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Study of the Level of Enzyme Muramidase and Some Biochemical Variable and Finding the Relationship between Them in Patients with Crohn`S Disease

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Abstract: Crohn's disease is a chronic inflammatory condition affecting any part of the gastrointestinal tract, causing symptoms like abdominal pain and diarrhea. It affects millions worldwide, with higher prevalence in North America and Europe and its incidence continues to rise globally.

Aim of study: The main objective of the current research study the relationship between specific biochemical parameters and Crohn` s disease in Mosul City. A cross study in a group of 70 as 48 males and 22 females participants with crohn` s disease and 70 healthy peoples 30 males, 40 females. Healthy participants by paid a visit outpatient clinics and private hospitals in Mosul city from the date of 19/1 to 25/2 of 2025. Whole blood samples were collected from all volunteers' patients and healthy to be analyze for serum Mur activity, TNF-α, MDA, GPx, PN and CAT. The results were showed that the majority patients with Crohn` s disease indicated higher levels of serum Mur activity in compared to healthy controls Also patients with Crohn` s disease having an increase of TNF, MDA, GPx,

PN, CAT and decreases in levels of Fe,Se and Mn in patients with Crohn s disease.

Keyword: crohn s disease, TNF, Mramidase

Introduction

Crohn's disease (CD) is a chronic inflammatory disease that primarily affects the digestive system. The disease is characterized by intermittent inflammations that can affect any part of the digestive tract starting from the mouth and extending to the anus. (Baumgart & Sandborn, 2012)

The mechanism of Crohn's disease (CD) is multifactorial involving a complex interaction between genetic factors, environmental factors (such as smoking, certain medications, and an unhealthy diet and immune factors. These interactions lead to an abnormal inflammatory response within the gastrointestinal tract causing damage to the intestinal lining or immune impairment which increases the risk of developing the disease. (Khor *et al*., 2011)

Crohn’s disease presents with a wide range of symptoms that vary depending on the location and severity of inflammation in the gastrointestinal tract. Common signs include abdominal pain and cramping often in the lower right abdomen persistent diarrhea that may contain bloo, fever, fatigue, loss of appetite, and unintended weight loss. Patients might also experience mouth ulcers, a sensation of incomplete bowel evacuation, rectal bleeding, and perianal complications such as fistulas or abscesses causing pain and discharge. Additional complications can involve bowel obstruction, ulcers, anal fissures, and malnutrition. Beyond gastrointestinal symptoms, extraintestinal manifestations may occur including joint inflammation, eye redness or pain, skin disorders like erythema nodosum, liver or bile duct inflammation delayed growth and puberty in children, iron deficiency anemia, and increased risk of blood clots and colorectal cancer. Symptoms can develop gradually or abruptly often alternating between flare-ups and remission periods. Since these symptoms are not exclusive to Crohn’s disease, proper medical assessment is essential for an accurate diagnosis. (Mayo Clinic, 2024)

Crohn`s disease is categorized into 3 principal patterns: (1) primarily inflammatory which after several years commonly evolves into (2) primarily stenotic or obstructing or (3) primarily penetrating or fistulizing.

These different clinical patterns dictate different therapeutic approaches. Some genetic studies suggest a molecular basis for this classification. (Walfish & Companioni, 2025)

Muramidase(Mur) E.C.3.2.1.17 , also known as lysozyme (Lyz) or N-acetyl muramoyl hydrolase, is a key enzyme in the human immune system. It plays a crucial role in defending the body against bacterial infections by breaking down the bacterial cell wall, thus helping to eliminate harmful bacteria. (Nawaz *et al*., 2022)

In humans, muramidase, or lysozyme, is composed of 130 amino acids. While its three-dimensional structure broadly resembles that found in chickens, there are distinct differences, particularly in the beta-sheet region. Human lysozyme features a double-stranded antiparallel beta-sheet along with four alpha-helices. In contrast, chicken lysozyme contains three strands of antiparallel beta-sheets. (Galvez-Iriqui & Plascencia-Jatomea, 2020)

In recent years, modern studies have shown a close correlation between the levels of this enzyme and Crohn's disease, which is one of the chronic inflammatory diseases affecting the digestive system. Research indicates that elevated levels of muramidase in the blood may be an indicator of the occurrence and severity of the disease, making it an important variable for assessing the clinical status of the condition. However, the role of Mur is not limited to Crohn's disease only; it also includes a wide range of diseases such as ulcerative colitis, sarcoidosis, tuberculosis,

diabetes and leukemia. (Klebanoff, 2005)

2. Materails and Methods

2.1 Case study

Study deals with the clinical study that include (140) males and females' participants. The participants were divided in to two groups, the first group included (70) patient with crohn's disease, including (48) males and (22) females, and their ages ranged from 12 to 56. The second group included (70) of healthy people (30) males and (40) females and their ages ranged from 18 to 50. The level of muramidase, tumor nicrosis factor–alpha, , Iron, Selenium, Manganese, malondialdehyde, glutathione peroxidase, peroxynitrate and catalase were estimated in the blood of patienst and healthy people.

2.2 Blood sample collection

5ml of venous blood was drawn and collected in gel tube ,leaved at room tempreture for 20 mintes then separated in centrefuge at 3000 rpm after separation ,serum was stored in an eppendrof at -10℃ for biochemical analysis.

2.3 biochemical analysis

Serum levels of muramidase (Mur),Tumer nicrosis factor-alpha (TNF-α), were measured using enzyme-linked immunosorbent assay (ELISA) technology from the Chinese company SUNLONG,Iron (Fe) from the Chinese company Mandri, Selenium(Se),Manganese(Mn)in Atomic absorption spectrometer,Malondialdehyde(MDA),Glutathion peroxidase(GPx) ,Peroxy nitrate(PN),Catalase(CAT) from Aldrich,Sigma,Fluka company.

3. Result and Discussion

The results of assessing the efficacy of the enzyme myuramidase in the serum of Crohn's disease patients showed a significant increase in its level in the patients compared to the control group at a probability level of (p=0.001) as shown in Table (1). This increase may be attributed to the activation of immune inflammatory pathways associated with Crohn's disease . (Pruzanski *et al*., 1977)

The results shown in Table (1) indicate a significant increase in the level of tumor necrosis factor-alpha (TNF-α) in Crohn's disease patients compared to healthy individuals at a probability level of (p = 0.008). The elevated level of TNF-α in Crohn's disease patients is due to an excessive and disorganized immune response arising from a complex interplay of genetic, immune, and environmental factors (Al-Hakeem & Al-Sadoon, 2024). Studies also suggest that genetic mutations, such as those affecting the NOD2 gene lead to impaired ability of macrophages to eliminate certain bacteria, allowing their accumulation within tissues and stimulating the production of large amounts of TNF-α. (Russell *et al*., 2011); (Al-Taii & Jankeer, 2025)

Table (1) indicates a significant decrease in iron levels in individuals with Crohn's disease compared to healthy controls at a probability level of (p = 0.001), suggesting the likelihood of anemia caused by inflammation or malabsorption.

Iron levels in Crohn's disease patients are lower than in healthy individuals due to the disease's effect on the intestines, where iron absorption is reduced because of chronic inflammation and ongoing bleeding within the gastrointestinal tract. Additionally, inflammation leads to an increase in the hormone hepcidin, which inhibits iron absorption and traps iron within cells, resulting in iron deficiency and anemia. Therefore, the decreased iron levels in these patients reflect the disease's impact on the body's ability to obtain and utilize iron normally(Widbom *et al*., 2020)(

The results presented in Table (1) indicate a significant decrease in selenium levels in Crohn's disease patients compared to healthy individuals at a probability level of p=0.001 suggesting a

deficiency in antioxidants. The lower selenium levels in Crohn's disease patients compared to healthy individuals are primarily due to malabsorption of nutrients resulting from chronic inflammation in the intestines. Additionally, the restricted diet followed by patients due to disease symptoms like diarrhea and abdominal pain contributes to this. Furthermore, persistent inflammation increases the body's need for antioxidants, leading to greater consumption of selenium. (Yan *et al.,* 2022)

The results shown in Table (1) indicate a significant decrease in manganese levels in Crohn's disease patients compared to healthy individuals at a probability level of ($p = 0.001$). The low manganese levels in Crohn's disease patients are mainly attributed to chronic inflammation of the intestines, which impairs the absorption of minerals and nutrients, leading to malnutrition and mineral deficiencies such as manganese. Additionally, inflammatory changes and the disease's impact on the gastrointestinal system contribute to manganese deficiency, which may affect the patient's overall health. (Huang *et al*., 2012); (Yaseen & Saeed, 2021)

The results shown in Table (1) indicate a significant increase in the concentration of malondialdehyde in the serum of Crohn's disease patients compared to healthy individuals at a probability level of ($p = 0.004$), reflecting increased oxidative damage in the cells. (Rezaie *et al*., 2007)

The elevated level of malondialdehyde in the blood of Crohn's disease patients reflects the increased damage resulting from chronic inflammation and oxidative stress in their bodies. Due to the persistent inflammation in the intestines, immune cells produce large amounts of free radicals that lead to lipid oxidation in body cells, causing damage and increasing the accumulation of malondialdehyde, which is an indicator of this damage. Therefore, malondialdehyde levels are higher in Crohn's disease patients compared to healthy individuals who do not experience such inflammation or oxidative damage.(Merino de Paz *et al*., 2024); (Abachi *et al*., 2022)

The results in Table (1) indicate a significant increase in glutathione levels in the serum of individuals with Crohn's disease compared to healthy controls at a probability level of ($p = 0.001$) suggesting an increased antioxidant activity as an attempt to counteract oxidative stress caused by chronic intestinal inflammation

The elevated levels of the enzyme glutathione peroxidase in the blood of Crohn's disease patients represent a natural response of the body to combat the damage resulting from chronic inflammation and oxidative stress in the intestines. This enzyme acts as an antioxidant that protects cells from damage caused by free radicals, reflecting the body's effort to enhance defense mechanisms against the effects of the disease. (Szczeklik *et al*., 2016)

Table (1) indicates a non-significant increase in peroxynitrite levels in Crohn's disease patients compared to healthy individuals at a probability level of ($p = 0.155$). The results showed that Crohn's disease patients have elevated levels of peroxynitrite in the blood compared to healthy individuals, reflecting increased inflammation and oxidative stress associated with the disease. This elevation suggests that peroxynitrite may play a role in intestinal tissue damage and disease progression. It is considered an important marker that can be utilized to assess disease severity and monitor treatment effectiveness, though it should be considered alongside other indicators to ensure accurate diagnosis and comprehensive care. (Liu *et al*., 2021)

Table (1) shows no significant differences in catalase levels between Crohn's disease patients and healthy individuals at a probability level of ($p = 0.770$) indicating that this marker may not be directly or markedly affected by the disease. (Alam *et al*., 2019)

Table (1) levels of muramidase and some biochemical parameters.

Biochemical parameters	Control N=70		Patient N=70		p-value
	M	SD	M	SD	
Muramidase(pg/ml)	0.8	0.94	8.99	1.99	0.001
Tumer nicrosis factor(pg/ml)	150.2	26.25	174.2	52.67	0.008
Iron(µmol/L)	17.45	4.09	12.20	5.48	0.001
Selenium(µmol/L)	79.53	5.96	46.10	5.89	0.001
Manganese(µmol/L)	0.79	0.12	0.51	0.11	0.001
Malondialdehyde (Mmol/L)	0.27	0.20	0.40	0.24	0.004
Glutathion peroxidase(U/L)	1.39	0.30	2.20	0.71	0.001
Peroxy nitrate (M/L)	39.31	12.94	43.83	18.02	0.15
Catalase (mKat/L)	62.98	13.24	62.23	12.07	0.77

P is significant at $p \leq 0.05$

The relationship between Muramidase and the other biochemical parameters

The statistical relationship using the Correlation Coefficient was employed to determine the association between the Muramidase and the biochemical variables measured in the serum of a group of patients with Crohn's disease. As shown in Table 2, the correlations did not reach significance levels. A positive correlation was observed between Muramidase and tumor necrosis factor-alpha (TNF-α), whereas an inverse correlation was found between Muramidase and each of catalase, peroxynitrite, iron, selenium and manganese in Crohn's disease patients. Meanwhile, the relationship between Muramidase and glutathione peroxidase at the significance level $p \leq 0.01$ reflects an increase in antioxidant activity in response to intestinal inflammation.

Table (2) Analysis of the correlation coefficient between the muramidase and the biochemical variables measured in the serum of patients with Crohn's disease

Table (2) correlation between muramidase and biochemical variables

Biochemical parameters	Muramidase
Tumer nicrosis factor-alpha (Pg/ml)	0.075
Iron(µg/L)	0.020-
Selenium(µg/L)	0.088-
Manganese(µg/L)	0.086-
Malondialdehyde(Mmol/L)	0.034
Glutathion peroxidase(U/L)	0.389**-
Catalase(mKat/L)	0.183-

**Correlation is significant at the 0.01 level (2-tailed)

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