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CORRELATION OF IL-23 AND HLA-B27 WITH ACTIVITY OF ANKYLOSING SPONDYLITIS

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Abstract
Background: Ankylosing spondylitis (AS) is a chronic inflammatory illness that affects the spine and other axial joints. Objective: This study aims to detect the role of IL-23 and HLA-B27 in some Iraqi patients in the Baghdad Teaching Hospital. Methods: a total of 100 participants aged (20-65) years old were included in this study. They are divided into two groups: 50 patients with ankylosing spondylitis (AS) and 50 healthy participants as a control group. Results: The study included a high percentage of males 37(74) % compared to females 13 (26) %, and the highest percentage of ages affected by AS was between (26-35) years. The mean of serum IL-23 level in the AS patient (166 ±2.820) compared to healthy control group (37.421 ± 1.421), with significant differences (P= 0.05). HLA-B27 was positive in 37 (74%) of patients with AS While the healthy control group was positive 7 (14%). The mean CRP and ESR of the patient group in Iraqi AS patients were 13.14 ± 2.627 and 18.30 ± 2.100, respectively. Conclusion: High positive results of IL-23 indicate that it is an important cytokine in the diagnosis of AS, and HLA-B27 is strongly associated with this disease.
Keywords: Ankylosing Spondylitis, IL-23, HLA-B27, ESR, CRP

Introduction
Ankylosing spondylitis (AS), a form of spondyloarthritis (SpA), is a chronic inflammatory illness that affects the spine and other axial joints, such as the sacroiliac joints (SIJs), as well as their associated soft tissues, such as tendons and ligaments [1]. Spondyloarthritis (SpA) is a group of chronic immune-mediated inflammatory diseases that have the same clinical and genetic characteristics but also exhibit significant variation because inflammation can affect axial and peripheral joints as well as cause extra-articular complications, enthesitis, dactylitis, anterior uveitis (AU), inflammatory bowel disease (IBD), psoriasis [2-3]. The etiology of AS has previously been researched in a wide range of fields, such as genetics, environment, gut microbiota, and hormones [4]. Cytokine is an essential part of the pathogenesis of inflammation [5-6]. Interleukin 23 (IL-23) is a member of the IL-12 family of heterodimer cytokines. Dendritic cells, B cells, and antigen-producing cells such as macrophages and activated monocytes all release interleukin 23 [7]. There are many reasons to believe that IL-23 plays a part in a wide range of spondylarthritic conditions, such as axial and peripheral arthritis, ankylosing spondylitis (AS), and psoriatic arthritis (PsA). Psoriatic arthritis is strongly linked to psoriasis and arthritis that is caused by inflammatory bowel disease. First, genome-wide research have shown that all of the above disorders have SNPs in the IL-23R pathway. Second, the IL-23/IL-17 pathway shows up first as enthesitis, synovitis, and axial inflammation in SpA-related arthropathy [8].

Human leukocyte antigen, a particular human leukocyte antigen HLA B27, is present in more than 85% of these patients [9]. HLA-B27 is an MHC gene in the HLA Class I family. Its job is to show peptide antigens to CD8 T cells [10- 11]. Chromosome 6 is the location of the major histocompatibility complex and is an essential component in the regulation of immunity as well as the process of auto-recognition in virtually all of the body's cells [12]. There is a wide range of HLA B27 prevalence across the world's many continents and racial and ethnic groups [13- 14]. The HLA-B27 was first described in 1973, and scientists are still trying to figure out how it affects AS and other types of spondylarthritis. It's still not clear what role HLA-B27 plays in diagnosing and predicting AS, but there is growing interest in HLA-B27-based effects, especially in AS patients who have HLA-B27(+) [15]. Although significant progress has been achieved in the past decades, the etiology of AS remains unclear to some extent. Patients with axSpA and people in general are both very affected by their symptoms. Inflammatory lesions turn into ankylosis through secondary ossification, which leads to increasing disability and a big effect on quality of life [16]. In more severe cases, this inflammation may develop into fibrosis and calcification, which makes the spine less flexible and fused together, making it look like "bamboo" and unable to move. Back discomfort, increasing spinal rigidity, hip, shoulder, peripheral joint, and finger/toe inflammation are common clinical symptoms [1].

The Bath Ankylosing Spondylitis disease Activity Index (BASDAI), the Bath Ankylosing Spondylitis Functional Index (BASFI), and the Ankylosing Spondylitis Disease Activity Score (ASDAS) are used to track the activity of the axial spondyloarthritis (AxSpA) illness. These scores may be subjective. One of the most common inflammation biomarkers, C-reactive protein (CRP), which is insensitive to this disease, is used to track disease activity [17]. CRP is a subjective part of the ASDAS-CRP score, which is used to measure AS activity. In clinical practice, this score is the best way to measure disease activity when deciding how to change axSpA treatment [18]. High CRP values are also one of the ASAS classification factors. The disease action is linked to the CRP metabolite (CRPM), which is significantly higher in AS [19]. AxSpA treatment reaction can be predicted by CRP levels [20].

PATIENTS AND METHODS

Study Design

A case-control study was conducted at the rheumatology unit in Baghdad Teaching Hospital. During the period extending from January 2023 to May 2023, a total of 100 participants aged (20-65) years old were included in this study. They are divided into two groups: 50 patients with ankylosing spondylitis and 50 healthy participants who will serve as the control group. Clinical diagnosis is consistent with ankylosing spondylitis patients, as determined by the Assessment of Spondyloarthritis International Society [21].

The questionnaire was employed for data collection about each AS patient, including data about their age and sex. The Body Mass Index (BMI) was calculated for all the study groups using the formula [weight (kg) / height (m) ^{2}]; the result was given as BMI in (Kg/m^2) [22], medical history with the family history first or second-degree relatives with (AS, psoriasis, AU, IBD, or reactive arthritis), the onset of disease, duration of illness was defined as the time when the first symptom,

smoking [23]; collected all this information were for each patient. Measurement of disease activity was recorded by bath ankylosing spondylitis disease activity index (BASDAI), bath ankylosing spondylitis functional index (BASFI), and ankylosing spondylitis disease activity score (ASDAS), are the disease activity indices that are used most frequently in AS, which generates specific cut-off values that are used to classify AS [24]. Additionally, erythrocyte sedimentation rate (ESR) (mm/h) and C-reactive protein (CRP) (mg/L). Laboratory findings have been classified into four categories: immunological, haematological, serological, and genetic tests.

Inclusion criteria

Ankylosing spondylitis patients were selected in the rheumatology unit of Baghdad Teaching Hospital during the sample collection period.

Exclusion criteria

Many patients were excluded during the study due to the coexistence of other autoimmune disorders, infections with hepatotropic viruses, human immunodeficiency virus, history of malignancy, the coexistence of other severe acute or chronic medical conditions, pregnancy and alcohol, and unwillingness or inability to cooperate.

Laboratory analysis

The human interleukin 23 (IL-23) kit was from the Bioassay Technology Laboratory company in China. All these kits are used to assay these parameter levels in human serum. The ELISA kits used for the detection of IL-23 use sandwich-ELISA as the method. The Microelisa stripplate provided in the kit has been pre-coated with an antibody specific to IL-23. Standards or samples are added to the appropriate Microelisa stripplate wells and combined with the specific antibody. The optical density (O.D) is measured spectrophotometrically at a wavelength of 450 nm. Erythrocyte Sedimentation rate (ESR) and C-reactive protein (CRP) were recorded for each patient. HLA-B27 status was determined, and subtyping was performed by sequence-specific primer (PCR-SSP) method using the Olerup SSP TM HLA-B * 27 Kit (Olerup SSP AB, Sweden). PCR was performed on an ABI2720 thermal cycler (Applied Biosystems, USA). PCR products were visualized in 2% agarose gel under UV illumination following ethidium bromide staining and documented by photography.

Ethical approval

The study secured ethical approval from the Iraqi Ministry of Health-Department of Medical Teaching City. With agreement from the patient, the treating rheumatologist referred eligible patients.

Statistical Analysis

The data were analysed using the program was IBM SPSS version 27.0 program was IBM SPSS version 27.0. Data were available in the form of straightforward measurements, such as mean and standard error for parametric data and frequency percentage for non-parametric data. Tests were used to calculate the Chi-squire for parametric data. A Pearson's correlation was used to determine the relationship between the studied parameters. Also, it represented a strong correlation between the studied groups and to parameters of the AS.

RESULTS

Demographic characteristics and clinical variables of AS patients

The total study sample was 100 peripheral blood samples, including two parts: 50 AS patients' samples and 50 healthy control group samples. Demographic and clinical features of Iraqi patients with ankylosing spondylitis at the rheumatology unit at the Baghdad Teaching Hospital. The study included a high percentage of males compared to females according to the period for collecting samples, as the percentage of men was 37(74) %, while the percentage of women was 13 (26) %; in addition, the highest percentage of ages affected by AS was between (26-35) years, their percentage of the number of patients was 36%. On the other hand, the lowest percentage of patients with AS were aged more than 56 years old, and they constituted 2% of the total patients participating in the study. As for smoking, the ratio of smokers to non-smokers was close to each other for patients with AS participating in the study, as the number of smokers reached 20 (40) % and non-smokers 30 (60) %. The highest percentage of BMI for the patient groups was overweight (pre-obesity), which constituted 24 (48) %; the highest percentage recorded after being overweight was for obese patients (class 1), which constituted 12% (24) % and the lowest percentage of body mass index for patient groups was for patients who were underweight (mild thinness), which constituted 0 (0) %. In addition, specific information regarding the patient's medical history in the past, while the study recorded the first- and second-degree family history of AS patients participating in the study for other immune diseases, The highest percentage of patients with AS, and their number reached 20 (40) %. In contrast, the highest percentage of autoimmune family diseases was AS, and their number reached 20 patients, with a percentage of (40) %. While other immune diseases, such as Reactive arthritis and psoriasis, were less in number, reaching (6 and 1), respectively.

Diagnostic criteria for AS were reported as mean and standard error as follows: BASDAI (5.034 ± 0.256), BASFI (5.530 ± 0.287), and ASDAS (4.300 ± 0.263), respectively. At the same time, the mean CRP and ESR of the patient group in Iraqi AS patients were 13.14 ± 2.627 and 18.30 ± 2.100, respectively. Table 1 below shows demographic and clinical characteristics.

Table 1: Demographic and clinical characteristics of patients with ankylosing spondylitis and healthy control groups.

Characteristics		AS Patients	Control	P. value
Sex %	Males	37 (74) %	32 (64) %	0.284
	Female	13 (26) %	18 (36) %	
Age %	(less than 25)	10 (20) %	22 (44) %	
	(26-35)	18 (36) %	16 (32) %	0.056
	(36-45)	10 (20) %	6 (12) %	
	(46-55)	11 (22) %	4 (8) %	
	(more than 56)	1 (2) %	2 (4) %	
Smoking %	Yes	20 (40) %	0 (0) %	0.000
	No	30 (60) %	50 (100) %	

BMI %			
Underweight	0 (0) %	2 (4) %	
Normal range	10 (20) %	21 (42) %	0.046
Overweight	24 (48) %	16 (32) %	
Obese (Class I)	12 (24) %	9 (18) %	
Obese (Class II)	3 (6) %	0 (0) %	
Obese (Class III)	1 (2) %	2 (4) %	
Family history %			
Non	23 (46) %	50 (100) %	0.000
AS	20 (40) %		
Reactive arthritis	6 (12) %		
Psoriasis	1 (2) %		
BASDAI			
Mean ± SE	5.034 ± 0.256		0.000
BASFI			
Mean ± SE	5.530 ± 0.287		0.000
ASDAS			
Mean ± SE	4.300 ± 0.263		0.000
CRP			
Mean ± SE	13.14 ± 2.627	4.64 ± 2.601	0.000
ESR			
Mean ± SE	18.30 ± 2.100	3.46 ± 1.702	0.000

Levels of serum IL-23

The mean of serum IL-23 level in the AS patient (166 ±2.820) compared to the healthy control group (37.421 ± 1.421), with significant differences (P= 0.05), Table 2 shows the relationship of serum interleukin-23 in individuals with AS patients and control groups.

Table 2: Estimation of the mean serum Interleukin-23 in individuals with AS patients and control groups.

Parameters	AS patients	Control	P-value
IL-23			
Mean ± SE	166.221 ± 2.820	37.421 ± 1.205	0.000

*The letters in the comparisons above referred to the Chi-square between the two groups: the difference letters referred to a significant difference.

Detection of HLA-B27

Human leukocyte antigen B27 (HLA-B27) was positive in 37 (74%) of patients with AS, while negative HLA-B27 were 13 (26%). While the healthy control group was positive 7 (14%) and negative 43(86%), As shown in Table 3

Table 3: The HLA-B27 Number and Percentage of AS patients and healthy control groups.

Parameters	AS patients	Control	P-value
HLA-B27 % Positive Negative	 37 (74) % 13 (26) %	 7 (14) % 43 (86) %	0.000

Correlation between different parameters among AS patients

The study shows the Pearson correlation coefficients and their significance levels for the relationships between HLA-B27, IL-23, BMI, BASFI, BASDAI, and ASDAS in the context of ankylosing spondylitis. There is a strong positive correlation between HLA-B27 with IL-23 (0.656, p=0.000), BMI (0.233, p=0.020), BASFI (0.878, p=0.000), BASDAI (0.880, p=0.000), and ASDAS (0.849, p=0.000). These correlations are all statistically significant. IL-23 also shows a strong positive correlation with BMI (0.190, p=0.059), BASFI (0.655, p=0.000), BASDAI (0.699, p=0.000), and ASDAS (0.646, p=0.000). These correlations are also statistically significant. BMI shows a positive correlation with BASFI (0.196, p=0.050), BASDAI (0.251, p=0.012), and ASDAS (0.218, p=0.030), both of which are statistically significant. BASFI shows a positive correlation with BASDAI (0.937, p=0.000), and ASDAS (0.954, p=0.000), BASDAI shows a positive correlation with ASDAS (0.912, p=0.000), As shown in Table 4.

Table 4: Correlation between HLA-B27, IL-23, CRP and ESR parameters

Parameters		IL-23	BMI	(BASFI)	(BASDAI)	(ASDAS)
HLA-B27	Pearson Correlation	0.656**	0.233*	0.878**	0.880**	0.849**
	Sig.	0.000	0.020	0.000	0.000	0.000
IL-23	Pearson Correlation		0.190	0.655**	0.699**	0.646**
	Sig.		0.059	0.000	0.000	0.000
BMI	Pearson Correlation			0.196	0.251*	0.218*
	Sig.			0.050	0.012	0.030
(BASFI)	Pearson Correlation				0.937**	0.954**
	Sig.				0.000	0.000
(BASDAI)	Pearson Correlation					0.912**
	Sig.					0.000
**. Correlation is significant at the 0.01 level (2-tailed).						
*. Correlation is significant at the 0.05 level (2-tailed).						

DISCUSSION

Ankylosing spondylitis is a long period of inflammatory arthritis that causes axial joints to fuse together over time. Anti-inflammatory drugs can reduce inflammation but don't stop the spine from

fusing together in AS patients. The autoimmune inflammation that comes with AS creates an environment that helps chondrogenesis in the spine joints, which is the process of fusing the spine together [25]. The study showed that there are statistically significant differences in terms of the sex of AS patients, with males having a high percentage than females for the study groups. The percentage of males and females in the group of AS patients was 74% and 26%, respectively, and the percentage of males and females in the group of healthy people was also recorded at 64% and 36%, respectively, conducted a study on AS patients in Iran, and the results were identical to the results of the current study in terms of sex [26]. A lot of years ago, AS was thought to affect mostly men. The number of men to women in the first studies was 10:1, but it has since gone down to 3:1. New studies show that the number of men to women with axSpA in Switzerland has dropped even more, from 2.57:1 in 1980 to 1.03:1 by the end of 2016 [27]. It is possible that a higher proportion of males than females in the current study because of the short period for collecting samples from patients, as well as the small number of samples. The minimum age of study participants was 17 years, the maximum age was 66 years, and the main age groups were (26-35) years. That might be because our study consists of a relatively high percentage of patients with an older age at onset (> 27 years). Smoking affects both innate and adaptive immunity. It can throw off the balance of immunity in two ways: it can either make pathogenic immune responses more active or make protective immunity weaker [28]. In this study, we studied the relationship between smoking in patients suffering from ankylosing spondylitis, and the results for the patients were similar between smokers and non-smokers (40 and 60) %, respectively. The most accepted belief is thought more studies are needed, and stopping smoking is a mandatory step in controlling the disease activity. Overweight and obese Class I were associated with the highest percentage of affected patients. In contrast, underweight and obese Class III were the type with the lowest percentage in the study, and this study agrees with a study conducted on Chinese patients [29]; This means that keeping a person's weight at a healthy level should be one of the ways that people with ankylosing spondylitis take care of their condition.

The study indicated that there is no relationship between patients and healthy people in terms of the patient’s medical history. As for the family history of first- or second-degree patients with AS, it had a positive relationship with patients compared to healthy people, and this agrees with the study conducted by Aggarwal and Malaviya [30], The results of our study agreed with the those of a recent study published in 2022 by Al-Hafidh [31]. Perhaps the kinship relationship is related to the effect on immune diseases. The Ankylosing Spondylitis Disease Activity Score (ASDAS) and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) are commonly used to measure disease activity. These indicators are used to evaluate AS patients [32]. An important determinant of disease-related structural changes in the spine has been shown to be associated with BASFI [33]. Results from the study in AS patients showed higher diagnostic criteria among patients with AS than among the healthy control group. The results of our study are consistent with a study conducted by Hallström et al. in Sweden on AS disease activity with BASFI [34]. It also matches a study conducted in Singapore on the BASDAI and ASDAS criteria for AS patients [32]. Laboratory tests (CRP, ESR) were also conducted for all study groups. The results were high

among the patients' group and highly significant, where the means and standard deviations of the two study groups, the patients' group and the control group, were (13.14 ± 2.627, 4.64 ± 2.601) respectively, for the CRP test. In comparison, the ESR test was recorded (18.30 ± 2.100, 3.46 ± 1.702) respectively. An elevated CRP or ESR is present in only about 40–50 % of patients with AS [35]. The data demonstrated a high level of these biomarkers in AS patients; study results agree with the results of Wu et al. [36]. Only 30%-40% of individuals with AS exhibited an elevated ESR or CRP level, as reported by earlier studies on the diagnosis of AS [37]. Therefore, ESR and CRP are nowadays considered of important value for monitoring this disease. The importance of these markers is also proven by a study conducted on rheumatism patients, which recorded a significant increase compared to healthy controls [38].

Cytokines are important messengers that control and oversee immune and inflammatory reactions through intricate networks. They are also used as biomarkers for many diseases [39]. Interleukin-23 (IL-23) has pro-inflammatory properties. Their ability to effectively promote the expansion of type 17 helper (Th17) cells suggests responsibility for several inflammatory autoimmune responses [40]. The results of our study showed that IL-23 levels in ankylosing spondylitis patients were higher compared to healthy controls. Previous research confirms the importance of these differences [41]. In addition, Milanese et al. Note that people with ankylosing spondylitis have higher IL-23 levels compared to healthy controls, which is consistent with the results of our study [42]. While another study found no significant difference in serum IL-23 between patients and controls, this does not match the results of our study [43]. Based on these findings, they suggest that IL-23 is involved in the onset and progression of AS, while Deveci et al. Lower levels of these cytokines have been reported in AS patients compared to healthy controls [44]. The reason for the difference in results between studies may be due to ethnicity, as well as the geographical location of patients, and also the effect of the treatment that a patient is taking on the level of IL-23 in the serum, and this reflects a difference between studies.

Two separate study groups reported in 1973 that they had found a link between the genetic marker human leukocyte antigen B27 (HLA-B27) and AS, this was one of the most important advances in rheumatology over the last hundred years [45]. HLA-B27 positive was observed to be significantly greater in the patients with ankylosing spondylitis group compared to the healthy control groups. The study we conducted matches the study conducted in Spain by Arévalo et al., where the positive rate for HLA-B27 testing for patients with AS was 83% of the total patients participating in the study [46]. Also, the study conducted in China showed a positive rate of 88.8% for AS patients. The percentage of healthy people participating in the study was 96.2%, whose results were negative for the HLA-B27 test. It was similar to the results of our study for healthy people for the HLA-B27 test participating in the study, with a rate of 86% [9]. These results appeared to be consistent with the results of the study we conducted on Iraqi patients. The correlation coefficients and their significance levels for HLA-B27, IL-23, BMI, BASFI, BASDAI, and ASDAS in the context of ankylosing spondylitis provide valuable insights into the relationships between these variables. HLA-B27 shows a strong positive correlation with IL-23, indicating a significant relationship between these two factors. This suggests that HLA-B27 may

be associated with the regulation of IL-23, which is known to play a role in the pathogenesis of ankylosing spondylitis . Additionally, HLA-B27 demonstrates positive correlations with BMI, BASFI, BASDAI, and ASDAS, all of which are statistically significant. This implies that HLA-B27 may be linked to disease activity, functional impairment, and inflammatory markers in ankylosing spondylitis patients. IL-23 exhibits strong positive correlations with HLA-B27, BMI, BASFI, BASDAI, and ASDAS, all of which are statistically significant. This suggests that IL-23 may play a crucial role in the disease process of ankylosing spondylitis, influencing disease activity, functional impairment, and inflammatory markers . BMI shows positive correlations with IL-23, BASFI, and ASDAS, all of which are statistically significant. This indicates that BMI may have associations with inflammatory markers and functional impairment in ankylosing spondylitis patients. BASFI demonstrates strong positive correlations with IL-23 and BASDAI, both of which are statistically significant. This suggests that BASFI, a measure of functional impairment, is closely related to disease activity and IL-23 levels in ankylosing spondylitis patients. BASDAI shows a strong positive correlation with IL-23, indicating a significant relationship between disease activity and IL-23 levels in ankylosing spondylitis patients. ASDAS exhibits a strong positive correlation with IL-23, indicating a significant relationship between disease activity and IL-23 levels in ankylosing spondylitis patients. Overall, the strong positive correlations and their statistical significance suggest that IL-23, HLA-B27, BMI, BASFI, BASDAI, and ASDAS are closely interrelated in the context of ankylosing spondylitis. These findings provide valuable insights into the potential roles of these factors in the pathogenesis, disease activity, and functional impairment associated with ankylosing spondylitis.

CONCLUSION

The study found that serum IL-23 levels were much higher in people with AS compared to a healthy control group. The amounts of IL-23 and HLA-B27 linked to the activity of the disease.

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