Study the Association of IL-18 on Crohn’s Disease in Iraq Population

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Abstract Crohn’s disease (CD) is an inflammatory bowel illness that can affect any portion of the GI tract, including the colon. The CD is caused by an abnormal immune response to microbiota in the gastrointestinal system, and it can develop in those genetically predisposed to it. Inflammatory bowel disease, on the other hand, is well-acknowledged to be a complex condition in which intrinsic genetic factors may play a role. Cytokine genes are thought to be crucial in inflammatory bowel disease. Interleukin 18, a newly discovered proinflammatory cytokine, has lately been related to CD and other inflammatory bowel diseases. In the current investigation, 79 biopsy samples were taken from CD patients and control subjects of both sexes and ages during the period from October 2022 to March 2023 from Teaching Hospital Gastroenterology and Hepatology, Medical City, Baghdad, of the study was to determine the levels of Interleukin -18 (IL-18), in CD individuals and compare with the healthy group and the risk of developing CD.

Key Words IL-18, Crohn’s disease

1. Introduction

Crohn’s disease (CD) is linked to an aberrant immune system’s reaction to the microbiota, especially in patients genetically predisposed to the ailment. It has been demonstrated that people with CD have changes in the composition of their gut bacteria community and a decrease in the number and diversity of their microbiota population. Researchers have discovered changes in Firmicutes, particularly Clostridia and Proteobacteria, in CD patients [1], [2]. Given the recent discovery that Proteus spp. may be a pathogen in the recurrence of CD after intestinal resection, we performed additional research to investigate the possibility of its function as a gastrointestinal pathogen. A recent study showed that postoperative ileal biopsies with detectable Proteus spp. are associated with a 14-fold higher risk of CD recurrence [3], [4].

Proteus mirabilis may interact with intestinal lining cells to induce an immune response. As part of the immune response to the presence of bacteria, cytokines such as IL-18 may be produced. Many cytokines, including Interleukin-18 (IL-18), have been studied recently in the etiology of CD. Because of the bacterium’s inflammatory properties, there has also been increased attention on the possible link between Proteus mirabilis infection and CD [5], [6].

Ethics Statement

After the Helsinki Declaration, the University of Al-Anbar Governorate’s Medical Ethics Committee in Ramadi, Iraq, approved this study (approval number 45, June 15, 2022). All participants in the study, whether they were patients or their parents, provided written informed consent.

2. Materials and Methods

Thirty-nine patients with CD between the ages of (14-75) years participated in this study. Name, age, sex, occupation, residence, and history were collected from all patients. These patients were selected from the Teaching Hospital Gastroenterology and Hepatology, medical city, Baghdad. Each case was selected through a colonoscopy conducted by an internist. All suggested inclusion criteria for CD cases were applied, and all patients underwent a full clinical evaluation. Forty healthy participants with no prior history of CD or any of the exclusion criteria were chosen as a control group for blood sample collection. Participants in the study supplied written informed consent.
Specimens
Blood specimen
Blood specimens were collected from the patients and control groups; five milliliters of the blood sample were collected by venipuncture and poured into a plastic tube after sterilization of the skin with antiseptic material; the blood samples were placed in a plastic plain tube and centrifuged in (a 3000 rpm for five minutes) then separate the serum. Serum samples were kept in tubes at −20 °C for use in immunoassays to measure serum IL18 levels from both groups using the ELISA method.

3. Results and Discussion
General Characteristics of the Patients and Control
This study included 39 Crohn’s disease patients, 23 (58.974%) of whom were male and 16 (41.0256%) female. As a control group, forty 40 healthy people participated in the study.

The ages of Crohn’s patients ranged from (14-75) years, and they were divided into four age groups. The results indicated that males were more prevalent in all groups, while the age groups (43-58 years) were more prevalent in females than males. The recent study showed that the age group (26-42) had the highest frequency compared to all other age groups of males, as shown in (Table 1).

The age group (26-42) increased significantly more than the other groups, followed by (less than 25) years, while the age group (more than 59) decreased significantly in the study groups. This result was consistent with [7], which showed that CD affects all age groups. Its percentage increases among young people, and the reason is due to frequent smoking, eating fast food, and significant exposure to stress and fatigue, which affects the body. It reduces the ability to tolerate diseases, particularly in light of Iraq’s current economic position [8], [9]. The most affected group was the category with an average standard of living. It decreases with age over 59 years, although advanced age is associated with a change in the immune system, as it changes with age. This age-related shift in immune function may influence the development of autoimmune diseases such as CD [10].

Interleukins (IL-18) in Serum are Measured by ELISA
The frozen serum samples were thawed and left to thaw at room temperature, centrifuged for a short period at 2000 rpm, and then processed using the ELISA method. The (Ltd.) The company manufactures the immunological kits used to assess the concentration of IL-18 in serum (Table 2).

The levels of IL-18 in the Patient and Control Groups’ Serum
In all serum samples taken from patients and controls, human serum (IL-18) was found in the patients at different levels, while no IL-18 was found in the control group.

Our findings showed IL-18 levels in Crohn’s patients’ serum were greater than in the control groups. The average IL-18 concentration in the serum of CD study participants was (40.5 _ 8.9) ng/mL, compared to (0.0 _50.6) ng/mL in the control group, with significant differences. Table 3 shows the statistical differences between patients and controls.

The pro-inflammatory cytokine IL-18 is generated in response to microbial assaults and other stimuli. Cells that make it include macrophages, dendritic cells, and epithelial cells. IL-18 is essential for Th1 immune responses and the generation of pro-inflammatory cytokines, including interferon-gamma (IFN-). In CD, higher levels of IL-18 have been found in inflamed intestinal tissues, implying that it may play a role in the illness’s etiology and immunological dysregulation.

My research showed a higher level of IL-18 in patients with CD than in healthy individuals. We found that IL-18 rises with disease relapse. Our findings also show that the level of IL-18 increases with increased support for disease activity through increased cytokine production. A major role for this cytokine in the pathogenesis of CD. This study’s findings are consistent with [12], [13].

Some studies have been presented regarding the effect of IL-18 on the structural integrity of the intestinal epithelium via its effect on tight connective tissues. This may be due to the presence of IL-18 receptors on intestinal epithelial cells, which may modify tight junction proteins, affecting barrier function, or induce pro-inflammatory cytokines, causing tissue inflammation and activating cell signaling pathways, which may affect epithelial cell turnover. These data suggest that IL-18 overproduction causes increased intestinal monolayer permeability, resulting in intestinal inflammation. This mechanism’s effective utilization can potentially improve CD symptoms [14].

The IL-18 receptor is composed of the constitutively expressed co-receptor IL-18R, which exhibits a relatively low affinity for the mature form of IL-18. Upon binding of IL-18 to its receptor, IL-18R, the interaction activates the toll/interleukin-1 receptor (TIR) domain, initiating signal transduction. This process leads to the recruitment of additional IL-18R units, thereby forming a high-affinity receptor complex. Subsequent to this assembly, the signaling domain of the receptor engages the adapter protein MyD88. This interaction triggers the activation of the NFκB pathway and initiates pro-inflammatory responses. Additionally, the extracellular interleukin 18 binding-inhibiting protein (IL-18BP) plays a crucial regulatory role. IL-18BP has a higher affinity for IL-18 compared to IL-18R, effectively sequestering soluble IL-18 and preventing its interaction with the IL-18 receptor, as depicted in Figure 1 (referenced in [15], [16]).

Natural killer (NK) cells and cells called Th1 that express IL18R can produce IFN in response to IL18 [17], [18]. Furthermore, by reciprocally triggering the expression of their respective receptors, IL18 and IL12 work together to increase the release of IFN by a range of cell categories; nonpolarized T cells, NKT cells, dendritic cells, macrophages, and B cells are examples of these cells [17]. It is widely understood that anti-CD40 and IL4 stimulation of B cells generates IgG1 and IgE. Combining IL-12 and IL-18 with B cells injected
Table 1: Distribution of Crohn’s patients according to the ages

<table>
<thead>
<tr>
<th>Age group</th>
<th>Crohns Count</th>
<th>% of Total</th>
</tr>
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<tbody>
<tr>
<td>&lt;= 25</td>
<td>12</td>
<td>15.2%</td>
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<tr>
<td>26 - 42</td>
<td>16</td>
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<tr>
<td>43 - 58</td>
<td>6</td>
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<tr>
<td>59+</td>
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<td>6.3%</td>
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<tr>
<td>Total</td>
<td>39</td>
<td>49.4%</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group</th>
<th>Normal Count</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;= 25</td>
<td>11</td>
<td>13.9%</td>
</tr>
<tr>
<td>26 - 42</td>
<td>13</td>
<td>16.5%</td>
</tr>
<tr>
<td>43 - 58</td>
<td>13</td>
<td>16.5%</td>
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<tr>
<td>59+</td>
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<td>3.8%</td>
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<tr>
<td>Total</td>
<td>40</td>
<td>50.6%</td>
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<table>
<thead>
<tr>
<th>Total Count</th>
<th>% of Total</th>
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<tbody>
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<td>47</td>
<td>59.5%</td>
</tr>
<tr>
<td>32</td>
<td>40.5%</td>
</tr>
<tr>
<td>79</td>
<td>100.0%</td>
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Table 2: Materials provided with the anti-IL18 ELISA Kit

<table>
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<th>Cass or control</th>
<th>ELISA</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohns</td>
<td>7(8.9%)</td>
<td>32(40.5%)</td>
<td>39(49.4%)</td>
</tr>
<tr>
<td>Normal</td>
<td>40(50.6%)</td>
<td>0(0.0%)</td>
<td>40(50.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>47(59.5%)</td>
<td>32(40.5%)</td>
<td>79(100.0%)</td>
</tr>
</tbody>
</table>

Figure 1: Pathogen-associated molecular patterns (PAMPS)

Figure 2: Role of interleukin-18 in the pathophysiology

Combined with IL-1, it helps boost the host’s immunity against infections by improving phagocyte antibacterial capacities and activating Th1 and Th17 responses by the immune system [23], [24]. Furthermore, polymorphisms involving a single nucleotide within the IL-18 genes that cause loss of function disturb the balance of Th1 and Th2 immune system responses, raising host susceptibility to CD development [25]. By increasing the generation of additional pro-inflammatory molecules like TNF-alpha and IL-1, IL-18 acts as an immune response amplifier.

Some results of Crohn’s patients’ negative IL18 (8.9%) could be due to the patient being treated with CD inhibitor medications like Remicade or pharmaceuticals that help reduce intestinal inflammation, such as balsalazide.

IL-18 interacts with other T-cell development and activation cytokines, like IL-12 and IL-23. In CD, cytokine imbalances can alter the inflammatory processes. Diet, exercise, and reducing anxiety and stress all affect the amount of IL18. These findings correspond with my previous research on CD patients [26], [27].

4. Conclusion

The study’s findings give evidence that IL-18 may have a significant pathogenesis role in Th1-mediated CD diseases. Th1 cytokines, on the other hand, are largely synthesized in CD tissues, and locally released molecules contribute to the proliferation of IFN-producing cells. IL-18 has a crucial role in promoting IFN-synthesis and Th1 cell proliferation. As a result, IL-18 may contribute to the local immune response in CD by encouraging the development of Th1-positive
intestine lymphoid cells, which increases IL-18 within the afflicted area.

**Conflict of interest**

The authors declare no conflict of interests. All authors read and approved final version of the paper.

**Authors Contribution**

All authors contributed equally in this paper.

**References**


