Value of Serum Leptin in patients with Rheumatoid arthritis; Its Correlation With disease activity and Musculoskeletal Ultrasound findings

Sherin H Hamza[1] and Takwa Badr Younes[2]

[1]Internal Medicine Department- Rheumatology Division, Faculty of Medicine, Ain Shams University Cairo Egypt
[2]Physical Medicine, Rheumatology and Rehabilitation Departments, Faculty of Medicine, Ain Shams University, Cairo, Egypt
drsherinehosny@hotmail.com, takwabadr@yahoo.com

Abstract: Aim of the work: Measurement of serum Leptin in rheumatoid arthritis patients (RA) and it's relation to clinical data, serological measures, disease activity, musculoskeletal US findings, and radiological joint damage score. **Subjects and Methods:** Our study included (40) patients with RA and (40) potentially healthy age and sex matched individuals as controls. Patients underwent full clinical assessment, assessment of disease activity by DAS28, measurement of serum leptin by ELISA. Plain x-ray hands and feet was done to all RA patients for evaluation of radiological joint damage using modified Larsen score which was done by an expert radiologist. Musculoskeletal ultrasound (MSUS) using the six joint power Doppler ultrasound (PDUS) scoring system for assessment RA joint findings**. Results:** There was no significant differences (p>0.05) between RA patients and controls as regards the mean level of serum Leptin with a mean of 22.60 ± 16.53 versus24.46 ± 17.06 respectively. There was no significant relation between mean serum leptin levels and the grades of disease activity of RA assessed by DAS28 (p>0.05). The mean serum leptin levels in RA patients showed no significant difference as regards the presence or absence of RF or the titer of anti CCP (p>0.05). The mean levels of serum cholesterol and LDL were higher in RA patients than the control with highly statistically significant difference (p<0.001). Also the mean levels of TG and HDL were higher in the patients than the controls with a statistically significant difference (p<0.05). There was a highly significant positive correlation between the mean serum leptin level and body mass index *(r= 0.686, p <0.001*), TG (r=0.80, p <0.001), cholesterol (r=0.76, p<0.001), and LDL (r=0,83, p<0.001) among our studied RA patients. There was no significant relation between radiological damage assessed by mean Larsen score and mean serum leptin level (p>0.05). Regarding the MSUS findings; tenosynovitis was found in 12 RA patients (30%), 36 patients (90%) had synovial hypertrophy, 18 RA patients (45%) had synovial effusion. Positive power Doppler ultrasound signal (PDUS) was found in 23 RA patients (57.5%)**,** showing grade 1 in 16 RA patients (40%), grade 2 in 7 RA patients (17.5%), PDUS score ranged from 0 to 10 with a mean of 3.08 ± 3.06.there was no significant relation or correlation as regard mean serum leptin level and the PDUS score (r=-0.008, p=0.095). **Conclusion:** Serum leptin levels have no significant correlation with RA clinical findings, disease activity score, serological data, musculoskeletal ultrasound findings and radiological joint damage in our RA patients. Factors as BMI, TG, cholesterol, and LDL influence serum leptin levels. So, according to our results, serum leptin doesn't seem to be a possible marker to monitor presence of inflammation or radiological joint damage in RA.

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1. Introduction

Rheumatoid arthritis (RA) as a common chronic inflammatory polyarthritis represent a health burden as it leads to disability and increase in mortality **(1).** It is characterized by outrage production of inflammatory cytokines such as TNF- α, IL-1, IL-6 that correlates with the disease activity and deterioration, as well as with a decrease of body cell mass **(2).**

Leptin is an adipokine secreted by adipose tissue into the circulation, reaching both the central and peripheral nervous systems regulating appetite and food intake, basal metabolism, bone mass, reproductive function and insulin secretion. Research linking regulation of adipose tissues has provided an intricate network to metabolism and immune homeostasis **(3).**

Serum leptin was reported in the immunopathogenesis of inflammatory diseases like RA and osteoarthritis as a proinflammatory signal, also leptin was found to upregulates the secretion of proinflammatory cytokines, such as (TNF-α) and (IL-6), which are found to correlate with disease activity and severity. Articular adipose tissue leptin (paracrine leptin) might affect the control of inflammatory events in RA ***(4).*** Moreover, a potential cause of rheumatoid cachexia may be due to increased inflammatory

cytokines caused by, hyperleptinemia. Some researches proposed that Leptin can be a possible biomarker that could have a possible association with RA disease activity ***(5).***

The present study was designed to measure the mean serum Leptin level with in rheumatoid arthritic patients and its relation with disease activity, modified Laresn's joint damage sore, and musculoskeletal US findings.

2. Subjects and Methods

Clinical evaluation

The current study was a case control cross sectional study that included (40) patients with RA fulfilling the ACR/EULAR 2010 classification criteria **(6)** and (40) potentially healthy age and sex matched individuals as controls. They were selected from Rheumatology outpatient clinic and inpatient department at Ain shams university hospitals. **Exclusion criteria included** Patients with Body mass index (BMI) less than (18) and more than (30), diabetics, patients taking medications known to affect nutritional status (e.g. cytotoxic, anti-Parkinson's drugs) or regulation of fat metabolism (e.g. Anti-diabetic, Thyroid Hormone drugs, etc……). The nature of the present study was explained to all participants. The laboratory and radiological procedures represent standard care and pose no ethical conflict. Verbal consent was obtained from all participants approved by local ethics committee of Ain Shams university hospitals.

All patients were subjected to full clinical assessment with special emphasis on musculoskeletal manifestations and measurement of body mass index (BMI). Assessment of disease activity was done by using DAS28 score ***(7).***

Laboratory Investigations

Seven ml of venous blood was withdrawn from each subject and was divided into two parts; 3 ml was placed in a tube with EDTA anticoagulant for determination of Erythrocyte sedimentation rate (ESR) in first hour by Westergren method, 4 ml was placed in another test tube and allowed to be clotted then centrifuged and were stored for t -20 °C prior to assay. Complete blood count (CBC) was done using coulter JS. Quantitative C- reactive protein (CRP) titer done by immunoturbidimetric method, with full blood chemistry, including renal and liver function tests. Fasting blood sugar, 2hours post prandial blood glucose, Lipid profile including total cholesterol, serum triglycerides, LDL, HDL. RF was measured by using Biotic RA factor latex agglutination slide for the qualitative and semi quantitative determination of RF in serum.**,** and anti- citrullinated peptide (anti CCP) by ELISA Kit.

Measurement of the serum leptin level using the DRG® leptin ELISA Kit (EIA-2395 USA) which is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The micro titer wells were coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule.

Radiological investigations

Plain x-ray of both hands, wrists and feet was done to all RA patients with assessment of radiological joint damage by modified Larsen scoring method which was done by expert radiologist**.** Interpretation*:* Grade 0: Intact bony outlines and normal joint space. Grade 1: Erosion less than 1 mm in diameter or joint space narrowing, Grade 2: One or several small erosions (diameter more than 1 mm), Grade 3: Marked erosions. Grade 4: Severe erosions: There is usually no joint space left; the original bony outlines are partly preserved. Grade 5: Mutilating changes: The original bony outlines have been destroyed with a maximum possible score of 160 ***(8).***

Musculoskeletal ultrasonographic imaging with power Doppler gray scale ultrasound (PDUS) by high resolution ultrasound equipment logiq P5UK (General Electric Healthcare system) using a gray scale US mode with frequency ranging from (10-13 MHz-frequency), was done by two expert rheumatologist to all patients. Hands were examined. Both longitudinal and transverse scans were performed by moving the transducer from radial to ulnar and from proximal to distal sides on the dorsal and volar aspects to enable maximum coverage of the anatomical surface areas. Synovial vascularization was assessed by power Doppler PDUS included the intraarticular, tenosynovial and intrabursal power Doppler (PD) signals that were graded on a semiquantitative scale from 0-3 where (grade 0= absence of color signal, no synovial flow, grade 1 = mild, or <3 isolated signals, grade 2=moderate, or>3 isolated PD signals or confluent signals in less than half of the synovial area; grade 3= marked, signals in more than half of the synovial area). These scores corresponded to the maximum score for PD signals obtained from any of the synovial sites evaluated at each joint. The sum of PD signal scores obtained from each joint was used as the PDUS score, as reported by ***Naredo et al., (*9**)**.** For better clinical availability we scanned six joints including; bilateral wrists and bilateral 2nd, 3rd MCP joints dorsal recess **(10)**. The six joint PDUS score was the sum of the six synovial sites. An individual joint was considered positive for synovitis if its max PDUS score was ≥1

The presence of effusion was diagnosed in each joint as hypoechoic or anechoic compressible intra-articular material, within synovial recesses. Synovitis was defined as echogenic non-compressible intra-

articular tissue, within synovial recesses, which were longitudinally scanned from the dorsal view with the joint in extension: maximum distance from the articular bony margin to the joint capsule >2 mm **(11)**.

Statistics

Statistical presentation and analysis of the present study was conducted, Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Unpaired Student T-test was used to compare between two groups in quantitative data. Chi-square: The hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship. Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables. Linear Correlation coefficient was used for detection of correlation between two quantitative variables in one group. ANOVA test was used for comparison among different times in the same group in quantitative data. The difference was considered insignificant if $p>0.05$, significant if $p<0.05$, and highly significant if $p<0.001$.

3. Results

The studied (40) RA patients included (33) females and (7) males, their age ranged from (22) to (58) years, with mean of (40.83±7.89) years and mean disease duration of (13.17 ± 8.5) years. Their BMI ranged from 19.8-29.9 kg/m^2 with a mean of 25.5 ± 2.76. Also, forty potentially healthy age and sex matched volunteers were selected as controls {29 females (72.5%) and 11 males (27.5%)}, age ranged from (22) to (60) years, with a mean of (38.75 ±9.03) years. Their BMI ranged from 19.7-29.5 kg/m^2 with a mean of 25.6 ±3.82. Six of our RA patients (15%) received NSAIDs alone, 31 patients (77.5%) received both steroids and DMARDs; (23 of them received methotrexate (MTX), 5 received leflunomide and 3 received hydroxychloroquine, and 3 patients (7.5%) received biologic agents (Etanercept) table 1. According to DAS28 score; 10 patients (25%) were in remission, 8 patients (20%) were in low activity, 12 patients (30%) were in moderate activity, and 10 patients (25%) were in high disease activity (table 1).

Regarding laboratory data; the mean serum levels of (ESR and CRP) as markers of disease activity were higher in the patients' group than in the controls group with highly statistically significant difference ($p<0.001$) and a statistically significant difference ($p<0.05$) respectively. Moreover, regarding lipid profile; the mean levels of serum cholesterol and LDL were statistically highly significantly increased ($p<0.001$) in RA patients group (being 162.0± 43.16 and 98.00 ± 27.78) than the control group (108.6 ±43.4, 76.88 ± 19.1). The mean levels of TG and HDL were statistically significantly higher ($p<0.05$) in patients group (158.6 ± 57.10, 39.43 ± 9.94) than control group (125.6 ±46.54, 32.4± 12.07) by t- test (table 1).

The mean level of serum Leptin in the patients and controls groups were 22.60 ± 16.53 and 24.46 ± 17.06 respectively with no statistically significant differences ($p>0.05$) by using t- test (table1, figure1).

By spearman correlation test there was a statistically significant highly positive correlation between mean serum leptin level and body mass index in our RA individuals *($r= 0.686$, $p <0.001$)*. While, there was no significant correlation between mean serum leptin level and age, disease duration, and clinical manifestations (table 4).

There was no statistically significant relation between serum leptin levels and the grades of RA disease activity assessed by modified DAS28 ($p>0.05$) using ANOVA test being 19.1±6.92 in patients in remission, 18.30±14.2 in low disease activity, 21.5±10.02 in moderate activity, and 34.00±29.1 in patients with severe activity (table 2). Moreover, there was no significant correlation between mean serum leptin level and DAS 28, laboratory markers of disease activity (ESR and CRP) (table 4).

The mean serum leptin levels in RA patients showed no statistically significant difference ($p>0.05$) as regards the presence or absence of RF (being 23.4±17.3 and 18.0±8.43 respectively) or the titer of anti CCP (negative 12.5±0.71, weak positive 20.83±7.25, positive 25.67±23.12, and strong positive 21.00±15.56anti –CCP) table 2.

Using spearman correlation test we found a statistically highly significant positive correlation between mean serum leptin and TG (r=0.800, p 0.001), cholesterol (r=0.76, p0.001), LDL (r=0.83, $p<0.001$) among our studied RA patients (table 4).

Radiological assessment by modified Larsen score ranged from 4-28 with a mean of 13.35±6.32 (table 1). No significant correlation was present between mean serum leptin level and modified Larsen score (r=-0.107, p=0.51) (table 4).

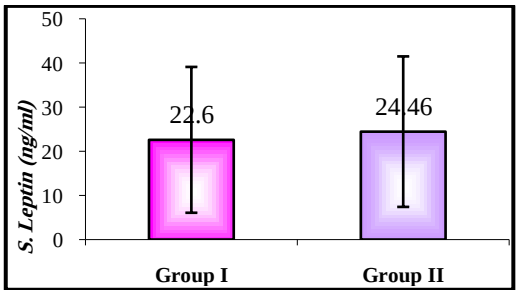

Figure (1): Comparison between group I (40 RA patients) and group 2 (40 controls) as regards the level of serum Leptin.

MSUS findings included the presence of tenosynovitis in 12 patients 30%, 18 patients (45%) had hand synovial effusion, 36 patients (90%) had synovial hypertrophy. As regards Power Doppler US grading score measured in six joints in our RA patients as an indication of active synovitis; Seventeen patients (42.5%) were grade 0 which means negative PDUS signal, 16 patients (40%) were grade 1, and 7 patients (17.5%) were grade 2, and none were grade 3(marked synovitis). The PDUS score ranged from 0-10 with a mean score of 3.08±3.06 (table 1). Patients with higher values of anti-CCP, ESR, CRP, DAS 28, had a statistically significant higher rates of PDUS signal score ($p < 0.05$). Also a highly significant positive correlation, with ESR, CRP, DAS 28 ($r = 0.824$, $p<0.001$, $r = 0.746$, $p<0.001$, $r=0.879$ $p<0.001$ respectively. Significant correlation as regards anti-CCP $r=0.450$, $p<0.004$. No significant relation or correlation was found as regard the mean serum leptin level and the mean power Doppler signal score ($r = -0.008$, $p = 0.959$) fig (2), table (3,4).

Table (1): Demographic, clinical, laboratory and radiographic characteristics among RA patients versus controls.

Variables			RA pts No. (40)		Controls No. (40)	T	P
Age (years) Mean ± SD			40.83 ± 7.8		38.7±9.03	1.09	0.27 NS
BMI (kg/m^2) Mean ± SD			25.5 ± 2.76		25.6 ± 3.8	1.13	0.26 NS
Disease duration (years) Mean ± SD			13.17 ± 8.5				
Gender	**Male (no,%)**		7	17.5%	1127.5	0.645	0.42 NS
	Female (no,%)		33	82.5%	2972.5		
Drug intake	**NSAID**		6	15%			
	Steroids + DMARDs		31	77.5%			
	Biologic Agents		3	7.5%			
Clinical manifestations	**Morning stiffness (min)**		31	77.5%			
	Tender joints		31	77.5%			
	Swollen joints		19	47.5%			
DAS28 score	**Remission ≤ 2.6**		10	25%			
	Low disease activity (> 2.6 ≤ 3.2)		8	20%			
	Moderate disease activity (> 3.2 ≤ 5.1)		12	30%			
	High disease activity > 5.1		10	25%			
MSUS Findings	**Presence of tenosynovitis**		12	30%			
	Presence of synovial hypertrophy Presence of synovial effusion		36 18	90% 45%			
	Positive PDUS signal		23	57.5%			
	Doppler grading	**0 (negative PDUS)**	17	42.5%			
		1	16	40%			
		2	7	17.5%			
		3	0	0%			
PDUS score (Mean ± SD)			3.08±3.06				
Modified Larsen score (Mean ± SD)			13.35±6.32				
ESR (mm/hr)			37.98 ±20.49		8.79 ± 5.61	8.68	**0.001HS**
CRP (mg/L)			2.07 ± 0.92		1.64 ± 0.81	2.24	**0.027HS**
Hb (gm/dL)			11.82 ± 1.26		14.60 ± 0.89	11.4	**0.001HS**
WBC (10^3 cells/mm^3)			5.52 ± 1.47		7.840 ± 1.95	5.99	**0.001HS**
Platelets (10^3 cells/mm^3)			187.67 ±22.8		305.7 ±87.3	8.27	**0.001HS**
AST (U/L)			26.18 ± 12.11		21.86±11.79	1.615	0.11 NS
ALT (U/L)			25.23 ± 11.25		21.19 ±11.3	1.600	0.11NS
S.Creatinine (mmol/L)			83.88 ± 12.99		85.15 ± 17.8	0.364	0.71NS
BUN (mg/dl)			14.25 ± 3.61		13.85 ± 4.10	0.469	0.64 NS
Triglyceride (mg/dL)			158.6 ± 57.10		125.6 ±46.54	2.833	**0.006S**
Cholesterol (mg/dL)			162.0± 43.16		108.6 ±43.4	5.509	**0.001HS**
LDL (mg/dL)			98.00 ± 27.78		76.88 ± 19.1	3.959	**0.001HS**
HDL (mg/dL)			39.43 ± 9.94		32.4± 12.07	2.812	**0.006S**
S. Leptin (ng/ml)			22.60 ± 16.53		24.4± 17.06	-0.496	0.621NS

Sig.: Significance NS: non-significant ($p>0.05$) S: significant ($p<0.05$) highly significant (HS)$P<0.001$

Table (2): Comparison between grades of disease activity, RF, and anti-CCP titer among the studied RA patients as regards serum leptin level.

		S. Leptin (ng/ml)	ANOVA	
		Mean±SD	F/t	P
DAS28	Remission	19.10 ± 6.92	1.758	0.173NS
	Low	18.30 ± 14.23		
	Moderate	21.50 ± 10.02		
	High activity	34.00 ± 29.12		
RF	positive	18.0±8.43	0.353	0.467NS
	negative	23.4±17.3		
Anti-CCP	Negative	12.50±0.71	0.510	0.678 NS
	Weak positive	20.83±7.25		
	Positive	25.67±23.12		
	Strong positive	21.00±15.56		

Table (3): Comparison between negative and positive Doppler signals according to other parameters.

	Power Doppler US signal		t-test	
	Negative Doppler	Positive Doppler		
	Mean±SD	Mean±SD	t	P
Anti. CCP	30.94±8.51	40.96±11.70	-2.988	**0.005**
Serum Leptin	21.82±15.37	23.17±17.66	-0.252	0.802
DAS28	2.70±1.03	5.03±1.49	-5.529	**<0.001**
BMI	25.87±2.43	25.87±3.12	-0.004	0.997
ESR	23.76±11.54	48.48±19.39	-4.671	**<0.001**
CRP (mg/L)	1.49±0.50	2.50±0.93	-4.042	**<0.001**

Table (4): Correlation between serum Leptin level and different clinical manifestations, some laboratory parameters, disease activity and radiological damage score among the studied RA patients

Variable	S. Leptin (ng/ml)		
	r	P	Sig.
Age (years)	0.086	0.448	NS
Disease duration (years)	0.044	0.788	NS
Morning stiffness (min)	0.048	0.768	NS
Number of tender joint	-0.091	0.576	NS
Number of swollen joint	-0.195	0.227	NS
BMI	0.686	**0.001**	**HS**
DAS28	-0.108	0.506	NS
Larsen score	-0.107	0.513	NS
Positive Power Doppler signal score	-0.008	0.959	NS
ESR (mm/hr)	-0.203	0.210	NS
CRP (mg/L)	-0.211	0.191	NS
Hb (gm/dL)	-0.133	0.415	NS
WBCs (10^3 cells/mm^3)	-0.149	0.360	NS
Platelets (10^3 cells/mm^3)	0.091	0.577	NS
AST (U/L)	-0.010	0.953	NS
ALT (U/L)	-0.055	0.738	NS
S. Creatinine (mmol/L)	-0.182	0.260	NS
BUN (mg/dL)	0.168	0.301	NS
Triglyceride (mg/dL)	0.800	**0.001**	**HS**
Cholesterol (mg/dL)	0.769	**0.001**	**HS**
LDL (mg/dL)	0.839	**0.001**	**HS**
HDL (mg/dL)	-0.197	0.222	NS
RF (IU/mL)	0.245	0.127	NS
Anti-CCP (IU/mL)	0.099	0.545	NS

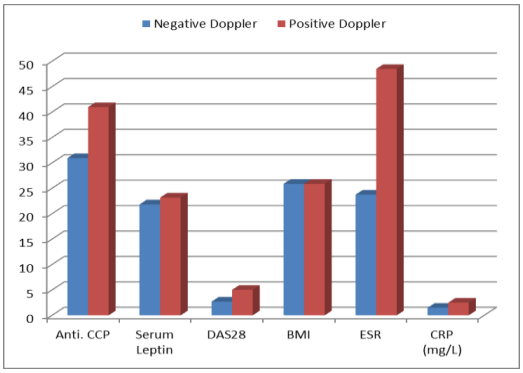

Figure (2): Comparison between negative Doppler and positive Doppler as regards laboratory findings and DAS 28 score.

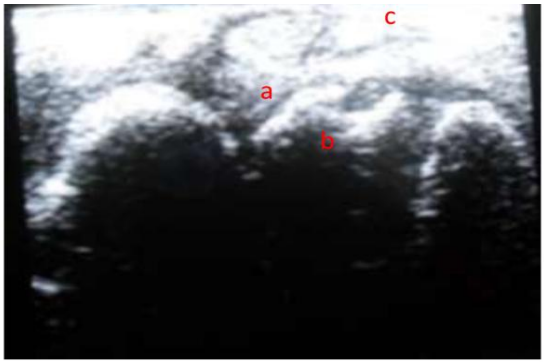

Figure (3): Showing a longitudinal scan on the dorsal aspect of the right wrist radioulnar joint of RA patient with moderate synovial hypertrophy as a hyperechoic signal (a) surrounded by a rim of hypoechoic compressible signal suggestive of synovial effusion. There is an underlining area of bone line discontinuity at the radial articular surface denoting erosion (b). Tenosynovitis along the extensor tendons with hypoechoic and hyperechoic signals (c).

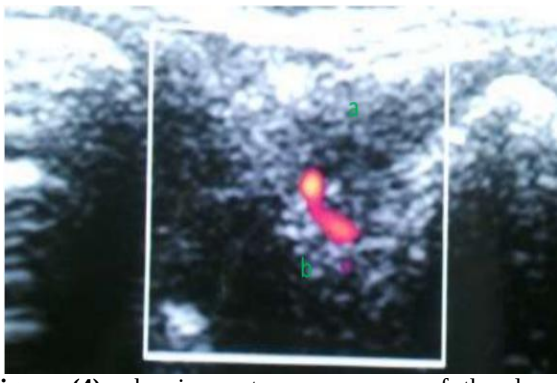

Figure (4): showing a transverse scan of the dorsal aspect of right wrist joint of RA patient with synovial hypertrophy (a), and mild vascularity is seen as power Doppler signal of grade 1 (b).

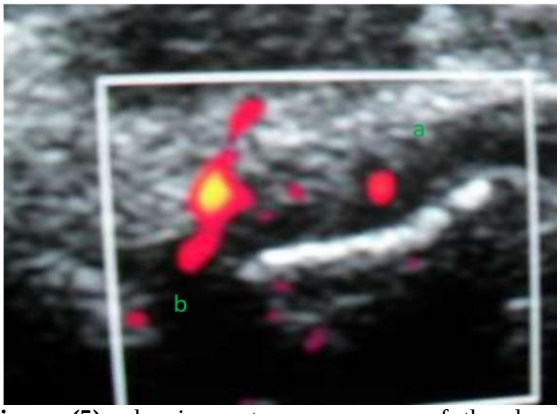

Figure (5): showing a transverse scan of the dorsal aspect of left wrist joint of RA patient with synovial hypertrophy (a), and moderate vascularity is seen as power Doppler signal of grade 2 (b).

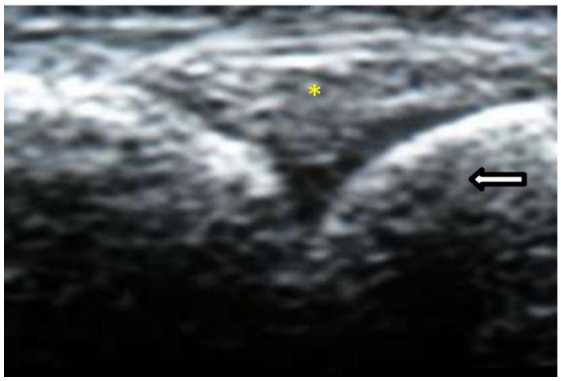

Figure (6): showing a longitudinal scan of the dorsal aspect of the left 2nd metacarpophalangeal joint (arrow) of RA patient with synovial tissue hypertrophy as an area of non compressible echogenicity raising the overlying joint capsule (star).

4. Discussion:

RA is often associated with an increased risk of cardiovascular and metabolic disorders. A strong correlation between the progressive course of RA and leptin levels have been reported by several studies. Some investigators reported increased leptin levels in RA patients. Other investigators stated that the ratio between leptin level in serum and in synovial fluid is correlated to disease duration, and other parameters of RA activity. Serum Leptin levels also correlated to IL-17 in plasma from patients with RA with conventional pharmacological treatment, which suggests that leptin could be a biomarker of long-term disease. The effects of leptin are not only related to articular tissues, but it also regulates innate and adaptative immunity. Leptin stimulates macrophage and monocytes proliferation and phagocytosis. It affects natural killer (NK) cells cytotoxicity and it regulates neutrophil chemotaxis. Regarding adaptive immunity, leptin induces proliferation of naive T cells, activates T helper 1 cell immune responses and suppress T helper 2 cell immune responses. Leptin is also able to modulate the activity of Treg cells, which are potent inhibitors of autoimmunity. Therefore; leptin is considered a link among metabolic function, the immune tolerance, and the autoimmune activity. The interference with leptin signaling could provide future new therapies for autoimmune disorders such as RA. (3).

The present study revealed no significant increase in the mean serum leptin level among RA patients compared to controls. This was in agreement with ***Otvos et al., (12), and Manole et al., (4)*** who found no statistically significant differences between serum leptin levels in RA patients and controls *(p>0.05).* ***Harle et al., (13)*** reported that serum leptin levels were lower in RA women than in healthy women. On the contrary, studies done by ***Šenolt et al.,* (14), *Yoshino et al., (15), Allam and Radwan (16), Tian et al., (17);*** and ***Abdalla et al.,*** in ***2014*(2)** showed that leptin plasma levels of RA patients markedly elevated in comparison with healthy controls. A meta-analysis that included thirteen studies of 648 RA patients, and 426 controls found that level of serum leptin level significantly increased in RA patients than controls ***(18)***. Moreover, ***Seven et al.,* (19)** found that the levels of serum and synovial fluid leptin were significantly increased among RA patients than controls *(p<0.05)* and had referred elevated leptin levels to the pro-inflammatory conditions in RA and to the stimulatory effect of TNF α and IL 1β on leptin activity**.** While, ***Tokarczyk-Knapik et al., (20)*** stated that chronic inflammation lower the leptin level in plasma concentration contrary to acute inflammation. The differences between these studies may be due inhibition of leptin and leptin mRNA production resulting from long-term stimulation of adipose tissue by TNF-α or IL-1 ***(21).***

Our study revealed significantly highly positive correlation between levels of serum leptin and BMI in RA patients group. A similar observation was reported by two previous studies ***(22), (17)***. While was in partial agreement with ***Targojska-Stepniak et al.,* (23)** who reported that the mean leptin level correlated positively with BMI in women with RA, but not in men. On the contrary, other studies found that serum leptin level did not correlate with BMI in RA patients, and suggested that regulation of leptinemia is complex and that weight is not the only major regulator **(24)**, **(20).**

We didn't find significant correlation between serum leptin and age, and this agrees with the results of ***Abdalla et al., (2)*** and ***Oner et al.,* (5)** who found no correlation between increase age of RA patients and serum leptin level. Also we found no correlation between serum leptin and disease duration (r= 0.044, p=0.788), also this goes with the results of ***Abdalla et al.,* (2)** and ***Oner et al., (5)*** who didn't find significant correlation between serum leptin level and disease duration of RA patients. On the other hand ***Olama et al.,* (25)** found a significant correlation between disease duration, parameters of RA activity and Serum leptin, also the ratio of synovial/serum leptin *(*for serum leptin *p= 0.018).*

Moreover, no correlation was found between mean serum leptin level and clinical manifestations of RA including morning stiffness, and this agree with ***Bokarewa et al., (26)*** who found no correlation between leptin and morning stiffness. The present study also showed no correlation between mean serum leptin and tender joints number *(p=0.576),* and swollen joints number *(p=0.227),* and this agree with the results of study done by ***Gunaydin et al.,* (22),** which showed that circulating leptin levels do not seem to reflect swollen and tender joint counts *(p=0.7).*

As regards RA activity as assessed by modified DAS 28, the mean serum leptin level didn't significantly differ in patients with remission or with low, moderate or high disease activity *(p= 0.173),* a similar observations have been reported in previous studies **(27), (19), (5).** Also, no significant correlation was found between serum leptin and disease activity by DAS28, this agrees with the results of studies done by ***Oner et al.,* (5)** and ***Popa et al., (24)***, who found no significant correlation between serum leptin level and disease activity of RA (p= 0.18). On the other hand, our results were not matching with those of ***Otero et al., (28)*** and ***Targojska-Stepniak et al., (23)*** who reported negative correlation between levels of leptin and DAS28, tender joint count in RA patients with erosive and long disease duration *(p=0.02).* Moreover, ***Rho and his colleges,* (29)** reported that there was a positive relation between leptin and disease activity. ***Lee and Bae (18)*** reported the presence of minimal positive significant correlation between leptin levels and RA disease activity (DAS 28 and CRP).

On correlating ESR and CRP as an inflammatory markers with serum leptin level we found non significant correlation. These results are similar to the results of ***Tokarczyk-Knapik et al., (20) Nishiya et al., in 2002(30) and Oner et al., (5).*** On the contrary, ***Targojska-Stepniak et al., (23)*** found a positive correlation between leptin levels and ESR in RA patients with long disease duration. It was reported that the inhibitory effect of CRP on the site of leptin binding receptors, leading to inhibition of further leptin signaling in vitro. This may contribute to the influence of higher CRP concentration on leptin resistance in RA (**29**).

The mean serum leptin level in our group of RA patients was not associated with any of disease specific autoantibodies namely RF and anti-CCP antibodies. A non-significant difference was found between RA patients with negative and positive rheumatoid factor as regards mean serum leptin levels (p>0.05). Similarly, ***Hizmelti et al., (31)*** reported no statistically significant difference (p >0.05) in serum leptin level between patients with negative and positive RF. Current study found no statistically significant difference between negative, weak positive, positive, and strong positive anti–CCP as regards serum leptin level (p>0.05), that agrees with ***Klein-Wieringa et al.,* (32)** who found that there was no association between negative, weak positive, positive, and strong positive anti–CCP as regards serum leptin level (p>0.05). Moreover, our study showed that there was no statistically significant correlation between RF, anti-CCP and serum leptin level these results were similar to the results of ***Targojska-Stepniak et al.,* (23)** who found no association between leptin levels and anti-cyclic citrullinated antibodies, rheumatoid factor, or extra-articular findings.

On correlating between mean serum leptin level and TG, cholesterol, LDL, and HDL levels, our results revealed a statistically significant positive correlation with the levels of TG, cholesterol, and LDL, while there was no correlation with HDL these results concord with the results of ***Kadowaki et al., (33)*** and ***Ekmekci and Ekmekci* (34)** who found that leptin concentrations associated strongly and favorably with all recorded lipid variables except for HDL cholesterol in RA subjects.

Moreover, our results showed that there was no significant correlation between mean Larsen score and serum Leptin level *(p= 0.313).* There are few studies about the effect of adipokines like leptin and visfatin on radiographic changes of involved joints in RA. A study done by ***Giles et al., (35)*** reported no association between the level of leptin and radiographic joint

damage *(p>0.05)*. Also, in the current study no correlation was found between serum leptin and radiological damage by Larsen score. These results also agree with those of ***Mirfeizi et al*** **(36)** who found that leptin does not have any effect on the joint damage process. While ***Ouchi and Walsh*** **(37)** found that higher leptin in RA truly deteriorates joint damage. Yet ***Batún-Garrido et al*** **(38)** showed independent association between high serum leptin level and RA disease activity, number of tender, number of swollen joints, the presence of metabolic syndrome.

In the present study there was no significant relation or correlation as regard the mean serum leptin level and the mean power Doppler score as a measurement of RA active synovitis, yet there was mild elevation of the mean serum leptin level in patients with presence of positive power Doppler signal than patients with negative power Doppler signal, but did not reach a statistical significant difference. This may be attributed to the small number of RA patient sample which could be further investigated in larger patient sample study in the future. To our knowledge we were the first to tackle these interesting findings that showed the relation between power Doppler ultrasound signal and the mean serum leptin level in RA patients.

We concluded that serum leptin levels have no significant correlation with RA clinical findings, disease activity score, serological data, musculoskeletal ultrasound findings and radiological joint damage in our RA patients. Also, we reported that elevated serum leptin level is not considered to be a potential threat for the occurrence of radiological joint damage. Factors as BMI, TG, cholesterol, and LDL influence serum leptin levels. So, according to our results, serum leptin doesn't seem to be a possible marker to monitor presence of inflammation or radiological joint damage in RA. This may be due to differences in ethnic, demographics and the limited number of RA patient's selection in our study.

Due to the uncertainty of the biological effects of leptin on the autoimmune response in RA, further studies on larger cohorts are recommended aiming to reach novel prospective focusing on the effect of nutrition for the modulation of the immune response to verify our results hoping for better immunological, clinical and radiological outcome among RA patients.

Corresponding Author:
Dr. Sherin H Hamza
Internal Medicine Department- Rheumatology Division, Faculty of Medicine, Ain Shams University Cairo Egypt
Email: drsherinehosny@hotmail.com

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