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Assessment of Serum Calprotectin (S-100 Protein) In Iraqi Patients with Ankylosing Spondylitis and Its Relation with Treatment and Disease Activity.

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Summary: ***Background:*** *Ankylosing Spondylitis (AS) Is A Chronic Inflammatory Disease Of The Axial Skeleton Manifested By Back Pain And Progressive Stiffness Of The Spine. It Characteristically Affects Young Adults With A Peak Age Of Onset Between 20 And 30 Years(1).There Is Evidence That Calprotectin Plays An Important Role In Regulating Inflammatory And Immune Processes Via Several Mechanisms. Calprotectin Promotes Migration Of Phagocytes To The Sites Of Inflammation And Is Recognized By Toll-Like Receptor-4 On Monocytes(2).In General, Calprotectin Has Been Considered To Be An Important Molecule Contributing To Inflammation (3).* ***Objective:*** *To Evaluate The Role Of Calprotectin (S-100 Protein) In Iraqi Patients With Ankylosing Spondylitis And Its Relation With Treatment And Disease Activity.****Results:*** *Ninety (90) Subjects Were Included In This Study, Sixty (60) Of Them (Patient Group) Were Diagnosed As Established AS Patients Who Were Attending The Rheumatology Outpatient Clinic Of Baghdad Teaching Hospital, Thirty (30) Patients Of Them Were On Conventional Treatment (Steroid And/Or Cytotoxic Drugs), While The Other Thirty (30) Patients Were On Biological Treatment(Infliximab Infusion) And Other Thirty (30) Were Apparently Healthy Control Group.Mean Serum Level Of Calprotectin Was Statistically Higher In Patient Group (89.23±73.97 Ng/Ml) Than Control Group (54.94±20.51 Ng/Ml), And Also Was Statistically Higherin Patients On Conventional Treatment (118.92 ± 95.19ng/Ml)Than Those On Biological Treatment (59.54±15.73ng/Ml). The Mean Serum Level Of Calprotectin Among Patients Whose Disease Activity Low Was Lower Than Those Their Disease Activity High And The Differences Was Statistically Not Significant.****Conclusion:****The Study Shown That Calprotectin Was Higher In Patient Group Than Control Group And Also Was Higher In Patients On Conventional Treatment Than In Patients On Biological Treatment.*

Keywords- *Ankylosing Spondylitis,Calprotectin.*

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I. Introduction

Ankylosing Spondylitis (AS) Is The Prototypic Form Of Spondyloarthritis (Spa), A Family Of Disorders Characterized By Inflammation Around The Entheses (The Sites Of Ligament Insertion Into Bone), An Association With The Human Leukocyte Antigen (HLA)-B27, And Radiographic Sacroiliitis (4).

John Ball At 1971 Was The First To Report That The Pathological Feature That Distinguished AS From Rheumatoid Arthritis Was That The Major Involvement In AS Was At The Entheses And Not At The Synovia. Entheses Are Located Where Ligaments, Tendons, And Joint Capsules Are Attached To Bone. At The Vertebrae, The Entheses Are At The Annulus Fibrosus. Two Processes Are Observed. One Is Bone Destruction, And The Other Is New Bone Formation, Which Can Occur In The Sacroiliac Joints, The Vertebrae (Syndesmophyte Formation), And Other Entheseal Sites. When The Syndesmophytes Bridge Across All The Vertebrae, The Spine Becomes The Classical, But Very Uncommon, Bamboo Spine Of AS (5).

Immunohistology Shows T Cells, B Cells, Bone Marrow Macrophages, And Cells Involved In Neoangiogenesis (6). Enthesitis Also Involves The Extremities. The Classical Site Is At The Achilles Tendon. Here, The Ligaments And Tendons Interdigitate Into Cancellous Bones Through Fibrocartilage Connections. The Enthesis At The Achilles Tendon Suffers Repeated Damage And Repair Even In Normal Subjects, But, In AS, The Process Becomes Clinically Significant (7). In Addition To Enthesitis, Peripheral Synovitis Is Also Present In Some Patients With AS. The Synovitis Is Characterized By Hypervascularity And Infiltration By Macrophages, T Cells, And B Cells (8).

Multiple Randomized Trials Have Shown That Biologic Agents That Specifically Inhibit TNF-Alpha Suppress The Symptoms Of AS, As Well As The Acute Phase Response. Hence, There Is No Doubt That TNF-Alpha Is A Critical Mediator Of Inflammation In AS (9). However, These TNF-Alpha Inhibitors Do Not Arrest The Progression Of Bone Erosions Or Syndesmophyte Formation (10).

The Standard Method For Evaluating Disease Activity In Ankylosing Spondylitis Is The Bath Ankylosing Spondylitis Disease Activity Index **(**BASDAI) Which Consist Of One Through Ten Scale (One Being No Problem And10 Being The Worst Problem)Used To Answer 6 Questions Predicating The 5 Majorsymptom Of AS(11).

The Bath Ankylosing Spondylitis Functional Index(BASFI) Is Afunctional Index Which Can Accurately Assess A Patient's Functional Impairmentdue To The Disease, As Well As Improvements Following Therapy(12).

II. Patients And Methods

Patients: Sixty Patients (55 Males And 5 Females), Their Mean Age ± Standard Deviation (SD) Was (40.05 ± 8.02 Years) Who Attended To Medical City, Baghdad Teaching Hospital, Department Of Rheumatology Outpatient Clinic And Biological Therapy Unit Were Included In This Study During Period From Beginning Of October 2016 Till End Of October 2017, Thirty (30) Patients Treated With Biological Agent (Intravenous Infusion Of Infliximab Of 5mg/Kg), And Other Thirty (30) Patients Treated With Conventional Treatment (Steroid And/Or Cytotoxic Drugs). The Patients Were Comparedto Thirty (30) Apparently Healthy Individualsfrom Central Blood Bank Who Were Randomly Selected As Control Group, Written Informed Consents For The Research Were Obtained From All The Enrolled Patients And Controls.

Methods: From Each Individualtwo (2) Ml Of Venous Blood Was Aspirated And Let Clot At Room Temperature, Then Centrifuged To Separate The Serum Which Was Collected In Aliquots To Store In (- 20 ºc) Until Needed For Investigation Of Calprotectin.

Kits And Reagents: Human Calprotectin ELISA Kit (*SHANGHAI YEHUA, China).*

Statistical Analysis: Statistical Analysis In This Study Was Done Using SPSS Version Computer Software 20. T Test Was Used To Analyze The Data, And Calculation Of Mean Difference, Fisher Exact And Chi-Square Test For Comparison Of Proportion, P-Value Of Less Than 0.05 Was Considered As Statistically Significant, P-Value < 0.01 As Highly Significant And P-Value < 0.001 As Extremely Significant.

III. Results

This Study Was Included Sixty (60) Patients With AS, Fifty Five 55 (91.6%) Males & Five 5 (8.4%) Females, The Male To Female Ratio Was 11:1 As Shown In Figure-1.

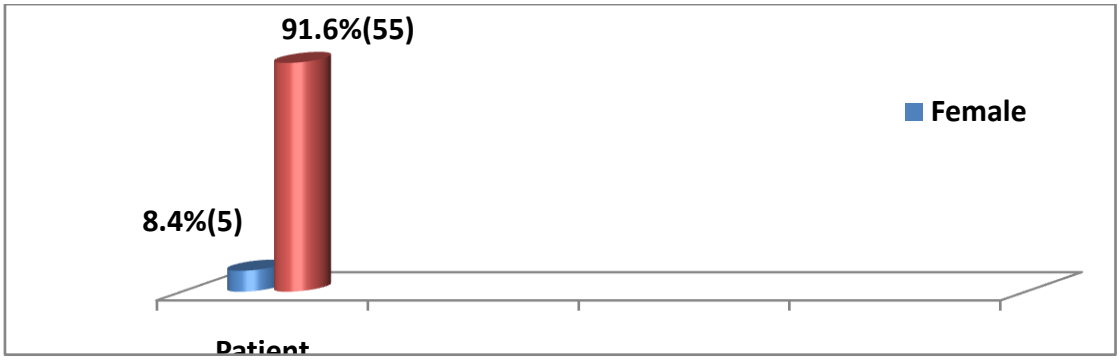

Figure 1: Distribution Of Patients According To Their Gender.

The Mean Age Of Patients Group Did Not Differ Significantly From Control Group (40.05 ± 8.02 Years Vs40.07 ± 7.20 Years Respectively, P > 0.05) As Shown In Table-1.

Table 1:

Group	Patients	Controls
Number	60	30
Mean (Yr.)	40.05	40.07
Standard Deviation	± 8.02	± 7.20
SEM	1.04	1.31
Df		88
P-Value*		0.99
		*Not Statistically Significant

Serum Levels Of Calprotectin (Ng/Ml) By Using ELISA Technique, The Mean Serum Level Of Calprotectin (Ng/Ml) Was Higher In Patient Group (89.23±73.97) Than Control Group (54.94±20.51) And This Difference Was Statistically Significant (P Value =0.0148) As Shown In Table-2.

Table-2: Results Of Serum Levels Of Calprotectin (Ng/Ml) In Patient And Control Groups.

Group	Patients	Controls
Number	60	30
Mean (Ng/Ml)	89.23	54.94
Standard Deviation	± 73.97	± 20.51
SEM	9.55	3.74
Df	88	
P-Value*	0.0148	
* Statistically Significant		

Table-3 Showed That Serum Level Of Calprotectin (Ng/Ml) Was Higher In Patients Treated With Conventional Treatment, Than In Patients Treated With Biological Treatment (Infliximab Infusion)And The Difference Was Statistically Significant (P Value =0.0013).

Table-3: Results Of Serum Levels Of Calprotectin (Ng/Ml) In Patients Treated With Conventional Treatment And Other Patients Treated With Biological Treatment.

Group	Conventional Treatment	Biological Treatment
Number	30	30
Mean (Ng/Ml)	118.92	59.54
Standard Deviation	± 95.19	± 15.73
SEM	17.38	2.87
Df	58	
P-Value*	0.0013	
*Very Statistically Significant		

Table-4 Showed That The Mean Serum Level Of Calprotectin (Ng/Ml) Among Patients Whose BASDAI Level < 4.0 Was Lower Than Those Their BASDAI Level ≥ 4.0 And The Differences Was Statistically Not Significant (Pvalue =0.4815).

Table 4: Results Of Mean Serum Levels Of Calprotectin (Ng/Ml) In Patients Whose BASDAI Level< 4.0 And Those Their BASDAI ≥ 4.0.

Group	BASDAI < 4.0	BASDAI ≥ 4.0
Number	40	20
Mean (Ng/Ml)	84.43	98.84
Standard Deviation	± 78.00	± 66.01
SEM	12.33	14.76
Df	58	
P-Value*	0.4815	

Table-5 Showed That The Mean Serum Level Of Calprotectin (Ng/Ml) Among Patients Whose BASFI Level < 4.0 Was Lower Than Those Their BASFI Level ≥ 4.0 And The Differences Was Statistically Not Significant (P Value =0.8399).

Table 5: Results Of Serum Levels Of Calprotectin (Ng/Ml) In Patients Whose BASFI Level< 4.0 And Those Their BASFI ≥ 4.0.

Group	BASFI < 4.0	BASFI ≥ 4.0
Number	31	29
Mean (Ng/Ml)	87.21	91.12
Standard Deviation	± 58.48	± 86.95
SEM	10.86	15.61
Df	58	
P-Value*	0.8399	
*Not Statistically Significant		

IV. Discussion

Ankylosing Spondylitis (AS) Is A Potentially Disabling Chronic Inflammatory Condition Affecting The Axial Skeleton That Is Manifested By Chronic Back Pain. The Onset Is Typically Before 45 Years Of Age [13]. This Study Included Sixty (60) Patients With Aswho Attended The Rheumatology Consultation Clinic Of Baghdad Teaching Hospital In The Period Between October 2016 To October 2017, Thirty (30) Of Them

Treated With Biological Treatment And Other Thirty (30) Treated With Conventional Treatment, And Thirty (30) Control Healthy Person. The Mean Age Of Patients Was 40.05 ± 8.02 Years, This Is In Accordance With The Results Of Demirdal S., Et Al Study Inturkey (2013)That Reported Mean Age Of Participated AS Patient Of 37.9±12.7years (14).

This Study Showed That Males Are More Predominant For AS Than Females With A Ratio Of 11:1 Whichdisagrees With Lee W., Et Al Who Showed Male: Female Ratio 3:1 (12) . Thisinconsistency Might Be Attributed To Low Sample Size Of The Present Study.

The Mean Serum Level Of Calprotectin (Ng/Ml) Was Higher In Patient Group (89.23±73.97) Than Control Group (54.94±20.51) And This Difference Was Statistically Significant (P Value =0.0148) As Shown In [Table-1]. This Is Because Calprotectin Likely Plays A Role In AS Pathogenesis And In The Disease Course. De Rycke Et Al. Demonstrated 10-Fold Higher Calprotectin In Inflamed Synovium Than In Serum [15].

[Table-2] Showed That Serum Level Of Calprotectin (Ng/Ml) Was Higher In Patients On Conventional Treatment, Than In Patients Treated With Biological Treatment (Infliximab Infusion) And The Difference Was Statistically Significant (P Value =0.0013).This Is Because The Number Of Synovial Monocytes And Granulocytes That Release Calprotectin Rapidly Decreases After TNF Treatment In Patients With AS [16].

[Table-3] Showed That The Mean Serum Level Of Calprotectin (Ng/Ml) Among Patients Whose BASDAI Level < 4.0 Was Lower Than Those Their BASDAI Level ≥ 4.0 (Worst Disease Activity) And The Differences Was Statistically Not Significant (P Value =0.4815), And Also [Table-4] Showed That The Mean Serum Level Of Calprotectin (Ng/Ml) Among Patients Whose BASFI Level < 4.0 Was Lower Than Those Their BASFI Level ≥ 4.0 And The Differences Was Statistically Not Significant (P Value =0.8399). This Is In Accordance With Recent Data From The GESPIC Cohort Indicate That Elevated Serum Calprotectin Is An Independent Predictor Of Disease Progression And Syndesmophyte Formation [17].

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