Measurement of interleukin-6 levels and some of the hematological factors in individuals that suffering from Crohn's disease in Iraq

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Abstract

The Crohn's disease (CD), which is state of recurrent systemic infections with gut symptoms that are related to immunological issues, this disease typically affects the digestive system. The CD is a type of Inflammatory bowel disease (IBD). Patients with CD have a genetic background and are affected by environmental variables and the gut microbiome. A blood sample of 30 patients from Iraq with CD and thirty samples of healthy people as control were taken in this study during the period from Jul 2022 to Dec 2022. Gastro-Enterology and Hepatology Hospital in Baghdad, Iraq is the place that the blood samples were taken from. Hematological test was performed on all samples and also measurement of IL-6 levels in serum. The results of this study show significant differences in hematological factors between patients and control also in levels of IL-6. The conclusion of this study is that interlukine-6 plays part in the progression of CD by neutrophils are drawn to the site of inflammation, and furthermore neutrophils cause more damage to the same site.

Keywords*:* IL-6, inflammatory bowel disease**,** Crohn's disease.

1. Introduction

The Crohn's disease (CD), which is state of recurrent systemic infections, primarily affecting the digestive system with symptoms that are related to immunological disturbances. The two primary forms of the inflammatory bowel illness are the ulcerative colitis and the Crohn's disease (Baumgart and Sandborn, 2012). Inflammatory bowel disease is becoming more common worldwide, with an estimated 3-8.5/100,000 cases occurring in European nations, and up to 2.2 million individuals afflicted with the disease. Although it can impact any section of the digestive system, including colon and terminal ileum are the most common locations (Schmitt et al., 2021). Many symptoms are related with CD, these symptoms including, bleeding in rectal, raise in body temperature, reduction in weight and also diarrhea. Many of the environmental elements and genetic have been coordinate with the CD development (Veauthier and Hornecker, 2018).

Although, CD can impact people of any age, it often manifests (diagnoses) in teens and young adults. To lessen the impact of CD, a variety of treatment strategies are employed, such as enhancing quality of life, maintaining symptomatic management, and reducing short- and long-term toxicity and consequences (Lichtenstein et al., 2009). The onset of Crohn's disease, including tissue inflammation that is provided by an uncontrollably excessive immune reactions to

luminal antigens of bacteria. Immune cells that penetrate CD patients' guts, including as T- helper cells, T- cytotoxic cells, B-cells monocytes, and natural killer (NK) cells, are involved in the immune reactions (Petagna et al., 2020).

The primary goal of reaching clinical recovery was changed from time to include endoscopic recovery, mucosal healing, and steroid-free recovery. These three outcomes are now essential to effective CD treatment, having replaced the earlier focus on lowering the level of clinical symptoms. First category of the drugs authorized on the management of CD were antibodies against TNF, such as certolizumab pegol, adalimumab, and infliximab. Over the following few years, CD treatment has been authorized for antibodies against interleukin 12 (IL12), interleukin 23 (IL23), and integrin alpha4beta7 (vedolizumab) via their common p40 subunit (Sandborn et al., 2013), (Klenske et al., 2019).

Since abdominal discomfort, such as rectal bleeding, fatigue, fever, diarrhea, and loss of weight are among the most prevalent symptoms of CD, it is linked to severe morbidity and negatively affects the life quality of the patient. Inflammation outside the intestines often appears in the skin, joints, the eyes, and the liver, indicating the systemic character of this crippling illness. Furthermore, most patients eventually experience structuring or penetrating issues that require additional procedures and leave them disabled (Harbord et al., 2016).

Gastrointestinal bleeding, it happened due to inflammation and ulceration of the gastrointestinal of the CD patients, which this bleeding may cues anemia. The anemia is a common and important manifestation of CD (Wilson et al., 2004).

The development and progression of CD is mediated by cytokines which play important role in this part. These cytokines are tiny proteins essential in signaling between the cells. These proteins function is to be involved in signaling through autocrine, paracrine, and endocrine pathways as immunomodulators. The Cytokines are formed and generated by diverse cell types, which includes, B lymphocytes, macrophages, T lymphocytes, neutrophils, and mast cells, plus other immune cells. Cytokines are essential to the emergence of inflammation and immunity and are engaged in several biological processes, including the stimulation of cells, growth, and differentiation. When an infectious agent or other danger signal is present, a rapid defense mounting can be achieved by the cells of the innate immune system such as, the macrophages and the monocytes, which these cells secrete cytokines that promote inflammation, such interleukin-1, interleukin -8, interleukin -6, and interleukin-12, and the tumor necrosis factor (TNF)-a. The T and B lymphocytes-mediated adaptive immunity is then developed under the guidance of the cytokine milieu. The CD4 T-helper lymphocytes, that produce interferon (IFN)-c, TNF-a and support delayed-type hypersensitivity, responses with granuloma development and macrophage activation, are the primary source for the Th1 immune responses, which are triggered by IL-12. The CD4 T helper cells, which produce interleukin-5, interleukin-13, interleukin-10, and interleukin-4 and stimulate humoral immunological reactions and allergic hypersensitivity reactions, are the main players in the T-helper-2 (Th2) immune response development (Papadakis and Targan, 2000), (Neurath, 2014).

The shocking discovery that scientists had cloned or purified the same protein shocked researchers who had been working on the identification of several seemingly unrelated biological growth factors almost ten years ago. Some names as hybridoma growth factor, plasmacytoma growth factor, hepatocyte stimulatory factor, hematopoietic factor, 26K factor, interferons, and T cytotoxic-cell differentiation factor were among the many

names given to the molecule that we now know as interleukin-6 (IL-6). Every name represented a different biological function that the same protein controls (Simpson et al., 1997).

Many cell types produce the interleukin-6 (IL-6), which has pleiotropic effects on multiple organ systems. The cytokine interleukin-6 is a model for maintaining balance. The interleukin-6 is generated and aids in defense of the host against such invaders, by triggering of the acute-phase, also the immunological reactions when infections or tissue damage disturbs homeostasis, however, many disorders such, the acute systemic inflammatory response syndrome and the chronic immune-mediated diseases, these two disorders can pathologically be impacted by dysregulated, excessive, and protracted interlukin-6 production (Tanaka et al., 2018). The Crohn's disease (CD) is defined by increased production of proinflammatory chemokines and cytokines as well as CD4+ cell infiltration into the inflammatory areas of the gut mucosa (Seegert et al., 2001).

The genomes of humans and mice, the IL-6 gene is found at chromosomes 5 and 7p21, respectively. Four introns and five exons are found in each of the cloned and sequenced IL-6 genes for humans, mice, and rats. Within the exon region, which is the protein-coding region of the gene, exon lengths, and cysteine residues within exons are preserved across species. Nevertheless, variations take place outside the coding region at the 5' and 3' boundaries of exons 1 and 5, respectively (Simpson et al., 1997).

2. Aim of this study

This study's objective is to determine the amount of interluken-6 in patients that have Crohn's disease in Iraq as well as hematological parameters such, red blood cells counts, white blood cells counts, neutrophils counts, and hematocrit.

3. Material and methods

3.1. Collection of samples

Based on clinical symptoms and endoscopic imaging 30 blood sample were collected have CD and 30 blood sample as healthy individual.

3.2. Time and place of samples collections

During the period from Jul 2022 to Dec 2022 the samples were collected. Iraq's the Gastro-Enterology and Hepatology Hospital which is the hospital that the blood samples were collected from for the study.

3.3. Technique of treating samples

The hematology analyzer Diagon-Cell 60 (Diagon, Hungary) was used to obtain a complete blood count (CBC), and the sandwich enzyme-linked immunosorbent assay (SUNLONG, China) was used to measure IL-6 levels.

3.4. Analytical statistics

The SAS (2012) software is utilized to identify the characteristics that differed among the parameters under study. Means comparison at probability threshold 0.01. For all outcomes, the mean ± standard deviation (SD) was utilized (Judge et al., 2001).

4. Results

According to the Table-1 there was notable variations in hematological factors between CD patients and control group.

Table 1: Hematological Factor Comparison between the Control Group and Individuals with Crohn's Disease.

Group	No.	Hematocrit (percentage) the Mean± SD	Neutrophils ($x10^9$/L) the Mean± SD	the White blood cells ($x10^9$/L) the Mean± SD
CD	30	35.08 ± 4.6	10.4 ± 3.7	7.5 ± 1.2
control	30	42.95 ± 4.2	4.7 ± 0.9	6.1 ± 1.1
p-value		0.0001	0.0001	0.01

There were significant differences in the level of interluken-6 between individuals with Crohn's disease and control group as in Table-2.

Table 2: Comparing the serum IL-6 levels of Individuals with Crohn's Disease and Controls.

Group	No.	IL-6 (pg/ml) theMean ± SD
CD	30	37.4 ± 1.3
control	30	24.9 ± 1.7
p-value		0.0001

5. Discussion

Many local mediators (cytokines) are produced from varying cells of the lymphoid and macrophage lineage, as well as epithelial and mesenchymal cells. All protocols of proliferation, cell activation, proliferation, and differentiation, as well as the development of inflammation and immunity, is coordinated by these local mediators. The innate immune system can quickly start and mount a defense by secreting many proinflammatory cytokines like interleukin (IL)-1, IL-12, IL8, IL-6 when an infectious agent or other danger signal is present.

Th1 immune responses are primarily induced by IL-12 and are mostly produced by CD4 T-helper cells, which release TNF-a, interferon (IFN)-c. T-helper-2 (Th2) which develop by immune reactions of T helper cells (CD4), that secrete interlukine-10, Interleukins -4, Interleukins -13, Interleukins -5 and support humoral immune responses and allergic hypersensitivity reactions. Patients with UC and CD, it shows that many of the cytokines that mediate inflammation (proinflammatory cytokines), such as, Interleukins-6, Interleukins-1, and tumor necrosis factor-a, were showed to be higher expression in the inflammatory mucosa. Moreover, chemokines and other lipid mediators of inflammation are generated. The activated endothelium recruits' blood-borne effector cells via chemokines and adhesion molecules, that are both increased by TNF-a, IFN-c, and IL-1. By this process amplifies the inflammatory cascade and releases more damaging enzymes, free radicals, and inflammatory mediators that injure tissue and are linked to the etiology of fibrosis, diarrhea, and mucosal permeability (Papadakis and Targan, 2000).

The current study shows a higher level of IL-6 in patient group compared to healthy individual group, the reason for that may be to the reaction with as pathogen-associated molecular patterns (PAMPs), which is microbial compounds, macrophages release IL-6. These PAMPs attach to pattern recognition receptors (PRRs), a vital class of molecules that are detected by the cells of the host's first line of defense system, and also contains Toll-like receptors (TLRs). They turn on intracellular signaling pathways that produce pro-inflammatory cytokines and are found on the cell surface and within intracellular compartments (Simpson et al., 1997). The results of this study are agreement with (Gross et al., 1992), which this study

used 70 consecutive patients with CD, the results show patients with CD had a mean blood level of IL-6 (mean, 6.8 -t 0.9 U/mL) that was higher from that of healthy people. Another study which isolates intestinal mononuclear cells by taking colonic biopsies.

The hematological factors used in this study are hematocrit, neutrophils and total white blood cells count. The results of this study show significant differences in hematological factors between CD patients and control group. The hematocrit is considered a measured part of a blood test, which is the amount of red blood cells by volume (RBCs) in whole blood. low present ofhematocrit was noticed in CD patients perhaps all the sample was taken have severe bleeding cases the results of this study agreement with (Belaiche et al., 1999) and (Veroux et al., 2003).

The result of this study shows up normal count of neutrophils and normal levels of white blood cells count in CD patients, neutrophils considered a type of white blood cells, the reason for that it may be the drawing in neutrophils to the area of inflammation by producing cytokines like IL-6 from surrounding cells in the intestine, neutrophils are essential for the first line of protection against the invasive pathogens, additionally to attracting and stimulating the other cells of the immune system. Neutrophils are able to directly fight microbes by the creation of neutrophil extracellular traps, degranulation (release of soluble anti-microbials), and phagocytosis (ingestion). Research on neutrophil responses to infection or damage indicates that the neutrophils must first stick to the blood artery endothelium, roll along the endothelium, and then tightly arrest at the afflicted locations. After being stopped, the cells move out of the vasculature to different infection sites. In summary, the family of selectin adhesion molecules E-selectin and P-selectin. these family of the selectin are expressed by endothelial cells, assists in the start of the attachment and rolling of neutrophils in the peripheral vasculature's postcapillary venules, while L-selectin promotes lymphocyte adhesion in the lymph nodes' high endothelial venules. Notwithstanding the significant function of selectins in promoting leukocyte rolling, a multitude of molecules possess the ability to mediate leukocyte recruitment in an independent manner from selectins. These consist of vascular adhesion protein 1, CD44, and α4 integrin (Hickey and Kubes, 2009). This is in agreement with what was found by some studies (Segal, 2018).

Conclusion

The conclusion of this study is that interlukine-6 levels contribute to the onset of CD by drawing in neutrophils to the area of inflammation, and furthermore neutrophils cause more damage to the same site.

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قياس مستويات الإنترلوكين ٦ وبعض العوامل الدموية لدى الأفراد المصابين بمرض كرون في العراق

الحسن طالب ولي: جامعة آشور/ كلية العلوم/ قسم علوم الحياة

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الخلاصة

مرض كرون وهو حالة من الالتهابات الجهازية المتكررة مع أعراض الأمعاء التي ترتبط بمشاكل مناعية، وعادة ما يؤثر هذا المرض على الجهاز الهضمي. وهو نوع من انواع مرض التهاب الامعاء. المرضى الذين يعانون من مرض كرون لديهم خلفية وراثية ويتأثرون بالمتغيرات البيئية والميكروبيوم المعوي. تم في هذه الدراسة أخذ عينة دم من ٣٠ مريضا من العراق مصابين بمرض كرون مع ثلاثين عينة من الأشخاص الأصحاء كمجموعة سيطرة خلال الفترة من تموز ٢٠٢٢ إلى كانون الأول ٢٠٢٢. مستشفى أمراض الجهاز الهضمي والكبد في بغداد، العراق هو المكان الذي تم فيه أخذ عينات الدم. تم إجراء اختبارات الدم على جميع العينات وكذلك قياس انترلوكين ٦ في مصل الدم. أظهرت نتائج هذه الدراسة وجود فروق ذات دلالة إحصائية في العوامل الدموية بين المرضى والسيطرة أيضا في مستويات الانترلوكين ٦. الاستنتاج من هذه الدراسة هو أن إنترلوكين ٦ يلعب دورًا في تطور مرض كرون عن طريق سحب العدلات إلى موقع الالتهاب، علاوة على ذلك تسبب العدلات المزيد من الضرر لنفس الموقع.