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Assessment of Xanthine Oxidoreductase level with Zinc and Magnesium in Sample of Patients with Inflammatory Bowel Disease in Babylon Province

Akan Khanjar Nasrullah College of Medicine, University of Babylon, Iraq
Email: arkan369@gmail.com
Ban Mahmood Shaker Al-joda Department of Biochemical, College of Medicine, University of Babylon, Iraq
E-mail: aljodaban@gmail.com
Hassan Salim Aljumaily, Department of Biochemical, College of Medicine, University of Babylon, Iraq
Email: hassanaljumaily310@gmail.com

Abstract

Background: Inflammatory bowel disease affects the colon and small intestine, with Crohn's disease and ulcerative colitis being the most frequent types. Xanthine oxidoreductase (XOR), a dehydrogenase enzyme, is expressed in all cells. Its activity is usually modest. During phylogenesis, the mammalian XOR enzyme regulates ROS and NO production.

Objective:

The objective of this study is to quantify the levels of Xanthine oxidoreductase and investigate any possible associations between zinc and magnesium in the context of inflammatory bowel disease in Babylon province.

Materials and Methods:

Xanthine oxidoreductase levels were evaluated in 100 people: 50 with inflammatory bowel disease and 50 with healthy people. The study participants and the healthy controls varied in age from 15 to 65. The enzyme-linked immunosorbent assay (ELISA) method measured xanthine oxidoreductase while Iron was in the spectrophotometer in the serum. And used a case-control analysis as study design.

Ethical Approval

a-Approval of the scientific committee in the Biochemistry Department of Babylon Medical College.
b-Approval of the scientific committee of Marjan Medical City in Hilla city, Babylon province.

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

Results:

The serum xanthine oxidoreductase was greater in IBD patients than in controls (p 0.01). In contrast, the current study discovered a negative association between zinc and magnesium in IBD patients (p 0.05) and a non-significant relationship between xanthine oxidoreductase, zinc and magnesium in IBD patients (p > 0.05).

Conclusion:

There is a significant association between IBD and high xanthine oxidoreductase levels in Babylon province patients.

Keywords:

Inflammatory bowel disease, Xanthine oxidoreductase, zinc, magnesium.

Introduction

Inflammatory Bowel Disease (IBD) is a collection of inflammatory conditions that affect the colon and small intestine. The primary types of IBD are Crohn's disease and ulcerative colitis. Crohn's disease impacts multiple parts of the gastrointestinal tract, including the small and large intestine, mouth, esophagus, stomach, and anus. In contrast, ulcerative colitis predominantly affects the colon and rectum [1].

Although Crohn's disease and ulcerative colitis are distinct conditions, they may exhibit similar clinical manifestations, such as abdominal discomfort, diarrhea, rectal bleeding, intense visceral cramps, pelvic muscle spasms, and weight loss. Inflammatory bowel disease (IBD) is commonly associated with anemia, which is the most prevalent extra-intestinal complication [2].

Inflammatory Bowel Disease (IBD) is a multifaceted ailment that emerges from the interplay of environmental and genetic factors, ultimately resulting in immunological reactions and inflammation within the intestinal tract [3].

The enzyme XOR is ubiquitously expressed across various cellular phenotypes, primarily functioning as a dehydrogenase. Its enzymatic activity is generally observed to be modest. Throughout the process of phylogenesis, the mammalian XOR enzyme has obtained a crucial regulatory role in the generation of reactive oxygen species (ROS) and nitric oxide (NO). The XOR enzyme has been found to play a role in various biological processes, including but not limited to inflammation, repair, and aging. Additionally, it is involved in physiological pathways such as cell growth, differentiation,

and mobility, as well as in the regulation of endothelial function and vascular tone. This information has been documented in previous research
[4]

The level of XOR expression is notably elevated in the intestinal epithelial cells, which have been suggested to serve as a barrier with microbicidal properties. The enzyme exhibits a high level of concentration in the initial segment of the gastrointestinal tract, goblet cells, and enterocytes of the small intestine, particularly in the basal and apical strata. Additionally, it is found within epithelial Paneth cells, which are acknowledged for their function in providing antimicrobial defense. The enzyme was detected in the apical cell layers of the epithelia of the esophagus and tongue.

Xanthine oxidoreductase (XOR) is typically localized in the cytosol, and has also been observed in peroxisomes. Moreover, XOR has been detected in extracellular compartments, including blood and milk, as reported in reference [5]. The XOR enzyme exhibits a broad range of activity and low substrate specificity, enabling it to perform oxidation and reduction reactions on both endogenous and exogenous compounds. This versatility allows XOR to function as a drug-metabolizing and detoxifying enzyme. XOR can be generated in a state that lacks molybdenum or sulfur at the post-translational level, leading to the enzyme's inactivity at the Moco site [6].

XOR 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 [7].

Zinc is a micronutrient that ranks second only to iron in relation to its concentration within the human body. The zinc content in adult humans ranges from 2 to 3 grams, although determining the precise amount can be challenging, particularly in cases of illness [8].

Zinc is an essential micronutrient that plays a crucial role in various physiological processes, including immune system regulation, cell proliferation and differentiation, wound healing, carbohydrate metabolism, insulin signaling, and sensory perception, particularly olfaction and gustation. The optimal growth and development during pregnancy, infancy, and childhood necessitates the presence of Zinc [9].

The process of zinc absorption occurs in the small intestine through a mechanism that is mediated by a carrier. The absorption of zinc presents a challenge due to its secretion into the gastrointestinal tract [10]. 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%. Studies 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 [11].

Magnesium is ranked fourth in terms of prevalence among minerals in the human body, following calcium, sodium, and potassium. Additionally, it is the second most abundant intracellular cation,

after potassium. On average, an individual weighing 70 kg possesses approximately 25 grams of magnesium in reserve. The distribution of this magnesium is as follows: 53% is located in bone, 27% in muscle, 19% in soft tissues, and less than 1% in serum. The regulation of serum Mg concentration (SMC) is highly precise, with an average serum value ranging from 75 to 95 mmol/L. However, certain studies suggest that serum levels below 85 mmol/L should be classified as deficient [12].

Magnesium functions as a cofactor for more than six hundred enzymes. Magnesium plays a crucial role in various physiological processes such as energy generation, carbohydrate metabolism, DNA replication, and protein synthesis. Magnesium functions as a calcium antagonist within the human body and is an essential element for the synthesis and activation of vitamin D [13].

A deficiency in magnesium has been found to elevate blood pressure, decrease insulin sensitivity, and induce neural excitation. The presence of multiple conditions, such as obesity, type 2 diabetes, cardiovascular disease, metabolic syndrome, and osteoporosis, have been linked to low levels of serum magnesium [14]. Mg 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 [15].

Materials and methods

Patients and control

One-hundred people participated in the current research. Fifty people with IBD (diagnosed using standard clinical, radiographic, endoscopic, and histological criteria) participated in the research; a complete medical history was collected from each participant, including their age, gender, family history, and smoking habits.

Fifty healthy people make up the second group (control group)

The study did not include individuals who met the following criteria: pregnancy, acute or chronic inflammatory illness, severe disease (such as heart failure, renal insufficiency, liver damage, or diabetes), or other related malignancies. These categories range in age from 15 to 65. The statistical analysis was performed using SPSS version 20. In the case of all findings shown as Mean SD, P values below 0.05 indicate statistical significance. Patients at the Gastro Intestinal and Hepatic facility at Merjan Medical City in Hilla, Babylon Province, Iraq, participated in this research. Starting in August 2022 and ending in January 2023.

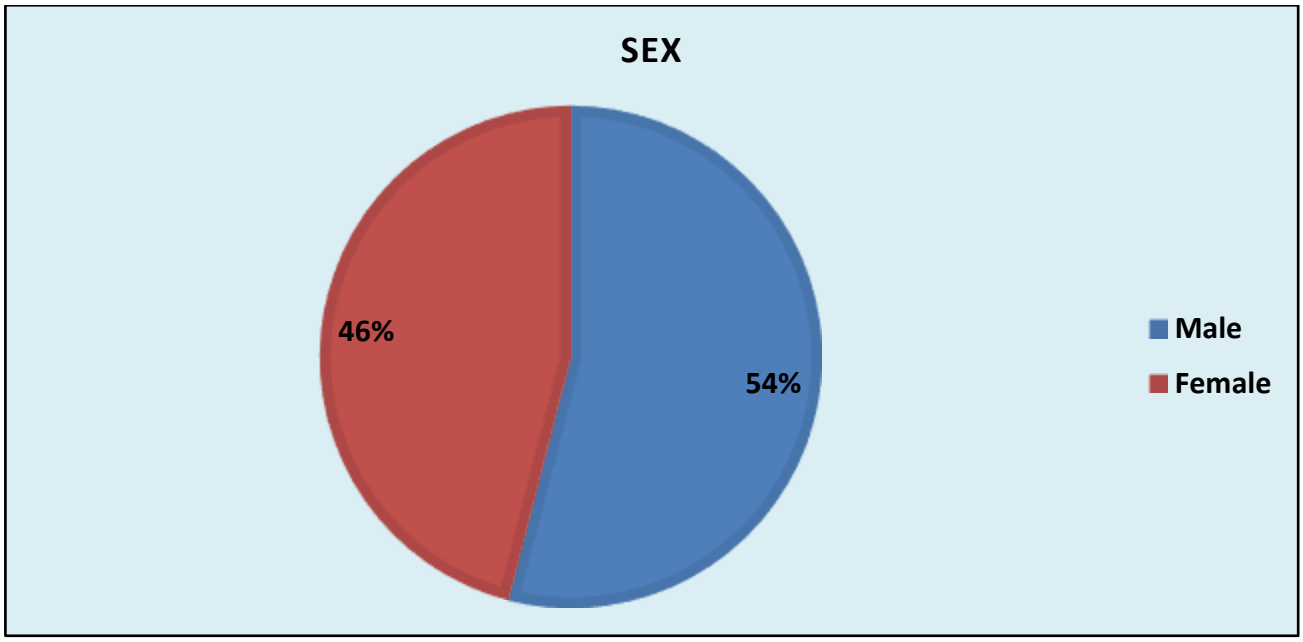

Figure (1): The ratio of females to males in IBD patients.

Chemicals and methods

1-Xanthine Oxidase concentration is measured by an enzyme-linked immunosorbent assay kit [Bioassay Technology Laboratory].

2- A colorimetric test with 5-Bromo-PAPS measures zinc concentration.

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

Ethical issues

According to the native ethics group, these studies were approved, and all patients who participated gave informed permission and provided information about the study's goal, according to document number 4 on 06/07/2022**.**

Results

The study groups consist of 100 adults designated into two categories:

1- Adults have inflammatory bowel disease (n=50)
2- Adults as the control group (n=50)

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Age

Table 1 shows no statistically significant difference in mean age between the control group and the group with inflammatory bowel disease. Patients with inflammatory bowel disease showed the following age distributions in terms of frequency: Figure 1 shows that there were ten patients aged 15–24 (representing 20%), 16 aged 25–34 (32%), 13 aged 35–44 (26%), six aged 45–54 (10%), and five aged 55 and above (10%).

Table-1: The Age of studied groups.

	Control (n=50) Means ±SD	IBD patients (n=50) Means ±SD	P value
Age (Years)	35.06 ± 12.34	39.32 ± 11.63	NS
Range	15-59	15-55	

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

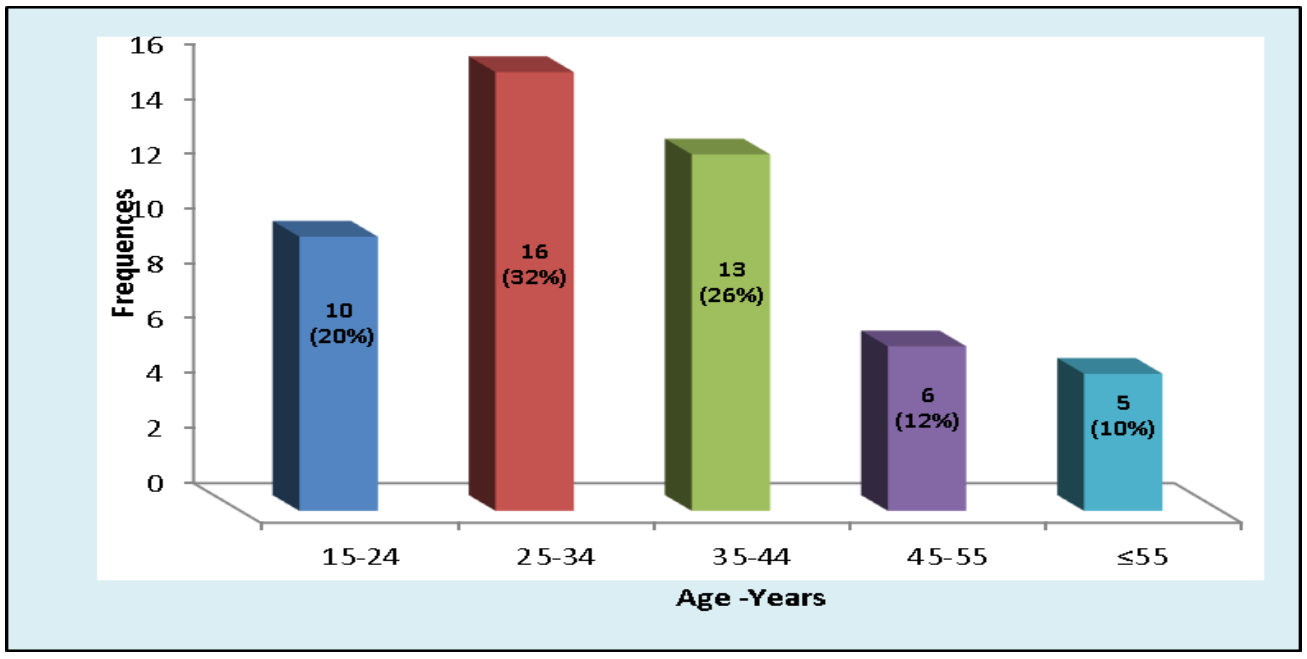

Figure (2): The frequency distribution of inflammatory bowel disease patients according to age.

Gender

Table-2 reveals that males were more likely to have inflammatory bowel disease than women were; the research included 50 patients with inflammatory bowel disease, 27 (54%) of whom were men and 23 (46%) of whom were women.

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

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

Association of Xanthine oxidoreductase level with zinc and magnesium of inflammatory bowel disease patients.

Mean differences between IBD patients and controls are seen in Table 3, with IBD patients having higher xanthine oxidoreductase concentrations and controls having lower zinc and magnesium concentrations.

Conversely, the present investigation found a significant (p-value< 0.05) inverse association between xanthine oxidoreductase and zinc and in patients with inflammatory bowel disease (IBD), as shown in Figures 3 and 4.

Table (3): Mean difference of xanthine oxidoreductase, Zinc, and Mg in IBD patients and control.

		XOR	ZINC	Mg
XOR	Pearson Correlation		-0.164	-0.341**
	P value		0.103	0.001
ZINC	Pearson Correlation			0.360**
	P value			0.0004

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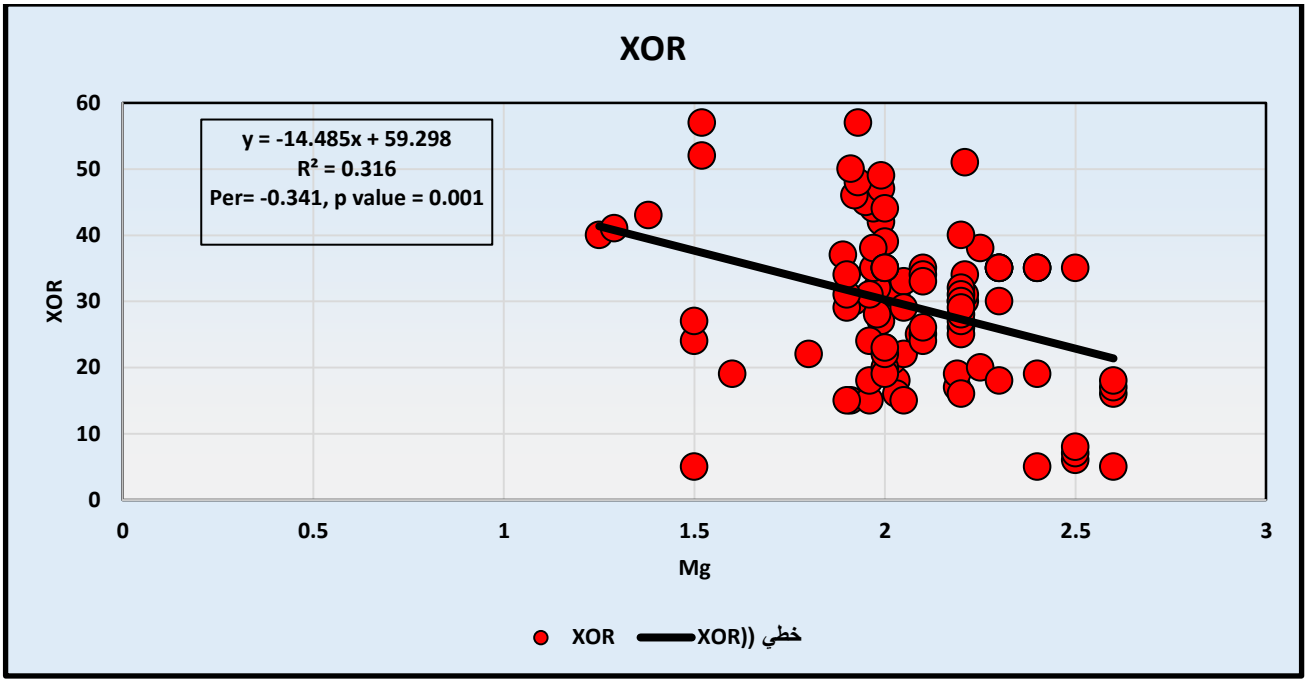

Figure 3: Correlation between Mg and XOR parameters

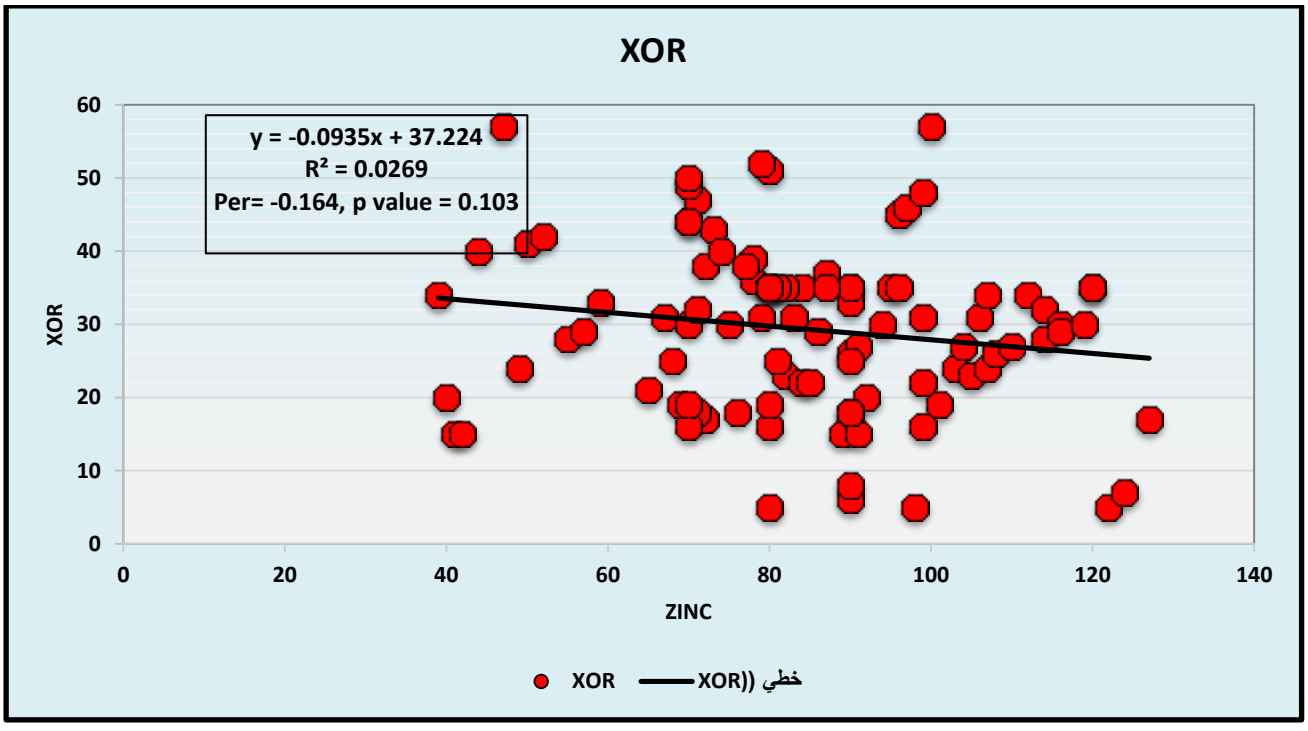

Figure 4: Correlation between Zinc and XOR parameters

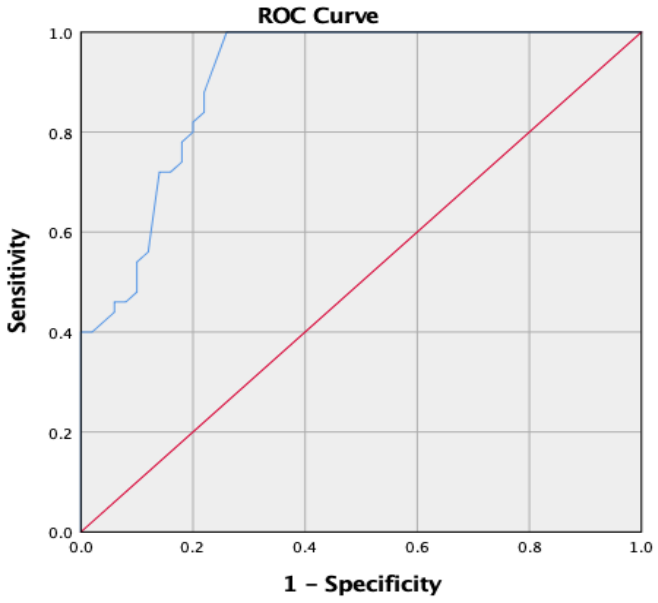

Figure (5): ROC curve for ZINC [Accuracy = 0.906, p value = 0.001

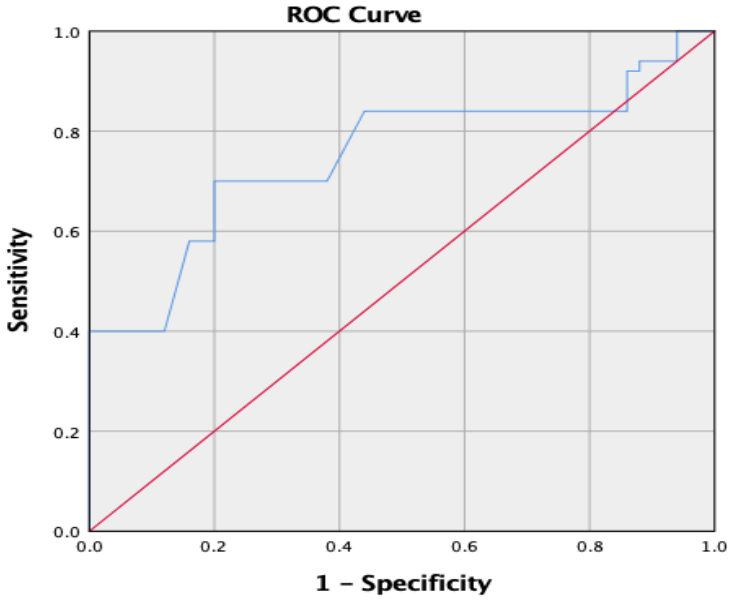

Figure (6): ROC curve for Mg [Accuracy = 0.75 , p value = 0.036]

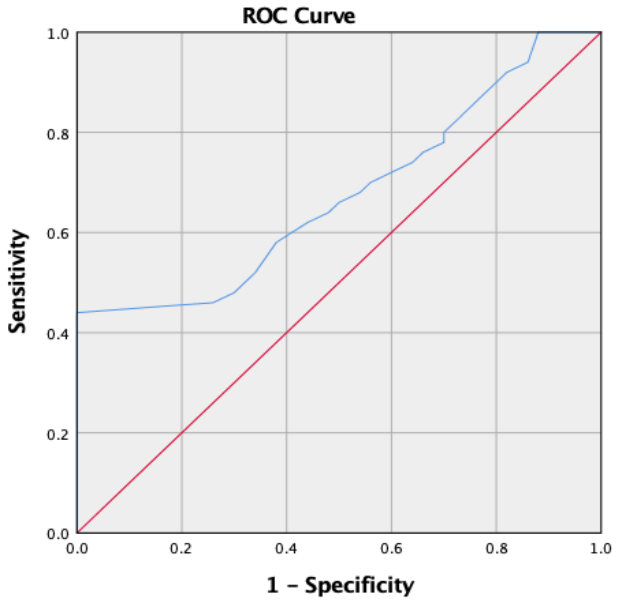

Figure (7): ROC curve for XOR [Accuracy = 0.676 , p value = 0.029]

A recent study has demonstrated a correlation between zinc deficiency and inflammatory status in individuals with inflammatory bowel disease (IBD). One plausible hypothesis for the anti-inflammatory function of zinc in inflammatory bowel disease (IBD) is its potential to mitigate trans-mucosal leakage in Crohn's disease by decreasing the quantity of pro-inflammatory cells and curtailing the production of proinflammatory cytokines [16].

Zinc is a crucial element for cellular proliferation with regards to immunity. The coenzyme function of this substance plays a crucial role in various immune response reactions, impacting both acquired and innate immunity. Additionally, it is essential for thymic hormone function and antioxidant response. This information is supported by reference [17].

The deficiency of Zinc has been observed to hinder or entirely inhibit the activity of phagocytes and lymphocytes, leading to an ineffective cytokine response. Additionally, it has been documented that zinc, as a constituent of activated macrophages, inhibits inducible nitric oxide synthase (iNOS) activity by approximately 90%, thereby impeding the generation of reactive oxygen and nitrogen species and mitigating cellular harm [18].

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

While Griffin IJ, et al. [19] 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.

Magnesium is an essential micronutrient that plays a crucial role in the enzymatic processes involved in carbohydrate and lipid metabolism. Additionally, it exhibits anti-inflammatory properties by regulating inflammatory pathways. Magnesium is a crucial element in the metabolism of skeletal tissue, transmission of neuromuscular signals, and functioning of the immune system. Moreover, several studies have indicated that magnesium has the potential to mitigate oxidative stress and free radical damage in living organisms [20].

The majority of pathological conditions linked to a deficient magnesium status have been identified as having a chronic inflammatory stress element. More than 75 years ago, it was indicated by evidence that a deficiency in magnesium leads to an inflammatory response. Research conducted over the last 25 years has provided evidence that restricting magnesium intake to less than 10% of the recommended daily allowance can lead to an inflammatory reaction that involves the activation of leukocytes and macrophages, the release of inflammatory cytokines and acute-phase proteins, and an overproduction of free radicals or oxidative stress [21].

Our study identified magnesium deficiency among IBD patients compared to healthy controls with significant mean difference ($P<0.05$),

This finding is concomitant greatly with Hekmatdoost A, et al. [22], who revealed reduced gastrointestinal absorption of Mg occurs in IBD patients. Patients with IBD consumed a lower amount of Mg than healthy adults.

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

The presence of chronic inflammatory stress and oxidative stress may be influenced by subclinical magnesium deficiency, potentially leading to various pathological conditions. The present study elucidates the inverse correlation between XOR and Mg.

Conclusion

Xanthine oxidoreductase levels are elevated in individuals with inflammatory bowel disease in Babylon province, suggesting a strong association between Xanthine oxidoreductase and IBD.

Recommendations

The correlation between Xanthine oxidoreductase levels and the severity of inflammatory bowel disease needs further investigation.

Second, further research (a therapeutic trial) is needed to determine whether or whether iron and Zinc supplements are helpful in the management of inflammatory bowel illnesses.

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