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Assessment of some Interleukins in Sample of Iraqi Patients with Ankylosing Spondylitis

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Abstract: Ankylosing spondylitis (AS) is a widespread persistent inflammation illness indicated by chronic inflammatory of the peripheral joints and vertebral column, which results in bone degradation consequently, has an obvious impact on the worth of the sufferers' lives. Cytokines have a key role in the pathophysiology of AS. During the progression of a disease, inflammation is likely caused by cytokines. This study included 100 AS male patients with a mean age (40.56 ± 10.19) and a disease duration of 9.04 ± 2.03 years. The age of the male control (38.78 ± 10.57) was matched (p= 0.227). The Bath AS(Ankylosing spondylitis) Disease Activity Index (BASDAI) and the Bath AS(Ankylosing spondylitis) Functional Index (BASFI) were (3.45 ± 2.03 and 3.84 ± 2.62) respectively. It was found that 57% of patients have HLA-B27 positive and the levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were (25.39 ± 19.59 mm/h and 18.34 ± 3.45 mg/l) respectively. This study evaluated the levels of interleukins (IL-20, IL-22, and IL-24) with the development of Ankylosing spondylitis (AS) in the Iraqi population by using ELISA. The differences in the Interleukins concentrations in serum between patients and healthy controls were included. The results for serum levels of (IL-20, IL-22, and IL-24) appeared higher significant differences (p <0.0001) in patients as contrasted to healthy control (194.0 ± 52.90 pg/mL *vs* 99.52 ± 11.99 pg/mL), (373.8 ± 102.2 ng/mL *vs* 132.0 ± 41.63 ng/mL), and (389.6 ± 180.7 ng/mL *vs* 186.9 ± 29.38 ng/mL) respectively. In conclusion, higher serum concentrations of IL-20, IL-22, and IL-24 were found in male patients with AS in comparison to healthy controls. It was concluded that might be considered as biomarkers in AS development.

Keywords: Autoimmune disease; ankylosing spondylitis; disease duration; disease activity.

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Introduction

Spondyloarthritis, also known as ankylosing spondylitis (AS), is a form of autoimmune disease with persistent inflammation that mostly influences the joints of the spine and consequently can cause severe pain and, in extreme cases, the fusion of the vertebrae. Autoimmune-mediated inflammation persists over time, primarily affecting the spine (1).The Subligamentous of new bone forms in AS, resulting in ankylosis of the vertebrae and sacroiliac joints (2). There is still a lack of knowledge about the molecular etiology of AS and the autoimmune trigger that causes it. There is, however, a compelling theory that the relationship between genetic, immunological, and viral components is crucial (3). Human leukocyte antigen (HLA)-B27 is the most prevalent risk factor associated with AS genetically and immunologically, vulnerability in individuals of many races; nevertheless, a minority of patients with manifest AS lack HLA-B27 (4). This might suggest that additional risk assessment elements influence the emergence of the disease. In addition, T helper (h) 1 and Th17 cells perform an essential function in two the (innate and adaptive) immunological responses implicated in the pathogenesis of AS (5). These cells employ soluble cytokines like TNF and IL (IL-17) to perform immunological activities, and these cytokines

contribute significantly to the pathophysiology of AS(6).

Cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29) with the following properties are presently classified as members of the IL-10 family by genomic layouts, etc. are identical. New IL-10 family members demonstrate proinflammatory activity (7). While the IL-10 family cytokines have an inflammatory mediator. Cytokines related to IL-10 may also promote autoimmune pathology, which has a massive part since the emergence of autoimmune diseases is controlled by an imbalance of both anti-inflammatory and pro-inflammatory cytokines. Autoimmune and inflammatory diseases have been linked to problems with how the body makes cytokines in the IL-10 family. such as psoriasis and rheumatoid arthritis (8).

In this study understanding the correlation between ILs (IL-20, IL-22, and IL-24) and AS in a group of Iraqi male patients was carried out

Materials and methods

Patients and controls

One hundred of male AS patients with mean age (40.5 ± 10.19) were chosen from the hematology department at Baghdad Hospital in Medical City. Patients have been diagnosed with AS after undergoing a variety of diagnostic testing, such as a complete blood count, C-reactive protein, magnetic resonance imaging, and X-ray. Each individual who took part in the research supplied an informed written agreement, as well as the investigation, which was authorized by the local ethics committee (CSEC/0122/0002). Also for AS patients, disease duration, special lifestyles, particularly having smoked, clinical diagnosis and history of administered therapy were obtained; C-reactive protein (mg/l) and erythrocyte sedimentation rate (mm/h) and disease activity indicators such as the Bath ankylosing spondylitis disease activity index (BASDAI) in order to take determine disease progression. The mobility and functional restrictions were used to make the Bath AS functional index (BASFI) (13). This research also included 100 healthy age-matched controls that had no history of autoimmune or infectious disease.

Blood collection

The concentration of interleukins was determined by collecting five-millilitre blood samples from each patient and control participants and placing them in a gel tube, after permitting the blood to coagulate for an hour at 4 degrees Celsius and centrifuging it at 3,000 g after ten minutes, serum can be taken out and was kept about -20°C till analysis in this study age, duration of disease, HLA-B27, erythrocyte sedimentation rate (ESR) and C- reactive protein(CRP) were took from patients data .

Measurement of interleukin concentrations

Measurements of interleukins (IL-20, IL-22, and IL-24) in serum samples were performed using ELISA sandwich kits (BT-Lab, China) following the manufacturer's instructions**.** The catalogue number of interleukins was: IL-20 (E2163Hu), IL-22(E0038Hu), IL-24(MBS2502285)

Results and Discussion

Baseline characteristics of ankylosing spondylitis patients and healthy control

Totally 100 as male patients including a mean age 40.56 ± 10.19 and disease duration of 9.04 ± 2.03 years. The age of the control (male) (38.78 ± 10.57) was matched (p= 0.227). The BASDAI and BASFI were (3.45 ± 2.03 and 3.84 ± 2.62) respectively. The result found that 57% of patients had HLA-

B27 positive and the ESR and CRP values were (25.39 ± 19.59 mm/h and 18.34 ± 3.45) respectively. Table 1 summarizes the clinical and demographic parameters and healthy controls.

Table (1): Baseline Analyses of the characteristics of patients with ankylosing spondylitis and healthy controls.

Characteristics	AS Patients (n =100)	Controls (n = 100)	p-value
Age (year)	40.56±10.19	38.78±10.57	0.227 NS
Disease duration (year)	9.04 ± 2.03	-	-
HLA-B27 positive	57%	-	-
BASDAI	3.45 ± 2.03	-	-
BASFI	3.84±2.62	-	-
ESR (mm/hour)	25.39±19.59	22.19 ±9.402	0.0880 NS
CRP (mg/L)	18.34±3.45	-	-

NS: non-significant.

Numerous researches have showed that ecological and hereditary variables have a function in the inclination and highly susceptible to AS 9. In reality, AS is related with HLA-B27 (human leukocyte antigen B27) for a long time, and it is a notable instance of a genetic marker's association with a disease (9). Ninety percent of AS patients express HLA-B27, but this gene was found in fewer than 8% of widespread society (10).Although pathogenic function of this gene has not yet been adequately elucidated. Several concepts explanations have been given for how HLA-B27 could cause AS: (1) The arthritogenic peptide hypothesis postulates that a molecular mimicry between pathogens and self-peptides could be the cause of cytotoxic T cell lines (CTLs) cross-reactivity, leading to autoimmune spondylitis ; (2) the HLA-B27 miss folding hypothesis states that the accumulation of aberrantly folded HLA-B27 in the endoplasmic reticulum (ER) activates an intracellular signaling, (3) Homodimerization of HLA-B27 on the cell surface is related with a diminished immune response, as suggested by the discovery that natural killer cells have killer immunoglobulin-like receptors on their surface and CD4+ T cells can detect these dimers(11).

Two functional indexes are most commonly used to measure functional capacity: the (BASFI) and the Dougados functional index, The 10 questions on the BASFI are answered using a VAS. The ultimate grade is the average of the questions' ratings, in the range of 0 (no restriction) to 10 (maximal functional restriction). Both functional indexes are valid and sensitive for distinguishing between groups of patients with varying physical function and/or improvement (12).

The CRP and ESR are the two indexes of disease activity utilized most frequently in clinical application and clinical investigation. However. They lack sensibility and particularity, and do not indicate the illness process in AS, The ASAS AxSpA categorization criteria and the ASDAS, an assessment of disease activity, both involve a high CRP, Nonetheless, only 40–50% of patients with AS exhibit an increased CRP or ESR, Consequently, a healthy ESR or CRP does not preclude AS and does not fully capture active illness. Patients with as have higher two acute phase reactant concentrations than those with non-radiographic axial Spa,

enhanced CRP were also related with greater radiographic abnormalities on X-rays of the spine and inflammatory signals on MRI of the sacroiliac region (13). In addition, elevated AS CRP and ESR values patients anticipate long term radiographic deterioration in the sacroiliac joints and spine. High sensitivity C-reactive protein (hsCRP) corresponds favorably in clinical disease activity than CRP indicators in individuals with axial SpA, according to (14).

Serum levels of Interleukins in patients with ankylosing spondylitis (AS)

Serum levels of IL-20, IL22, and IL-24 have been evaluated using ELISA, mean levels of IL-20, IL22, and IL-24 in AS patients and control group were (194.0 ± 52.90 pg/mL vs 99.52 ± 11.99 pg/mL), (373.8 ± 102.2 ng/mL vs 132.0 ± 41.63 ng/mL) and (389.6 ± 180.7 ng/mL vs 186.9 ± 29.38 ng/mL) respectively as shown in table 2 and figure1. The results statistically revealed high significant differences ($p<0.0001$) between the level of ILs of patients and healthy control group.

Table (2): Serum levels of interleukins in control and patients with ankylosing spondylitis.

ILs	(mean± SD)		Significant	*P* value
	Control n(100)	Patients n (100)		
IL-20(pg/mL)	99.52±11.99	194.0±52.90	**	<0.0001
IL-22 (ng/mL)	132.0±41.63	373.8±102.2	**	<0.0001
IL-24 (ng/mL)	186.9±29.38	389.6±180.7	**	<0.0001
** ($p\leq0.01$)				

Significantly **($p \leq 0.01$).

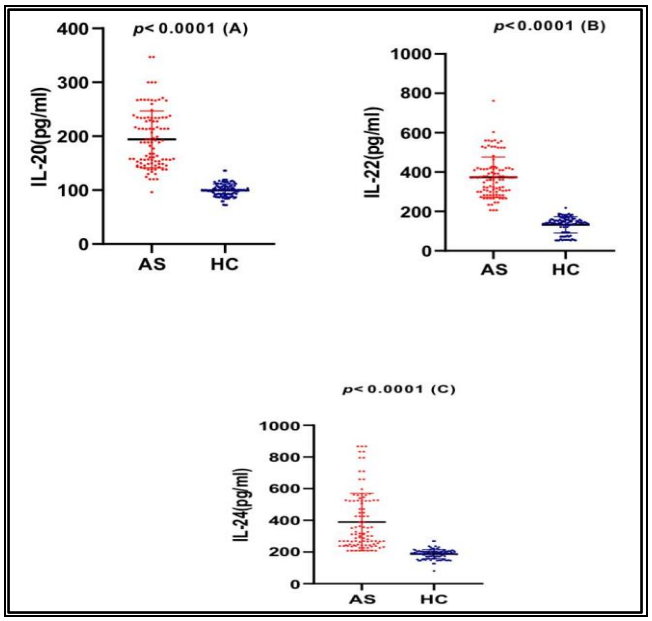

Figure (1): Serum levels of A) IL-20, B) IL-22and C) IL-24 of ankylosing spondylitis (AS) patients and healthy controls (HC).

These results were agreed with (15)who found that Patients had vastly greater levels of IL-22 with AS than in control groups. IL-22 levels in axSpA patients were substantially higher than in healthy participants. Consequently, the diagnostic and prognostic value of IL-22 in axSpA has been postulated.

(16). In addition, it's been shown that number of IL-22+ mucosal-associated persistent T cell was greater in AS patients than in healthy controls. (17). Even though there isn't a lot of adequate data, it seems most probability IL-22 has surfaced as a possible immunological role in the cause of AS that needs more exploration. IL-22, previously known as IL-TIF (IL-10-associated T-cell-derived stimulating factor), is belongs to the IL-10 cytokine family that was recently identified. There are five other cytokines in the family (IL-10, IL-19, IL-20, IL-24, and IL-26) (18).

Several members of the IL-10 cytokine family were included in this investigation, which revealed more statistically significant differences in patients than healthy controls. Initially, IL-22 was discovered as a cytokine generated by Th17 cells, a subgroup of CD4+ T cells defined predominantly by the creation of IL-17A. Nevertheless, Th22 cells are recognized as a separate subgroup of CD4+ T lymphocytes that act primarily by producing large quantities of IL-22 without the co-production of IL-17A. (19). Th17 and Th22 cells act a substantial function in mediating inflammatory reactions via IL-17A and IL-22 (20). The presence of IL-20 and IL-24 in the synovial joint of patients with rheumatoid arthritis (RA) and (SpA) indicated that these cytokines specifically target cells at these positions, however, the exact subset of cellular targets for IL-20 and IL-24 may be challenging to determine. Two cytokines share a functional receptor formed of two receptor subunits, and both cytokines can utilize either of the two receptor complexes IL-20R1/IL-20R2 or IL-22R/IL20R. It is unknown how IL-20 and IL-24 competing for the identical receptors on their respective target cells (21). Additionally, intricacy was heightened by the fact that receptor complex IL-20R1/IL-20R2 is a potential signaling pathway for IL-19 (22).

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