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ORIGINAL ARTICLE

Study of Tumor Necrosis Factor -β (Lymphotoxin) and Cell Death Receptor (TNFR-2) in Rhumatiod Arthritis with Type 2 Diabetes Mellitus

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ABSTRACT

The aimed of this study is to detect diabetes type 2 in rheumatoid arthritis patients early. Thus, this study determine TNF-β, TNFR-2 in rheumatoid arthritis patients with and without diabetic type 2 and compare results with a control group in order to assess their use as biochemical markers for early diagnosis of rheumatoid arthritis. As well as to find a correlation of TNFR-2 with FBS, AST, ALT, ALP and TNF-β in all subjects in the current study. Age ranged from 35 to 55. The study's participants were separated into three groups: (G1) control (30), (G2) rheumatoid arthritis (30), and (G3) rheumatoid arthritis with T2DM (30). In (G2) and (G3), the levels of AST, ALT, and ALP were considerably higher than in (G1). In addition, there was a highly significant increase in (G3) when compared to (G2). The F.B.S results revealed a non-significant increase in (G2) when compared to (G1), but a highly significant increase in (G3) when compared to (G2) and (G1). When compared to control (G1), the mean value of serum TNF-β was found to be significantly higher in (G2) and (G3). In contrast, the mean value of serum TNF-β in G3 was significantly higher than in G2. In comparison to control (G1) , there was a significantly significant rise in TNFR-2 with (G2) and (G3). In addition a significantly substantial increase in (G3) as comparing to (G2) was found. The relationship between TNFR-2 and all parameters was investigated for all groups. For each group, the correlation coefficient was calculated. TNFR-2 was found to have a strong positive or negative connection. In comparison to G1 (control group)., the study found substantial changes (increase or decrease) in TNFR-2 in G2 and G3 (patient groups) .Furthermore, there are substantial changes in G3 findings when compared to G2. These findings suggested that these measures could be employed as biochemical indicators for detecting diabetes patients with rheumatoid arthritis. Furthermore, a substantial positive or negative correlation of TNFR-2 with all parameters for all groups revealed a good linked biomarker with these patients, indicating that the best medicine and therapy will be available.

Keywords: TNF-β, TNFR-2, rheumatoid arthritis, rheumatoid arthritis with T2DM.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease characterized by chronic synovial inflammation that causes cartilage and bone deterioration. Inflammation appears to be a major component in the onset and progression of diabetes, according to growing data. Systemic inflammation linked to RA may increase the chance of acquiring diabetes later in life. Other classic risk factors for type 2 diabetes mellitus (T2DM) are also common in patients with RA, and may contribute to the diabetes risk. Physical inactivity is widespread in persons with RA as a result of persistent pain, edema, and stiffness in the joints, which contributes to T2DM[1,2] .

Tumor necrosis factor (TNF), a pleiotropic cytokine produced mostly by activated macrophages, that affects a variety of biological actions in a number of tissues and cells, including cell proliferation, inflammation, carcinogenesis, viral replication, septic shock, and autoimmunity[3].and have an important role in the pathogenesis of various autoimmune disorders, including RA. Although TNF-β has been linked to autoimmune and inflammatory illnesses and is the closest homolog to TNF-α, there is little evidence that TNF-β has a function in RA[4]. TNF was discovered to increase cell proliferation in fibroblast-like synovial cells (FLS) from RA patients in a similar way to TNF-β. NF-κB was activated, and gene expression of IL-6, IL-8, and MMP-3 was stimulated. These findings add to the growing body of evidence supporting the therapeutic benefit of lowering TNF- in some RA patients[5].

The death receptor (tumour necrosis factor receptor) (TNFR) is a protein superfamily of cytokine receptors that has an external cysteine-rich domain that can bind tumor necrosis factors (TNFs)[6]. TNF receptors are found in a number of organs throughout mammals, particularly in leukocytes[7]. Although TNFR-2 has proinflammatory properties, it also has potent anti-inflammatory properties and protects oligodendrocytes, cardiomyocytes, and keratinocytes. TNFR-2 targeting, both stimulatory and inhibitory, has sparked great interest in the therapy of autoimmune disorders [8].

This study aimed to determine TNF-β, TNFR-2 in rheumatoid arthritis patients with and without diabetic type 2 and compare results with a control group in order to assess their use as biochemical markers for early diagnosis of rheumatoid arthritis. As well as to find a correlation of TNFR-2 with FBS, AST, ALT, ALP and TNF-β in all subjects.

SUBJECT & METHODS

All individuals in the current study aged from 35 to 55. Blood collecting between December (2020) to August (2020).Patients newly diagnosed (before therapy) by rheumatologists at the Baghdad teaching hospital and the Al- Yarmuk teaching hospital (2021). The participants were separated into three groups: group1 as control (30), group 2 as rheumatoid arthritis (30), and group 3 as rheumatoid arthritis with T2DM (30). All patients underwent a personal interview utilizing a specially prepared questionnaire format that covered a complete medical history as well as specific information. Each patient and control had four millilitres of venous blood drawn and immediately transferred to a transparent dry plain tube. The blood was allowed to clot for at least 10-15 minutes at room temperature after the needle was removed. After that, centrifuged for fifteen minutes at (3500 rpm). The serum is withdrawn and tested right away, then the samples are stored until the measurements are completed. The following biochemical tests were performed: serum TNF levels, serum TNFR-2 levels, fasting blood sugar (F.B.S), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline Phosphatase levels (ALP).

The data analysed using the SPSS-21 statistical program that was available (Statistical Packages for Social Sciences-version 21). The data was shown using simple standard error values. The Students' t-test was used to assess the significance of the difference between two independent means (quantitative data). Statistical significance was considered when the P-value was equal to or less than (0.05)[9]. The relation between two quantitative variables was obtained using Pearson's correlation, and the significance of the correlation was assessed using the t-test.

RESULTS & DISCUSSION

Data in Table-1 demonstrated TNF-β levels in rheumatoid arthritis (G2) (26.43±0.73) ng/L and (G3) (44.10± 1.27) ng/L which were a highly significant increased compared to serum TNF-β in (G1) (14.47±0.63). In addition a highly significant increased levels in (G3) comparing to (G2).

The TNF-β is a key regulator in the stimulation of the NF-κB signaling pathway and its controlled proteins involved in

inflammation, matrix disintegration, and mortality in human chondrocytes in vitro [10]. In the RA synovium, IL-6 and IL-8 are known to stimulate and recruit macrophages, whereas MMP3 contributes directly to RA joint destruction[11].Tumor necrosis factor-β was also detected in tissue sections of RA patients' synovial tissue [12]. The recent discovery that pre-treatment with a monoclonal anti-TNF-β in the collagen-induced arthritis mice model dramatically improved the disease outcome offered important evidence for TNF-β pro-inflammatory function in RA [13]. The discovery that TNF-β may act directly on RA FLS to increase the hyperplasic and inflammatory environment indicates that this cytokine may play a role in disease progression. The current study agreement with previous observations of elevated levels of TNF-β in RA patients [14].

Additionally, the present study demonstrated that TNF-β levels was significantly elevated in RA with T2DM patients. Increasing evidence shows that the innate immune system and proinflammatory cytokines are involved in pancreatic beta cell death and impaired insulin action in people with typical kinds of type 2 diabetes [15].

Evidence suggesting the innate immune system and proinflammatory cytokines are involved in the pathophysiology of type 2 diabetes and obesity supports the possibility that the TNF-β locus is implicated in the pathophysiology of type 2 diabetes and obesity [16].

Results of Table-1 revealed a highly significant elevation in TNFR-2 levels in (G2) (4.41± 0.13) ng/ml and (G3) (11.13± 0.14) ng/ml compared to control (G1) (1.70± 0.33) ng/ml , as well as a highly significant increase in TNFR2 levels in (G3) comparing to (G2) was found.

In the current study there were s a significant increase TNFR-2 levels in RA patients compared to healthy control. The results of this study agreement with previous study [17].TheTNFR-2 is primarily expressed on activated T cells in the immune system, and it is particularly important in the modulation of immunological responses via signaling in regulatory T cells (Tregs), a specialized immune modulatory lymphocyte subpopulation that suppresses the development of autoimmune disorders [18].

The TNF and TNF-β can be bound and neutralized by the TNFR-2 cystallizeble fragment fusion protein. TNF-β is equally effective as TNF in triggering RA FLS, according to Calmon et al, which is important evidence for the potential benefit of suppressing TNF-β in select groups of RA patients [19]. The level of TNFR-2 in body fluids is extremely important. A concentration that is too high may cause widespread cell death. Low TNFR-2 concentrations, caused by receptor shedding or other mechanisms, might be a compensatory strategy for reducing inflammation and ensuring normal T-cell selection. T cells do, in fact, shed sTNFR-2 through proteolytic cleavage of the TNFR-2 extracellular component. It binds to TNF in the extracellular fluid, decreasing the amount of TNF that may be bound by functioning T cells [20].

Table 1 : Mean ±SE of serum TNF-β and TNFR-2 levels in G1, G2 and G3

parameter	mean± SE			p-value		
	G1 (n=30)	G2 (n=30)	G3 (n=30)	G1&G2	G1&G3	G2&G3
TNF-β (ng/L)	14.47±0.63	26.43±0.73	44.10±1.27	HS**	HS**	HS**
TNFR2 (ng/ml)	1.70±0.33	4.41±1.33	11.13±0.14	HS**	HS**	HS**

**HS: Highly significant at the 0.01 level

The current study also included the determination of the levels of F.B.S .The results of F.B.S showed a non-significant elevation in (G2) (88.03±1.58) comparing to (G1) (89.43±1.20) while there are highly significant elevation in (G3) (216.57 ±5.34) comparing to (G2) and (G1). The findings are similar with those of Yeasmin et al,who showed significant differences in the mean of F.B.S between rheumatoid arthritis with T2DM and the control group[21]. Patients with RA had a higher risk of type 2 diabetes, according to previous research based on healthcare utilization data [22].

It's probable that the obesity-related confounding impact explains some of the link between rheumatoid arthritis and type 2 diabetes. Despite the fact that obesity is a well-known risk factor for type 2 diabetes [23]. Hyperlipidemia or hypertension raises the incidence of diabetes in RA patients, according to a study. Hyperlipidemia and hypertension together raise the risk of diabetes by 23 times. All of these risk factors are linked to a sedentary lifestyle [24].

In addition to inflammatory mediators released by adipose tissue, liver immune cells that produce pro-inflammatory cytokines. A number of these pro-inflammatory cytokines are important in the development of RA [25].

Data in Table 2 illustrate levels of serum liver enzyme AST, ALT and ALP in the three studied groups. Results showed a highly significant increase in AST levels in G2 and G3 comparing to G1, also there was a highly significant increase in G3 compared to G2. Moreover, there was a highly significant increase in mean of ALT levels in G2 and G3 comparing to G1 and there was a highly significant increase in G3 compared to G2.

Additionally, a highly significant elevation in serum ALP levels was noticed in G2, G3 compared to G1, also there was a significant increase in serum ALP levels in G3 compared to its mean in G2.

Table 2: Liver Enzyme Levels in G1, G2 and G3.

parameter	mean± SE			p-value		
	G1 (n=30)	G2 (n=30)	G3 (n=30)	G1&G2	G1&G3	G2&G3
S AST (U/L)	26.40±0.68	53.27±1.25	59.97±1.46	HS**	HS**	HS**
S ALT(U/L)	26.47±0.79	52.93±1.35	61.27±1.70	HS**	HS**	HS**
Serum ALP (U/L)	199.50±7.47	307.53±3.91	327.37±6.33	HS**	HS**	HS**

G1: Control, G2: Rheumatoid arthritis patients, G3: Rheumatoid arthritis with T2DM patients, **HS: highly significant

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been found to be useful indicators for measuring liver health, the existence of hepatic insulin resistance, and the risk of T2DM.

Results of this study agree with previous study [26]. That revealed a significant increase in AST and ALT levels in RA patients. Increased levels of liver enzymes Damage to liver cells, which increases the effectiveness of ALT/AST enzymes in the blood, may be due to the building of glycogen in liver cells as a result of fat accumulation, leading to cirrhosis of the liver, and also abnormal changes in hepatocytes, which lead to the release of liver enzymes in the blood. In addition to the effect of rheumatism therapy on AST and ALT levels, where a prior study found that MTX treatment increased AST and ALT levels in RA patients [27].

Elevated concentration of these enzymes might be owing to fatty acids' severe hepatotoxic action on hepatocytes, since they are generated in excess amounts as a result of chronic and relative insulin resistance. Aggregation of free fatty acids, high-concentration cell membrane disruption, mitochondrial malfunction, toxin production, and oxidative stress are all mechanisms that lead to a rise in pro-inflammatory cytokines such tissue necrotic factor [28].

Additionally, the results in Table 2 showed that ALP levels increase significantly between RA patients and control. Results were in agreement with other studies that showed increase in ALP in RA patients[29]. This study's finding of elevated ALP is comparable to that of Finzel et al., [30]. Osteoblastic activity, which indicates accelerated bone turnover, has been linked to an increase in ALP in rheumatoid arthritis. It is well understood that increasing disease activity causes more aggressive bone resorption. Concomitant bone growth and an increase in serum ALP accompany activated bone resorption. Because serum ALP corresponds with acute phase response, it might be elevated by inflammatory indicators like Interleukin-1 [31].

Crystallization is supported by the ALP, which catalyzes the synthesis of phosphate from pyrophosphate. Several investigations have found that blood ALP levels are significantly higher in people with RA, which might be due to a compensatory bone regeneration mechanism and osteoblastic proliferation aimed at restoring the damaged bones in the joints [32].

Serum TNFR-2 Correlation

Correlation relation of TNFR-2 were studied with all studied parameters.

Correlation coefficient (r) determined for all groups for all parameters as shown inTable-3.

Table-3: Correlation of TNFR-2 to clinical and biochemical parameters in G1, G2 and G3

Parameters		TNFR-2		
		r_1	r_2	r_3
F.B.S (mg/dL)	r	0.013	-0.202	-0.113
	P	0.945	0.284	0.551
AST (IU/L)	r	0.063	0.169	0.087
	P	0.74	0.373	0.647
ALT (IU/L)	r	-0.032	0.29	-0.068
	P	0.866	0.12	0.72
ALP (U/L)	r	0.079	-0.476**	-0.184
	P	0.68	0.008	0.331
TNF-β (ng/l)	r	-0.084	-0.184	0.135
	P	0.66	0.33	0.477

G1: Control, G2: Rheumatoid arthritis, G3: Rheumatoid arthritis with T2DM

*Correlation is significant at the 0.05 level ** Correlation is highly significant at the 0.01 level.

Results in Table-3 revealed a non- significant negative correlation between TNFR-2 and F.B.S in both G2 and G3 (r_2= -0.202), (r_3= 0.133), in contrast to G1 which showed a positive correlation (r_1= 0.013).

This study showed a non- significant positive correlation between TNFR-2 levels and ALT in all of three groups (r_1= 0.063) , (r_2= 0.169) and (r_3= 0.087) , as shown in Table 3. Also, there were a non-significant negative correlation between TNFR-2 and AST in both G1 and G3 groups (r_1= - 0.032) , (r_3= -0.068) respectively , while a positive correlation in G2 (r2= 0.169) .Results illustrated a non-significant negative correlation between TNFR-2 levels and ALP in G3 (r_3= - 0.184) in contrast to G1 which showed a positive correlation (r_1= 0.079).While a highly significant negative correlation was observed in G2 (r_2= - 0.476) .

The present study also, showed a negative correlation between TNFR-2 levels and TNF-β in both G1 and G2 ($_{r1}$= -0.084), (r_2= -0.064). While a positive correlation was found in G3 (r_3= 0.080).

CONCLUSIONS

Study concluded a significant changes (increase or decrease in TNF-β, TNFR-2, F.B.S, AST, ALT and ALP in G2 and G3 (patients groups) comparing to G1 (control group). In addition to, a significant differences in results in G3 comparing to G2. This results revealed that these parameters could be used as a biochemical markers for early diagnosis of diabetic in rheumatoid arthritis patient's .Also a significant positive or negative correlation of TNFR-2 with all parameters for all groups showed a good related biomarkers with these patients that optimal drug and treatment will be possible.

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