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College of Medicine
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Evaluation of Serum Xanthine oxidoreductase, Galectine-3 and Some Biochemical Parameters levels in Adult Patients with Inflammatory Bowel Diseases in Babylon Province – Iraq

A Thesis
Submitted to the Council of the College of Medicine / University of Babylon as a partial Fulfillment for the Requirements of the Degree of Master in Science / Clinical Biochemistry Science

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Supervisor Certification

We certify that this thesis entitled " **Evaluation of Xanthine oxidoreductase, Galactine-3 and some Biochemical markers in adult patients with (IBD) in Babylon – Iraq**" was carried out 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 review of the available recommendation, I forward this thesis for debate by the examining committee.

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Dedication

This work is dedicated to:

To…… my lovely father and mother

To…… my dear wife

To…… my lovely sons

To…… all members of my family

To…… all friends and lovers

To…… every knowledge student

To my great supervisor (Dr. Ban Mahmood Shaker Al-joda)

To…… my great country

Arkan

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Summary

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine, Crohn's disease and ulcerative colitis being the principal types. IBD is a complex disease which arises as a result of the interaction of environmental and genetic factors leading to immunological responses and inflammation in the intestine.

This study was designed to investigate the role of xanthene oxidoreductase and galectin-3 in the development of inflammatory bowel diseases in Babylon province. Also, measuring zinc, iron, phosphorus, magnesium and vitamin E in these patients and study the relationship between parameters.

The present study included 100 subjects with age range (15-65) years old with normal BMI.

The subject in this prospective case-control study included two groups as following:

Group (I): included (50) patients with inflammatory bowel disease.

Group (II): included (50) apparently healthy subjects as control.

Galectin-3 concentration, Xanthine oxidoreductase and vitamin E were determined by enzyme linked immunosorbent assay (ELISA) technique, where the level of iron, zinc magnesium and phosphorus were measured by colorimetric method according to manufactured manual or kits.

Xanthine oxidoreductase concentration and Galectin-3 were increased significantly ($p<0.05$) in inflammatory bowel diseases individuals (compared to the control group). On the other hand, there were a significant positive and negative in the levels of iron, zinc, magnesium and vitamin E. While there was non-significant changes ($p>0.05$) of serum Phosphorus concentrations.

The present study concluded that inflammatory bowel diseases patients in Babylon province have higher serum level of Xanthine oxidoreductase and this might be attributed to its role in the promotion of inflammatory state and

the presence of galectins-3 in serum of this patients may result from leakage from tissue, thereby making it as a possible biomarker for inflammatory bowel diseases, also may serve as prognostic serum biomarker.

On the other hand, there are several reasons for serum iron, zinc and magnesium deficiency in inflammatory bowel diseases patients including low gastro intestinal absorption, increased loss, and reduced intake due to anorexia.

While decreased the level of vitamin E supported that oxidative stress which is caused by increased free radicals generation with impairment of antioxidants, plays an important role in pathogenesis of inflammatory bowel diseases.

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List of Abbreviations

Abbreviation	Meaning
Abs	Absorbance
CD	Crohn's disease
CRDs	carbohydrate recognition domains
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
ELISA	Enzyme linked immune sorbent assay
ESR	Erythrocyte sedimentation rate
FAD	Flavin adenine dinucleotide
Gal-3	Galectin-3
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
M	Mean
Mg	Magnesium
NADH	Nicotinamide adenine dinucleotide
NS	Nitric Oxide
PO4	Phosphorus
r	Correlation-coefficient
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SD	Standard deviation
SMC	serum Mg concentration
TPN	Total parenteral nutrition
UC	Ulcerative colitis
UC	uric acid
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

Chapter One

Introduction and Literature Review

1. Introduction and Literature Review

1.1 Gasterointestenal Tract

The gastrointestinal tract (GI tract, digestive tract, alimentary canal) is the tract or passageway of the digestive system that leads from the mouth to the anus. Gastrointestinal is an adjective meaning of or pertaining to the stomach and intestines **[1]**.

The human gastrointestinal tract consists of the esophagus, stomach, and intestines, and is divided into the upper and lower gastrointestinal tracts. The complete human digestive system is made up of the gastrointestinal tract plus the accessory organs of digestion (the tongue, salivary glands, pancreas, liver and gallbladder), Figure [1-1] **[2]**.

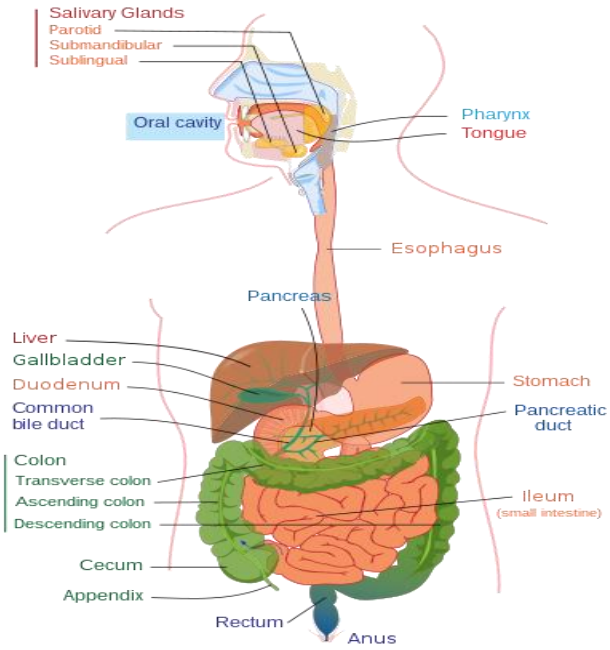


Figure 1-1: Upper and lower human gastrointestinal tract [2]

The gastrointestinal tract contains the gut microbiota, with some 4,000 different strains of bacteria having diverse roles in maintenance of immune health and metabolism, and many other microorganisms **[3]**.

Cells of the GI tract release hormones to help regulate the digestive process. These digestive hormones, including gastrin, secretin, cholecystokinin, and ghrelin, are mediated through either intracrine or autocrine mechanisms, indicating that the cells releasing these hormones are conserved structures throughout evolution **[4]**.

1.2 Inflammatory bowel diseases

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine, Crohn's disease and ulcerative colitis being the principal types. Crohn's disease affects the small intestine and large intestine, as well as the mouth, esophagus, stomach and the anus, whereas ulcerative colitis primarily affects the colon and the rectum **[5]**.

In spite of Crohn's and UC being very different diseases, both may present with any of the following symptoms: abdominal pain, diarrhea, rectal bleeding, severe internal cramps/muscle spasms in the region of the pelvis and weight loss. Anemia is the most prevalent extra-intestinal complication of inflammatory bowel disease **[6]**.

Inflammatory bowel disease is a complex disease which arises as a result of the interaction of environmental and genetic factors leading to immunological responses and inflammation in the intestine **[7]**.

Crohn's disease is a type of inflammatory bowel disease (IBD) that may affect any segment of the gastrointestinal tract. Many people with Crohn's disease have symptoms for years before the diagnosis. The usual onset is in the teens and twenties, but can occur at any age **[8]**.

Symptoms often include abdominal pain especially in the lower right abdomen, Flatulence, bloating, and abdominal distension are additional symptoms and may also add to the intestinal discomfort. Pain is often accompanied by diarrhea, fever and weight loss among older individuals, usually related to decreased food intake, since individuals with

intestinal symptoms from Crohn's disease often feel better when they do not eat and might lose their appetite **[9]**.

People with extensive small intestine disease may also have malabsorption of carbohydrates or lipids, which can further exacerbate weight loss **[10]**.

Crohn's disease can lead to several mechanical complications within the intestines, including obstruction, fistulae, and abscesses. Also increases the risk of cancer in the area of inflammation **[11]**.

Major complications of Crohn's disease include bowel obstruction, abscesses, free perforation and hemorrhage, which in rare cases may be fatal **[12]**.

Individuals with Crohn's disease are at risk of malnutrition for many reasons, including decreased food intake and malabsorption. The risk increases following resection of the small bowel. Such individuals may require oral supplements to increase their caloric intake, or in severe cases, total parenteral nutrition (TPN) **[13]**.

While the exact cause or causes are unknown, Crohn's disease seems to be due to a combination of environmental factors and genetic predisposition. In this view, the chronic inflammation of Crohn's is caused when the adaptive immune system tries to compensate for a deficient innate immune system **[14]**.

The later hypothesis describes impaired cytokine secretion by macrophages, which contributes to impaired innate immunity and leads to a sustained microbial-induced inflammatory response in the colon, where the bacterial load is high **[15]**.

The increased incidence of Crohn's in the industrialized world indicates an environmental component. Crohn's is associated with an increased intake of animal protein, milk protein, increased ratio of omega-6 to omega-3 polyunsaturated fatty acids, smoking and hormonal contraception

[16]. Although stress is sometimes claimed to exacerbate Crohn's disease **[5]**.

Ulcerative colitis (UC) is a long-term condition that results in inflammation and ulcers of the colon and rectum. People with ulcerative colitis usually present with diarrhea mixed with blood, of gradual onset that persists for an extended period of time (weeks). Additional symptoms may include Weight loss, fever, anemia, fecal incontinence, increased frequency of bowel movements, mucus discharge, and nocturnal defecations **[17]**.

The disease is classified by the extent of involvement, depending on how far the disease extends: proctitis (rectal inflammation), left sided colitis (inflammation extending to descending colon), and extensive colitis (inflammation proximal to the descending colon) **[18]**. Furthermore UC is also characterized by severity of disease **[19]**.

Ulcerative colitis is characterized by immune dysregulation and systemic inflammation, which may result in symptoms and complications outside the colon **[20]**. Commonly affected organs include: eyes, joints, skin liver and mouth **[21].**

The risk of blood clots increases by Ulcerative colitis **[22]** and significant association with primary sclerosing cholangitis (PSC) **[23]**.

Ulcerative colitis is an autoimmune disease characterized by T-cells infiltrating the colon. Factors such as genetics, environment, and an overactive immune system play a role **[24]**.

A genetic component to the cause of UC can be hypothesized based on aggregation of UC in families, variation of prevalence between different ethnicities, genetic markers environmental factors **[25]**.

1.3 Epidemiology

In the world, IBD resulted in a global total of 55,000 deaths in 1990, 51,000 deaths in 2013 and 47,400 people deaths in 2015. The increased incidence of IBD since World War 2 has been correlated to the increase in meat consumption worldwide, supporting the claim that animal protein intake is associated with IBD **[26,27]**.

Inflammatory bowel diseases are increasing in Europe. Incidence and prevalence of IBD has risen steadily for the last decades in Asia, which could be related changes in diet and other environmental factors **[28]**.

Around 0.8% of people in the UK have IBD. Similarly, around 270,000 (0.7%) of people in Canada have IBD, with that number expected to rise to 400,000 (1%) by 2030 **[29]**.

The geographic distribution of UC and Crohn's disease is similar worldwide, with the highest number of new cases a year of UC found in Canada, New Zealand and the United Kingdom **[30]**.

The disease is more common in North America and Europe than other regions. In general, higher rates are seen in northern locations compared to southern locations in Europe and the United States **[31]**.

Together, ulcerative colitis and Crohn's disease affect about a million people in the United States. While, UC and Crohn's disease affected about 11.2 million people as of 2015 in the world **[32]**.

The incidence of IBD is increasing exponentially in most of the Arab countries; our pooled estimates revealed that the incidence of UC and CD is approximating 2.33 and 1.46 per 100,000 persons per year, respectively. The highest incidence of IBD appears to be in Kuwait and KSA. On the other hand **[33]**, UC is more prevalent than CD in Iraqi and Iranian population **[34]**

1.4 Diagnosis of IBD

The diagnosis is usually confirmed by biopsies on colonoscopy. Fecal calprotectin is useful as an initial investigation, which may suggest the possibility of IBD, as this test is sensitive but not specific for IBD**[35]**.

Imaging such as x-ray or (CT) scan to evaluate for possible perforation or toxic megacolon. Blood and stool tests serve primarily to assess disease severity, level of inflammation and rule out causes of infectious colitis **[36]**.

Liver functions tests are often elevated in inflammatory bowel disease, and are often mild and generally return spontaneously to normal levels.The most relevant mechanisms of elevated liver functions tests in IBD are drug-induced hepatotoxicity and fatty liver. Electrolyte studies and kidney function tests are done, as chronic diarrhea may be associated with hypokalemia, hypomagnesemia and kidney injury **[37]**.

A complete blood count may reveal anemia, which commonly is caused by blood loss leading to iron deficiency or by vitamin B_{12} deficiency, usually caused by ileal disease impairing vitamin B_{12} absorption. Ferritin levels help assess if iron deficiency is contributing to the anemia. Erythrocyte sedimentation rate (ESR) and C-reactive protein help assess the degree of inflammation, which is important as ferritin can also be raised in inflammation. Serum iron, total iron binding capacity and transferrin saturation may be more easily interpreted in inflammation **[38]**.

1.5 Treatment

1- Surgery

Crohn's disease and ulcerative colitis are chronic inflammatory diseases, and are not medically curable. However, ulcerative colitis can in most cases be cured by proctocolectomy, although this may not eliminate extra-intestinal symptoms. Surgery cannot cure Crohn's disease but may be needed to treat complications such as abscesses, strictures or fistulae **[39]**.

2- Medical therapies

Medical treatment of IBD is individualised to each patient. The choice of which drugs to use and by which route to administer them (oral, rectal, injection, infusion) depends on factors including the type, distribution, and severity of the patient's disease, as well as other historical and biochemical prognostic factors, and patient preferences **[40]**.

3- Nutritional and dietetic therapies

Exclusive enteral nutrition is a first-line therapy in pediatric Crohn's disease with weaker data in adults **[41]**.

4- Microbiome

There is preliminary evidence of an infectious contribution to inflammatory bowel disease in some patients that may benefit from antibiotic therapy, such as with rifaximin. The evidence for a benefit of rifaximin is mostly limited to Crohn's disease with less convincing evidence supporting use in ulcerative colitis **[41]**.

5- Alternative medicine

Complementary and alternative medicine approaches have been used in inflammatory bowel disorders. Evidence from controlled studies of these therapies has been reviewed; risk of bias was quite heterogeneous. The best supportive evidence was found for herbal therapy **[42]**.

6- Novel approaches

Stem cell therapy is undergoing research as a possible treatment for IBD.

7- Psychological interventions

Currently, there is no evidence to recommend psychological treatment, such as psychotherapy, stress management and patient's education, to all adults with IBD in general **[42]**.

1.6 Galectin-3

Galectins are a large family of widely distributed carbohydrate- binding proteins **[43]**.

Galectins are galactoside-binding lectins in modulating "cell-to-cell" and "cell-to-matrix" interactions in all organisms by binding to endogenous glycans. Mammalian galectins have either one or two highly conserved carbohydrate recognition domains (CRDs), recognizing-galactoside residues, to form complexes that crosslink glycosylated ligands. To date, 12 galectin-related proteins have been identified in humans **[44]**.

Galectins have been classified into three subgroups according to their CRD number and function:

(1) Proto-type galectins are generally smaller (14-16 KDa) than other and containing a single CRD .

(2) tandem-repeat galectins (galectin-4, -6, -8, -9, and -12), are generally larger (between 35-39 kDa) than other galectins. These galectins contain two distinct CRDs **[45]**.

(3) chimera-type galectin (galectin-3), which contains one CRD like the prototype group, but the CRD in Gal-3 is connected to a unique N-terminal domain of about 120 amino acids that are rich in proline and glycine. Gal-3 can form homo-dimers and oligomers through its N-terminal domain depending on the concentration and availability of the ligands. Furthermore, Gal-3 function depends not only on its oligomeric state in the extracellular space but also on its subcellular location, where Gal-3 monomers can be found either in the cytoplasm or nucleus **[46].**

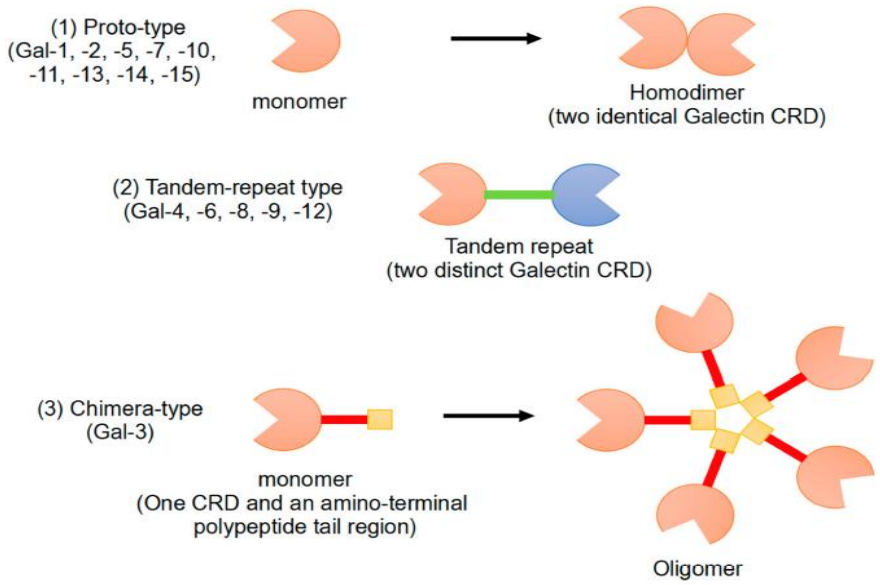


Figure 1-3: Schematic diagram of Galectin family and structure [50].

The N-terminal domain of Gal-3 is essential for its multimerization and may participate in the interaction with other intracellular proteins. The C-terminus is the CRD, consisting of about 135 amino acid residues; this is what defines the molecule as a galectin and is responsible for its interaction with glycol conjugates containing N-acetyl lactos amine **[47]**.

All members of galectin family were numbered consecutively by order of discovery (Figure 1-3), Galectins are ubiquitously present in vertebrates, invertebrates, and also protists **[48]**.

Note that Gal-3 is the only chimera-type galectin. Human Gal-3 is a 35-kDa protein that is coded by a single gene, LGALS3, located on chromosome 14, and is expressed in the nucleus, cytoplasm, mitochondrion, cell surface, and extracellular space **[49]**.

In relationship to inflammatory processes, galectin-3 has been mainly described as promoting inflammation **[50]**.

Galectins can also tailor adaptive immunity by influencing T-cell signaling and activation, modulating T-cell survival, controlling the suppressive function of regulatory T cells (Tregs) depending on its

intracellular or extracellular localization, respectively, altering the cytokine balance and regulating B-cell maturation and differentiation **[51]**. Galectin-3 (Gal-3) is a multifunctional protein that plays essential roles in various biological functions such as cell proliferation, apoptosis, migration, immune responses, cell adhesion, inflammation, angiogenesis and pathological phenomena such as cardiovascular remodeling, in various autoimmune and inflammatory processes, tumor progression as well as metastasis. The activities of galectins differ depending on the specific tissue and subcellular location **[52]**.

Galectin-3 are synthesized in the cytoplasm and found in both nuclear and cytoplasm compartment they are also secreted to the outer plasma membrane and the extracellular matrix (ECM) and are present in the circulation, suggesting a multi functionality of this molecule.

Extracellular Gal-3 modulates important interactions between epithelial cells and extracellular matrix glycoproteins or glycolipids, thanks to its collagen-specific protein sequence will bind their appropriate glycol receptor at the cell surface; initiating a signal transduction pathway, which drives gene expression of factors important for various biological functions **[53]**. In contrast, intracellular Gal-3 is important for cell survival due to its ability to block the intrinsic apoptotic pathway. In the nucleus, galectin‑3 promotes pre‑mRNA splicing and regulates gene transcription that affects cell proliferation, and cell cycle regulation, [figure 1-2].

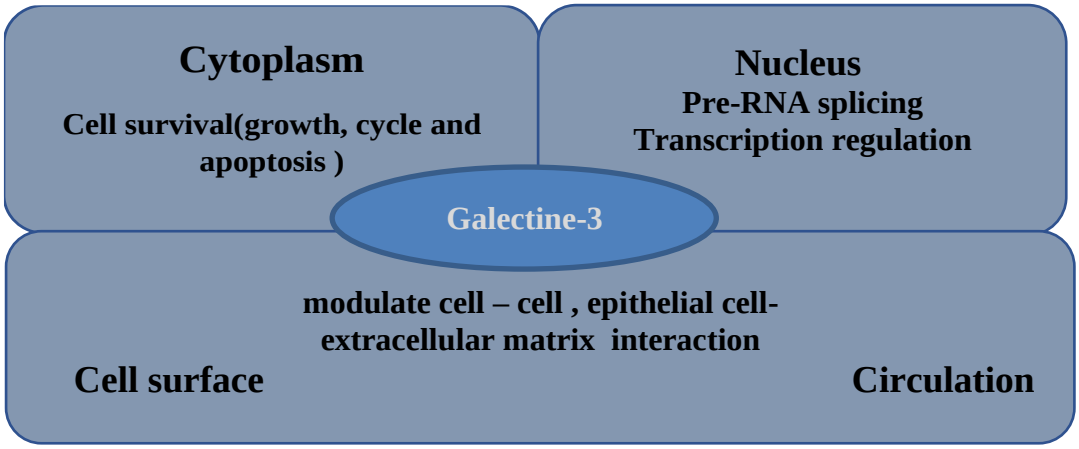


Figure1-2: Functions of Galection-3 in different locations including the cytoplasm, nucleus, cell surface and circulation [47].

Furthermore, many studies have revealed that Gal-3 expression is useful for detecting very early stage of many disease conditions, such as heart disease, kidney disease, diabetes mellitus, viral infection, autoimmune disease, neurodegenerative disorders, and tumor formation **[54]**.

1.7 Xanthine oxidoreductase

Xanthine oxidoreductase (XOR) is a member of a highly conserved family of molybdo-flavoenzymes that are widely distributed from prokaryotic to eukaryotic organisms and are hypothesized to derive from a common ancestral progenitor. In most living beings, the catabolism of hypoxanthine and xanthine to uric acid is ensured by the xanthine dehydrogenase activity (XDH, EC 1.17.1.4), but only mammals possess the xanthine oxidase (XO, EC 1.17.3.2), whose activity in milk was already described at the end of the 19th century **[55]**.

Xanthine oxidoreductase is present in all cell types, acting mainly as dehydrogenase and in most cases with a low level of activity. During phylogenesis, mammalian XOR has acquired the important regulatory function of producing ROS and NO. Thus, XOR is implicated in many biological processes, such as inflammation, repair and aging, in physiological pathways, such as cell growth, differentiation and mobility, and also in the regulation of both endothelial function and vascular tone **[56]**.

Expression of XOR is high in intestinal epithelial cells, where a barrier, microbicidal role has been proposed. The enzyme is particularly abundant in the first part of the digestive tract, goblet cells and enterocytes of the small intestine, especially in the basal and apical layers. It is also present in epithelial Paneth cells, which are recognized as cells that play an antimicrobial defensive role . The enzyme was also present in the apical cell layers of epithelia of the esophagus and tongue.

The normal subcellular localization of XOR is the cytosol, but it was also found in peroxisomes. In addition, XOR can be found in extracellular compartments, such as blood and milk **[57]**.

Mammalian XOR protein is a homodimer of approximately 300 kDa, in which each subunit has three domains (connected by unstructured hinge regions) [Figure 1- 4] **[58]**.

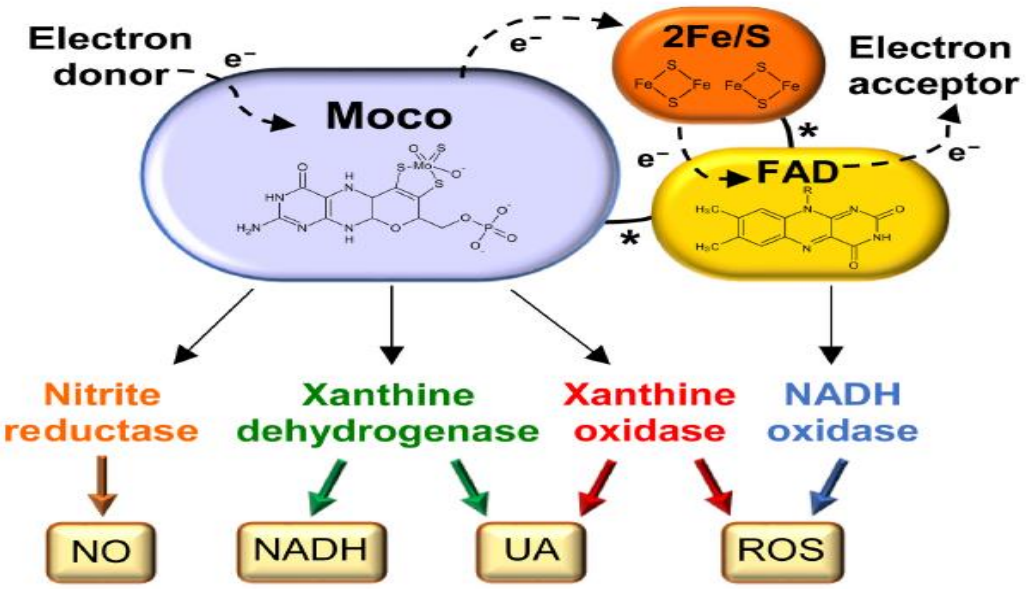


Figure 1-4: The structure and functions of mammalian xanthine oxidoreductase (XOR): consist of 20-kDa N-terminal domain (orange) has two non-identical iron-sulfur clusters (2Fe/S), the 40-kDa intermediate domain (yellow) has a flavin adenine dinucleotide (FAD) cofactor and the 85-kDa C-terminal domain (lilac) has a molybdopterin cofactor containing a molybdenum atom (Moco). [58].

Xanthine oxidoreductase shows low substrate specificity and highly versatile activity, which allow it to oxidize and reduce a number of endogenous and exogenous products, thus acting as a detoxifying and drug-metabolizing enzyme **[59]**.

Moreover, mammalian XOR is constitutively an NAD+ dependent dehydrogenase, which can be transformed in oxidase in a reversible way through the oxidation of two cysteine residues or irreversibly through a partial proteolysis of the fragment containing such cysteine groups. The transition from XDH to XO includes an intermediate XOR form with both dehydrogenase and oxidase activities, depending on the oxidation of only one of the two crucial sulfhydryl groups, when the enzyme is released from the cell into gastrointestinal lumen and urinary tract, as well as in biological fluids, such as milk and serum **[60]**.

In physiological conditions, human plasma contains only traces of

XOR, deriving from dead cells, in particular from hepatocytes, which release XOR into circulation, where the transition occurs from dehydrogenase to the oxidase form **[61]**.

Xanthine oxidoreductase binds with high affinity to the glycosaminoglycans on the surface of the endothelium. The XOR bound to endothelial cells acts as a systemic modulator of redox balance, setting some important endothelial functions. Endothelium-linked XOR generates reactive oxygen (ROS) and nitrogen (RNS) species, which activate endothelial cells and contribute to their permeabilization and the formation of phlogistic exudate **[62]**.

Xanthine oxidoreductase-derived oxidants also induce the expression of adhesion molecules on the inner surface of the microcirculation, thus promoting leukocyte activation, diapedesis and migration towards the phlogistic stimulus. Activated phagocytes release cytokines, increase XOR expression and produce cytocidal oxidant products. The contribution of XOR activity and products to inflammation is shown in [Figure 1-5] **[63]**.

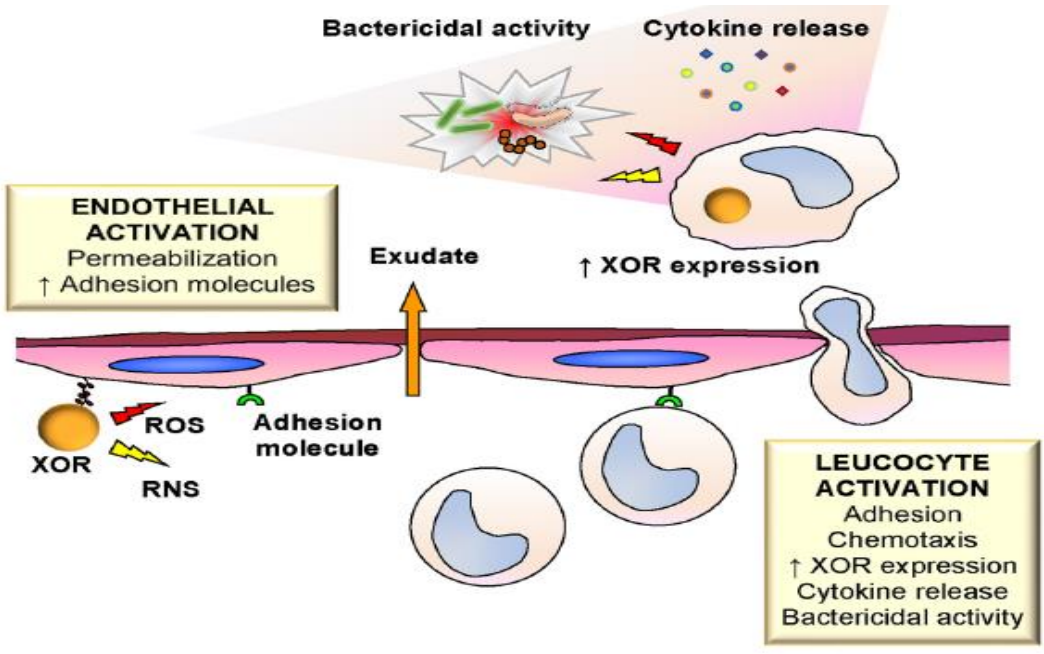


Figure 1-5: Activities of endothelium-linked human xanthine oxidoreductase and its products [63].

1.8 Iron (Fe)

One of the important trace essential minerals is iron, controls the differentiation and development of living cells, as well as Iron may interact and carry oxygen to various areas of the body via transfer of electrons across cells, which has an influence on genome synthesis, as well as contribute in a variety of metabolic process that are necessary to life **[64]**.

The liver is a major storage organ for iron and plays an important role in iron metabolism. It is the major production site of the iron regulatory peptides: ferritin, transferrin, and hepcidin. Liver derangements, therefore, have a direct effect on iron regulation **[65]**.

Most cases of anemia in patients with IBD result from functional or absolute iron deficiency. Functional iron deficiency is a state in which there is insufficient availability of iron for incorporation into erythroid precursors despite normal or increased body iron stores. In patients with absolute iron deficiency, iron is stored in the bone marrow. Other parts of the monocyte macrophage system in the liver and spleen become depleted, making iron unavailable for normal or increased rates of erythropoiesis. This may occur as the result of poor dietary intake of iron, reduced iron absorption, and/or increased blood loss **[66]**.

Anemia in IBD patients involves multiple pathogenic mechanisms resulting in low hemoglobin levels and compromised quality of life. Although ongoing blood loss from chronically inflamed intestinal mucosa and micronutrient deficiency (iron and B12) are the main mechanisms underlying the development of anemia in patients with IBD, chronic inflammation, hemolysis, and medication-induced myelosuppression may also play important roles in both the development of anemia and the management of this condition **[67]**.

1.9 Zinc (Zn)

Zinc is an essential trace mineral that is only second to iron in terms of body content. Adult humans have 2 to 3 grams of zinc, but it's difficult to know how much zinc they have, especially when they're sick **[68]**.

Zinc is required for the proper functioning of the immune system, cell division, cell growth, wound healing, carbohydrate breakdown, insulin action enhancement, and the sense of smell and taste. Zinc is required for optimal growth and development during pregnancy, infancy, and childhood **[69]**.

Zinc is absorbed in the small intestine by a carrier-mediated mechanism. The fraction of zinc absorbed is difficult to determine because zinc is also secreted into the gut **[70]**.

Loss of zinc through gastrointestinal tract accounts for approximately half of all zinc eliminated from the body. Considerable amount of zinc is secreted through the biliary and intestinal secretions, but most of it is reabsorbed.

Other routes of zinc excretion include urine and surface losses (desquamated skin, hair, sweat). Measurements in humans of endogenous intestinal zinc have primarily been made as fecal excretion; these indicate that amounts excreted are responsive to zinc intake, absorbed zinc and physiologic need **[71]**.

Zinc deficiency is common in patients with inflammatory bowel disease (IBD), during both active and remission phases, with a prevalence ranging from 15% to 40%. Studie on animal models and translational studies proved that decreased serum zinc concentrations may enhance inflammation through various pathophysiological mechanism, including disruption of epithelial barrier, altered mucosal immunity, and increased pro-inflammatory cytokines **[72]**.

A possible explanation for zinc playing an anti-inflammatory role in IBD could be related to its role in reducing the trans-mucosal leak in Crohn's disease, by decreasing the number of pro-inflammatory cells and reducing pro-inflammatory cytokine production **[73]**.

Zinc is a trace element known for its role in cell turnover and repair systems, several studies showing that correcting zinc deficiency can lead to restoring intestinal permeability in CD patients, probably due to its ability to modulate tight junctions both in the small and the large bowel **[74]**.

1.10 Phosphorus (P)

Phosphorus, an essential mineral, is naturally present in many foods and available as a dietary supplement. Phosphorus is a component of bones, teeth, DNA, and RNA. In the form of phospholipids, phosphorus is also a component of cell membrane structure and of the body's key energy source, ATP. Many proteins and sugars in the body are phosphorylated. In addition, phosphorus plays key roles in regulation of gene transcription, activation of enzymes, maintenance of normal pH in extracellular fluid, and intracellular energy storage **[75]**.

The absorption of phosphate in the intestinal epithelial cells occurs via a co-transport mechanism through active sodium/phosphate (Na+/Pi) co-transporters **[76]**. Upon intake of natural (non-enhanced) food, approximately 60% of the dietary phosphorus is absorbed in the intestine as phosphate. In normal adults, dietary phosphorus load is counterbalanced by a phosphorus excretion in urine that equals its net intestinal absorption. Urinary phosphorus excretion has been proposed as a recovery biomarker of total dietary phosphorus intake. An important assumption in the use of urinary phosphorus as a recovery biomarker is that dietary phosphorus intake is excreted at a constant rate in the urine for all individuals, regardless of dietary or non-dietary factors **[77]**.

Phosphate is essential in human physiology. An individual with a low serum phosphate level (<0.65 mmol/L) is considered to be at risk of experiencing clinical symptoms including fatigue, proximal muscle weakness, and bone pain. However, only a few case reports have documented specific acute clinical symptoms associated with hypophosphatemia. Furthermore, these symptoms are difficult to distinguish from the clinical manifestations of IBD and iron deficiency/iron deficiency anemia **[78]**.

Inflammatory bowel diseases (IBD) may affect bone metabolism and are frequently associated with osteopenia and osteoporosis.

hypophosphatemia can cause diarrhea and calcification (hardening) of organs and soft tissue, and can interfere with the body's ability to use iron, calcium, magnesium, and zinc **[79]**.

Malabsorption makes it difficult to absorb necessary nutrients in the small intestine. It can be caused by inflammation in the intestines.
The degree of malabsorption depends on how much of the small intestine is affected. Malabsorption and nutrient deficiencies are often more significant if larger sections of the small intestine are inflamed or have been surgically removed **[80]**.

1.11 Magnesium (Mg)

Magnesium is the fourth most common mineral in the human body after calcium, sodium, and potassium and is the second most common intracellular cation after potassium. Within the frame of a 70 kg individual, there is an average of 25 grams of Mg in reserve with 53% in bone, 27% in muscle, 19% in soft tissues, and less than 1% in the serum. Although serum Mg concentration (SMC) is tightly controlled with a normal serum value of 75–95 mmol/L, some research would indicate that serum levels less than 85 mmol/l should be considered deficient **[81]**.

Magnesium serves as a cofactor for over 600 enzymes. Most notably, magnesium is required for energy production, carbohydrate metabolism, DNA and protein synthesis. Magnesium is also an antagonist of calcium in the body and is required for vitamin D synthesis and activation **[82]**.

Magnesium deficiency increases blood pressure, reduces insulin sensitivity, and causes neural excitation. Consequently, low serum magnesium levels are associated with a wide variety of conditions, including obesity, type 2 diabetes, cardiovascular disease, metabolic syndrome, and osteoporosis **[83]**.

Magnesium deficiency is a frequent complication of inflammatory bowel disease (IBD) demonstrated in 13-88% of patients. Decreased oral intake, malabsorption ,increased intestinal losses and surgical resection (removal) of portions of the intestines, especially the small intestine are the major causes of Mg deficiency.

The complications of Mg deficiency include: cramps, bone pain, delirium, acute crises of tetany, fatigue, depression, cardiac abnormalities, urolithiasis, impaired healing and colonic motility disorders **[84]**. Serum Mg is an insensitive index of Mg status in IBD. Twenty-four-hour urinary excretion of Mg is a sensitive index and should be monitored periodically. Parenteral Mg requirements in patients with IBD are at least 120 mg/day or more depending upon fecal or stromal losses. Oral requirements may be as great as 700 mg/day depending on the severity of malabsorption **[85]**.

1.12 Vitamin E

Vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols. Vitamin E deficiency, which is rare and usually due to an underlying problem with digesting dietary fat rather than from a diet low in vitamin E, can cause nerve problems. Vitamin E is a fat-soluble antioxidant which may help protect cell membranes from reactive oxygen species. and protects membrane lipids from peroxidation by scavenging peroxyl, oxygen and superoxide anion radicals **[86]**.

Vitamin E has also been shown to have prominent anti-inflammatory effects. With its high antioxidant capacity and anti-inflammatory activity, vitamin E would be expected to reduce injury and/or improve tissue after injury from ulcerative colitis **[87]**.

Tocotrienols and tocopherols, are absorbed from the intestinal lumen, incorporated into chylomicrons, and secreted into the portal vein, leading to the liver. Absorption efficiency is estimated at 51% to 86%, and that applies to all of the vitamin E family–there is no discrimination among the vitamin E during absorption **[88]**. Unabsorbed vitamin E is excreted via feces. Additionally, vitamin E is excreted by the liver via bile into the intestinal lumen, where it will either be reabsorbed or excreted via feces, and all of the vitamin E are metabolized and then excreted via urine **[89]**.

Oxidative stress is associated with inflammatory bowel diseases, which increases the need for antioxidants in these patients, especially during exacerbation of the disease. Vitamin E is component protects the DNA against damage. In addition, it has anti-inflammatory and anti-edema effects on the skin, which may be particularly beneficial in the case of extra-intestinal symptoms of inflammatory bowel diseases **[90]**.

Aim of the study

This study was aimed to:

1- Measuring serum level of Galectin-3 and xanthene oxidoreductase in patients with inflammatory bowel diseases and healthy control.

2- Measuring zinc, iron, phosphorus and magnesium in patients with inflammatory bowel diseases and healthy control.

3- Measuring serum level of vitamin E (as antioxidant) in patients with inflammatory bowel diseases and healthy control.

4- Study the relationship between parameters.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Materials and Patients

2.1.1 Chemicals and kits

Chemicals and kits that used in this study were listed in table (2-1):

Table 2-1: Chemical and kits used in the study

No.	Chemicals	Origin
1.	Human Galectin-3 ELISA kit	BT Laboratory (China)
2.	Human Vitamin E ELISA kit	BT Laboratory (China)
3.	Human XOR ELISA kit	BT Laboratory (China)
4.	Iron kit	Liquicolor/ China
5.	Magnesium Kit	Abbott/Germany
6.	Phosphorus Kit	Abbott/Germany
7.	Zinc Kit	Spectrum/ China

2.1.2 Instruments and Tools

The instruments and equipment used in this study are shown in table (2-2):

Table 2-2: Instruments and equipment used in the study

No.	Instruments and Materials	Origin
1.	Centrifuge EBA 20	Hettich (Germany)
2.	Deep Freeze	GFL / Germany
3.	Distillator	Bibby science (England)
4.	Mico Plate Reader	Bio-tech instruments (USA)
5.	Eppindorf tube (0.5 ml)	China
6.	Gel tube	Al-Rawan/ Iraq
7.	Incubator	Fisher scientific (germany)
8.	Magnetic Stirrer with Hot plate	Grant / England
9.	Multiple micropipettes	Watson Nexty (Japan)
10.	Spectrophotometer	Cecil 7200 (Germany)
11.	Vortex (Electronic)	Kunkel /Germany

2.1.3 Study Design

This is a case control study.

The present study included 100 subjects with age range (15-65) years old with normal BMI.

The subjected in this case-control study included two groups as following:

Group (I): included (50) patients with inflammatory bowel disease.

Group (II): included (50) apparently healthy subjects as control.

2.1.4 Research and Sampling Ethics

Depends on the following:

a- Approval of scientific committee in Chemistry and Biochemistry Department , in College of Medicine at University of Babylon.

b- Approval of scientific committee of Merjan Medical City in Hilla City, Babylon Province.

c- The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance.

2.1.5 Patients selection

This study was carried out on patients attended to Gastro Intestinal and Hepatic center in Merjan Medical City, Hilla city, Babylon province, Iraq. During the duration from August 2022 to January 2023. All patients were diagnosed based on standard clinical, radiological, endoscopic, and histological criteria. The practical side of the study was performed at the laboratory of Biochemistry Department/ College of Medicine in University of Babylon .

2.1.6 Exclusion criteria

patients have the following conditions were excluded :

- Pregnancy.
- Concomitant acute or chronic inflammatory disorders
- Chronic diseases, including heart failure, renal insufficiency, liver injury, diabetes.
- Patients with other associated malignancy in the body.

2.1.7 Questionnaire

The socio- demographic characteristics that composed of age, sex, family history and smoking habits.

2.1.8 Sample collection

Five milliliters of venous blood was obtained from inflammatory bowel disease patients and control, by 5ml disposable syringe, the blood was drained into gel plain tube and were left for 15 minutes at room temperature to clot. After that , the blood samples were centrifuged for 10 minutes. Then the serum were aspirated and stored at (-20 ºC) until time of use.

2.2 Biochemical measurement

2.2.1 Determination of human Galectin-3 concentration

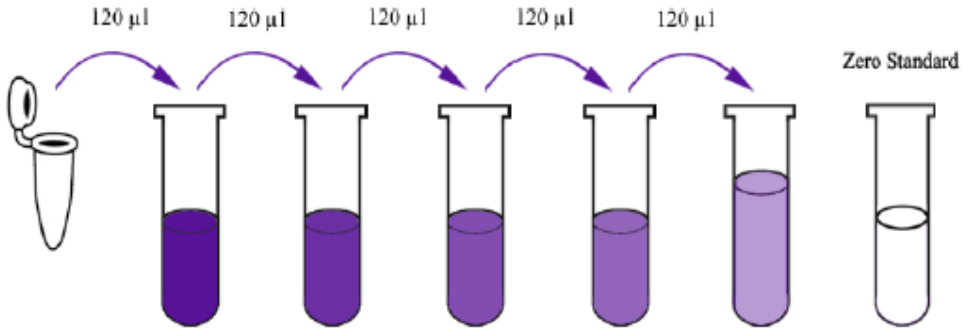
A. Principle :

This ELISA kit uses sandwich–ELISA as the method for the accurate quantitative detection of human Galectin-3. The plate has been pre-coated with human Galectin-3 antibody. Galectin-3 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human Galectin-3 Antibody is added and binds to Galectin-3 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated Galectin-3 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human Galectin-3. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm **[91]**.

B.Reagents Preparation

Wash buffer: A volume of 20 ml of concentrated wash buffer was diluted into 480 ml of distilled water to yield 500 ml of washing buffer.

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with 120µl of reference standard & sample diluent. The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 2400pg/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: 1200, 600, 300, 150, 75 and 0 pg/ml. The standard was prepared within 15 minute before use.



2400 pg/ml 1200 600 300 150 75 0

Figure 2-1: The standard reference of dilution method for galectin-3

C- <u>Procedure</u> :

1- All reagents were prepared at room temperature before use .

2-Fifty microliter of the each standard was added to the standard well.

3- A volume of 40 µl of sample and then 10 µl of anti-Galectin-3 antibody were added to testing sample wells.

4- A volume of 50 µl of streptavidin -HRP reagent was dispensed into each well .Covered with a sealer and incubated for 60 minutes at 37 $_{o}$C .

5- The wash process was repeated for five times by filling each well with wash buffer (approximately 400 µl).

6- The liquid was removed at each step was essential to good performance then remaining wash solution was removed by aspirating and the plate was invert and blot it against clean paper towels .

7- Substrate solution A (50µl) was added to each well and then added 50µl substrate solution B to each well

8-The microplate wells covered with a new sealer then incubated for 10 minutes at 37 $_{o}$C .

9-The reaction was stopped by adding 50 µl of stop solution to each well . The color was changed from blue to yellow color .

10-The absorbance was read the optical density at 450 nm using microtiter plate reader within 10 minutes. The standard curve is depicted in figure 2-2.

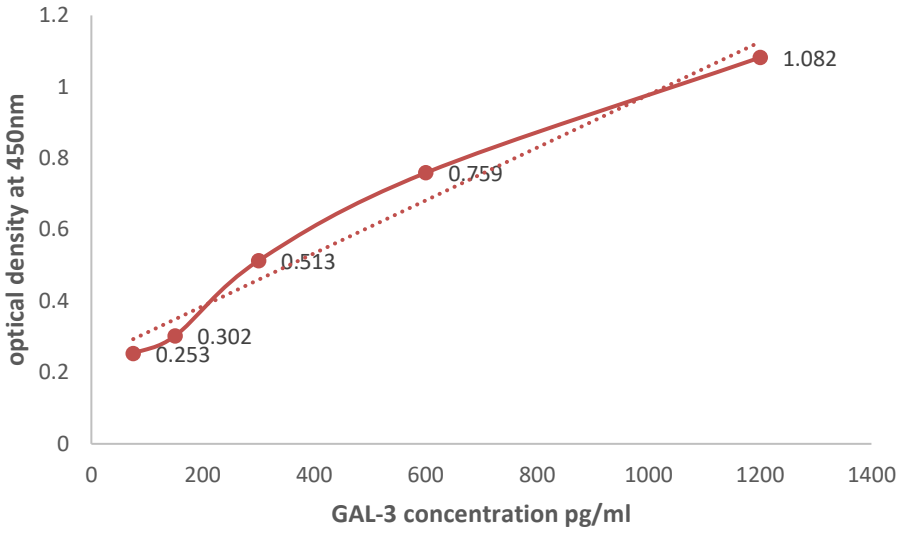


Figure 2-2 : **Standard curve for Galectin-3 concentration by ELISA**

2.2.2 Determination of human Xanthine Oxidase concentration

A. Principle and procedure are mentioned in 2.2.1

B.Reagents Preparation

Wash buffer: A volume of 20 ml of concentrated wash buffer was diluted into 480 ml of distilled water to yield 500 ml of washing buffer.

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with 120µl of reference standard & sample diluent. The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 128 ng/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: 64, 32, 16, 8, 4 and 0 ng/ml. The standard was prepared within 15 minute before use.

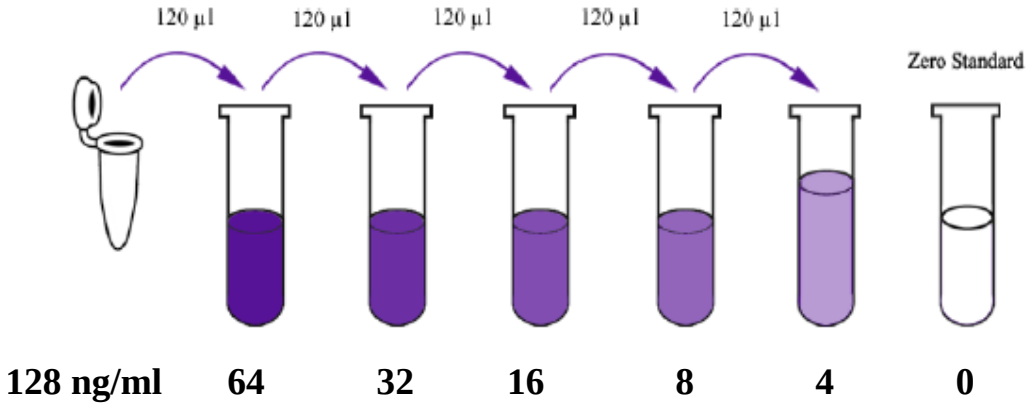


Figure 2-3: The standard reference of dilution method for Xanthine Oxidoreductase

C- Calculate :

The standard curve is depicted in figure 2-4.

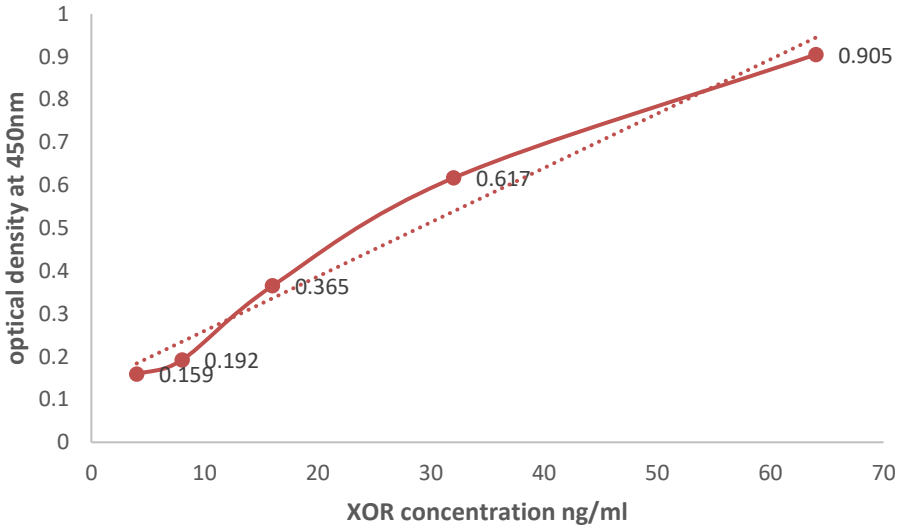


Figure 2-4 : **Standard curve for Xanthine Oxidase concentration by ELISA**

2.2.3 Spectrophotometric Determination of serum Iron Concentration

Iron concentration was measured by colorimetric test with Lipid Clearing Factor (LCF) CAB Method.

Principle

Iron (lll) reacts with chromazurol B (CAB) and cetyltrimethylammonium bromide (CTMA) to form colored complex. This complex has an absorbance at 623 nm. Intensity of the complex formed was directly proportional to the amount of iron present in the sample.

Reagent Composition

-Standard (Iron) 100 µg/dl

-CAB 0.18 mmol/L

-CTMA 2.2 mmol/L

-Guanidinium chloride 2.6 mol/L

-sodium acetate Buffer (PH 4.7) 45 mmol/L

Reagent Preparation: Reagents are ready to use.

Procedure

Table 2-3: The Additions

Addition Sequence	B	S	T
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	---	50 µl	---
Sample	---	---	50 µl

The Additions were mixed well and incubated at (25ºC) for 15 mins. The absorbance was measured of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank .

Calculations

$$\text{Iron in } \mu g/dl = \frac{\text{Abs.T}}{\text{Abs.S}} \times 100 \ \mu g/dl \text{ concentration of S.}$$

Expected Values:

Male 59-158 µg/dl

Female 37-145 µg/dl

2.2.4 Spectrophotometric Determination of serum Zinc Concentration

Zinc concentration was measured by colorimetric test with 5-Bromo-PAPS.

Principle

Zinc in an alkaline medium reacts with 5-Bromo-PAPS to form a purple colored complex. Intensity of the complex formed was directly proportional to the amount of zinc present in the sample.

Reagent Composition

-Standard 200µg/dl

Reagent (R)

-5-Br-PAPS 0.02 mmol/L

-Bicarbonate Buffer Ph 9.8 200 mmol/L

-Sodium citrate 170 mmol/L

-Dimethylglyoxime 4 mmol/L

-Detergent 1%

Reagent Preparation: Reagents are ready to use.

Procedure

Table 2-3: The Additions

Addition Sequence	B	S	T
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	---	50 µl	---
Sample	---	---	50 µl

The Additions were mixed well and incubated at (25ºC) for 10 mins. The absorbance was measured of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank .

Calculations

$$\text{Zinc in µg/dl} = \frac{\text{Abs.T}}{\text{Abs.S}} \times 200 \text{ µg/dl concentration of S.}$$

Expected Values:

Male 72.6-127 µg/dl

Female 70.8-114 µg/dl

2.2.5 Determination of serum Phosphorus Concentration

Princple:

Inorganic Phosphate react with ammonium molybdate to form a heteropolyacid complex (phosphomolybdate). This complex has an absorbance in ultraviolet range and is measured at 340 nm. Intensity of the complex formed was directly proportional to the amount of inorganic phosphorus present in the sample **[94]**.

Reagent Composition

R1: Sulphuric acid 665 mmol/L

R2: Ammonium molybdate 2.3 mmol/L

Sulphuric acid 665 mmol/L

Reagent Preparation: Reagents are ready to use.

Procedure and Calculation:

The analyzer automatically calculates the analytic concentration of each sample**.**

Expected Values:

For Adult 2.3 - 4.7 (mg/dl) or 0.74 - 1.52 (mmol/L)

2.2.6 Determination of serum Magnesium Concentration

Magnesium concentration was measured by ARCHITECT Systems and the AEROSET System [ABBOTT clinical chemistry].

Princple:

This method utilizes an arsenazo dye which binds preferentially with magnesium to form arsenazo-magnesium complex. This complex has an absorbance in ultraviolet range and was measured at 572 nm. Intensity of the complex formed was directly proportional to the amount of magnesium present in the sample **[94]**.

Reagent Composition:

-TRIS Buffer 100 mmol/L
-Arsenazo Dye 0.13 mmol/L
-Sodium Azide 0.1%

Reagent Preparation: Reagents are ready to use.

Procedure and Calculation:

The analyzer automatically calculates the analytic concentration of each sample**.**

Expected Values:

For Adult 1.6 - 2.6 (mg/dl) or 0.66 - 1.07 (mmol/L)

2.2.7 Determination of human Vitamin E concentration

A. Principle and procedure are mentioned in 2.2.1

B.Reagents Preparation

Wash buffer: A volume of 20 ml of concentrated wash buffer was diluted into 480 ml of distilled water to yield 500 ml of washing buffer.

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with 120µl of reference standard & sample diluent. The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 160 nmol/L. Then serial dilutions were made as needed. The recommended concentrations were as follows: 80, 40, 20, 10, 5 and 0 nmol/L. The standard was prepared within 15 minute before use.

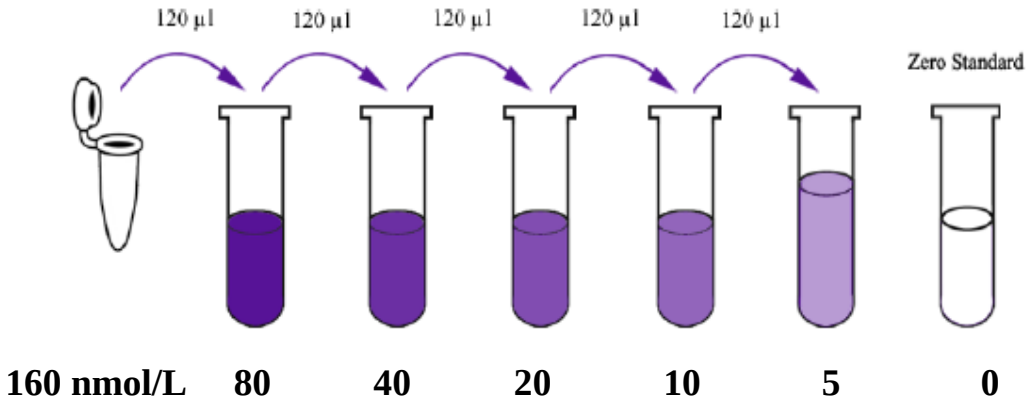


Figure 2-5: The standard reference of dilution method for vitamin E

C- Calculate:

The standard curve is depicted in figure 2-6.

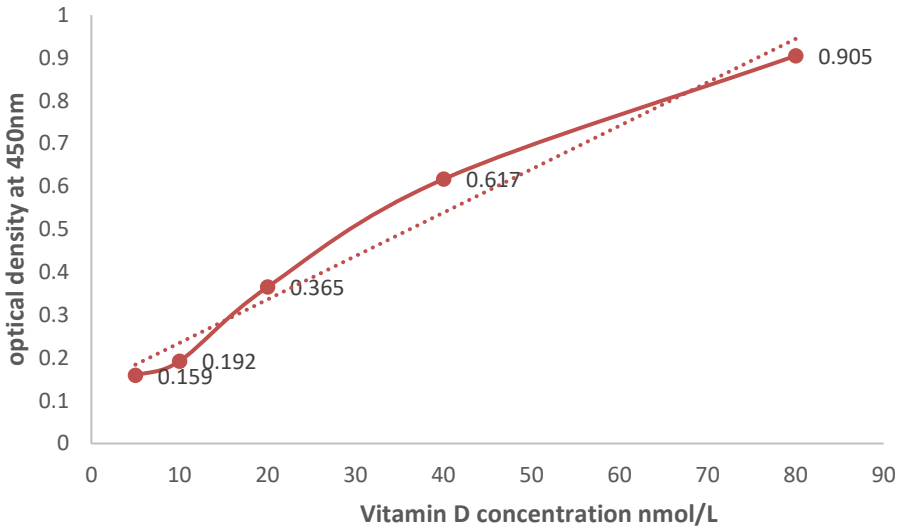


Figure 2-6 : **Standard curve for Vitamin E concentration by ELISA**

2.3 Statistical Analysis

Statistical analysis was carried out using SPSS version 22. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Analysis of variance (ANOVA), student's t-test and the linear regression analysis were used for the evaluation of data . A p-value of ≤ 0.05 was considered as significant.

Chapter Three

Results and Discussion

3.Result and Discussion

3.1 General Characteristic of the Study group.

3.1.1 Age

The present case control study included 50 patients with inflammatory bowel diseases with a mean age 39.32 ± 11.63 years and an age range of 15-65 years. Besides, the study included 50 apparently healthy individuals with a mean age of 35.06 ± 12.34 years and an age range of 15-59 years, shown in Table 3-1.

Table 3-1: Independent sample t test to compare between ages for IBD patients and control groups.

A variable Parameter	Control (n=50) Means ± SD	IBD patients (n=50) Means ± SD	P-value
Age (years)	35.06 ± 12.34	39.32 ± 11.63	p > 0.05 (NS)
Range	15-59	15-65	

SD: standard deviation; NS: non-significant at p > 0.05.

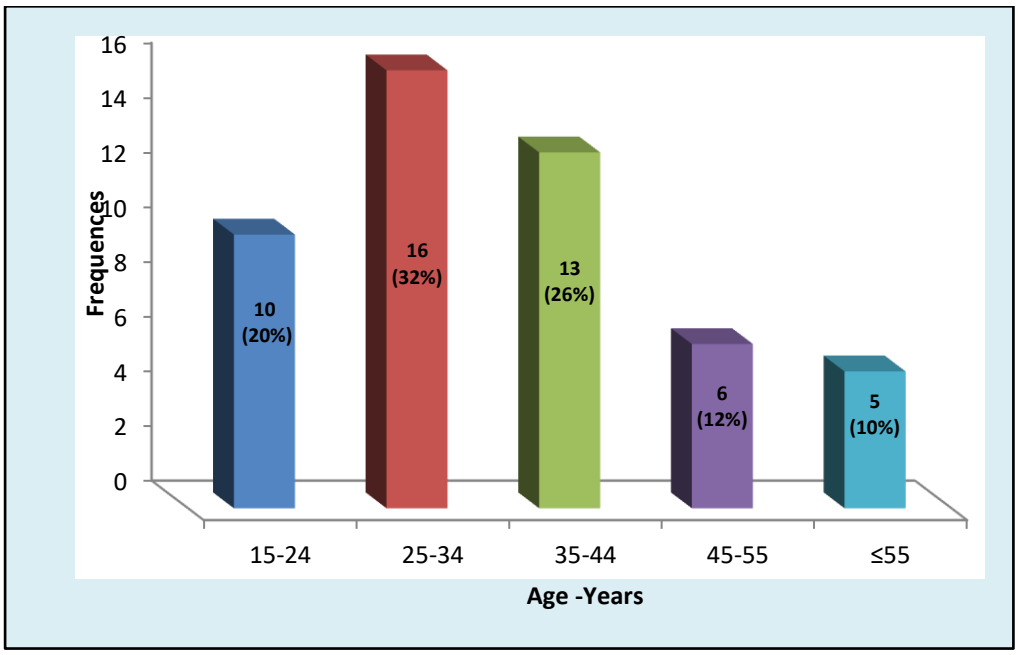


Figure 3-1 : The frequency distribution of age

Age distribution of present study (figure 3-1), showed that most people were in the age range of 25-34 years, and this was relabeled with other reviews like Shubbar A H, et al. **[95]** 35.5 year in Iraq, Feshareki R, et al. **[96]** 35 year in Korea and by Leong RW, et al. **[97]** 34 year in China**,** while it is lower in study done by Morita N, et al. **[98]** 24 year in Japan.

The study demonstrated that the mean age of patients with inflammatory bowel diseases was 39.32 years old, and this finding is near the mean age of Cibor D, et al **[99]**.

Besides, several studies **[100,101]** demonstrated that the mean and median ages at the time of diagnosis for patients with CD are, in general, 5–10 years earlier than those of patients diagnosed with UC, while previous study **[102]** reported that Crohn's disease affects mainly people within age of 15–30 years and the peak incidence of Ulcerative colitis between 15-25 years.

3.1.2. Sex

The study includes 50 patients with inflammatory bowel diseases of both sexes, 27 (54%) men and 23 (46%) women. Men were at higher risk to get inflammatory bowel diseases as compared with women, as shown in table (3-2) and figure (3-2).

Table 3-2: Distribution of sex according to studied group

Sex	Control (n=50)	IBD patients (n=50)
Female n(%)	20 (40%)	23 (46%)
Male n(%)	30 (60%)	27 (54%)

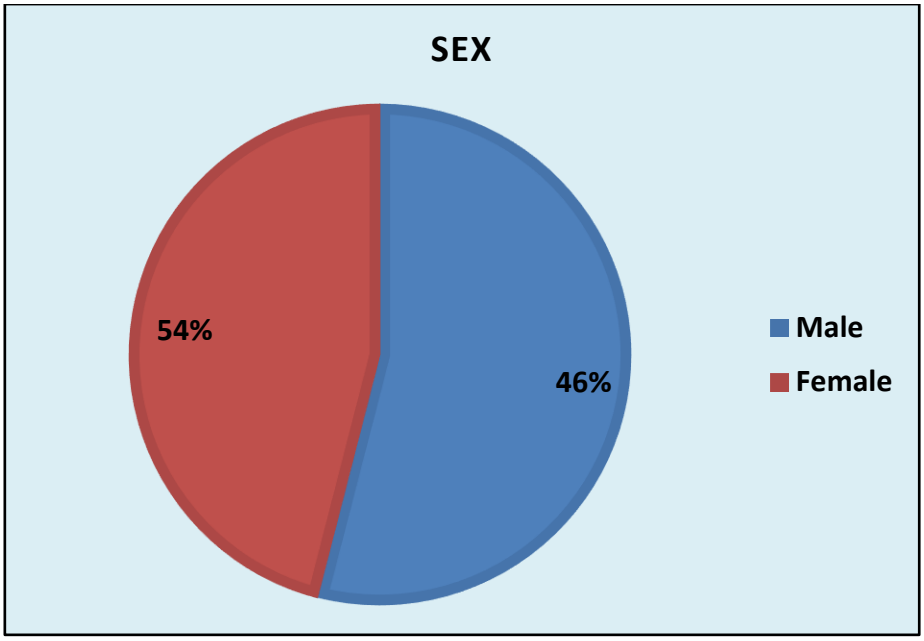


Figure 3-2: The ratio of females to males in IBD patients.

Our finding was in agreement with the study of Fallahi G H et al. **[103]** in Iran in which male patients are predominant and the ratio was (1.5/1). While Amira et al. **[95]** in Iraq and Vahedi H et al. **[104]** in Iran show a slight female sex predominance for IBD patients.

3.1.3 Smoking and inflammatory bowel diseases

According to the history of IBD patients participated in this study 11 male patients was a heavy smoker for a long time. The rate of smokers to a nonsmoker in patients and control groups is represented in Table (3-3):-

Table 3-3: Distribution of smoking according the study group

Groups	Control (n=50)	IBD patients (n=50)
Smokers n(%)	20 (40%)	11 (22%)
Non –smokers n(%)	30 (60 %)	39 (78%)

Cigarette smoking is the most risk factor for Crohn's disease **[105]** demonstrated that smoking cigarettes definitely increase the risk of Crohn's disease, In contrast, smoking is not a risk factor for ulcerative colitis and may be protective of the development of it.

The risk of Crohn's disease increased gradually by smoking duration **[106]** Smoking produces a shift in the normal balance between oxidants and antioxidants to impact an oxidative stress systemically. Oxidants included in cigarette smoke can directly injure cells and tissues, inactivate defense mechanisms, and initiate inflammation, which further elevates oxidative stress **[107]** .

3.2 Galectin-3 concentration in patients and control group

Results in Table (3-4) shows increase concentration of Gal-3 in inflammatory bowel diseases patients compared with the control with significant mean differences between them.

Table 3-4: Independent sample t test to test the difference of Galectin-3 in IBD patients and control.

Control (n=50) Means ±SD	IBD patients (n=50) Means ±SD	T test	P value
179.88 ± 66.24	245.52 ± 39.7	6.010	<0.001 (Sig.)

SD: standard deviation; Sig.: significant difference between groups at (P value < 0.05)

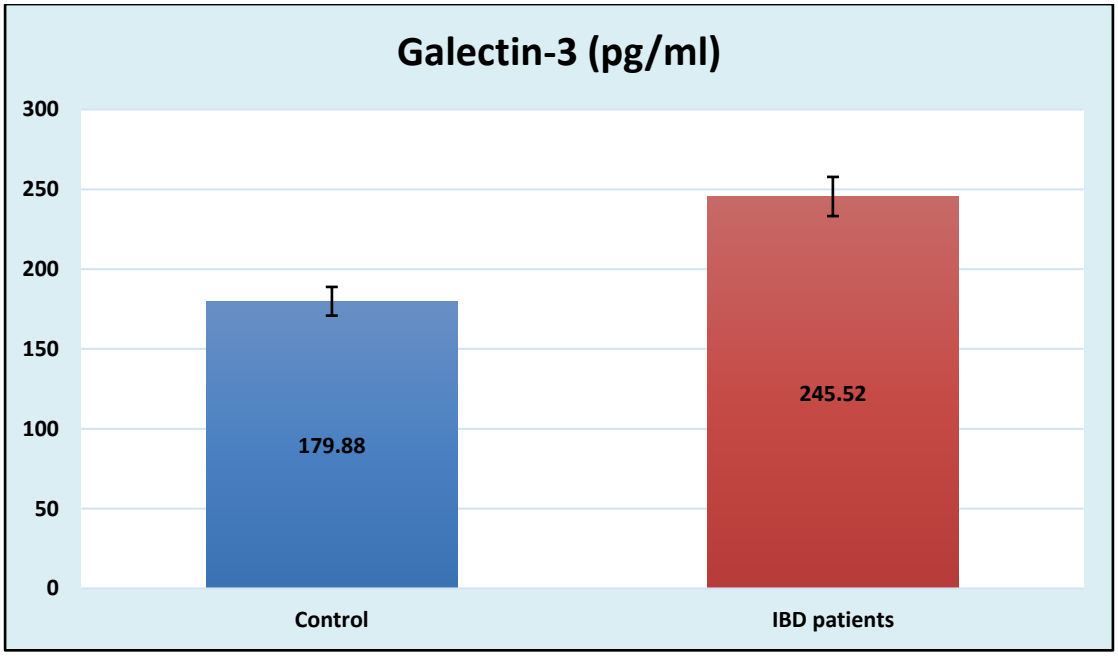


Figure 3-3: Compare Galectin-3 for both control and IBD patients' group.

Galectin‑3 may be used as prognostic biomarker for various types of disease, and therefore several studied have investigated the prognostic value of gal-3 in IBD.

Galectin-3 is a member of the galectin gene family that is expressed at elevated levels in a variety of neoplastic cell types and has been associated with cell growth, cellular adhesion process, cell proliferation, transformation, metastasis, and apoptosis **[108]**. Thus the levels of serum Gal-3 was measured in patients with IBD and control to evaluate its role in IBD. Results in Table (3-4) shows increase concentration of Gal-3 in inflammatory bowel diseases patients compared with the control with significant mean differences between them.

Johannes L, et al. **[109]** suggested that the presence of galectins in serum may result from leakage from tissue, thereby making it a possible disease biomarker. Our findings consistent with the results of Frol'ova L et al, **[110]** who showed an elevation of plasma Gal- 3 activity in both UC and CD patients compared to levels in controls.

Several studies of IBD mouse models have reported the importance of galectins in the inflammatory process. Papa Gobbi, et al. did not find any difference in Gal-3 expression between IBD patients and the control group, or between CD and UC patients **[111]**.

This discrepancy might have resulted from the occurrence of some differences in factors that affected the levels of Gal-3 such as age, renal function, and concomitant diseases as well as various sources of commercial ELISA kits used for Gal-3 measurement **[99]**.

Gal-3 is a chemoattractant and adhesion factor, which activates monocytes/macrophages, promotes adhesion of neutrophils, and takes part in eosinophil recruitment **[112]**.

While Xue F M, et al. reported that expression of CD98+ eosinophils in intestine biopsies was upregulated in IBD patients **[113]**. After activation, dendritic cells express Gal-3, which is a ligand for CD98. Gal-3 binds CD98, resulting in activation of eosinophils, which release inflammatory mediators and induce inflammation in the intestine.

3.3 Xanthine Oxidoreductase concentration in patients and control group

Present study revealed that XOR concentration are higher in IBD patients with significant different when compared with the control group, as shown in Table 3-5.

Table 3-5: Independent sample t test to test the difference of XOR in IBD patients and control.

Parameter	Control (n=50) Means ±SD	IBD patients (n=50) Means ±SD	T test	P value
XOR (ng/ml)	25.3 ± 9.5	33.36 ± 11.95	5.348	<0.001 (Sig.)

SD: standard deviation; Sig.: significant difference between groups at (P value < 0.05)

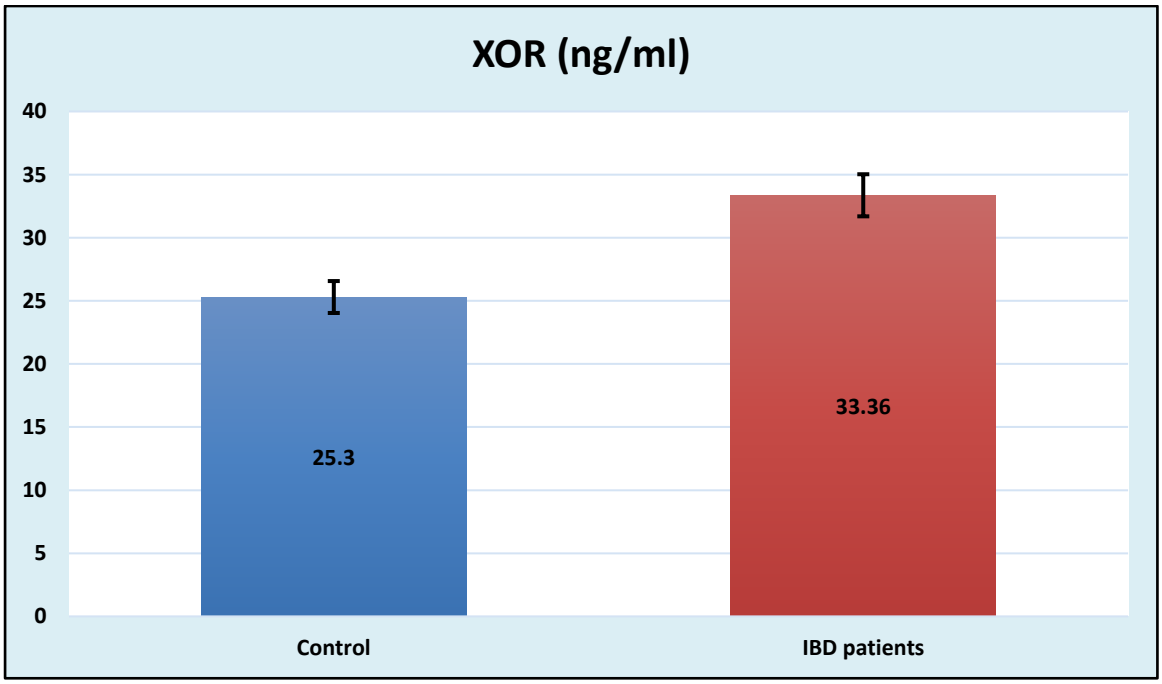


Figure 3-4: Compare XOR for both control and IBD patients' group.

The current results are consistent with Meijer B**,** et al. **[114]** who observed a high XOR-activity in IBD patients, while Gibbings S, et al.**[115]** found that XOR activity was not affected in IBD patients, and this is opposite present finding.

XOR products can promote a pro-oxidant and pro-inflammatory state by regulating endothelial function, since ROS and RNS increase the permeability of the vascular lining **[116]**.

Expression of XOR is high in intestinal epithelial cells, where a barrier, microbicidal role has been proposed. The enzyme is particularly abundant in the first part of the digestive tract, and it is found in goblet cells and enterocytes of the small intestine, especially in the basal and apical layers. It is also present in epithelial Paneth cells, which are recognized as cells that play an antimicrobial defensive role **[117]**. In ultrastructural studies of the rat digestive tract, Van den Munckhof et al. detected XOR activity in enterocytes, in goblet cells, and in the mucus of the duodenum **[118]**.

The enzyme was also present in the apical cell layers of epithelia of the esophagus and tongue. Bacteria, apparently in the process of being destroyed, were clearly seen to be surrounded by XOR in the cornified layer. These authors proposed that XOR has an antimicrobial function in the gut resulting from the generation of ROS and RNS.

Thus, Increased XOR activity could induce oxidative stress through ROS, RNS and uric acid production **[119]**.

3.4 Iron, Zinc, Magnesium and Phosphorus concentration in patients and control group

It appears that nutrition is important in both the prevention and treatment of IBD. Abundant evidence shows high prevalence of malnutrition (deficiency of macro- and micronutrients) in patients with IBD, due to the greater extent of gastrointestinal involvement **[120]**.

Micronutrients are involved in the pathogenesis of IBD through affecting the immune responses, their involvement in the identification and response to pathogens and maintenance of the integrity of the GI tract **[121]**.

Iron is crucial to humans' biological functions and cellular biochemical processes. Both iron deficiency and iron overload pose significant and potentially fatal health risks, and the homeostasis of iron is tightly regulated. When this regulation is disrupted leads to cellular toxicity, tissue injury, and organ fibrosis. Such harmful effects are mediated through the deposition of iron in parenchymal cells of a number of vital organs, including the heart, pancreas, and liver **[122]**.

In this study, serum iron concentration was detected in both groups, serum iron decreased significantly in IBD patients compared with healthy control ($P<0.05$), as in Table 3-6.

Table 3-6: Independent sample t test to test the difference of iron, Zinc, magnesium and phosphorus in IBD patients and control.

Parameters	Control (n=50) Means ±SD	IBD patients (n=50) Means ±SD	T test	P value
Iron (mg/dl)	111.38 ± 21.96	85.24 ± 36.05	4.378	<0.001 **(Sig.)**
Zinc (mg/dl)	97.76 ± 14.07	71.08 ± 16.16	8.802	<0.001 **(Sig.)**
Magnesium (mg/dl)	2.17 ± 0.26	1.94 ± 0.25	4.290	<0.001 **(Sig.)**
Phosphorus (mg/dl)	3.26 ± 0.71	3.59 ± 0.69	2.33	>0.05 **(Non-Sig.)**

SD: standard deviation; Sig.:Non- significant difference between groups at (P value > 0.05)

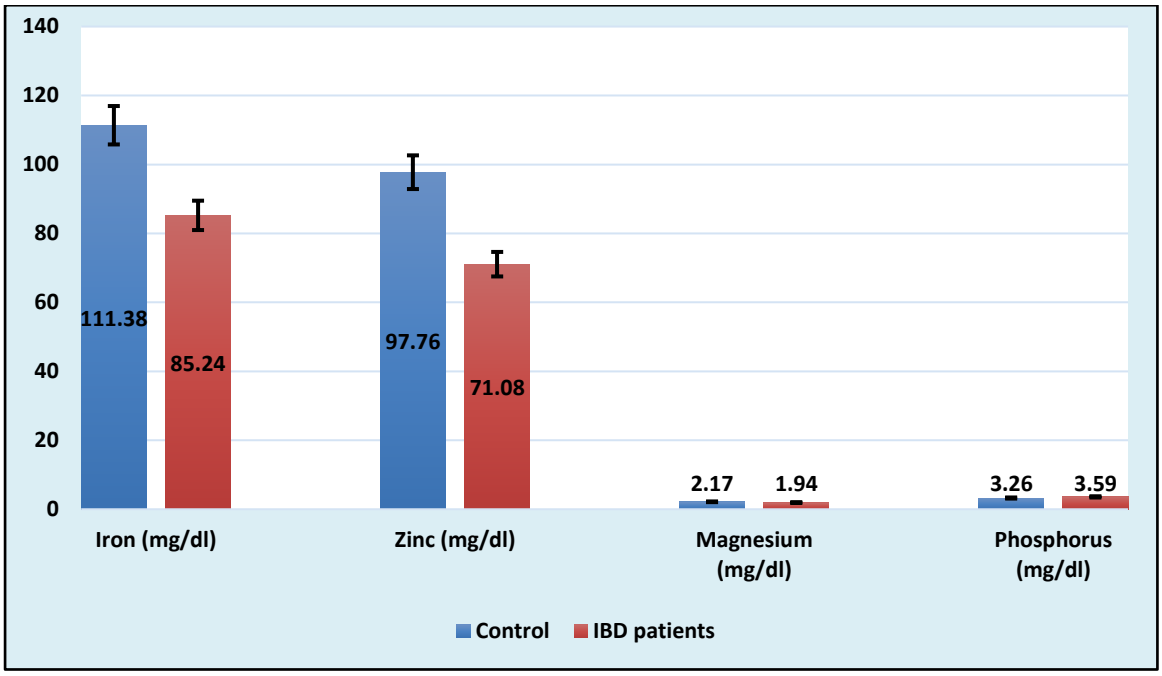


Figure 3-5: Compare Iron, Zinc, Magnesium, Phosphorus for both control and IBD patients' group.

This result was explained by Capellini, et al. **[123]** who found that Iron deficiency, even in the absence of anemia, can be debilitating, and exacerbate any underlying chronic disease, leading to increased morbidity and mortality. Iron deficiency is frequently concomitant with chronic inflammatory disease.

Iron deficiency affects between 13 and 90% of patients with IBD, depending on the population that is studied, as well as severity of the disease. The main causes of iron deficiency in IBD arise from impaired GI iron absorption due to chronic inflammation, bowel resection (especially in Crohn's disease), disease triggered malnutrition and (mainly chronic) blood loss **[124]**.

Zinc is an essential micronutrient that appears to play a role in IBD pathogenesis. This trace element is a cofactor for a number of enzymes, which is involved in the development of innate immunity, and has anti-inflammatory effects. Zinc deficiency has been associated with diarrhea and risk of GI infectious diseases **[125]**.

The present study confirmed significant zinc deficiency in IBD patients compared to control ($P<0.05$), table [3-6]. Current study is in agreement with Vagianos K, et al who revealed low serum zinc levels have been reported in nearly one third of CD patients**[126]** .

While Griffin IJ, et al. demonstrated non-significant difference in zinc level between patients and control and there are several reasons for zinc deficiency in IBD including low GI absorption, increased loss, and reduced intake due to anorexia**[127]** .

Chronic latent Mg deficiency is an important underlying pathology in many clinical conditions.

Present study identified magnesium deficiency among IBD patients compared to healthy controls with significant mean difference ($P<0.05$), table [3-6].

This finding is concomitant greatly with Hekmatdoost A, et al., who revealed reduced gastrointestinal absorption of Mg occurs in IBD patients. Patients with IBD consumed a lower amount of Mg than healthy adults **[128]**.

In contrast, Perez J C, et al. did not identify significant differences between IBD and healthy children regarding magnesium hair levels **[129].**

Magnesium absorption is reduced with aging by as much as 30%. Space flight and microgravity environments result in accelerated aging with decline of cardiovascular function (increased oxidative stress, insulin resistance, inflammation, and mitochondrial damage). Mg protects against these adverse effects and the shortening of telomeres seen with lower Mg and a reduction of life expectancy **[130]**.

There was a correlation between magnesium concentration and altered psychological status, anxiety, and depression.

Magnesium deficiency has an impact on psychological well-being in addition to the physical manifestations such as fatigue, muscular cramps and arrhythmia. Hypomagnesemia has previously been linked to depression in people with IBD **[131]**.

Phosphate homeostasis is important for life and mammals to have a precise system to regulate it during evolution. Phosphate is the main component of nucleic acids adenosine triphosphate and phospholipids of the membrane and plays an important role in cytoplasmic cellular signaling **[132]**.

In this study , there were non-significant difference ($p>0.05$) between means of serum phosphorus concentration in IBD patients and control group, table [3-6].

Our finding is not in harmony with Kaźmierczak I K, et al. **[133]**, who found that IBD patients had low phosphate levels more frequently (the difference was significant in the UC group) when compared with controls.

Low phosphate levels are an indicator of inflammation, malnutrition or underlying disease, although in the presence of very low levels, also cardiopulmonary and leukocyte function may be impaired **[134]**.

Osteopenia and osteoporosis seem to be one of the most important systemic complications of IBD. Moreover, the majority of IBD patients have vitamin D deficiency, this problem might result from insufficient sunlight exposure, it could also be caused by inflammatory lesions in the gastrointestinal wall—the site of vitamin D absorption and elimination of foods that are a source of vitamin D. Similarly, gastrointestinal tract lesions may lead to a poorer absorption of calcium and phosphate **[135]**.

So calcium and phosphate metabolism disturbances in these diseases, are directly related to vitamin D deficiency **[133]**.

3.5 Vitamin E concentration in patients and control group

In the present study, vitamin E concentration were significantly decreased ($p < 0.05$) in IBD patients compared to the control as in Table 3-7.

Table 3-7: Independent sample t test to test the difference of Vitamin E in IBD patients and control.

	Control (n=50) Means ±SD	IBD patients (n=50) Means ±SD	T test	P value
Vitamin E (nmol/L)	48.44 ± 0.95	41.13 ± 1.49	8.348	<0.001 **(Sig.)**

SD: standard deviation; Sig.: significant difference between groups at (P value < 0.05)

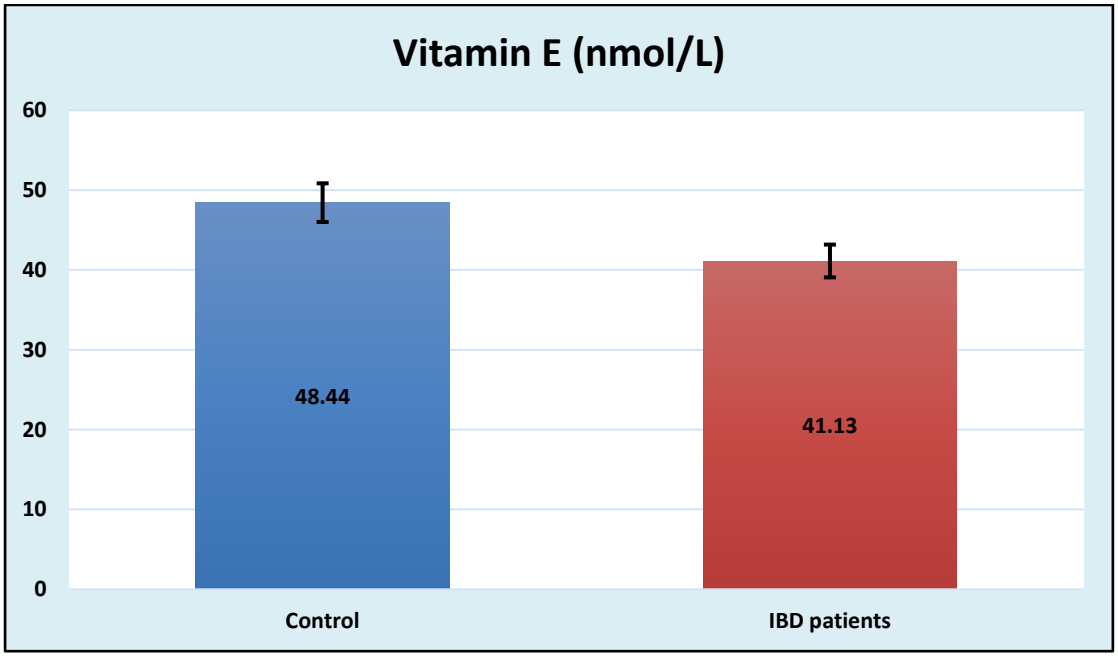


Figure 3-6: Compare vitamin E for both control and IBD patients' group.

Reactive oxygen species and subsequent lipid peroxidation decrease cellular antioxidant capacity, resulting in prominent colonic inflammation. Excessive inflammation and oxidative stress have pivotal roles in IBD pathogenesis. Inflammatory mediators are known to be secreted from migrated granulocytes in the inflamed mucosa in this disease. Inhibition of

lipid peroxidation or scavenging of oxygen free radicals would provide an important protective and therapeutic treatment for IBD. Hence, vitamin E has been postulated to diminish the risk of IBD **[136]**.

It is well established that vitamin E is a major antioxidant in cellular membranes and protects membrane lipids from peroxidation by scavenging peroxyl, oxygen and superoxide anion radicals. Vitamin E has also been shown to have prominent anti-inflammatory effects **[137]**.

These findings are in agreement with those of previous studies Bitiren M, et al. they revealed that free radical production and subsequent macro- and microscopic damages were significantly associated with decreased the level of vitamin E **[138]**.

In developing countries, the most common cause of Vit.E deficiency is inadequate dietary intake, treatment, disorders that cause fat malabsorption, and a rare genetic form of Vit.E deficiency **[139]**.

These findings also consistent with previous case-control studies in Jordan that found a protective effect of dietary Vit. E consumption **[140]**.

Vitamin E decreased the production of IL-1β, IL-6 and TNF-α in the colonic tissue. Thus, vitamin E produced a potent anti-inflammatory effect by scavenging radicals and eliminating cytokines causing colonic inflammation in this model. With its high antioxidant capacity and anti-inflammatory activity, vitamin E would be expected to reduce injury and/or improve tissue after injury from ulcerative colitis **[141]**.

Increasing attention has been given to the role of free radicals in IBD. There have been numerous studies stating the potent role of vitamin E as a major free radical scavenger and antioxidant that protects cellular membrane lipids from peroxidation. Recent studies suggested that vitamin E also has an anti-inflammatory effect that is related to inhibition of neutrophil function or cytokine production in colon mucosa **[87]**.

3.6- Correlation Analysis

Table (3-8) revealed the Pearson correlations coefficient (r) and regression line equation among biomarkers included in the study. This table showed that there was a highly significant positive correlation (P <0.05) between the levels of galectin-3 and XOR (r= 0.323), our own data shows that levels of XOR increase with increases levels of galectin-3, as in Figure (3-7a), and significant positive correlation for (zinc & iron).

Table 3-8: Pearson Correlation Coefficient (r) among biomarkers included in the study

parameters	r. p	xor	ZINC	Mg	P	IRON	VITAMIN E
Galectin (pg./ml)	Pearson Correlation	**0.363***	**-0.25****	-0.202	.081	**-.290***	**0.217**
	P value	**0.02**	**0.01**	-0.156	0.421	**.036**	**-0.13**
XOR (ng/ml)	Pearson Correlation		0.217	**-0.341****	.248	0.253	0.009
	P value		0.13	**0.021**	0.083	0.080	0.721
ZINC (mg/dl)	Pearson Correlation			0.132	0.074	**0.299****	.0.018
	P value			0.36	0.609	**0.036**	0.901
Mg (mg/dl)	Pearson Correlation				0.165	0.195	0.038
	P value				-0.251	-0.175	.0.793
Phosphate (mg/dl)	Pearson Correlation					0.003	0.178
	P value					0.981	0.216
IRON (mg/dl)	Pearson Correlation						0.123
	P value						-0.395

Mg= magnesium; PO4= phosphorus; significant correlation if (p value < 0.05)
****. Correlation is significant at the 0.01 level (2-tailed).**
***. Correlation is significant at the 0.05 level (2-tailed).**

The current study observed significant (p value < 0.05) negative correlation for (galectin-3 &Zinc), (galectin-3 & iron) and (XOR & Mg). Other serum biomarkers showed non-significant correlations. Figure (3-3) shown some of correlation between biomarkers.

Previous studies revealed that Gal-3 has been shown to be an important contributor to inflammation and markers of systemic inflammation through macrophages activation promotion and monocytes attraction. Analysis of group of symptomatic subjects with IBD showed that Gal-3 levels were higher in patients with IBD as compared to control.

This finding indicate that Gal-3 play an important role in many phases of acute and chronic inflammatory response **[142,143]**.

Compelling evidence highlights major roles for galectins in controlling innate and adaptive immune responses. These lectins may influence the capacity of innate immune cells [e.g., neutrophils, dendritic cells (DCs), monocytes/macrophages, eosinophils, and mast cells] to respond to chemotactic gradients, migrate across endothelial cell surfaces, synthesize and release pro- or anti- inflammatory cytokines, and recognize, engulf, and kill microbes and damaged cells. In this regard, some galectins trigger innate immune responses, while others influence the resolution of acute inflammation [**144]**. And this relabels with study of Shimura T, et al **[145]**.

In order to prevent harmful effect of accumulation of damaged DNA, protein and lipids. Cell has a complex and very effective antioxidant defense system that permit an immediate response to OS **[146,147]**.

Clinical trials have revealed that oxidative stress may increase free Oxygen Reactive Species (ROS) formation and reduce antioxidant defenses. However, oxidative stress plays an important role in the development of many pathologies **[148]**.

Epidemiological studies reveal that low levels of antioxidants are associated with an increased risk of different type of disease. Antioxidant depletion in the circulation may be due to the scavenging of lipid peroxides as well as sequestration by tumor cells **[148]**.

Zinc deficiency was recently shown to correlate with inflammatory status in IBD. A possible explanation for zinc playing an anti-inflammatory role in IBD could be related to its role in reducing the trans-mucosal leak in Crohn’s disease, by decreasing the number of proinflammatory cells and reducing proinflammatory cytokine production **[149]**.

In terms of immunity, zinc is essential for cell proliferation and influences both the acquired and innate immunity by also acting as a

coenzyme in many key reactions of the immune response, being essential for antioxidant response and thymic hormone function **[150]**.

Zinc deficiency leads to impairing or even completely suppressing the phagocyte and lymphocyte activity, determining an inefficient cytokine response. Moreover, it has been reported that, in activated macrophages, zinc, as a component thereof, suppresses the activity of inducible nitric oxide synthase (iNOS) by about 90%, preventing the production of reactive oxygen and nitrogen species and cellular damage **[151]**.

Galectin-3 promote superoxide production by human neutrophils demonstrating the direct role of Galectin-3 in oxidative processes, extracellular Galectin-3 can exhibit strong pro-apoptotic activity through inducing oxidative stress and mitochondrial permeability transition **[152]**.

To our interest, Lala R, *et al*. (2018) observed that the plasma level of Galectin-3 in patients with CHF is closely connected with markers for oxidative stress, which confirms its participation in these pathogenic processes **[153]**.

All the above explain the negative correlation for galectin-3 with zinc and this finding is in accordance with the previous study **[154,155]**, they found a significant effect of Zinc intake on the reduction of serum Gal-3.

Healthy mammals have low levels of circulating XOR. In the case of humans, these levels can dramatically increase in response to a range of diseases. It is possible that the increased levels of serum XOR seen in IBD simply reflect damage to intestinal epithelial cells, which are known to be rich in the enzyme. Moreover, circulating XOR, with its capacity to generate ROS and RNS, can be viewed as potentially pathogenic **[156]**.

It has the capacity to bind to glycosylaminoglycans on the surface of vascular endothelial cells and, thus concentrated, to initiate oxidative damage in distal organs. In this context, it is of interest that anti-XOR antibodies are also present in mammalian serum **[157]**.

In fact, the levels can be remarkably high, accounting for as much as 6% of the total immunoglobulin M antibodies in healthy human subjects. It is conceivable that such antibodies constitute a natural defense against excess levels of XOR, clearing them from the circulation in the form of immune complexes. A further complication is that XOR catalyzes production of uric acid, a powerful scavenger of ROS, casting the enzyme once again in a protective role rather than a destructive role **[158]**.

In a broader context, it has been proposed that the enzyme is central to the evolution of the innate immune system in general **[119]** and this explain the positive correlation of galectin-3 with XOR **[159]**.

Magnesium is a necessary micronutrient for enzymes that contribute to carbohydrate and lipid metabolism and has anti-inflammatory agents activity by modulating inflammatory pathways. Mg is also essential in skeletal tissue metabolism, neuromuscular transmission and immunity mechanism. In addition, numerous researches reported that Mg could reduce oxidative stress and free radicals in organisms **[160]**.

Most pathological conditions associated with a low magnesium status have been characterized as having a chronic inflammatory stress component. Over 75 years ago, evidence was presented that suggested magnesium deficiency results in an inflammatory response. Evidence obtained in the past 25 years, has confirmed that severely limiting magnesium intake to less than 10% of the requirement results in an inflammatory response characterized by leukocyte and macrophage activation, release of inflammatory cytokines and acute-phase proteins, and excessive production of free radicals or oxidative stress **[161]**.

Subclinical magnesium deficiency may play a contributory role in many pathological conditions by affecting the severity or presence of chronic inflammatory stress, which also results in oxidative stress. This explains the negative correlation of XOR with Mg presented in this study.

The negative correlation of zinc with iron conducted by the study of Cappellini M D, et al. **[123]**, which included, that Iron and zinc combined supplementation trials in humans and animal models have revealed negative interactions, but there are conflicting data on the direction and magnitude of these interactions. On the other hand, zinc deficiency, investigated via experimental animal models and in vitro studies, gives rise to iron deficiency anemia, tissue and cellular iron accumulation.

Cross-sectional studies in humans reveal a positive association of serum zinc levels with hemoglobin and markers of iron status **[162]**.

Studies in intestinal cell culture and experimental animal models have also demonstrated modulation of iron transporter expression and iron regulatory proteins by zinc **[163]**.

Previous study demonstrated that zinc induces iron uptake and transcellular transport in intestinal cells **[164]**. Therefore, zinc appears to be a key modulator of intestinal iron absorption and tissue iron distribution, and this consistent with our result revealed that levels of iron decrease with decreases levels of zinc.

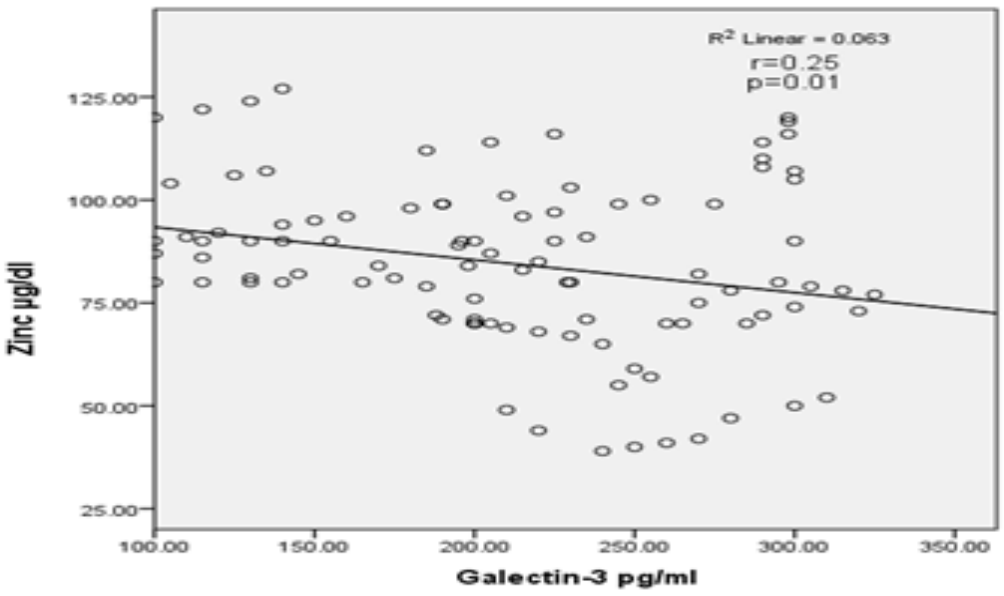


Figure 3-7-a : Correlation between galectin-3 and Zinc parameters

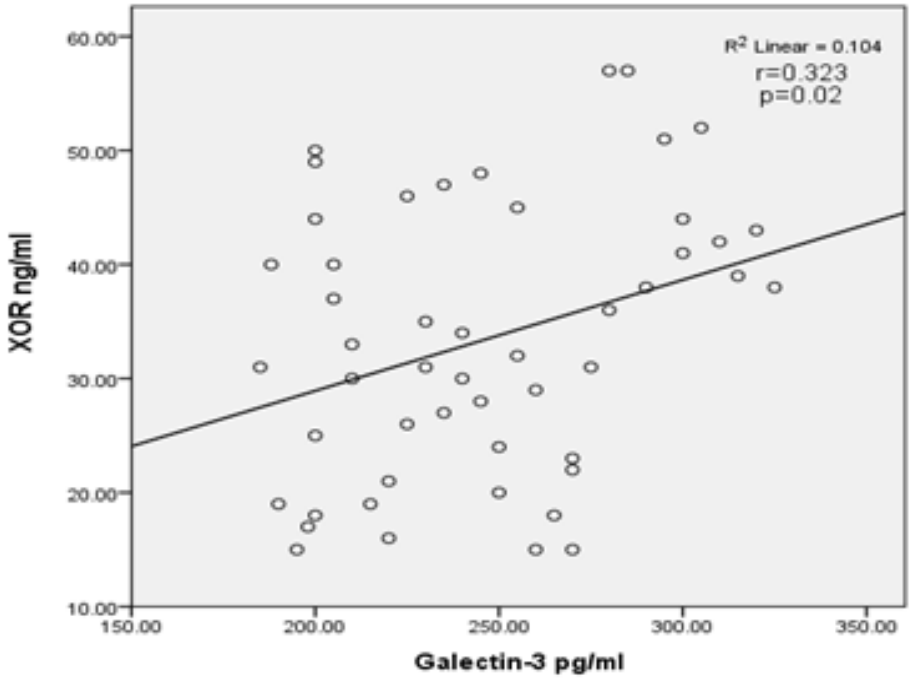


Figure 3-7-b : Correlation between XOR and Galectine-3parameters

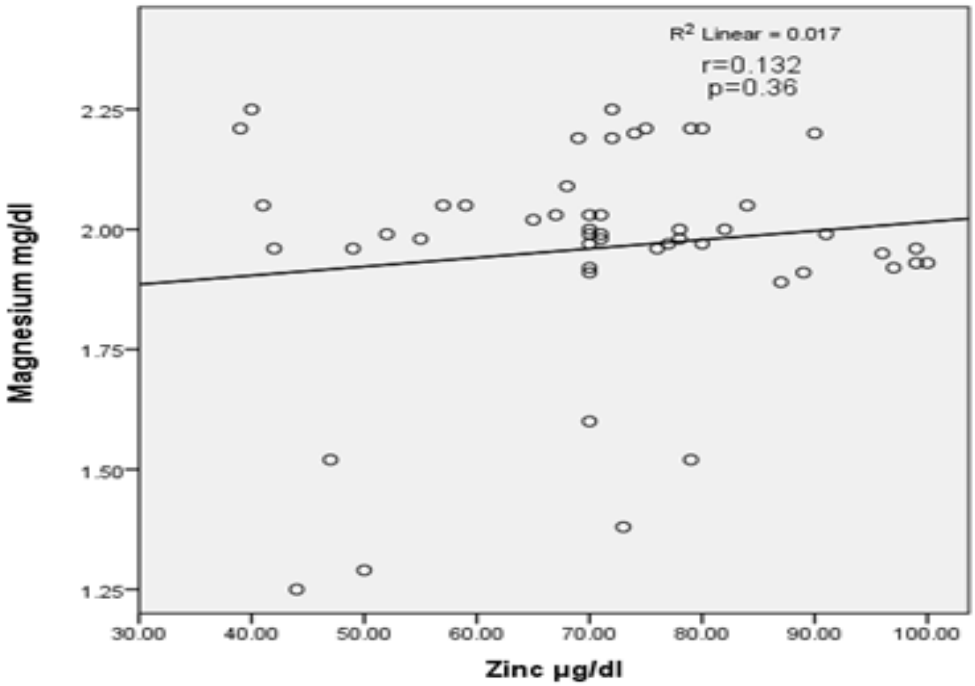


Figure 3-7-c : Correlation between Magnesium and Zinc parameters

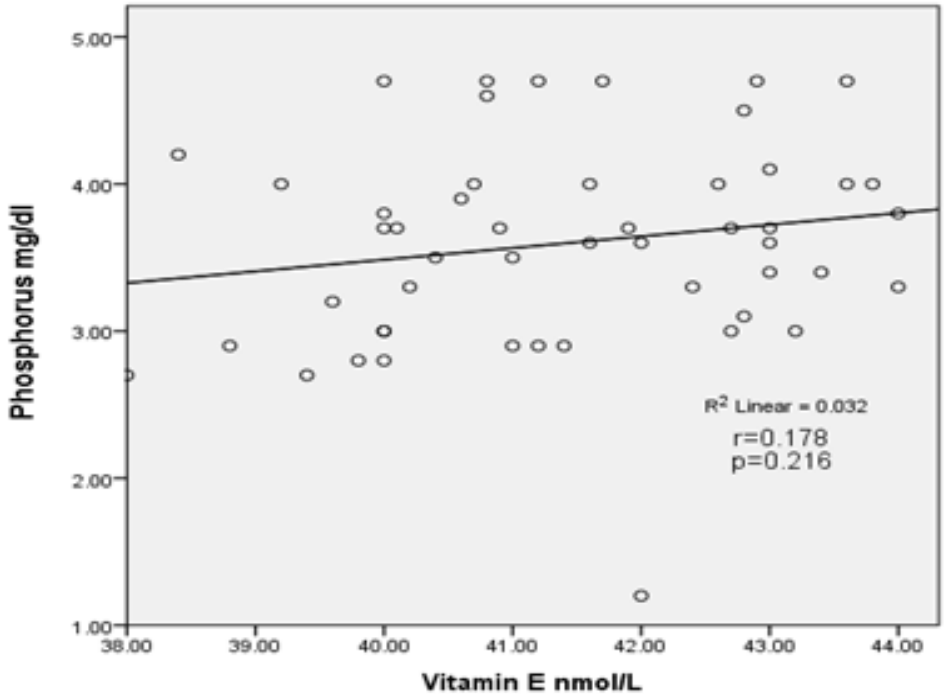


Figure 3-7-d : Correlation between Vitamin E and PO4 parameters

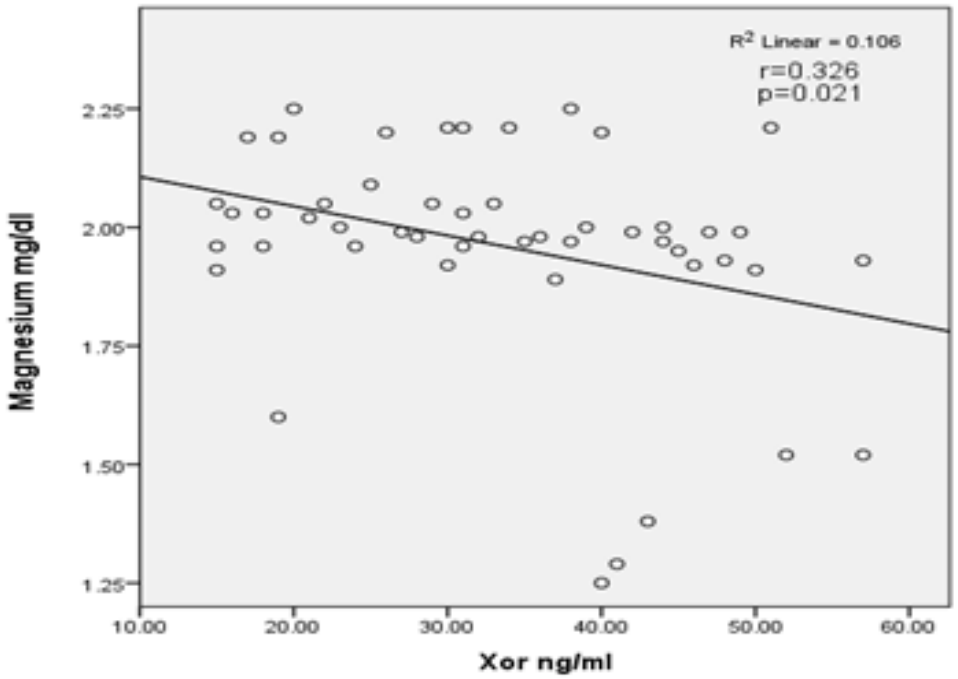


Figure 3-7-e : Correlation between Magnesium and XOR parameters

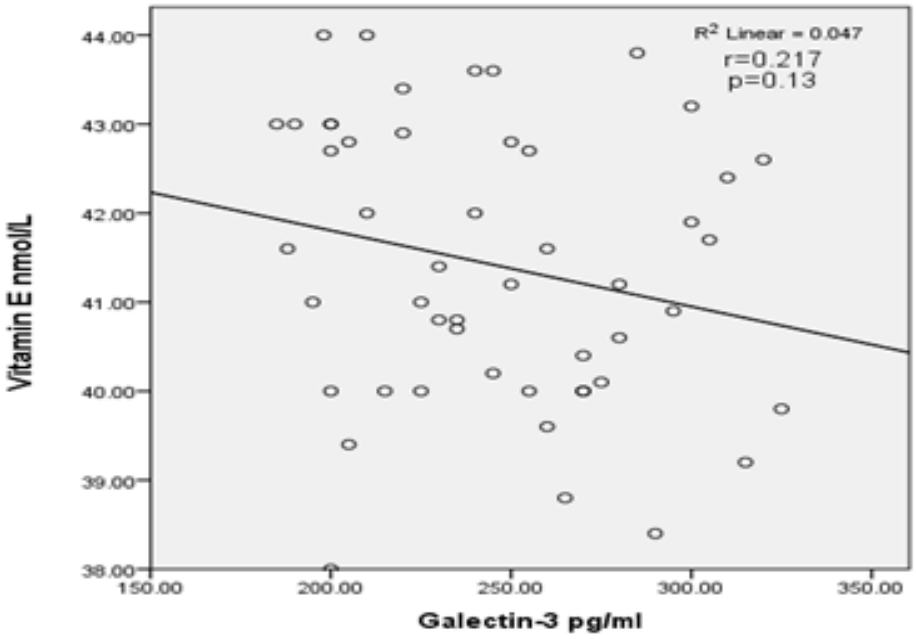


Figure 3-7-f : Correlation between Vitamin E and Galectine-3 parameters

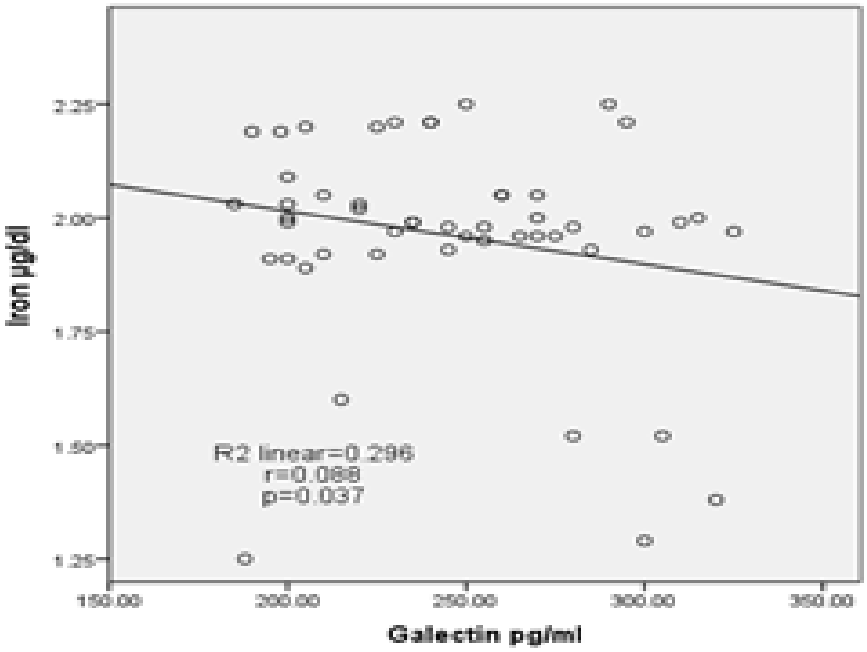


Figure 3-7-g : Correlation between Iron and Galectine-3 parameters

3-7 Conclusion

1. Inflammatory bowel diseases patients in Babylon province have higher serum level of Galectin-3 comparing to normal subjects. Concluded that the presence of Galectins-3 in serum may result from leakage from tissue, thereby making it as a possible biomarker for inflammatory bowel diseases, also may serve as prognostic serum biomarker.

2. Serum level of Xanthene oxidoreductase elevated significantly in Inflammatory bowel diseases patients and this might be attributed to the role of Xanthene oxidoreductase in the promotion of inflammatory state in this patients.

3. Serum iron, zinc and magnesium were significantly decreased in all Inflammatory bowel diseases patients, while there was non-significant changes of serum Phosphorus concentrations.

4. Decreasing level of vitamin E support that oxidative stress which is caused by increased free radicals generation with impairment of antioxidants, plays an important role in pathogenesis of Inflammatory bowel diseases.

5. There were a significant positive correlation for XOR with galectin-3 and significant positive correlation for zinc & iron.

7. The current study observed significant negative correlation for (galectin-3 &Zinc), (galectin-3 & iron) and (XOR & Mg). While other serum biomarkers showed non-significant correlations.

3.8 Recommendations

1. Further study need of specific link between Galectin-3 levels and the activity of Inflammatory bowel diseases.
2. Further study (therapeutic study) to evaluate the benefit supplementation of zinc and vitamin E in the treatment of Inflammatory bowel diseases.
3. Determine the genotyping of Inflammatory bowel diseases patients to give a complete picture about the gene responsible for the disease in Iraqi population.
4. Study the association between hormonal change, like leptin and ghrelin in Inflammatory bowel diseases patients.
5. Study the role of smoking in the prevention of ulcerative colitis.

References

1. Kastl A J, Terry N A, Wu G D, et al. The Structure and Function of the Human Small Intestinal Microbiota: Current Understanding and Future Directions. Cell. Mol. Gastroenterol. Hepatol. 2020; 9:33–45.

2. Avelar Rodriguez D, Ryan P M, Toro Monjaraz E M, et al. Small Intestinal Bacterial Overgrowth in Children: A State-Of-The-Art Review. Front. Pediatrics. 2019; 7: 363-370.

3. Vonaesch P, Morien E, Andrianonimiadana L, et al. Stunted Childhood Growth Is Associated with Decompartmentalization of the Gastrointestinal Tract and Overgrowth of Oropharyngeal Taxa. Proc. Natl. Acad. Sci. USA. 2018; 115: 8489–8498.

4. Deng Z, Luo X M, Liu J, et al. Quorum Sensing, Biofilm, and Intestinal Mucosal Barrier: Involvement the Role of Probiotic. Front. Cell. Infect. Microbiol. 2020; 10: 538-77.

5. Talley N. Clinical examination : a systematic guide to physical diagnosis. Chatswood, N.S.W: Elsevier Australia. 2018; 227-230.

6. Dandrieux JR. Inflammatory bowel disease versus chronic enteropathy in dogs: are they one and the same?. The Journal of Small Animal Practice. 2016; 57(11): 589–599.

7. Tang Q, Jin G,Wang G, et al. Current Sampling Methods for Gut Microbiota: A Call for More Precise Devices. Front. Cell. Infect. Microbiol. 2020; 10: 151

8. Ashton JJ, Gavin J, Beattie RM. Exclusive enteral nutrition in Crohn's disease: Evidence and practicalities. Clinical Nutrition. 2019; 38 (1): 80–89.

9. Charlebois A, Rosenfeld G, Bressler B . The Impact of Dietary Interventions on the Symptoms of Inflammatory Bowel Disease: A Systematic Review". Critical Reviews in Food Science and Nutrition. 2018; 56(8): 1370–8.

10. Lopetuso LR, Napoli M, Rizzatti G, Gasbarrini A. The intriguing role of Rifaximin in gut barrier chronic inflammation and in the treatment of Crohn's disease. Expert Opinion on Investigational Drugs. 2018; 27(6): 543–551.

11. Sunkara T, Rawla P, Ofosu A, Gaduputi V. Fecal microbiota transplant - a new frontier in inflammatory bowel disease. Journal of Inflammation Research. 2018; 11: 321–328.

12. Gilardi D, Fiorino G, Genua M, et al. Complementary and alternative medicine in inflammatory bowel diseases: what is the future in the field of herbal medicine?. Expert Review of Gastroenterology & Hepatology. 2018; 8(7): 835–46.

13. Dave M, Mehta K, Luther J,et al. Mesenchymal Stem Cell Therapy for Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. Inflammatory Bowel Diseases. 2015;21 (11): 2696–707.

14. Wayne M, Gregory T C M, et al. Crohn's & Colitis Australia inflammatory bowel disease audit: measuring the quality of care in Australia. Internal Medicine Journal. Wiley. 2019;49 (7): 859–866.

15. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. The American Journal of Gastroenterology. 2012; 107 (10): 1474–82.

16. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. ACG Clinical Guideline: Ulcerative Colitis in Adults. The American Journal of Gastroenterology. 2019;114 (3): 384–413.

17. Cristiana P, Daniela G, João P T,et al. Oxidative Stress and DNA Damage: Implications in Inflammatory Bowel Disease. Inflammatory Bowel Diseases. 2015; 21 (10): 2403–2417.

18. Colìa R, Corrado A, Cantatore FP. Rheumatologic and extraintestinal manifestations of inflammatory bowel diseases. Annals of Medicine. 2016; 48 (8): 577–585.

19. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. ACG Clinical Guideline: Ulcerative Colitis in Adults". The American Journal of Gastroenterology. 2019;114 (3): 384–413.

20. Vavricka SR, Schoepfer A, Scharl M, et al. Extraintestinal Manifestations of Inflammatory Bowel Disease. Inflammatory Bowel Diseases. 2015; 21 (8): 1982–1992.

21. Roncoroni L, Gori R, Elli L, et al. Nutrition in Patients with Inflammatory Bowel Diseases: A Narrative Review. Nutrients. 2022;14(4):751.

22. Rashvand S, Behrooz M, Samsamikor M, et al. Dietary patterns and risk of ulcerative colitis: a case-control study. Journal of Human Nutrition and Dietetics. 2018;31(3): 408–412.

23. Ryan F J, Ahern A M, Fitzgerald R S, et al. Colonic microbiota is associated with inflammation and host epigenomic alterations in inflammatory bowel disease. Nature Communications. 2020; 11 (1): 1512.

24. Limketkai BN, Sepulveda R, Hing T, et al. Prevalence and factors associated with gluten sensitivity in inflammatory bowel disease. Scandinavian Journal of Gastroenterology. 2018; 53 (2): 147–151.

25. Politis DS, Papamichael K, Katsanos KH, et al. Presence of pseudopolyps in ulcerative colitis is associated with a higher risk for treatment escalation. Annals of Gastroenterology. 2019;32 (2): 168–173.

26. GBD 2013 Mortality Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015; 385 (9963): 117–71

27. Kaplan GG, Bernstein CN, Coward S, et al. The Impact of Inflammatory Bowel Disease in Canada 2018: Epidemiology. Journal of the Canadian Association of Gastroenterology. 2018;2 (1): 6–16

28. Park J, Cheon JH. Incidence and Prevalence of Inflammatory Bowel Disease across Asia. Yonsei Medical Journal. 2021; 62(2): 99–108.

29. Coward S, Clement F, Benchimol EI, et al. Past and Future Burden of Inflammatory Bowel Diseases Based on Modeling of Population-Based Data. Gastroenterology. 2020;156 (5): 1345–1353.

30. Patil DT, Odze RD. Backwash Is Hogwash: The Clinical Significance of Ileitis in Ulcerative Colitis. The American Journal of Gastroenterology. 2017; 112 (8): 1211–1214.

31. Jess T, Gamborg M, Munkholm P, et al. Overall and cause-specific mortality in ulcerative colitis: meta-analysis of population-based inception cohort studies. The American Journal of Gastroenterology. 2007;102 (3): 609–617.

32. Theo V, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2015; 388 (10053): 1545–1602.

33. Mosli M, Alawadhi S, Hasan F, et al. Incidence, Prevalence, and Clinical Epidemiology of Inflammatory Bowel Disease in the Arab World: A Systematic Review and Meta-Analysis. Inflamm Intest Dis. 2021;6:123–131

34. Hassan JT, Delmany AS. Epidemiological and clinical characteristics of patients with inflammatory bowel disease in Erbil City. Med J Babylon 2018;15:281-5.

35. Adam G C, Julia E, Mendieta L, et al. Ranking microbiome variance in inflammatory bowel disease: a large longitudinal intercontinental study. Gut. 2021;70 (3): 499–510.

36. Ananthakrishnan AN, Kaplan GG, Bernstein CN, et al. International Organization for Study of Inflammatory Bowel Diseases. Lifestyle, behaviour, and environmental modification for the management of patients with inflammatory bowel diseases: an International Organization for Study of Inflammatory Bowel Diseases consensus. Lancet Gastroenterol Hepatol. 2022; 26 (22): 00021-8.

37. Fedorak RN. Probiotics in the management of ulcerative colitis. Gastroenterology & Hepatology. 2010; 6 (11): 688–690.

38. Ghouri YA, Richards DM, Rahimi EF, et al. Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease. Clinical and Experimental Gastroenterology. 2014;**7**: 473–487.

39. D'Haens GR, Panaccione R, Higgins PD, et al. The London Position Statement of the World Congress of Gastroenterology on Biological Therapy for IBD with the European Crohn's and Colitis Organization: when to start, when to stop, which drug to choose, and how to predict response?. The American Journal of Gastroenterology. 2011;106 (2): 199–212.

40. Forbes A, Escher J, Hébuterne X, et al. ESPEN guideline: Clinical nutrition in inflammatory bowel disease. Clinical Nutrition. 2017;36 (2): 321–347

41. Walus M, Knowles A, Keefer S R, et al. Controversies Revisited: A Systematic Review of the Comorbidity of Depression and Anxiety with Inflammatory Bowel Diseases. Inflammatory Bowel Diseases. 2017;22 (3): 752–762.

42. Kookhwan C, Jaeyoung C, Kyungdo H, et al. Risk of Anxiety and Depression in Patients with Inflammatory Bowel Disease: A Nationwide, Population-Based Study. Journal of Clinical Medicine. 2019;8 (5): 654.

43. Dings RPM, Miller MC, Griffin RJ, Mayo KH. Galectins as molecular targets for therapeutic intervention. Int J Mol Sci. 2018;19(3):905.

44. Brinchmann MF, Patel DM, Iversen MH. The role of galectins as modulators of metabolism and inflammation. Mediators Inflamm. 2018

45. Cibor D, Szczeklik K, Brzozowski B, et al. serum galectin 3, galectin 9 and galectin 3-binding proteins in patients with active and inactive inflammatory bowel disease. Original article. 2019;1:6

46. Wang Y, Liu S,Tian Y, et al. Prognostic role of galectin‑3 expression in patients with solid tumors: a meta‑analysis of 36 eligible studies. Cancer Cell Int. 2018; 18:172

47. Sciacchitano S, Lavra L, Morgante A, et al. Galectin-3: One Molecule for an Alphabet of Diseases, from A to Z. Int. J. Mol. Sci. 2018; 19: 379.

48. James R. Vinnai. The Association Between Oxidative Stress, Cellular Differentiation And Galectins In Human Promyelocytic Leukemia Cells (HL-60). Electronic Thesis and Dissertation Repository. 2016;4343.

49. Farhad M, Rolig AS , Redmond WL. The role of Galectin-3 in modulating tumor growth and immunosuppression within the tumor microenvironment. Oncoimmunology. 2018; 7(6): 1-8.

50. Chetry M, Thapa S, Hu X, et al .The Role of Galectins in Tumor Progression, Treatment and Prognosis of Gynecological Cancers. Journal of Cancer. 2018; 9: 4742-4755.

51. Dong R, Zhang M, Hu Q, et al. Galectin‑3 as a novel biomarker for disease diagnosis and a target for therapy (Review). International Journal Of Molecular Medicine. 2018; 41: 599-614.

52. Chou FC , Yi Chen H , Chi Kuo C, et al. Role of Galectins in Tumors and in Clinical Immunotherapy. Int. J. Mol. Sci. 2018; 19: 430.

53. Hara A , Niwa M , Noguchi K , et al. Galectin-3 as a Next-Generation Biomarker for Detecting Early Stage of Various Diseases. Biomolecules .2020;10(389): 2-19.

54. Szczeklik K, Krzysciak W, Domagala-Rodacka R, et al. Alterations in glutathione peroxidase and superoxide dismutase activities in plasma and

saliva in relation to disease activity in patients with Crohn's disease. J Physiol Pharmacol 2016; 67: 709-715.

55. Cecerska-Hery´c E, Jesionowska A, Klaudyna S, et al. Xanthine oxidoreductase reference values in platelet-poor plasma and platelets in healthy volunteers, Oxid. Med. Cell. Longev. 2015; 34:1926- 34

56. Terao M, Garattini E, Rom˜ao M J, et al. Evolution, expression, and substrate specificities of aldehyde oxidase enzymes in eukaryotes, J. Biol. Chem. 2020;295: 5377–5389,

57. Battelli M G, Abbondanza A, Stirpe F. Effects of hypoxia and ethanol on xanthine oxidase of isolated rat hepatocytes: conversion from D to O form and leakage from cells, Chem. Biol. Interact. 1992; 83: 73–84,

58. Medellin N C, Kelley E E. Xanthine oxidoreductase-catalyzed reduction of nitrite to nitric oxide: insights regarding where, when and how, Nitric Oxide. 2013; 34:19–26,

59. Furuhashi M. New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity, Am. J. Physiol. Endocrinol. Metab. 2020;319: 827–834.

60. Manaman L M. Xanthine oxidoreductase mediates membrane docking of milk-fat droplets but is not essential for apocrine lipid secretion, J. Physiol. 2016;594: 5899–5921.

61. Battelli M G, Polito L, Bortolotti M, et al. Xanthine oxidoreductase in cancer: more than a differentiation marker. Cancer. 2016;5: 546–557.

62. Al-Shehri S S, Duley J A, Bansal N. Xanthine oxidase-lactoperoxidase system and innate immunity: biochemical actions and physiological roles. Redox Biol. 2020; 34: 101-524.

63. Lima W G, Martins-Santos M E, Chaves V E. Uric acid as a modulator of glucose and lipid metabolism, Biochimie. 2015; 116: 17–23.

64. Jingfang L, et al. Iron metabolism and type 2 diabetes mellitus: A meta-analysis and systematic review. Journal of diabetes investigation. 2020; 11(4): 946-955.

65. Tey T T, Yiu R, Leow W Q. Hepatitis B-Associated Symptomatic Iron Overload, with Complete Resolution after Nucleoside Analogue Treatment. Case Reports in Gastrointestinal Medicine. 2021.

66. Thomas DW, Hinchliffe RF, Briggs C, et al. Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol. 2013; 161: 639-648

67. Sindhu Kaitha, Muhammad Bashir, Tauseef Ali. Iron deficiency anemia in inflammatory bowel disease. World J Gastrointest Pathophysiol. 2015; 6(3): 62-72.

68. Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol. 2006; 20(1) :3-18.

69. Wessels I, Maywald M, Rink L. Zinc as a Gatekeeper of Immune function. Nutrients. 2017;9(12): 1-19.

70. 2nd ed. Bangkok, Thailand: 2004. FAO/WHO. Expert Consultation on Human Vitamin and Mineral Requirements, Vitamin and mineral requirements in human nutrition: Report of joint FAO/WHO expert consolation; p. 341.

71. Brown KH, Rivera JA, Bhutta Z, et al. International Zinc Nutrition Consultative Group (IZiNCG) technical document1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004;25:S99–203.

72. Mayer L S, Uciechowski P, Meyer S, et al. Differential impact of zinc deficiency on phagocytosis, oxidative burst, and production of pro-inflammatory cytokines by human monocytes. Metallomics. 2014; 6(7): 1288–1295.

73. Weisshof R and Chermesh I. Micronutrient deficiencies in inflammatory bowel disease. Current Opinion in Clinical Nutrition & Metabolic Care. 2015; 18(6): 576–581.

74. Michielan A and D'Inc`a R. Intestinal permeability in inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of leaky gut. Mediators of Inflammation. 2015; 628157:1-10.

75. Phosphorus H R, Erdman JW, Macdonald IA, et al. Present Knowledge in Nutrition. 10th ed. Washington, DC: Wiley-Blackwell; 2012:447-58.

76. Berns JS. Niacin and Related Compounds for Treating Hyperphosphatemia in Dialysis Patients. Semin Dial. 2008;21:203–205.

77. Shinozaki N, Murakami K, Asakura K, et al. Dietary phosphorus intake estimated by 4-day dietary records and two 24-hour urine collections and their associated factors in Japanese adults. Eur J Clin Nutr. 2018;72:517–25.

78. Kaźmierczak I K, Szymczak A, Tomczak M, et al. Calcium and phosphate metabolism in patients with inflammatory bowel diseases. Polskie Archiwum Medycyny Wewnętrznej. 2015; 125: (7-8).

79. Jakubowski A, Zagórowicz E, Kraszewska E, et al. Rising hospitalization rates for inflammatory bowel disease in Poland. Pol Arch Med Wewn. 2014; 124: 180-190.

80. Detlie T E, LindstrømJ C, Jahnsen M E, et al. Incidence of hypophosphatemia in patients with inflammatory bowel disease treated with ferric carboxymaltose or iron isomaltoside. Aliment Pharmacol Ther. 2019;50:397–406.

81. Schwalfenberg G K and Genuis S J. The Importance of Magnesium in Clinical Healthcare. Hindawi Scientifica. 2017; 1-14 .

82. Piovesan D, Profiti G, Martelli P L, et al. The human 'magnesome': detecting magnesium binding sites on human proteins. BMC Bioinformatics. 2012;13(14):10-15.

83. Hasiec E K, Studzinska M M, Czajkowski M, et al. Plasma magnesium concentration in patients undergoing coronary artery bypass grafting. Annals of Agricultural and Environmental Medicine. 2017; 24(2): 181–184.

84. Maier J A, Castiglioni S, Locatelli L, et al. Magnesium and Inflammation: Advances and Perspectives. Semin. Cell Dev. Biol. 2021;115: 37–44.

85. Gommers L M M, Ederveen T H A,Wijst J, et al. Low Gut Microbiota Diversity and Dietary Magnesium Intake are Associated with the Development of PPI-induced Hypomagnesemia. FASEB J. 2019; 33: 11235–11246.

86. Fabisiak N, Fabisiak A, Watala C, et al. Fatsoluble vitamin deficiencies and inflammatory bowel disease. Journal of Clinical Gastroenterology. 2017; 51(10): 878–889.

87. Wa´sko-Czopnik D and Paradowski L. An influence of deficiencies of essential trace elements and vitamins on the course of crohn's disease," Advances in Clinical and Experimental Medicine. 2012;21(1): 5–11.

88. Aghdassi E, Wendland B E, Steinhart A H, et al. Antioxidant vitamin supplementation in Crohn's disease decreases oxidative stress a randomized controlled trial. American Journal of Gastroenterology. 2003; 98(2): 348–353.

89. Zieli´nska A and Nowak I. Tokoferole i tokotrienole jako witamina E. Chemik. 2014; 68(7): 585–591.

90. De Moreno de LeBlanc A, Levit R, De Giori G S,et al. Vitamin producing lactic acid bacteria as complementary treatments for intestinal inflammation. Anti-Inflammatory & Anti-allergy Agents in Medicinal Chemistry. 2018; 17(1): 50–56.

91. Bioassay technology laboratory. Human Galectin-3 ELISA Kit user manual. Bioassay technology laboratory, Shanghai, China.

92. Bioassay technology laboratory. Human Xanthine Oxidase ELISA Kit user manual. Bioassay technology laboratory, Shanghai, China.

93. Bioassay technology laboratory. Human Vitamin E ELISA Kit user manual. Bioassay technology laboratory, Shanghai, China.

94. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders; 2018:1909–10.

95. Shubbar A H, Makki H fayadh HH, AL-Akayshi H R. Chronic Colitis: Clinical, endoscopic & histological Evaluation of 130 Iraqi patients. IJGE. 2001;1: 11-17.

96. Feshareki R, Soleimani H. Crohn's disease in Isfahan; report of a case. Pahlavi Medical Journal. 1976; 7: 565 - 575.

97. Leong RW, Lau JY, Sung JJ. The epidemiology and phenotype of Crohn's disease in the Chinese population. Inflamm Bowel Dis. 2004; 10: 646-651.

98. Morita N, Toki S, Hirohashi T, et al. Incidence and prevalence of inflammatory bowel disease in Japan: nationwide epidemiological survey during the year 1991. J Gastroenterol. 1995; 30: 1-4.

99. Cibor D, Szczeklik K, Brzozowski B, et al. Serum Galectin 3, Galectin 9 and galectin 3-binding proteins in patients with active and inactive inflammatory bowel disease. journal OF Physiology And Pharmacology. 2019;70(1): 95-104.

100. Burisch J, Pedersen N, Cukovic-Cavka S, et al. East–west gradient in the incidence of inflammatory bowel disease in Europe: the ECCO-EpiCom inception cohort. Gut. 2014;63:588–97.

101. Vind L, Riis T, Jess E, et al. Increasing incidences of inflammatory bowel disease and decreasing surgery rates in Copenhagen City and County, 2003–2005: a population-based study from the Danish Crohn colitis database. Am J Gastroenterol. 2006;101:1274–82

102. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. ACG Clinical Guideline: Ulcerative Colitis in Adults. The American Journal of Gastroenterology. 2019;114 (3): 384–413.

103. Fallahi GH, Moazzami K, Tabatabaeiyan M, et al. Clinical characteristics of Iranian pediatric patients with inflammatory bowel disease. Acta Gastroenterol Belg. 2009; 72: 230-234.

104. Vahedi H, Merat S, Momtahen S, et al. Epidemiologic characteristics of 500 patients with inflammatory bowel disease in Iran studied from 2004 through 2007. Arch Iran Med. 2009; 12: 454-460.

105. Alshekhani M A M, Salim B F, Mohialdeen Z N. clinical characteristic of inflammatory bowel disease diagnosed in kurdistan center for gastroenterology and hepatology (kcgh) – asulaimaiyah-iraqi kurdistan-iraq. Gastroint hepatol dig dis. 2020; 3(2):1-7

106. Van Osch FH, Jochems SH, van Schooten FJ, et al. Significant role of lifetime cigarette smoking in worsening bladder cancer and upper tract urothelial carcinoma prognosis: a meta-analysis. The Journal of urology. 2016;195(4):872-9.

107. Fischer BM, Voynow JA, Ghio AJ. COPD: balancing oxidants and antioxidants. International journal of chronic obstructive pulmonary disease. 2015;10:261.

108. Zhang D, Chen ZG, Liu SH, et al. Galectin-3 gene silencing inhibits migration and invasion of human tongue cancer cells in vitro via down-regulating β-catenin. Acta Pharmacol Sin. 2013;34:176–184.

109. Johannes L, Jacob R, Leffler H. Galectins at a glance. J Cell Sci. 2018; 131: 208-884.

110. Frol'ova L, Smetana K, Borovska D, et al. Detection of galectin-3 in patients with inflammatory bowel diseases: new serum marker of active forms of IBD? Inflamm Res.2009; 58: 503-512.

111. Papa Gobbi R, De Francesco N, Bondar C, et al. A galectinspecific signature in the gut delineates Crohn's disease and ulcerative colitis from other human inflammatory intestinal disorders. *Biofactors* 2016; 42: 93-105.

112. Gao P, Simpson JL, Zhang J, Gibson PG. Galectin-3: its role in asthma and potential as an anti-inflammatory target. Respir Res 2013; 14: 136.

113. Xue FM, Zhang HP, Hao HJ, et al. CD98 positive eosinophils contribute to T helper 1 pattern inflammation. PLoS One. 2012; 7

114. Meijer B, Seinen M L , Hosman T, et al. High inter-individual variability of serum xanthine oxidoreductase activity in IBD patients. Nucleosides Nucleotides Nucleic Acids. 2018;37(6):317-323.

115. Gibbings S, Elkins N D, Fitzgerald H, et al. Xanthine Oxidoreductase Promotes the Inflammatory State of Mononuclear Phagocytes through Effects on Chemokine Expression, Peroxisome Proliferator-activated Receptor-Sumoylation, and HIF-1. The journal of biological chemistry. 2011; 286(2): 961–975.

116. Battelli M G, Polito L, Bortolotti M, Bolognes A. Xanthine oxidoreductase-derived reactive species: physiological and pathological effects, Oxidative Med. Cell. Longev. 2016; 35 (2):7579.

117. Morita, Y.Identification of xanthine dehydrogenase/oxidase as a rat Paneth cell zinc-binding protein. Biochim. Biophys. Acta. 2001; 1540:43–49.

118. Van den Munckhof R. J. M., H. Vreeling-Sindelarova J. P. M. Schellens,C. J. F. Van Noorden, and W. M. Frederiks. Ultrastructural localization of xanthine oxidase activity in the digestive tract of the rat. Histochem. J.1995; 27:897–905.

119. Vorbach, C., Harrison R, and Capecchi M. Xanthine oxidoreductase is central to the evolution and function of the innate immune system. Trends Immunol. 2003;24:512–517.

120. Forbes A, Escher J, Hébuterne X, et al. ESPEN guideline: Clinical nutrition in inflammatory bowel disease. Clin Nutr. 2017;36:321-347.

121. Ananthakrishnan AN, Khalili H, Song M, et al. Zinc intake and risk of Crohn's disease and ulcerative colitis: a prospective cohort study. Int J Epidemiol. 2015;44:1995-2005.

122. Tavill AS. Hemochromatosis In: Schiff L, Schiff ER, editors. Diseases of the liver. Philadelphia: JB Lippincott. 2022; 669–691.

123. Cappellini M D, Comin-Colet J, Francisco A, et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and Management. Am J Hematol. 2017;92:1068–1078.

124. Peyrin-Biroulet L, Williet N, Cacoub P. Guidelines on the diagnosis and treatment of iron deficiency across indications: a systematic review. Am J Clin Nutr. 2015;102:1585–1594.

125. Ohashi W, Fukada T. Contribution of Zinc and Zinc Transporters in the Pathogenesis of Inflammatory Bowel Diseases. J Immunol Res 2019;2019

126. Vagianos K, Bector S, McConnell J, et al. Nutrition assessment of patients with inflammatory bowel disease. J Parenter Enteral Nutr. 2007;31:311-319.

127. Griffin IJ, Kim SC, Hicks PD, et al. Zinc metabolism in adolescents with Crohn's disease. Pediatr Res. 2004;56:235-239.

128. Hekmatdoost A, Vahid F, Rashvand S, Sadeghi M. The association between index of nutritional quality and ulcerative colitis: A caseâcontrol study. Journal of Research in Medical Sciences. 2018;23(67): 555-17.

129. Perez J C, Shin D, Zwir I, et. Evolution of a Bacterial Regulon Controlling Virulence and Mg2+ Homeostasis. PLoS Genet. 2009; 5: 100-428.

130. Bian X, Wu W, Yang L, et al. Administration of Akkermansia Muciniphila Ameliorates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Mice. Front. Microbiol. 2019; 10: 2259.

131. Razzaque, M.S. Magnesium: AreWe Consuming Enough? Nutrients 2018; 10: 1863.

132. Fernandez-Martin JL, Dusso A, Martinez-Camblor P et al. Serum phosphate optimal timing and range associated with patients survival in haemodialysis: the COSMOS study. Nephrol Dial Transplant. 2019; 34: 673–681

133. Kaźmierczak I K, Szymczak A, Tomczak M , et al. Calcium and phosphate metabolism in patients with inflammatory bowel diseases. Polskie Archiwum medycyny Wewnętrznej. 2015; 125: 7-8.

134. Lee JE, Lim JH, Jang HM et al. Low serum phosphate as an independent predictor of increased infection-related mortality in dialysis patients: a prospective multicenter cohort study. PLoS One 2017; 12: 185-853.

135. Ulitsky A, Ananthakrishnan AN, Naik A, et al. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity
and quality of life. J Parenter Enteral Nutr. 2011; 35: 308-316.

136. Chen J X, Liu A, Lee M, et al. *δ*- and *c*-tocopherols inhibit phIP/DSS-induced colon carcinogenesis by protection against early cellular and DNA damages. Molecular Carcinogenesis.2017; 56(1): 172–183.

137. Kania-Dobrowolska M, Baraniak J, Kujawski R and˙zarowski M. Nutricosmetic – new subgroup of dietary supplements. Post Fitoter. 2017; 18(2): 132–138.

138. Bitiren M, Karakilcik AZ, Zerin M, et al. Protective effects of selenium and vitamin E combination on experimental colitis in blood plasma and colon of rats. Biol Trace Elem Res. 2010;136:87-95.

139. Isozaki Y, Yoshida N, Kuroda M, et al. Effect of a novel water-soluble
vitamin E derivative as a cure for TNBS-induced colitis in rats. Int J Mol Med. 2006;17:497-502.

140. Tayyem R F, Bawadi H A , Shehadah I N, et al. Macro- and Micronutrients Consumption and the Risk for Colorectal Cancer among Jordanians. Nutrients. 2015; 7:1769-1786.

141. Fabisiak N, Fabisiak A, Watala C, and Fichna J. Fatsoluble vitamin deficiencies and inflammatory bowel disease. Journal of Clinical Gastroenterology. 2017;51(10): 878–889.

142. Iwona S, Wlazel RN, Migala M, et al. The association between Galectin-3 and occurrence of reinfarction early after first myocardial infarction treated invasively. Biomarkers. 2013;18(8):655-9.

143. Tunón J, Blanco-Colio L, Cristóbal C, et al. Usefulness of a combination of monocyte chemoattractant protein-1, galectin-3, and N-terminal probrain natriuretic peptide to predict cardiovascular events in patients with coronary artery disease. Am J Cardiol. 2014;113(3):434-40

144. Johannes L, Jacob R, Leer H. Galectins at a glance. J. Cell Sci. 2018;131, 1-9.

145. Shimura T, Shibata M, Gonda K, et al. Association between circulating galectin-3 levels and the immunological, inflammatory and nutritional parameters in patients with colorectal cancer. Biomedical reports. 2016;5: 203-207.

146. Revin VV, Gromova NV, Revina ES, et al. The Influence of Oxidative Stress and Natural Antioxidants on Morphometric Parameters of

Red Blood Cells , the Hemoglobin Oxygen Binding Capacity , and the Activity of Antioxidant Enzymes. 2019.

147. Islam MO , Bacchetti T , Ferretti G. Alterations of Antioxidant Enzymes and Biomarkers of Nitro-oxidative Stress in Tissues of Bladder Cancer. Oxidative Medicine and Cellular Longevity. 2019

148. Sharma A, Rajappa M, Saxena A, Sharma M. Antioxidant status in advanced cervical cancer patients undergoing neoadjuvant chemoradiation.Br J Biomed Sci. 2007;64(1): 23–27.

149. Weisshof R and Chermesh I. Micronutrient deficiencies in inflammatory bowel disease. Current Opinion in Clinical Nutrition & Metabolic Care.2015; 18(6): 576–581.

150. Mohammadi E, Qujeq D, Taheri H, and Hajian-Tilaki K. Evaluation of Serum Trace Element Levels and Superoxide Dismutase Activity in Patients with Inflammatory Bowel Disease: Translating Basic Research into Clinical Application. Biological Trace Element Research. 2017; 177(2): 235–240.

151. Malavolta M, Piacenza F, Basso A, et al. Serum copper to zinc ratio: Relationship with aging and health status. Mechanisms of Ageing and Development. 2015;151: 93–100.

152. Suzuki Y, Inoue T,Yoshimaru T, Ra C. Galectin-3 but not galectin-1 induces mast cell death by oxidative stress and mitochondrial permeability transition. Biochimica et Biophysica Acta . 2008;1783: 924–934.

153. Lala R, Lungeanu D ,Darabantiu D, Pilat L, Puschita M .Galectin-3 as a marker for clinical prognosis and cardiac remodeling in acute heart failure. Herz. 2018 ; 43:146–155

154. Schnurr TM, et al. Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: a case-cohort study. 2020; 1–9.

155. De Silva NMG, et al. Liver function and risk of type 2 diabetes: bidirectional mendelian randomization study. Diabetes. 2019;68(8):1681–91.

156. Radi R, Rubbo H, Bush K, and Freeman B A. Xanthine oxidase binding to glycosaminoglycans: kinetics and superoxide dismutase interactions of immobilised xanthine oxidase-heparin complexes. Arch. Biochem. Biophys. 1997; 339:125–135.

157. Wang J, Praagh A, Hamilton E, et al. Serum xanthine oxidase: origin, regulation, and contribution to control of trypanosome parasitemia. Antioxid. Redox Signal.2002; 4:161–178.

158. Hooper D C, Scott G S, Zborek T A, et al. Uric acid, a peroxynitrite scavenger, inhibits CNS inflammation, blood CNS barrier permeability changes, and tissue damage in a mouse model of multiple sclerosis. FASEB J. 2000;14:691–698.

159. Harzand A, Tamariz L, Hare J M. Uric acid, heart failure survival, and the impact of xanthine oxidase inhibition. Congest. Heart Fail. 2012;18:179–182.

160. Forrest H Nielsen. Magnesium deficiency and increased inflammation: current perspectives. Journal of Inflammation Research. 2018;11: 25–34.

161. Saleh A Naser, Almatmed Abdelsalam, Saisathya Thanigachalam, et al. Domino effect of hypomagnesemia on the innate immunity of Crohn's disease patients. World J Diabetes. 2014; 5(4): 527-535.

162. Ergul A B, Turanoglu C, Karakukcu C,et al. Increased Iron Deficiency and Iron Deficiency Anemia in Children with Zinc Deficiency. Eurasian J. Med. 2018; 50:34–37.

163. Houghton L A, Parnell W R,Thomson C D, et al. Serum Zinc Is a Major Predictor of Anemia and Mediates the E_ect of Selenium on

Hemoglobin in School-Aged Children in a Nationally Representative Survey in New Zealand, 2. J. Nutr. 2016; 146:1670–1676.

164. Kondaiah P, Aslam M F, Mashurabad P, et al. Zinc induces iron uptake and DMT1 expression in Caco-2 cells via a PI3K/IRP2 dependent mechanism. Biochem. J. 2019; 476: 1573–1583 .

وزاره التعليم العالي
والبحث العلمي
جامعه بابل
كليه الطب
فرع الكيمياء والكيمياء الحياتيه

تقييم الزانثين اوكسيدوردكتيس و كلاكتين-3 و بعض مؤشرات الكيمياء الحيوية في المرضى البالغين المصابين بالتهاب الامعاء في بابل

رسالة
مقدمة إلى مجلس كلية الطب في جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في العلوم / الكيمياء الحياتية السريرية

من قبل

أركان خنجر نصرالله سايل

بكالوريوس كليه العلوم / جامعة ذي قار (2016)

اشراف

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الخلاصة

مرض التهاب الأمعاء هو مجموعة من الحالات الالتهابية للقولون والأمعاء الدقيقة، ومرض كرون والتهاب القولون التقرحي هما النوعان الرئيسيان. مرض التهاب الأمعاء هو مرض معقد ينشأ نتيجة تفاعل العوامل البيئية والوراثية التي تؤدي إلى استجابات مناعية والتهاب في الأمعاء. اشتملت الدراسة الحالية على 100 شخص تتراوح أعمارهم بين (15-65) سنة مع مؤشر كتلة الجسم الطبيعي.

اشتمل الموضوع في دراسة الحالات والشواهد هذه على مجموعتين على النحو التالي:

المجموعة(الأولى) : وتضم (50) مريض بمرض التهاب الأمعاء.

المجموعة (الثانية): ضمت (50) من الأشخاص الأصحاء على ما يبدو كمجموعة تحكم.

تم تصميم هذه الدراسة لمعرفة دور الزانثين اوكسيدو ردكتيس والجالكتين- 3 في تطور أمراض الأمعاء الالتهابية في محافظة بابل. كما يتم قياس الزنك والحديد والفوسفور والمغنيسيوم وفيتامين هـ في هؤلاء المرضى ودراسة العلاقة بين المعلمات الكيميائيه.

تم تقدير تركيز الكالكتين-3 و الزانثين اوكسيدو ردكتيس و فيتامين هـ بواسطة تقنيه الاليزا. و تم قياس مستوى الحديد والزنك والمغنيسيوم والفوسفور بطريقة القياس اللوني حسب دليل التصنيع. لوحظ وجود زياده معنويه في تركيز الزانثين أوكسيدوريدكتيس و الكالكتين($p < 0.05$) -3 في الأفراد المصابين بأمراض التهاب الأمعاء (مقارنة مع مجموعة السيطرة). من ناحية أخرى كان هناك تباين معنوي في مستويات الحديد والزنك والمغنيسيوم وفيتامين هـ بينما كانت هناك تغيرات غير معنوية ($p> 0.05$) في تراكيز الفوسفور في الدم.

خلصت الدراسة الحالية إلى أن مرضى أمراض الأمعاء الالتهابية في محافظة بابل لديهم مستوى مصل أعلى من الزانثين أوكسيدوريدكتيس وهذا قد يعزى إلى دوره في تعزيز الحالة الالتهابية ووجود الكالكتين-3 في مصل هؤلاء المرضى قد ينتج عن التسرب من الأنسجة ، مما يجعلها علامة بيولوجية محتملة لأمراض الأمعاء الالتهابية ، قد تكون أيضًا بمثابة علامة بيولوجية تنبؤية في المصل.

من ناحية أخرى ، هناك عدة أسباب لنقص الحديد والزنك والمغنيسيوم في الدم في مرضى أمراض الأمعاء الالتهابية مثل انخفاض امتصاص الجهاز الهضمي وزيادة الفقد وانخفاض المدخول بسبب فقدان الشهية.

في حين أن انخفاض مستوى فيتامين (هـ) يرفع نسبة الإجهاد التأكسدي الناجم عن زيادة توليد الجذور الحرة مع ضعف مضادات الأكسدة ،مما يجعله يلعب دورًا مهمًا في التسبب في أمراض الأمعاء الالتهابية.