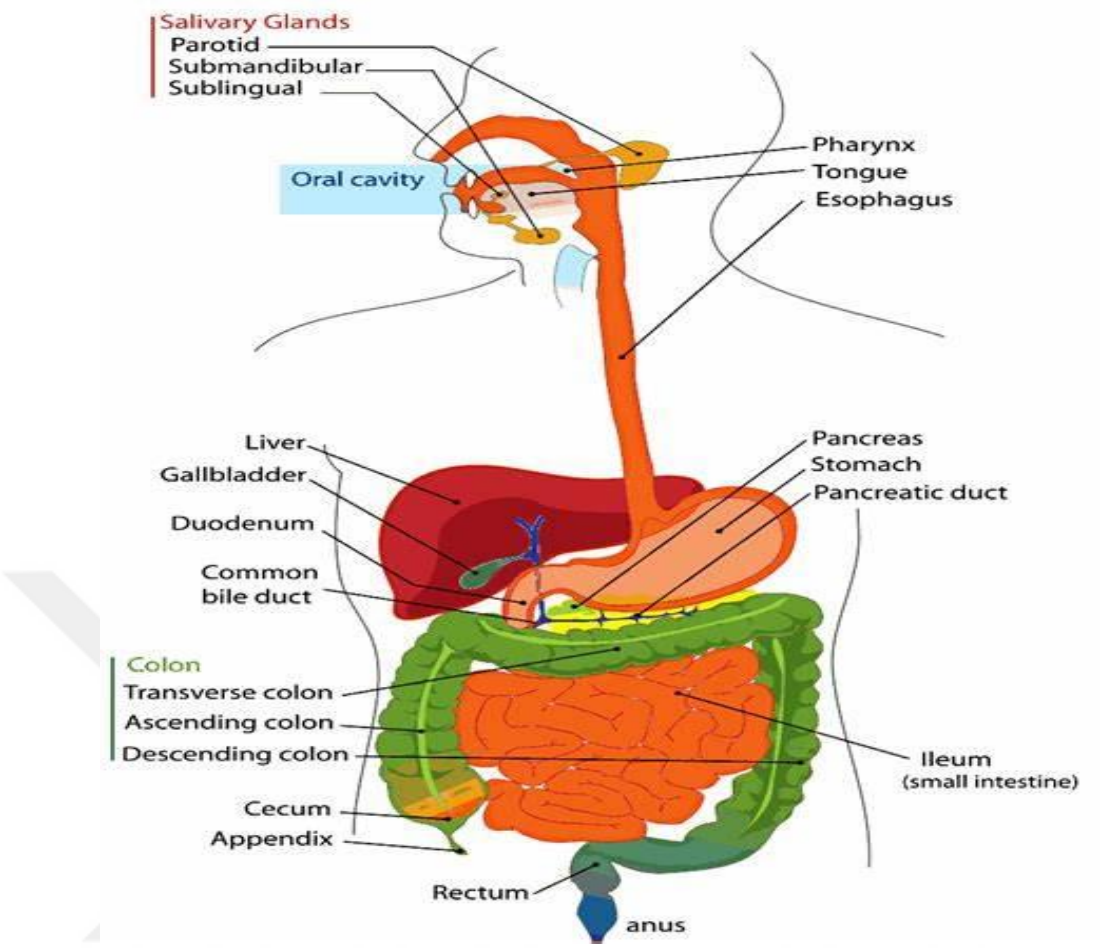

Figure 2.1 The digestive system

The digestive system runs from the mouth to the anus. It consists of the mouth, also known as the oral cavity, which has teeth for grinding food and a tongue for mixing food and saliva; the throat, also known as the pharynx; the esophagus; the stomach; the small intestine, which is made up of the duodenum, the jejunum, and the ileum; and the large intestine, which is made up of the cecum, a closed-end sac connecting with the ileum; the ascend The salivary glands, the gastric glands in the stomach lining, the pancreas, the liver, and the livers supporting organs the gallbladder and bile ducts all contribute digestive fluids. The physical and chemical breakdown of ingested food and the eventual evacuation of nondigestible wastes are both facilitated by each of these organs and glands. In this section, their structures and roles are step-by-step explained (Hillman *et al.* 2017).

2.2.1 Introduction to the upper gastrointestinal tract

The mouth, throat, and stomach are GI (upper gastrointestinal) tract organs in addition to the esophagus. When food is being digested, it goes through a tube made up of these hollow organs that are all connected. The pharynx and esophagus' sole function in digestion is to transport food through the GI tract. Contrarily, the stomach, and mouth are the organs where food is also broken-down during digestion. In both of these organs, food is chemically and mechanically broken down into smaller pieces (mechanical digestion) (chemical digestion). It should be mentioned that although the duodenum, the first segment of the small intestine, is sometimes regarded as a part of the upper GI tract, this approach is not used here. The concept Lower Gastrointestinal Tract contains information on the small (and big) intestine (Treuting *et al.* 2018, Sakata *et al.* 2019).

2.2.2 Oral and pharyngeal structures

The structure mouth (Figure 2.2): Actually, just a small amount of food is digested in the mouth. However, food is prepared in the mouth for transportation via the upper digestive system into the stomach and small intestine, where the primary digestive processes take place, through the process of mastication, or chewing. The first mechanical step that food goes through is chewing. The muscles of mastication cause the lower jaw to move when chewing (the masseter, the temporal, the medial and lateral pterygoids, and the buccinator). The force of the bite is controlled by the sensitivity of the periodontal membrane that surrounds and supports the teeth, not by the strength of the masticatory muscles (Huch *et al.* 2013).

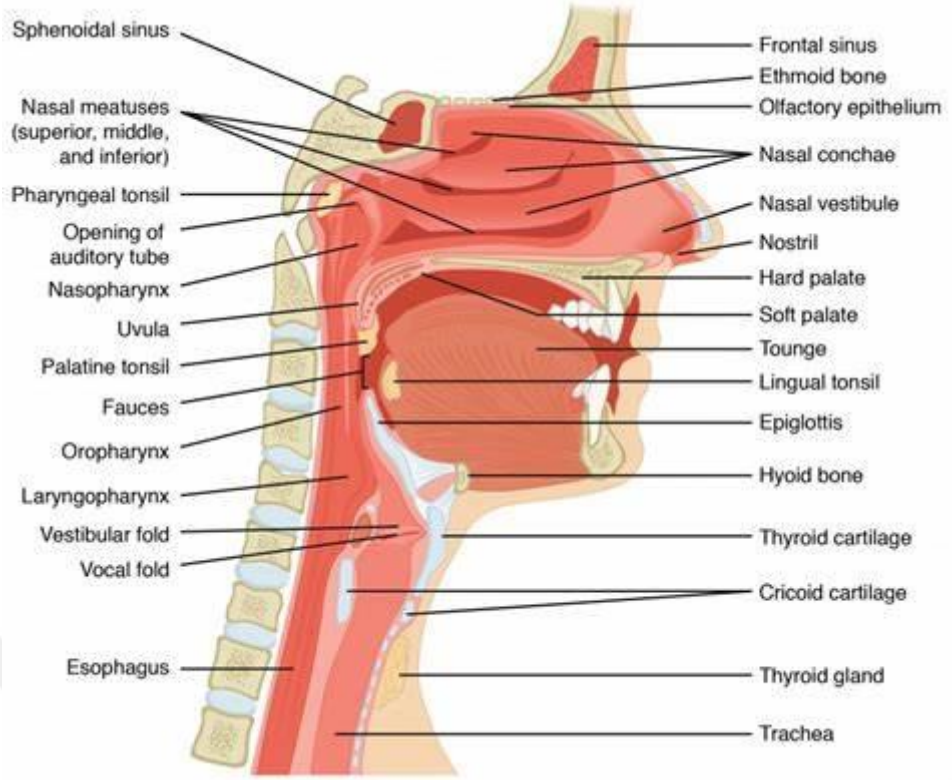

Figure 2.2 Oral and pharyngeal structures

For sufficient digestion, mastication is not necessary. However, by breaking down food into smaller pieces and combining it with saliva produced by the salivary glands, chewing does help digestion. Dry food is lubricated and moistened by saliva, which is then distributed throughout the food mass while you chew. A spherical lump of food, or bolus, is formed by the tongue moving against the hard palate and the cheeks (Pedersen *et al.* 2018).

The mouth's roof. The hard and soft palates combine to form the concave roof of the mouth. The horizontal sections of the two palatine bones and the palatine portions of the maxillae, or upper jaws, combine to produce the hard palate. The mucous membrane covering the hard palate is thick and slightly pale; it is continuous with the gums and is attached to the upper jaw and palate bones by tough fibrous tissue. The front hard palate continues the front soft palate. It continues with the mucous membrane that covers the nasal cavity's floor in the back. The palatine aponeurosis, the glossopalatine and pterygopalatine muscles, and a robust, thin, fibrous layer make up the soft palate. The

soft palate's back has a little protrusion called the uvula that hangs freely (Kalabis *et al.* 2012).

The submandibular gland is the second largest of the three main salivary glands, which also include the parotid and sublingual glands. The submandibular glands are paired with major salivary glands that lie in the submandibular triangle. The glands have a superficial and deep lobe separated by the mylohyoid muscle.

The Wharton duct, the submandibular gland's primary excretory duct, drains into the oral cavity at the sublingual caruncle. The sublingual caruncle is a papilla located medial to the sublingual gland and lateral to each side of the frenulum linguae. The submandibular gland produces approximately 70% of the saliva in the unstimulated state. However, the parotid gland's saliva production predominates once the salivary glands become stimulated. (Grewal *et al.* 2022)

2.2.3 Epidemiology of the gastrointestinal tract

In tropical regions, digestive disorders are among the most frequent health issues. They frequently present as diarrhea, abdominal pain, distention, gastrointestinal hemorrhage, intestinal blockage, malabsorption, or malnutrition. Children's morbidity and death are significantly influenced by infectious diarrheal illnesses. A variety of disorders that affect the mouth, esophagus, stomach, hepatobiliary, and pancreatic systems, as well as the small and large bowels, rectum, and anus, are covered in this chapter along with acute and chronic diarrheal syndromes. Special focus is placed on diseases that are more prevalent in tropical regions, such as intestinal intussusception, tropical enteropathy, tropical sprue, duodenal ulcer, gastroenteritis, TB of the stomach and intestine, malabsorption, and tropical enteropathy. Many infectious diseases have been burdened less by improved sanitation and socioeconomic conditions, but this is also accompanied by the rise of previously uncommon diseases in the tropics such as celiac disease and inflammatory bowel diseases (Ananthakrishnan and Xavier 2020).

2.2.4 Gastrointestinal diseases

Many diseases and health problems may affect the digestive system (For example Ulcerative colitis) and affect the nature of its work, and these health disorders may affect various parts of the digestive system and the alimentary canal, and their severity ranges from severe or simple disorders that last for short periods such as mild cases of heartburn, and severe or Chronic diseases that may threaten the life of the infected person in some cases, such as perforating ulcers, and Gastrointestinal inflammatory diseases are mainly induced by bacteria, and have risen due to the emergence of antibiotic-resistant strains. In the lack of effective treatments, phage therapy has been proposed as a clinical alternative to restore intestinal eubiotics, thanks to its immunomodulatory and bactericidal effect against bacterial pathogens, such as *Clostridioides difficile* in ulcerative colitis and invasive adherent *Escherichia coli* in Crohn's disease. In addition, genetically modified temperate phages could be used to suppress the transcription of bacterial virulence factors. In this review, we will highlight the latest advances in research in the field, as well as the clinical trials based on phage therapy in the area of gastroenterology. (Zumbach *et al.* 2002, Gutiérrez *et al.* 2020).

The different symptoms, which may be very disturbing in some cases, such as flatulence, diarrhea, constipation, and other digestive disorders, so many people suffer from these symptoms, In turn, it led to the production of many drugs that contribute to the elimination or alleviation of these symptoms, and in the following is a statement of some common diseases and health problems that may affect the digestive system: And this may happen during intermittent periods even in some healthy people, but if stomach acid reflux is repeated in a simple, moderate or severe manner, Once a week, or if it causes damage to the esophagus, or the appearance of various symptoms, gastroesophageal reflux disease is diagnosed, and it is worth noting that after food moves through the esophagus to the stomach in normal cases, the circular muscle known as the lower esophageal sphincter (Lower esophageal sphincter) contracts. In short, the LES is located between the esophagus and the stomach to prevent food from returning back into the esophagus, while this muscle is in a state of relaxation during

eating to allow food to enter the stomach. Esophagus (Gordon *et al.* 2001, Iacono *et al.* 2005).

It should be noted that in many cases, gastroesophageal reflux disease can be controlled by making some lifestyle changes and using some types of medications that do not need a prescription to alleviate the symptoms associated with the disease, but in some severe cases, the infected person may need to Performing surgery or using some medications that require a prescription to help control the disease and its accompanying symptoms, and in the following a statement of some of these symptoms: night after lying down to sleep. chest pain difficulty swallowing Burping up bits of food or sour liquid. Stomach acid reflux during sleep, which in turn may lead to coughing, sleep disturbances, laryngitis, and asthma (Kang *et al.* 2017, Demeter and Pap 2004).

One of the most prevalent chronic, dangerous, and sneaky diseases affecting people is chronic gastritis. Numerous hundreds of millions of individuals worldwide may have chronic gastritis in one way or another, according to estimates that more than half of the world's population has this disease to some degree or extent. Although chronic gastritis has been known about and studied since the early 20th century, Warren and Marshall's discovery of Helicobacter pylori in 1982 was what brought it more widespread notice. With the probable exception of autoimmune gastritis, it is now abundantly known that the vast majority of instances of gastritis are caused by bacteria. The elimination of H. pylori has therefore made it clear that chronic gastritis is curable, at least in situations where gastritis has not progressed to the atrophic (atrophic gastritis) end stages, leading to normalization of the stomach mucosa (Koivurova *et al.* 2021, Telaranta-Keerie *et al.* 2010).

Even though it is evident that gastritis has a role in the etiology of common peptic ulcers and stomach malignancies, the relevance of chronic gastritis as a dangerous disease is usually underestimated in clinical practice. According to estimates, millions of people worldwide die prematurely each year as a result of cancer and ulcers, which are chronic gastritis side effects. Either the non-atrophic or atrophic forms of chronic gastritis might be seen. These gastritis phenotypes and morphologies indicate several

stages of the same chronic condition. Because gastritis's documented morphological manifestations are so similar over the world, chronic gastritis and its complications appear to be the same condition everywhere (Sipponen and Maaroos 2015).

Even though the fundamentals of chronic gastritis are well understood, there are still many unsolved concerns. For instance, we are unsure of the role that genetics or immunology play in the onset and progression of chronic H. pylori gastritis. The molecular mechanisms underlying chronic gastritis, as well as the effects of environmental factors including nutrition and other bacteria besides H. pylori, are largely unclear. We are unable to accurately anticipate either who will develop chronic gastritis and advance to atrophic end stages and fatal consequences, or who will not. This confusion also applies to the specifics of how gastritis causes peptic ulcers or stomach cancer to manifest itself. However, we are aware that common peptic ulcers and stomach cancer are uncommon without the presence of concurrent chronic gastritis or atrophic gastritis (Shi *et al.* 2020, Arkkila *et al.* 2006).

2.2.5 Ulcerative colitis

History of ulcerative colitis: For many years, little was understood about ulcerative colitis and Crohn's disease. Two English doctors named Wilks and Moxon originally identified ulcerative colitis in 1875 and separated it from infectious diarrheal illnesses. Before the Civil War, there were reports of a condition that shared symptoms with ulcerative colitis, albeit it wasn't labeled as a separate illness until 1875 (Akkol *et al.* 2020, Suciangto and Syahrir 2022).

While Aretaeus reported a variety of diarrheal diseases, including one with "foul evacuations," which primarily affected older children and adults, Hippocrates realized that diarrhea was not a single disease entity. Roman doctors, including Ephesus in the eleventh century, reported what appeared to be "ulcerative colitis." For centuries, "noncontagious diarrhea" flourished under a variety of names, including Thomas Sydenham's "bloody flux" in 1666. U.S. Army doctors first characterized the symptoms

of an "ulcerative colitis-like" disease in 1865. (A few of these examples lean more toward ulcerative colitis than Crohn's disease (Aniwan *et al.* 2021, Park *et al.* 2007).

Numerous doctors from England, France, Germany, Italy, and other European nations described ulcerative colitis in the 1880s and 1890s. W. Hale-White first noted the link between ulcerative colitis and liver illness in 1895, and R. F. Weir performed an appendectomy to help ulcerative colitis patients with "colonic discharge" in 1920. J. P. Lockhart-Mummery highlighted the diagnostic significance of sigmoidoscopy in 1907 when he described colon cancer in individuals with ulcerative colitis. W. H. Allchin may have identified the first case of "familial" ulcerative colitis in 1909. At the 1913 Paris Congress of Medicine, additional cases were reported, and that same year, J. Y. Brown may have made the first ileostomy suggestion for the surgical treatment of ulcerative colitis (Selby 1997, Kirsner 1995).

Several reports, including those by H. Rolleston, C. E. Smith, J. M. Lynch and J. Felsen, A. F. Hurst, and E. Spriggs, were among the many that were published during the 1920s. Perhaps Hermann Strauss was the first to suggest blood transfusions for ulcerative colitis. J. A. Bargen published his research in 1924, linking the diplostreptococcus to ulcerative colitis theory that was later disproved. His 1946 investigation into the symptoms, complications, and treatment of "thrombo-ulcerative colitis" was more significant. The psychogenic features of ulcerative colitis were first brought up by C. D. Murray in 1930, which led to a period of strong psychiatric research on the disease from 1930 to 1960 (Park *et al.* 2007, Chaudhary and Truelove 1961).

The International Congress of Gastroenterology in 1935 brought the condition to the attention of the world, and the volume of literature grew quickly following this. Ulcerative colitis was more frequently diagnosed than Crohn's disease by the 1940s. However, Crohn's disease had increased in prevalence by the end of World War II. The more prevalent form of Crohn's disease has appeared concurrently with the apparent stability of ulcerative colitis in the United States (Magro *et al.* 2017).

2.2.6 Inflammation and symptoms of ulcerative colitis

Inflammation and ulcers of the colon and rectum are symptoms of the chronic illness known as ulcerative colitis (UC). Abdominal pain and bloody diarrhea are the main signs of an active illness (hematochezia). There may also be anemia, fever, and weight loss. Symptoms frequently appear gradually and might be minor to severe. Symptoms often come and go, with intervals between flare-ups when there are no symptoms. Colon cancer, liver inflammation, aberrant colon dilation (megacolon), and joint, eye, or joint inflammation are all possible complications (Shentova-Eneva and Yankov 2022, Feakins 2014).

There is no known cause of UC. Genetics, modifications to the healthy gut flora, immune system malfunction, and environmental influences are among the theories. Some claim that because of a Western diet and lifestyle or less exposure to intestinal illnesses, rates are often higher in the industrialized world. Early appendix removal might offer some protection. Usually, a colonoscopy with tissue samples is used for diagnosis. It belongs to the same class of inflammatory bowel diseases (IBD) as Crohn's and microscopic colitis (Sartor 2006).

A recurrent, chronic mucosal condition that affects just the colon, ulcerative colitis (UC) spreads continuously proximally from the rectum. An aberrant cell-mediated immune response to commensal enteric bacteria in the large intestine may play a role in the hereditary cause of UC. Environmental factors like nutrition are implicated by the disorder's relapse/remission cycle and the substrate-driven nature of microbial metabolism in the large bowel (Magee *et al.* 2005, Travis *et al.* 2012).

Despite being a very uncommon side effect of ulcerative colitis, colorectal cancer is more common in people with ulcerative colitis than in the overall population (Sugita *et al.* 1991).

Physicians use a variety of factors, including symptoms, levels of test biomarkers, as well as clinical and endoscopic findings, to assess the activity of ulcerative colitis (UC) in a particular patient. There is frequently little connection between endoscopic severity and patient complaints, and colonoscopy has the disadvantages of being invasive, expensive, and time-consuming (Hanauer *et al.* 2000, Sandborn *et al.* 2007). As a result, several laboratory biomarkers have been assessed to track the activity of endoscopic ulcerative colitis. Leukocyte migration to the gut lumen and an acute phase reaction are both signs of active inflammation in ulcerative colitis. As a result, increased protein levels can be seen in serum and feces. Increased levels of C-reactive protein have been linked to endoscopic and clinical ulcerative colitis activity. An important biomarker for tracking the progression of inflammatory bowel disease (IBD) is fecal calprotectin. 60 percent of the cytosolic proteins in granulocytes are made up of fecal calprotectin (Mazlam *et al.* 1992, Johne *et al.* 1997). The migration of neutrophils to the gastrointestinal mucosa is proportional to elevated fecal amounts of this protein. At room temperature, fecal calprotectin is stable for up to a week. IBD may be accurately distinguished from non-inflammatory bowel illnesses like irritable bowel syndrome by measuring the levels of this biomarker (IBS). Additionally, it has been suggested that elevated levels of fecal calprotectin are a sign of clinical recurrence in IBD patients. Fecal calprotectin levels have been shown in numerous studies in pediatric and adult cohorts to be indicative of the degree of mucosal inflammation and, as a result, can aid in the distinction between active and inactive IBD. Additionally, hematologic changes including thrombocytosis and anemia are linked to ulcerative colitis activity (Schoepfer *et al.* 2008, Larsen *et al.* 2002).

Using several biomarkers to evaluate endoscopic disease activity can be essential for early relapse diagnosis and for adjusting individual therapy. According to recent scientific research, it is crucial to pursue mucosal healing as a therapeutic goal in addition to clinical remission because it is linked to a decreased need for hospitalization and UC-related surgery. As a result, the evaluation of appropriate biomarkers has become increasingly important in the clinical practice of managing UC patients (Arnot *et al.* 2002, Lewis 2011).

Classification of ulcerative colitis: A persistent condition called ulcerative colitis may impact several regions of the colon and rectum. Depending on the severity of your condition and the type of ulcerative colitis you have, there is a wide range of symptoms and problems. Being told that you have a chronic, lifelong illness is terrifying (Kim *et al.* 2014).

2.2.7 Ulcerative proctitis

Bowel inflammation in ulcerative proctitis is restricted to the rectum. This ailment usually only affects the first six inches of the rectum, and it doesn't raise the chance of developing cancer. Symptoms may include:

- Rectal bleeding
- Rectal pain
- Urgency in your bowel movements

2.2.8 Left-sided colitis

This type of ulcerative colitis features ongoing inflammation that starts at the rectum and progresses all the way to the splenic flexure, a bend in the colon next to the spleen. Proctosigmoiditis, which affects the rectum and the lower portion of the colon directly above the rectum known as the sigmoid colon, is also a component of left-sided colitis. Symptoms may include:

- Loss of appetite
- Weight loss
- Bloody diarrhea
- Pain on the left side of the abdomen

2.2.9 Extensive colitis

The entire colon is affected by this kind of ulcerative colitis. The rectum is the site of ongoing inflammation that continues past the splenic flexure. Symptoms may include:

- Loss of appetite
- Bloody diarrhea
- Abdominal pain
- Weight loss (Conrad *et al.* 2014, Stange *et al.* 2008).

2.3 Biological Therapy

The treatment known as biological or biologic therapy aims to boost or restore the body's immune system's (natural internal defense) capacity to combat illness and infection. Numerous cancers, as well as ulcerative colitis and other conditions, are frequently treated with biological therapy, also known as biotherapy or immunotherapy (Yanai and Hanauer 2011). A form of cancer treatment known as biological therapy employs the body's immune system to eradicate cancer cells. Many different types of cancer are treated with biological therapy to stop or slow the growth of tumors and stop the spread of the disease. In comparison to other cancer treatments, biological therapy frequently has fewer harmful side effects (Schirrmacher 2019).

2.3.1 The working of biological therapy

A method of treatment known as biological therapy makes use of the body's immune system to combat disease. The body can be protected from some of the negative effects of particular medicines via biological therapy. Biological response modifiers are frequently used during biological therapy (BRMs). Small amounts of these substances are typically produced by the body in response to illness and infection. BRMs can be produced in vast quantities in contemporary laboratories for use in the treatment of

cancer and other illnesses like rheumatoid arthritis and Crohn's disease (Mishra *et al.* 2013, Yanai and Hanauer 2011). Biological therapy can also target particular molecules on cancer cells to kill them, or it might target proteins that promote the development of cancer cells. Biological therapy can be administered orally, intravenously, or as an injection, depending on the agent (Subramani *et al.* 2009).

Biological medicines often function by:

making cancer cells vulnerable to immune system action. This objective can be accomplished through biological therapy treatments in several ways. Chemicals that activate immune system cells, for instance, could be injected into the body. Another option is to train a sample of the immune system cells in a lab to destroy cancer cells before reintroducing them to the body. This makes it simpler for the immune system to identify cancer cells. Biological therapy can also specifically target cancer cells by activating or deactivating cell signals that aid in their ability to evade immune system cells. For instance, medications referred to as immune checkpoint inhibitors can target particular chemical receptors on cancer cells, preventing the cancer cells from sending signals that would otherwise suppress the immune system (Xiao *et al.* 2021, Giovannini *et al.* 2019).

2.3.2 The application of biological therapies

The field of cancer research is particularly dynamic and intriguing in the area of biological therapy. Biological therapies include the use of monoclonal antibodies, interferon, interleukin-2 (IL-2), and various colony-stimulating factors (CSF, GM-CSF, G-CSF). Examples of BRMs being investigated for the treatment of advanced malignant melanoma are interleukin-2 and interferon. A typical sort of biological therapy for a variety of malignancies and other diseases uses monoclonal antibodies. These antibodies were created in a lab to target particular proteins expressed by aberrant cells. Rituximab, which is used to treat non-lymphoma, Hodgkin's alemtuzumab (Campath), which is used to treat chronic lymphocytic leukemia (CLL), and ipilimumab (Yervoy),

which is used to treat metastatic melanoma are some examples of monoclonal antibody medicines (Schirrmacher 2019, Jilaveanu *et al.* 2009).

Other varieties of monoclonal antibodies that are used to treat cancer focus on the proteins necessary for cell division. Bevacizumab (Avastin), which targets the vascular endothelial growth factor (VEGF), cetuximab (Erbitux), panitumumab (Vectibix), and trastuzumab (Herceptin), which targets the human epidermal growth factor receptor. (Moradi-Kalbolandi *et al.* 2018, Yanai and Hanauer 2011).

Many diseases, including rheumatoid arthritis and Crohn's disease, are being treated with biological therapy techniques that involve preventing the action of particular proteins that cause inflammation, known as tumor necrosis factor (TNF). For people with severe rheumatoid arthritis, commercially available injectable TNF-blocking medications include etanercept (Enbrel) and infliximab (Remicade) (Keilholz *et al.* 2002, Kay and Rahman 2009).

2.4 Lab. Diagnosis

2.4.1 Fecal calprotectin

Neutrophils produce a protein called calprotectin. Higher levels indicate increased inflammation; the presence of fecal calprotectin is a sensitive biomarker of gastrointestinal inflammation. The use of fecal calprotectin testing has been authorized by the U.S. Food and Drug Administration to help diagnose inflammatory bowel disease (IBD) in adults and children who present with gastrointestinal symptoms and to distinguish IBD from irritable bowel syndrome (Petryszyn *et al.* 2019).

For the diagnosis of IBD, the fecal calprotectin assay manufacturer suggests a diagnostic threshold of greater than 50 mcg per g. The diagnostic performance of a fecal calprotectin assay was compared with the reference standard of diagnostic colonoscopy

in a comprehensive evaluation of 19 studies on diagnostic accuracy, which comprised 5,032 individuals older than 16 years (Petryszyn *et al.* 2016, Holtman *et al.* 2016).

Fecal calprotectin testing was compared with one of two reference standards: endoscopy or 12 months of clinical follow-up without an IBD diagnosis in two prospective cohorts of kids in the Netherlands with chronic diarrhea, recurrent stomach discomfort, or both. The authors of the trial added the requirement of clinical follow-up because they believed it would be unethical to undertake an intrusive treatment on a large number of kids who had a low risk of having an organic gastrointestinal disease. A calprotectin cutoff of greater than 50 mcg per g was used to test 13 patients who were initially treated in primary care, but none of them had IBD. This resulted in a specificity of 0.87 (95% CI, 0.80 to 0.92) for the group of 114 patients. Fecal calprotectin testing revealed a sensitivity of 1.00 (95% CI, 0.81 to 1.00) and a specificity of 0.84 (95% CI, 0.74 to 0.91) in 85 individuals who were referred to a specialist (Holtman *et al.* 2016).

Because of its high sensitivity and negative predictive value, the diagnosis of IBD is unlikely in adults and children with a normal fecal calprotectin value of less than 50 mcg per g. A combination of a higher fecal calprotectin threshold of more than 250 mcg per g, a higher C-reactive protein level of more than 1 mg per dL (10 mg per L), and a hemoglobin level less than two standard deviations below the mean for age and sex increased the specificity to 0.97 in children with symptoms suggestive of IBD, while the sensitivity remained 100%. This helped prevent the need for unnecessary endoscopy (Van *et al.* 2020, Yang *et al.* 2014).

The chemical structure of calprotectin: The S-100 protein (Figure 2.3) family member calprotectin has a wide range of intra- and extracellular activities. Among the protein's appealing qualities for usage as a medicinal agent are its anticancer properties, antibacterial actions, and capacity as a disease marker. The application of protein medicines is hampered by several factors, including proteolytic breakdown, brief circulation half-life, limited solubility, and immunogenicity.

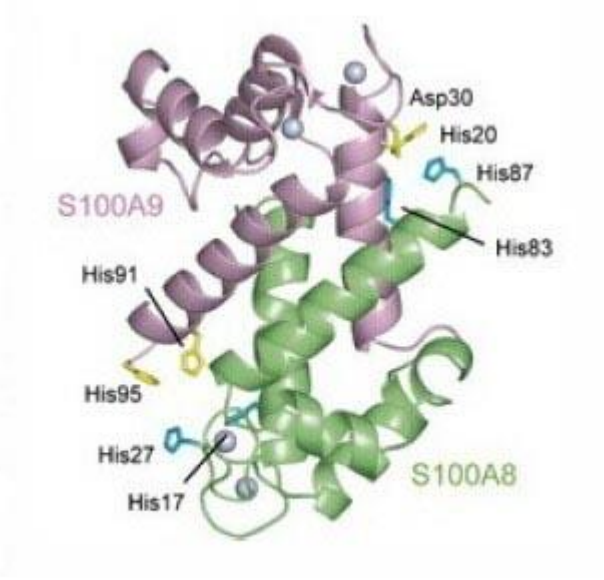

Figure 2.3 The S-100 protein

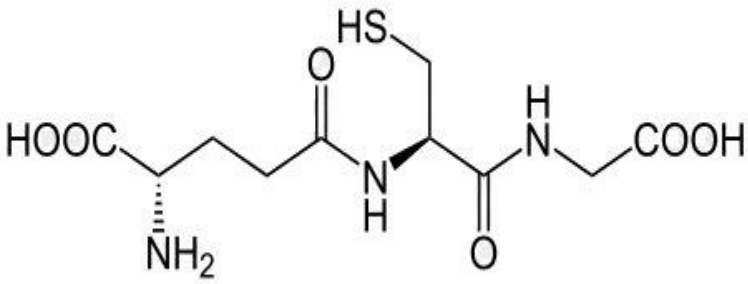

Figure 2.4 The structure of calprotectin

The S100 calcium-binding protein family's MRP8 and MRP14 two tiny anionic proteins form the heterodimer known as calprotectin. Numerous potentials use for calprotectin (Figure 2.4) have been proposed. It is also known as L1 antigen, cystic fibrosis antigen, calgranulin A-calgranulin B, MRP8-MRP14, and S100A8-S100A9. Calprotectin levels

significantly rise in serum and bodily fluids during inflammatory conditions such as rheumatoid arthritis and cystic fibrosis, indicating a regulatory function in inflammatory responses. In mouse and human cell lines, purified calprotectin slows cell development and causes apoptosis. Myelomonocytic and keratinocyte cell lineage development stages are also correlated with calprotectin expression. Lastly, due to its broad-spectrum antibacterial properties, calprotectin may shield epithelia against infection and support innate immunity (Nisapakultorn *et al.* 2001, Shahsavari *et al.* 2016).

Calprotectin's pegylation, recombinant expression, and purification would all improve its pharmacodynamic and pharmacokinetic qualities. Our findings from fluorescence spectroscopy and far ultraviolet-optical density suggest that pegylation changed the calprotectin's physical and structural characteristics to make it more stable and functionally active. This study would open the way for better in vitro and in vivo validations of calprotectin usage in medical practice since pegylation of the calprotectin improved its pharmacological and pharmacokinetic features (Clohessy and Golden 1995, Shahsavari *et al.* 2016).

Calprotectin, an essential component of the innate immune response, is a heterodimer of the calcium-binding proteins S100A8 and S100A9. Calprotectin (CP) is a ligand for various pattern-recognition cell surface receptors, such as the cluster of differentiation 33, toll-like receptor 4, and receptor for advanced glycation end products (RAGE) (CD33). Through the NF-kB pathway, the receptors start kinase signaling cascades that activate inflammation. When CP activates a receptor, both the receptor and its ligand are upregulated, creating a positive feedback loop linked to particular chronic inflammatory disorders. Therefore, it has been suggested that CP and its two constituent homodimers could be suitable targets to inhibit several chronic inflammatory diseases. Over the past 30 years, several CP and other S100 protein inhibitors have been studied, but none have made substantial progress in clinical trials. Here, recent developments in the creation of CP-directed chemotherapeutics are discussed along with the current understanding of how CP interacts with its receptors (Garcia *et al.* 2022, Eggers *et al.* 2011).

2.4.2 Interleukins (IL-6)

A pleiotropic cytokine with an important role in the control of the immune system is interleukin-6 (IL-6). IL-6 is a powerful cytokine that promotes inflammation and is essential for the host's defense against infections and acute stress. However, IL-6 expression that is either elevated or dysregulated plays a crucial role in the etiology of many human disorders. The pathogenic involvement of the IL-6 pathway in inflammation, autoimmune, and cancer has been identified in numerous preclinical and clinical investigations (Yao *et al.* 2014).

In Figure 2.5, interleukins (IL-6), tumor necrosis factor (TNF), and anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor, or TGF), among others, are released during this process. After surgery or trauma, hepatocytes release interleukin-6 (IL-6), the main and initial mediator involved in the activation and regulation of acute-phase protein synthesis. The most reliable and significant biomarker for pain is IL-6, which is also the most pertinent indicator of tissue damage following surgical procedures. Through local and systemic effects, the balance between proinflammatory and anti-inflammatory cytokines regulates the postoperative immune response, infection, and wound healing (Tantri *et al.* 2023).

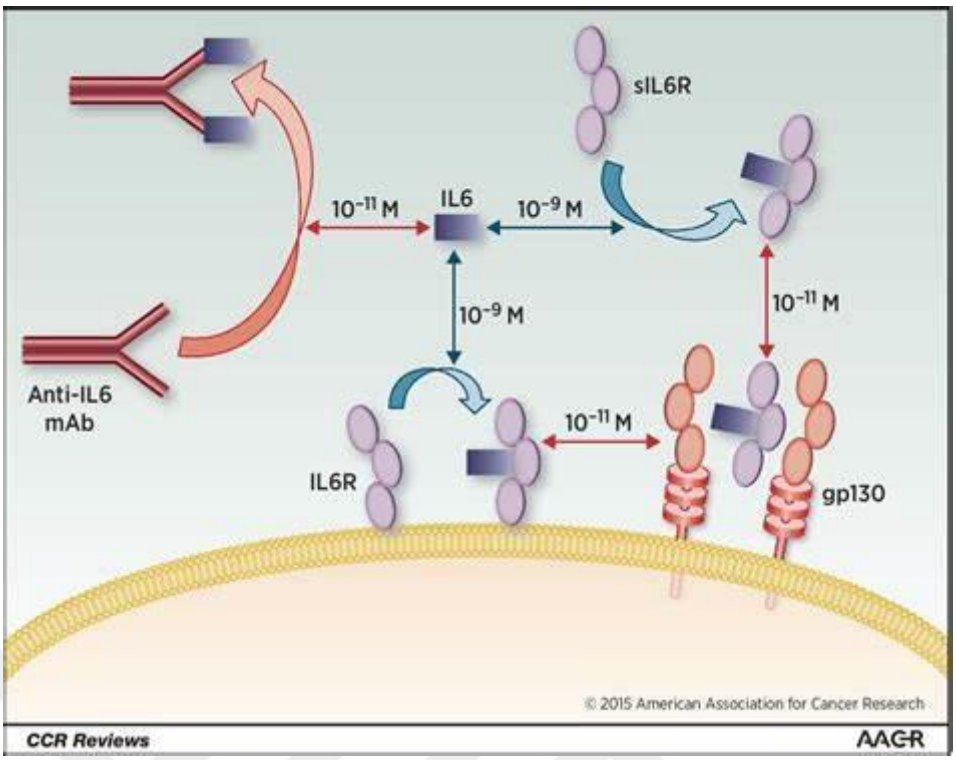

Figure 2.5 Interleukins (IL-6)

The stracuture of IL-6: An inflammatory cytokine with pleiotropic effects is interleukin-6 (IL-6). Chronic inflammatory conditions and multiple autoimmune diseases are linked to its dysregulation. It mediates its biological functions through a hexameric complex called IL-6/IL-6R/gp130, which is made up of IL-6, IL-6R, and glycoprotein 130 (Kaur et al. 2020). To carry out numerous biochemical operations, this complex in turn activates several signaling systems (classical and trans-signaling). Several pathological pathways, including JAK/STAT3, Ras/MAPK, PI3K-PKB/Akt, and regulation of CD4+ T cells and VEGF levels, are activated by the trans-signaling mechanism and lead to diseases like cancer, multiple sclerosis, rheumatoid arthritis, anemia, inflammatory bowel disease, Crohn's disease, and Alzheimer's. IL-6 is a key target for the therapy of these complex disorders due to its involvement in their pathogenesis (Kaur et al. 2020, Yao et al. 2014). Even while several anti-IL-6 monoclonal antibodies are utilized in medicine, their high price, need for parenteral administration, and potential for immunogenicity have restricted their application and prompted the creation of novel, tiny, non-peptide compounds that act as IL-6 inhibitors. All compounds identified as IL-6 inhibitors in the literature have been categorized in the current report as IL-6

production, IL-6R, and IL-6 signaling inhibitors. Critical analysis of the reports currently available identifies significant and prominent structural characteristics shared by these compounds. Through knowledge- and/or computer-based methodologies, these analyses would help medicinal chemists develop new and effective IL-6 production and signaling inhibitors for the treatment of complicated multifactorial disorders (Kaur *et al.* 2020).

2.4.3 Hs- CRP

The liver produces the protein (Figure 2.6) known as C-reactive protein (CRP). When there is bodily inflammation, the level of CRP rises. The C-reactive protein level can be checked with a quick blood test. In comparison to a regular C-reactive protein test, a high-sensitivity C-reactive protein test (hs-CRP) is more sensitive. In comparison to a standard test, the high-sensitivity test can detect lower C-reactive protein increases. The risk of developing coronary artery disease can be determined with the hs-CRP test. The arteries in the heart narrow when there is coronary artery disease (Joppa *et al.* 2006, Mackenzie and Woodhouse 2006). A heart attack may result from narrowed arteries. aid in the identification of a chronic inflammatory illness like lupus or rheumatoid arthritis. Find out your risk for heart disease. The risk of heart attacks has been linked to blood levels of hs-CRP that are high. Additionally, those who have already experienced a heart attack are more likely to experience another one if their hs-CRP level is high. However, when their hs-CRP level is within the normal range, their risk decreases. Not everyone should take a hs-CRP test. The test cannot identify what is causing the inflammation. Thus, having a high hs-CRP level is achievable without having any negative effects on the heart. People who have a 10% to 20% likelihood of having a heart attack over the next 10 years may benefit the most from a hs-CRP test. The risk level here is intermediate. Using tests that consider lifestyle choices, family history, and general health, a healthcare professional can ascertain the risk (Su *et al.* 2020, Mackenzie and Woodhouse 2006).

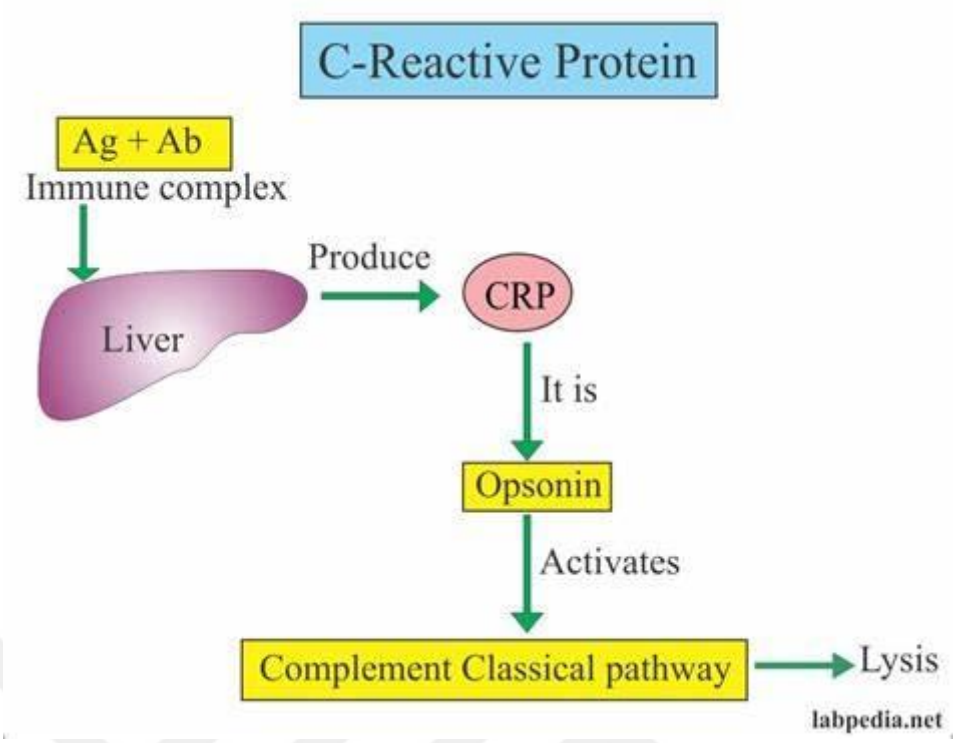

Figure 2.6 C-Reactıve protein pathway

An abrupt increase in the C-reactive protein level can result from strenuous exercises, such as heavy lifting or a lengthy marathon. Before the test, the doctor could advise against doing such things. Some drugs may have an impact on CRP levels. Include over-the-counter medications in the list of medications to be disclosed to the healthcare professional. They might need to fast before the test if the blood sample will be utilized for additional tests. For instance, you might schedule a cholesterol test, which calls for fasting, at the same time as a Hs-CRP test to monitor for heart disease (Albert *et al.* 2002, Harris *et al.* 1999).

Milligrams per liter (mg/L) is the unit used to test C-reactive protein. Results of 8 mg/L or 10 mg/L or higher are regarded as high. Depending on the lab performing the test, the range values change. An elevated test result indicates inflammation. It could be brought on by a severe accident, prolonged illness, or infection. One risk factor for coronary artery disease is the Hs-CRP level. An increased risk of getting heart disease is not always associated with having a higher hs-CRP level. Results from other tests may be used to estimate the risk (Wilkins *et al.* 1998, Mac Giollabhui *et al.* 2020).

2.4.4 Menaril (CL, NA and K)

Intracellular fluid (ICF) and extracellular fluid are two parts of the total body water (ECF). The main ion of the ICF is potassium (K+), and the activity of the Na/K-ATPase enzyme, which is hampered by low oxygen and energy levels, determines how much potassium is present intracellularly. Infants that are born prematurely are more susceptible to intracellular and extracellular compartmental abnormalities. During body growth, the overall amount of intracellular water rises along with the number and size of body cells. ECF is separated into intravascular, extravascular, and "third space" components. The "third space" refers to free fluid in preformed body compartments under physiological and pathological settings. The sodium (Na+) ion is the main ion in ECF. Growth causes a reduction in ECF. The blood volume in newborns ranges from 85 to 100 mL per kilogram of body weight, but it is only 60 to 70 mL in adolescents and adults (Linshaw1991, Nicholson 1996).

Many of the regulatory processes involved have limitations in patients because of immaturity or limited efficacy in patients. The renal glomerular surface area available for filtration is small in preterm and term neonates compared to that in older infants and adults. In term infants, GFR increases significantly during the first week of life and continues to rise over the first two years of life. Immaturity of the distal nephron with an anatomically shortened loop of Henle leads to reduced ability to concentrate urine(Nicholson 1996, Jobe *et al.* 1985). Maximum urinary concentrations are up to 550 mosm/l in preterm infants, and 700 mosm/l in term infants, compared to 1200 mosm/l in adults. Neonates may be placed at risk for volume depletion when a high renal solute load cannot be compensated for by the ability to produce concentrated urine. Although hormonal factors. the renin-angiotensin-aldosterone system and arginine-vasopressin are mature early in gestation, the effects are limited by renal immaturity. A lower plasma oncotic pressure and higher permeability of the capillary wall in preterm infants compared to term infants and adults enhances the shift of water from the intravascular to the interstitial compartment, with an increased risk of edema, especially under pathologic conditions such as sepsis (Modi and Hutton 1990, Haycock and Aperia 1991).

The sodium:

Sodium is one of the most crucial electrolytes in the extracellular fluid because it is an osmotically active cation. It controls the modulation of the membrane potential of cells as well as the volume of extracellular fluid. As part of active transport, sodium, and potassium are transferred across cell membranes. The primary cation in extracellular fluid, sodium (Na+), controls how the intravascular and interstitial volumes are maintained. Consuming sodium might affect the ECF volume. Urine is the main route by which sodium is eliminated, but perspiration and feces are also involved (Schielke*et al.* 1991, Eshetu*et al.* 2020).

The most typical electrolyte issue is hyponatremia. When the serum sodium level is less than 135 mmol/L, a diagnosis is made. There are neurological effects of hyponatremia. Patients may exhibit delirium, headaches, confusion, and/or nausea. When serum sodium levels exceed 145 mmol/L, hypernatremia develops. Tachypnea, trouble falling asleep, and agitation are all signs of hypernatremia. Rapid adjustments to the salt level might lead to dangerous side effects such as cerebral edema and osmotic demyelination syndrome (Eshetu *et al.* 2020, Kronzucker*et al.* 2013).

An anion that is mostly present in the extracellular fluid is chloride. Chloride levels in the serum are primarily regulated by the kidneys. Both proximal and distal tubules reabsorb the majority of the chloride, which is filtered by the glomerulus, by active and passive transport. The loss of gastric bicarbonate might result in hyperchloremia. Hypochloremia might manifest as vomiting or gastrointestinal loss or as a water retention condition like congestive heart failure (Pfortmueller*et al.* 2018, Yunos*et al.* 2010).

The kidneys regulate sodium levels. The majority of sodium reabsorption occurs in the proximal tubule. Sodium is reabsorption in the distal convoluted tubule. Through sodium-chloride symporters, which are regulated by the hormone aldosterone, sodium is transported in the body (Schlesinger *et al.* 2020). The main extracellular fluid anion found in plasma, lymph, connective tissue, cartilage, and bone is chloride (Cl). At

various ages, the exchangeable chloride per unit of body weight stays largely constant. Usually, chloride intake and output follow those of sodium, but external losses and excretion can happen on their own, usually in equilibrium with bicarbonate status. Chloride is changed frequently each day. Tubular reabsorption of 60–70% of the filtrated chloride results in renal conservation (Curry and Alan 2018, Schlesinger *et al.* 2020, Geilfus 2018).

The main intracellular cation is potassium (K+), and lean body mass and the potassium pool have a good correlation. 10% of the potassium body pools cannot be exchanged (bone, connective tissue, cartilage). Consumption of potassium varies greatly. The relationship between intracellular and extracellular potassium concentrations is not constant. Mostly an intracellular ion, potassium. The sodium-potassium adenosine triphosphatase pump, which pumps out sodium in exchange for potassium, which goes into the cells, is primarily responsible for controlling the balance between sodium and potassium. The glomerulus in the kidneys is where potassium is filtered. At the thick ascending loop of Henle and the proximal convoluted tubule, potassium is absorbable. The distal convoluted tubule is where potassium secretion takes place. Potassium secretion is increased by aldosterone. At the apical membrane, potassium is also secreted through potassium channels and potassium-chloride cotransporters (Cohn*et al.* 1980, Römheld and Kirkby 2010).

Cardiac arrhythmias are associated with potassium problems. Low serum potassium levels, or hypokalemia, are characterized by weakness, exhaustion, and twitching of the muscles. Arrhythmias may develop as a result of hyperkalemia, which happens when serum potassium levels are above 5.5 mmol/L. Myoglobinuria, rhabdomyolysis, muscle spasms, and weakness are some of the symptoms of hyperkalemia (Yurinskaya*et al.* 2005, Römheld and Kirkby 2010).

2.4.5 CBC

The CBC includes the number of platelets, red and white blood cells, and other biochemical measurements. These variables are denoted by the letters RDW, HCT,

MCHC, and Hemoglobin (HGB) is one of them. Red blood cells contain hemoglobin, an iron-rich protein made in the bone marrow. By adhering to the hemoglobin in the blood, oxygen from the air that enters the lungs is transported to the body's tissues. The hemoglobin binds to the carbon dioxide produced in the tissues, which travels to the lungs where it is expelled from the body. With the logistical assistance of the hemoglobin protein, a vital cycle that occurs in the body can proceed. Red blood cells are protected, take on the shape of a disc, and pass through the body more easily thanks to the hemoglobin protein. Hemoglobin is the first parameter examined in cases of anemia or iron deficiency issues since it reflects the body's iron content. The protein complex known as ferritin is in charge of storing and using the iron mineral that is ingested through diet. Iron is dissolved, stored, and released by ferritin, which is often referred to as an iron store. The ferritin test is used to assess the body's iron stores (Theil 2011, Herman *et al.* 2018).

2.4.6 Ferritin

The mineral iron plays a crucial role in metabolism and is ingested by the body through a variety of foods. Red blood cells make up the majority of the blood's cells (erythrocytes, red blood cells). The movement of oxygen and carbon dioxide throughout the body is carried out by erythrocytes. The iron mineral affects the erythrocyte's capacity to carry out this function. It is sometimes referred to as iron storage since ferritin is a protein that stores iron in the body and regulates its release. The ferritin protein contains 4,500 iron molecules in total. To determine whether the body has enough iron, a ferritin test can be performed (Wawer*et al.* 2018).

The doctor can recommend ferritin levels of 20–200 mL/ng for women and 20–500 mL/ng for men, although the ideal ferritin value varies depending on factors including age and gender. Regular monthly menstrual bleeding in women is the clear cause of the low rate. A low ferritin ratio in the body, which is below optimal values, is an indication of a low iron ratio. Anemia is the term used to describe iron deficiency in individuals. Iron deficiency anemia develops because it will have a detrimental impact on the creation of red blood cells (Chen*et al.* 2006).

Excessive tea and coffee consumption, erratic eating patterns, heavy menstrual cycles, pregnancy, frequent childbirth, frequent consumption of processed meat products, celiac disease, vitamin C deficiency, chronic diseases, blood loss from various injuries, and digestive and intestinal issues can all contribute to low ferritin levels. Organs and tissues that cannot obtain enough oxygen because of iron shortage begin to malfunction. This creates an uncomfortable situation. Lethargy, anxiety, lack of appetite, desires for earth and lime, dizziness and pain, a persistent feeling of cold, forgetfulness, difficulties concentrating, hair loss issues, nauseating skin color, brittle nails, and numbness in fingers are all common symptoms. Due to a low ferritin ratio, feelings of sex drive and restless legs syndrome may develop (Hosseini*et al.* 2018, Moisidis-Tesch and Shulman 2022).

3. MATERIALS AND METHODS

3.1 Patients Material and Methods

3.1.1 Patients

Samples of 150 patients (males and females) were collected from patients being referred to the gastroenterology clinic of Azadi Teaching Hospital and private Kirkuk clinic for upper and lower GIT endoscopy in Kirkuk city from October 2021 to June 2022 with clinical symptoms of bloody diarrhea with rectal urgency, rectal bleeding, frequent stools, and mucus discharge from the rectum, tenesmus, and lower abdominal pain and a clinical trial (randomLy) study were done. Interviews were done using a questionnaire that looked for the causal factors of ulcerative colitis (Appendix). The purpose of this investigation was to learn more about ulcerative colitis and its diagnosis methods and monitor biological therapy. The main objective was to determine the role of fecal calprotectin with other immunological, biochemical, and hematological parameters in diagnosis and monitor biological therapy compared with patients who didn't receive the biological therapy and the extent of the relationship between them with some vitamins. We will mention in this chapter the materials and devices used in this study. We will also briefly mention the methods of collecting samples and methods of preserving them until the final results are obtained.

3.1.2 Sample collection

Selected eligible patients were subjected to the examination of stool and blood. Exclusion criteria patients suffering from a colon and rectum disorder.

3.1.3 Stool samples

Stool samples were collected from 150 patients in clean, dry, and water proof containers. The stool assay was performed by using fecal calprotectin for alegria, stored at -20˚C.

3.1.4 Blood samples

Collected blood samples from (100) patients who had a positive result from Fecal calprotectin and (50) patients who had Negative Fecal calprotectin. About (5 mL) which is aseptically drawn by venipuncture, (2 mL was placed in an EDTA tube) used for CBC which was performed immediately, and (3 mL) of blood was placed in a gel tube, and centrifuged for 5min. at 4000rpm to separate the serum, which was separated and divided into two Eppendorf tubes and stored at -20˚C for the determination of biochemical, immunological, and vitamins study.

3.1.5 Study design

The study groups were divided into three groups, which were divided according to whether or not biological treatment was taken. The division was made as follows:

A- The control group (The healthy group),

B- Group B patients with intestinal ulceration and receiving biological therapy,

C- Group C patients with ulcerative colitis without biological therapy.

3.2 Materials

3.2.1 Instruments and equipment

The equipment and kits that were used in the research and as mentioned in Table 3.1.

Table 3.1 The study kits

Kits	Manufacturing Company	Country
Fecal calprotectin Alegria	Orgentec Diagnostika GmbH	Germany
IL-6 ECLIA	Nipigon Health corp	Canada
Hs-CRP ECLIA	Nipigon Health corp	Canada
PCT ECLIA	Nipigon Health corp	Canada
Fuji dri- chem Slide (Na- K-Cl)	Fujifilm Europe GmbH	Japan
Ferritin ECLIA	Nipigon Health corp	Canada
25-OH Vitamine D ECLIA	Nipigon Health corp	Canada
Vitamin B12 ECLIA	Nipigon Health corp	Canada
Folate ECLIA	Nipigon Health corp	Canada
Swelab AlfaDiluent	Boule Medical AB	Sweden
Swelab AlfaLyse	Boule Medical AB	Sweden

3.2.2 Calprotectin

Is an ELISA-based test system for the quantitative measurement of calprotectin in human stool samples to be used in the assessment of intestinal inflammatory disorders, using kits from Orgentec Diagnostika GmbH, Germany, catalog No. REF 2114293 Table 3.2.

Table 3.2 Kit content

Components	Ingredients
Alegria Test Strips	modules of 8 wells
Code on barcode	Calprotectin on printout: Calpro
Wash Buffer	1x 20 mL Tris, detergent, preservative sodium azide 0.09%; 50 x concentrate.
System fluid	1x 2.5 mL System Fluid, contains acid; 1000 x concentrate.
Extract	1x 20 mL Stool Extraction Medium; containing TBS, stabilizing protein, extraction reagent, and preservative sodium azide 0.09%. Ready to use.

Description of the fecal Calprotectin Alegria Test Strips: Sufficient for 24 determinations AlegriaTest Strips are modules of 8 wells each composed of Table 3.3:

Table 3.3 Test strips

Wells	Reagents
1 + 2	Empty and not coated (wells for the sample dilution)
3+4	Coated with antibody (reaction wells)
5	Control; yellow; containing Calprotectin, PBS, BSA, detergent, preservative sodium azide 0.09%, and ProClin 300 0.05%.
6	Enzyme Conjugate; light red; containing anti-Calprotectin antibodies, HRP labeled; PBS, BSA, detergent, preservative ProClin 300 0.05%.
7	Sample Buffer: yellow; containing PBS, BSA, detergent, preservative sodium azide 0.09% and ProClin 300 0.05%.
8	TMB Substrate: clear; containing 3,3', 5,5'- Tetramethylbenzidin. Anti-Calprotectin antibodies are coated onto microwells.

Preparation of reagents:

Wash : Dilute the content of the Wash Buffer concentrate (50x) with distilled or deionized water to a final volume of 1000 mL before use. Transfer the diluted Wash Buffer into the instrument reagent container. If only one Alegria run is to be performed on one day we recommend transferring only 500 mL of diluted Wash Buffer.

System fluid: Dilute the content of the System Fluid concentrate (1000x) with distilled or deionized water to a final volume of 2500 mL before use. Transfer the diluted System Fluid into the instrument reagent container.

Principle:

Calprotectin contained in a stool sample has to be released from the stool with an extraction medium. Then, extracted Calprotectin can be analyzed with the Alegria.Calprotectin assay.

The Alegria. assay features barcoded 8-well-microstrips, called Alegria.Test Strips. Each strip is designed for a single determination of one patient sample. The Alegria. Test Strip holds a complete set of reagents. Included are enzyme conjugate, enzyme substrate, sample buffer, and a test-specific control. Furthermore, each strip has two

antibody-coated wells which serve as reaction wells for one control and one patient sample.

The determination is based on an indirect enzyme-linked immune reaction with the following steps: Calprotectin present in control / positive samples binds to the surface of the reaction wells forming an antibody-antigen-complex. After incubation, the first washing step removes unbound and unspecifically bound molecules. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complex. After incubation, a second washing step removes unbound enzyme conjugate. The addition of enzyme-substrate solution results in hydrolyzation and color development during incubation. The intensity of the blue color correlates with the concentration of the antibody-antigen complex and can be measured photometrically at 650 nm. The Alegria. Test Strip is based on the proprietary SMC-Technology (Sensotronic Memorized Calibration): information about the assay, analysis, and evaluation, and the lot-specific expiry date is contained on the barcode printed on each Alegria.

The Alegria. Test Strip can be used with the diagnostic instrument Alegria- a fully automated Random Access Analyser. Using SMC-Technology data encoded on the barcode are transferred from the Alegria. Test Strip to the instrument and the assay is automatically processed and evaluated. The instrument reads the date of expiry and rejects further processing if the Alegria. The test Strip is out of date.

Procedure:

Alegria test Strips with SMC technology are used with the diagnostic instrument Alegria. Detailed information about operating the instrument can be taken from the Instrument User Manual.

1. Take 15 mg of stool sample into plain tube.
2. Add 0.75 mL of extract into the plain tube and vortex for 30 sec at 1800 rpm.
3. Further, homogenize for 15 min at top speed on a rocking shaker.

4. Open the tube and transfer the homogenate to a microtube.
5. Centrifuge for 2 min at 3000 rpm. Transfer clear fluid to another microtube and test for Calprotectin immediately.
6. Remove the foil from the empty wells 1 to 4 of the AlegriaTest Strip. Do not remove foil with printed barcode, covering wells 5 to 8.
7. 10 µL Pipette extracted stool sample at the bottom of well 1.
8. Insert the strip into the Sys Tray.
9. Place loaded Sys Trays into the correct position in the Alegriainstrument and start running.
10. All further steps will be done automatically. The test run is completed when the instrument starts printing the results Figure 3.1.

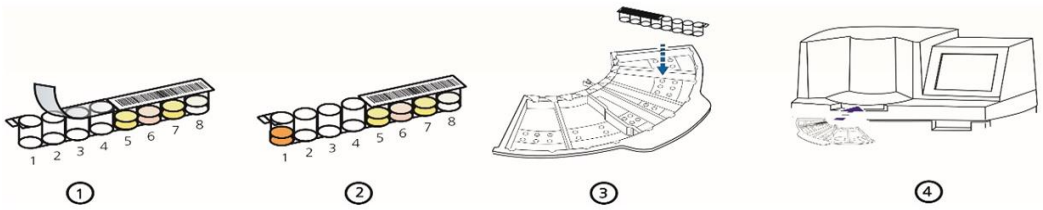

Figure 3.1 The instrument test

Calculation:

Using SMCTechnology (Sensotronic Memorized Calibration), all test data are transferred to the system through individual barcodes on the AlegriaTest Strip. Calculation and interpretation of results will be performed automatically.

3.2.3 Interleukin-6 (IL-6)

Immunoassay for in vitro quantitative determination of interleukin-6 (IL-6) In human serum and plasma, using kits from Nipigon Health Corp, Canada, Catalog No. REF 0320502304 Table 3.4.

Table 3.4 Kit content

Components	Ingredients	Volume50T	Volume100T
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; 0.05% ProClin^{Tm} 300	1×5.0 mL	1×3.0 mL
(RB)	Anti-IL-6-Ab~biotin, 1.0mg/L:25mM EPBS;0.05% ProClin^{TM} 300	1×7.5 mL	1×4.5 mL
(RA)	Anti-IL-6-Ab~Ru(bpy)$_3^{2+}$,1.0 mg/mL:25mM EPBS;0.05% ProClin^{TM} 300	1×7.5mL	1×4.5 mL
Calibrator (High)	Equinum serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Equinum serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material(High)	Equinum serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material (Low)	Equinum serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL

Principle:

Sandwich principle. The total duration of the assay had to be taken 18 minutes:

1st incubation: The biotinylated monoclonal IL-6-specific antibody and monoclonal IL-6-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2nd incubation: After the addition of streptavidin-coated microparticles, the complex binds to the solid phase via the interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the IL-6 rack pack had been put into the correct analyzer slot and automatically suspended magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.4 High-Sensitivity C-reactive Protein (Hs-CRP)

Immunoassay for in vitro quantitative determination of C-reactive protein in human serum and plasma, using kits from Nipigon Health Corp, Canada, catalog No. REF 0320502104 Table 3.5.

Table 3.5 Kit content

Components	Ingredients	Volume50T	Volume100T
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; 0.05% ProClin^{Tm} 300	1×3.0 mL	1×5.0 mL
(RA)	Anti-CRP-Ab~Ru(bpy)$_3^{2+}$ 1.0μg/mL:0.1M PBS;0.05% ProClin^{TM} 300	1×5.25mL	1×9.0 mL
(RB)	Anti-CRP-Ab~Ru(bpy)$_3^{2+}$ 1.0μg/mL:0.1M PBS;0.05% ProClin^{TM} 300	1×5.25mL	1×9.0 mL
Diluent	0.1M PBS;0.05% ProClin^{TM} 300	1×7.5 mL	1.13.5 mL
Calibrator (High)	Calf serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Calf serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material(High)	Calf serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material (Low)	Calf serum, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL

Principle:

Sandwich principle. The total duration of the assay: 18 minutes

1st incubation: The biotinylated monoclonal CRP-specific antibody and monoclonal CRP-specific antibody labeled with a ruthenium complex were added to the first reaction cup. In the second reaction cup, the sample diluent and sample were diluted 25 times, and then the diluted sample was added to the first reaction cup and incubated together to form a sandwich complex.

2nd incubation: After the addition of streptavidin-coated microparticles, the complex binds to the solid phase via the interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the HS-CRP rack pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.

- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.5 Procalcitonin (PCT)

Immunoassay for in vitro quantitative determination of PCT (Procalcitonin) in human serum and plasma, using kits from Nipigon Health Corp, Canada, catalog No.0320502204 Table 3.6.

Table 3.6 Kit content

Components	Ingredients	Volume50T	Volume100T
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; ProClin^{Tm} 300	1×5.0 mL	1×3.0 mL
(RB)	Anti-PCT-Ab~ biotin, 1.5 mg/L:0.1M PBS; ProClin^{TM} 300	1×9.0 mL	1×5.5 mL
(RA)	Anti-PCT-Ab~ $Ru(bpy)_3^{2+}$,0.5mg/L:0.1M PBS; ProClin^{TM} 300	1×9.0mL	1×5.5 mL
Calibrator (High)	Horse serum, ProClin^{TM} 300 lyophilized	1×4.0 mL	1×4.0 mL
Calibrator (Low)	Horse serum, ProClin^{TM} 300 lyophilized	1×4.0 mL	1×4.0 mL
Control Material(High)	Horse serum, ProClin^{TM} 300 lyophilized	1×4.0 mL	1×4.0 mL
Control Material (Low)	Horse serum, ProClin^{TM} 300 lyophilized	1×4.0 mL	1×4.0 mL

Principle:

Sandwich principle. The total duration of the assay: 18 minutes

1st incubation: Antigen in the sample, a biotinylated monoclonal PCT-specific antibody, and a monoclonal PCT-specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2nd incubation: After the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via the interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the PCT rack pack into the correct analyser slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analysed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analytic concentration using a particular algorithm.

3.2.6 Blood test for electrolytes (Na^+, K^+, Cl^-)

Quantitative measurement of sodium, potassium and chloride ion concentration in serum. Using kits FUJIFIL ME urope GmbH, Germany, catalog No. REF 147208 Table 3.7.

Table 3.7 The test for electrolytes

Components per slide	Ingredients
Common to Na, K, Cl	Sliver 0.50 mg, sliver chloride 0.26 mg
Na	NaCl 0.52 mg, methyl monensin 0.31 mg
K	NaCl 0.51 mg, valinomycin 0.14 mg
Cl	Tri-alkyl ammonium chloride 0.95 mg

Principle of the measurement:

50 µLofreferencefluidand50 µL of whole blood, plasma or serum is deposited on a FUJI DRI-CHEM SLIDE Na-K-Cl at the same time on the reference side and the sample side respectively. After depositing, the reference fluid and the specimens spread along the distribution device and also towards each other on the special thread bridge to form a stable ionic junction. A differential potential is generated between the two half-cells. The potential difference is proportional to the logarithm of each ion concentration ratio of the two fluids. The slide is incubated for a fixed time in the FUJI DRI-CHEM ANALYZER and the potentiometric difference between the reference and the specimen is measured. The potentiometric value is then converted into each of the electrolyte's concentrations using a calibration curve preinstalled in the analyser.

Procedure:

- Set slideson FUJIDRI-CHEMANALYZER.
- Set a sample tube in the specified sample rack.

- Set the reference fluid in the specified position.
- Input a sequence No. and a sample ID if appropriate.
- Press the “START” key to initiate testing.

For further details of the operation procedure, consult“INSTRUCTION MANUAL” for FUJIDRI-CHEMANALYZER.

3.2.7 Ferritin

Immunoassay for in vitro quantitative determination of Ferritin in human serum and plasma, using kits from Nipigon Health Corp, Canada, catalog No.032050104 Table 3.8.

Table 3.8 Kit content

Components	Ingredients	Volume50T	Volume100T
(MB)	Streptavidin-coated microparticles preservative	1×1.75 mL	1×3.5 mL
(RB)	Anti-Ferritin-Ab~ biotin, PBS; preservative	1×3.5 mL	1×7.0 mL
(RA)	Anti-Ferritin -Ab~ $Ru(bpy)_3^{2+}$,PBS; preservative	1×3.5mL	1×7.0 mL
Calibrator (High)	Bovin serum, preservative	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Bovin serum, preservative	1×1.0 mL	1×1.0 mL
Control Material(High)	Bovin serum, preservative	1×1.0 mL	1×1.0 mL
Control Material (Low)	Bovin serum, preservative	1×1.0 mL	1×1.0 mL

Principle:

Sandwich principle. The total duration of the assay: 18 minutes

1st incubation: a sample, biotinylated monoclonal Ferritin antibody, and monoclonal Ferritin - antibody labeled with a ruthenium complex react to form a sandwich complex.

2nd incubation: After the addition of streptavidin-coated microparticles, the complex binds to the solid phase via the interaction of biotin and streptavidin.

Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the ferritin rack pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.8 25-OH vitamin D total

Immunoassay for in vitro quantitative determination of Total 25-OH Vitamine D in human serum and plasma, using kits from Nipigon Health Corp, Canada, catalog No.0320504804 Table 3.9.

Table 3.9 Kit content

Components	Ingredients	Volume50T	Volume100T
PT2	1.4% Dithiothreitol	1×3.0 mL	1×4.0 mL
PT3	0.25 M Sodium Hydroxide	1×2.5 mL	1×3.0 mL
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; 0.05% ProClin^{Tm} 300	1×3.0 mL	1×5.0 mL
(RB)	Anti-Vitamine D -Ab~ $Ru(bpy)_3^{2+}$0.05M:0.1M BIS- Tris: 0.05% ProClin^{TM} 300	1×5.0mL	1×8.0 mL
(RA)	Anti-25-OH Vitamine D -Ab~ Biotin 0.05M:0.1M MES;0.05% ProClin^{TM} 300	1×5.5mL	1×9.0 mL
Calibrator (High)	Horse serum, 0.05% ProClin ^{TM} 300 lyophilized	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Horse serum, 0.05% ProClinTM 300 lyophilized	1×1.0 mL	1×1.0 mL
Control Material (High)	Horse serum, 0.05% ProClinTM 300 lyophilized	1×1.0 mL	1×1.0 mL
Control Material (Low)	Horse serum, 0.05% ProClinTM 300 lyophilized	1×1.0 mL	1×1.0 mL

Principle:

Competition principle. The total duration of the assay: 27 minutes

1st incubation: By incubating the sample with pretreatment reagents 1 and 2, bound 25-OH Vitamine D is released from the Vitamine D binding protein.

2nd incubation: By incubating the pretreated sample with the ruthenium-labeled monoclonal vitamin D -specific antibody, a complex between the 25-OH vitamin D and the ruthenate vitamin D monoclonal antibody is formed.

3rd incubation: After the addition of streptavidin-coated microparticles and 25-OH vitamin D labeled with biotin, the unbound ruthenium-labeled vitamin D monoclonal antibody becomes occupied. A complex consisting of the ruthenate vitamin D is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the 25-OH vitamin D rack pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.9 Vitamin B12

Immunoassay for in vitro quantitative determination of Vitamin B12 in human serum and plasma, using kits from Nipigon Health corp, Canada, catalog No. REF 0320500210 Table 3.10.

Table 3.10 Kit content

Components	Ingredients	Volume50T	Volume100 T
(MB)	Streptavidin-coated microparticles 0.75mg/mL; 0.1M PBS; 0.05% ProClin^{Tm} 300	1× 1.8mL	1×3.5 mL
(RB)	Vitamin B12~Biotin: 0.05M phosphate buffer,0.05% ProClin^{TM}	1×2.0mL	1×4.0 mL
(RA)	Intrinsic factor~ $Ru(bpy)_3^{2+}$.0.05M phosphate buffer,0.05% ProClin^{TM}	1×3.3mL	1×6.5 mL
(RD)	40g/L Sodium hydroxide	1×1.0 mL	1.2.0 mL
Calibrator (High)	Vitamin B12,0.05M phosphate buffer, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Vitamin B12,0.05M phosphate buffer, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material(High)	Vitamin B12,0.05M phosphate buffer, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL
Control Material (Low)	Vitamin B12,0.05M phosphate buffer, 0.05% ProClin^{TM} 300	1×1.0 mL	1×1.0 mL

Principle:

Competition principle. The total duration of assay:27 minutes

1st incubation: By incubating the sample with pretreatment reagent 1, bound vitamin B12 is released from the vitamin B12 binding protein.

2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed

3rd incubation: After the addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium-labeled intrinsic factor become occupied. A complex consisting of the ruthenate intrinsic factor and the biotinylated vitamin B12 is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the Vitamin B12 rack pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.10 Folate

Immunoassay for in vitro quantitative determination of Ferritin in human serum and plasma, using kits from Nipigon Health corp, Canada, catalog 0320505204 Table 3.11.

Table 3.11 Kit content

Components	Ingredients	Volume50T	Volume100T
Pretreatment reagent II PT2	Sodium 2-mercaptoethanesulfonate (MESNA)	1×0.8 mL	1×1.6 mL
Pretreatment reagent III PT3	Sodium Hydroxide	1×0.6 mL	1×1.1 mL
(MB)	Streptavidin-coated microparticles 0.75mg/mL; Preservative	1×1.5 mL	1×3.0 mL
(RB)	Biotin-labeled folate coupled BSA, HEPES, Preservative	1×2.0mL	1×4.0 mL
(RA)	Folate binding protein ~$Ru(bpy)_3^{2+}$, HEPES, Preservative	1×3.0 mL	1×6.0 mL
Calibrator (High)	Horse serum, HEPES, Preservative	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Horse serum, HEPES, Preservative	1×1.0 mL	1×1.0 mL
Control Material(High)	Horse serum, HEPES, Preservative	1×1.0 mL	1×1.0 mL
Control Material (Low)	Horse serum, HEPES, Preservative	1×1.0 mL	1×1.0 mL

Principle:

Competition principle. The total duration of the assay: 27 minutes

1st incubation: By incubating the sample with pretreatment reagents II and III, bound folate is released from endogenous folate binding protein.

2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed the amount of which is dependent upon the analyte concentration in the sample.

3rd incubation: After the addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied. with the formation of ruthenium-labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via the interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer.

Application of a voltage to the electrode then induces chemiluminescent emission which is immediately measured by a photomultiplier.

Results are determined via a calibration curve which is specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Procedure:

- Put the Folate rack pack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.
- Enter the sample ID.
- Select the sample flame to be used, and click [sample request].
- Select the position of the sample to be analyzed.
- In the item list, select the test items.
- Click OK and start the sample test.

Calculation:

The system software automatically calculates the analyte concentration using a particular algorithm.

3.2.11 Blood parameters (Haemoglobin estimation, WBC, and platelet)

Principle:

The measuring principles of the Swelab Alfa Plus analyzer are based on impedance and spectrophotometer principles.

Total white blood cell count and platelet count were based on the impedance principle. WBC concentration values are determined by counting cells in whole blood dilutions of 1:400 for the WBC.

The hemoglobin is determined from the same dilution as the WBC. For each sample a blank is measured as a reference, this means that any drift in reagent, cuvette-absorption, or diode is eliminated. The photometer system consists of a photodiode, a cuvette with a length of 15 mm, and a filter at a wavelength of 535 nm (bandwidth 20 nm). The HGB readings are slightly corrected for turbidity in case of extreme WBC counts.

Procedure:

- Go to start Menu.
- Choose the Blood tab, in the upper right-hand corner, for sample type.
- Enter sample ID.
- Aspirate the sample through the sample probe by gently inserting the sample probe into the sample tube and then press the whole blood start plate behind the sample probe.
- Sample results will be displayed after 45 Second.

3.3 The Statistical Analysis

The statistical analysis of the results of the study was carried out to find the averages, the differences, and the average standard deviation among the variables of the study using the SPSS 25 program. The ANOVA one-way test was used to find the differences and averages between the variables at the level of significance P = 0.05, and the Pearson test was used to find the correlations between the variables studied.

4. RESULTS AND DISCUSSION

To understand the role of fecal calprotectin with some immunological, biochemical, and haematological variables in the diagnosis and control of biological therapy for patients with ulcerative colitis and its association with the rest of the study variables, we clarified by interpreting the results statistically and clinically. Also, a study of their relationship with the results of the Fecal Calprotectin test for patients who take biological therapy and patients who do not take biological therapy, and their relationship with healthy subjects, and an indication of the extent to which parameters are affected by these microbes. The results of the study were as follows:

4.1 Study of the Relationship of the Fecal Calprotectin Test with Age

The results of the stool calprotectin test's relationship with age in our study indicated that there was a significant difference between the control group (25.36 ± 9.48) and the patients in the biological therapy group (27.80 ± 7.22). On the contrary, there was a significant difference between the control group (25.36 ± 9.48) and the patients without the biological therapy group (36.36 ± 5.19). These age differences can be explained as follows: The relationship between stool calprotectin levels and age is an essential factor to consider when interpreting test results. As individuals age, differences in the gut microbiome, immune response, and inflammation can affect the levels of calprotectin in the stool in people taking biologics versus those not taking biologics. Our study indicated that younger individuals, especially infants, and children, may naturally exhibit higher levels of calprotectin with biological therapy due to their developing immune system and the evolution of their gut flora. In turn, the elderly may experience an age-related decline in immune function and intestinal permeability, resulting in lower levels of calprotectin Table 4.1, Table 4.2 and Figure 4.1.

However, it is crucial to note that elevated stool calprotectin levels in any age group may indicate underlying gastrointestinal issues, such as inflammatory bowel disease, infection, or even malignancy. Therefore, age-specific reference ranges should be employed to ensure an accurate interpretation of test results. By considering the patient's

age-related variations in stool calprotectin levels, healthcare providers can better assess the need for further investigation or intervention, ultimately leading to more accurate diagnoses and tailored treatment plans.

Table 4.1 The relationship of the study between groups for age in ulcerative colitis

Descriptives Result for Age							
Test and Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control	25.36	9.48	1.89	21.44	29.27	7.00	50.00
Patients with biological therapy	27.80	7.22	2.01	23.68	31.92	15.0	63.00
Patients without biological therapy	36.36	5.19	2.36	31.60	41.11	16.0	72.00
Total (n=150)	31.11	7.16	1.39	28.34	33.88	7.00	72.00

Table 4.2 The Parameter and study between groups for age in ulcerative colitis

ONEWAY ANOVA FOR AGE					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2461.152	3	1230.576	6.837	.002
Within Groups	17999.450	147	179.994		
Total	20460.602	150			

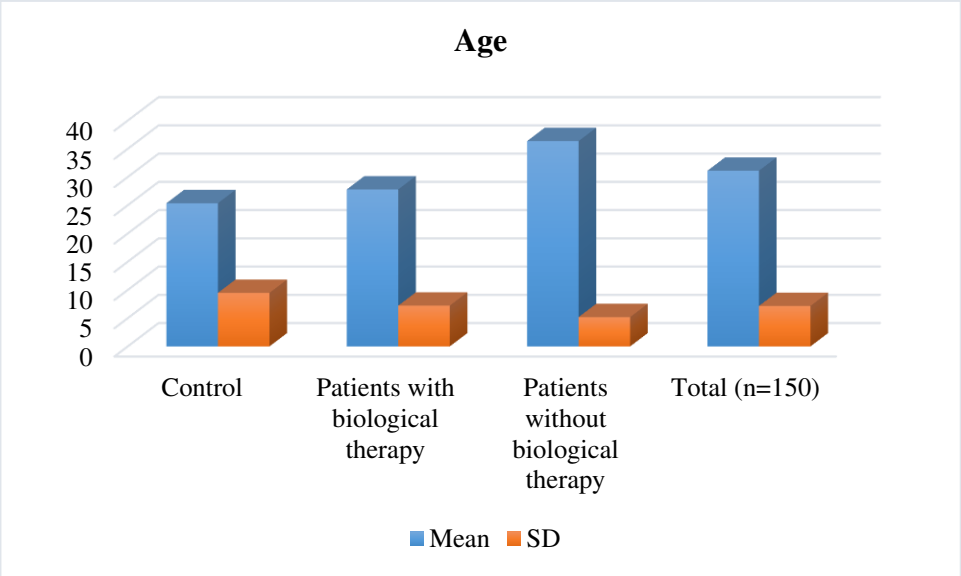

Figure 4.1 The mean and SD for age in patients with UC

4.2 Comparison of Age with Ulcerative Colitis (Previous Research)

Age at the beginning, duration, and anatomic extent of ulcerative colitis have all been proposed as potential risk factors for the development of colitis-associated colorectal cancer. These elements' relative relevance is still up for debate. Clarifying the factors linked to the elevated risk of colorectal cancer is especially important given the rising usage of surveillance programs. Only once these elements have been precisely specified can a logical and scientific surveillance strategy be suggested (Sugita *et al.* 1991).

There were differences in the effect of biological treatment in the study when comparing the results of children with the results of adults, and these results confirm that the negative effect of biological treatment is greater the older the patient's age (Merchant *et al.* 2010)

At diagnosis, a considerably higher prevalence in males was seen that rose with age (P = 0.0002), which appears to be related to the condition of being an ex-smoker, which is most usually found in males. The second rise in incidence in old age may also be partially explained by the higher frequency of ex-smokers. Younger patients and women have been observed to have greater colitis severity, greater clinical activity, and

increased use of steroids as the first therapeutic step. The same is true for symptoms like diarrhea, fever, and weight loss that primarily indicate clinical severity (Riegler *et al.* 2000).

In the colon, hypermethylation frequently begins in healthy mucosa as a result of aging and is noticeably elevated in malignancy. We examined the methylation patterns of five genes in the normal and dysplastic mucosa of patients with ulcerative colitis (UC), a condition that is significantly associated with an increased risk of colon cancer, to test the hypothesis that individuals at increased risk of colon cancer have higher levels of methylation in their nonneoplastic mucosa. These findings support the theory that the field deficit reflecting acquired propensity to colorectal neoplasia is marked (and may be causes) by age-related methylation (Issa *et al.* 2001).

Although numerous mucosal types have been linked to the development of ulcerative colitis (UC), the illness itself. The study's findings showed that older patients are more prone to infection, which suggests that patients' ages have a significant bearing on their health (Fite *et al.* 2013). Increased risk of infections in older patients on biologic therapy compared to both younger biologic users and older non biologic users (Borren *et al.* 2019)

4.3 Study of the Relationship of the Fecal Calprotectin Test with Gender

The results of Table 4.3 revealed that men were more sensitive to infection with intestinal ulceration disease recorded at 58 % while women recorded 42 %, The percentage of infection in our study of Table 4.3 showed that was greater among women in patients with biological therapy group which recorded 58 % than men, while patients without biological therapy group revealed the upper value of infection was recorded with men which recorded 56%. These results indicate that women are more affected than men when taking biological therapy. The level of fecal calprotectin can be used to predict the onset of inflammatory bowel disease with high accuracy and precision. By using measures of fecal calprotectin, we evaluated the cost-effectiveness of identifying adults and children who require endoscopic confirmation of ulcerative colitis.

Females have lower inpatient prevalence of UC, complications, and economic burden compared to males. This reinforces the slight male predominance that was seen in the older. One can speculate that the difference in complications, costs, charges, and LOS indicate that females have less severe disease at hospital admission. Numerous studies have examined the effect of gender on disease severity and outcomes, and there continues to be conflicting data (Kroner *et al.* 2019). Men are less likely to achieve clinical remission, mucosal healing, and clinical response compared to women during induction treatment with biological therapy for UC (Agrawal *et al.* 2023).

Table 4.3 The relationship of the Fecal Calprotectin test with gender in UC

Descriptives Result for Gender				
Group	**Men**		**Women**	
	No	**percentage**	**No**	**percentage**
Control	38	76%	12	24%
Patients with biological therapy	21	42%	29	58%
Patients without biological therapy	28	56%	22	44%
Total (n=150)	87	58.0%	63	42.0%

4.4 The Result of the Fecal Calprotectin Test

The results of Table 4.4 showed that the two patients' groups (patients with and without biological therapy) significantly elevated the mean of calprotectin levels which recorded 291.76 and 835.96 µg / mg as compared with 25.0µg / mg for the control group (Table 4.4 and Figure 4.2). The elevation in calprotectin levels of patient's groups indicates an inflammatory condition of patients with ulcerative colitis disease and the level of calprotectin was increased during disease activity in ulcerative colitis patients. Patients with ulcerative colitis taking biological therapy have the effect of reducing the level of fecal calprotectin (Shimoyama *et. al.* 2018).

The role of fecal calprotectin diagnosis and monitoring biological therapy of ulcerative colitis patients is of critical importance in ensuring accurate and effective treatment. By measuring calprotectin levels in stool samples, doctors can quickly determine the presence and extent of inflammation in the colon. This helps to guide the selection and

monitoring of biological therapies, which have become an increasingly popular treatment option in recent years. Patients with elevated fecal calprotectin levels may be candidates for more aggressive therapy, while those with low levels may require less intensive treatment.

This test allows medical professionals to quickly and accurately measure the levels of calprotectin in a patient's stool sample, which can be a key indicator of inflammation in the intestine. Elevated calprotectin levels often correspond to conditions such as inflammatory bowel disease (ulcerative colitis), including Crohn's disease and ulcerative colitis.

The level of fecal calprotectin can be used to predict the onset of inflammatory bowel disease with high accuracy. By using measures of fecal calprotectin, we evaluated the cost-effectiveness of identifying adults and children who require endoscopic confirmation of ulcerative colitis.

Table 4.4 The relationship of the Fecal Calprotectin test within UC

Descriptives Result for Calprotectin							
Test and Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control	25.00	0.15	0.18	23.00	25.00	21.00	25.00
Patients with biological therapy	291.76	39.15	57.50	174.32	409.19	11.00	1000
Patients without biological therapy	835.96	92.27	42.63	750.18	921.77	25.00	1100
Total (n=150)	475.33	43.21	42.78	390.47	560.20	11.00	1100

Table 4.5 The Parameter and study between groups in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	12227155.892	3	6113577.946	87.282	.000
Within Groups	7004424.175	147	70044.242		
Total	19231580.067	150			

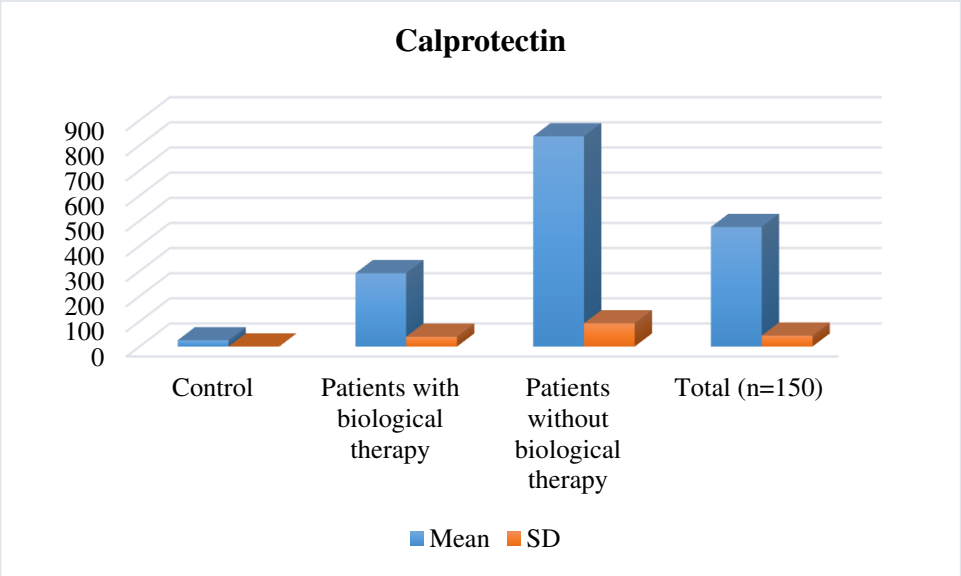

Figure 4.2 The mean and SD for calprotectin in patients with UC

4.5 Comparison of Fecal Calprotectin with Ulcerative Colitis (Previous Research)

A promising biomarker of endoscopic activity in ulcerative colitis has been identified as fecal calprotectin (FC). An extremely sensitive indicator of intestinal inflammation, fecal calprotectin helps distinguish between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). In IBD, fecal calprotectin is used to diagnose the condition, track disease activity, provide treatment advice, and forecast disease relapse and postoperative recurrence. An extraction stage is followed by an immunoassay for quantifying the feces calprotectin. Manufacturers have created several tests and extraction tools, including point-of-care techniques, over the past few decades (Pathirana *et. al.* 2018).

A study states that FC assessment following 8 weeks of treatment with all biological therapy may serve as a promising early indicator of therapeutic response in terms of mucosal repair as a result, FC has an early predictive value in patients receiving all currently known biological treatments for the treatment of moderate/severe UC. However, the study recommends the use of FC to get a quick assessment of how well a treatment is working (Bertani *et al.* 2020). The FC concentrations were able to

distinguish between various levels of endoscopic severity and were directly connected with both clinical and endoscopic activity (P 0.01). A study finds resoundingly endorses the use of FC for UC decision-making, relapse prediction, and staging disease activity (Zhu *et. al.* 2023). Fecal calprotectin concentration was considerably greater in patients with active ulcerative colitis compared to controls and patients with inactive ulcerative colitis. The individuals with inactive ulcerative colitis did not significantly vary from the controls ($p>0.05$) (Önal *et al.* 2012).

4.6 Study of the Relationship of Fecal Calprotectin with Ferritin

The results of Table 4.6 showed that there is a significant difference between the three groups studied (Control, patients with biological therapy, and patients without biological therapy), the two patient's groups (patients with and without biological therapy) significantly elevated the mean of ferritin levels which recorded 223.42 and 311.27 ng / dL as compared with 78.27 ng / dL for the control group (Table 4.6 and Figure 4.3). The elevation of ferritin levels in patient groups may be due to inflammatory conditions which may lead to elevated ferritin levels. Moreover, patients with biological therapy recorded normal values of ferritin level and which means that the biological therapy has a positive effect on intestinal ulceration of patients, while patients without biological therapy recorded high levels of ferritin which means that patients have too much iron in their bodied.

According to the results of our study, the fecal calprotectin test and the ferritin test are among the basic diagnostic tools for the evaluation and monitoring of various health conditions. The fecal calprotectin test can be used primarily to identify inflammation in the gastrointestinal tract and help doctors differentiate between inflammatory bowel diseases (ulcerative colitis) such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome (IBS). On the other hand, the results of ferritin were elevated in the subjects taking the biological therapy and the subjects not taking the biological therapy when compared with the control group while ferritin level was higher in patients without biological therapy. The ferritin test measures the level of ferritin in the blood (an essential protein that stores iron), which helps diagnose conditions related to iron

deficiency, such as anemia, or iron overload, such as hemochromatosis. It may be related to changes in blood levels. Calprotectin levels. These tests provide valuable insights into a patient's overall health and well-being, guiding healthcare professionals in designing appropriate treatment plans and monitoring progress. The combination of the results of these tests provides a more comprehensive understanding of a person's digestive and iron health, ensuring timely and effective interventions. The results of Table 4.7 showed that there is a positive signification correlation between the ferritin level of the groups studied which recorded 0.978** at a level of 0.01.

Table 4.6 The relationship of the Fecal Calprotectin test with ferritin in UC

Groups studied	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
Control	78.5380	17.97805	2.54248	51.10	99.40
Patients with biological therapy	223.4260	15.18261	2.14715	201.50	262.70
Patients without biological therapy	311.2716	11.25934	1.59231	300.50	353.40
Total	204.4119	97.43636	7.95565	51.10	353.40

Table 4.7 Correlation between ferritin level and groups studied

		Groups studied	|Ferritin level
Groups studied	Pearson Correlation	1	.978**
	Sig. (2-tailed)		.000
	N	150	150
|Ferritin level	Pearson Correlation	.978**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

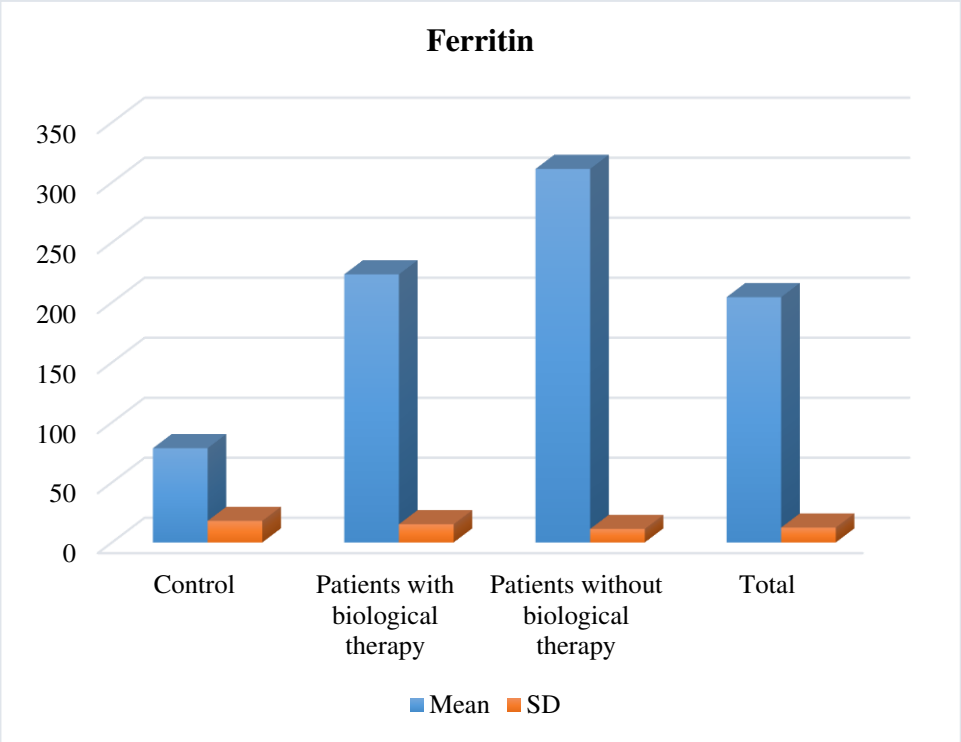

Figure 4.3 Ferritin level of groups studied

4.7 The Previous Research for Fecal Calprotectin with Ferritin in Ulcerative Colitis

In patients with ulcerative colitis, the biomarkers could consistently differentiate between ferritin, the calprotectin level ferritin, and healthy controls (Dai *et al.* 2015). Due to the ongoing inflammatory process that interferes with the usefulness of several recognized indicators, such as ferritin and transferrin, the interpretation of the iron status indices in patients with IBD is extremely challenging. Therefore, researchers had to create new parameters for this purpose to meet the demand for more practical indicators that might more consistently distinguish the group of individuals with IDA. In patients with infection, inflammation, or cancer, where serum ferritin is not a reliable sign of iron shortage, sTfR appears to be particularly helpful for the diagnosis of IDA. Chronic inflammation and liver damage had no impact on sTfRs, unlike ferritin and transferrin, which should make them a more trustworthy criterion than serum ferritin for identifying IDA in patients with IBD (Daude *et al*. 2020).

Children with IBD have significantly lower ferritin levels, which are indicative of the persistence of defective adipose tissue and its propensity to contribute to chronic inflammation. To fully understand the involvement of these adipokines in the etiology of IBD, more research is required (Foltenovaand Karasek 2022).

4.8 Study of the Relationship of Fecal Calprotectin and IL-6 with UC

The results of Table 4.8 revealed the two patient groups with and without biological therapy significantly elevated the mean IL-6 level as compared with the control group. The results indicated that there were clear statistical differences between the control group (3.69 ± 2.24) and the second group (patients with biological therapy) which recorded (7.36 ± 1.59). There were also significant statistical differences between the control group (3.69 ± 2.24) and the third group (patients without biological therapy) which recorded 20.53 ± 5.19. These results indicate that the fecal calprotectin test combined with the measurement of interleukin 6 (IL-6) is a very valuable tool in the medical field for the detection and monitoring of Crohn's disease and ulcerative colitis. The results indicated that the levels of interleukin-6 increased to double in patients taking biological therapy, while it increased to four times in people with ulcerative colitis and without taking biological therapy when comparing the results with the control group. These non-invasive tests provide insight into the level of inflammation present in the digestive tract, allowing for more accurate diagnoses and targeted treatment plans. Because inflammation is a major factor in UC, monitoring levels of these biomarkers can greatly help clinicians determine the severity of the condition and evaluate the effectiveness of therapeutic interventions. Furthermore, the combined use of fecal calprotectin and interleukin-6 tests could help reduce the need for more invasive diagnostic procedures, such as colonoscopies, while providing reliable and informative data for the management of UC in patients. Ultimately, this approach contributes to improving the quality of life for those affected by these debilitating conditions.

Table 4.8 The relationship of the Fecal Calprotectin test with IL-6 in UC

Descriptives Result for IL-6							
Test and Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control	3.692080	2.2463	0.449	2.764	4.619	.022	7.638
Patients with biological therapy	7.363677	1.59359	0.825	5.678	9.048	1.50	18.99
Patients without biological therapy	20.53066	5.1997	2.217	16.06	24.99	5.48	89.01
Total (n=150)	12.48074	2.9743	1.278	9.945	15.01	0.02	89.01

Table 4.9 The Parameter and study between groups for IL-6 in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	5788.384	3	2894.192	25.429	.000
Within Groups	11381.659	147	113.817		
Total	17170.044	150			

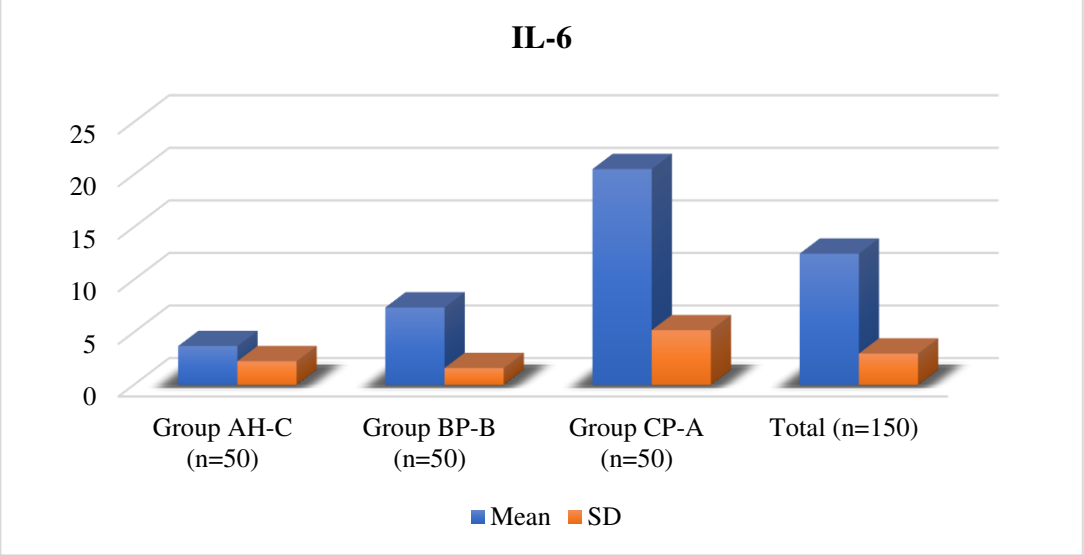

Figure 4.4 The mean and SD for IL06 in patients with UC

4.9 The Previous Research for Fecal Calprotectin with IL-6 in UC

Interleukin-6 (IL-6) levels, erythrocyte sedimentation rate (ESR), platelet, hemoglobin, white blood cell, and p-ANCA levels, as well as fecal calprotectin levels, were all found in the serum. The receiver operating characteristic (ROC) curve was used to assess the clinical utility of each index in predicting the severity of UC. Results Children in the remission stage had significantly lower disease progression, IL-6, PCT, CRP, ESR, p-ANCA, and calprotectin levels than children in the active stage, according to statistical comparisons (P0.05 or 0.01). There were statistical changes in the IL-6, CRP, ESR, white blood cell, and calprotectin (P 0.01 or 0.05) (Zhou *et al.* 2020).

Ulcerative colitis is characterized as a persistent inflammation of the gastrointestinal tract. ulcerative colitis etiopathogenetic mechanisms are still poorly known, and the therapeutic approaches employed up to this point have mostly relied on empirical principles. IL-6 may influence the creation of novel therapeutic approaches for UC treatment. Recent investigations have also mentioned the role of IL-6 in UC treatment that after 6 weeks of biological therapy, the IL-6 level significantly lowed with the patient having UC (Bertani *et. al.* 2020).

4.10 Study of the Relationship of the Fecal Calprotectin Test with Vitamin D

In Table 4.10 and Figure 4.5, the results of the relationship of the fecal calprotectin test with vitamin D in our study indicated a slight increase in the standard deviations level, and there were significant differences in vitamin D level between the control group recorded (22.9447 ± 6.1704) and the patient's group with biological therapy which recorded (26.3233 ± 8.2883). There were also significant statistical differences between the control group (22.9447 ± 6.1704) and the patients without biological therapy group which recorded (20.7572 ± 4.1340) at P = 0.05. These vitamin D differences can be explained as follows:

The fecal calprotectin test, along with the vitamin D assessment, takes a holistic approach to understand the health of the gastrointestinal tract and the general health of an individual. The results of vitamin D did not have any statistically significant changes in patients with ulcerative colitis, whether they were taking biological therapy or not. The fecal calprotectin test did not have clear statistical indicators in diagnosing inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. By measuring levels of the protein calprotectin in the stool, healthcare professionals can determine the presence of inflammation in the intestine. Similarly, according to the results of our study, measuring vitamin D test levels may not be as important as the GI course in assessing bone health and immune system function. Adequate levels of Vitamin D are essential for absorbing calcium and maintaining strong bones, as well as supporting a healthy immune system. The combination of these two tests can provide valuable information about a person's gut health. Both fecal calprotectin and vitamin D tests can be performed simultaneously, allowing for a comprehensive approach to monitoring patient health and wellness.

Table 4.10 The relationship of the Fecal Calprotectin test with vitamin D in UC

Descriptives Result for Vitamin D							
Test and Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control	22.9447	6.1704	3.2340	16.269	29.61	3.00	70.89
Patients with biological therapy	26.3233	8.2883	2.9254	20.348	32.29	3.00	66.30
Patients without biological therapy	20.7572	4.1340	2.2075	16.313	25.20	3.00	77.21
Total (n=150)	22.9634	6.7646	1.5533	19.882	26.04	3.00	77.21

Table 4.11 The parameter and study between groups for vit. D in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	578.723	3	289.361	1.168	.315
Within Groups	24770.833	147	247.708		
Total	25349.555	150			

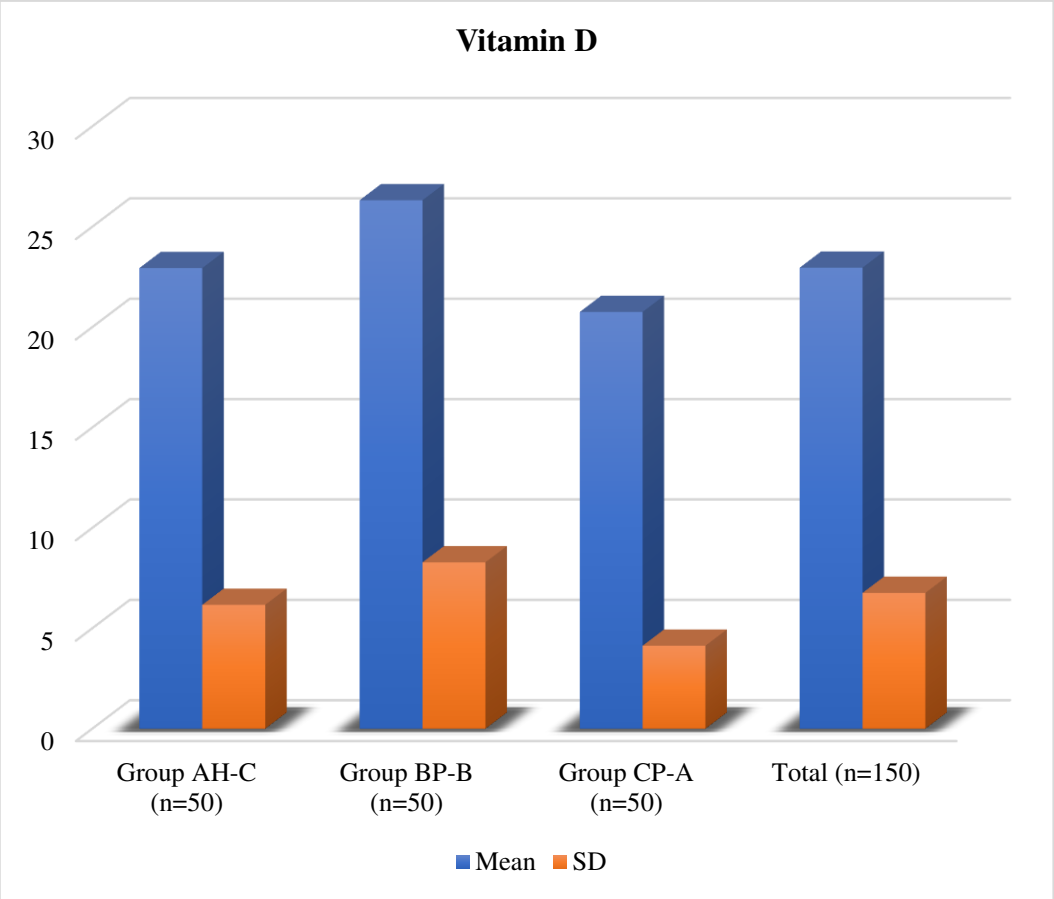

Figure 4.5 The mean and SD for vitamin D in patients with UC

4.11 The Previous Research for Fecal Calprotectin with Vitamin D in UC

Low serum vitamin D levels in ulcerative colitis (UC) patients can cause issues like reduced bone mineral density. It may also represent the severity of underlying diseases. In comparison to controls, individuals with UC had reduced mean vitamin D levels (54.6 nmol/L vs. 80.7 nmol/L; p 0.0001). Patients with severe UC had the lowest mean vitamin D levels (E3; p = 0.0001). Across treatment groups, there was no discernible difference in serum vitamin D levels (p = 0.876). Amongst patients using calcium and vitamin D supplements, there was no statistically significant difference in vitamin D levels (p = 0.35), and there was no connection (p = 0.22) (Zammit *et al.* 2019).

Due to malabsorption, inadequate dietary intake as a result of ulcerative colitis symptoms, low solar exposure, difficulties converting the vitamin to its useable form, or

increased excretion, people with ulcerative colitis tend to have low vitamin D levels. Because of this, some experts think that treating ulcerative colitis with vitamin D supplements could be a simple and affordable procedure (Mathur *et al.* 2017).

4.12 Study of the Relationship of the Fecal Calprotectin test with Vitamin B12

In Table 4.12 and Figure 4.6, the results of the relationship of fecal calprotectin test with vitamin B12 in our study indicated that the level of vitamin B12 of patient's groups (with biological therapy and without biological therapy) studied showed a significant decrease in the mean of this vitamin which recorded 197.15 and 148.58 pg/mL as compared with 219.77 pg/mL for the control group. The decrease in vitamin B12 in patients may be due to produce abnormally large red blood cells that do not function correctly and lead to anaemia and this deficiency can cause a whole host of symptoms including ulcers.

The Fecal Calprotectin test, in conjunction with a Vitamin B12 assessment, can provide critical insights into an individual's gastrointestinal health. Calprotectin is a protein found in white blood cells, and its presence in fecal matter often indicates inflammation in the intestines. The results of vitamin B12 had statistically significant changes in patients with colon ulcers who were taking biological therapy when comparing the results to the control group. On the other hand, Vitamin B12 is an essential nutrient that plays a crucial role in maintaining the health of nerve cells, red blood cell production, and DNA synthesis. By assessing Vitamin B12 levels, doctors can identify deficiencies that may be indicative of malabsorption issues, pernicious anemia, or other health conditions affecting the gastrointestinal tract. Combined, these two tests create a comprehensive starting point for evaluating an individual's overall digestive health and determining appropriate treatment strategies.

Table 4.12 The relationship of the Fecal Calprotectin test with vitamin B12 in UC

Groups studied	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
Control (n=50)	219.7760	13.27029	1.87670	201.40	255.90
Patients with biological therapy (n=50)	197.1540	6.78201	.95912	184.50	211.40
Patients without biological therapy(n=50)	148.5236	9.06223	1.28159	132.50	175.80
Total(n=150)	188.4845	31.45961	2.56867	132.50	255.90

The results of Table 4.13 revealed that there is a negative significant correlation between vitamin B12 levels and the groups studied which recorded -0.928** at a level of 0.01.

Table 4.13 Correlation between vitamin B12 level and groups studied

		Groups studied	Vitamin 12 level
groups studied	Pearson Correlation	1	-.928**
	Sig. (2-tailed)		.000
	N	150	150
vitamin 12 level	Pearson Correlation	-.928**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

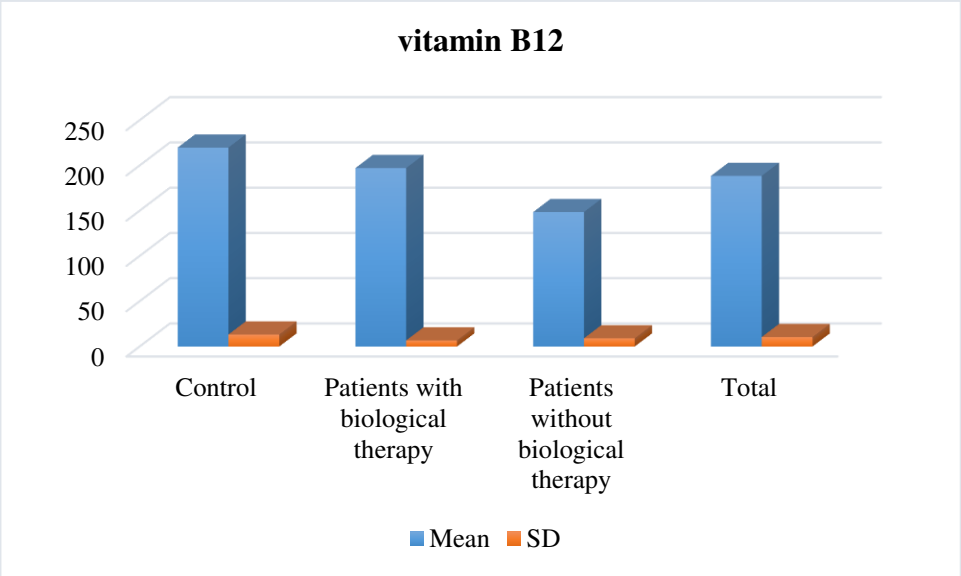

Figure 4.6 The mean of vitamin B12 in patient with UC

4.13 Study of the Relationship of the Fecal Calprotectin Test with Vitamin B12

UC typically results in inflammation of the large intestine's inner lining. Other IBD types, including Crohn's disease, typically have an impact on the small intestine, which is where vitamins are absorbed. Those with UC are also at risk for B12 insufficiency, while Crohn's disease patients are more likely to experience it. A study found that 33% of people with Crohn's disease had B12 deficiencies, compared to 16% of people with UC. Nonetheless, because of malnutrition and the symptoms of this illness, patients with UC may still experience issues absorbing nutrients. A person may lose nutrients and develop new problems if they have severe diarrhea or have blood in their stool. For instance, iron anemia may result from iron loss. ulcerative colitis development may also be influenced by vitamin levels, according to certain research. For instance, serum levels of folate and vitamin B12 have an impact on the emergence including UC (Zheng *et al.* 2017).

Vitamin B12 must first combine with our stomach acid in the small intestine before it can be absorbed into the small intestine's cells. Nonetheless, there are some IBD (ulcerative colitis) cases. If this is the case, stomach acid may not mix with vitamin B12 adequately. When it reaches the small intestine, this might make it less able to be

absorbed. This may eventually result in a B12 shortage. Within the small intestine, vitamin B12 is absorbed. The small intestine may become severely inflamed in Crohn's disease, which can impair the absorption of numerous nutrients, including vitamin B12. Surgery may be used to remove severe cases' portions of the small intestine, including the terminal ileum, which increases the likelihood that a Crohn's patient would experience malabsorption. Absorption of B12 will be lowered with UC. In addition, there is a higher chance of malabsorption if some of the inflammatory terminal ileum is removed. A UC may eventually have a B12 deficiency as a result of this (Lurz *et al.* 2020).

4.14 Study of the Relationship of the Fecal Calprotectin Test with Folate

The results of the relationship of the fecal calprotectin test with folic acid in our study indicated that there were clear statistical differences towards a decrease between the control group which recorded (10.18 ± 2.92) and the patients in the biological therapy group which recorded (7.18 ± 1.81). There were also significant statistical differences towards a decrease between the control group (10.18 ± 2.92) and the patients without biological therapy group which recorded (6.83±1.44), as well as a decrease in folate concentrations in patients without biological therapy when compared to the patients with biological therapy at P = <0.05 as in Table 4.14 and Figure 4.7. These folate differences can be explained as follows:

The fecal calprotectin test, combined with the folic acid test, offers a comprehensive approach to understanding the health of your digestive system. The results of folic acid had slight statistical changes in patients with colon ulcers who were taking biological therapy, slightly less than patients taking biological therapy when comparing the results to the control group. On the other hand, folate testing measures levels of an essential vitamin in the blood. Changes in folate levels highlight potential deficiencies that can lead to anemia and other health complications. By combining these tests, healthcare professionals can obtain a more accurate picture of a patient's overall gut health and develop appropriate treatment plans to effectively address any identified issues. This

dual-testing methodology ensures that patients receive comprehensive care that is tailored to their specific digestive needs.

Table 4.14 The relationship of the Fecal Calprotectin test with folate in UC

Descriptives Result for Folate							
Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control	10.1892	2.92706	0.985	6.1554	12.22	0.60	20.00
Patients with biological therapy	7.18000	1.81531	0.864	4.4137	8.946	0.60	20.00
Patients without biological therapy	6.83190	1.44404	0.648	3.5270	8.136	0.23	18.94
Total (n=150)	7.75156	1.73569	0.476	4.8064	8.696	0.23	20.0

Table 4.15 The Parameter and study between groups for folate in ulcerative colitis

ONEWAY ANOVA						
Parameter and study between group		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Folate	Between Groups	198.440	3	99.220	4.537	.013
	Within Groups	2186.718	147	21.867		
	Total	2385.158	150			

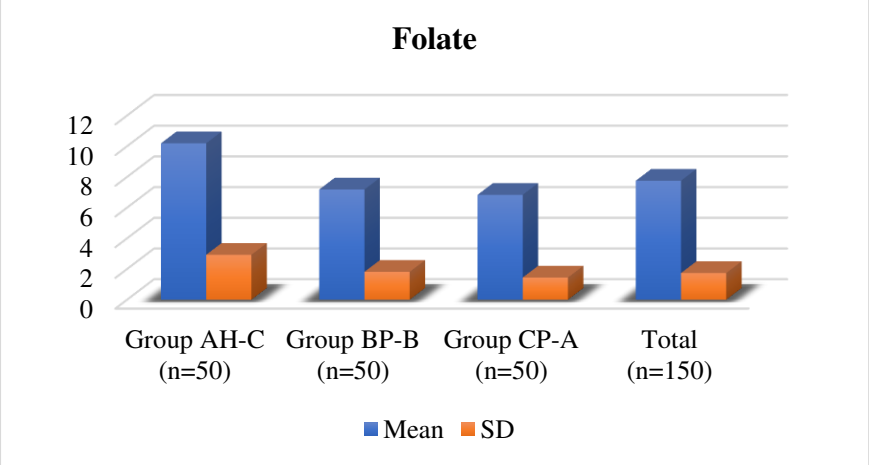

Figure 4.7 The mean and SD for folate in patients with UC

4.15 The Previous Research for Fecal Calprotectin with Folate in UC

Patients with UC have significantly lower levels of folate than normal. Impaired reabsorption may contribute to reduced folate levels. Vitamin B12 levels may be decreased in UC patients due to chronic inflammation, which causes a hypermetabolic condition in the metabolism of amino acids and carbohydrates. Given that vitamin B12 plays a consistent and pervasive function in metabolic activity, dietary treatment for UC patients, including the use of water-soluble vitamins, appears to be necessary. (Lwakawa *et. al.* 2019).

The primary method by which folates are absorbed in the intestine and the root cause of hereditary folate malabsorption is the proton-coupled folate transporter (PCFT; SLC46A1) (Matherly *et al.* 2022).

A meta-analysis including folate concentration in patients with UC was significantly lower than that in control It has been proved that folate plays a role in regulating reactive oxygen species production and reducing oxidative stress by acting directly as an antioxidant. The majority of the UC centers around deficiencies in folate were relatively overlooked. Current European Society for Clinical Nutrition and Metabolism (ESPEN) recommendations suggest that micronutrient levels need to be assessed annually in UC patients, with the correction of any deficit with supplementation. Limited evidence makes specific recommendations on nutrient supplementation difficult. Circulating micronutrients of antioxidants, minerals, and vitamins are causally linked to the development of UC, which would contribute to the research field of nutrient prevention for UC (Chen *et. al.* 2023).

4.16 Study of the Relationship of the Fecal Calprotectin Test with HsC-RP

The results of the stool calprotectin protein C relationship test in our study (Table 4.16) indicated that there were significant differences between the control group which recorded (5.78 ± 0.81) and the patients without biological therapy group which recorded

(10.80±2.13). The Fecal Calprotectin test, when combined with hsC-RP (high-sensitivity C-reactive protein), provides valuable information to healthcare professionals in diagnosing and managing inflammatory bowel disease (ulcerative colitis). The results of hsC-RP had high statistical changes in patients with colon ulcers who were not taking biological therapy, while there were no significant statistical differences in patients taking biological therapy when comparing the results to the control group. These two tests work together to offer a more comprehensive picture of a patient's gastrointestinal health, as Fecal Calprotectin specifically targets inflammation in the gut while hsC-RP detects systemic inflammation throughout the body. This combination of tests allows for the early detection of ulcerative colitis and helps doctors assess the severity of inflammation, monitor responses to treatments, and predict possible relapses. Furthermore, these non-invasive tests can prevent unnecessary procedures such as colonoscopies, ultimately improving patient care and comfort.

Table 4.16 The relationship of the Fecal Calprotectin test with hsC-RP in UC

Descriptives Result for Hs-CRP							
Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control (n=50)	5.7856	0.812	1.1571	1.423	6.200	0.15	29.18
Patients with biological therapy(n=50)	5.4770	0.179	0.9837	2.170	6.188	0.15	25.18
Patients without biological therapy(n=50)	10.804	2.133	1.5760	5.961	12.30	0.15	51.58
Total (n=150)	8.7136	1.350	0.8585	4.647	8.053	0.15	51.58

Table 4.17 The parameter and study between groups for hsC-RP in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	671.243	3	335.622	4.745	.011
Within Groups	7073.348	147	70.733		
Total	7744.592	150			

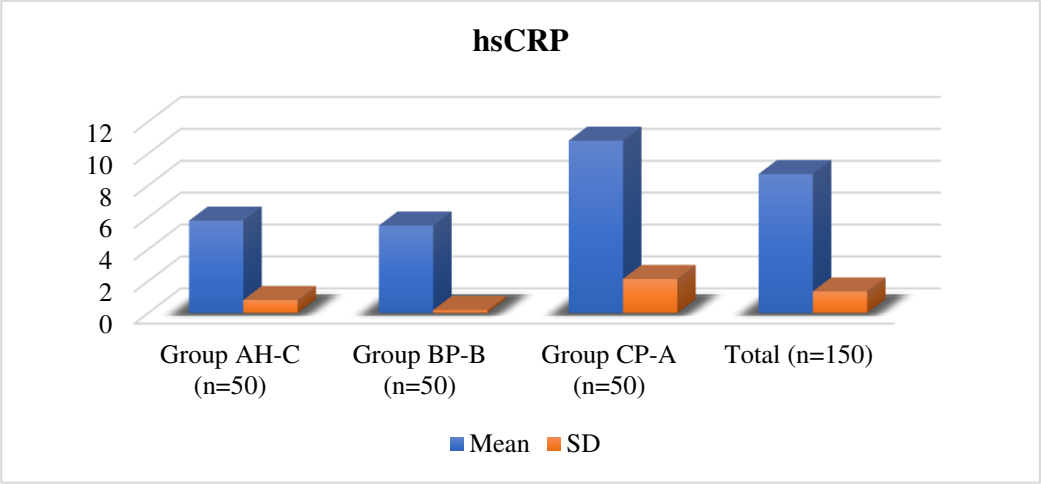

Figure 4.8 The mean and SD for hsC-RP in patients with UC

4.17 The Previous Research for Fecal Calprotectin with hs-CRP in UC

Patients had higher levels of fecal calprotectin and C-reactive protein. In ulcerative colitis, there was a substantial relationship between the fecal calprotectin concentration and the C-reactive protein (Önal *et al.* 2012). The biological therapy is a viable alternative therapy that helped half of our patients. In our investigation, the hsCRP result was a crucial statistic. More information is required, particularly in long-term follow-up (Eronen *et al.* 2022).

CRP activates complement, induces the production of pro-inflammatory cytokines, and induces apoptosis which, together with the inflammatory status during the disease. (Mosquera *et al.* 2021). Fecal calprotectin (FC) and serum C-reactive protein (CRP) are biomarkers of disease activity in ulcerative colitis (UC), disease activity is reduced by taking biological therapy. In UC both high FC and high CRP predict 'incident'. Combined FC and CRP values also predict an 'incident' (Engström *et al.*2019)

Plasma levels of IL-6 and hs-CRP before diagnosis are associated with the risk of incident UC. Subclinical levels of systemic inflammation may be a feature of an early disease state that precedes the development of symptomatic IBD (Lochhead *et al.* 2016)

4.18 Study of the Relationship of the Fecal Calprotectin Test with PCT

The results of the stool calprotectin test with Procalcitonin (PCT) in our study indicated that there were statistical differences between the groups studied, the two patient's groups significantly increased the mean of procalcitonin levels which recorded 0.21 and 0.34 ng/mL for patients with biological therapy and patients without biological therapy respectively as compared with the control group which recorded 0.04 ng/mL (Table 4.18 and Figure 4.9). The results of the procalcitonin test statistically revealed significant differences in the mean level of procalcitonin in patients with colon ulcers who were not taking biological therapy. Also, there is a significant statistical difference in patients taking biological therapy when comparing the results to the control group.

Table 4.18 Procalcitonin level of groups studied

Groups studied	Mean (Ng/mL)	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum
Control	0.0470	50	0.01991	0.00282	0.01	0.10
patients with biological therapy	0.2127	50	0.05893	0.00833	0.01	0.30
patients without biological therapy	0.3455	50	0.05973	0.00845	0.26	0.48
Total	0.2017	150	0.13214	0.01079	0.01	0.48

The results of Table 4.19 showed that there is a positive significant correlation between procalcitonin levels and the groups studied which recorded 0.925** at a level of 0.01.

Table 4.19 Correlation between procalcitonin levels of groups studied

		Groups studied	Procalcitonin level
Groups studied	Pearson Correlation	1	.925**
	Sig. (2-tailed)		.000
	N	150	150
Procalcitonin level	Pearson Correlation	.925**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

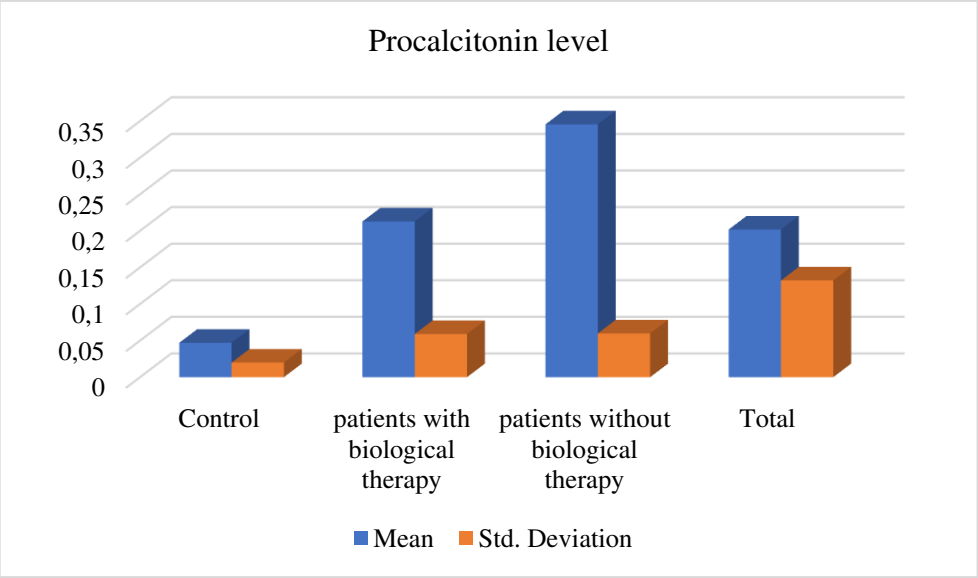

Figure 4.9 Procalcitonin level of groups studied

4.19 The Previous Research for Fecal Calprotectin with PCT in UC

Serum PCT and the Ulcerative Colitis Endoscopic Index of Severity showed a strong correlation. A combination of PCT and FC may increase the predictive value of serum PCT, which may be a possible early non-invasive predictive biomarker for UC patients (Wu*et al.* 2019).

Procalcitonin is a good marker for separating a severe flare from mild and moderately active disease, even if its level does not rise to the same extent in UC patients as it does in those with infective colitis. Procalcitonin has already been examined as an ASUC patient outcome predictor (Mishra *et al.* 2022).

The gold standard for determining intestinal inflammation is a colonoscopy with biopsy, but it is intrusive, uncomfortable, and expensive. There is currently no endoscopic or histological score that can accurately predict the clinical recurrence of UC. Fecal biomarkers are more sensitive and specific than blood biomarkers due to their proximity to the site of intestinal inflammation and direct contact with the intestine. Nowadays, fecal calprotectin is the most researched and significant fecal biomarker. As novel biomarkers, lactoferrin, PCT, and S100A12 perform equally as well as FC at predicting

the return of UC. Despite being the most promising prognostic marker at the moment, FC lacks a reliable cut-off value. The focus of UC remission management will be on combining patient symptoms, adding various indications to build a UC recurrence prediction model, and creating tailored treatment programs for individuals with a high recurrence risk (Ge*et al.* 2022). Objective To determine the clinical use of fecal calprotectin and perinuclear antineutrophil cytoplasmic antibody (p-ANCA) in predicting the severity of pediatric ulcerative colitis (UC). Procalcitonin (PCT), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, platelet, hemoglobin, white blood cell, and p-ANCA serum levels were identified, as well as fecal calprotectin. The receiver operating characteristic (ROC) curve was used to assess the clinical utility of each index in predicting the severity of UC (Zhou *et al.* 2020).

4.20 Study of the Relationship of the Fecal Calprotectin Test with Sodium

In Table 4.20 and Figure 4.10, the results of the relationship of fecal calprotectin test with sodium (Na) in our study indicated that there were no statistical differences between the first group (138.104 ± 4.3839) and the second group (145.764 ± 5.0018) at P = <0.05. There were no statistically significant differences in sodium (Na) concentrations between the first group (138.104 ± 4.3839) and the third group (138.570 ± 18.863), also, sodium (Na) concentrations did not change in the third group when compared to the second group at P = <0.05. These Na differences can be explained as follows:

The Fecal Calprotectin test with sodium (Na) is a significant advancement in the field of diagnostics for gastrointestinal disorders. By utilizing the sodium component in this test, it enhances the accuracy and reliability of the results, making it easier for physicians to determine the exact cause of a patient's abdominal discomfort or irregular bowel movements. Early detection and diagnosis of these conditions is critical in order to initiate the proper course of treatment, and the Fecal Calprotectin test with sodium is proving to be an invaluable tool in facilitating this process. The results of sodium (Na) had statistically slight changes in patients with colon ulcers who were not taking

biologic therapy, and there were no statistically significant differences in patients taking biologic therapy when comparing the results to the control group.

Table 4.20 The relationship of the Fecal Calprotectin test with Na in UC

Groups studied	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
Control (n=50)	138.10	4.3839	0.87678	135.0	154.00
Patients with biological therapy (n=50)	145.76	5.0018	0.89835	138.0	154.00
Patients without biological therapy (n=50)	138.57	18.863	2.7515	15.00	154.00
Total (n=150)	140.62	13.560	1.33615	15.00	154.00

Table 4.21 The parameter and study between groups for Na in ulcerative colitis

ONEWAY ANOVA						
Parameter and study between group		**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Na	Between Groups	1176.192	3	588.096	3.345	.039
	Within Groups	17580.242	147	175.802		
	Total	18756.434	150			

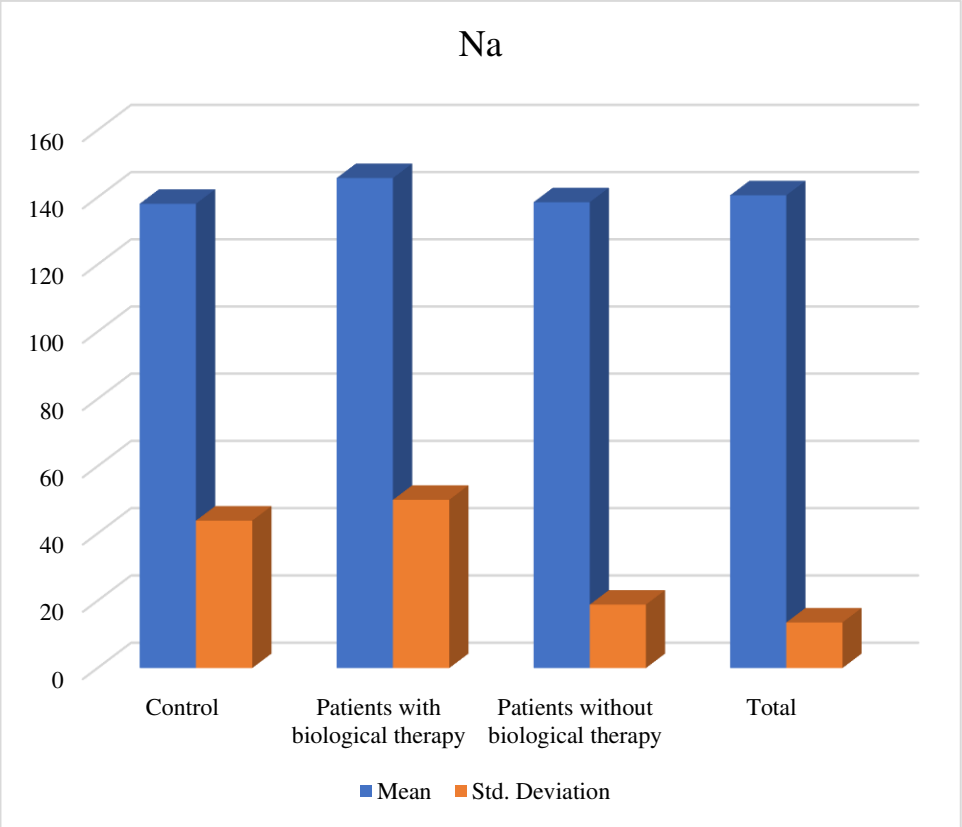

Figure 4.10 The mean and SD for Na in patient with UC

4.21 The Previous Research for Fecal Calprotectin with Na in Ulcerative Colitis

This study looked at how oral microencapsulated sodium butyrate (BLM) treatment affected a group of ulcerative colitis patients' residual symptoms, inflammatory markers, and remission rates of (UC). When compared to the control group, the patient group experienced therapeutic success (defined as Mayo partial score 2 and faecal calprotectin (FC) 250 g/g at 12 months of follow up; p = 0.022) (Vernero *et al.* 2020).

Water and electrolyte flux rates across the human colonic epithelium were measured in vitro, and the results showed that in UC, the colon becomes less absorptive and more secretory. Particularly, the colon absorbs less salt and water during the active phase of UC (Archampong *et al.* 1992).

Bilateral sodium isotope flux studies in distal colonic mucosa demonstrated decreased net sodium absorption in untreated UC patients due to a reduced mucosa-to-serosa unidirectional flux. In vitro measurements of the net transport and simultaneous bidirectional flux rates of water and electrolytes across the human colonic epithelium demonstrated that in UC the colon becomes less absorptive and more secretory. Specifically, in the active phase of UC colon absorbs less water and sodium (Tyagi *et al.* 2019).

colon is an important target of a variety of disease states, including diarrhea, cancer, and IBD, all of which involve dysregulation of complex ionic transport systems. Due to the colon's terminal location in the gut and its ability to be the final intestinal area to modulate secretion and absorption of salt and water. Recently, the colonic transport proteins have been evaluated as new targets for IBS ,as the colon can modulate the body's ability to concentrate or dilute fluid and electrolytes.(Negussie *et al.* 2022).

4.22 Study of the Relationship of the Fecal Calprotectin Test with Potassium

In Table 4.22 and Figure 4.11, the results of the stool calprotectin test relationship with potassium (K) in our study indicated that there were no statistical differences between the first group (4.01320 ± 0.15415) and the second group (4.10709 ± 0.40378) at P = <0.05. Also, there were no significant statistical differences in the potassium concentrations between the first group (4.01320 ± 0.15415) and the third (4.16116 ± 1.15970). Likewise, the potassium concentrations did not change in the third group when compared to the second group at P = <0.05. The results of potassium had statistically slight changes in patients with colon ulcers who were not taking biological therapy. Also, there were no statistically significant differences in patients taking biological therapy when comparing the results to the control group. These K differences can be explained as follows:

Table 4.22 The relationship of the Fecal Calprotectin test with K in UC

Descriptives Result for K							
Group	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
				Lower Bound	**Upper Bound**		
Control (n=50)	4.01320	0.15415	0.03083	3.949	4.076	3.60	4.400
Patients with biological therapy (n=50)	4.10709	0.40378	0.07252	3.958	4.255	3.07	4.800
Patients without biological therapy (n=50)	4.16116	1.15970	0.16916	3.820	4.501	3.03	11.20
Total (n=150)	4.10897	0.81460	0.08026	3.949	4.268	3.03	11.20

Table 4.23 The parameter and study between groups for K in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	.357	3	.179	.265	.767
Within Groups	67.328	147	.673		
Total	67.686	150			

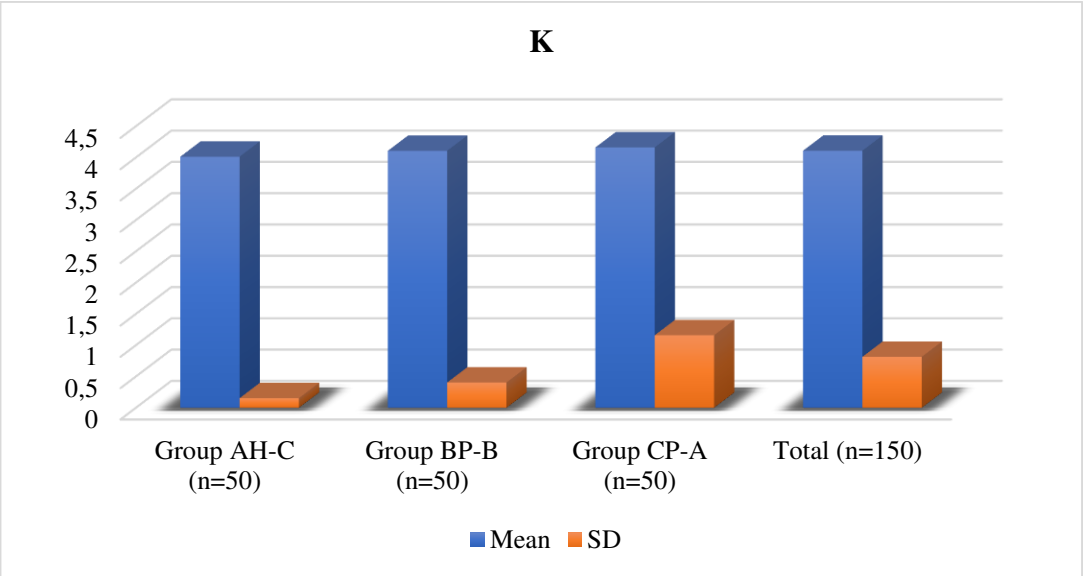

Figure 4.11 The mean and SD for K in patients with UC

4.23 The Previous Research for Fecal Calprotectin with K in Ulcerative Colitis

At various stages of the illness, the rate of potassium secretion exhibited little variation. The nearly normal secretion of potassium in UC with a PD of almost zero suggested that potassium loss to the lumen was relatively excessive, in contrast to how normally the PD with the lumen negatively charged would tend to facilitate the flux of potassium into the lumen and lead to the establishment of an intraluminal steady-state concentration significantly greater than that of blood (Khalil *et al.* 2016).

In actuality, UC patients' mucus included a disproportionately nearly high normal amount of potassium. Overall, compared to healthy people, ulcerative proctocolitis patients had reduced colonic absorption of water and increased production of potassium. Crohn's disease and ulcerative colitis are the two main conditions that make up the chronic inflammatory intestinal ailment known as inflammatory bowel disease (ulcerative colitis). Intestinal inflammatory mechanisms enhance potassium production while decreasing salt, chloride, and calcium absorption. Water and electrolyte flux rates across the human colonic epithelium were measured in vitro, and the results showed that in UC, the colon becomes less absorptive and more secretory. Particularly, during the active stage of UC, the colon secretes more potassium and absorbs less water and sodium (Suss *et al.* 2020).

According to an in vitro study, when the potassium content of normal mucosal cells was intentionally decreased and then returned to an appropriate environment, they quickly regained potassium, in contrast to UC mucosal cells, which continued to lose potassium, suggesting that mucosal cells in UC "leak" potassium (Barkas*et al.* 2013). the first evidence that KV1.3 potassium channels could serve as a marker of disease activity and as a potential treatment target in active UC in vivo (Hansen *et al.* 2014).

4.24 Study of the Relationship of the Fecal Calprotectin Test with Chloride

The results of the stool calprotectin test relationship with chloride (CL) in our study indicated that there were significant differences between the groups studied, patients with and without biological therapy significantly reduced the mean of chlore concentration which recorded 108.272 and 104.059 mmol/mL respectively as compared with 110.012 mmol/mL. Inflammatory bowel disease (IBD) is a chronic inflammatory intestinal disorder encompassing two major entities Crohn's disease and ulcerative colitis. Intestinal inflammatory processes reduce the absorption of sodium, chloride, and calcium, while they increase potassium secretion. The decrement in chloride concentration of patients with ulcerative colitis may be due to decreasing chloride salt absorption in the stool of patients. The results of Table 4.24 revealed that patients with biological therapy reduced. *in vitro* flux studies conducted in the colonic mucosa and rectum of UC patients exhibited a significant reduction in net chloride absorption rather than enhanced chloride secretion (Sandle *et al.* 1986). Further, the transmucosal electrical potential difference was reported to be either significantly low or absent (Sandle *et al.* 1990) in the mucosa of UC patients. A decrease in electrical potential difference is consistent with defective chloride absorption. Roediger et al. (1986) reported that in UC patients, Cl^- uptake by the colon is significantly diminished, which goes well with the elevated levels of colonic and fecal Cl^- content. Along the same lines, Farkas et al. (2011) reported significantly lower Cl^-/HCO^-_3 exchange activity in the colonic crypts isolated from UC patients. Collectively, all these studies reflect the notion that impaired Cl^- absorption in IBD represents one of the critical events in IBD-associated diarrhea.

Table 4.24 The relationship of the Fecal Calprotectin test with CL in UC

Groups studied	Mean	Std. Deviation	Std. Error of Mean	Minimum	Maximum
Control (n=50)	104.059	5.3002	0.7731	100.00	118.70
Patients with biological therapy (n=50)	110.012	5.8338	1.0477	100.00	119.00
Patients without biological therapy (n=50)	108.272	3.5912	0.7182	104.00	118.00
Total (n=50)	107.672	5.2993	0.52215	100.00	119.00

Table 4.25 Correlation between Cl concentration and groups studied

		Groups studied	Cl concentration
Groups studied	Pearson Correlation	1	.980**
	Sig. (2-tailed)		.000
	N	150	150
Cl concentration	Pearson Correlation	.980**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

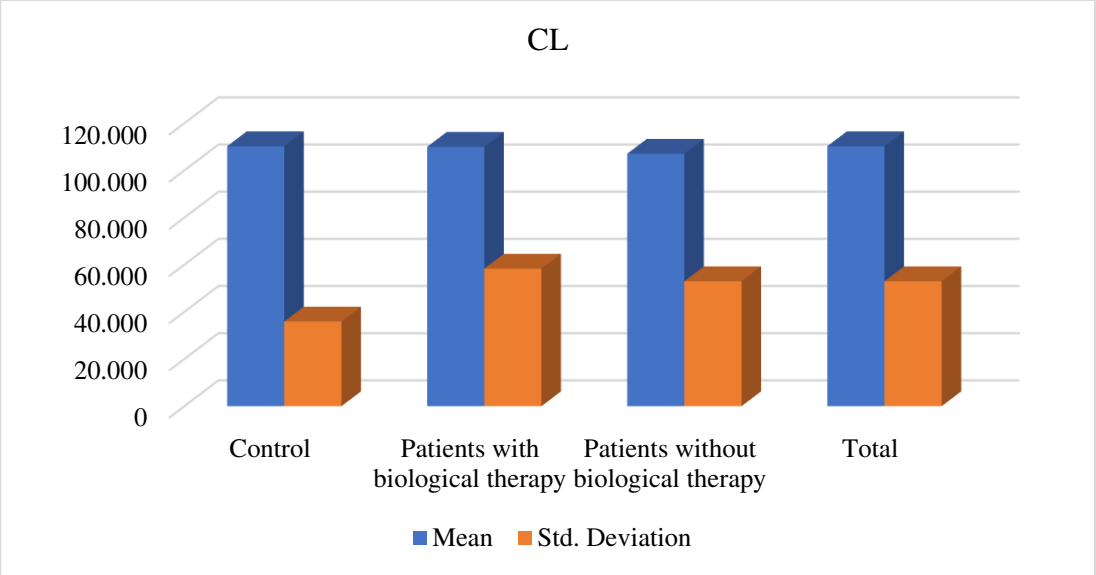

Figure 4.12 CL in patients with UC

4.25 The Previous Research for Fecal Calprotectin with CL at UC

The typical colon secretes potassium and bicarbonate while absorbing water, sodium, and chloride. Water, salt, and chloride absorption are altered in ulcerative colitis (UC), whereas potassium secretion is left unaffected. The literature doesn't seem to provide any information on bicarbonate secretion. As an anion exchange mechanism couples around 25% of chloride absorption with bicarbonate secretion, a decrease in chloride absorption should be accompanied by changes in bicarbonate secretion. The results of this study indicate that patients with active UC have decreased fecal excretion of bicarbonate. This finding could have been explained by the mucosa's inflammation impairing the colon's anion exchange system. It is also assumed that biological anions

play a part. The reduction in fecal bicarbonate loss does not appear to have a direct impact on the acid-base balance (Capriili*et al.* 1986).

4.26 Study of the Relationship of the Fecal Calprotectin Test with Hb

In Table 4.26 and Figure 4.13, the results of the relationship of stool calprotectin test with Haemoglobin in our study indicated that there were no statistical differences between the control group which recorded (12.79 ±1.51), and the patients in the biological therapy group which recorded (11.96 ±1.98) and the patients without biological therapy group which recorded (12.46 ± 1.66) at P = <0.05. This can be explained. Also, there were no statistical changes in the results of haemoglobin in patients with colon ulcers who were taking biological therapy, and there were no significant statistical differences in patients who did not take biological therapy when comparing the results with the control group. The differences in Hb are as follows:

Table 4.26 The relationship of the Fecal Calprotectin test with haemoglobin in UC

Descriptives Result for Haemoglobin							
Group	Mean g/dL	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	**Upper Bound**		
Control (n=50)	12.7960	1.5178	0.303	12.169	13.422	10.2	15.70
Patients with biological therapy (n=50)	11.964	1.9886	0.357	11.234	12.693	6.48	15.80
Patients without biological therapy (n=50)	12.460	1.6665	0.243	11.970	12.949	8.60	15.50
Total (n=150)	12.392	1.7480	0.172	12.050	12.733	6.48	15.80

Table 4.27 The parameter and study between groups for Hb in ulcerative colitis

ONEWAY ANOVA					
Parameter and study between group	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	9.975	3	4.987	1.653	.197
Within Groups	301.701	147	3.017		
Total	311.676	150			

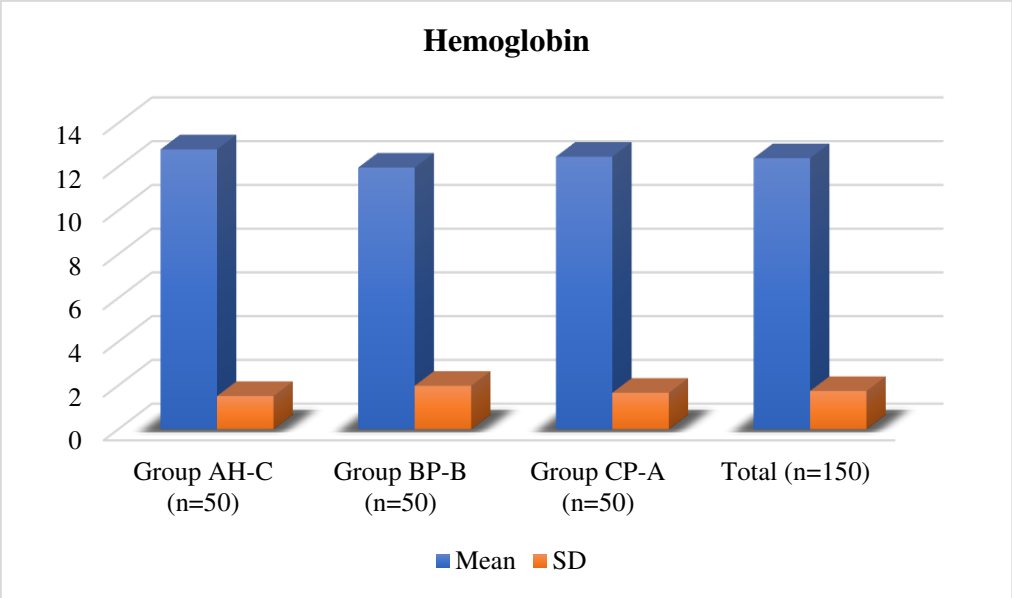

Figure 4.13 The mean and SD for haemoglobin in patients with UC

4.27 The Previous Research for Fecal Calprotectin with Hb in Ulcerative Colitis

Haemoglobin levels rose from 8.3 to 11.9 g/dl in the first phase (mean haemoglobin difference: 3.6 2.3 g/dl, p 0.001). A full response was seen in 15 patients (75%) (Mean haemoglobin difference: 4.5 1.5 g/dl), a partial response was seen in 1 patient (5%) (Haemoglobin difference: 2.1 g/dl), and no response was seen in 4 patients (mean haemoglobin difference: 0.4 1.8 g/dl), with one patient requiring blood transfusions. Erythropoietin was very effective in the second experimental phase for those who had previously failed to respond (mean haemoglobin difference 3.3 1.9 g/dl). One patient experienced a partial response, and their haemoglobin level increased slightly (by 1.0 g/dl). The majority of individuals with ulcerative colitis-related anaemia get better with intravenous iron alone. Erythropoietin works well for people who don't respond (Gasche*et al.* 1999).

4.28 Study of the Relationship of the Fecal Calprotectin Test with WBC

In Table 4.28 and Figure 4.14, the results of the relationship of stool calprotectin test with White Blood Cells in our study indicated that there is a statistically significant difference between the groups studied The results showed that patients without

biological therapy group significantly increased the mean of WBC count as compared with other groups studied which recorded 15.58 × 10 / L as compared with 5.94 × 10 / L for the control group and 11.95 × 10 / L for patients with biological therapy group (table 4.28 and figure 4.14). Some researchers have found that a WBC count is one of the best ways to predict how severe someone's ulcerative colitis is. The higher your WBC count is, the more aggressive your condition may be. May have higher amounts of inflammation and increased symptoms and white blood cells that play a role in fighting infections and inflammation is common and is often related to allergies, parasitic infections, or autoimmune disorders.

Table 4.28 WBC count of groups studied

Groups studied	**Mean $\times 10^9$ /L**	**Std. Deviation**	**Std. Error of Mean**	**Minimum**	**Maximum**
Control **(n=50)**	5.9480	1.01161	0.14306	4.20	8.80
Patients with biological therapy **(n=50)**	11.9560	0.78615	0.11118	9.20	13.70
Patients without biological therapy **(n=50)**	15.5860	1.22591	0.17337	13.40	18.70
Total **(n=150)**	11.1633	4.11534	0.33602	4.20	18.70

Table 4.29 Correlation between WBC count and groups studied

		Groups studied	WBC count
Groups studied	Pearson Correlation	1	.959**
	Sig. (2-tailed)		.000
	N	150	150
WBC count	Pearson Correlation	.959**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

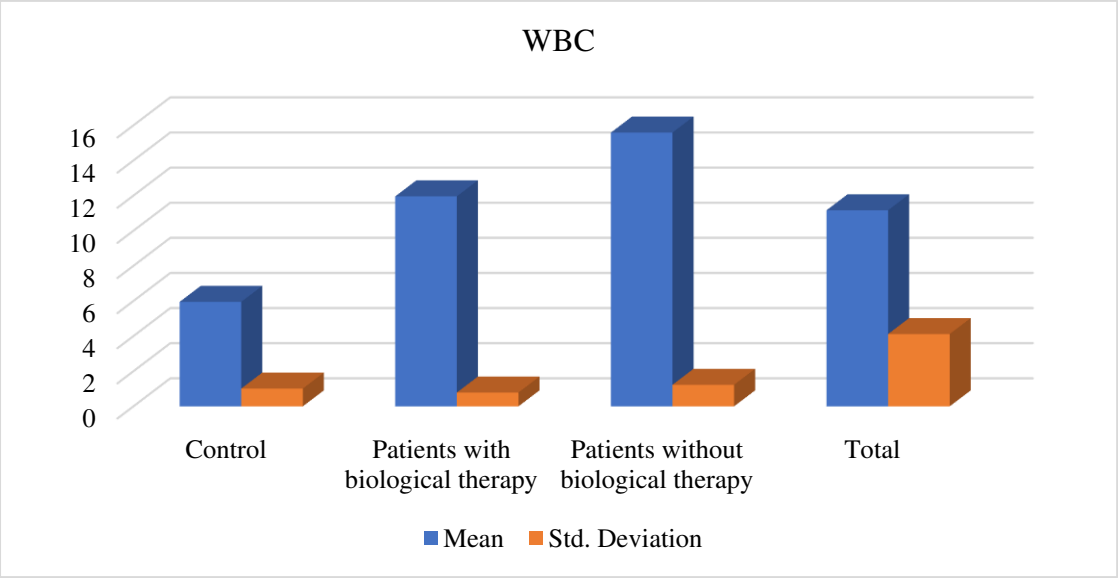

Figure 4.14 The mean and SD for WBC in patients with UC

4.29 The Previous Research for Fecal Calprotectin with WBC in UC

Elevated fecal calprotectin levels in the patients with WBC. In ulcerative colitis, there was a substantial relationship between the fecal calprotectin concentration and the WBC. The variable of greatest statistical significance for separating severe clinical and endoscopic mucosal illness across all laboratory markers was WBC. The one exception was determining the degree of colonic involvement, where it lagged behind ESR and Alb in terms of relevance. The top three tests tested with the highest relative relevance for separating severe from no severe clinical and mucosal illness using classification tree analyses are the total WBC plus ESR and often PLT. Despite having the highest numerical relative relevance, WBC does not statistically outperform the others according to any test. On the other hand, WBC and ESR always appear in all categorization trees, whereas PLT appears in three of the four. The strongest were WBC, ESR, and PLT specifically (Mack *et al.* 2020)

Fecal calprotectin with leukocytes had a better sensitivity and specificity for predicting UC relapse than fecal lactoferrin. Patients with quiescent UC on maintenance therapy with mesalamine appeared to have a substantial relapse risk indicator in their feces, according to the study (Yamamoto *et al.* 2014).

In inflammatory bowel disease, biological therapy has a major impact on how lymphocytes adhere to endothelium surfaces and move toward areas of inflammation. In the management of ulcerative colitis (UC), treatments that target this pathway seem to be a promising therapeutic strategy (Picardo *et al.* 2019).

Traditional colonoscopy with white blood cells in combination with histopathological biopsy is regarded as the gold standard for identifying and measuring intestinal inflammation in UC (Langhorst *et al.* 2016).

4.30 Study of the Relationship of the Fecal Calprotectin Test with Platelet

The results of the relationship of fecal calprotectin test with Platelets in our study indicated that there were significant differences between the patient groups studied (patients with biological therapy and without biological therapy) and the control group which recorded 412.56 and 490.18 platelet $\times 10^3$ /µL while control group recorded 168.10 platelets $\times 10^3$ /µL.

The symptoms which are observed in the course of ulcerative colitis are an increase in the number of leukocytes and blood platelets, an increase in the concentration of IL-6, and anemia. Blood platelets are the key element, linking the processes of hemostasis, inflammation, and the repair of damaged tissues (Polinsk *et. al.* 2011). The accumulation of immune cells in inflamed tissue can also lead to the generation of adenosine diphosphate (ADP), which activates platelets and promotes the degranulation of both dense granules and α-granules (Stafford *et al.* 2003).

Table 4.30 The relationship of the Fecal Calprotectin test with platelet in UC

Groups studied	Mean $\times 10^3$ /µL	Std. Deviation	Std. Error of Mean	Mini.	Max.
Control (n=50)	168.108	14.53200	2.05514	102.40	190.0
Patients with biological therapy (n=50)	412.560	9.59360	1.35674	387.00	433.0
Patients without biological therapy (n=50)	490.180	29.69071	4.19890	418.00	542.0
Total (n=150)	356.949	139.10677	11.35802	102.40	542.0

Data from Table 4.30, Table 4.31 and Figure 4.15 showed that there is a positive significant correlation between platelet count and groups studied which recorded 0.948** at a level of 0.01.

Table 4.31 Correlation between platelets count of groups studied

		Groups studied	Platelets count
Groups studied	Pearson Correlation	1	.948**
	Sig. (2-tailed)		.000
	N	150	150
Platelets count	Pearson Correlation	.948**	1
	Sig. (2-tailed)	.000	
	N	150	150
**. Correlation is significant at the 0.01 level (2-tailed).			

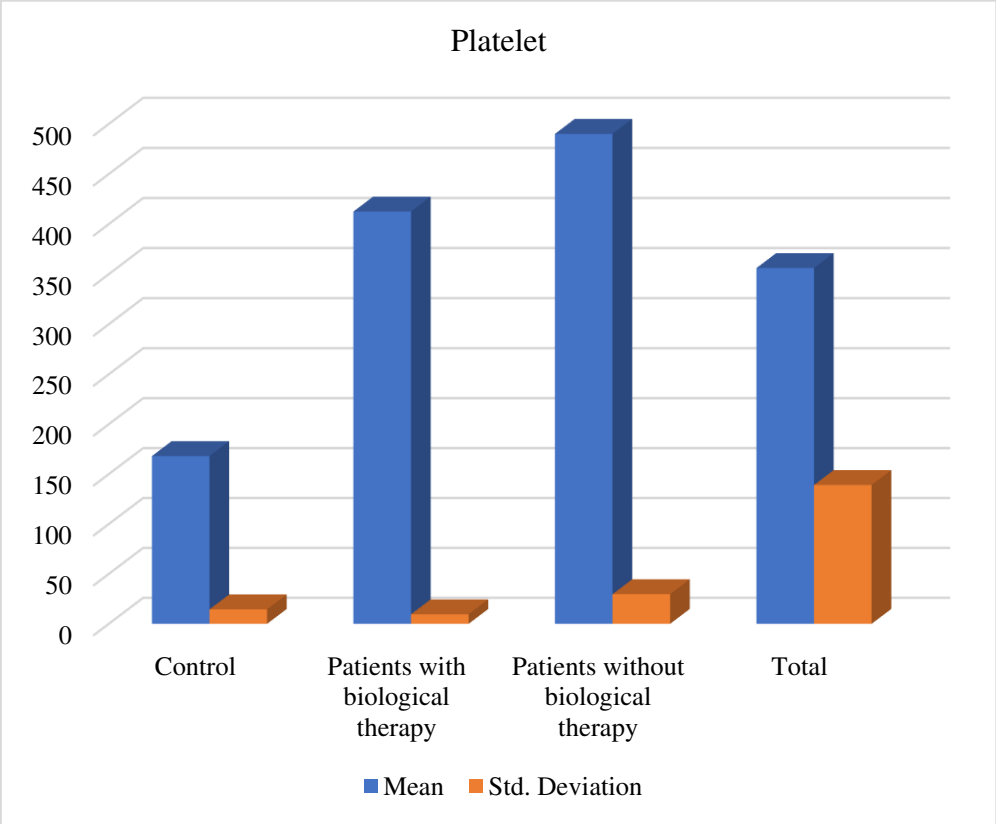

Figure 4.15 The mean and SD for platelet in patients with UC

4.31 The Previous Research for Fecal Calprotectin with Platelet in UC

The previous research for Fecal Calprotectin with platelet in UC Several non-invasive procedures have been researched for the diagnosis and assessment of the level of activation of inflammatory bowel disease (ulcerative colitis). Yet, a perfect test has not been discovered. When there is inflammation, it affects mean platelet volume (MPV). A few studies have found that ulcerative colitis is associated with decreased platelet volume. The ESR, CRP, white blood cell count, and mean platelet volume assays were run on each patient. Patients with UC were shown to have a statistically significant decrease in platelets when compared to healthy controls (Yüksel*et al.* 2009).

To maintain haemostasis, platelets (PLTs) are essential. A growing body of research, however, points to their critical function as inflammatory amplifiers in chronic inflammatory disorders. Poor platelet activation may result in ongoing mucosal irritation. According to earlier research, PLTs controlled thrombosis and coagulation as well as contributed to the onset and progression of intestinal inflammation in IBD. According to our research, PLT levels in UC patients were considerably higher than in controls. This infers that gut inflammatory responses activate and raise the number of PLTs by stimulating them. There is a lack of knowledge regarding the mechanisms driving the aberrant increase in PLT count in IBD patients (Chen *et al.* 2020).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Ulcerative colitis is a chronic inflammatory condition that affects the colon, it's typically the first that manifests in people of various ages. The illness has a relapsing-remitting nature, causes significant impairment, and has large direct and indirect costs. However, both the incidence and prevalence are rising globally. According to our study's findings, the proportion of positive changes in immunological, biochemical, and haematological variables in patients taking the biological therapy group compared with patients without the biological therapy group was observed that indicate the level of health improvement with patients receiving a biological treatment that increased compared with those who didn't receive the biological therapy in patients having UC. provide earlier diagnosis and therapy and so provide the patients with earlier enhanced health as more biologic medicines become available for IBD patients. For fewer surgical procedures and better long-term clinical and patient-reported outcomes biological therapies should be used. Patients with ulcerative colitis have benefited from biological therapies during the induction phase, and these effects are maintained during maintenance therapy that enhances the treatment procedure and improves the patient's quality of life.

High accuracy and precision can be achieved when predicting the development of inflammatory bowel disease (UC) using the level of fecal calprotectin (FC). We assessed the cost-effectiveness of identifying adults and children who need endoscopic confirmation of UC by employing measurements of FC.

The results of the stool calprotectin test relationship with age in our study indicated that there were no high statistical differences between the control group with the patient with biological therapy and patient without biological therapy group. However, it is crucial to note that elevated stool calprotectin levels in any age group may indicate underlying gastrointestinal issues, such as inflammatory bowel disease (ulcerative colitis), infection, or even malignancy.

The level of fecal calprotectin can be used to predict the onset of inflammatory bowel disease with high accuracy and precision. By using measures of fecal calprotectin, we evaluated the cost-effectiveness of identifying adults and children who require endoscopic confirmation of This test allows medical professionals to quickly and accurately measure the levels of calprotectin in a patient's stool sample, which can be a key indicator of inflammation in the intestine. Elevated calprotectin levels often correspond to conditions such as inflammatory bowel disease (UC), including Crohn's disease and ulcerative colitis.

According to the results of our study, the fecal calprotectin test and the ferritin test are among the basic diagnostic tools for the evaluation and monitoring of various health conditions. The fecal calprotectin test can be used primarily to identify inflammation in the gastrointestinal tract, and help doctors differentiate between inflammatory bowel diseases (ulcerative colitis) such as Crohn's disease, ulcerative colitis, and irritable bowel syndrome (IBS).

Because inflammation is a major factor in IBD, it is considering the fecal calprotectin test combined with the measurement of interleukin 6 (IL-6) is a very valuable tool in the medical field for the detection and monitoring of ulcerative colitis (UC), such as Crohn's disease and ulcerative colitis. These non-invasive tests provide insight into the level of inflammation present in the digestive tract, allowing for more accurate diagnoses and targeted treatment plans, monitoring levels of these biomarkers can greatly help clinicians determine the severity of the condition and evaluate the effectiveness of therapeutic interventions.

According to the results of our study, measuring vitamin D test levels may not be as important as the GI course in assessing bone health and immune system function.

The results of the stool calprotectin test with Procalcitonin (PCT) in our study indicated that there were statistical differences between the groups studied.

The Fecal Calprotectin test, in conjunction with a Vitamin B12 assessment, can provide critical insights into an individual's gastrointestinal health. On the other hand, Vitamin B12 is an essential nutrient that plays a crucial role in maintaining the health of nerve cells, red blood cell production, and DNA synthesis. By assessing Vitamin B12 levels, doctors can identify deficiencies that may be indicative of malabsorption issues, pernicious anemia, or other health conditions affecting the gastrointestinal tract.

Changes in folate level highlight potential deficiencies that can lead to anemia and other health complications. By combining these tests, healthcare professionals can obtain a more accurate picture of a patient's overall gut health and develop appropriate treatment plans to effectively address any identified issues.

The Fecal Calprotectin test, when combined with Hs-CRP (high-sensitivity C-reactive protein), provides valuable information to healthcare professionals in diagnosing and managing ulcerative colitis (UC). As Fecal Calprotectin specifically targets inflammation in the gut while Hs-CRP detects systemic inflammation throughout the body. This combination of tests allows for the early detection of IBD and helps doctors assess the severity of inflammation, monitor responses to treatments, and predict possible relapses.

As for the blood parameters tests, the hemoglobin test was not a clear statistical variable, in contrast to the blood cells, in which there were clear differences with the white blood cells. Likewise for platelet.

5.2 Recommendations

- Future studies are needed to determine what contributes to the variation in disease prevalence and course by gender, and possibly better tailor treatment regimens by gender
- Utilizing fecal Calprotectin level for predicting the development of inflammatory intestinal ulceration disease,

- Investigate fecal Calprotectin and other biomarker which may prevent unnecessary colonic endoscopy,
- IL-6 is a good marker for the detection of intestinal ulceration infection in human,
- Hs-CRP (high-sensitivity C-reactive protein), provides valuable information to healthcare professionals in diagnosing and managing inflammatory bowel disease (ulcerative colitis),
- Procalcitonin is a good marker for the detection of intestinal ulceration infections in humans,
- Early detection of ferritin levels may be useful for identifying people with ulcerative colitis,
- It is necessary of conducting comprehensive blood tests, especially red blood cells, haemoglobin, white blood cells, and platelets, for early detection of ulcerative colitis.

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CURRICULUM VITAE

Personal Information

Name and Surname : Nuha Hamza Khedhir KHEDHIR

Education

PhD	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2020-2023
MSc	Tikrit University Graduate School of Natural and Applied Sciences Department of Biology	2017-2018
Undergraduate	Kirkuk University Medical Technology Department of Medical laboratory Technology	2003-2004