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**Ministry of Higher Education and**
**Scientific Research**
**University of Babylon**
**College of Medicine**

# Assessment of Serum Levels of Vitamins D3 , B12 , Zinc, Selenium and Fecal calprotectin in Adult Patients with Inflammatory Bowel Disease in Babylon –Iraq

A Thesis

Submitted to the Council of the College of Medicine, University of Babylon as a Partial Fulfillment of the Requirements for the Degree of Master in Science / clinical Biochemistry

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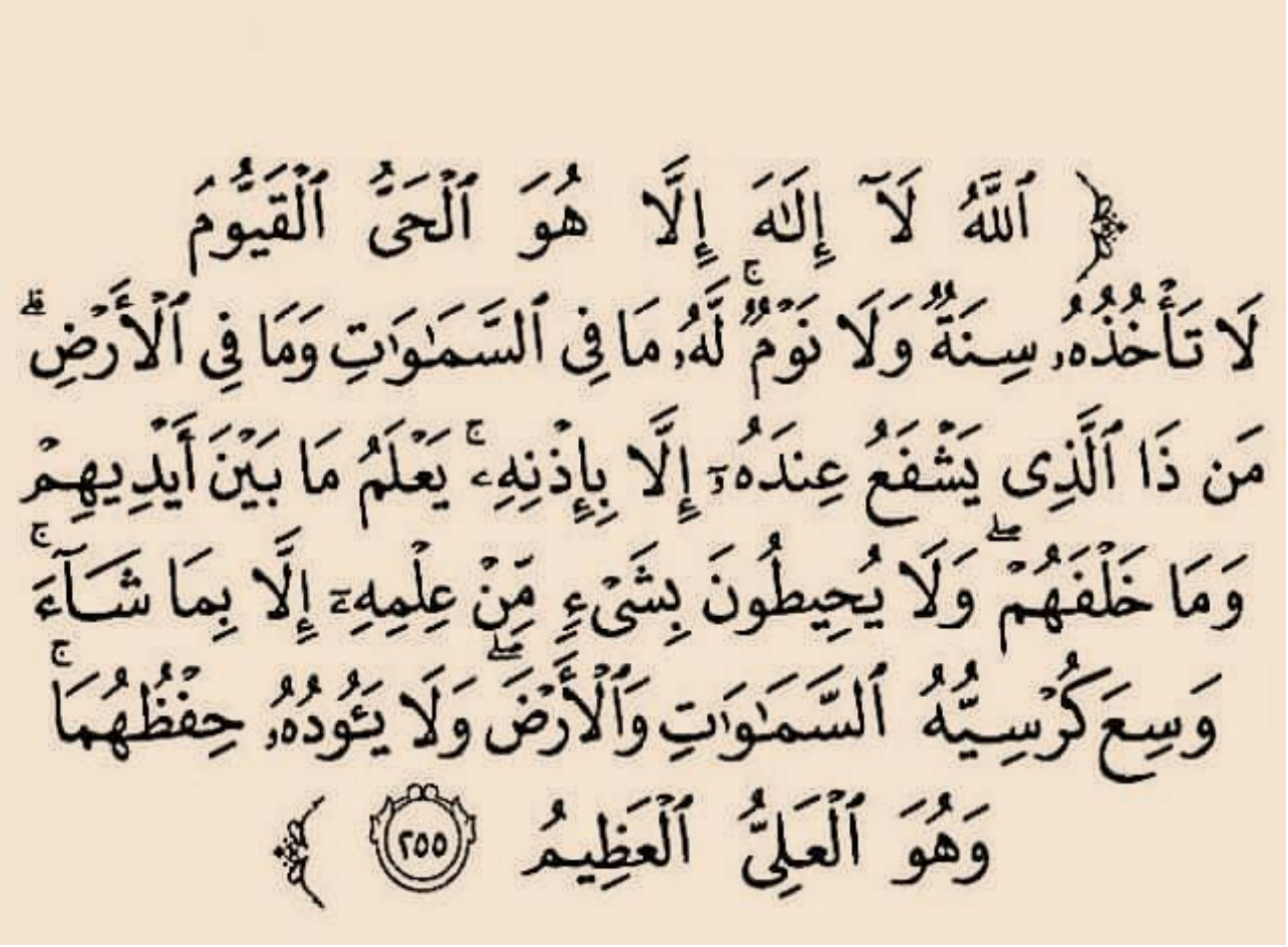


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سورة البقرة (اية 255)

# Dedication

To…my father

To…my mother

To…..my best friend, my support,

my love, my wife

To…. my brothers and my sisters

*Ali Hamid Jawad*

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# Supervisors Certification

We certify that we have read this thesis entitle **"Assessment of serum levels of vitamins D3 ,B12 , Zinc , Selenium and fecal calprotectin in adult patients with Inflammatory Bowel disease in Babylon –Iraq"** was carried under our supervision at the College of Medicine, University of Babylon, as a partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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In view of the available recommendation, I forward the present Thesis for debate by the examining committee.

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# Decision of discussion committee

We certify that we have read this thesis entitle Comparism **Assessment of Serum Levels of Vitamins D3 , B12 , Zinc, Selenium and Fecal calprotectin in Adult Patients with Inflammatory Bowel Disease in Babylon –Iraq**." and as examining committee examined the student " **Ali Hamid Jawad**" in its content and in our opinion it is adequate with "Excellance" as a thesis for the degree of master in Science of Clinical Biochemistry

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## Summary

Inflammatory bowel disease (IBD) is a group of gastrointestinal disorders that cause prolonged and chronic inflammation of the gastrointestinal tract. The major IBD phenotypes are Crohn's disease (CD) and ulcerative colitis (UC). Ulcerative colitis is a disease of unknown etiology which characterized by inflammation of the mucosa and sometimes the submucosa of the colon.while Crohn's disease cause transmural inflammation that affect any portion of the GIT (commonly the terminal ileum or the perianal region) in a noncontinuous type. The obtained results compared to the healthy group indicate.

This study was conducted from November 2020 till March 2021.The entire samples collected from peoples attended Marjan Medical City in Babylon Province, Hilla city. The laboratory work was done in the Department of Chemistry and Biochemistry, College of the Medicine, University of Babylon.

The presented study aims to evaluate of the levels of Zinc, Selenium , Vitamin D and vitamin B12 and fecal calprotectin in patients with Inflammatory bowel diseases by measuring serum levels and comparing the results with the results of the control group in Babylon-Iraq , to find the correlation of Zinc, Selenium , Vitamin D and vitamin B12 and fecal calprotectin with lipid profile and other parameters in these patients and compared with apparently healthy as case – control study .The study included (50) patients as patients group diagnosed with IBD (G1) and (50) as apparently control group(G2) ,The age of all studied groups from (14) years old or above and BMI with (18.5 – 24.9) Kg/m2.

Serum was used in determination of fasting blood sugar (FBS), lipid profile, C- reactive protein (CRP), vitamin D , Vitamin B12 , zinc and Selenuim and whole blood was used in determine ESR level while stool sample was taken for the faecal calprotectin test.The results revealed decreased in Total Cholesterol (TC), Low density lipoprotein cholesterol (LDL-c) ,High Density Lipoprotein Cholesterol (HDL-c) were seen in patients groups when comparing to healthy control.

while FBG and Triglyceride (TG) elevation in patients group comparing to control group . a results showed significant decreased in vitamin D , Vitamin B12 , zinc and Selenuim levels in patients group when comparing to control group ,while ESR ,CRP and faecal calprotectin significant evelation in patients group comparing to control group.

A significant positive correlation was found between vitamin D , Vitamin B12 , zinc , Selenuim and HDL, and found significant negative correlation between vitamin D ,ESR ,TG and faecal calprotectin while a non-significant correlation was found in patients group between vitamin D, TC, and LDL-c.

A significant positive correlation was found between Zinc and Vitamin B12 , vitamin D, Selenuim and HDL, and found significant negative correlation between Zinc, ESR ,TG, FBS and faecal calprotectin while a non-significant correlation was found in patients group between Zinc, TC, and LDL-c.

A significant positive correlation was found between Zinc and Vitamin B12 , vitamin D, Selenuim and HDL, and found significant negative correlation between Zinc, ESR ,TG, FBS and faecal calprotectin

A significant positive correlation was found between Vitamin B12 ,Selenuim and HDL, and found significant negative correlation between Vitamin B12, ESR ,TG, and faecal calprotectin .

A significant positive correlation was found between Selenuim and HDL, and found significant negative correlation between Selenuim, ESR ,TG,FBS, and faecal calprotectin .

A significant positive correlation was found between faecal calprotectin FBS,TG and ESR, and found significant negative correlation between faecal calprotectin and HDL.

The conclusion from this study found that :-

1.The vitamin D concentration in patients with IBD should be examined routinely, since IBD constitutes a risk factor of vitamin D deficiency.

2.Zinc levels are often low in in patients with chronic diarrhea or malabsorptive disorders.

3. Selenium deficiency is a common finding in patients with IBD and correlates with disease severity.

4. Inflammatory bowel disease  patients may be at risk of vitamin B12 insufficiencies,

5.the fecal calprotectin is an ideal non-invasive biomarker to identify intestinal inflammation throughout the gastrointestinal tract.

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# List of abbreviations

| Abbreviation | Full name |
|---|---|
| 5-ASAs | 5-aminosalycilates |
| ACD | Anemia of chronic disease |
| Alb | Albumin |
| BMI | Body mass index |
| BMP-6 | Bon Macrophage protein-6 |
| CBC | Complete blood count |
| CD | Crohn's disease |
| CRP | C-reactive protein |
| CT | Computer tomography |
| DMT-1 | Divalent metal transporter |
| EPO | Eosinophil peroxidase |
| ER | Endoplasmic reticulum |
| ERAD | ER-associated protein degradation |
| ESR | Erythrocyte sedimentation rate |
| GIT | Gastrointestinal tract |
| Glob | Globulin |
| GPx | Glutathione peroxidase |
| GSH | Glutathione |
| HPLC | High performance liquid chromatography |
| IBD | Inflammatory bowel disease |
| IL-6 | Interleukin-6 |
| IMA | Ischemia modified albumin |
| iNOS | Inducible nitric oxide synthase |
| LPO | Lactoperoxidase |
| MDA | Malondialdehyde |

| | |
|---|---|
| **MPO** | **Myeloperoxidase** |
| **MRI** | **Magnetic resonance imaging** |
| **mROS** | **Mitochondrial reactive oxygen species** |
| **NADPH oxidase** | **Adenine dinucleotide phosphate oxidase** |
| **NO** | **Nitric oxide** |
| **NOD2** | **Nucleotide-binding oligomerization domain-containing protein2** |
| **NOS** | **Nitric oxide synthase** |
| **NSAIDs** | **Non-steroidal anti-inflammatory drugs** |
| **ODC** | **Ornithine decarboxylase** |
| **OPO** | **Ovoperoxidase** |
| **OS** | **Oxidative stress** |
| **OSI** | **Oxidative stress index** |
| **PAs** | **Polyamines** |
| **PET** | **Position emission tomography** |
| **PUT** | **Putrescine** |
| **RBCs** | **Red blood cells** |
| **RNS** | **Reactive nitrogen species** |
| **ROS** | **Reactive oxygen species** |
| **SOD** | **Superoxide dismutase** |
| **1,25(OH)2 D3** | **1,25 dihydroxyvitamin D** |
| **SPECT** | **Single photon emission computed tomography** |
| **FGF23** | **Fibroblast Growth Factor 23** |
| **TAC** | **Total antioxidant capacity** |
| **PTH** | **Parathyroid hormone** |
| **TNF** | **Tumor necrosis factor** |
| **CYPs** | **Cytochromes P450** |

| | |
|---|---|
| **FBS** | **Fasting blood suger** |
| **CYP27B1** | **Cytochrome P450 Family 27 Subfamily B Member 1** |
| **UC** | **Ulcerative colitis** |
| **UIBC** | **Unsaturated iron binding capacity** |
| **CYP2R1** | **Cytochrome P450 Family 2 Subfamily R Member 1** |
| **SNP** | **single-nucleotide polymorphism** |
| **RDA** | **Recommended Dietary Allowance** |
| **Hcy** | **Homocysteine** |
| **MMA** | **methylmalonic acid** |
| **IF** | **Intrinsic factor** |
| **Cb1** | **cobalamin** |
| **IBS** | **Irritable bowel syndrome** |
| **Zn** | **Zinc** |
| **Se** | **selenium** |
| **UV** | **Ultraviolet** |
| **UVB** | **Under ultraviolet B light** |

# Chapter one

## Introduction and Literature review

## 1.1 : The gastrointestinal tract

The gastrointestinal tract (GIT), are a group of organs that includes the mouth, esophagus, pancreas, stomach, liver, gallbladder, intestines and rectum (end of large intestine)(1). There are about 100 trillion of different microbial organisms [ eg. bacteria, viruses, and fungi], which constitute the microbiota (microbial flora) reside in human gut(2). It is estimated that the collective genome of intestinal microbes contains about 100 times more genes than the human genome(3). In healthy people, the gut microbiota consists of five dominant bacterial phyla, which include ***Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria,*** **and** ***Verrucomicrobia***, and less abundant phyla such as***Cyanobacteria, Fusobacteria,*** and others(4)**.** This community is generally referred to the hidden metabolic organ due to their immense influence on the wellbeing of human, including host metabolism, physiology, immune function, andnutrition(5).

The small intestine consists of three parts: the duodenum, jejunum, and ileum, while the **large intestine** (or colon) is the final segment of the GIT in the digestive system, it is about 5 feet long and about 3 inches in diameter. It involves each of: cecum, ascending (right) colon, transverse colon, descending (left) colon, and sigmoid colon (which is connected to the rectum), as shown in **Figure (1.1)(6,7)**.

The colon is recognized as a metabolically active organ that plays a significant role in keeping the overall health of the human body by saving unabsorbed nutrients (formation and storage of residue in the form of feces and its removal through defecation) and absorbing electrolytes and water(8). In addition, the colon contributes to energy and the nitrogen metabolism, either directly or indirectly by means of the microbial. Additionally, the colon is an immunologically active tubular cavity playing a central portion in host immune responses and defense against diseases pathogens(9).

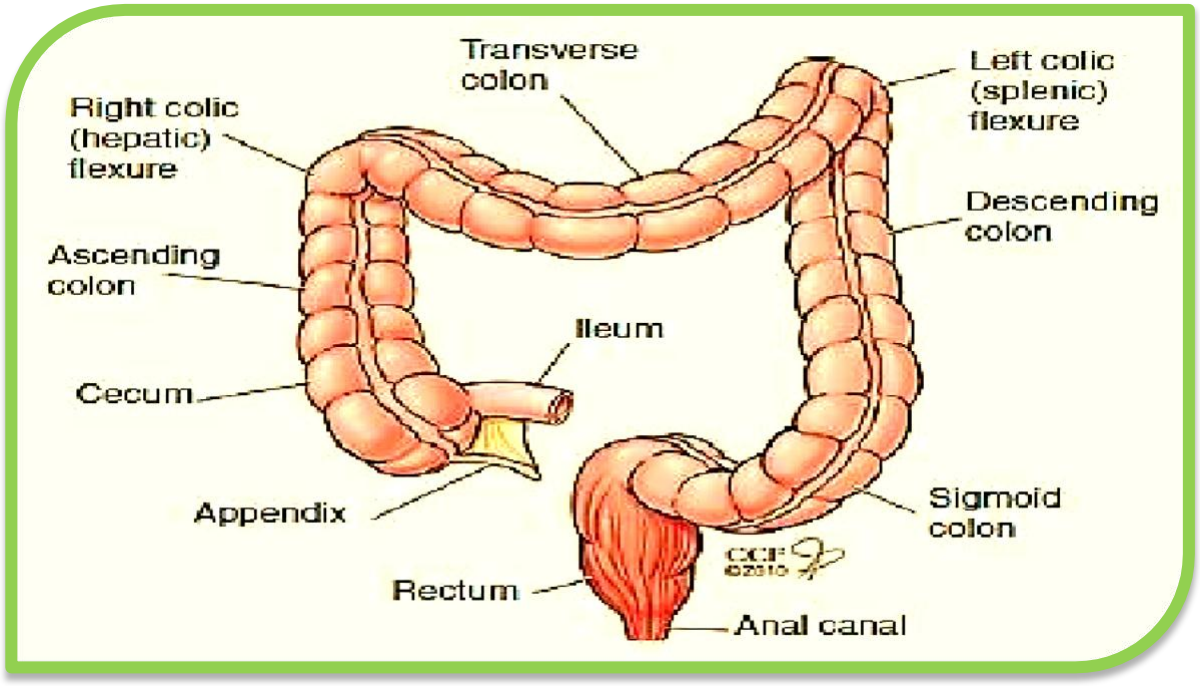



**Figure (1.1): Anterior view of the large intestine**(7)**.**

## 1.2 : Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of gastrointestinal disorders that cause prolonged and chronic inflammation of the GIT(10).

### 1.2.1 : Classification of inflammatory bowel disease

The major IBD phenotypes are Crohn's disease (CD) and ulcerative colitis (UC)(11). Although CD and UC share a many of the clinical features, there are important uniqueness in the incidence patterns, disease localization, histopathology and endoscopic features that suggests that there are variations in the underlying pathways that lead to each disease(12,13).

Ulcerative colitis **(UC)** is a disease of unknown etiology which characterized by inflammation of the mucosa and sometimes the submucosa of the colon. This disease can be broadly divided into distal and more extensive colitis. Distal disease refers to colitis limited to the rectum (proctitis) or involved the rectum and sigmoid colon (proctosigmoiditis). More extensive disease refers to "left sided colitis" (up to splenic flexure), "extensive colitis" (up to hepatic flexure) and "pancolitis" (involved the whole colon)(14). The most typical endoscopic features in UC patients include edematous mucosa, loss of vascular

markings, erythema, and mucosal friability. More severe cases may be associated together with erosions, ulcers, and spontaneously bleeding(15).

Crohn's disease **(CD)** cause transmural inflammation that affect any portion of the GIT (commonly the terminal ileum or the perianal region) in a noncontinuous type(16). The typical endoscopic features in CD include discontinuous distribution of longitudinal ulcers {defined as ≥4 - 5 cm ulcers},a cobblestone appearance, and/or small aphthous ulcers arranged in a longitudinal fashion(15). The differences CD and UC are illustrated in **Table (1.1)**(17).

**Table (1.1): Comparison between Crohn's disease and ulcerative colitis.**

| Variable | CD | UC |
|---|---|---|
| **Location** | **Affect whole GIT from mouth to anus** | **Colon and Rectum** |
| **Distribution** | **Patchy areas of inflammation (Skip lesions)** | **A continuous area of inflammation** |
| **Depth of inflammation** | **Submucosal or transmural** | **Inflammation limited to the mucosa** |
| **Strictures** | **Often Present** | **Absent** |
| **Granulomas** | **Often Present** | **Absent** |

## 1.2.2 : Epidemiology

The prevalence of IBD varies extremely among different ethnic groups, but the highest prevalence has been recorded in the Western world, while much lower average has been observed in developing countries and Asia(18)**.** The prevalence of UC is approximately 235 per 100,000 populations in North America, Western Europe, and United Kingdom; while in the developing countries, the prevalence is much lower (approximately 20 per 100,000 populations). In the situation of CD, it is less frequent than UC, and its estimated

prevalence is about 200 per 100,000 populations in North America, and again, it is much lower (10 per 100,000 populations) in developing countries(19).

It is also worth to mention that the incidence and prevalence of CD and UC in Western world are stabilizing, but the rates for two diseases are still increasing in Southern Europe, developing countries, and Asia. Additionally, for several ethnic groups it has been shown that after migration to a different geographical area, the prevalence of IBD in the ethnic group becomes equal to the local level of prevalence within one to two generations. This phenomenon, along with the increasing level of IBD in developing countries, implies that factors in theWestern lifestyle and environment have an effect on IBD risk(20). CD is slightly more common in females than males (Male: Female ratio = 1:1.2) and occurs at an earlier age (mean age 26 years) than UC (Male: Female ratio = 1.2:1; (mean age 34 years). Furthermore, the incidence between ages 60s and 80s is more commonly observed in CD than UC(21). In Iraq , the number of patients in last few year was raised , in 2018 the number of IBD patients was 14866 male and 16027 female , in 2019 the number of IBD patients was 18473 male and 20100 female, while in 2020 there are a 18912 male and 20396 female patient with IBD (Ministry of Health in Iraq).

### 1.2.3 : Causes and pathogenesis of inflammatory bowel disease

IBD results from a confluence of genetic, microbial, and environmental factors. Further factors include diet, use of antibiotics and non-steroidal anti- inflammatory drugs (NSAIDs), stress, and infections. All leading to a dysregulation in the mucosal immune system that mediates the clinical and endoscopic findings observed in IBD patients(22). **Figure (1.2)**(23).

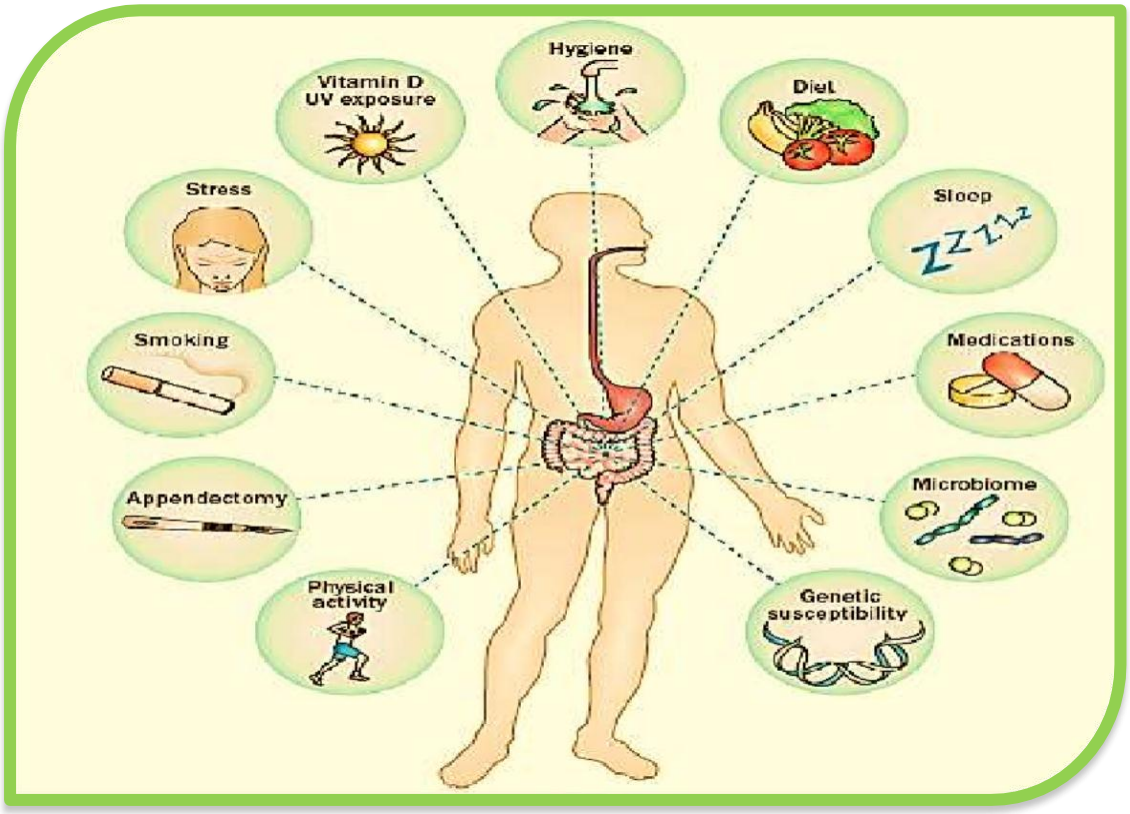



**Figure (1.2): The pathogenesis of inflammatory bowel disease**(23).

### 1.2.4 : Symptoms of inflammatory bowel disease.

The typical symptoms of **UC** present with bloody diarrhea, abdominal pain, urgency, and tenesmus. Rarely, patients may present with weight loss or other systemic symptoms, such as a low-grade fever. The disease typically starts gradually and progresses for several weeks(24). On the other hand, **CD** is clinically recognized by the following symptoms: persistent diarrhea, rectal bleeding, urgent need to move bowels, abdominal cramps and pain, sensations of incomplete evacuation (tenesmus), and constipation(25).

### 1.2.5 : Diagnosis of inflammatory bowel disease.

There is no single diagnostic test currently available for diagnosis ofIBD. The diagnosis of IBD based on a combination of clinical symptoms, laboratory

tests, imaging data, and endoscopy(26). The laboratory tests include complete blood count (CBC), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal calprotectin, and lactoferrin(27). The imaging data such as ultrasound, magnetic resonance imaging (MRI), computer tomography (CT), X-ray, position emission tomography (PET) and single photon emission computed tomography (SPECT) are also used as a methods for diagnosis of IBD(28). The endoscopic diagnosis (Colonoscopy) is the great essential diagnostic method to diagnose and differentiate between CD and UC(29). During endoscopy, multiple biopsies are often necessary to confirm the diagnosis(30), **Figure (1.6)**.

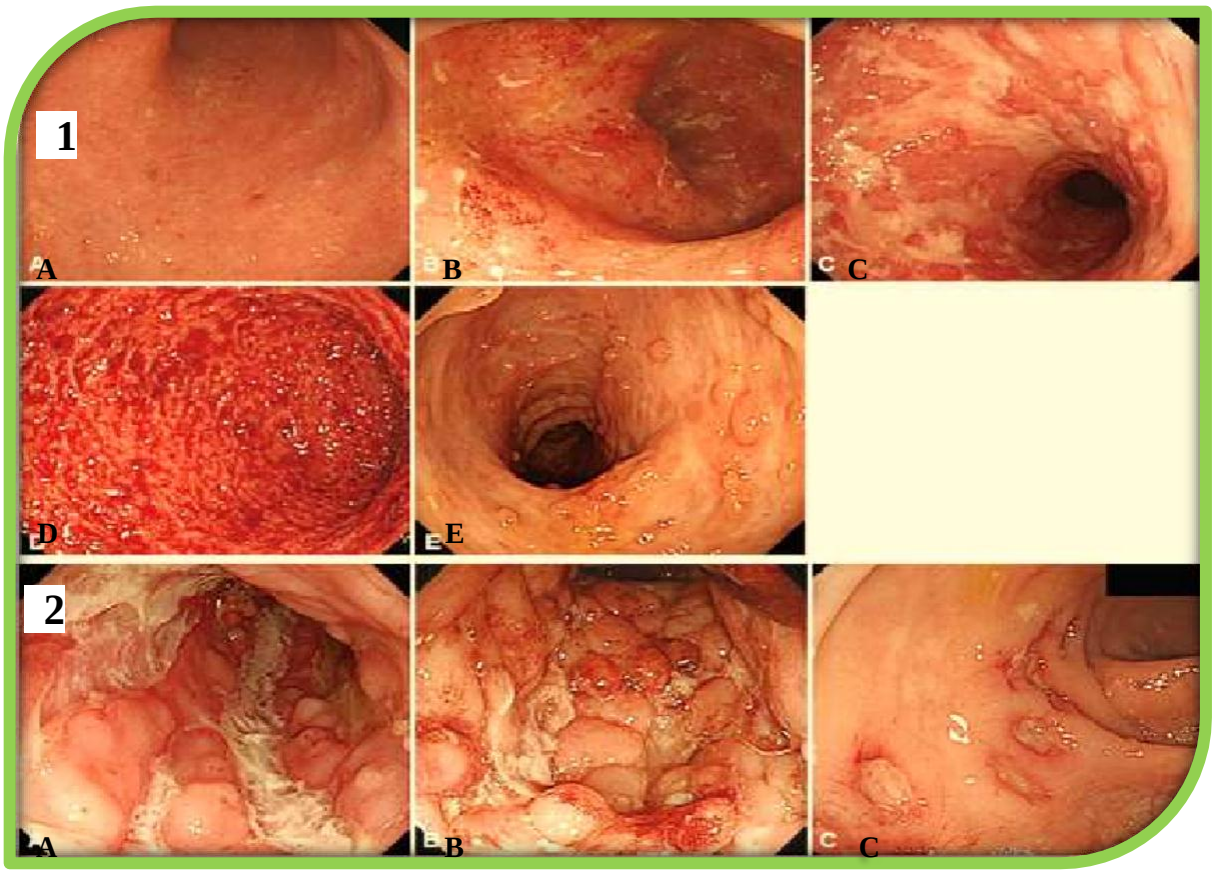



**Figure (1.6): Endoscopic features of IBD. {1} Typical endoscopic features of ulcerative colitis. (A) Mild: mucosal erythema, fine granularity, decreased vascular marking. (B) Moderate: marked erythema, loss of vascular marking, erosions. (C) Severe: ulcers. (D) Severe: spontaneous bleeding. (E) Luminal narrowing with pseudopolyps. {2} Typical endoscopic features of Crohn's disease. (A) Longitudinal ulcers, (B) cobblestone appearance, (C) aphthous ulcers showing longitudinal array**(15)**.**

## 1.2.6 : Treatment of inflammatory bowel disease

The appropriate treatment options are given depending on the severity of the disease. The goal of medical therapy is to induce and keeping clinical remission, avoid complications, and improve the quality of life for patients(31), 5-aminosalycilates (5-ASAs) is the initial medical treatment option in UC. In cases of mild to moderate disease 5-ASAs can be managed orally, rectally or in combination. The combination is more effective(32). The remission state may be induced via corticosteroids followed by transitioning of patients to steroid-sparing agents, generally a thiopurine and/or the antitumor necrosis factor (anti-TNF), anti-TNF targeted the TNF alpha protein that play major role in IBD(33,34). In moderate to severe IBD the biological anti-TNF therapies such as (Infliximab, adalimumab and certolizumab ) can be used to keep and induce remission statein IBD patients who have a contraindication to corticosteroids, in those whom oral corticosteroid therapy is failing, in those whom thiopurine therapy has failed, or in lieu of thiopurines. Recent data suggest that the combination of azathioprine (Imuran) and infliximab is more effective than either agent alone (reduce the likelihood of developing an allergic reaction)(35,36). The treatments for CD can include:

antibiotics, aminosalicylates, corticosteroids, immunomodulators, anti-TNF-antibodies and other novel biological drugs. Exclusive enteral nutrition is recommended in children with acute disease as it provides the greatest gains for weight and growth with the least quantity of side effects(37).

## 1.2.7 : Burden of inflammatory bowel disease

The individuals with IBD also experience additional tremendous personal cost, that is, the burden of having a disease. The unpredictable and fluctuating nature of IBD can make it highly difficult to plan for the future, negatively affecting choices with respect to career, travel and other

personal choices(38). Life is severely affected by the severity of the disease(39). Moreover, women patientswith IBD more likely to experience a lower live quality than women withoutIBD(40), the life quality reduced even in patients who do not have physical symptoms (due to fluctuating disease or drug-induced remission)(41). Patients withIBD suffer from poor sleep quality, regardless of whether their illness is active or remmision(42). The life quality of children with IBD is reduced, however, theteenagers patients with IBD are most affected(43).

### 1.2.8 : Complication of inflammatory bowel disease

The intestinal complications that arise from IBD include hemorrhage, strictures, colon perforation, anal fistulas, toxic megacolon, and colon cancer, while the extra intestinal complications include osteoporosis, deep vein thrombosis, anemia, gallstones, arthritis and iritis(44). The bowel obstruction and stricture, fistula, perforation, and abscess are the most potential complications of CD, the complications that may arise from UC include toxic megacolon and perforated bowel(45,46).

The high levels of C-reactive protein (CRP), homocysteine, and cytokines in IBD patients may lead to endothelial dysfunction, an early signal of atherosclerosis and, therefore, an increased risk of cardiovascular disease(47). But the most dangerous thing is that patients with IBD have a higher risk of increased colorectal carcinoma compared to the general population, (the average risk for life is 3-5%). The cancers arising in IBD occur at an early age and frequently have a fiat appearance, with extensive submucosal spread. Because of this, these tumors are often hard to visualize endoscopically and are frequently diagnosed at an advanced stage(48).

## 1.3 : vitamin D (Vit.D)

Vitamin D is one of fat-soluble vitamins, considered as steroid hormone. It is regulate and responsible for improving the metabolism and absorption of calcium, iron, magnesium, zinc, and phosphate in small intestine. Vitamin D is an organic chemical compound or a group of related compounds, the most important compounds of this group is vitamin D3 (Cholecalciferole) and vitamin D2 (Ergocalciferol), both of them can be obtained from food and dietary supplements(49). Vitamin D creates (specifically Cholecalciferole) in the skin, it is the main natural source of this vitamin, which creates from cholesterol. This process depends on exposure to sunlight (UV specifically), so vitamin D called as sun-ray vitamin. Vitamin D which comes from diet or through synthesis in the skin by effect of sun's ray is not biologically active. The activating process of this vitamin require enzymatic conversion processes (hydroxylation) which is made by two steps, first in the liver and the second in the kidneys(50) . Vitamin D is considered as a hormone because it is synthesized in a place and organizes effects on organs, glands, and places in various parts of the body(51)**.** It is unique because it can be taken as Cholecalciferole vitamin D3 or Ergocalciferol vitamin D2. In addition, like other vitamins, in the modern world is adding vitamin D to the basic foodstuffs, such as milk, to avoid diseases caused by deficiency(52).

Vitamin D is a lipophilic hormone synthesised in the skin under the influence of ultraviolet (UV) sunlight. Although most foods contain little vitamin D, it can be obtained from the diet to a lesser extent. The most important sources of this vitamin are fatty fish and egg yolk(53,54).

UV type B light exposure causes 7-dehydrocholesterol transformation into cholecalciferol or vitamin D3. However, this UV-mediated conversion varies

with time of the year, latitude and altitude(55). Cholecalciferol is hydroxylated in the liver into 25-hydroxyvitamin D3 (25(OH)D3) and subsequently in the kidney into 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), or calcitriol, the active metabolite(56).

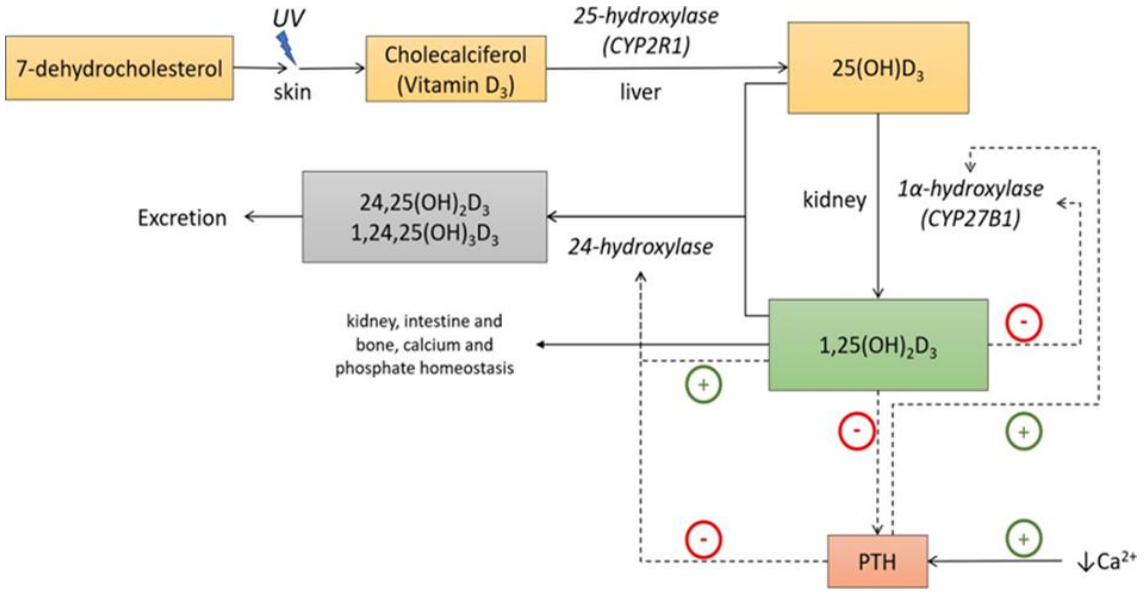
**(Figure 1.7)**These two steps are catalysed by the enzymes 25-hydroxylase (CYP2R1) and 1α-hydroxylase hydroxylase (CYP27B1) that belong to the family of cytochrome P450 mixed function oxidases(CYP27B1) that belong to the family of cytochrome P450 mixed function oxidases (CYPs). CYP2R1

(CYPs). CYP2R1 has only been identified in the microsomal fraction of liver. However, although the has only been identified in the microsomal

fraction of liver. However, although the kidney is the kidney is the main

source of 1,25(OH)2 D3, several other tissues also express CYP27B1. The regulation main source of 1,25(OH)2 D3, several other tissues also express CYP27B1. The regulation of the of the renal CYP27B1 differs from that of the extrarenal version(57).

In this way, renal Cytochrome P450 Family 27 Subfamily B Member 1 (CYP27B1) is activated by the parathyroid hormone(PTH) and inhibited by fibroblast growth factor 23 (FGF23) as well as by 1,25(OH)2D3 itself. Elevated calcium suppresses CYP27B1 by means of the suppression of PTH, and elevated phosphate suppresses CYP27B1 by means of FGF23 stimulation, although these ions can have direct e_ects on renal CYP27B1 on their own(58). Thus, it is known that CYP27B1 is expressed in numerous tissues where vitamin D3 can act as an intracrine or paracrine signal. In this way, several immune system cell types express both CYP27B1 and the vitamin D receptor, with CYP27B1 production controlled by a number of immune-specific inputs(59).




**Figure( 1.7) Vitamin D metabolism and functions. Under ultraviolet B light (UVB) exposure,7-dehydroxycholesterol is converted to vitamin D3 in the skin. First, hydroxylation occurs in the liver where it is converted to 25-hydroxyvitamin D3. 25-hydroxyvitamin D3 is further converted in the kidney to its active metabolite, 1α,25-dihydroxyvitamin D3. In the kidney, 1α-hydroxylase is stimulated by the parathyroid hormone (PTH) and feedback inhibited by 1α,25-dihydroxyvitamin D3. 1α,25-dihydroxyvitamin D3 targets the intestine, kidney and bone to regulate calcium and phosphate homeostasis(60).**

The half-life of 1,25(OH)2D3 is very short, ranging from 20 to 40 hours(61). therefore, its serum determination is not clinically relevant. However, the 25(OH)D3 half-life ranges from 12 to 19 days(62), so it appears to be the most reliable source of systemic vitamin D. 1,25(OH)2D3 has its ect on the classic target organs such as bone, intestine and kidney and stimulates calcium transport from these organs to the peripheral blood stream(63).

## 1.3.1:Influence of Vitamin D on inflammatory bowel disease

Vitamin D deficiency It is one of a limited set of variables credibly proposed to mediate the observed association between environmental exposures and the inflammatory bowel diseases (IBD), Crohn's disease (CD)

and ulcerative colitis (UC)(64).

Serum vitamin D levels have been associated with inflammatory diseases, such as inflammatory bowel disease (IBD), rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, atherosclerosis, and asthma. Regarding inflammation, it has become clear that vitamin D inhibits production of proinflammatory cytokines like IL-6 or TNF in monocytes.

via the inhibition of p38 MAP kinase(65), and it can promote anti-inflammatory T-cell pathways to stimulate the antimicrobial ects of macrophages(66). In terms of gut homeostasis, vitamin D plays a role in protecting the intestinal epithelial barrier, immunity and microbiota, all of which are directly involved in IBD(67). In-vitro 1,25(OH)2D3 treatment can protect the intestinal epithelial barrier in ulcerative colitis (UC) patients by regulating various pathways involved in tight junction proteins(68) Similar studies have proposed that lack of VDR leads to hyperfunction of Claudin-2. Claudin-2 is an epithelial protein that forms a water channel to permit the paracellular passage of water through its pores in the epithelium, and its expression is restricted to colonic proliferative cells. An overexpression of Claudin-2 permits intestinal leakage, and is associated with active disease in IBD and reduction of intestinal VDR(69). Other genetic changes in the VDR gene can contribute to a greater risk for CD. Patients with Crohn's disease (CD) who are homozygous for a certain single nucleotide polymorphism (SNP) in the VDR gene have lower levels of VDR protein expressed in serum monocytes. These individuals have an overactivation of lymphocytic adhesion molecules and a higher risk of developing a penetrating phenotype of CD(70). Vitamin D has ects on both innate and adaptive immune pathways. 1,25(OH)2D3 modulates the expression of cationic antimicrobial peptides like

defensins and cathelicidins. VDRsignals through distal enhancers in the NOD2 gene, leading to the expression of defensin beta 2 in macrophages. However, this response was absent in macrophages from patients with CD homozygous for nonfunctional NOD2 variants(71). It should be noted that NOD2 is also known as IBD1. .

This gene is located on chromosome 16, in the susceptibility locus for CD, which is responsible for familial aggregation of this disease(72). It has also been reported that DNA copy number of the beta-defensin gene cluster is highly polymorphic within the healthy population, whereas CD patients appear to present a lower copy number. This results in a diminished beta-defensin expression, predisposing patients to major susceptibility to colonic CD(73). An induction of human cathelicidin antimicrobial peptide gene was observed under the influence of 1,25(OH)2D3 in colon cancer cells and other tissues(74,75) Vitamin D is also involved in immune regulation by modulating the role of dendritic cells and macrophages through two methods: firstly, 1,25(OH)2D3 inhibits IL-12, a pivotal interleukin in Th1 development(76); secondly, it promotes IL-10 to stop maturation from dendritic cells to serum monocytes and leads to apoptosis in mature dendritic cells(77). Regarding adaptive immunity, 1,25(OH) 2D3ects T-cell polarisation by inhibiting T helper 1(Th) (IFN-gamma production) and promoting Th2 cell development (IL-4, IL-5, and IL-10 production) from T-cells(78). It has been demonstrated in controlled studies that higher vitamin D levels in UC patients were associated with protective anti-inflammatory serum cytokine profiles(79). Gut microbiota have become the main research topic in intestinal homeostasis. One recent study demonstrated that individuals with significant intake of vitamin D had dierent microbiota strains from those without a significant vitamin D intake. These patients had plenty of Bacteroidetes, Prevotella and Megasphera strains,

traditionally associated with a non inflammatory status(80) .In another study, administration of vitamin D to CD patients resulted in significant change in the gut microbiota with a high abundance of some strains such as Alistipes and Roseburia that were not observed in healthy controls(81). Roseburia genii are part of commensal bacteria that produce short-chain fatty acids, especially butyrate,ecting colonic motility, immunity maintenance and anti-inflammatory properties(82) .Therefore, administration of vitamin D might havea positive ect on CD by modulating the intestinal bacterial composition and also by increasing the abundance of potential beneficial bacterial strains [103]. Regarding the epidemiology of vitamin D deficiency in IBD, there is a systematic review and meta-analysis that defined this ase activity and inflammatory markers in IBD. Due to chronic malabsorption, IBD patients and particularly those with CD are at greater risk of certain nutritional deficiencies.

Micronutrients, including hydro and lipophilic vitamins, iron, calcium and zinc, are the most common problems(83).Malabsorption, maldigestion and upper protein requirements are directly related to clinical disease activity. It is unknown why quiescent IBD has a greater prevalence of vitamin D deficiency than other risk groups(84). Even in patients with IBD in clinical remission, vitamin D malabsorption has been confirmed. This fact led to controversy among researchers about oral intake as standard supplementation. According to several prospective studies, IBD patients with vitamin D deficiency are at greater risk of relapse, hospitalisations and flare-ups(85). described a correlation between the use of a TNF-alpha inhibitor and higher vitamin D levels in CD patients. This correlation could be explained by a better disease control of these patients(86). However, they detected a correlation between a higher disease activity and lower vitamin D levels in UC but not in CD. Moreover, the same authors found that in CD located in the small intestine, CD patients after small intestine resections

showed significant lower vitamin D levels. Other resections did not lead to changes in the vitamin D levels; therefore, the small intestine plays an important role in vitamin D absorption, particularly in IBD(87).

## 1.4: Zinc(zn)

Zinc, the 23rd most abundant element in the earth's crust, having atomic number 30 and atomic weight 65.39, is vital in the living world. Pure zinc is a bluish-white, shiny metal, and is amphoteric in nature(88) . Zinc, being colorless and diamagnetic, is invisible to most spectroscopic methods. Plasma zinc has a rapid turnover rate and it represents only about 0.l% of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye (4.2mmol/g or 274mg/g) and in prostatic fluids (4.6–7.7 mmol/l or 300–500mg/l)(89) .

It is indispensable for the development of living organisms. Zinc can be found in all body tissues and fluids. In mammals, 85% of the whole body Zn is in muscle and bone, and 11% in skin and the liver with the remaining 4% in all the other tissues. It is the second most abundant trace 7 element in the human body following Fe. The Recommended Dietary Allowance (RDA( of Zn is 11 mg for males and 8 mg for females. Doses that are larger than 25 mg may cause anemia and Cu deficiency(90).

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity. Zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression(88).

Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humsoral immunity(88).

Zinc is a food component classed as a micronutrient. is element is necessary for the proper functioning of certain enzymes and hormones. It is an essential component for the synthesis of proteins and erythrocytes. It is also responsible for the proper functioning of the immune system. Its absorption takes place in the small intestine. Absorption of the component from food products only takes place up to 40%. In food, many nutrients have an effect on the uptake of zinc. absorption of zinc is increased by the presence of an appropriate amount of complete protein in the diet. In contrast, polyphosphates, phytates, or excessive consumption of such elements as iron or calcium may reduce the absorption of this micronutrient(91,92). An insufficient supply of zinc in the diet has been shown to cause a number of abnormalities in the body. It can lead to stunted growth and a reduced rate of development. Deficiency also correlates with depressed mood and depression, especially in people with IBD Deficits may also cause skin lesions or worsened wound healing(93).

Effect of zinc on the immune system is significant. A reduced concentration of the element contributes to reduced monocyte adhesion activity. In addition, its deficiency may increase the secretion of proinflammatory cytokines, mainly IL-6. is state in the body also contributes to atrophy of the thymus as well as reducing the number of mature T lymphocytes(91). An appropriate level of zinc is important for the activation of T cells and their proper functioning(92). In animal studies, zinc deficiency has been shown to reduce the production of IL-2, a factor that influences the growth of mainly cytotoxic T lymphocytes(94). A deficiency may cause apoptosis of mature B lymphocytes

and reduce the number of immature B lymphocytes. -is leads to a reduction in the production of immunoglobulins, which has a significant effect on the lowering of immune system functioning(95). Its concentration also affects NK cells that belong to the lymphocyte family. IL-12 is a factor that increases the effect of NK cells. It has been observed that decreased zinc concentration reduces NK activity. Low values of the element in the body may lead to the disruption of the phagocytic capacity of macrophages(96).

### 1.4.1: Influence of Zinc on inflammatory bowel disease

In patients with IBD, zinc deficiency may be caused by chronic diarrhoea and intestinal inflammation, as a result of which the element cannot be absorbed in sufficient quantity. Reduced amounts have been shown in the plasma of patients. . showed that higher intake of zinc was associated with a reduction in the risk of CD but had no effect on the occurrence of UC. A proper amount of zinc may also contribute to the prolongation of a remission period of the disease(97).

## 1.5: Vitamin B12 (cobalamin)

Vitamin B12 (B12; also known as cobalamin) is one of eight B vitamins and its role in cellular metabolism is closely intertwined with that of folate,. Since the discovery and characterization of B12 more than 60 years ago and the recognition of its central role in preventing the serious disease known as pernicious anaemia much has become known about B12 deficiency(98,99).

It consists of a corrin ring made up of four pyrroles with cobalt at the center of the ring(100). There are several vitamin B12-dependent enzymes in bacteria and algae, but no species of plants have the enzymes necessary for

vitamin B12 synthesis. This fact has significant implications for the dietary sources and availability of vitamin B12. In mammalian cells there are only two vitamin B12-dependent enzymes(101). One of these enzymes, methionine synthase, uses the chemical form of the vitamin which has a methyl group attached to the cobalt and is called methylcobalamin . The other enzyme, methylmalonyl CoA mutase, uses vitamin B12 with a 5'-adeoxyadenosyl moiety attached to the cobalt and is called 5'-deoxyaldenosylcobalamin, or coenzyme B12. In nature there are two other forms of vitamin B12: hydroxycobalamin and aquacobalamin, where hydroxyl and water groups, respectively, are attached to the cobalt. The synthetic form of vitamin B12 found in supplements and fortified foods is cyanocobalamin, which has cyanide attached to the cobalt. These three forms of B12 are enzymatically activated to the methyl- or deoxyadenosylcobalamins in all mammalian cells. Most microorganisms, including bacteria and algae, synthesise vitamin B12, and they constitute the only source of the vitamin(102).

Milk from cows and humans contains binders with very high affinity for vitamin B12, . Omnivores and carnivores, including humans, derive dietary vitamin B12 from animal tissues or products (i.e., milk, butter, cheese, eggs, meat, poultry, etc.). It appears that no significant amount of the required vitamin B12 by humans is derived from microflora, although vegetable fermentation preparations have also been reported as being possible sources of vitamin B12(103).

### 1.5.1: Influence of vitamin B12 on inflammatory bowel disease

inflammatory bowel diseases (IBD), are chronic inflammatory gastrointestinal disorders commonly thought to predispose to vitamin B12

(cobalamin, Cbl) deficiency. The most important clinical manifestations of Cbl deficiency include nervous system dysfunction and megaloblastic anemia(104,105). Dietary Cbl must bind gastricderived intrinsic factor (IF) for its absorption in the distal ileum(106). CD can affect any segment of the gastrointestinal tract, whereas UC is confined to colon. One third to half of the patients with CD have isolated ileal disease and 30% to 55% have ileocolonic disease(107,108).

Because ileal disease or resection may mediate Cbl malabsorption, patients with CD are assumed to be at greater risk for deficiency. Aside from ileal disease or resection, other postulated mechanisms for Cbl deficiency in CD include fistulas, small bowel bacterial overgrowth, reduced alimentary intake, increased physiologic requirements, protein losing enteropathy, and hepatic dysfunction[108,109,110].

Isolated low serum Cbl levels differ from biochemical deficiency. Biochemical Cbl deficiency is defined as low serum Cbl levels with confirmatory biomarker elevations (methylmalonic acid [MMA] and homocysteine [Hcy])(111). These biomarker elevations confirm intracellular Cbl deficiency because they are substrates of reactions catalyzed by Cbl. Clinical deficiency is defined as a biochemical deficiency with megaloblastic anemia or neurological symptoms(112,113). The majority of Cbl deficiency is found in asymptomatic individuals without megaloblastic anemia[135] .This has been termed subclinical Cbl deficiency but is referred to as isolated biochemical deficiency here. Clinical deficiency arises from severe malabsorptive diseases such as autoimmune gastritis, which accounts for 94% of clinical deficiency(114,115).

## 1.6: Selenium (Se)

Selenium (Se) is an essential trace element. It is vital for human normal development, growth, male fertility, and thyroid hormone metabolism(116). Exerts its biological effect through over 35 selenoproteins identified in humans, some of which have important enzymatic functions. These selenoproteins include several enzymes with antioxidant capacity such as glutathione peroxidase (GSH-Px), phospholipid hydroperoxides glutathione peroxidase (PHGPx) and thioredoxin reductase (Tr). Selenium deficiency is associated with a wide range of health outcomes such as an impairment of thyroid hormone metabolism, cancer, degenerative affections, cardiovascular diseases, viral infections, inflammatory conditions, immune function, chronic renal failure, and alcoholism(117). The body contains complex antioxidant systems that require adequate intake of selenium for normal physiological functions and selenium can be incorporated into the body by ingesting foods such as carrots, cabbage, garlic, mushrooms, cheese, meats, and grains and selenium-containing supplements. Using GSH as the reducing equivalent, this enzyme plays a significant role in detoxification of peroxides induced by oxygen radicals(118,119).

### 1.6.1:Influence of Selenium on inflammatory bowel disease

Selenium deficiency has been described in patients with IBD. Most studies showed low mean selenium level(120). Experiments with selenium deficient human models showed exacerbation of the colitis with reduced dietary intake of selenium, and reduction in the severity of the inflammation with addition of selenium(121,122). Absorption of selenium is poorly , but is believed to occur most avidly in the ileum, followed by the jejunum and

large intestine. selenium levels were found to be significantly lower in both UC and CD patients, compared with controls(123,124). A deficiency of this element can lead to muscle damage, dystrophy of articular cartilage, or reduction of the body's immunity through, among others, B-lymphocyte dysfunction or decreased NK cell activity(125). However, an excess of selenium supply may lead to selenosis, which is manifested by depression, vomiting, or inflammatory skin conditions. IBD is characterized by ongoing chronic inflammation, which generates, among others, oxidative stress. Selenium deficiency has been demonstrated during both exacerbation and remission in patients with UC and CD(126). Researchers describe selenium deficiencies in patients with CD, which may lead to the reduction of the body's immune response to the development of inflammation. Han et al.2013 indicate female sex, corticosteroid use, and inflammation as the main factors that may predispose toward deficiencies of this element(127).

## 1.7 : Fecal Calprotectin

Calprotectin is a 36-kDa calcium and zinc binding protein found in human neutrophils, monocytes, and macrophages(128,129). It is a heterodimer of two calcium binding proteins— S100A8 and S100A9, and the name was derived from both its calcium binding properties (cal) and antimicrobial activity (protect) *in vitro*(130,131). Calprotectin was first isolated from human granulocytes in 1980 by Fagerhol et al2016. Calprotectin constitutes between 30–60% of neutrophil cytosolic proteins(132). It is released from activated neutrophils during periods of active inflammation, as well as upon neutrophil damage or death(133,134). Although calprotectin may be isolated and measured in multiple body fluids including plasma, urine, cerebrospinal fluid, synovial fluid, and pleural

fluid, it is primarily clinically useful to gastroenterologists through measurement of fecal concentrations as a direct marker of mucosal inflammation(135) .

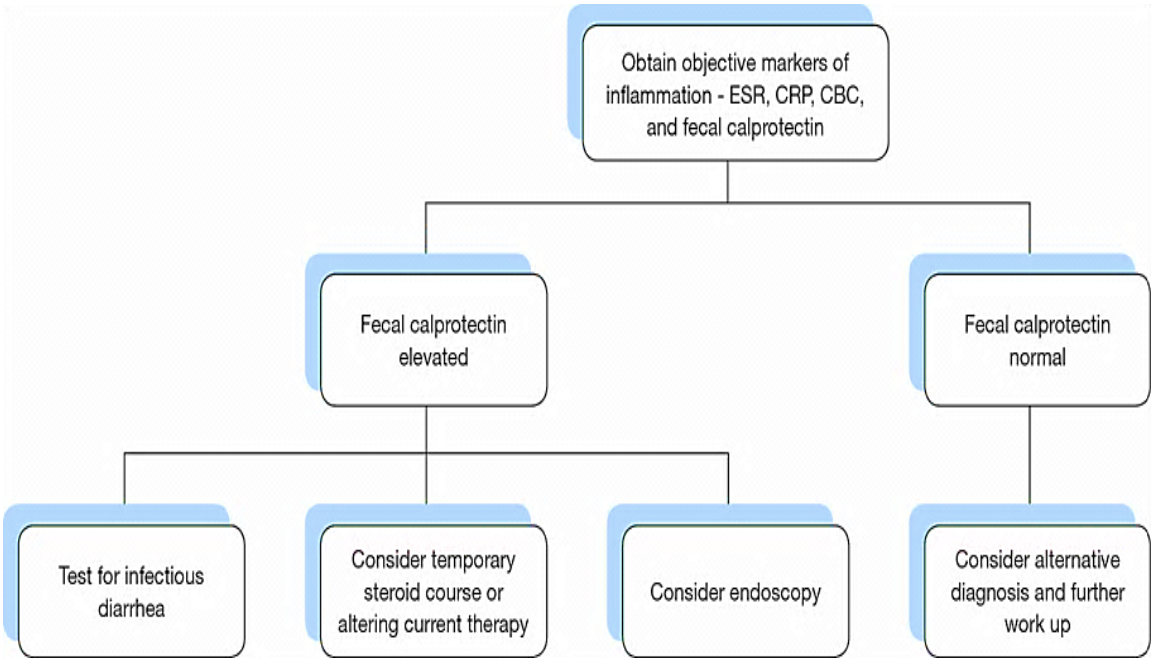
Fecal calprotectin elevation can be seen in both multiple primary gastrointestinal as well as extraintestinal disease processes(136). Fecal calprotectin can be measured via enzyme-linked immunosorbent assay (ELISA) testing in stool samples to detect intestinal inflammation of any etiology(137). It has a reported stability at room temperature in stool samples for up to one week, although at seven days there has been a noted significant variance in intra-sample concentrations(138). Analysis of a single stool sample is adequate for measurement of fecal calprotectin as good correlation has been seen between one time sampling values and those obtained from 24 hours collections(139) .

The presence of an elevated fecal calprotectin level, although sensitive for mucosal inflammation, is nonspecific and there are a number of infectious, inflammatory, and neoplastic processes that may lead to an elevation in the fecal calprotectin concentration . Utilizing newer assays, current recommendations for an upper limit of normal have increased to 50 μg/g, with even better diagnostic precision for active inflammatory bowel disease (IBD) seen at levels of greater than 100 μg/g(140,141).

Most often in today's clinical practice, fecal calprotectin is used in the initial investigation and subsequent monitoring during therapy of patients with IBD. One benefit for patients of using fecal calprotectin to assess disease activity is that it is a noninvasive marker, not requiring phlebotomy, exposure to radiation (such as with cross sectional imaging), or the risks inherent with endoscopy (including exposure to sedation and the risks of bleeding, perforation, etc.), to provide objective data regarding the presence

or activity of luminal inflammation. A large 2010 meta-analysis found measurement of fecal calprotectin had a sensitivity of 93% and specificity of 96% for detecting inflammation in adult patients, which could be used to help stratify which patients would benefit from more urgent endoscopic evaluation of their gastrointestinal symptoms(142). A strategy of initial screening with fecal calprotectin and referral for colonoscopy versus colonoscopy avoidance using predefined cut-off levels has been shown to be effective both in reducing health care costs and number of invasive procedures(143). Fecal calprotectin plays an mportant role both in helping gastroenterologists differentiate IBD from functional or visceral hypersensitivity disorders such as irritable bowel syndrome (IBS), and subsequently in assessing the degree of inflammation in patients with IBD to help tailor or optimize medical management(144).

Fecal calprotectin is not only effective in differentiating IBD from IBS, but also in differentiating active IBD from inactive IBD(145) This kind of objective symptom evaluation is often useful because even patients with a known diagnosis of IBD may have gastrointestinal complaints due to other coexistent processes that may not represent inadequate control or a true flare of their disease (Figure 1.8). An elevated fecal calprotectin value may be evenmore accurate than an elevated C-reactive protein (CRP) level for predicting active, endoscopically visual mucosal inflammation(146). Fecal calprotectin is also an inherently gut-specific test compared to the CRP, which can be elevated due to any etiology of systemic inflammation. In patients with known IBD, fecal calprotectin levels correlate with mucosal healing(147,148). There may be a role going forward for monitoring fecal calprotectin in asymptomatic patients, as there is evidence that persistent elevations portend a future relapse, whereas normal levels suggest sustained remission(149).




**Figure( 1.8) Possible algorithm for evaluation of symptom exacerbation in IBD**

Abnormal levels of fecal calprotectin can also be found in severl other gastrointestinal conditions, although its specificity for any particular disease process will be limited in this setting. In patients with microscopic colitis, for example, patients may have a mildly elevated fecal calprotectin level compared to control patients, but the absolute level in one study was still relatively low, with amedian of 48 g/g(150). Up to 38% of patients with active histologic microscopic colitis may have normal levels of fecal calprotectin, as well, further limiting its sensitivity(151). Fecal calprotectin can also be elevated in patients taking Non-steroidal anti-inflammatory drugs( NSAIDs), likely due to NSAID induced enteropathy(152). Fecal calprotectin elevation is not commonly seen with upper gastrointestinal tract lesions such as Barrett's esophagus or gastric ulceration, and although some patients may have elevated levels compared to controls, levels in this population are likely to be lower than the common cutoff value of 100 g/**g**(153,154). Levels of fecal calprotectin also

appear to be elevated in patients with cirrhosis compared to matched control patients, demonstrating intestinal inflammation that may play a role in suspected gut lumen bacterial translocation leading to infectious complications in these patients(155). Fecal calprotectin elevation can also be seen in patients with acute diverticulitis, and continued elevations after clinical resolution can be predictive of recurrence(156,157).

# The Aims of the study

Determination of Zinc, Selenium , VitaminsD3 ,B12 and fecal calprotectin in patients with Inflammatory bowel diseases (IBD) by measuring serum levels and comparing the results with the results of the control group.

# Chapter Two
## Materials and methods

# 2.Materials and Methods:

## 2.1 Chemicals and Suppliers

### 2.1.1 Chemicals

Kits that are used in this study with their sources are listed in the Table 2-1.

**Table 2-1: Kits and their Sources:**

| No. | Chemicals | Company andcountry |
|---|---|---|
| 1 | C-reactive protein kit | NycoCard, Alere, Norway |
| 2 | Glucose kit | Linear /France |
| 3 | High density lipoprotein cholesterolkit | BIOLABO / France |
| 4 | Human Calprotectin Stool ELISA Kit | Creative Diagnostics/USA |
| 5 | selenuim Kit | Linear /France |
| 6 | Total cholesterol kit | BIOLABO / France |
| 7 | Triglyceride kit | BIOLABO / France |
| 8 | Vitamin D kit | Spectrum Diagnostics Egypt |
| 9 | Vitamin B12 Kit | Creative Diagnostics/USA |
| 10 | Zinc Kit | Creative Diagnostics/USA |

### 2.1.2 Instruments and Tools

All instruments and tools that have been used in this study arelisted in table 2-2:

**Table 2-2: Instruments and their Suppliers.**

| No. | Instruments | Company andcountry |
|---|---|---|
| 1 | Centrifuge tube | Sigma (Germany) |
| 2 | ELISA washer, reader andprinter | BioTek /USA |
| 3 | Freezer | Liebhe (Austria) |
| 4 | Micropipettes 100-1000 µL | XINKANG / China |
| 5 | Micropipettes 5-50 µL | XINKANG / China |
| 6 | Rotter | Netherland |
| 7 | Spectrophotometer | England |
| 8 | Vortex mixer | Korea |
| 9 | Water bath | Grand (Germany) |

## 2.2 Patients Selection & Blood Sampling

This study included 100 Iraqi subjects with aged ranged age (14) years old or above & BMI with (18.5 – 24.9) Kg/m2. The (50) patients with Inflammatory bowel diseases (IBD) which considered group one (G1) attended Marjan Medical City in Babylon Province, Hilla city, Digestive Disease Center, from November 2020 till March 2021. Apparently healthy control group consist of (50) subjects which considered as group two (G2). The disease was clinically diagnosed by the consultant medical staff at the hospital, which was based on a clinical evaluation using colonoscopy and a histopathological examination of a biopsy, and under the supervision of this staff, information sheet was filled. Moreover, disease activity has been confirmed by symptoms, high levels of erythrocyte sedimentation rate (ESR), positive C-reactive protein (CRP) and faecal calprotectin in addition to colonoscopy.

1. Ten milliliters of blood were collected after an overnight fasting (12 hr.) from all subjects by venipuncture. A liquate of 2 ml of whole blood (EDTA tube) was used in determination of ESR, The other part was incubate at 37 °C for 15 min to clot then centrifuge at 4000 xg for 10 min . The serum which obtained was freeze until analysis.
2. A stool sample was taken for the Faecal calprotectin test.
3. **Ethical issues**

   This study was performed with permissions from Babylon University, Faculty of Medicine, Digestive Center, Marjan Hospital. The consent of all patients was taken and they were informed that the samples were for research purposes.

## 2.3 Study Design

A case-control study.

## 2.4 Exclusion criteria

1- Carcinoma colon (CA).

2- Other systemic autoimmune disease like diabetes , thyroid diseases and pernicious anemia .

3- Multiple sclerosis

4- Hyperlipidemia and Hypertension

5- Age less than 14 .

6- Pregnant female patients .

## 2.5 Inclusion criteria:

Age (14) years old or above and Normal BMI.

## 2.6 Estimation of Body Mass Index (BMI)

Body mass index is a simple index of weight for height that is ordinarily used distinguish underweight, normal, overweight and obesity as show in Table 2-3. It is calculated by dividing the weight in kilograms on the height square in metes ($kg/m^2$) according to the following equation (158) BMI =weight (kg)/(Height) $^2$ (m) $^2$.

**Table 2-3: Body mass index classification**

| | |
|---|---|
| Underweight | <18.50 |
| Severe thinness | <16.00 |
| Moderate thinness | 16.00 - 16.99 |
| Mild thinness | 17.00 - 18.49 |
| Normal range | 18.50 - 24.99 |
| Overweight | ≥25.00 |
| Pre-obese | 25.00 - 29.99 |
| Obese | ≥30.00 |
| Obese class I | 30.00 - 34.99 |
| Obese class II | 35.00 - 39.99 |
| Obese class III | ≥40.00 |

## 2-7 Methods

## 2.7.1 Determination of Fasting Blood Glucose Concentration

### 2.7.1.1 Principle:

Glucose was oxidised to gluconate by glucose oxidase (GOD), with the formation of hydrogen peroxide ($H_2O_2$). In the presence of peroxidase (POD), a mixture of phenol and 4-amino antipyrine (4-AA) was oxidised

by $H_2O_2$ to form red quinonimine dye. This dye is equivalent to the level of glucose in. sample, such as the following reactions(159).

$$\text{Glucose} + O \xrightarrow{\text{GOD}} H_2O_2 + \text{gluconate}$$

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} 4H_2O + \text{Quinonimine.}$$

### 2.7.1.2 Reagents:

| Reagents | Composition | |
|---|---|---|
| **Reagent 1 (Buffer)** | **Tris buffer pH 7**<br>**Phenol** | **100 mmol/L**<br>**0.3 mmol/L** |
| **Reagent 2 (Enzymes)** | **Glucose oxidase**<br>**Peroxidase**<br>**4-amino-antipyrine** | **10000 U/L**<br>**1000 U/L**<br>**2.6 mmol/L** |
| **Reagent 3(Standard)** | **Glucose 100 mg/dL** **or** | **5.56 mmol/L** |

### 2.7.1.3 Procedure:

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (Buffer), then mixed gently until complete dissolution to prepare working reagent. The procedure was carried out as in the following:

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| **Working reagent** | 1 mL | 1 mL | 1 mL |
| **Standard** | - | 10 µL | - |
| **Sample** | - | - | 10 µL |
| The tubes were mixed, then let stands for 5 minutes at 37˚C. the absorbance was read at 505 nm at room temperature (25˚C), by using cuvette of 1 cm light path. | | | |

### 2.7.1.4 Calculations

$$\textbf{Glucose (mg/dl)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100$$

### 2.7.1.5 Reference Range(160,161).

| Result | Fasting Plasma Glucose (FPG) |
|---|---|
| **Normal** | less than 100 mg/dl |
| **Prediabetes** | 100 mg/dl to 125 mg/dl |
| **Diabetes** | 126 mg/dl or higher |

## 2.7.2 Determination of Serum Total Cholesterol Concentration

### 2.7.2.1 Principle

Cholesterol concentration was determined enzymatically according to the method described by Allain C. *et al*.2017 As shown in the following reactions(162).

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholesten 4 one 3} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinonimine (pink)} + 4\ H_2O$$

### 2.7.2.2 Reagents

| Reagents | Composition | |
|---|---|---|
| Reagent 1 (Buffer) | Phosphate buffer Chloro-4-phenol Sodium chloride Triton x 100 Preservative | 100 mmol/L 5.0 mmol/L 2.3 mmol/L 1.5 mmol/L |
| Reagent 2 (Enzymes) | Cholesterol oxidase<br>Cholesterol esterase<br>peroxidase<br>PAP<br>PEG 6000 | 100 IU/<br>170 IU/<br>1200 IU/<br>0.25 mmol/<br>167 µmol/L |
| Reagent 3 (Standard) | Cholesterol 200 mg/dL | or 5.17 mmol/L |

The content of vial reagent 2 (Enzymes) was added to vial reagent 1 (buffer), mix gently until complete dissolution (approximately 2 minutes) to prepare work reagent.

### 2.7.2.3 Procedure

| **Reagents** | **Blank** | **Standard** | **Sample** |
|---|---|---|---|
| Reagent | 1ml | 1ml | 1mL |
| Demineralized water | 10µL | - | - |
| Standard | - | 10µL | |
| Sample | - | - | 10µL |

The tubes were mixed and then let stands for 5 minutes at 37˚C. Absorbance at 500 nm (480-520) against blank was read. The color is stable for 1 hour

### 2.7.2.4 Calculation

$$\text{Cholesterol (mg/dl)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200^{*}$$

* Concentration of standard

**2.7.2.5 Reference Range** (American Academy of Pediatrics, 1998)

| Age | Diagnosis | Range (mg/dl) | Range (mmol/L)* |
|---|---|---|---|
| **Adult** | **Desirable** | **< 200** | **< 5.18** |
| | **Borderline** | **200 -239** | **5.18 –6.19** |
| | **High** | **≥ 240** | **≥ 6.22** |

*To convert results from mg/dl to mmol/L multiply mg/dl by 0.0259.

## 2.7.3 Determination of Serum Triglycerides Concentration.

### 2.7.3.1 Principle

Triglycerides concentration was determined enzymatically according to the method described by Allain F. and Prencip method associated with Trinder reaction, as shown in the following reactions(163).

$$\text{Triglycerides} \xrightarrow[\text{Lipase}]{} \text{Glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[\text{Glycerol Kinase}]{} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-P + O2} \xrightarrow[\text{Glycerol-3- Phosphate Oxidase}]{} \text{Dihydroxyacetone-p + H2O2}$$

$$H_2O_2 + \text{4-chlorophenol + PAP} \xrightarrow[\text{Peroxidase}]{} \text{Quinonimine (pink)} + 4\ H_2O$$

The absorbance of the colored complex (quinonimine), proportionalto the amount of triglycerides in the specimen

### 2.7.3.2 Reagent

| Reagents | Composition | |
|---|---|---|
| **Reagent 1 (Buffer)** | PIPES<br>Magnesium chloride<br>Chloro-4-phenol | 100mmol/L<br>9.8mmol/L<br>3.5mmol/L |
| **Reagent2 (Enzymes)** | lipase<br>Peroxidase<br>Glycerol-3-p-oxidase<br>Glycerol kinase<br>PAP<br>ATP | 1000 IU/L<br>1700 IU/L<br>3000 IU/L<br>660 IU/L<br>0.5 mmol/L<br>1.3 mmol/L |
| **Reagent 3 (Standard)** | Triglycerides 200 mg/dl or 2.28 mmol/L | |

### 2.7.3.3 Procedure

The content of vial reagent 2 (Enzymes) was added to vial reagent 1(Buffer), mixed gently until complete dissolution (approximately 2 minutes) to prepare work reagent. The procedure was carried out as in the following:

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| **Reagent** | 1mL | 1mL | 1mL |
| **Demineralized water** | 10µL | - | - |
| **Standard** | - | 10µL | |
| **Sample** | - | - | 10µL |

The tubes were mixed, and then let stands for 5 minutes at 37˚C. Absorbance at 500 nm against blank was read. Color stable for 1 hour.

### 2.7.3.4 Calculation

$$\text{Triglyceride (mg/dl)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 200 *$$

* 200 is standard concentration.

### 2.7.3.5 Reference range(164)

| Diagnosis | Range(mg/dL) | Range(mmol/L)* |
|---|---|---|
| Normal | < 150 | < 1.70 |
| Borderline High | 150 to 199 | 1.70 to 2.25 |
| High | 200 to 499 | 2.26 to 5.64 |
| Very High | ≥ 500 | ≥ 5.65 |

*To convert results from mg/dl to mmol/L multiply mg/dl by 0.0113.

## 2.7.4 Determination of Serum HDL-Cholesterol Concentration

### 2.7.4.1 Principle:

Low density lipoproteins (LDL), very density lipoproteins (VLDL) and chylomicron from specimens were precipitated by phosphor tungstic acid and magnesium chloride. HDL-cholesterol obtained in supernatant after centrifugation is then measured with total cholesterol reagent(165).

### 2.7.4.2 Reagents

| Reagents | Composition |
|---|---|
| Reagent 1 (precipitant) | Phosphotungstic acid 13.9mmol/L Magnesium chloride pH 6.2, 570 mmol/L |
| Reagent 2 (Standard) | Cholesterol 100 mg/dl or 2.58 mmol/L |

### 2.7.4.3 Procedure

The procedure was carried out as in the following

| Reagents | Volume |
|---|---|
| Serum | 0.5ml |
| Precipitant | 50 µL |

The tubes were mixed vigorously, then let stand for 10 minutes at room temperature. Centrifuge for 15 minutes at 1400 x g . Then was applied next procedure which includes measurement of cholesterol insupernatant.

| Reagents | Blank | Standard | Sample |
|---|---|---|---|
| **Reagent** | 1ml | 1ml | 1ml |
| **Distilled water** | 25µL | - | - |
| **Standard 2.58mmol/L** | - | 25µL | |
| **Supernatant** | - | - | 25µL |

The tubes were mixed, and then let stands for 5 minutes at 37˚C. Absorbance at 500 nm against blank was read. The color is stablefor 1 hour.

### 2.7.4.4 Calculation

$$\text{HDL-cholesterol (mg/dl)} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times 100^{*}$$

* Standard concentration

### 2.7.4.5 Reference Range(166):-

| Diagnosis | Range (mg/dL) | Range (mmol/L) |
|---|---|---|
| **Major risk factor for heart disease** | **<40** | **>1.04** |
| **Negative risk factor for heart disease** | **≥60** | **≤1.55** |

## 2.7.5 Determination of serum Low and very Low Density Lipoprotein cholesterol (LDL-c) and (VLDL-c)

### 2.7.5.1 Principle

Low and very low density lipoprotein cholesterol are a risk factor of coronary heart diseases, and the estimation of their concentrations are most commonly by Friedewald's equation for triglyceride (TG) level less than 400 mg/dL(167,168).

**LDL-cholesterol (mg/dl) =**Total-cholesterol – [ TG /5 + HDL-c](169)

### Reference Range of Serum/Plasma (LDL-c)

| Diagnosis | Range mg/dl. | Range mmol/L* |
|---|---|---|
| **Optimal** | **<100** | **<2.59** |
| **Near/above optimal** | **100-129** | **2.59-3.34** |
| **Borderline high** | **130-159** | **3.37-4.12** |
| **High** | **160-189** | **4.15-4.90** |
| **Very high** | **>189** | **>4.90** |

* To convert results from mg/dl to mmol/L multiply mg/dl by 0.0259

VLDL-cholesterol concentration was calculated by dividing TG value by 5(170).

**VLDL-cholesterol (mg/dl)** = TG/5

## 2.7.6 Determination of C-reactive protein

C-reactive protein (CRP) is one of the cytokine induced acute phase proteins, the levels of which rise during a general, unspecific response to infections and non-infections inflammatory processes. In healthy persons the serum or plasma CRP levels are below 5 mg/l. This threshold is often exceeded within four to eight hours after an acute inflammatory event, with CRP values reaching approximately 20 to 500mg/l. As elevated CRP levels are always associated with pathological changes, the CRP assay provides information for the diagnosis, therapy and monitoring of inflammatory diseases(171).

### 2.7.6.1 Test principle

NycoCard CRP single test is a solid phase, Sandwich-Format, immunometric assay. In the test well of the devise there is a membrane coated with immobilized CRP-specific monoclonal antibodies. A diluted sample is applied to the test device. When the sample flows through the membrane, the C-reactive proteins are captured by the antibodies. CRP trapped on the membrane will then bind the gold- antibody conjugated added, in a sandwich-type reaction. Unbound conjugate is removed from the membrane by the washing solution.

A paper layer underneath the membrane absorbs excess liquid. In the presence of a pathological level of CRP in the sample, the membrane appears red-brown with color intensity proportional to the CRP concentration of the sample. The color intensity is measured quantitativelywith the NycoCardTM READER.

### 2.7.6.2 Test procedure

**1-** Five microliters of patient sample was added to the tube with R1/Dilution Liquid.

The tube was closed and mixed well for 10 seconds.

**2-** Fifty microliters of diluted sample was applied to the TD/Test Device. The sample was allowed to soak into the membrane (waiting for 30 second).

**3-** One drop R2/Conjugate was applied to the TD/Test Device. The reagentwas allowed to soak into the membrane (waiting for 30 second).

**4-** One drop R3/Washing solution was applied to the TD/Test Device.

**5-** The reagent was allowed to soak into the membrane (waiting for 20 second).

**6.** The result was read by the NycoCardTM READER **II**.

### 2.7.8.3 The reference Range CRP :- < 5 mg/L

## 2.7.7 Determination of Erythrocyte sedimentation rate (ESR)

The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body. It reflects the tendency of red blood cells to settle more rapidly in the face of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins. Changes in red cell shape or numbers may also affect the ESR.

### 2.7.7.1 Principle

When anticoagulated whole blood is allowed to stand in a narrow vertical tube for a period of time, the RBCs – under the influence of gravity - settle out from the plasma. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).There are two main methods used to measure the ESR: the Westergren method and the Wintrobe Method.

### 2.7.7.2 Procedure

The Westergren method requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tube is placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour, is the ESR.

**2.7.7.3** The normal reference range for ESR results is 1–13 mm/hr for males and 1–20 mm/hr for females. These values can also vary depending on the person's age.

## 2.7.8 Determination Serum Vitamin B12 concentration

### 2.7.8.1 Principle

Delayed Competitive Enzyme Immunoassay (TYPE 9)

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

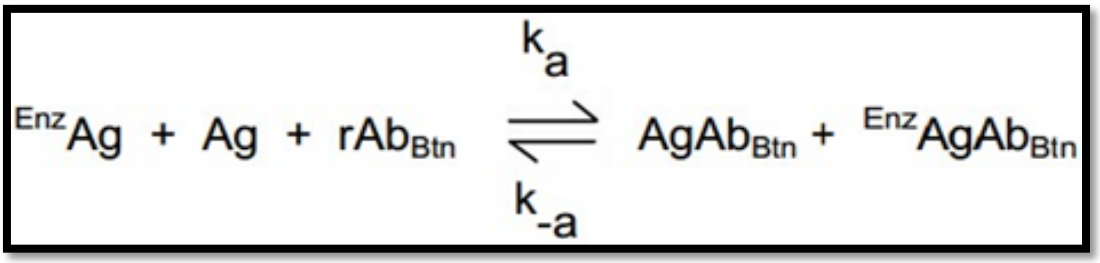
AbBtn = Biotinylated antibody

Ag = Antigen (Variable Quantity) AgAbBtn = Immune Complex

After a short incubation, the enzyme conjugate is added (this delayed addition permits an increase in sensitivity for low

concentration samples). Upon the addition of the enzyme conjugate,

competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

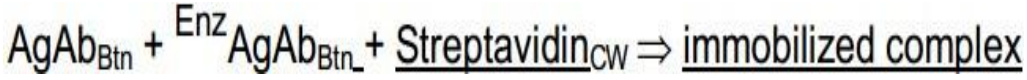



EnzAg = Enzyme-antigen Conjugate (Constant Quantity) EnzAg AbBtn = Enzyme-antigen Conjugate -Antibody Complex rAbBtn = Biotinylated antibody not reacted in first incubation

ka = Rate Constant of Association

k-a = Rate Constant of Disassociation K = ka/k-a = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.




StreptavidinCW = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

### 2.7.8.2 Reagent preparation

1. Wash Buffer

Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container.Diluted buffer can be stored at 2-30°C for up to 60 days.

Note 1: Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

2. extraction agent

was Added an aliquot of the stabilizing agent in order to prepare a 1/40 (stabilizing agent/releasing agent) dilutesolution. For example, to make 4ml (4000µl), add 0.100ml (100µl) stabilizing agent to 3.9ml (3900µl) releasing agent.

3. sample extraction (see note 3)

Obtain enough test tubes for preparation of all patient samples, controls, and serum references. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µL) of the prepared extraction agent to each test tube, shaking (see note 3) after each addition. Let the reaction proceed for 15 min. At endof the 15 min, dispense 0.050ml (50µL) of the neutralizing buffer, shaking (see note 3) after each addition, tofinish the extraction.

volume with a calibrated pipette and byadding near the bottom of the glass tubes at an angle while touching the side of the tubes.

### 2.7.8.3 Procedure

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to roomtemperature (20 - 27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates' wells for each serum reference calibrator,

control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050ml (50µL) of the appropriate extracted Vitamin B-12 calibrator, control or specimen into theassigned well.

3. Add 0.050ml (50µl) of the Vitamin B-12 Biotin Reagent to all wells.

4.Mix the microplate gently for 20-30 seconds to mix.

5.Cover and incubate for 45 minutes at room temperature.

6. Was Added 0.050ml (50µl) of Vitamin B-12 Enzyme Reagent to all wells. Add directly on top the reagents dispensed in the wells.

7.Mix the microplate gently for 20-30 seconds to mix.

8.Cover and incubate for 30 minutes at room temperature.

9.Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

10.was Added 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two

(2) additional times.

11.Was Added 0.100ml (100µl) of substrate reagent to all wells.

Always add reagents in the same order to minimize reaction time differences between wells.

do not shake the plate after substrate addition

12.Incubate at room temperature for twenty (20) minutes.

13.Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize

reaction time differences between wells.

14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. The results should be read within fifteen (15) minutes of adding the stop solution.

Note: Dilute the samples suspected of concentrations higher than 2000pg/ml 1:5 and 1:10 with Vitamin B-12 '0' pg/ml calibrator and re-assay

### 2.7.8.4 Calculation

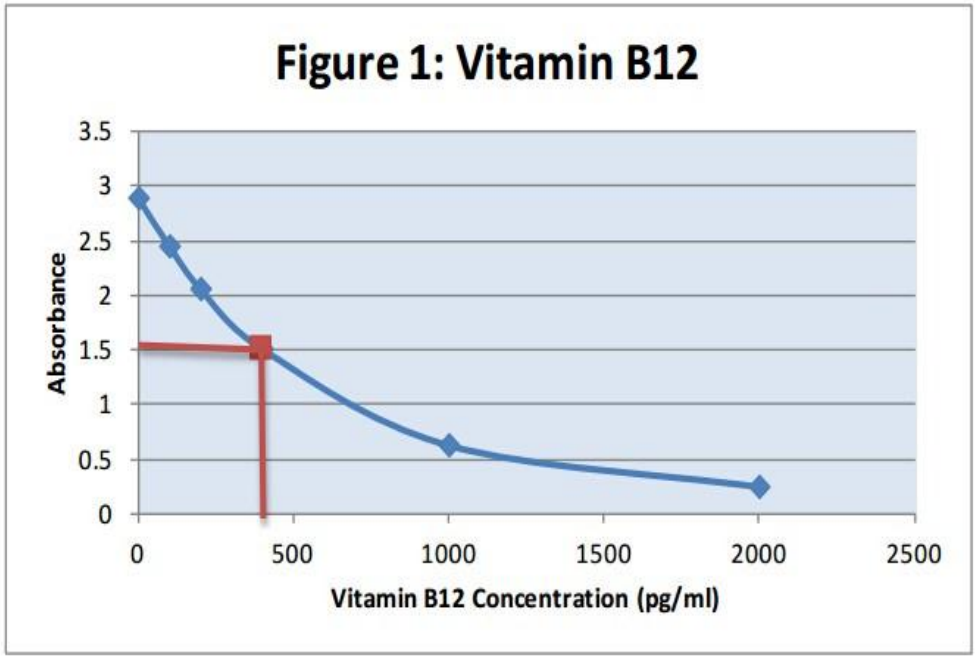



**Figure 2-2: The standard curve for Serum Vitamin B12 concentration**

## 2.7.11 Determination of Serum Vitamin D concentration

### 2.7.11.1 Principle:

This test kit is a competitive protein binding assay for the measurement of 25-OH Vit D. It is based on the competition of 25-OH Vit D present in the sample with 25-OH Vit D tracer, for the binding p°Cket of vitamin D binding protein (VDBP, Gc-globulin). Since all circulating 25-OH Vit D is bound to VDBP in vivo, samples have to be precipitated with precipitation reagent to extract the analyte. The supernatant can be used

without further treatment within the test. In the first incubation step, sample, calibrator, control, the vitamin D binding protein and the VDBP-Antibody, an antibody specific for this protein is added to the solid phase. 25 -OH Vit D present in the sample then competes with the tracer, coated on the well for the specific binding site of the binding protein and the VDBP Antibody is bound to the vitamin binding protein. Hence, with increasing concentrations of 25-OH Vit D in the sample, the amount of binding protein, immobilized to the well via the tracer, is reduced. After a washing step to remove unbound components, the quantitation of VDBP is achieved by incubation with a host specific peroxidase labeled antibody using TMB (tetramethylbenzidine) as enzyme substrate. An acidic stopping solution is then added to stop the reaction. The color converts to yellow. The intensity of the yellow color is indirectly proportional to the concentration of 25-OH Vitamin D in the sample. A dose response curve of the absorbance unit vs. concentration is generated using the results obtained from the calibrators. Concentrations of 25-OH Vitamin D, present in the specimen samples, are determined directly from this curve

### 2.7.11.2 Reagents preparation:

1. To run the assay more than one time, make sure that the reagents are carefully stored at conditions stated on the label. Prepare just the appropriate amount necessary for the assay. The kit can be used up to 4 times within the expiry date stated on the label.
2. Reagents with the less than 100 µL volume should be centrifuged before use to avoid loss of volume.
3. The WASHBUF (wash buffer concentrate) should be diluted with aqua bidest. 1:10 before use (add 900 mL of a. bidest. to 100 mL concentrate), mix well. Crystals could occur due to high salt

concentration in the stock solutions. The crystals have to be redissolved at room temperature or at 37°C using a water bath before dilution of the buffer solutions. The buffer concentrates are stable at 2-8°C until the expiry date stated on the label. Diluted buffer solution could be stored in a closed container at 2-8°C for 1 month.

4. Incubated the ASYBUF (Assay Buffer) for 10 minutes at 37 °C in a water bath before use.
5. Dissolve vitamin D binding protein (VDBP, blue cap) in 11 mL of Assay Buffer, mix well (no vortexer) and leave at room temperature for 30 min. Reconstituted VDBP is stable at -20°C until expiry date stated on the label. Avoid freezing/thawing cycles. We recommend to aliquot the VDBP (use glass containers). Frozen VDBP have to incubate for 10 minutes at 37 °C in a water bath before use. Avoid repeated freeze-thaw cycles!
6. All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2 –8 °C.

### 2.7.11.3 Procedure:

1. Prior to used in the assay allow all reagents and samples to come to room temperature (18 - 26 °C) and mix gentle, avoided foam formation.
2. Marked the positions of STD (Standards)/SAMPLE/CTRL (Control) on a Protocol sheet.
3. Taken microtiter strips out of the kit. Stored unused strips covered at 2-8° C. Strips are stable until the expiry date stated on the label.
4. Puted the microtiter wells and the precipitated STD/SAMPLE/CTRL on a cool block or ice. The microtiter plate and the

precipitated STD/SAMPLE/CTRL must be cooled during the complete pipetting step.

5. Was added 20 µL of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well.
6. Was added 100 µL VDBP (binding protein) into each well, except the NSB.
7. Was Added 100 µL ASYBUF (Assay Buffer) to the NSB wells.
8. Was added 100 µL AB (anti-VDBP antibody) into each well.
9. Cover the plate tightly and incubate for 3 hours at 8 - 10 °C in the dark.
10. Aspirated and wash the wells 5x with 250 µL of diluted Wash Buffer, remove remaining Wash Buffer by hitting the plate

against paper towel after the last wash.

11. Was added 200 µL CONJ (Conjugate) into each well.
12. Cover the plate tightly and incubate for 1 hour at 8 - 10 °C in the dark.
13. Aspirated and wash the wells 5x with 250 µL of diluted Wash Buffer), remove remaining Wash Buffer by hitting the plate

against paper towel after the last wash.

14. Was added 200 µL of SUB (Substrate) into each well.
15. Incubate for 20 - 30 minutes at room temperature (18-26°C) in the dark.
16. Was added 50 µL of STOP (Stop Solution) into each well, shake well.
17. Determined the absorption with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wave length is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (690 nm) as a reference.

### 2.7.11.4 Calculation of the results:

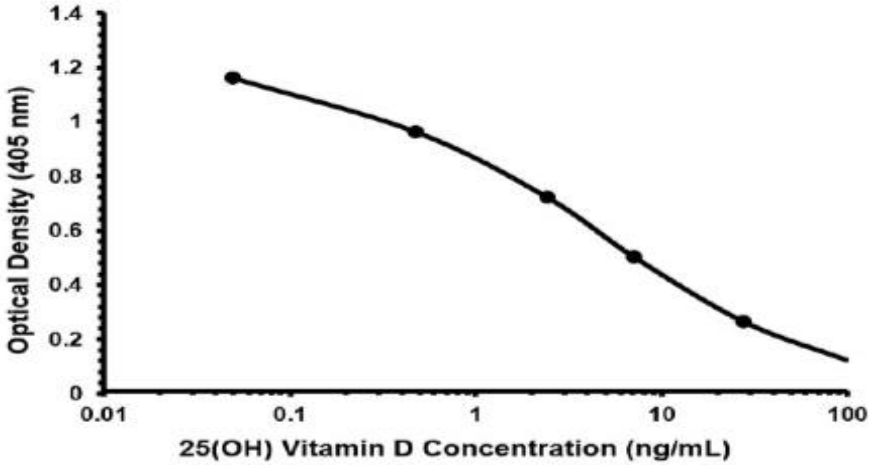



**Figure 2.3**: the standard curve serum vitamin D 25(OH) concentration

### 2.7.12 Determination of Calprotectin in Stool

### 2.7.12.1 Principle:

The assay utilizes the two-site sandwich ELISA technique with two selected monoclonal antibodies that bind to human calprotectin.

Standards, controls and diluted patient samples which are assayed for human calprotectin are added to wells of microplate

coated with a high affine monoclonal anti- human calprotectin antibody. During the first incubation step, calprotectin in the

samples is bound by the immobilized antibody. Then a peroxidase labeled conjugate is added to each well and the following

complex is formed: capture antibody - human calprotectin - Peroxidase conjugate. Tetramethylbenzidine (TMB) is used as a

substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to

yellow. The intensity of the yellow color is directly proportional to the calprotectin concentration of sample. A dose response

curve of the absorbance unit (optical density, OD) vs. concentration is

generated, using the values obtained from standard.

Calprotectin present in the patient samples is determined directly from this curve.

### 2.7.12.2Reagents preparation:

To runed assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each run. The kit can be used up to 4 times within the expiry date stated on the label.

Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

Preparation of the wash buffer: The wash buffer concentrate (WASHBUF) should be diluted with ultra pure water 1:10 before use (100 mL WASHBUF + 900 mL ultra pure water), mix well. Crystals could occur due to high salt con- centration in the stock solutions. The crystals must be redissolved at room temperature or in a water bath at 37°C before dilution of the buffer solutions.

The WASHBUF is stable at 2-8°C until the expiry date stated on the label. Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2-8°C for one month.

Preparation of the extraction buffer: The extraction buffer concentrate Extract must be diluted with ultra pure water 1:2.5 before use (100 mL Extract + 150 mL ultra pure water ), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be redissolved at 37°C in a water bath. The Extract is stable at 2-8°C until the expiry date stated on the label. Extraction buffer (1:2.5 diluted Extract) can be stored in a closed flask at 2-8°C for three months.

The lyophilized STD (standards) and CTRL (controls) are stable at 2-

8°C until the expiry date stated on the label. The STD (standards) and CTRL (controls) must be reconstituted with 500 µL of ultra pure water. Allow the vial content to dissolve for 10 minutes at room temperature, and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards and controls can be stored at 2-8°C for 4 weeks.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

### 2.7.12.3Procedure:

For automated ELISA processors the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or CD AG.

We recommend to carry out the tests in duplicate.

1. Prepared all reagents and samples to room temperature (15-30°C) and mix well.
2. Marked the positions of STD/SAMPLE/CTRL (standard/samples/controls) on a protocol sheet.
3. Taked as many microtiter strips as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until expiry date stated on the label.
4. Was added 100 µL of STD/SAMPLE/CTRL into respective well.
5. Cover plate tightly and incubate for 30 minutes at room temperature (15-30°C).
6. Aspirated the contents of each well. Wash each well 5 x with 250 µL of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.

7. Was added 100 µL CONJ (conjugate) into each well.

8. Cover plate tightly and incubate for 30 minutes at room temperature (15-30°C).

9. Aspirated the contents of each well. Wash each well 5 x with 250 µL of wash buffer. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.

10. Was added 100 µL of SUB (substrate) into each well.

11. Incubated for 10-20 minutes at room temperature (15-30°C) in the dark*.

12. Was added 100 µL of STOP (stop solution) into each well, mix thoroughly.

13. Determined absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference.

* The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

### 2.7.12.4 Reference Values:

1 g stool is equivalent to 1 mL.

The median value in healthy adults is about 25 mg/kg. Samples with a calprotectin concentration < 50 mg/kg are regarded as negative. Samples with a calprotectin concentration between 50 mg/kg and 100 mg/kg are regarded as borderline positive. We recommend repeating the measurement at a later time point in order to confirm the result. Samples with a calprotectin concentration > 100 mg/kg are regarded as positive.

## 2.7.13Determination of Serum Zinc concentration

### 2.7.13.1 Principle:

This method describes the determination of zinc in blood serum and plasma. Samples are diluted with deionized water. The analysis is performed against standards prepared in glycerol to approximate the viscosity characteristics of the diluted samples.

zinc can also be determined using the method (BC-3) for the determination of serum iron. This procedure is based on the precipitation of serum protein before analysis

### 2.7.13.2Reagents preparation:

For the determination of serum zinc, dilute the sample 1:5 with deionized water

**Standard Atomic Absorption Conditions for Zn**

| Wavelength (nm) | Slit (nm) | Relative Noise | Characteristic Concentration (mg/L) | Characteristic Concentration Check (mg/L) | Linear Range (mg/L) |
|---|---|---|---|---|---|
| 213.9 | 0.7 | 1.0 | 0.018 | 1.0 | 1.0 |
| 307.6 | 0.7 | 0.38 | 79.0 | 3500.0 | --- |

1. Recommended Flame: air-acetylene, oxidizing (lean, blue)
2. Data obtained with a standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer or impact bead will typically provide a 2-3 × sensitivity improvement.
3. Characteristic Concentration with a $N_2O$-$C_2H_2$ flame at 213.9 nm: 0.084 mg/L
4. Table contains HCL data. EDL sensitivity values approximately the same.

# Standard Flame Emission Conditions Zn

| Wavelength(nm) | Slit(nm) | Flame |
|---|---|---|
| **213.9** | **0.2** | **Nitrous oxide - acetylene** |

## 2.7.13.3Stock Standard Solution

**zinc, 500 mg/l. dissolve 0.500 g of zinc metal in a minimum volume of (1+1) Hc1 and dilute to 1 liter with 1% (v/v) Hcl.**

## 2.7.13.4Light Sources

Both Electrodeless Discharge Lamps (EDLs) and Hollow Cathode Lamps are available for zinc. EDLs provide greater light output and longer life than Hollow Cathode Lamps. For zinc, both EDLs and Hollow Cathode Lamps provide approximately the same sensitivity anddetection limit

## 2.7.13.5Procedure

Determine the concentration of zinc using the conditions listed in the "Standard Conditions" section. . Zinc standards are prepared by diluting the stock standard solution, described in the "Standard Conditions" for zinc, with 5% (v/v) glycerol. A 5% (v/v) glycerol solution should be used as a blank solution when determining zinc.

## 2.7.13.6Normal Serum Levels

**0.5-1.2mg/L or 50-120mg%**

## 2.7.14 Determination of Serum selenium concentration

### 2.7.14.12Reagents preparation

**Standard Atomic Absorption Conditions for Se**

| Wavelengh (nm) | Slit (nm) | Relative Noise | Characteristic Concentration Mg/L | Characteristic concentration Mg/L | Linear Range Mg/L |
|---|---|---|---|---|---|
| 196.0 | 2.0 | 1.0 | 0.59 | 30.0 | 200.0 |
| 204.0 | 0.7 | 0.60 | 2.9 | 150.0 | ……. |
| 206.3 | 0.7 | 0.44 | 12.0 | 600.0 | ……. |
| 207.5 | 0.7 | 0.43 | 40.0 | 2000.0 | ……. |

1. Recommended Flame: air-acetylene, oxidizing (lean, blue)

2. Data obtained with a standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer or impact bead will typically provide a 2-3 ´ sensitivity improvement.

3. Characteristic Concentration with a N2O-C2H2 flame at 196 nm: 2.7 mg/L

4. Table contains EDL data. HCL sensitivity values are more than 25% poorer. 5. Use 0.7 nm slit on Models 3100 and 3110.

### 2.7.14.2 Stock Standard Solution

selenium, 1000 mg/l. dissolve 1.000 g of selenium metal in a minimum volume of concentrated hno3 . evaporate to dryness, add 2 ml water and evaporate to dryness 2 or 3 additional times. dissolve in 10% (v/v) hcl and dilute to 1 liter with 10% (v/v) Hcl.

### 2.7.14.3 Light Sources

Both HCL and EDL sources are available for Se. EDLs, which are

more intense, provide better performance and longer life.

### 2.7.14.4 Interferences

The air-acetylene flame absorbs or scatters more than 50% of the light source radiation at the 196.1 nm selenium line. Flame absorption is reduced with the use of the nitrous oxide-acetylene flame, although sensitivity is reduced also. Use of background correction is recommended, as it will correct for flame absorption and thus improve the signal to noise ratio. It will also correct for nonspecific absorption caused by samples with high total salt content.

## 2.8 Statistical Analysis

The results are expresses as mean ± SD , p value . Statistical analyses were performed with SPSS version 23.0 software and Microsoft excel version 2016. A p value of $< 0.05$ was considered to be statistically significant.

**1.** Descriptive analysis is use to show the mean and standard deviation of variables.

**2.** The significance of difference between mean values are estimate by p value.

**3.** The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant

`

# Chapter Three

## Results&Discussions

## 3.1: Information of studied groups

Fifty patients with IBD were enrolled in this study. They were divided into two groups, 23 patients (17 male & 6 females) with Crohn's disease (CD) and 27 patients (15 male & 12 females) with ulcerative colitis (UC). The healthy control group (HC) consist of 50 healthy volunteers (32 male & 18 females). The data of the three groups are summarized in **Table (3.1)**.

**Table (3.1): Characteristics of Crohn's disease, ulcerative colitis and healthy control groups.**

<table>
<tr><th colspan="2" rowspan="2">Characteristic</th><th colspan="3">Study groups</th></tr>
<tr><th>Healthy control (n=50) No. (%)</th><th colspan="2">Case group (n=50) No.(%)</th></tr>
<tr><td rowspan="4">gender</td><td rowspan="2">male</td><td rowspan="2">32 (64)</td><td colspan="2">32(64)</td></tr>
<tr><td>15(47) UC</td><td>17(53) CD</td></tr>
<tr><td rowspan="2">female</td><td rowspan="2">18 (36)</td><td colspan="2">18(36)</td></tr>
<tr><td>12(67) UC</td><td>6(33) CD</td></tr>
</table>

### 3.1.1 Gender

The percentage of males and females was almost approximated in UC and CD patients; and therefore, there was no significant variation ($P > 0.05$) in their distribution in the two groups of IBD patients (Table 3-1).

The present sample of IBD patients revealed that male:female ratio was almost approximated, and there was no gender-related differences in the distribution of UC and CD patients. These findings are in a good agreement with previous studies carried in European patients (Steven et al., 2008; Lani et al., 2012); although, other European studies reported a slight female predominance for CD and a male predominance in UC, but the differences were not significant (Loftus, 2004; Bernstein et al., 2006). . It seemed to be from these studies and the present study that gender is not of

a major concern in UC and CD; although most autoimmune diseases show a female preponderance with the exception of ankylosing spondylitis, in which sex hormones might have an effect (González et al., 2010); therefore, it would be better to characterize UC and CD patients in terms of their sex hormone profile in order to reach a better understanding of gender effect in the etio-pathogenesis of both diseases.

### 3.1.2 Age at Presentation

Age from (14) years,or Above with a mean of (36.88 ± 12.32) years in inflammatory bowel disease (IBD) patients (ulcerative colitis; UC and Crohn's disease, CD) groups ,while the mean age in Healthy control group was(31.72 ± 9.96) years . concerning age, the results showed no significant difference between the groups. as shown in table (3-2).

**Table (3.2): the age comparison between IBD patients and healthy control group.**

| Group | | N | Mean | SD | SEM | Mean Difference | t | P-value |
|---|---|---|---|---|---|---|---|---|
| Age | Control | 50 | 31.72 | 9.96 | 1.41 | | | |
| | Case | 50 | 36.88 | 12.32 | 1.74 | -5.16 | -2.30 | 0.23433 |

Although no significant difference was observed regarding age at presentation Concerning other world studies, the profile of age at presentation is also a matter of controversy. European studies showed a median age of onset for CD of 20 - 30 years and for UC, it was higher (30 – 40 years) (Loftus et al., 2004; Cosnes et al., 2011). Consistent with findings in European patients, CD in Asia has been shown to be diagnosed at a younger age than UC (Yang et al,. 2008 b; Niriella et al., 2010; Subasinghe et al., 2011),

and the median age of CD diagnosis was 22.5 years in two Korean studies (Yang et al,. 2008 b; Ye et al., 2010), while for UC, a similar or slightly higher age range at diagnosis (35 – 44 years) than European range was reported in

Asian patients (Park et al., 2007;Yang et al., 2008 b; Asakura et al., 2009; Niriella et al., 2010; Subasinghe et al., 2011). Further studies from Asia did not confirm that (Ouyang et al., 2005; Subasinghe et al., 2011)

## 3-2 Descriptive Parameters:

The mean ± SD and p value of descriptive parameters in both studied groups with age (14) years or more and BMI ranged between (18.5 - 24.9) Kg/m2 are presented in Table 3-3.

**Table 3-3: Descriptive Parameter for G1 and G2.**

| Group / parameters | G1(No.50) Mean ±SD | G2(No. 50) Mean ± SD | P-value |
|---|---|---|---|
| **TC (mg/dL)** | **207.64 ± 54.36** | **214.84±62.51** | **0.540** |
| **TG (mg/dL)** | **83.64±26.325** | **130.34 ± 43.495** | **0.986** |
| **HDL-c (mg/dL)** | **52.440± 7.7963** | **57.284± 10.0232** | **0.008** |
| **LDL-c(mg/dL)** | **124.98± 33.72** | **128.50± 38.80** | **0.629** |
| **FBS (mg/dL)** | **95.16 ± 14.77** | **89.92 ±10.70** | **0.045** |
| **ESR (mm/hh)** | **75.90±20.16** | **15.42±3.79** | **0.000** |

significant differences when p value < 0.05.

**Group (1):** consist of (50) IBD as Patients.

**Group (2):** consist of (50) with apparently healthy as control.

Lipids are one of the complex molecules that are important in human health and play an important role in a group of diseases, including cholesterol, triglycerides, high-density Lipid, low-density Lipid, and very-low-density Lipid, they were measured for the two groups(172) .

The results showed that the mean of cholesterol level patients with IBD was (207.64±54.36) in compared with control group(214.84±62.51),

there was no significant difference between in IBD group and control group .

The results of cholesterol level agree with few studies, such as a study by (Soh, Hosim, *et al.*,2020) conducted in Korea on 7058 patients with inflammatory bowel disease, this study showed a lower level of cholesterol in people with inflammatory bowel disease than in people without this disease.

Some study showed that high blood cholesterol was associated with a lower risk of IBD, as cholesterol levels in the blood were in reverse associated. The higher cholesterol level, the lower the risk. Cholesterol levels are correlated to the systemic inflammatory status in IBD patients

Other studies have examined the relationship between dietary cholesterol and IBD , where studies showed total cholesterol were lower in the active UC and CD than in the healthy subjects and were correlated with the systemic inflammatory status.( giovanna.romanato *et al.*,2008).

The results showed that the mean of Triglyceride level patients with IBD was (83.64±26.325) in compared with control group(130.34±43.495), there was no significant difference between in IBD group and control group the P-value was (0.986) . Triglycerides are concentrated in adipose tissue. They are the main energy storage (Berg et al., 2002), play an important role in the transport of proteins and serve as sources of energy obtained from dietary fats (National Cholesterol Education Program, 2002).

The results of Triglyceride level agree with few studies, such as a study by (Soh, Hosim, *et al.*,2020) conducted in Korea on 7058 patients with inflammatory bowel disease, this study showed a lower level of Triglyceride in people with inflammatory bowel disease than in people without this disease.

Other studies have examined the relationship between triglyceride and IBD study by (Romanato, G., *et al*.2009) this study showed a lower level of Triglyceride in people with inflammatory bowel disease than in people without this disease.

The results showed that the mean of patients HDL cholesterol with IBD was (52.440 ± 7.7963) in compared with control group(57.284 ± 10.0232), there was significant difference between in IBD group and control group the P-value was (0.008) .

studies have examined the relationship between serum HDL-C and IBD hese results agreed with lower serum HDL-C levels were associated with higher incidence of CD , but not UC. (Soh, Hosim, *et al.*,2020)

In terms of its role in controlling levels of inflammatory stimulating cytokines and modifying oxidative stress, this type of fat is important, but what scientists have proven is a major function for this type of fat that deserves more study.

In patients with IBD, the average amount of harmful cholesterol (LDL) in the blood is (124.98± 33.72) in case with IBD,( 128.50 ± 38.80) in control since there is no significant difference between case and control (p-value is 0.629)

studies have examined the relationship between serum LDL-C and IBD hese results Lower serum LDL-C levels were associated with higher incidence of CD, but not UC(Soh, Hosim, *et al.*,2020).

The results showed that the mean of fasting blood suger level patients with IBD was (95.16±14.77) in compared with control group(89.92±10.70), and p –value (0.045) there was significant difference between in IBD group and control group .

Some studies showed the patients with IBD are at increased risk of and diabetes. Thus, regular monitoring of biomarkers of blood glucose levels should be considered for the early detection of diabetes in IBD patients.( Li, Zhihui, et al. 2021)

Inflammatory bowel disease may have endocrinal and metabolic associations in the form of; lipid abnormalities and insulin resistance. Also, insulin

resistance and hyperglycemia may be due to steroid use as steroid upgrades (hepatic gluconeogenesis, inhibition of glucose uptake in adipose tissue, and impairment of insulin action). There is no epidemiological evidence that Inflammatory bowel disease is a definite risk factor for diabetes.

The results showed that the mean of erythrocyte sedimentation rate (ESR) patients with IBD was (75.90 ±20.16) in compared with control group(15.42±3.79), and p –value (0.000) there was significant difference between in IBD group and control group .

ESR is the rate at which erythrocytes migrate through the plasma. Inevitably, ESR will depend on the plasma concentration and on the number and size of the erythrocytes. Conditions such as anaemia, polycytemia, and thalassemia affect ESR(173). Compared with CRP, ESR will peak much less rapidly and may also take several days to decrease, even if the clinical condition of the patient or the inflammation is ameliorated. Increases in ESR with age have been described(174).

Some study (Vermeire, et al. 2006) ESR proved to be the second best marker, with 85% of CD and 23% of UC patients positive compared with Healthy people(175).

The study showed that 50% of patients with inflammatory bowel disease, the result of the CRP test was positive, while the result of the non-infected people was negative.

CRP is a pentameric protein consisting of five monomers and is one of the most important acute phase proteins in humans. Under normal circumstances CRP is produced by hepatocytes in low quantities (,1 mg/l). However, following an acute phase stimulus such as inflammation, hepatocytes rapidly increase production of CRP under the influence of interleukin (IL)-6, tumour necrosis factor a (TNF-a), and IL-1b, and may reach peak levels of 350–400 mg/l. Generally, CRP levels of 10–40 mg/l are found in cases of mild inflammation or viral infections. Severe active inflammation or bacterial

infection will typically generate CRP levels of 50– 200 mg/l, and very high levels of .200–250 mg/l are only found in severe conditions and burns(176). CRP has a short half life (19 hours) compared with other acute phase proteins and will therefore rise early after the onset of inflammation and rapidly decrease after resolution of the inflammation(177-180).

CRP is an objective marker of inflammation and correlates well with disease activity in Crohn's disease (CD). Increased CRP levels are associated with better response rates and normal CRP levels predict high placebo response rates in clinical trials with biologicals. However, despite the advantages of CRP over other markers, it is still far from ideal. Furthermore, CRP correlates less well with disease activity in patients with ulcerative colitis (UC) as compared with CD. (Vermeire, *et al.* 2006)

Although CRP is upregulated in most inflammatory diseases, including IBD, there is remarkable heterogeneity in the CRP response between CD and UC. Whereas CD is associated with a strong CRP response, UC has only a modest to absent CRP response(181-191). This is an important feature to keep in mind when using CRP in clinical practice. There is no good explanation for this heterogeneity given that in UC increased amounts of IL-6, IL-1b, or TNF-a are also detected. However, in the study of Gross et al, serum IL-6 concentrations were significantly increased in patients with CD compared with UC and healthy controls and 68.5% of CD patients had serum IL-6 concentrations of >4 U/ml compared with only 21.7% of UC and 0% of healthy controls(192). Another explanation may lie in the fact that in UC the inflammation is confined to the mucosa whereas in CD it is transmural. However, this is unlikely to explain all of the differences. Recent studies have suggested that polymorphisms in the CRP gene, located on the long arm of chromosome 1 (1q23–24), account for interindividual differences in baseline CRP production in humans(193-195). Results are however conflicting and one recent study investigating CRP polymorphisms in IBD patients showed

no clear association with serum CRP levels(196).

**Table 3-4: Levels of vitamin D ,vitamin B12 ,Zinc ,Selenium and Faecal calprotectin in G1 and G2**

| groups / Parameters | G1(No.50) Mean ±SD | G2(No.50) Mean ±SD | P value |
|---|---|---|---|
| vitamin D | 11.41±4.01 | 54.80±16.17 | 0.000 |
| vitamin B12 | 155.66± 33.53 | 597.22± 192.37 | 0.000 |
| Zinc | 41.12± 14.43 | 89.04± 12.08 | 0.000 |
| Selenium | 30.86± 9.90 | 92.60± 15.07 | 0.000 |
| Faecal calprotectin | 346.30± 118.86 | 23.62± 10.07 | 0.000 |

significant differences when p value < 0.05.

Results in Table 3-3 showed that the mean of vitamin D level patients with IBD (G1) was (11.41±4.01) decrease in compared with control group(G2) (54.80±16.17) there was significant difference between in IBD group and control group the P-value was (0.000) .These results agreed (Gubatan, *et al.* 2018) suggest that Vitamin D deficiency is prevalent among IBD patients. In a systematic review and meta-analysis of 14 observational studies comprising of 938 patients with IBD, Del Pinto et(197) · demonstrated that the prevalence of vitamin D deficiency (defined as a serum 25(OH)D 25 ng/ml) was 38.1% in Crohn's disease and 31.6% in ulcerative colitis. The authors also showed that IBD patients had a higher odds of vitamin D deficiency [odds ratio (OR) 1.64, 95% confidence interval (CI) 1.30–2.08] compared with controls. When stratified by IBD type, Crohn's disease (OR 1.63, 95% CI 1.24–2.13) and ulcerative colitis (OR 2.28, 95% CI 1.18–4.41) had significantly higher odds of vitamin D deficiency compared with controls. The prevalence in IBD is higher than in other groups at risk of vitamin D deficiency. A study in Spain

noted that the prevalence of vitamin D deficiency was almost twice as high as in patients with asthma or postmenopausal women from the same region(198). In a multicultural IBD cohort(199), independent predictors of vitamin D deficiency included IBDrelated surgery and non-white ethnicity in Crohn's disease and non-white ethnicity in ulcerative colitis. In an IBD cohort from Mississippi(200), African-American ethnicity and a BMI greater than 30 kg/m2 predicated vitamin D deficiency, while age greater than 65 predicted vitamin D sufficiency. In another IBD cohort(201), non-white ethnicity was associated with an increased risk of vitamin D insufficiency (<30 ng/ml). In this same cohort, a diagnosis of ulcerative colitis was associated with an increased risk of vitamin D insufficiency, while women were 1.7 times more likely to have 25(OH)D levels less than 20 ng/ml compared with men. In a recent cross-sectional study by Lee et al(201). involving 102 pregnant patients with IBD and 574 pregnant patients without IBD, pregnancy was associated with increased vitamin D deficiency. In their study population, 32.3% of Crohn's disease patients and 58.3% of ulcerative colitis patients were vitamin D insufficient despite vitamin D supplementation. Their study suggests that current vitamin D supplementation guidelines may be inadequate for pregnant IBD patients.

Results in Table 3-3 showed that the mean of vitamin B12 level patients with IBD (G1) was (155.66± 33.53) decrease in compared with control group(G2) (597.22± 192.37) there was significant difference between in IBD group and control group the P-value was (0.000) .These results agreed (Kyle Kilby, *et al.* 2019) Vitamin B12 is essential in the metabolism of macronutrients, synthesis of DNA, and nerve function(202). Deficiency is associated with macrocytic anemia via impaired folate metabolism, neurologic damage, and hyperhomocysteinemia(203). While serious, these complications are commonly reported in IBD patients. In order to be absorbed, vitamin B12 requires activation by intrinsic factor produced in the stomach. Following activation, vitamin B12 is only absorbed via specific

receptors present in the terminal ileum(204). This location makes its deficiency a particular concern in CD, as a significant proportion of CD patients develop ileal disease(205). Numerous studies exist that assess B12 status in IBD patients and the results vary(206,207,208). In recent years, both a systemic review and meta-analysis of the literature have concluded that IBD is not associated with significantly lower serum B12 levels(209). However, surgical resection of the ileum is associated with B12 deficiency(210). Meta-analysis by Pan et al., 2017 suggests that patients with ileal resections were disproportionately underrepresented in the literature surrounding B12 deficiency(211). Considering that ileal disease is common in CD, and upwards of 50% of CD patients require surgerywithin 10 years of diagnosis, patients with ileal resection, and therefore B12 deficiency, likely represent a larger group than described(212).

Another study( Ratajczak, Alicja Ewa, et al. 2020 )A deficiency in vitamin B12 is due to insucient intake, abnormal absorption, or increasing demand. Furthermore, cobalamin connects with an intrinsic factor, allowing for the absorption of vitamins in the ileum, . Both of these vitamins are not absorbed in the large intestine. The risk of B12 deficiency is lower in ulcerative colitis than in Crohn’s disease, which may stem from the fact that CD cana ect all parts of the gastrointestinal tract, including the small intestine, whereas inflammation in UC predominantlyaects the large intestine(213). However, the study did not demonstrate a significant dierence in serum B12 levels between the patients and the control group(214). Kim et al. pointed out a negative correlation between the serum homocysteine level and BMD in women under 50 years of age(215). Women with a normal homocysteine concentration consumed a larger amount of vitamin B12 than women with a higher level. In the same group, subjects with a normal lumbar spine BMD presented a lower serum homocysteine level and higher folic acid concentration in erythrocyte than women with low BMD or osteoporosis. There was no correlation between the serum vitamin B12 level and the risk of

fractures in women and men. However, an association was found between homocysteine concentration and the risk of a femoral neck fracture, particularly in women(216). Salari et al. investigated the influence of folic acid supplementation (1 mg/day) on the serum osteocalcin and homocysteine level in postmenopausal women. they observed a significant dierence in osteocalcin concentration between the study and control group after 6 months. It was found that the serum homocysteine level decreased in both groups, although the changes were not significant within the groups or between them(217). Hyperhomocysteinemia (diagnosed in 60% of patients) was associated with osteoporosis and low BMD in CD patients, whereas bone disordersa ected 90% ofsubjects(218). .

Results in Table 3-3 showed that the mean of Zinc level patients with IBD (G1) was (41.12± 14.43) decrease in compared with control group(G2) (89.04± 12.08) there was significant difference between in IBD group and control group the P-value was (0.000) .These results agreed (Kyle Kilby, *et al.* 2019) As a cofactor, zinc is intimately involved in the regulation of both the innate and adaptive arms of the immune system(219) Importantly, zinc downregulates NF-$_k$B signaling through the zinc-finger protein A20, leading to decreased expression of its downstream products (namely IL-6 and TNF-)(220-223). It is worth noting that certain mutations in the gene that encodes A20 (TNFAIP3) are positively associated with the risk of developing numerous inflammatory conditions, including IBD(224). zinc exertsananti-oxidative ect through its inhibition of NADPH oxidase, and its role as a cofactor for superoxide dismutase(225). The relationship between zinc and IBD on both a theoretical and genetic level, make it a micronutrient of interest to explore further. Zinc deficiency is present in a significant portion of IBD patients (15.2–65%)(227,228). Zinc levels are balanced through absorption in the proximal small intestine, and excretion in intestinal and pancreatic secretions(229). Losses are increased in association with chronic

diarrhea, high output ostomies, and high output fistulas, which often pose a problem for IBD patients. These increased losses combined with the malabsorptive state of intestinal inflammation are likely responsible for zinc deficiency in IBD patients. Clinically, an association has also been observed between zinc deficiency and IBD. A prospective study of 170,756 women over 26 years found that increasing zinc intake was inversely corelated with CD risk(230). Furthermore, an American cohort study of 773 CD and 233 UC patients highlighted the relationship between zinc deficiency and adverse patient outcomes. They found that zinc deficiency was associated with a significant increase in hospitalizations(231). In patients who were able to achieve normal zinc levels within 12 months, the risk of adverse outcomes returned to baseline(232) .Although zinc deficiency is associated with disease activity and worse outcomes, a single small cross-over trial (n = 14) that explored the effect of daily zinc supplementation did not show changes in the level of pro-inflammatory cytokines, or disease activity(233).Given the positive results seen by Siva et al., 2017, larger trials following zinc supplementation may be a reasonable step forward(234).

Results in Table 3-3 showed that the mean of Selenium level patients with IBD (G1) was (30.86± 9.90) decrease in compared with control group(G2) (92.60± 15.07) there was significant difference between in IBD group and control group the P-value was (0.000) .

These results agreed (Kyle Kilby, *et al.* 2019) Much like zinc, selenium is integral to the immune function. Selenium is an antioxidant and a cofactor for numerous enzymes in the human body. Additionally, it decreases inflammation and inactivates osteoclasts(235),.Selenoproteins, through which selenium Exerts ect, encompass a group of proteins with diverse pro- and anti-inflammatory Relevant to IBD, glutathione peroxidases (GPx) utilize selenium to mitigate the e ect of ROS(236). Whereas loss of the genes encoding GPx proteins results in increased severity of disease(237). Selenium deficiency

is common in IBD patients with a recent study reporting 30.9% of patients as deficient, and numerous others observing levels that were significantly decreased compared to control(238-244). selenium deficiency leads to increased severity of disease and upregulation of pro-inflammatory TH1 cytokines [257,258]. In humans, selenium deficiency has been investigated for its possible role in cardiovascular disease, however a recent review by Benstoem et al., 2015 describes the literature as"inconclusive"(245).

A another study showed athat the serum selenium level was lower in CD patients than in healthy adults. In men with UC, the selenium concentration depended on the location of the inflammatory lesions(246)

Results showed that the mean of Faecal calprotectin level patients with IBD (G1) was (346.30± 118.86) increase in compared with control group(G2) (23.62± 10.07) there was significant difference between in IBD group and control group the P-value was (0.000) .

These results agreed (McMahon,, *et al.* 2018) Fecal calprotectin levels have been shown to correlate well with clinical disease activity indices, endoscopic indices, and other biomarkers, and normalization of levels are highly predictive of complete mucosal healing in UC patients(247). One study demonstrated that a fecal calprotectin level ≤60 μg/g could predict deep remission from an endoscopic, histological, and Patient Reported Outcome (PRO2 score) standpoint with >85% sensitivity and specificity in UC patients(248). Fecal calprotectin can also be utilized in patients in remission to predict future episodes of relapse, with one study showing values of ≥170 μg/g being >75% sensitive and specific for relapse in the next year(249). Other data has shown an even higher sensitivity (90%) for predicting relapse in CD and UC patients at fecal calprotectin values ≥50 mg/L

fecal calprotectin has also shown to be highly sensitive in predicting active small bowel inflammation on capsule endoscopy in patients with CD and normal colonoscopy.This finding demonstrates another beneficial use of fecal

calprotectin—the ability to assess for the presence of small bowel inflammation in patients in a noninvasive manner in a location that would not be visualized on histopathology obtained during ileocolonoscopy, the gold standard in IBD diagnosis and surveillance for disease activity. An elevated fecal calprotectin also seems to correlate well with computed tomography enterography (CTE) findings of active inflammation in small bowel CD.

Another study showed (Vermeire, *et al.* 2006) Faecal calprotectin has been shown to enable diagnosis of IBD. In this respect, a cut off of 30 mg/g had 100% sensitivity in discriminating active CD from IBS in the study of Tibble and colleagues. In the study by Fagerberg and colleagues, people with symptoms and suspected inflammation of the colon were subjected to stool analysis for faecal calprotectin and an ileocolonoscopy. Twenty two patients showed inflammation on endoscopy (of whom 20 were later diagnosed with IBD), and calprotectin levels were much higher in these patients than in people without inflammation on endoscopy.

The authors concluded that faecal calprotectin is helpful in the detection of colonic inflammation in people with gastrointestinal symptoms suggestive of IBD and that a positive test may prioritiseendoscopy. Interestingly, increased faecal calprotectin has been described in healthy first degree relatives of patients with CD. Follow up of these individuals will determine if faecal calprotectin may identify relatives at risk of developing IBD.

## 3-3 Relationships and Correlation Coefficients:

## 3-3-1 Relationship between vitamin D versus lipid profile,Zinc, selenuim, fecal calprotectin, and ESR

Correlation coefficients (r) between vitamin D levels and lipid profile(TG,HDL,LDL,VLDL,TC), vitamin B12 ,Zinc, selenuim, faecal calprotectin,FBS, and ESR for all studied groups are shown in Table 3-5.

**Table 3-5: Correlation coefficient and P-value between Vitamin D levels and other Parameters.**

| Group / Parameters | ( No. 100 ) | |
|---|---|---|
| | Correlation coefficients (r) | P -value |
| **Zinc** | **0.775** | **0.000** |
| **vitamin B12** | **0.779** | **0.000** |
| **Selenium** | **0.843** | **0.000** |
| **faecal calprotectin** | **-0.794** | **0.000** |
| **FBS** | **-0.148** | **0.000** |
| **ESR** | **-0.815** | **0.000** |
| **TG** | **-0.462** | **0.000** |
| **TC** | **0.087** | **0.389** |
| **HDL** | **0.284** | **0.004** |
| **LDL** | **0.034** | **0.738** |
| **VLDL** | **0.147** | **0.143** |

significant differences when p value < 0.05.

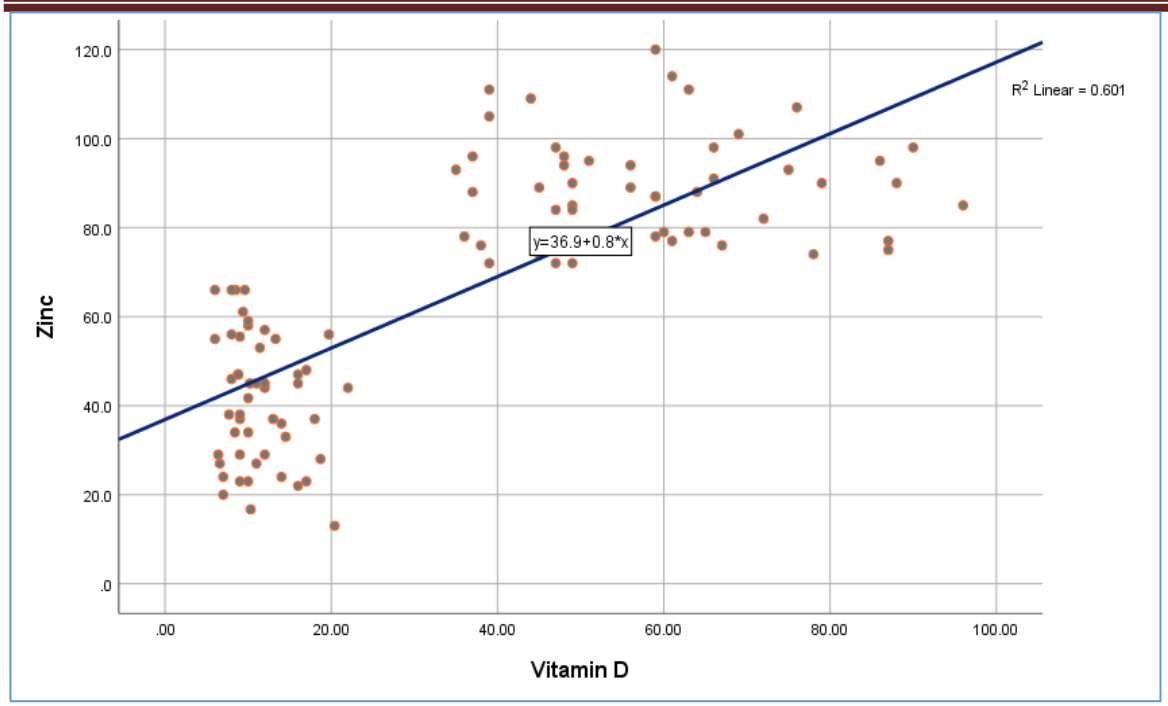



**Figure 3-1: Correlation between vitamin D and zinc**

A significant positive correlation was found between **vitamin D and zinc** (r =0.775, p value = 0.000) as shown in figure 3-1.

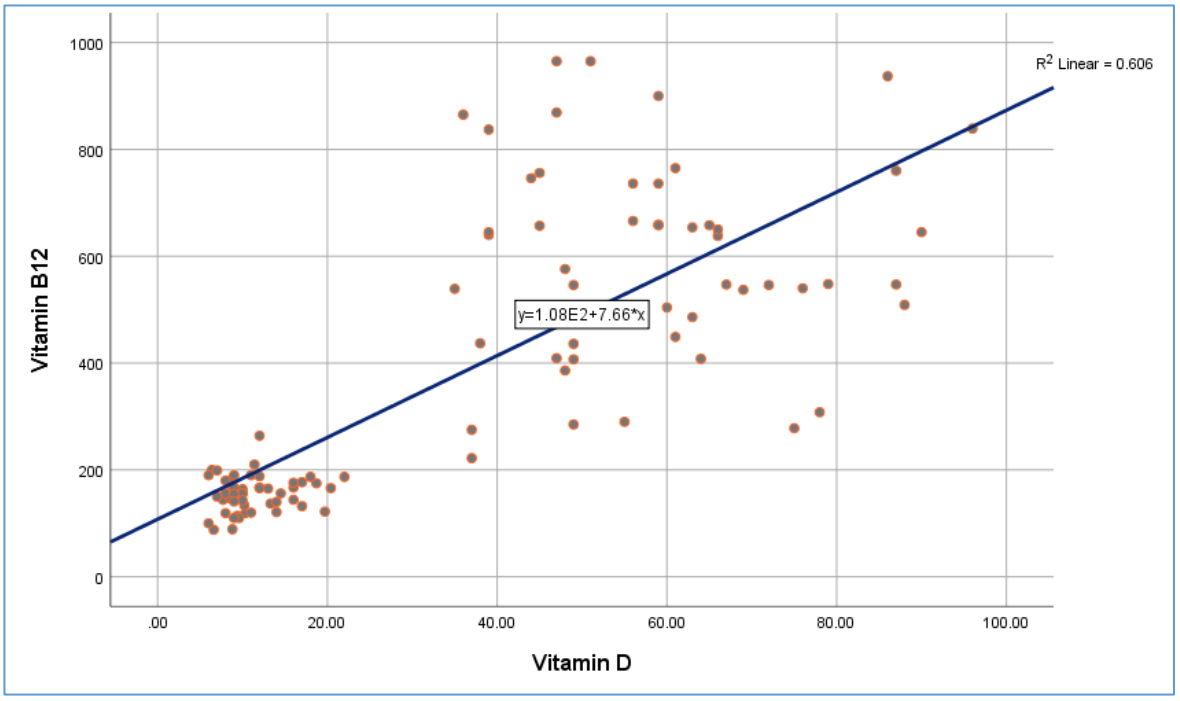



**Figure 3-2: Correlation between vitamin D and vitamin B12**

A significant positive correlation was found between vitamin D and vitamin B12 (r =0.779, p value = 0.000) as shown in figure 3-2.

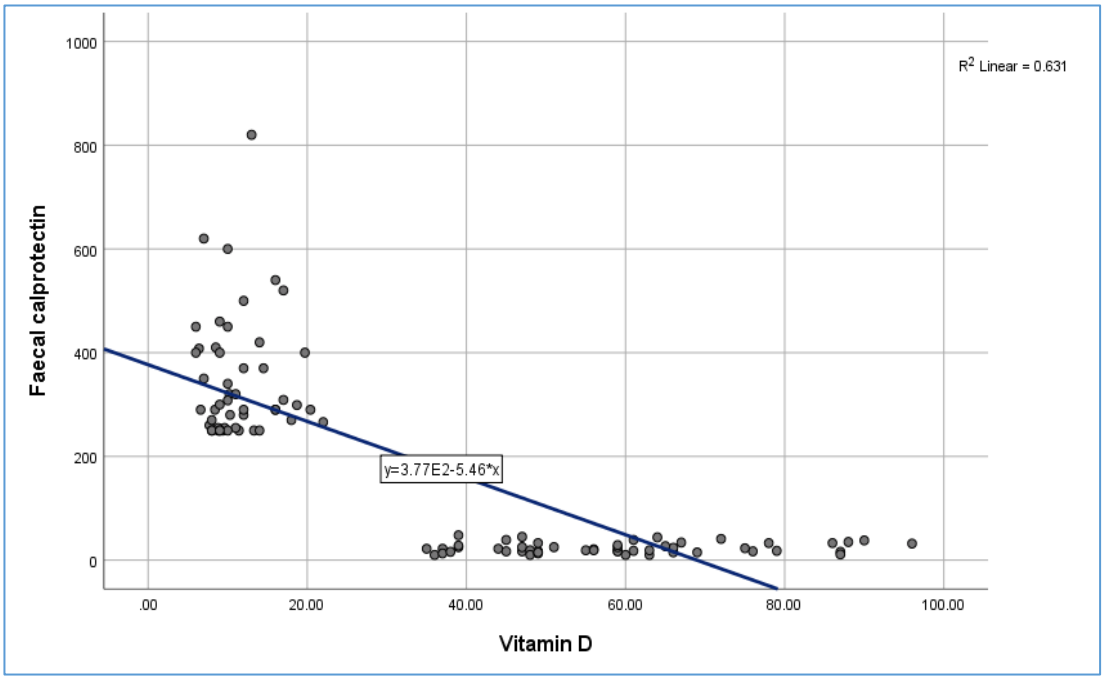



**Figure 3-3: Correlation between vitamin D and faecal calprotectin**

A significant negative correlation was found between vitamin D and faecal calprotectin(r = - 0.794, p value = 0.000) as shown in figure 3-3.

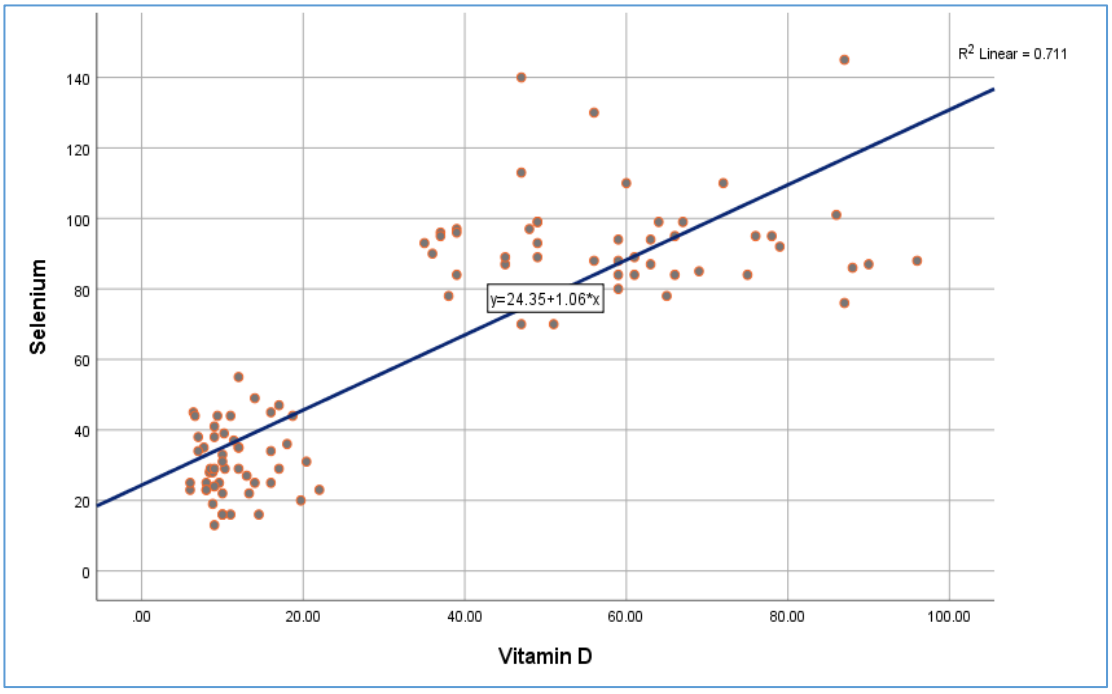



**Figure 3-4: Correlation between vitamin D and selenium**

A significant positive correlation was found between vitamin D and selenium(r =0.843, p value = 0.000) as shown in figure 3-4.

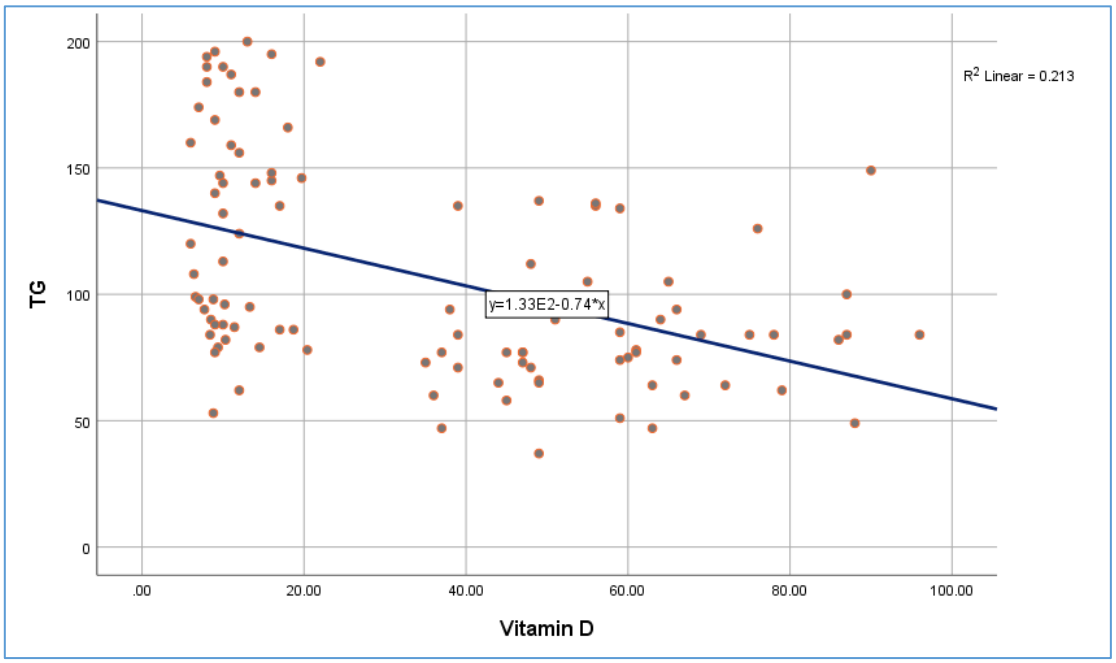



**Figure 3-5: Correlation between vitamin D and TG**

A significant negative correlation was found between vitamin D and TG (r = - 0.462, p value = 0.000) as shown in figure 3-5.

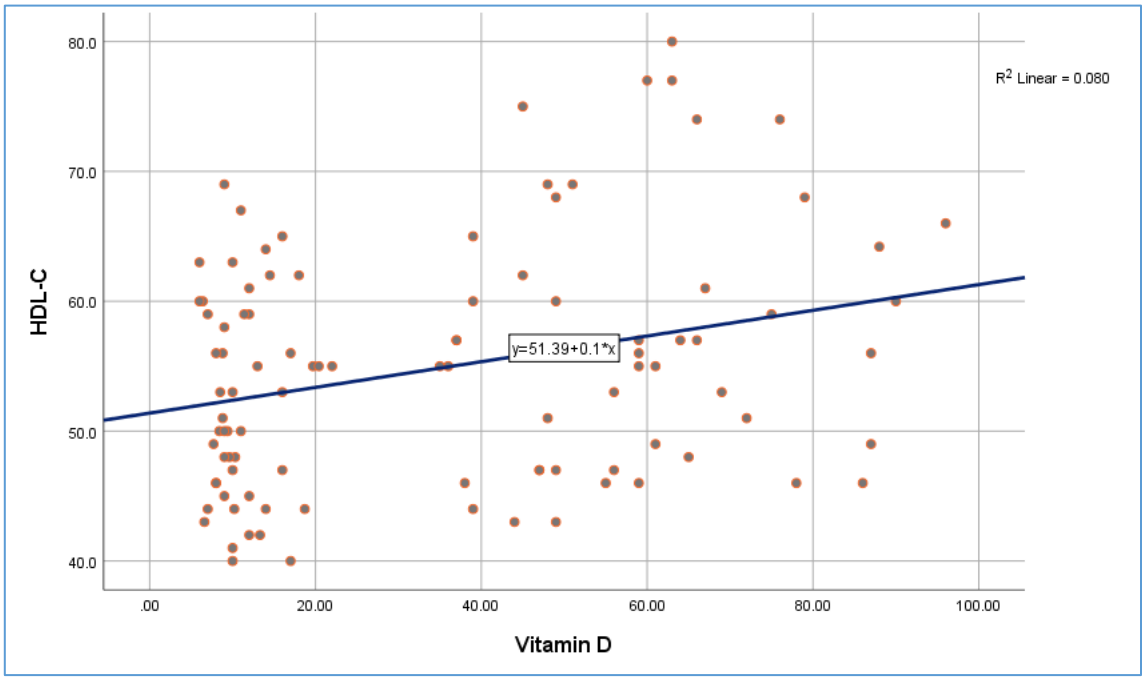



**Figure 3-6: Correlation between vitamin D and HDL-C**

A significant positive correlation was found between vitamin D and HDL-C(r =0.284, p value = 0.000) as shown in figure 3-6.

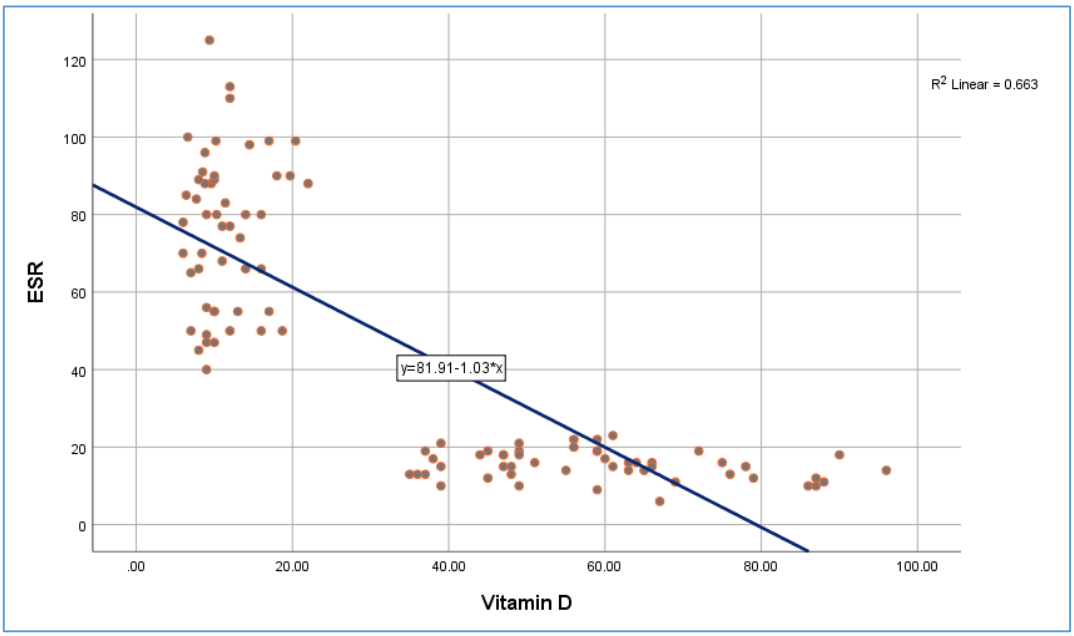



**Figure 3-7: Correlation between vitamin D and ESR**

A significant negative correlation was found between vitamin D and ESR (r = - 0.815, p value = 0.000) as shown in figure 3-7.

## 3-3-2 Relationship between Zinc versus lipid profile, vitamin D,Vitamin B12, selenuim, Fecal calprotectin, ,FBS and ESR

Correlation coefficients (r) between Zinc levels and lipid profile(TG,HDL,LDL,VLDL,TC), vitamin B12 , vitamin D, selenuim, faecal calprotectin,FBS, and ESR for all studied groups are shown in Table 3-6

**Table 3-6: Correlation coefficient and P-value between zinc levels and other Parameters.**

| Group / Parameters | ( No. 100 ) | |
|---|---|---|
| | Correlation coefficients (r) | P -value |
| Vitamin D | 0.775 | 0.000 |
| vitamin B12 | 0.762 | 0.000 |
| selenium | 0.794 | 0.000 |
| faecal calprotectin | -0.802 | 0.000 |
| FBS | -0.225 | 0.024 |
| TC | 0.074 | 0.467 |
| HDL | 0.260 | 0.009 |
| LDL | 0.023 | 0.819 |
| VLDL | 0.092 | 0.364 |
| TG | -0.427 | 0.000 |
| ESR | -0.776 | 0.000 |

**significant differences when p value < 0.05.**

A non significant correlation was found between Zinc,vitamin D,LDL-C,VLDL,TC while A significant positive correlation was found between zinc and vitamin B12 (r =0.762, p value 0.000 ) as shown in figure 3-8.

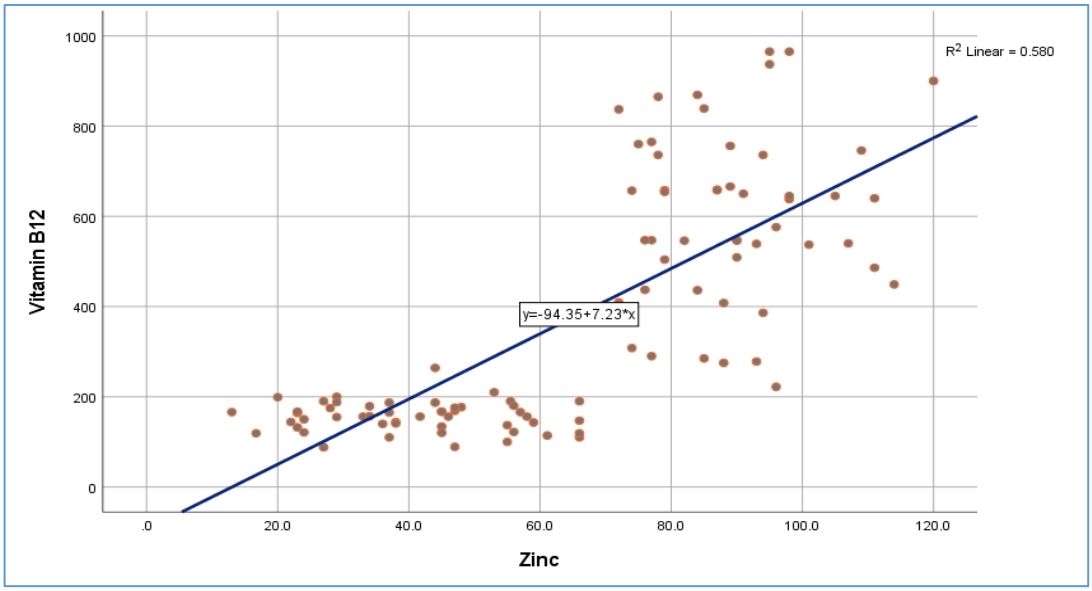



**Figure 3-8: Correlation between zinc and vitamin B12**

significant positive correlation was found between zinc and selenium (r = 0.794, p value 0.000 ) as shown in figure 3-9

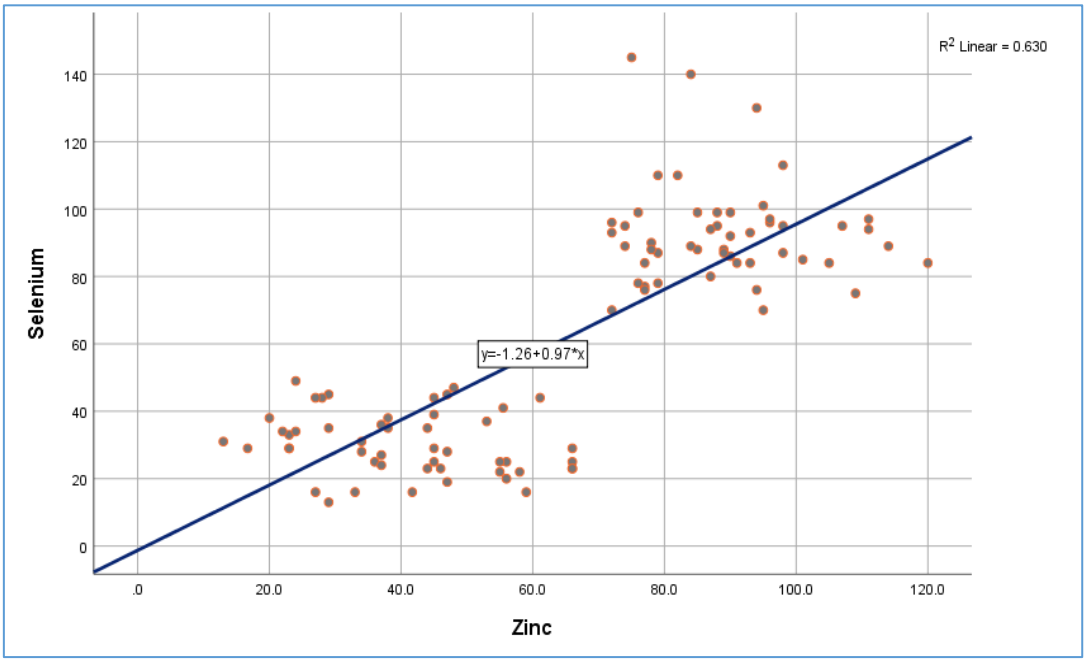



**Figure 3-9: Correlation between zinc and Selenium**

A significant negative correlation was found between zinc and Faecal calprotectin (r = - 0.802, p value 0.000 ) as shown in figure 3-10.

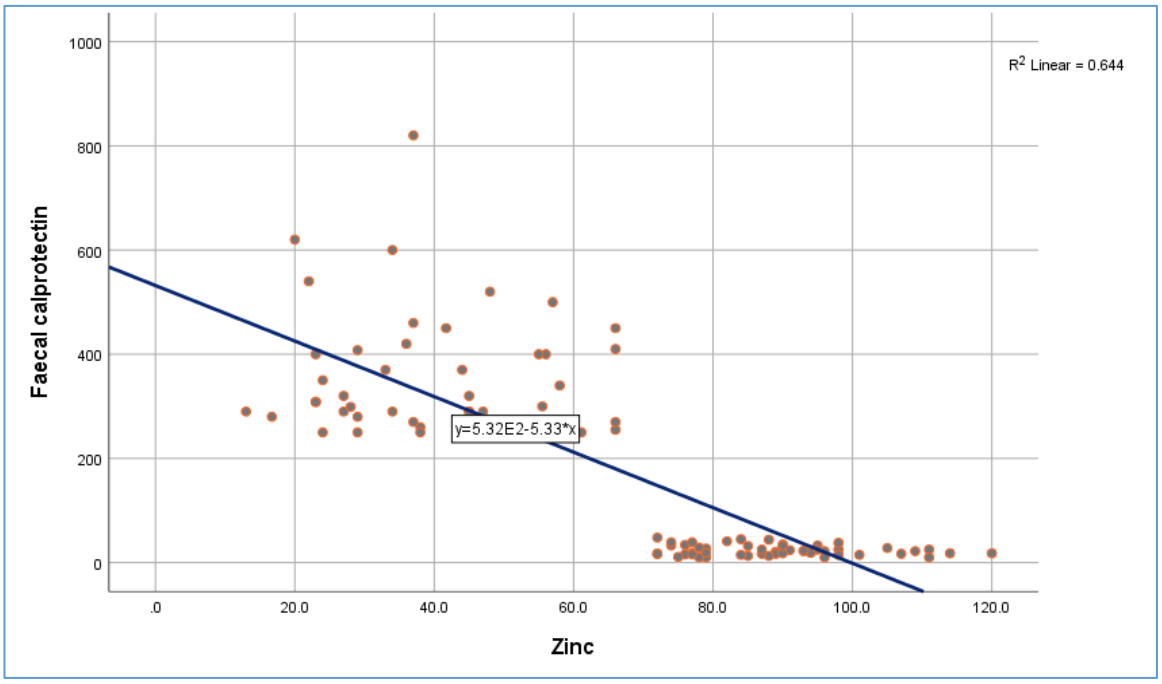



**Figure 3-10: Correlation between zinc and Faecal calprotectin**

A significant negative correlation was found between zinc and FBS (r = - 0.225, p value 0.024 ) as shown in figure 3-11.

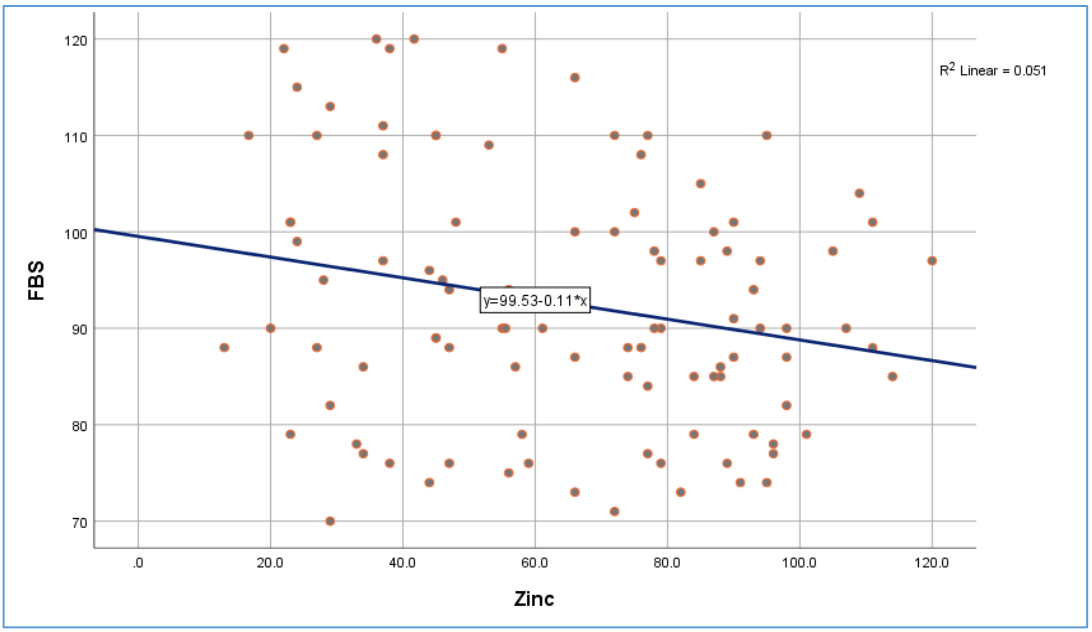



**Figure 3-11: Correlation between zinc and FBS**

A significant positive correlation was found between zinc and HDL-C (r = 0.260, p value 0.009 ) as shown in figure 3-12.

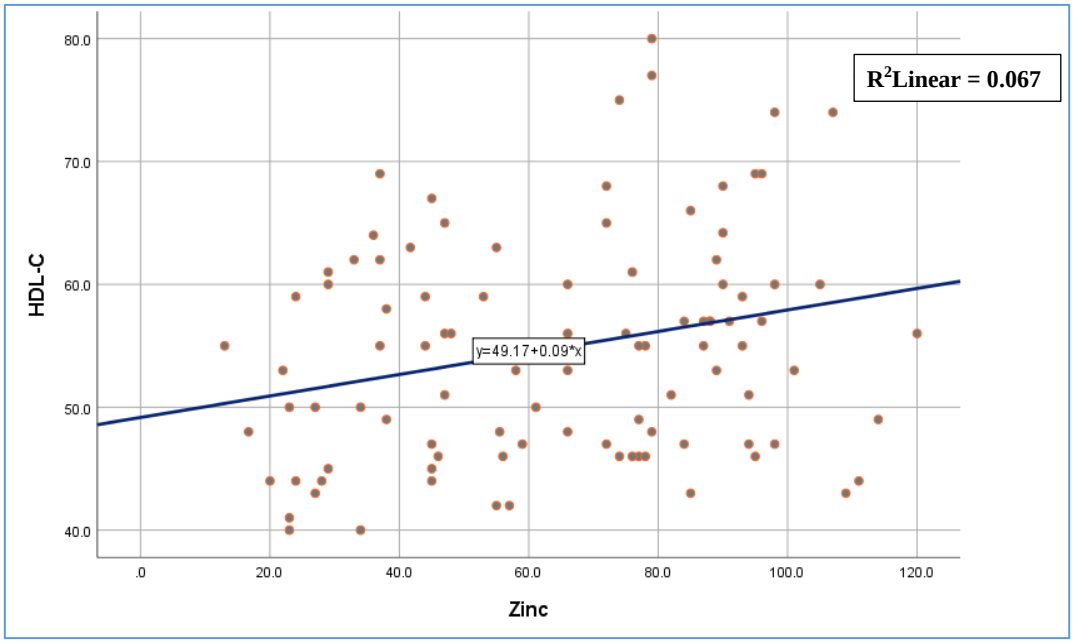



**Figure 3-12: Correlation between zinc and HDL-C**

A significant negative correlation was found between zinc and TG (r = - 0.427, p value 0.000 ) as shown in figure 3-13.

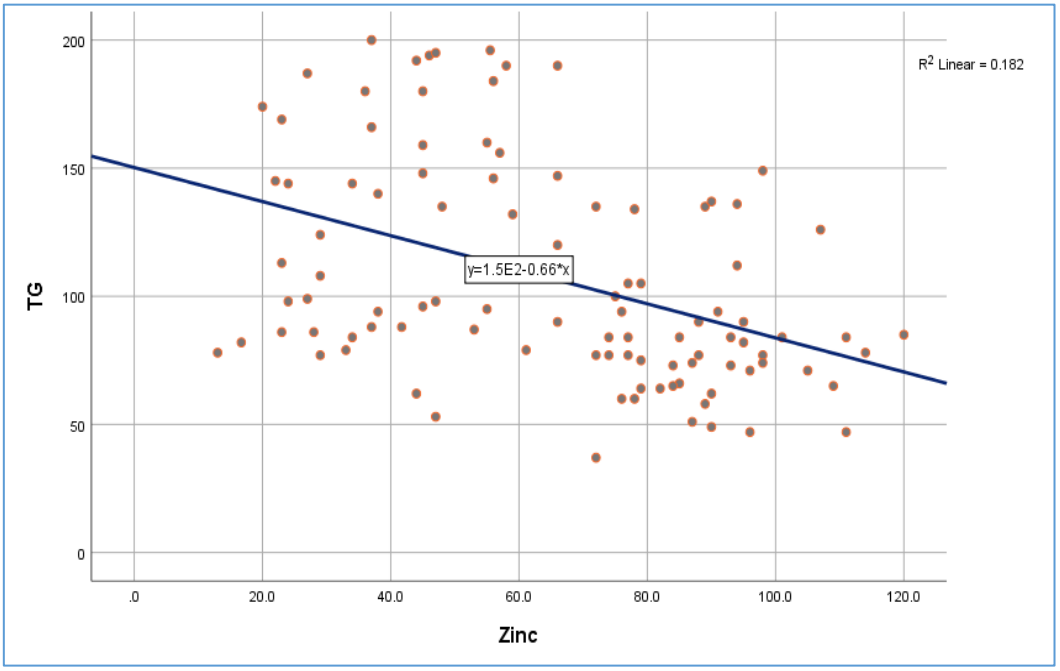



**Figure 3-13: Correlation between zinc and TG**

A significant negative correlation was found between zinc and ESR (r = - 0.776, p value 0.000 ) as shown in figure 3-14.

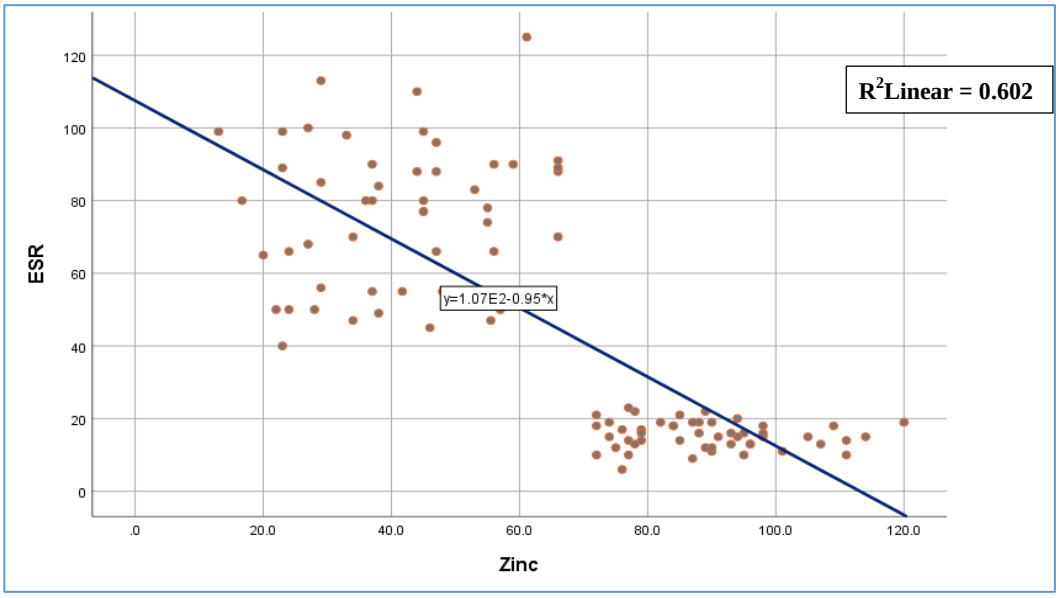



**Figure 3-14: Correlation between zinc and ESR**

## 3-3-3 Relationship between Vitamin B12 versus lipid profile, vitamin D,Zinc, selenuim, Fecal calprotectin, ,FBS and ESR

Correlation coefficients (r) between vitamin B12 levels and lipid profile(TG,HDL,LDL,VLDL,TC), Zinc, vitamin D, selenuim, faecal calprotectin,FBS, and ESR for all studied groups are shown in Table 3-7

**Table 3-7: Correlation coefficient and P-value between Vitamin B12 levels and other Parameters.**

| Parameters \ Group | ( No. 100 ) | |
|---|---|---|
| | Correlation coefficients (r) | P -value |
| Vitamin D | 0.779 | 0.000 |
| Zinc | 0.762 | 0.000 |
| selenium | 0.820 | 0.000 |
| Faecal calprotectin | - 0.744 | 0.000 |
| FBS | - 0.097 | 0.339 |
| TC | 0.038 | 0.707 |
| HDL | 0.260 | 0.009 |

| | | |
|---|---|---|
| **LDL** | **0.067** | **0.506** |
| **VLDL** | **0.131** | **0.194** |
| **TG** | **- 0.424** | **0.000** |
| **ESR** | **- 0.770** | **0.000** |

**significant differences when p value < 0.05.**

A non significant correlation was found between vitamin B12,vitamin D,LDL-C,VLDL,Tc and FBS while A significant positive correlation was found between vitamin B12 and selenium (r =0.820, p value 0.000 ) as shown in figure 3-15

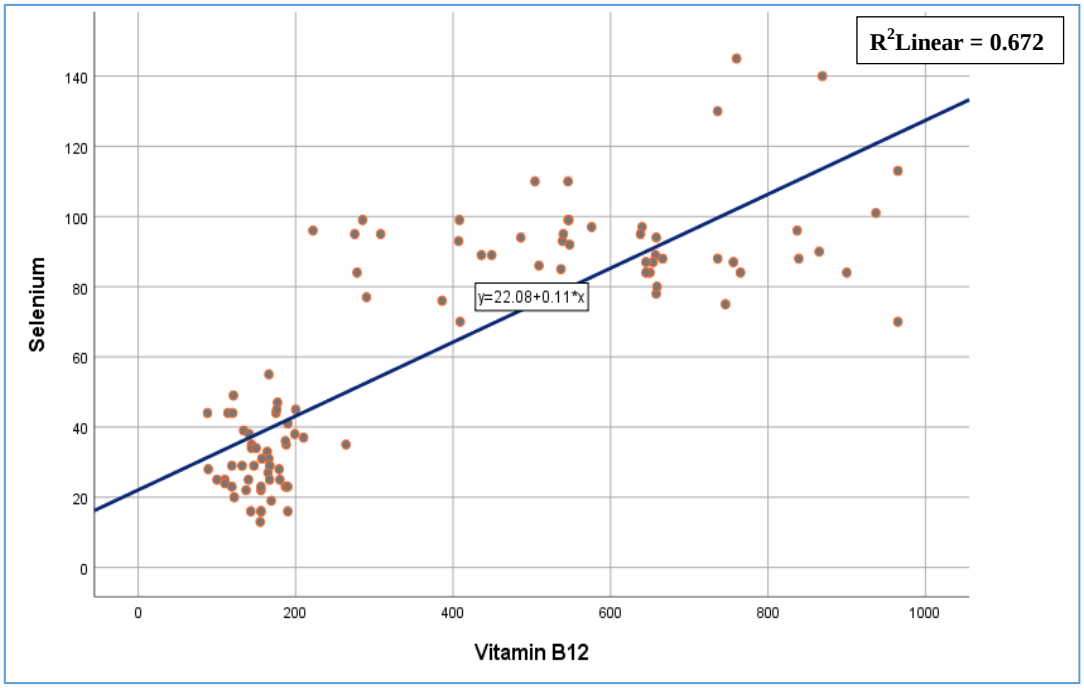



**Figure 3-15: Correlation between Vitamin B12 and Selenium**

A significant negative correlation was found between vitamin B12 and Faecal calprotectin (r = - 0.744, p value 0.000 ) as shown in figure 3-16

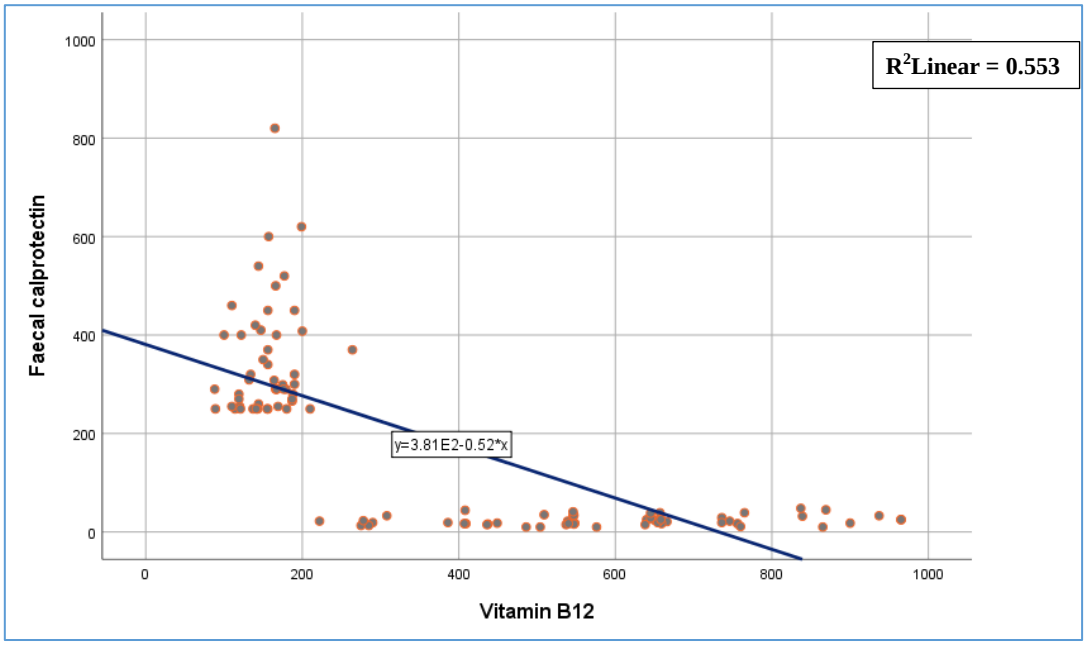



**Figure 3-16: Correlation between Vitamin B12 and Faecal calprotectin**

A significant positive correlation was found between vitamin B12 and HDL-C(r =0.267, p value 0.009 ) as shown in figure 3-17

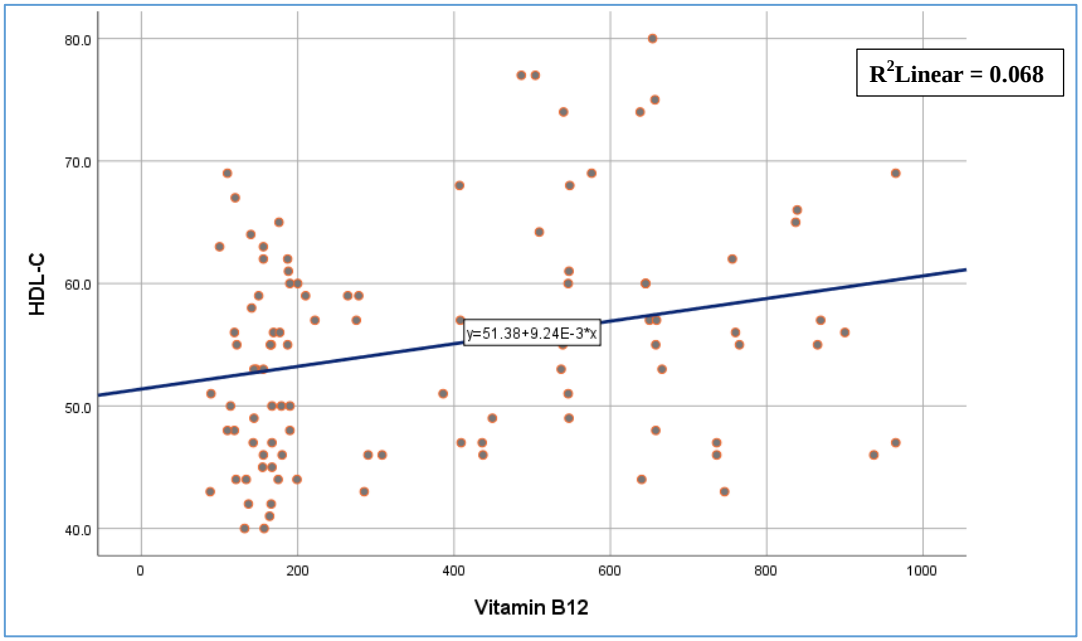



**Figure 3-17: Correlation between Vitamin B12 and HDL-C**

A significant negative correlation was found between vitamin B12 and TG (r = - 0.424, p value 0.000 ) as shown in figure 3-18

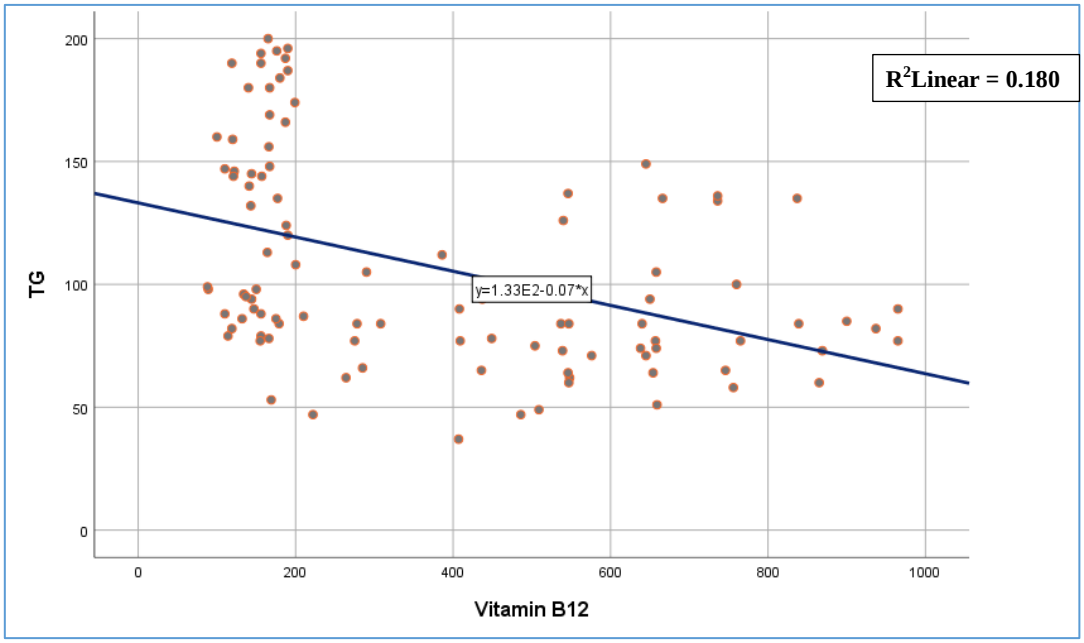



**Figure 3-18: Correlation between Vitamin B12 and TG**

A significant negative correlation was found between vitamin B12 and ESR (r = - 0.770, p value 0.000 ) as shown in figure 3-19

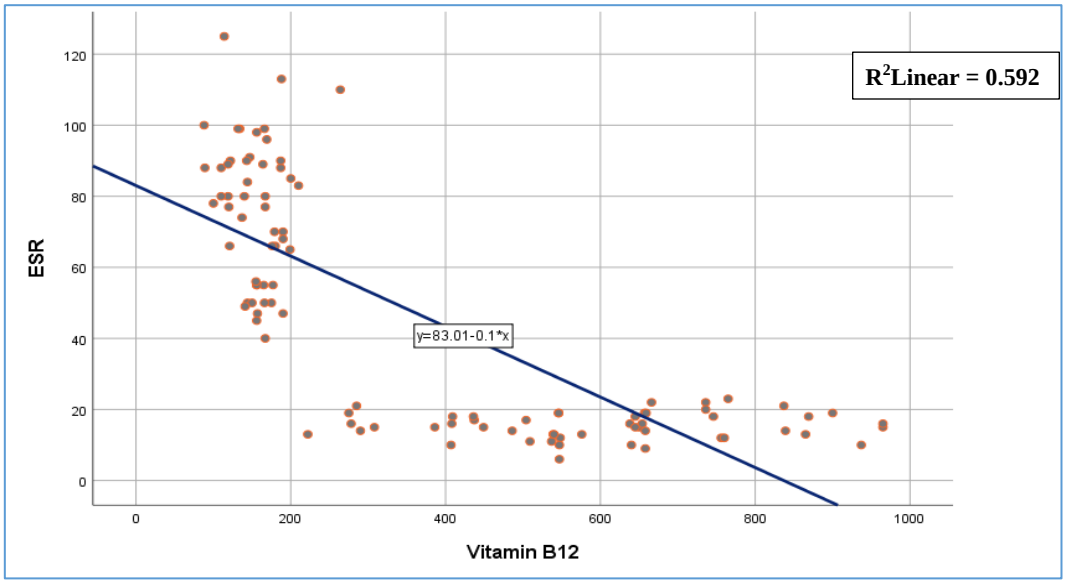



**Figure 3-19: Correlation between Vitamin B12 and ESR**

### 3-3-4 Relationship between selenuim versus lipid profile, vitamin D,Zinc, Vitamin B12, Fecal calprotectin, ,FBS and ESR

Correlation coefficients (r) between selenuim levels and lipid profile(TG,HDL,LDL,VLDL,TC), Zinc, vitamin D, vitamin B12, faecal calprotectin,FBS, and ESR for all studied groups are shown in Table 3-7

**Table 3-8: Correlation coefficient and P-value between selenuim levels and other Parameters.**

| Group / Parameters | ( No. 100 ) | |
|---|---|---|
| | Correlation coefficients (r) | P -value |
| Vitamin D | 0.843 | 0.000 |
| vitamin B12 | 0.820 | 0.000 |
| Zinc | 0.794 | 0.000 |
| faecal calprotectin | -0.815 | 0.000 |
| FBS | -0.203 | 0.043 |
| TC | 0.040 | 0.696 |
| HDL | 0.242 | 0.015 |
| LDL | 0.069 | 0.496 |
| VLDL | 0.163 | 0.105 |
| TG | -0.497 | 0.000 |
| ESR | -0.841 | 0.000 |

**significant differences when p value < 0.05.**

A non significant correlation was found between selenium, vitamin B12,vitamin D,LDL-C,VLDL,Tc and while A significant negative correlation was found between selenium and Faecal calprotectin (r = - 0.815, p value 0.000 ) as shown in figure 3-20

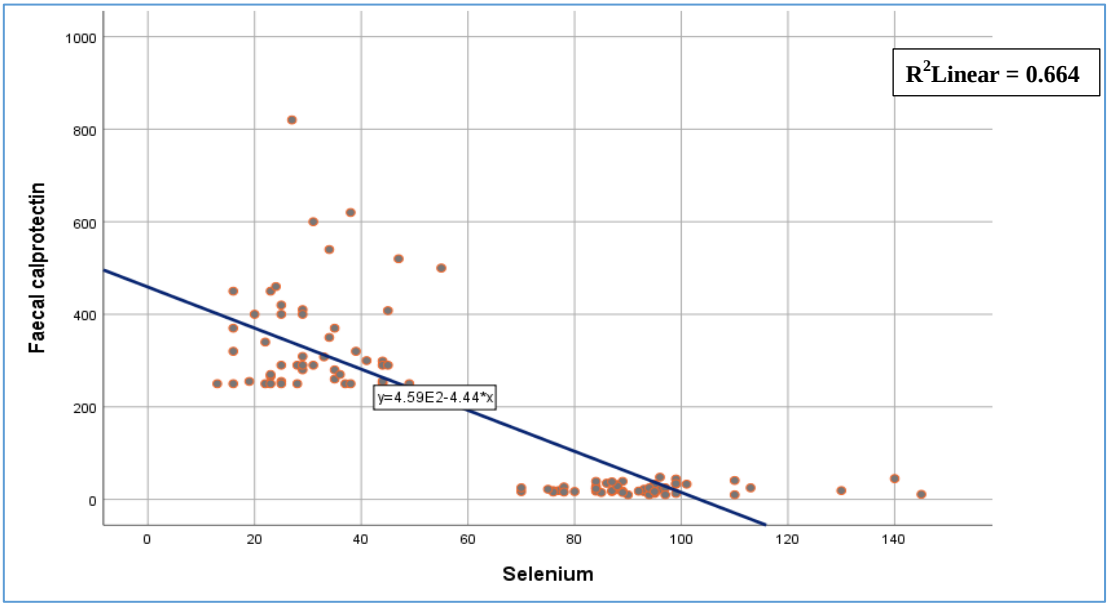



**Figure 3-20: Correlation between selenium and Faecal calprotectin**

A significant negative correlation was found between selenuim and FBS (r = - 0.203, p value 0.043 ) as shown in figure 3-21

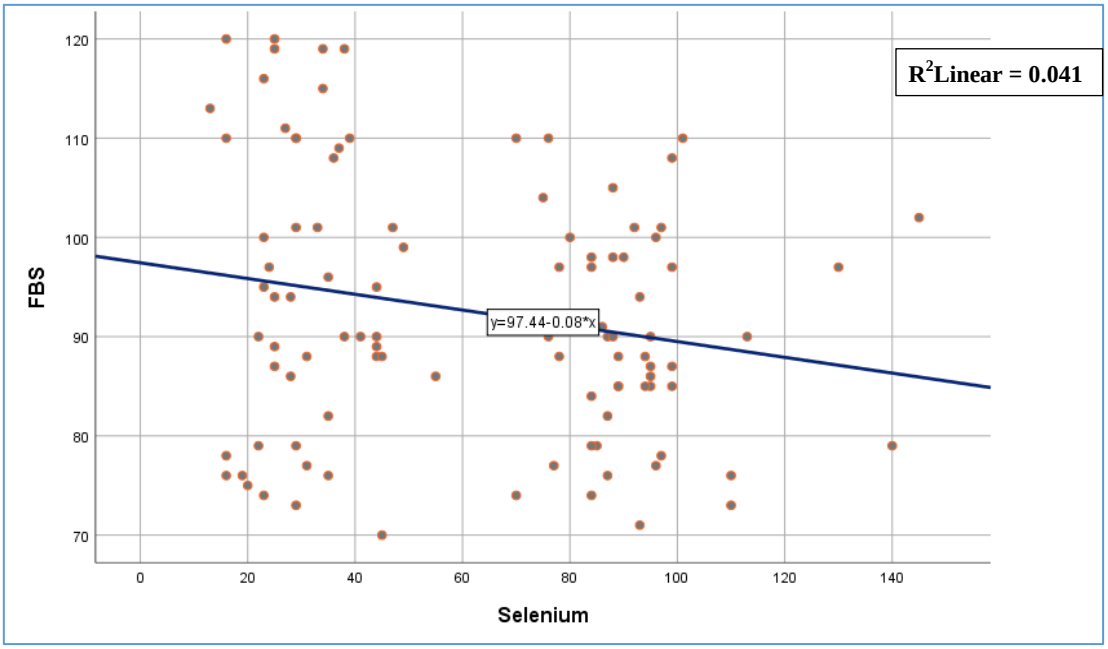



**Figure 3-21: Correlation between selenium and FBS**

A significant positive correlation was found between selenuim and HDL-C (r = 0.242, p value 0.015 ) as shown in figure 3-22

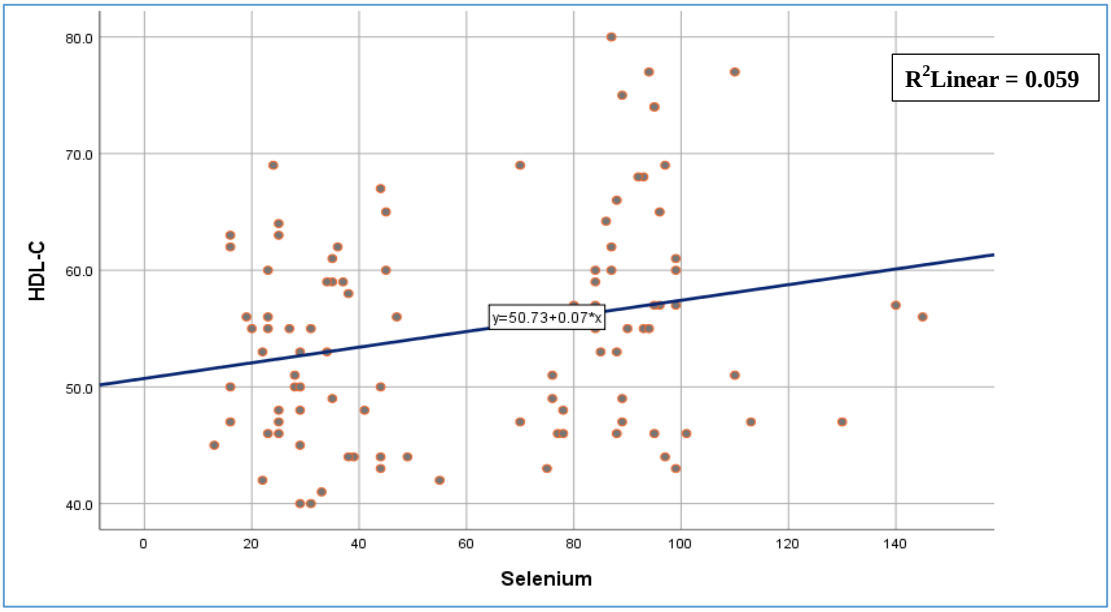



**Figure 3-22: Correlation between selenium and HDL-C**

A significant negative correlation was found between selenuim and TG (r = - 0.497, p value 0.000 ) as shown in figure 3-23

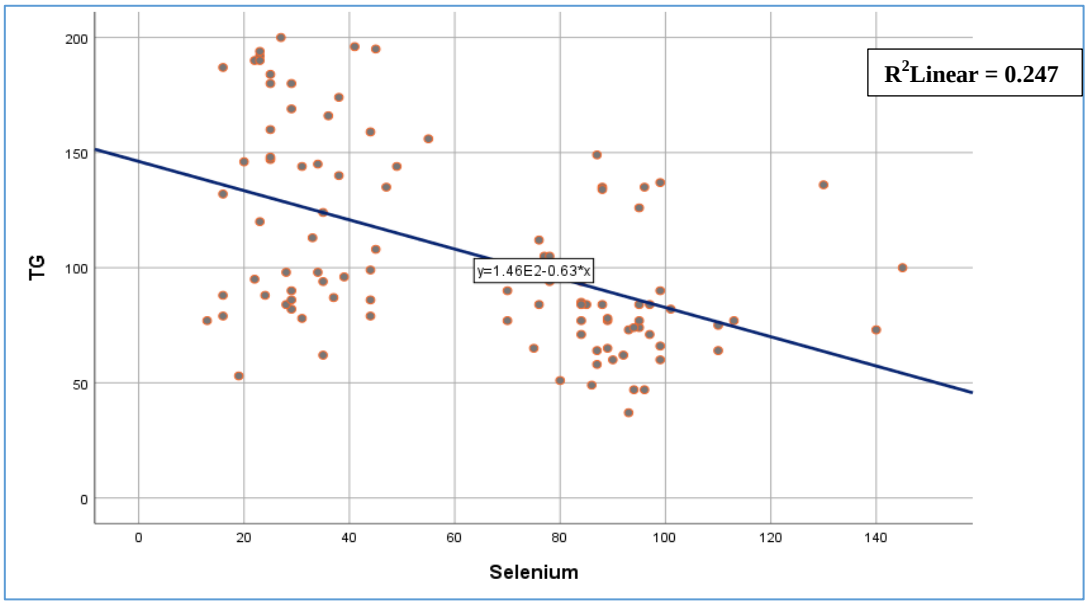



**Figure 3-23: Correlation between selenium and TG**

A significant negative correlation was found between selenuim and ESR (r = - 0.841, p value 0.000 ) as shown in figure 3-24

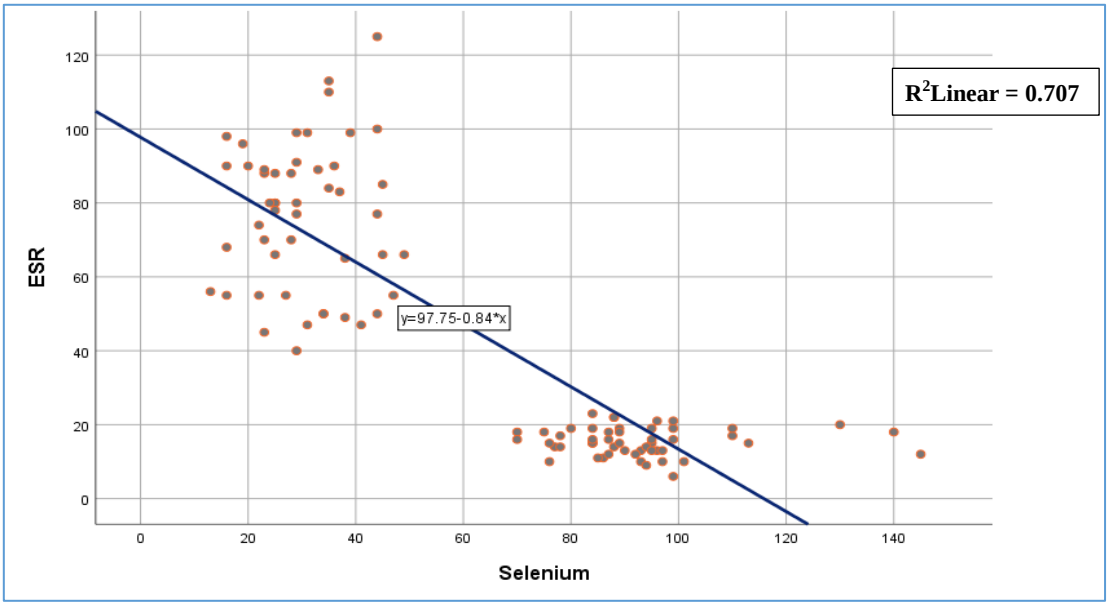



**Figure 3-24: Correlation between selenium and ESR**

## 3-3-5 Relationship between Fecal calprotectin versus lipid profile, vitamin D,Zinc, Vitamin B12, selenuim, ,FBS and ESR

Correlation coefficients (r) between faecal calprotectin levels and lipid profile(TG,HDL,LDL,VLDL,TC), Zinc, vitamin D, vitamin B12, selenuim,FBS, and ESR for all studied groups are shown in Table 3-8

## Table 3-9: Correlation coefficient and P-value between levels Fecal calprotectin and other Parameters

| Group / Parameters | ( No. 100 ) | |
|---|---|---|
| | Correlation coefficients (r) | P-value |
| vitamin D | - 0.794 | 0.000 |
| vitamin B12 | - 0.744 | 0.000 |
| Zinc | - 0.802 | 0.000 |
| Selenium | -0.815 | 0.000 |
| FBS | 0.226 | 0.024 |
| TC | - 0.048 | 0.637 |
| HDL | -0.209 | 0.037 |

| | | |
|---|---|---|
| **LDL** | **- 0.010** | **0.918** |
| **VLDL** | **0.028** | **0.780** |
| **TG** | **0.558** | **0.000** |
| **ESR** | **0.734** | **0.000** |

significant differences when p value < 0.05.

A non significant correlation was found between Faecal calprotectin, vitamin B12,vitamin D,LDL-C,VLDL,Tc ,zinc,and selenuim while A significant positive correlation was found between Faecal calprotectin and FBS(r = 0.226, p value 0.024 ) as shown in figure 3-25

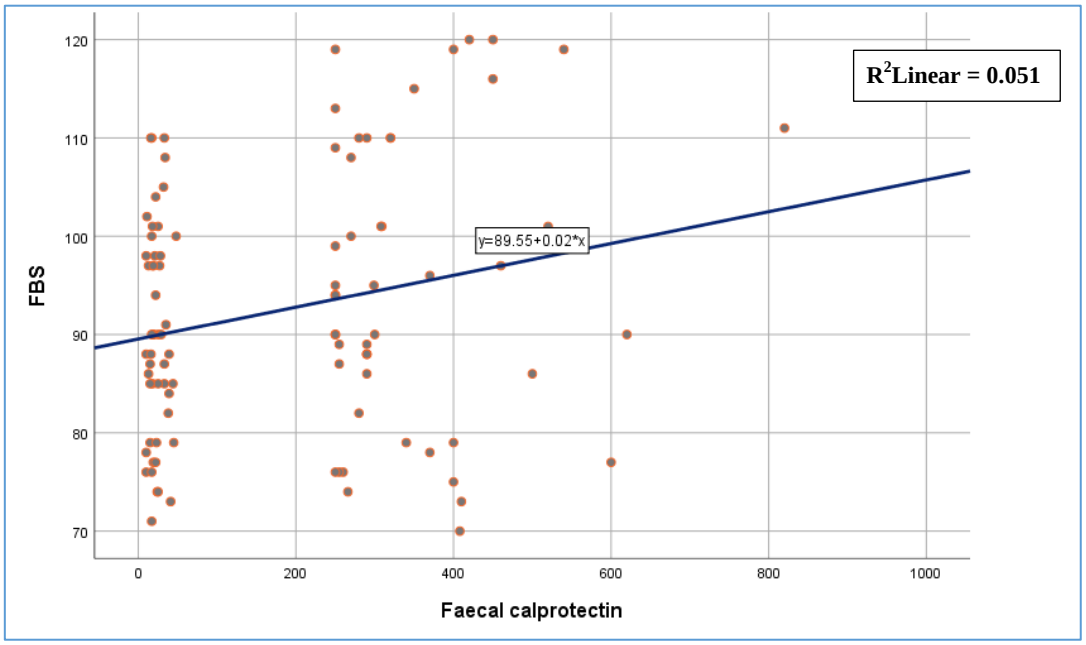



**Figure 3-25: Correlation between Faecal calprotectin and FBS**

A significant negative correlation was found between Faecal calprotectin and HDL-C (r = - 0.206, p value 0.037 ) as shown in figure 3-26

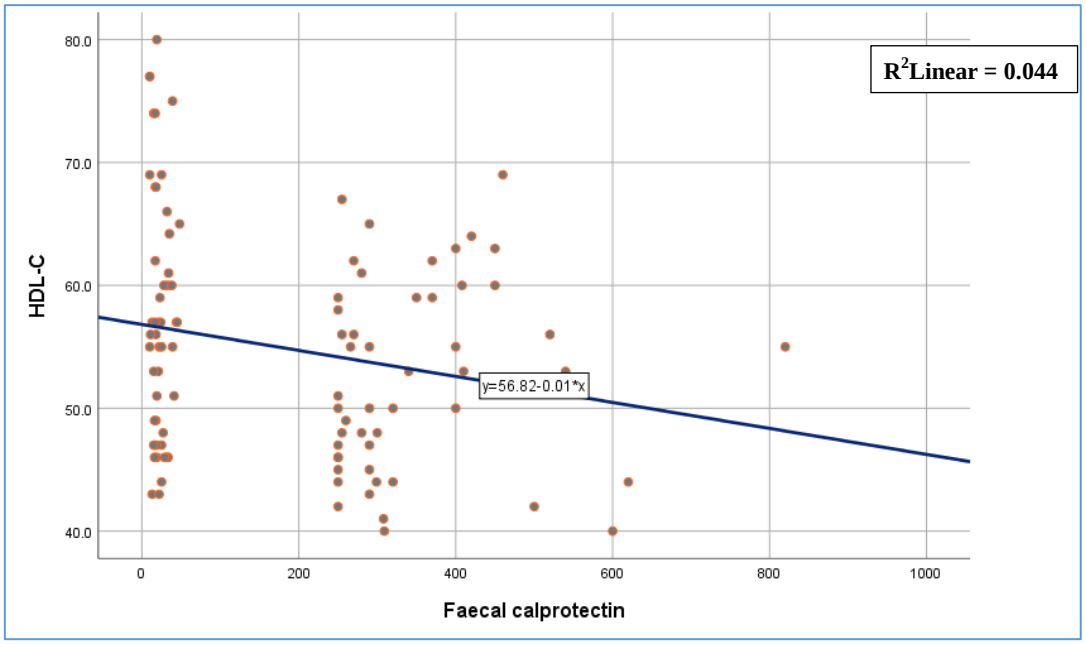



**Figure 3-26: Correlation between Faecal calprotectin and HDL-C**

A significant positive correlation was found between Faecal calprotectin and TG(r = 0.558, p value 0.000 ) as shown in figure 3-27

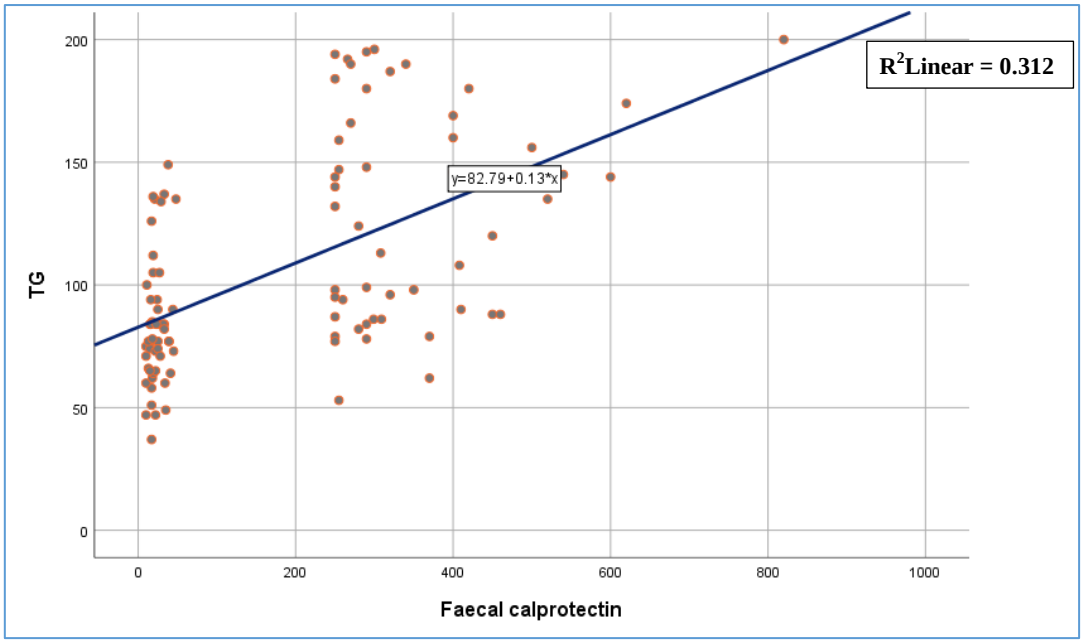



**Figure 3-27: Correlation between Faecal calprotectin and TG**

A significant positive correlation was found between Faecal calprotectin and ESR(r = 0.734, p value 0.000 ) as shown in figure 3-28

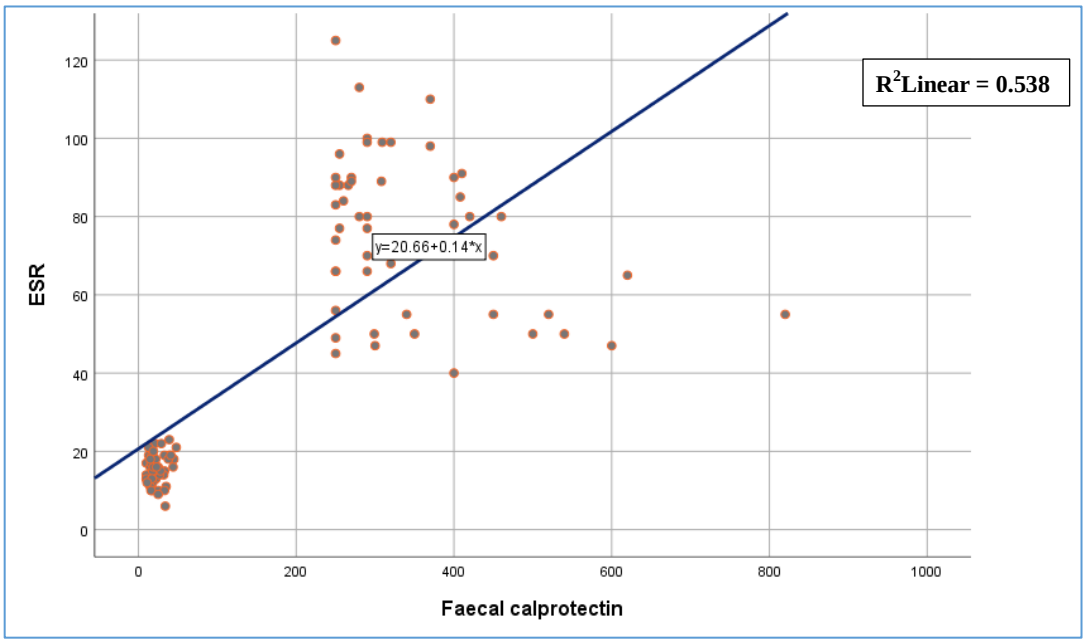



**Figure 3-28: Correlation between Faecal calprotectin and ESR**

# Conclusions& Recommendation

# CONCLUSION & RECOMMENDATION

## Conclusions:

The overall results of the present study could highlight several conclusions which include:-

1. The vitamin D concentration in patients with IBD should be examined routinely, since IBD constitutes a risk factor of vitamin D deficiency. Individual doses of vitamin D are recommended. Vitamin D deficiency is now recognised as predictor of clinical disease activity . This study demonstrates that low serum circulating 25(OH)D3 is associated with intestinal inflammation (FC) in IBD and . Deficiency of 25(OH)D3 is associated with more hospitalisations, flare-ups, use of steroids and escalating treatment.

2. Zinc levels are often low in in patients with chronic diarrhea or malabsorptive disorders. zinc deficiency is common in patients with inflammatory bowel disease (IBD) during disease and in remission, with a prevalence ranging from 15% to 40% .

3. Selenium deficiency is a common finding in patients with IBD and correlates with disease severity. In addition, patients with IBD have a lower abundance of protective gut microbiota, and selenium can increase it. Many studies established the protective effect selenium on IBD.

4. Inflammatory bowel disease patients may be at risk of vitamin B12 insufficiencies, as these micronutrients are absorbed in the small intestine, which is affected by IBD.

5. Inflammatory alterations in the intestine, as they occur in Crohn`s disease and ulcerative colitis, lead to elevated calprotectin concentrations in stool. Thereby the severity of intestinal inflammation correlates well with the concentration of calprotectin in feces. Thus, fecal calprotectin is an ideal non-invasive biomarker to identify intestinal inflammation throughout the gastrointestinal tract. It has the ability to distinguish organic disease like IBD from non-organic disorders such as Irritable Bowel Syndrome (IBS) in patients suffering from gastrointestinal

symptoms like diarrhea or chronic abdominal pain.

## Future works and Recommendations:

The following works were suggested for the future:-

1. Determination of vitamin D levels in patients with inflammatory bowel disease This is because IBD causes people to have low levels of vitamin D. Then the low levels of vitamin D might increase the risk of an immune response. The immune response can lead to either a flare-up of IBD or in developing a form of IBD in the first place.

2. measurement of vitamin B12(Cbl) levels in patients with inflammatory bowel disease This is because IBD causes people to have low levels of vitamin B12. Serum( Cbl) below the reference range (<148) was observed in CD and UC patients, respectively. Patients with ileal resections more than 30 cm had lower mean Cbl concentrations.

3. Monitoring of The ESR and CRP levels are used as serologic markers for inflammation, but they are not specific for IBD. measuring such inflammatory markers also aids in monitoring disease activity and response to treatment.

4. Determination of serum Zinc and selenuim levels in IBD patients.

Zinc and selenium are among the most important trace elements that have significant anti-inflammatory and antioxidant properties. studies have shown the importance of these trace elements in inflammatory bowel disease.

5. measuring calprotectin levels in stool is a good tool to monitor disease activity and assess the treatment success in IBD patients. Therefore, the number of invasive colonoscopies, which are time consuming and uncomfortable for patients, can be reduced for disease monitoring.

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Appendix 1

## Questionnaire

| | | |
|---|---|---|
| CD ☐ UC ☐ | | |
| Sample No: | | |
| Patient name: | Age: | Gander: |
| Weight: | Height: | |
| Smoking: | Drinking Alcohol: | |
| Other Disease: | | |
| Having Treatment | How long? | |
| Duration of disease: | | |
| Location of disease: | | |

**الخلاصة :-**

مرض داء الامعاء الالتهابي هو مجموعة من الاضطرابات المعدية المعوية التي تسبب التهابًا طويل الأمد ومزمنًا في الجهاز الهضمي. الأنماط الظاهرية الرئيسية لمرض التهاب الأمعاء هي مرض كرون والتهاب القولون التقرحي. التهاب القولون التقرحي هو مرض مجهول السبب يتميز بالتهاب الغشاء المخاطي وفي بعض الأحيان تحت المخاطية في القولون ، بينما يتسبب مرض كرون في التهاب عبر الجهاز الهضمي يؤثر على أي جزء من الجهاز الهضمي (عادةً الدقاق أو المنطقة حول الشرج) في نوع غير متصل .

تشير النتائج المتحصل عليها مقارنة بالمجموعة الصحية. أجريت هذه الدراسة في الفترة من تشرين الثاني (نوفمبر) 2020 حتى آذار (مارس) 2021 ، وحضرت جميع العينات المأخوذة من السكان إلى مدينة مرجان الطبية مركز الجهاز الهضمي في محافظة بابل ، مدينة الحلة. تم العمل المخبري في قسم الكيمياء والكيمياء الحيوية ، كلية الطب ، جامعة بابل. تهدف الدراسة المقدمة إلى تقييم مستويات الزنك والسيلينيوم وفيتامين د وفيتامين ب 12 وكالبروتكتين البرازي في مرضى أمراض الأمعاء الالتهابية عن طريق قياس مستويات المصل ومقارنة النتائج مع نتائج المجموعة الضابطة في بابل العراق ، للعثور على علاقة الارتباط بين الزنك والسيلينيوم وفيتامين د وفيتامين ب 12 وكالبروتكتين البراز مع خصائص الدهون وغيرها من العوامل في هؤلاء المرضى ومقارنتها بدراسة حالة صحية ظاهريًا ، وشملت الدراسة (50) مريضًا كمجموعة مرضى تم تشخيصهم بمرض التهاب الأمعاء (المجموعه الاولى). ) و (50) كمجموعة ضابطة ظاهريا (المجموعه الثانية) ، عمر جميع الفئات المدروسة من (14) سنة فأكثر ومؤشر كتلة الجسم (18.5 - 24.9) كغم / م 2.

تم استخدام المصل في تحديد نسبة السكر في الدم أثناء الصيام ، ونسبة الدهون ، والأنسولين ، والبروتين التفاعلي ، وفيتامين دي، وفيتامين بي12، والزنك ، والسيلينويم ، وتم استخدام الدم الكامل في تحديد مستوى معدل سرعة ترسيب الخلايا الحمراء بينما تم أخذ عينة من البراز اختبار كالبروتكتين البرازي. أظهرت النتائج انخفاضًا في إجمالي الكوليسترول ، وكوليسترول البروتين الدهني منخفض الكثافة ، وكوليسترول البروتين الدهني عالي الكثافة في مجموعات المرضى عند مقارنتها بمجموعة التحكم الصحية. بينما ارتفاع مستويات السكرو الدهون الثلاثيه في مجموعة المرضى مقارنة بمجموعة التحكم.

أظهرت النتائج انخفاضًا معنويًا في مستويات فيتامين د وفيتامين ب 12 والزنك والسيلينويم في مجموعة المرضى عند مقارنتها بالمجموعة الضابطة ، بينما أظهرت نتائج ارتفاعا في مستوى

معدل سرعة ترسيب الخلايا الحمراء و البروتين التفاعلي والكالبروتكتين البرازي معنويًا في مجموعة المرضى مقارنة بالمجموعة الضابطة.

أظهرت نتائج وجود ارتباط إيجابي معنوي بين فيتامين د وفيتامين ب 12 والزنك وسيلينويم و ،البروتين الدهني عالي الكثافه ووجدت علاقة سلبية معنوية بين فيتامين د و مستوى معدل سرعة ترسيب الخلايا الحمراء و الدهون الثلاثية والكالبروتكتين البرازي بينما وجدت علاقة غير معنوية في مجموعة المرضى بين فيتامين د والكوليسترول. ، و البروتين الدهني قليل الكثافة.

وتشير النتائج وجود ارتباط إيجابي معنوي بين الزنك وفيتامين ب 12 وفيتامين د وسيلينويم و البروتين الدهني عالي الكثافة وجد ارتباط سلبي معنوي بين الزنك ومعدل سرعة ترسب الخلايا الحمراء و الدهون الثلاثية و سكر الدم وكالبروتكتين البراز بينما وجدت علاقة غير معنوية في مجموعة المرضى بين الزنك والكوليسترول. ، و البروتين الدهني قليل الكثافة. وجد ايضا ارتباط إيجابي معنوي بين سيلينويم و البروتين الدهني عالي الكثافة وجدت علاقة سلبية معنوية بين السيلينيوم و كالبروتكين البراز وسكر الدم ومعدل سرعه ترسب الخلايا الحمراء والدهون الثلاثية.

وتشير النتائج وجود ارتباط إيجابي معنوي بين فيتامين ب 12 وسيلينويم و البروتين الدهني عالي الكثافة وجدت علاقة سلبية معنوية بين فيتامين ب 12 ، معدل سرعة ترسب الخلايا الحمراء والدهون الثلاثية ، وكالبروتكتين البراز. وتشير النتائج ايضا وجود علاقة إيجابية معنوية بين الكالبروتكتين البراز وسكر الدم والدهون الثلاثية ومعدل سرعة ترسب الخلايا الحمراء ، وجدت علاقة سلبية معنوية بين كالبروتكتين البرازي و البروتين الدهني عالي الكثافة

**نستنتج من هذه الدراسة إلى أن: -**

**1-** يجب فحص تركيز فيتامين (د) بشكل روتيني في مرضى داء الأمعاء الالتهابي ، لأن مرض التهاب الأمعاء يشكل عامل خطر لنقص فيتامين (د).

**2-** يعتبر نقص الزنك شائعًا في المرضى الذين يعانون من مرض داء الامعاء الالتهابي أثناء المرض وفي مغفرة غالبًا ما تكون مستويات الزنك منخفضة في المرضى الذين يعانون من الإسهال المزمن أو اضطرابات سوء الامتصاص..

**3-** نقص السيلينيوم هو نتيجة شائعة في المرضى الذين يعانون من مرض التهاب الأمعاء ويرتبط مع شدة المرض. بالإضافة إلى ذلك ، فإن المرضى الذين يعانون من مرض التهاب الأمعاء لديهم وفرة أقل من جراثيم الأمعاء الواقية ، ويمكن أن يزيد السيلينيوم من ذلك.

**4**. قد يكون مرضى التهاب الأمعاء معرضين لخطر نقص فيتامين ب 12 ، حيث يتم امتصاص هذه المغذيات الدقيقة في الأمعاء الدقيقة التي تتأثر بمرض التهاب الأمعاء.

**5-** التغيرات الالتهابية في الأمعاء ، كما تحدث في مرض كرون والتهاب القولون التقرحي ، تؤدي إلى ارتفاع تركيزات الكالبروتكتين في البراز. وبالتالي فإن شدة الالتهاب المعوي ترتبط بشكل جيد بتركيز كالبروتكتين في البراز. وبالتالي ، فإن كالبروتكتين البرازي هو علامة بيولوجية مثالية غير جراحية لتحديد الالتهاب المعوي في جميع أنحاء الجهاز الهضمي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الطب

# تقييم مستويات فيتامينات د3 ، ب12،الزنك،السيلينيوم والكالبروتكتين في البراز لدى المرضى البالغين المصابين بداء الامعاء الالتهابي المزمن في محافظة بابل - العراق

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم

/الكيمياء الحياتية السريرية

من قبل

**علي حامد جواد جاسم**

بكالوريوس تقنيات التحليلات المرضية/ كلية المستقبل الجامعة – بابل (2016)

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