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Evaluation of INFγ in serum of Crohn's patients by using ELISA technique

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***Abstract*— Crohn's disease, a chronic inflammatory bowel disorder that can cause gradual intestinal damage and an impairment. CD can affect people of all ages, from children to the elderly, and can causes a considerable morbidity and reduce the quality of life. The main aim of this study was to explore the serum level and evaluate the predictive role of immunological parameters of Interferon gamma (INF-γ) for the detection of an active disease in Crohn's patient. The current investigation, 70 blood samples acquired from Crohn's disease patients and 70 apparently healthy individuals were used. A sandwich ELISA kit (No: E-EL-H0108) U.S.A. was used to determine serum levels of INF-γ that contribute to disease progression. A total of 140 samples (70 cases and 70 controls) were collected in a 1:1 ratio, with each participant was screened base on inclusion and exclusion criteria. This study encourages employing INF-γ as a predictive biomarker for Crohn's disease.**

***Keywords*— Crohn's disease, Interferon gamma, Inflammatory Bowle Disease.**

I. INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory gastrointestinal condition that might cause a progressive bowel damage and disability. The exact cause of Crohn's disease is unknown; The research indicates that in genetically predisposed people, multiple events lead to innate immune system dysregulation. Aspects of Crohn's disease that involve inflammation during Crohn's illness, inflammation, which commonly involves the distal ileum and proximal colon, is often non-contiguous and patchy, and is also segmental and transmural [1]. CD is regarded as a global health concern, continuing to rise in incidence in newly industrialized countries and with high prevalence in western countries. The etiology and pathogenesis of CD help to inform the clinical diagnosis and treatment, and to improve the clinical outcomes [2]. CD disease exhibits a wide spectrum of severity and clinical progression. Common symptoms often involved abdominal pain, chronic diarrhea, fatigue, reduced appetite, unintended wight loss, and anemia. Additionally, extraintestinal complications may arise, significantly impacting patient's quality of Life (QOL). Currently, there is no cure for Crohn's disease, making life long essential management [3]. Although the degree of symptoms varied and may wax and wane over the disease course, patients can require persistent immunosuppression and procedures to address the disease symptoms, [4]. Studies strongly point out that impaired mucosal immune homeostasis is the cause of inflammatory bowel disease (IBD). An aberrant reaction to the gut microbiota as the immune system weakens can lead to a sequence of inflammatory events that damage the intestinal wall, causing the intestinal mucosal barrier to collapse and accelerate the progression of IBD [5]. IBD appears in patients who have a genetic predisposition to the disorder and have immune system abnormalities, which are often associated to specific aspect of the environment. They are distinguished by active and remission periods of illness. Depending on the location and degree of inflammation, the condition can vary in people in term of course and severity [6]. Medical care should be adopted to variety of characteristics, including disease subtype, severity, behavior, and location. The time on diagnosis the size of the lesions and extra-intestinal symptoms are other factors to consider. In actually, none of the medications used in the therapy of CD has been proven to be curative or completely safe [7]. The first-line treatment in clinical practice and for many years was infliximab [IFX] and adalimumab [ADA], which are anti-tumor necrosis factor-α (anti-TNF) medications. As expertise increased, doctors were adjusted to and at ease using these drugs [8]. Since cytokines are essential to the inflammatory cascade, they are thought to be significantly engaged in the pathogenic process as was mentioned by Li and Shi, 2018 [9].According to the conventional knowledge, Th1 cytokines are the primary cause of CD development, and the illness caused by problems of the acquired immune system. Only a few of them, such as IL1, 2, 6, 12, 18, TFN-α, and INF-γ, could be used to treat CD.

The current study surveyed the role of immunological marker INF-γ in CD activity from immune regulation, with the goal of providing new insights into disease diagnosis and activity control. In this study, we presented a framework for understanding variations in cytokine levels, as well as presenting available information on how aberrations in these components causes an immunological imbalance, which may affect on therapeutic approaches.

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II. METHOD

A. Study setting

The samples were collected from 140 Iraqi patients divided equally between cases and controls. The 70 patient admitted to the hospitals, after clinical confirmation with Crohn's disease from the 1st of November, 2024 to the 1st of February, 2025. Regarding sex, 38 patients were male and the rest were female. The study was conducted at the Gastroenterology and Hepatology Hospital, Medical City in Baghdad. The inclusion criteria of the case group were clinically confirmed cases of Crohn's disease. Inclusion criteria of the control group was apparently healthy patient without Crohn's disease. The Exclusion criteria include patient with other inflammatory bowel disease (IBD) like Ulcerative Colitis (UC) or indeterminate colitis, patient with Gastrointestinal disease condition such as celiac disease or diver colitis or Irritable Bowel Syndrome (IBS), Patient with infectious disease such as HCV, HBV. The isolated sample's serums were divided into five 0.5ml Eppendorf tube, frozen at -80℃ until ELISA analysis.

B. Ethical Consent

This study approved by the Ethics Committee of AL-Iraqia University, Baghdad, Iraq (No. FM.SA.182).

C. Measurement of serum INF-γ from patients with Crohn's disease by using ELISA kit

This assay employed a sandwich enzyme-linked immunosorbent assay (ELISA) technique for the specific detection of human INF- γ (kit No: E-EL-H0108 U.S.A.). The optical density (OD) measured at 450nm (±2nm). The levels of INF-γ was measured according to instruction of ELISA leaflet. INF- γ concentration was determined by comparing sample OD to standard curve in quantification of data (Fig. 1).

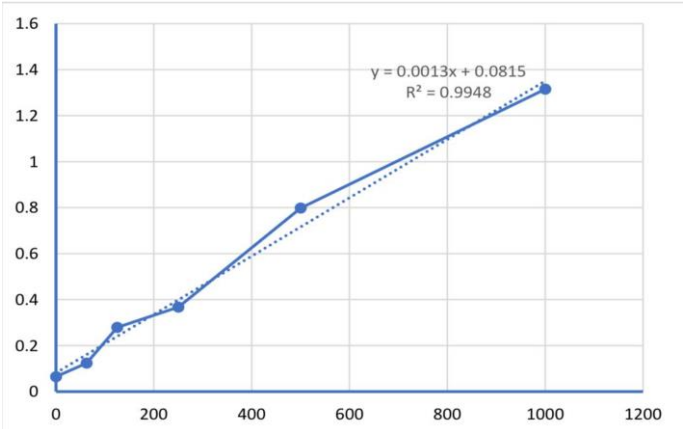

Fig.1: The relation between INF-γ Con and OD. It shows that the linear range where absorbance (OD) correlates best with concentration. Whereas the R^2 value indicates the goodness fit was ideal.

D. Statistical Analysis

The study data were entered, checked, and analyzed using computer software's programs of Statistical Package of Social Science (SPSS) version 26 and STATISTICA version 12. Descriptive statistics that consist of the frequency distribution tables, number and percentage were used for qualitative data, whereas that of the mean, standard deviation and range were used for quantitative data. Unpaired independent-samples student T-test, and Chi-square test were used to identify the differences between study groups of cases and controls regarding different quantitative and qualitative (categorial) parameters respectively. The univariate logistic regression model and Roc curve were used to identify the optimal cut off value of immunological parameters of Interferon gamma (IFN-γ) as predictive diagnostic markers for Chron's disease. A *P*-value of < 0.05 was used as statistical significance criterion throughout study.

III. RESULTS

A total of 140 samples (70 cases and 70 controls) were collected in a 1:1 ratio, with each participant was screened based on inclusion and exclusion criteria. The age of participants ranged from 10 to 56 years old, with a mean age of 31.61 ± 11.594. The largest proportion of participants (30.7%) fell within the 20–29 age group.

The mean SD of patient group was 29.21 ± 10.813 years old with a majority at the age group of 20-29 years old (40%), and that of controls was 34.00 ± 11.926 years old mostly at the age group of 40-49 years (28.6%) with significant mean differences among them (t= -2.487, df: 138, *P*= 0.014) as well as significant differences were identified regarding age groups of study samples ($x2$: 10.467, df: 4, *P* = 0.033).

Regarding the sex, the overall sample was female dominant, with a female to male ratio of 1.25:1 (55.7%: 44.3%). There were no significant differences (*P* > 0.05) between the cases and control groups, which were also female dominant, with female to male ratios of 1.33:1 (57.1%; 42.9%) and 1.18:1 (54.3%; 45.7%) respectively (Table 1).

Table 1. The study's sample's baseline characteristics (n=140)

Characteristics	Study groups (Chron's disease)			
	Cases (Yes, n=70)	Control (No, n=70)	Total (n=140)	Significancy
	Age (years)			
Mean ± SD	29.21 ± 10.813	34.00 ± 11.926	31.61 ± 11.594	t= -2.487, df: 138, P= 0.014a
Range (min-max)	42 (13- 55)	46 (10- 56)	46 (10- 56)	
	Sex			
Female	40 (57.1)	38 (54.3)	78 (55.7)	x^2: 0.116, df: 1, P = 0.734 b
Male	30 (42.9)	32 (45.7)	62 (44.3)	

a: Unpaired T-Test, b: Chi-Square Test.

Regarding immunological parameters among the groups examined, it has been shown that the mean level of INF-γ was found substantially elevated within case groups of study's samples in comparison to the controls group (568.039 ± 115.4206 vs. 134.576 ± 19.8671) respectively with a significant difference of 433.4629 (t= 30.965, df:138, *P*= 0.000) (Table2) (Fig.2).

Table 2. The mean level of INF-γ in patients and control groups (n=140)

Immunological Parameters (Mean ± SD)	Study groups (Chron's disease) (n=140)		Mean differences	Significance [a]
	Cases (Yes, n=70)	Control (No, n=70)		
Interferon gamma (IFN-γ)	568.039 ± 115.4206	134.576 ± 19.8671	433.4629	*t*= 30.965, df:138

Significant deference between groups *P*= 0.000 a: Unpaired T-Test

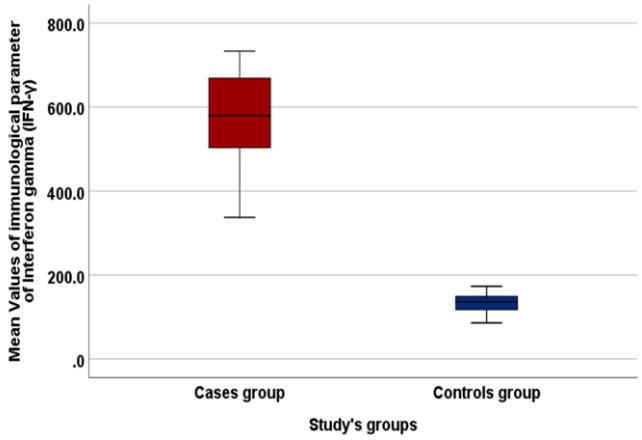

Fig. (2): The Mean comparison of immunological parameter of Interferon gamma (IFN-γ) among study's groups (n=140). It appears to present comparative data on INF-γ levels across different study groups where the X-axis represents the study group, while the Y-axis represents the mean INF-γ concentration. Significant Differences (*P*=0.000).

A. Immunological parameter (INF-γ) as a potential indicator for Chron's diseases progression.

Between 140, the ideal cutoff value of interferon gamma (IFN-γ) in the study sample to identify persons at higher risk of developing Chron's illnesses was **172.350** with a sensitivity of 100%, a specificity of 98.6%, and perfect area under the ROC curve (AUC) of 1 (*P*= 0.000) (Fig. 3).

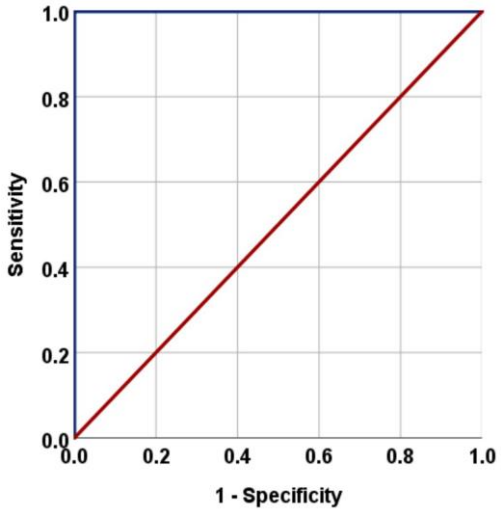

Fig. (3): The sensitivity and the specificity of ELISA test to measure the IFN- γ

IV. DISSCUION

The current study assess the serum levels of immunological marker Interferon Gamma (INF-γ).
Interferon gamma (IFN-γ), is a soluble cytokine secreted by immune cells and epithelial cells. It regulates numerous cellular processes, including immune modulation, leukocyte migratio, cell proliferation and apoptosis, as well as antimicrobial and anticancer activities. Additionally, IFN-γ exerts aprotumor effect by activating the janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway. IFNγ R is essential for IFN-γ biologic activity and signaling [10]. As a result, INFs are now to be understood as crucial cytokine in autoimmune hepatitis, rheumatoid arthritis, and inflammatory bowl disease (IBD). The typical immune mediated inflammatory disease is IBD, that disturbs the gastrointestinal system and become more mutual worldwide [11]. So, IFN-γ elevated levels contribute to chronic inflammation, tissue damage, and impaired mucosal healing. The presence of IFN-γ in mucosal tissue and serum making it a biologically plausible biomarker for predicting disease outcomes.

Interestingly, the current study showed that serum level of INF-γ among patient had a respectable relation with number of Crohn's disease. Data on baseline INF-γ concentration were available for 70 patient, the mean level of immunological parameter of Interferon gamma (IFN-γ). showed to be noticeably greater among the study's samples' case group when compared to the controls group (568.039 ± 115.4206 vs. 134.576 ± 19.8671) in respectively with significant difference of 433.4629 (t= 30.965, df:138, P= 0.000).

Patients with CD have significantly higher systemic quantities of the cytokines such as IFN-γ, IL-1β and TNF-α compared to healthy controls, indicating greater inflammation. A study by Xu *et al*., 2021 [12] suggested using a cytokine cocktail of TNF-α, IFN-γ, and IL-1β (20 ng/mL each) to create a more physiological environment.

The intestinal inflammation is associated with aberrant production of these cytokines in CD. The expression levels of TNF-α, IFN-γ, and IL-1β receptors were analyzed in intestinal organoids derived from Crohn's patient. Our findings suggest that, IFN-γ have a significant impact on intestinal barrier function, in addition to their role in regulating inflammation.

CD mucosa overexpresses macrophage-derived IL-12, IL-18, and TNF-α, leads to a Th1 immune response and increased production of IL-2 and IFN-. This response is thought to trigger intestinal inflammation. These soluble compounds will produce inflammation in the CD mucosa. Furthermore, disrupt mucosal and immunological homeostasis, contributing to the pathogenesis of IBD. Mesenchymal Stem Cells have a well-established role in the regulation of inflammatory responses and have a significant pathogenic impact on intestinal barrier function [13]. Patient with higher levels of IFN-γ experiences more severe disease courses which could help early detection, prevent treatment failure and allowing for timely adjustment in therapy.

V. CONCLUSION

Elevated INF- γ levels in patients with Crohn's disease than that in control groups, revealed its contribution to chronic inflammation, tissue damage, and impaired mucosal healing. This makes it reasonable biomarker for predicting disease outcomes, primely autoimmune disease including Crohn's disease.

CONFLICT OF INTEREST

The authors affirm that they have no conflicts of interest.

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