Studying of Some Immunological Parameters in Patients with Inflammatory Bowel Disease (IBD) in Al-Anbar Province

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ABSTRACT

The serum levels of interlukine-17A and interlukine-17F were determined using ELISA technique for quantitative determination of IL-17A and IL-17F concentrations in serum. A comparison in Immunological parameters between the two groups (patients and control) showed that there was a significant difference in levels of IL-17 A and IL-17F with p-value <0.05. The means were: IL-17 A (79.29 pg/ml), 18.72 pg/ml and IL-17F (226.4 pg/ml), 29.09 pg/ml respectively. Also, the serum levels of pANCA and c ANCA were determined using Enzyme-linked immunosorbent assay ELISA technique for quantitative determination of pANCA and c ANCA concentrations in human serum. There was a significant difference in levels of perinuclear antineutrophil cytoplasmic antibodies (pANCA) and cytoplasmic antineutrophil cytoplasmic antibodies (c ANCA) with p-value<0.05, the means were: pANCA (60.93 ng/ml), (10.31 ng/ml), and c ANCA (35.78 ng/ml), (8.34 ng/ml) respectively.

1. INTRODUCTION

Immunity is one of the physiological processes that can be split into innate and adaptive immunity in human beings. To recognize 'self' and 'non-self' elements, the human body relies on immunity; therefore, the immunity protects humans against invasion of pathogens. The use of a wide collection of different pattern recognition receptors and a mechanism for random and non-selective production of specific antigen receptors are two major processes of innate immunity. Adaptive immunity has been seen as a supplement to innate immunity and a conclusive solution to pathogen recognition [1].

Cells are related to inflammatory bowel disease pathogenesis as they are inherently plastic to the provocation of the surrounding state [2].

A potent pro-inflammatory factor in IBD is the Th17 cells that manufacture IL-17. In the study, IL-17 expression and IL-17A and IL-17F levels in the mucosa and serum of IBD patients were higher than in healthy controls [3,4].

Various Th17-related cytokines were found to be upregulated compared to normal subjects in UC and CD patients, but they were higher in UC patients [5,6].

The antigen P-ANCA responds to is usually myeloperoxidase (MPO) [7]. Other neutrophil elements, including the material granules azurophil (elastase, cathepsin G and antimicrobial cationic protein), minor granules (lactoferrin) and cytosolic proteins such as a-enolase, may be targets of autoantibodies [8,9].

Myeloperoxidase (MPO) is a large cationic molecule consisting of a heterodimer with a molecular weight (MW) of about 140kDa, which represents about 5% of the total protein content of neutrophils. The enzyme is characterized by a powerful bactericidal function, the peroxidase activity of which is physiologically inhibited by ceruloplasmin [10].

Proteinase 3 (PR3) is a serine protease within the azurophil granules of neutrophils and monocyte lysosomes with elastin lytic and other functional activities. Proteinase 3 is almost always the C-ANCA reaction antigen [8,9,11].

Proteinase 3 (PR3) is a weak molecular weight cationic protein (MW) (29-30 kDa) that belonging to the serine proteinase family of trypsin. PR3 is synthesized as a preproenzyme and subsequently processed into mature form in four stages. It is stored in neutrophil azurophilic granules but can also be located inside the secretory vesicle membrane, and it is physiologically inhibited by α1-antitrypsin [12].

In conjunction with IBD, a third main subgroup of ANCA, often denoted to as "atypical" which is now known as xANCA This is a form of P-ANCA that is not
myeloperoxidase-reactive. Usually demonstrates a pattern of combined perinuclear and cytoplasmic staining, a broad array of non-MPO p-ANCA antigen specificities are described (e.g. elastase, lysozyme), each with subtle variations in staining (which may class them as atypical) [8,9,11,13].

The term atypical p-ANCA should be considered as a misnomer and substituted by the more appropriate term antineutrophil nuclear antibodies (ANNA) [14].

As a mixture of c-ANCA and p-ANCA, the atypical pattern of ANCA is characterized as fine rim-like staining with intranuclear foci or large non-homogeneous rim-like staining of the nuclear periphery [15,16].

Atypical ANCA testing may be a useful test in this particular population to distinguish CD from UC. Previous studies indicated that atypical p-ANCA is a useful parameter to differentiate CD from UC [17].

In 1966, Faber and Elling first documented IBD-associated ANCA, which described the existence of leukocyte-specific antinuclear factors in patients with ulcerative colitis [17].

This discovery was not generalized until 1983, when granulocyte-specific antinuclear antibodies were also found in patients with ulcerative colitis and Crohn's disease by Nielsen and associates [19].

Perinuclear staining is produced by P-ANCA. C-ANCA and P-ANCA react with cytoplasmic antigens by definition. The perinuclear distribution of staining caused by P-ANCA on alcohol-fixed neutrophils is the product of antigen rearrangement from the cytoplasm to the nucleus during substrate preparation [20].

C-ANCA and P-ANCA (including IBD P-ANCA) stain neutrophil cytoplasm when neutrophils are fixed with formalin that immobilizes the antigens, rather than with alcohol. However, the role of ANCA in IBD may still be controversial, particularly in ambiguous cases. A previous study found a link with disease activity. ANCA may predict the development of IBD years before it is clinically diagnosed at the early age of the onset of disease, fibrostenosis, internal fistulas, and perianal disease and ileal involvement [21,22,23].

2. METHODOLOGY

2.1. Patients and Controls

The study included (60) patients of different ages (15-60) who were suffering of inflammatory bowel disease, and attended general teaching hospital in Ramadi city in Al-Anbar governorate during the period extended from the 1st of May 2020 to the 1st of January 2021. The samples of patients were selected according to the diagnosis of gastroenterologists. While control included (40) healthy persons of different ages (15-60) years. They were classified as a negative test group because after examination by gastroenterologists they did not show a history of inflammatory bowel disease.

From an acceptable vein, 10 ml of venous blood was obtained. Tourniquet was applied above the selected venipuncture site around (4-5) finger width and disinfected by 70% of ethanol for 30 seconds and allowed to dry completely, the blood was divided into two type of tubes, the first one; 2.5 ml whole blood was dispensed in tow tubes with ethylene diamine tetra acetic acid tube (EDTA-tube) and mixed gently. In the second tube; the residual part of the blood sample was transferred to it (free of anticoagulation) and allowed to coagulate for serum separation using centrifuge at (4000 rpm) for 5 minutes. The separated serum was collected in a sterile clean white tube for serological studies. The tubes were then placed in an aseptic cool-box and deposited in the freezer at (-20°C) before further processing [24].

2.2. Diagnostic Kits and Chemical Reagents

The following diagnostic kits and chemical reagents were used:

- Serum levels of interleukine-17A and interleukine-17F commercially available Enzyme Linked Immuno Sorbent Assay (EIISA) kits (Biosource InC, USA) were used for quantitative determination of IL-17A and IL-17F concentrations by micro plate Enzyme immunoassay in human serum which based on similar principle. Human Perinuclear Anti-Neutrophil Cytoplasmic Antibody (pANCA) ELISA Kit (Abbexa InC, UK) was used for quantitative measurement of pANCA concentration by micro plate Enzyme immunoassay in human serum. Human anti-neutrophil cytoplasmic antibody (cANCA)sandwich ELISA Kit (Abbexa InC, UK) was used for quantitative determination of human anti-neutrophil cytoplasmic antibody (also known as cANCA) concentration by micro plate Enzyme immunoassay in human serum.

3. RESULTS AND DISCUSSION

The total number of persons included in the study was (100) samples which included (60) patients were found to have inflammatory bowel disease (cases) and (40) normal persons as control.

3.1. Immunological Data Analysis

3.1.1. levels of IL-17A and IL-17 F

The present study showed significant increase in IL-17A levels in patients with IBD (cases) compared with persons without IBD (control) with p-value (<0.05) and the mean for IL-17A incases higher than control The means for their groups of IL-17A were (79.29 pg/ml ), ( 18.72 pg/ml) as shown in figure(3-1), respectively.
The present study showed significant increase in IL-17F levels in patients with IBD (cases) compared with persons without IBD (control) with p-value (<0.05) and the mean for IL-17F in cases higher than control the means for their groups of IL-17F were (226.4 pg/ml ), (29.09 pg/ml) respectively as shown in figure(3-2).

Fig. (3-2): Mean levels of IL-17F (pg/ml) in patients and controls. Std. Deviation: Control=9.359, patient =49.78

The present study showed significantly differences in level of IL-17A and F in cases and control.

IL-17A and IL-17F share (60%) among the members of the IL-17 family of cytokines. They are normally expressed by Th17 [25].

IL-17A and IL-17F share similar functions as members of the IL-17 family, especially in terms of their ability to induce the expression of chemokine which is important for neutrophil recruitment and activation [26].

The most studied cytokines which are responsible for the pathogenic activity of Th17 cells are IL-17A and IL-17F [27]. The present study is concordant with the study of [28] which showed in patients with UC, the IL-17 concentration was substantially higher than that in controls. The present study is also in agreement with the study of Jiang et al. [29] that showed an increased in serum and intestinal tissue levels in IL-17 and Th17 cells in IBD patients.

Several studies have moreover recently indicated that raised serum IL-17 levels are related to an enlarged risk of UC [28] that does consistent with the present study. In inflammatory bowel disease (IBD) immunopathogenesis, cytokines plays an important role, including Crohn's disease and ulcerative colitis, where many aspects of intestinal inflammation are driven and regulated. The imbalance between proinflammatory and anti-inflammatory cytokines that occur in IBD contributes to disease progression and damage to tissue and limits inflammation resolution [30]. By macrophage activation, IL-17 can stimulate the development of cytokines and chemokines in endothelial cells, thus contributing to neutrophil recruitment and inflammation [31]. In addition, serum IL-17 levels have been significantly correlated with the severity of IBD, suggesting that serum IL-17 may be closely linked to the progression of the disease to inflammation and reasonable explanations for this association may be that IL-17 mutations change the stability of a higher number of related pro-inflammatory cytokines secreted into the serum of UC patients [32].

### 3.1.2. Levels of p-ANCA and c-ANCA

The present study showed significantly increase in levels of p-ANCA in cases compared with control with p-value was (< 0.05) and the means of p-ANCA for two groups were (60.93 ng/ml), (10.31ng/ml) respectively as shown in figure (3-3).

Fig. (3-3): Mean levels of pANCA (ng/ml) in patients and control. Std. Deviation: Control= 3.189, patient = 17.41

The present study showed significantly increase in levels of c-ANCA in cases compared with control with p-value was (< 0.05), and the means of c-ANCA for two groups were (35.78 ng/ml), (8.341ng/ml) respectively as shown in figure (3-4).
ANCAs are autoantibodies against neutrophil granules and monocyte lysosomes, including p-ANCA and c-ANCA, and serum p-ANCA was first identified as a diagnostic tool to diagnose and treat IBD [33].

ANCA test might be a good test for the diagnosis of UC and differentiating UC from CD. The specificity of ANA for diagnosing was 97% [34]. The present study is consistent with the study of Prideaux et al. [35] which showed the highest prevalence of p-ANCA was demonstrated in patients with IBD.

The outcome of multiple comparisons showed that the seropositivity of pANCA was substantially higher in patients with moderate and serious disease than in those with mild disease [36].

Nevertheless, the prevalence of pANCA was 50 (70%) in UC patients, 6 (20%) in CD patients and 1 (2.5%) in healthy individuals [37].

Several studies showed a correlation between p-ANCA serum levels and UCA intensity [38] that are consistent with current study.

Atypical p-ANCA are found at high prevalences in patients with IBD, they represent a potentially valuable diagnostic seromarker for these disorders [39] that does also consistent with present study.

The present study is also consistent with the study of Rutgeerts and Vermeire, [40] which showed in the serum of patients with IBD, p-ANCA has been observed, the prevalence of positive p-ANCA in UC ranges from 40 to 80%.

Another study showed with extensive colitis and positive C-ANCA serology the cases were eight patients (more than 40%) were also PR3-positive, whereas the other 10 were PR3-negative [41].

The presence of many forms of antibodies in the serum of IBD patients demonstrates the immune-mediated existence of IBD; tissue damage and antibody development occur in IBD as a result of gene mutation-based dysregulated innate immunity; several antibodies with distinct antigenic specificities are generated, the role of these antibodies in IBD pathogenesis is uncertain. To date, serological antibodies tend to be likely to be indicators of an irregular immune response rather than direct effectors involved in disease pathogenesis [42].

Autoimmunity to neutrophils can facilitate tissue damage via numerous mechanisms [43].

In patients with these complaints, ANCA can cause a respiratory burst and degranulation of neutrophils which have been primed by cytokines; The preactivation or priming of neutrophils is associated with the translocation of certain granule contents to the surface membrane, including ANCA target antigens, where autoantibodies are available. Degranulation and release of neutrophils can then directly damage or contribute to the development of an immune complex in the vascular endothelium; proteinase 3 can also provide a proliferative T cell response [44].

In addition, ANCA may be a predictor of inherited susceptibility to ulcerative colitis, demonstrated first by the discovery of an increased prevalence of ANCA in the unaffected family members of sera-using patients with ulcerative colitis. ANCA recognition is also a potential predictor of the immunological disorder underlying these conditions [45].

Nevertheless, previous studies have indicated that p-ANCA may be a responsive and specific test for diagnosing UC, separating it from Crohn's disease and other colitides, and offering a prognosis for medical care response and risk of pucausies after the pelvic pouch [46].

While the present study is not consistent with the study of Peeters et al. [47] which showed there was no relationship between the patients and control of ANCA tests.

Moreover, the study of Abu-Freha et al. [48] founded prevalence of ANCA and pANCA in Arab Bedouin IBD patients.

By contrast this study does not agreement with the study of Saibenniad Ghorsi [49]. Which confirms the low prevalence of p-ANCA observed in ulcerative colitis patients. That does not consist with present study.

The present study also not consistent with the study of Zhou et al. [50] which showed there was no statement on ANCA prevalence in Taiwanese patients with IBD.

4. CONCLUSIONS

A significant increase in IL-17A and IL-17F in patients with IBD more than control. Significant increase was noticed in p ANCA and c ANCA in patients more than control.

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دراسة بعض المؤشرات المناعية لدى مرضى التهاب الأمعاء (IBD) في محافظة الأنبار

ذكرى ماجد محمد فرج العلي وحسن علي مطر وثائر عبد القادر صالح

الخلاصة:
تم تحديد المستويات المصلية لكل من الحركي الخلوي IL-17A و IL-17F و الحركي الغليظ IL-17A و IL-17F باستخدام تقنية الامتصاز المناعي المرتبط بالإنزيم (ELISA)، حيث أجريت مقارنة المتغیرات المناعیة بين مجموعتي المرضى والسيطرة، حيث بنيت النتائج هناك زيادة معنوية بين مجموعتي المرضى ومجموعة السيطرة لكلا من الحركي IL-17A (79.29 pg/ml), (18.72 pg/ml) عند مستوى معنیة (P < 0.05) ذا كان المتوسط العام لكلا من المتغیرات: IL-17F (226.4 pg/ml), (29.09 pg/ml). حيث اظهرت هذه الدراسة أيضا وجود فرق معنی بين المجموعتي جهان من الأجسام المناعیة IL-17A و pANCA و c ANCA عند مستوى معنیة (P < 0.05) ذا كان للمتوسط العام لكل من المتغیرات: pANCA (60.93 ng/ml), (10.31 ng/ml), c ANCA (35.78 ng/ml), (8.341 ng/ml).