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ORIGINAL ARTICLE

Interleukin1β and Tumor necrosis factorα as Candidate Biomarkers for Discriminating Rheumatoid Arthritis in Iraqi Patients

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ABSTRACT

Background: *Rheumatoid arthritis (RA) is a systemic, Auto immune and inflammatory disease, which produces chronic synovial inflammation leading to progressive joint damage and functional deficit.* ***Objective:*** *The aim of this study was to determine the value serum IL-1β and TNF-α level for the diagnosis of RA in the Iraqi population.* ***Methodology:*** *75 patients with RA were compared with 75 age and sex matched healthy control, in a case control study. Serum from all subjects was obtained and the levels of IL-1β and TNF-α were determined by ELISA.* ***Results:*** *The serum levels of IL-1β and TNF-α were markedly elevated in RA patients compared with HCs (p7. 09 ng/mL, with a sensitivity of 72% and specificity of 89.3%, and a TNF-α cutoff value of >1578.26 ng/mL, with a sensitivity of 57.3% and specificity of 90.7%, for diagnosing RA. Cytokine levels were not significantly associated with the disease of study subjects or their gender (ps> 0.05).* ***Conclusion:*** *This results illustrates that serum concentrations of IL-1β and TNF-α in the Iraqi population can be considered as useful biomarkers for diagnosis of patients with RA.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disorder ,distinguished by persistent synovial inflammation resulting in relentless joint destruction, functional deficiency, and decreased quality of life[1,2]. Rheumatoid arthritis (RA) affects around 1% of the world's population, is more frequent in women, and onset is usually between the fourth and sixth decades of life, although RA can present at any age[3-6]. Nevertheless, it is generally believed that the disease results from a complex interaction between genetic susceptibility, environmental stimuli and abnormal immune responses[7,8]. Several loci have been identified by genetic association studies to be associated with an elevated risk for RA the most prominent being the human leukocyte antigen (HLA) [9]. Environmental factors including smoking, obesity, and specific viral infections have also been suggested as possible stimuli that can activate autoimmune responses in persons who are genetically predisposed to develop autoimmunity[10]. In the pathogenesis of RA, adaptive immune responses, such as the production of autoantibodies (e.g., rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs)) and infiltration of inflammatory cells (including T and B lymphocytes and macrophages), into the synovial membrane, are commonly found[11,12]. This inflammatory environment stimulates proliferation of the synovium, new vessel formation and the secretion of degradative enzymes that ultimately leads to the destruction of the cartilage, erosion of the bony structures and deformation of the joints[13,14]. The proinflammatory cytokines, interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are essential molecular mediators participating in the inflammation of RA[15]. IL-1β is involved in the initiation of the inflammation and maintenance through induction of prostaglandins, leukotrienes and Matrix MetalloProteinases (MMPs) , as well as in synovial fibroblast proliferation and in osteoclast differentiation, promoting joint destruction and bone erosion[16,17]. TNF-α is predominantly from activated macrophages and T-cells, and it, is a central proinflammatory molecule in RA that induces leukocyte recruitment and upregulates adhesion molecules and chemokines and also activates synovial fibroblasts to produce MMPs and other proinflammatory mediators[18,19]. The current study was designed to assess the circulating levels of IL-1β and TNF-α in Iraqi RA patients as diagnostic aid in confirming the diagnosis of RA.

METHODOLOGY

Patients

Seventy-five patients with RA based on the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria and 75 age- and sex-matched healthy controls

were included in this case-control study. The RA participants were aged from 20 to 72 years old and the referent participants were aged from 22 to 72 years old. Sex ratio: males/females ratio among the RA group and control group was 28/47 and 32/43, respectively. All patients were recruited from the unit of rheumatology at Al-Diwaniyah Teaching Hospital, Iraq from January to July 2024.

Ethical Approval

Approval for the study was obtained from the Institutional Ethics Committee of the Faculty of Science, University of Al-Qadisiyah according to Administrative order No.5 in 20\4\2025

Determination of Serum IL-1β and TNF-α

Peripheral vein blood was collected from all subjects. serum levels of IL-1β and TNF-α in serum were measured with commercially

available enzyme-linked immunosorbent assay (ELISA) kits (BT. LAB, China) as recommended by the manufacture with Catalogue number E0143Hu and E0082Hu respectively. These kits are based on the sandwich enzyme-linked immunosorbent assay technique. A capture antibody specific for IL-1β or TNF-α is pre-coated onto the wells. Samples and standards are added, and the target protein binds to the antibody. A biotin-conjugated detection antibody and streptavidin-HRP are then added, followed by a TMB substrate. The intensity of the color is proportional to the concentration of the target in the sample and is measured at 450 nm.

Statistical Analysis

Analysis was done with SPSS v25.0. Continuous variables are presented as median value with corresponding interquartile range and depending on the distribution compared with Independent Samples t-tests or Mann-Whitney U tests. Frequencies and proportions were used for categorical data and x2 test for analysis. ROC curve analysis was used to evaluate the diagnostic value of serum IL-1β and TNF-α in the discrimination of RA patients from healthy controls, and optimal cutoff values were obtained.

RESULTS

Median serum level of interleukin-1 beta (IL-1β) was significantly raised in patients with RA (8.33 ng/mL) compared to controls (4.33 ng/mL; p <0.001). Likewise, all RA patients significantly had higher TNF-α serums level (1614 ng/mL) versus healthy controls (1114.ng/mL; p < 0.001) Out of total study population a median of TNF-a serums level was acquired to be 1310. ng/ml. (Table 1).

Table 1: Comparison of results of serum IL-1β and TNF-α between patients and control groups

Characteristic	Patients group *n* = 75	Control group *n* = 75	*P*
IL-1β			
Median (IQR)	8.33 (3.46)	4.33 (3.80)	< 0.001 M ***
Range	3.85 -11.82	0.15 -9.97	
TNF-α			
Median (IQR)	1614.7 (688.15)	1114.7 (742.47)	< 0.001 M ***
Range	723.69 -2306.44	223.69 -1806.44	

IQR: inter-quartile range; **M**: Mann Whitney U test; ***: significant at $p \leq 0.001$

Diagnostic Efficacy of Serum IL-1β and TNF-α

Receiver operating characteristic (ROC) curve analysis revealed that the ideal cutoff value of serum IL-1β for diagnosing RA was 7.09 ng/mL with a sensitivity of 72% and specificity of 89.3% (area under the curve [AUC] = 0.881; P < 0.001; Figure 1). For serum TNF-α, highest accuracy was achieved with a cutoff value of 1578.26 ng/mL with a sensitivity of 57.3% and a specificity of 90.7% (AUC = 0.789; p < 0.001) (Figure 2 and Table 2).

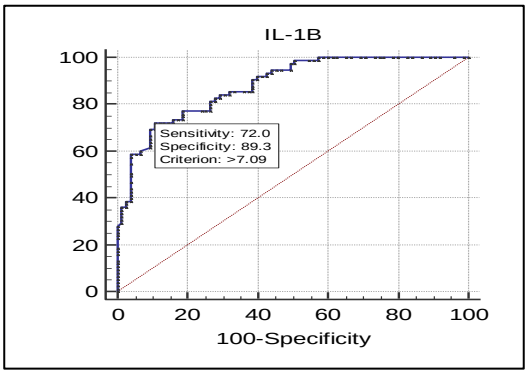

Fig.1: ROC curve of the diagnostic value of serum IL-1β levels in predicting rheumatoid arthritis, with sensitivity, specificity, and cutoff value.

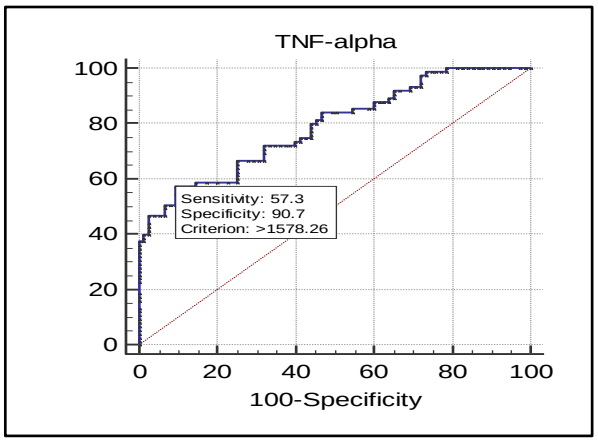

Fig.2: ROC curve showing the diagnostic accuracy of TNF-α in serum for the diagnosis of RA, with sensitivity, specificity, and corresponding cutoff value.

Table 2: The results of ROC curve analysis concerning the serum levels of IL-1 β and TNF-α

Characteristic	IL-1β (ng/ml)	TNF-α(ng/ml)
Cutoff value	>7.09	>1578.26
AUC	0.881	0.789
95 % CI	0.818 to 0.928	0.715 to 0.852
P	<0.001 ***	<0.001 ***
Sensitivity %	72.0	57.3
Specificity %	89.3	90.7
Accuracy %	88.1	78.9

AUC: area under curve; **CI**: confidence interval; **IL-1β**: interleukin-1 beta; **TNF-α**: tumor necrosis factor-alpha; ***: significant at $p \leq 0.001$.

The correlations of IL-1β and TNF-α to age and sex in patients group and control group are shown in table 3. It is obvious that none of these correlations was significant ($p > 0.05$).

Table 3: The correlation of IL-1β and TNF-α to age and sex in patients and control group

Characteristic	Correlation index	Patients group		Control group	
		Age	Sex	Age	Sex
IL-1β	*R*	0.034	-0.225	-0.117	0.048
	P	0.773	0.052	0.319	0.683
TNF-α	*R*	0.034	-0.225	0.103	0.091
	P	0.773	0.052	0.381	0.438

r **:** Correlation coefficient *p***:** *P value*

DISCUSSION

The current study showed that IL-1β and TNF-α levels are significantly higher in sera of Iraqi patients with rheumatoid arthritis (RA) compared to healthy individuals. These findings are consistent with the known involvement of these pro-inflammatory cytokines in the pathophysiology of RA[20]. The elevated level of IL-1β in sera of RA patients agrees with previous findings which suggest its central role in synovial inflammation, cartilage destruction and bone loss[21]. Blockade of IL-1 signaling has been studied as a therapeutic strategy and IL-1 receptor antagonists have shown promising clinical results[22,23]. Likewise, the high serum levels of TNF-α yielded in the present investigation also support the pivotal importance of that cytokine in the RA inflammatory pathway. TNF-α drives disease through the recruitment and activation of immune cells—including T cells and macrophages—and through the production of other pro-inflammatory mediators, which ultimately results in joint destruction[24,25]. ROC was used to analyze the diagnostic value of IL-1β and TNF-α (IL-1β>7.09 ng/mL and TNF-α>1578.26 ng/mL). There were high sensitivity and specificity with these cutoffs, which would be valuable for diagnostic biomarkers in clinical[26,27]. These results are in accordance with studies that have examined the diagnostic utility of IL-1β and TNF-α in RA[28]. Additionally, the lack of correlation between cytokine concentrations and demographic factors such as age and gender imply that IL-1β and TNF-α may be reliable diagnostic biomarkers irrespective of patient specific, characteristics[29]. The major advantages of this study are the recruitment of a well-characterized RA cohort, the presence of an age- and sex-matched healthy control group, and the use of strict statistical analyses[30,31]. There are some limitations. As a cross-sectional study, causality cannot be inferred with respect to cytokine levels and the development of RA. Moreover, the single-center nature of the study might decrease the external validity of the present results to the entire Iraqi population[32,33]. However, the current study provides important information about the inflammatory profile of RA in this population and highlights the fact that IL-1β and TNF-α could be clinically relevant biomarkers for diagnosis and management–a result that is in line with previous literature[34,35].

CONCLUSION

In the current study, the data that show an association between high serum levels of IL-1β and TNF-α to RA in an Iraqi population were described. The diagnostic value of these cytokines suggests that they could be used as effective diagnostic biomarkers of RA, which is important for early detection and intervention, and to minimize the risk of long-term joint damage.

These findings remain to be validated by additional studies with a focus on signaling intermediates, highlighting the therapeutic potential of targeting pro-inflammatory mediators, along with the significance of these biomarkers in tracking disease progression and evaluating treatment response.

Conflict of interest

The authors insured there was no conflict of interest in this study.

Patient Declarations

All patients in the study (including controls for blood sampling from healthy individuals) provided written consent. This research was completed with great cooperation between them.

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