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Serum Macrophage Migration Inhibitory Factor Level In Patients With Ankylosing Spondylitis And Its Relation To Disease Activity

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ABSTRACT

Background: Ankylosing Spondylitis (AS) is a chronic inflammatory form of arthritis that predominantly affects the axial joints and has a global prevalence of 1%. It is the most commonly occurring form of spondyloarthropathy. Macrophage migration inhibitory factor (MIF) is a potent pro-inflammatory cytokine implicated in several diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic vasculitis and ankylosing spondylitis
Objective: To determine the serum levels of macrophage migration inhibitory factor in ankylosing spondylitis patients and to find out its possible correlation with disease activity.
Patients and methods: This study was a case-control study conducted on 52 subjects (21 Ankylosing Spondylitis "AS"), and 21 healthy controls; to determine the serum levels of macrophage migration inhibitory factor (MIF) in ankylosing spondylitis patients and to find out its possible correlation with disease activity.
Results: Our results showed statistically significant difference between the studied groups regarding MIF (significantly higher in AS group).
Conclusion: MIF appears to have the unique ability to drive inflammation and could play an important role in the pathogenesis of AS.

Key words: Ankylosing Spondylitis (AS), Macrophage migration inhibitory factor (MIF), spondyloarthropathy

1. INTRODUCTION

Ankylosing Spondylitis (AS) is a chronic inflammatory form of arthritis that predominantly affects the axial joints and has a global prevalence of 1%. It is the most commonly occurring form of spondyloarthropathy [(1)].

It is a complex disease linked to several risk factors, both genetic and environmental.Its tendency to repeat within families, first documented in some detail in 1961 [(2)].

Human leukocyte antigen (HLA-B27) was the first genetic factor to be related with ankylosing spondylitis, and it confers the utmost vulnerability to this disease. The link of HLA-B27 with spondyloarthritis has crossed ethnic and geographic barriers [(3)].

Macrophage migration inhibitory factor (MIF) is a highly conserved 12.5-kDa protein that exhibits a unique combination of hormone-like, cytokine-like, and Thioredoxin-like properties and is now recognized to be a multipotential cytokine involved in the regulation of immune and inflammatory responses [(4)].

MIF is a potent pro-inflammatory cytokine implicated in several diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and systemic vasculitis [(4)].

CD74, expressed on the surface of antigen-presenting cells, is high-affinity receptor for macrophage migration inhibitory factor. Binding of MIF to CD74 initiates a downstream signaling pathway through extracellular signal-regulated kinasas (ERK-1/2) leading to cell proliferation [(5)].

CD74 has also been reported to undergo regulated intramembrane proteolysis involving a series of proteolytic cleavages in endocytic vesicles that release its cytosolic N-terminal fragment, which localizes into the nucleus and activates "(nuclear factor kappa-light-chain-enhancer of activated B cells)" (NF-KB) [(4)].

Autoantibodies have previously been reported in AS. High titers of autoantibodies to the invariant chain (CD74) of major histocompatibility complex (MHC) class II are seen in spondyloarthritis. It plays a key role in the development of chronic colitis in mouse models. Elevated serum MIF levels have been reported in AS [(6)].

2. PATIENTS AND METHODS

A total number of 42 subjects were included in the study after fulfillment of the inclusion and exclusion criteria, this study was conducted from January 2020 through September 2020 in patients clinics at Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University Hospitals Rheumatology Department, Kobry El-Kobba Military Hospital and Maadi Military Hospital and followed by Case- control study, group I: 21 patients with ankylosing spondylitis (AS) who diagnosed according to modified New York criteria for AS, group II: 21 Healthy volunteers (age and sex matched with the patients group served as controls. Assuming that MIF level in AS patients is 31.0±11.4, and in control group 22.1±8.3, at confidence level 95%, power 80%, so, total sample size was 42 (21 in every group) calculated by open Epi.

Our Inclusion criteria were: Patients with ankylosing spondylitis (AS) who met the modified New York criteria for AS. To meet the criteria for a diagnosis of AS, a person must have grade 2-4 sacroiliitis on both sides or grade 3-4 sacroiliitis on one side as well as at least one of the clinical criteria.

Our exclusion criteria were: Patients with Endocrinal disorders. Eg (DM), metabolic disorders. eg (metabolic syndrome), any other autoimmune disease, malignancies, infection, renal failure, other seronegative spondyloarthropathy diseases

3. METHODS:

This study was approved by the local ethical committee of Zagazig university hospitals. Written knowledgeable consent was taken from patients or their first-degree relatives. On admission: General examination was done to all patients with specialized maneuvers to examine the back (Straight leg test), schober test was done in the standing position, sacroiliac joint examination, assessment patient's "Ankylosing Spondylitis Disease Activity Score (ASDAS) was done, C-Laboratory investigations for patients and controls included: (CBC, ESR CRP Random Blood sugar and Human leukocyte antigen (HLA-B27)), imaging of patients included Plain x-ray sacroiliac joints: widening, narrowing, erosion, ankylosing, MRI sacroiliac joints: on MRI, findings included both active inflammatory lesions (primarily bone marrow edema) and structural lesions (eg, bone erosions, new bone

formation, sclerosis and fat infiltration). Serum of macrophage migration inhibitory factor was measured by ELISA.

4. ETHICAL APPROVAL:

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University approved the study protocol. An informed consent was obtained from all participants or their first-degree relatives and they were told about the aim of the study, and were informed that the data would be used for scientific purposes only.

5. STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium).Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) for parametric and median and range for non-parametric data. Independent T test and Mann Whitney test were used to calculate difference between quantitative variables in two groups for parametric and non-parametric variables respectively. One way ANOVA test was used to compare between more than two dependent groups of normally distributed variables. Pearson's and Spearman's correlation coefficient were used for correlating normal and non-parametric variables respectively.

6. RESULTS

Demographic data of the two studied groups: There was statistically non-significant difference between the studied groups regarding age or gender **(Table 1).**

Disease specific characters of the two studied groups: Patients had ankylosing spondylitis for period ranged from 2 to 24 years with mean 15.05±6.69 years. About 71.4% received NSAIDs (15 patients) while 95.2% are treated by DMARDs (20 patients) and 57.1% took TNFi(12 patients).Thirteen patients (61.9%) had positive HLA B27 (**Table 2).**

MIF values of the two studied groups: There is statistically significant difference between the studied groups regarding MIF (significantly higher in AS group) ($P < 0.001$) **(Table 3).**

Relation between disease activity and imaging findings. There was significant relation between MRI findings and disease activity (ASDAS ESR was significantly higher in patients with sacroiliitis).There was non-significant relation between x-ray findings and disease activity **(Table 4).**

MIF and disease activity among the studied patients: There was significant positive correlation between ASDAS ESR and MIF among the studied patients **(Table 5).**

Performance of MIF among the studied patients: The best cutoff of MIF was ≥ 41.3 with area under curve 0.625, sensitivity 80%, specificity 50%, positive predictive value 33.3%, negative predictive value 88.9% and accuracy 57.1% ($p > 0.05$) **(Table 6).**

Table (1) Comparison between case and control groups regarding demographic characteristics:

Demographic characteristics	Groups		Test	
	AS group	Control group	χ^2 /t	p
	N=21 (%)	N=21 (%)		
Gender:				
Female	6 (28.6)	6 (28.6)	0	1
Male	15 (71.4)	15 (71.4)		
Age (years):				
Mean ± SD	38.29 ± 6.48	37.24 ± 8.93	0.435	0.666
Range	26 – 53	20 - 55		

t Independent sample t test χ^2 Chi square test p>0.05 is statistically non-significant

Table (2) Disease-specific characteristics of the studied patients with AS:

Disease-specific characteristics	N=21	%
Disease duration (years) **Mean ± SD** **Median (Range)**		**15.05 ± 6.69** **14 (2 – 24)**
Disease activity **Mean ± SD Range**		**2.78 ± 0.68** **1.6 – 3.9**
HLA B27: **Absent** **Present**	 **8** **13**	 **38.1** **61.9**
Drug used: **NSAIDs** **DMARDs** **TNFi**	 **15** **20** **12**	 **71.4** **95.2** **57.1**

Table (3) Comparison between the studied groups regarding MIF value:

Laboratory data	Groups			Test
	AS group (n=21)	Control group (n=21)	Z	p
	Mean ± SD	Mean ± SD		
MIF (ng/dl)	43.56 ± 15.68	11.37 ± 3.16	-5.548	<0.001**

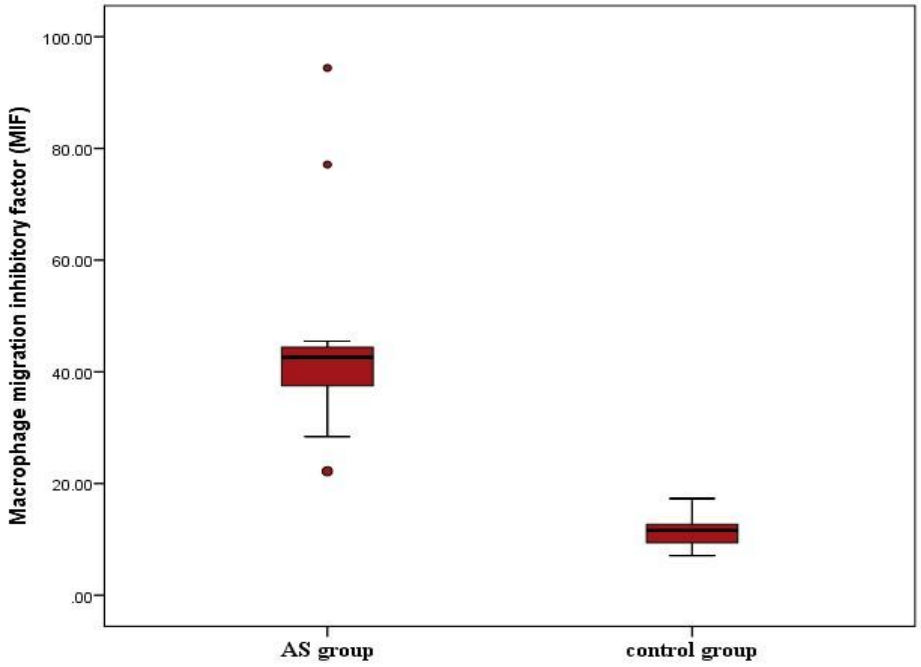

Figure (1) Boxplot showing MIF level among the studied groups

Table (4) Relation between MIF and imaging findings among the studied patients:

Radiological manifestations	MIF(ng/dl)	Test	
	Mean ± SD	**T**	**p**
MRI **Sacroilitis** (21 cases)	27.46 ± 19.75	0	>0.999
X ray: **Sacroilitis** (13 cases)	41.25 ± 16.58	-0.761	0.447

Table (5) Relation between disease activity and imaging findings:

	Disease activity (21 cases)			Test
	Mean ± SD	**Range**	**t/KW**	P
X-ray sacroiliac: **Absent (8 cases)** **Present (13 cases)**	 2.47 ± 0.51 2.73 ± 0.62	 1.8 – 3.6 2.1 – 3.9	-0.29	0.772
MRI sacroiliac: **Sacroiliitis (21 cases)**	2.69 ± 0.56	2.2 – 3.9	-3.044	0.007*

Table (6) Performance of MIF among the studied patients:

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	p
≥41.3	0.625	80%	50%	33.3%	88.9%	57.1%	0.409

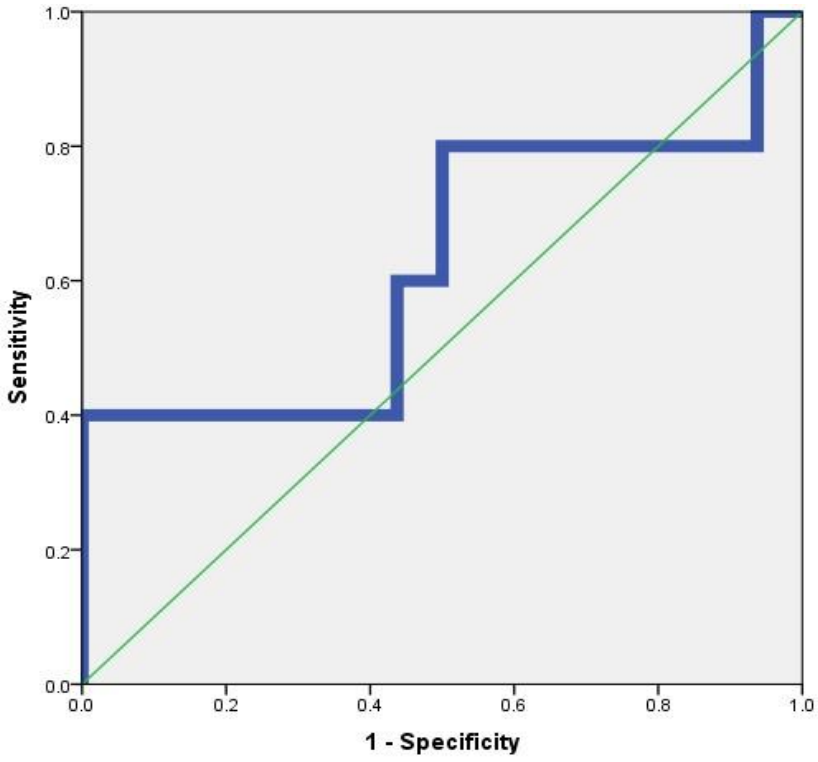

Figure (2) ROC showing performance of MIF.

7. DISCUSSION

Macrophage migration inhibitory factor (MIF) appears to have the unique ability to drive both inflammation and new bone formation and could play an important role in the pathogenesis of AS. Serum MIF levels could be considered a predictive of progressive spinal damage in AS patients [(4)].

Also, Fiorillo et al., 2019 in his article about ankylosing spondylitis and related immune-mediated disorders, reported that, CD74 is the receptor of the macrophage migration inhibitory factor (MIF) which is involved in the pathogenesis of AS and mediates its pro-inflammatory functions and mediated erosive process over time. [(7)].

The mean disease duration of AS patients was (15.05 ± 6.69) years; while the mean ASDAS score was (2.78 ± 0.68). Our results were higher than those of Kozaci et al., 2010, who reported that, the mean disease duration of the AS subjects was 11.5 ± 8.8 years [(8)].

MIF not only promotes inflammation, but also triggers osteoblastic activity, suggesting a novel pathogenic role for this pleiotropic cytokine in AS agreed with our findings [(9)].

We also found that 71.4% of AS patients received NSAIDs, 95.2% received DMARDs and 57.1% received TNFi. While Kozaci et al., 2010, reported that, most of his

patients were receiving nonsteroidal anti-inflammatory drugs and sulfasalazine but no one receiving anti-TNF-a blocking agents [8].

Regarding MIF levels, a highly significant increase in MIF levels in AS groups compared to control group. Our results agreed with Ranganathan et al., [4] who reported that MIF levels were significantly elevated in AS patients compared to healthy controls. Also, Onuora [10] demonstrated that serum levels of MIF were raised in patients with AS as compared with healthy individuals and Baykara et al., [11] reported that MIF levels are shown to be higher in AS patients compared to controls.

There was significant relation between MRI findings and disease activity (ASDAS/ESR) that was significantly higher in patients with sacroiliitis. This comes along with the study of Zhang et al. [12] which was done to analyse the correlations between clinical activity indices and MRI sacroiliitis in ankylosing spondylitis.

On contrary, MacKay et al. [13] demonstrated non-significant correlation between MRI and clinical DAS in a general axSpA outpatient population. There was no significant difference in MR DAS in individuals with high or low clinical DAS. There are two possible reasons for the lack of correlation: Firstly, clinical DAS may be more reflective of cumulative (chronic) disease activity, with MR DAS reflective only of the acute disease. Secondly, examinations were performed on one of four MR platforms, which could have introduced variability; however, this is reflective of the heterogeneity encountered in clinical practice.

8. CONCLUSION

In this study, we found that serum MIF levels are elevated in AS patients. MIF appears to have the unique ability to drive inflammation and could play an important role in the pathogenesis of AS.

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