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By Duha Adil Jasim

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Role the (IL1, IL6, and TNF) with chronic autoimmune diseases in Mosul City, Iraq

Duha Adil Jasim*, Anmar A. Al Taie

Department of Biology, College of Science, University of Mosul, Iraq

duha.23scp70@student.uomosul.edu.iq

ABSTRACT

Objective. Our study examined the relationship of the levels of cytokines IL1, 6, and TNF in three different immune diseases with age and sex.

Material and methods. The samples of the study were obtained from in Mosul city hospitals for the period from October 2023 to February 29, 2024. The study included 90 different medical cases suffering from immune diseases, 50 of whom were suffering from rheumatoid arthritis, 16 were suffering from erythematosus, and 24 were suffering from Celiac disease. The ages of the study participants were between 10 years and 65 years.

Outcomes. Serum samples were examined for IL1, 6, and TNF using the ELISA technique. The results of ELISA revealed that Concentrations of Interleukins and TNF were low in all patients because the patients in the chronic phase of Autoimmune Diseases take treatment to reduce the cytokines which is hyperactive Therefore, cytokines concentrations appeared lower than standard concentrations. However, the results show a significant association between measuring IL-1 for a CD and measuring IL-6, TNF for an SLE among Autoimmune disease patients and their sex, and age.

Conclusion. Significance and impact of study: IL1, 6, and TNF are one of the most common cytokines on the globe. It has been connected directly or indirectly with various disorders, including autoimmune diseases. However, the link between IL1, 6, TNF and Autoimmune Diseases is still controversial. Our results indicate that they play a possible role in the development of autoimmune diseases; however, more studies on a larger scale are required to confirm the results.

Keywords: ELISA, Autoimmune diseases (SLE, RA, CD), Cytokines (IL1, 6, and TNF)

Introduction

The immune system's principal role is to defend the body from infectious agents such as viruses and bacteria. The body's cells and molecules do not often induce an immune response due to several mechanisms that promote self-tolerance [1]. Autoimmunity is a situation in which the body's structures are attacked as if they were foreign [2]. A collection of complex illnesses with unclear etiologies is known as autoimmune diseases. Although certain autoimmune diseases may present clinically similarly, each autoimmune disease has its unique characteristics. For example, people patients with RA are mostly affected by polyarthritis of the hand joints, while the extra-articular major organ, such as the kidney, is less commonly affected. Unlike RA, patients with SLE may have multiple organ involvement due to the overproduction of various kinds of autoantibodies and the deposition of antibody-antigen immune complexes in numerous organs, such as the kidney, resulting in organ damage. Therefore, although oligo arthritis may appear in limited populations of people with Inflammatory Bowel Disease (IBD), the major manifestation of IBD is chronic intestinal inflammation [3]. Autoimmune illnesses are caused by a lack Having tolerance towards self (i.e., the immune system's inability to recognize differences between self and non-self) and are characterized

by autoantibody production and hyper activation of T cells, resulting in organ damage. Thus, autoimmune diseases can be classified as either organ-specific or systemic [4]. The immune system is in the role of recognizing and carrying out appropriate reactions to remove non-self-antigens and prevent a damaging response to self-antigens, known as immunological tolerance. Immune tolerance is defined as the immune system's inability to respond to particular substances or tissues that would typically trigger an immunological response. Autoimmunity is caused by the failure or breakdown of tolerance, which is necessary for normal immunological balance [5]. Autoimmune illness occurs after More immune system problems in both innate and adaptive immune systems [6]. Microbial antigens, external antigens, and cytokine dysregulation can all cause self-reactive cells. In addition, hyper activation of T and B cells can occur along with a change in the duration and quality of their response, affecting the immune system's homeostasis [6].

Lymphocytes, macrophages, natural killer (NK) cells, mast cells, and stromal cells release cytokines, which are soluble proteins with low molecular weight. Show Figure 2-1 They play a crucial role as mediators in the immune system's communication network [4]. Cytokines can be classified into several types based on their function, TNFs, interleukins (ILs), lymphocytes, monokines, IFNs, and transforming growth factors. Cytokines can be classified as pro- or anti-inflammatory. Pro-inflammatory cytokines contribute to the initiation and propagation of autoimmune inflammation, while anti-inflammatory cytokines enable inflammation regression and recovery from the acute phases of the autoimmune disease [4]. Cytokines play an important role in the pathophysiology of autoimmune disorders by regulating inflammation and immunity, immune cell differentiation, and self-tolerance breakdown, serving as potential biomarkers for diagnosis and treatment [7]. Cytokines play a significant role in the creation and

progression of autoimmune diseases by contributing to tissue-specific inflammation, immune dysregulation, and chronic inflammation [8]. Cytokines play a crucial role in autoimmune diseases like SLE by contributing to self-tolerance failure, autoantibody production, inflammation perpetuation, and tissue damage promotion throughout disease progression [9].

MATERIALS AND METHODS

Studying Groups

Samples of serum were collected from 90 Autoimmune Disease groups of patients (females and males) from private laboratories and Ibn-Sina, Al-salam teaching hospitals in Mosul city over 4 months from October 2023 to 29 of February 2024. They involved 50 cases of Rheumatoid Arthritis (RA), 16 cases of Systemic Lupus Erythematous (SLE), and 24 cases of Celiac Disease (CD) of ages ranging from (10-65) years. All the patients were diagnosed based on clinical indications, symptoms, and Laboratory diagnosis by using the immune technique. The patients were tested for Cytokines detection as a biomarker using an ELISA-specific antigen kit (IL 1, IL6, and TNF).

Blood sample collecting

Each patient provided 2.5 ml of fresh blood, which was collected in a gel tube. The serum was then obtained by centrifuging the sample at 3000 rpm for 3 minutes, divided into 2-3 aliquots in sterile Eppendorf's, and stored at -20 ◦C.

Cytokines detection by ELISA:

IL1, IL6, and TNF ELISA kits from SUNLONG (Biotech Co., LTD) were used, according to the manufacturer's manual. Then the results were recorded as absorbance A0 at 450 nm using a Micro Titer Plate Reader.

Outcomes

Serologically IL1, IL6, and TNF were evaluated in Autoimmune Diseases (n = 90) groups of patients SLE, RA, and CD by ELISA technique. It was clear that the results of ELISA revealed that Concentrations of interleukins were low in all patients as shown in Table 1.

Table (1): Number of patients whom were positive according to Standard values for IL1, 6 and TNF levels under study

IL / diseases	RA	SLE	CD	Total
IL1 < 180.3 U/mL	50	16	24	90
IL6 < 60.0 u/mL	50	16	24	
TNF < 480 ng/L	50	16	24	

Note the standard value Concentration about IL1 is 180.3 U/mL as IL6 is 60.0 U/mL and TNF is 480 ng/L.

It has been done a comparison was made between male and female patients for each of the three parameters in each disease by Using statistical methods as shown in Table 2.

Table (2): Shows the comparison between male and female patients in each IL1,6 and TNF

Type of diseases	Cytokines	Test	Sample	Median	Statistic	P-value
RA	IL-1 concentration u/ml	Independent-Samples Median Test	Male	95.25	0.442	0.710
			Female	90.50		
	IL-6 concentration u/ml		Male	17.75	0.036	0.917
			Female	18.00		
	TNF concentration ng/l		Male	125	2.815	0.166
			Female	138		
SLE	IL-1 concentration u/ml		Male	93.50	1.485	0.311
			Female	87.00		
	IL-6 concentration u/ml		Male	20.50	1.485	0.311
			Female	15.00		

	TNF concentration ng/l		Male	477	1.485	0.311
			Female	128		
CD	IL-1 concentration u/ml		Male	88.25	7.535	0.010
			Female	84.00		
	IL-6 concentration u/ml		Male	19.00	3.948	0.067
			Female	13.50		
	TNF concentration ng/l		Male	160.50	0.829	0.462
			Female	130		

Also, it was completed Comparison between age groups in patient samples for each of the three parameters in each disease Using statistical methods as shown in Table 3.

Table (3): Show Comparison between age groups in patient samples for each of IL1, 6 and TNF.

Type of diseases	Cytokines	Age	Median	Statistic	P- value
RA	IL-1 CONCENTRATION u/ml	10- 25	87.75	5.008	0.286
		26-35	99.50		
		36-45	90.50		
		46-55	94.50		
		56-65	133.00		
	IL-6 CONCENTRATION u/ml	10-25	18.00	3.046	0.550
		26-35	19.75		
		36-45	18.50		
		46-55	17.50		
		56-65	27.50		
	TNF CONCENTRATION ng/l	10-25	137.50	3.584	0.465
		26-35	146.00		
		36-45	123.00		
		46-55	140.00		
		56-65	800.00		
SLE	IL-1 CONCENTRATION u/ml	10-25	81.25	8.973	0.062
		26-35	97.50		
		36-45	85.25		
		46-55	87.00		
		56-65	93.50		
	IL-6 CONCENTRATION u/ml	10-25	14.50	14.986	0.005
		26-35	12.50		
		36-45	19.00		
		46-55	15.00		
		56-65	20.50		
	TNF CONCENTRATION ng/l	10-25	94.50	9.307	0.054
		26-35	106.50		
		36-45	164.00		
		46-55	123.00		

		56-65	486.00		
CD	IL-1 CONCENTRATION u/ml	10-25	88.75	2.965	0.397
		26-35	85.50		
		36-45	85.25		
		46-55	79.50		
		56-65	-		
	IL-6 CONCENTRATION u/ml	10-25	19.00	1.565	0.667
		26-35	15.50		
		36-45	13.75		
		46-55	14.00		
		56-65	-		
	TNF CONCENTRATION ng/l	10-25	578.00	9.836	0.020
		26-35	160.00		
		36-45	109.50		
		46-55	121.00		
		56-65	-		

DISCUSSION:

This study focused on Autoimmune Disease patients in Mosul city. Autoimmune Diseases are usually presented in three categories, Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Celiac Diseases (CD) cases as used in this study, the results of ELISA revealed that Concentrations of Interleukins and TNF were low in all patients as shown in table 1 because of the patients in chronic phase of the Autoimmune Diseases so they take treatment to reduce the cytokines Which its hyper Therefore, cytokines concentrations appeared lower than standard concentrations, This matches the results of research conducted on patients with autoimmune diseases whereas In individuals with RA, blood concentrations of IL-6 family cytokines were considerably raised and lowered with medical treatment [10]. The [11]. showed that IL-6 concentrations decreased significantly in responders to infliximab therapy in CD, this matches the results of my research. In addition, a Methotrexate (MT) treatment for individuals with RA improves the sustained elimination of clinical illness symptoms and inhibits the production of pro-inflammatory cytokines: TNF, IL-1 and IL-6 [12]. Like decreases in serum levels of TNF, IL-6, and IL-1 after add-on hydroxyl-chloroquine treatment in SLE patients

[13]. However, other research contradicted this result, as it was conducted on patients with autoimmune disease without treatment it demonstrates that the only effective treatment is to take a gluten-free diet (GFD)., albeit Complete adherence is difficult to keep up, and inadvertent gluten exposures are unavoidable for most patients. As a result, there is significant Interested in drug research in CD, and many potential therapeutics are under evaluation [14]. Testing the differences between the three diseases [(RA), (SLE), (Celiac)] for each of the three parameters [(IL-1 Concentration U/mL), (IL-6 Concentration U/mL), (TNF Concentration ng/L)] for every from male and female using statistical methods as shown in table 2 where it was found Significant differences between male and female in measuring IL-1 For a CD only, this is indicative of p-value to test Independent-sample median which reached 0.010 and it is less than 0.05 It has been demonstrated that the IL-1 ligand is more closely associated with acute and chronic inflammation than any other cytokine family. It was discovered that a one-unit increase in IL-1 serum increases the incidence of CD by 1.13 times [15]. When we carried out a in a meta-analysis, we found that women had a higher risk for celiac disease than men did in an undiagnosed population (Randomly) [16].

Testing the differences between the three diseases [(RA), (SLE), (Celiac)] for each of the three parameters [(IL-1 Concentration U/mL), (IL-6 Concentration U/mL), (TNF Concentration ng/L)] for ages using statistical methods as shown in table 3, where it was found Significant differences between ages in measuring IL-6 For an SLE, this is indicative of p-value to test Independent-sample median which reached 0.005 and it is less than 0.05, also found Significant differences between ages in measuring TNF For a CD, this is indicative of p-value to test Independent-sample median which reached 0.020 and it is less than 0.05.

When searching, the risk of Autoimmune Diseases is significantly higher in females between (40 and 65) years more likely than the males this matches Kronzer and others in 2021 According to reports, females have a significantly higher risk of developing autoimmune disorders than males, which is attributable to hormonal, genetic, environmental, and ages [17].

Serum interleukin-6 levels are A meta-analysis of serum IL-6 levels in SLE patients and healthy controls found that serum IL-6 levels were considerably higher in SLE patients compared to healthy controls [18].

Late-onset systemic lupus erythematosus (SLE), with an increased after the age of 50, has been associated with a different prevalence of clinical symptoms, disease activity, and mortality than early-onset SLE [19].

TNF contributes to Celiac Disease by promoting a pro-inflammatory environment, leading to increasing Gluten intolerance and other pro-inflammatory agents [20]. Celiac disease presentation varies with age; children show digestive symptoms, while adults exhibit extra digestive manifestations like anemia and Weight loss [21].

Conclusion

This study aimed to investigate the Cytokines (IL1, 6, and TNF) among 89 chronic Autoimmune Diseases patients (49 cases of Rheumatoid Arthritis (RA), 16 cases of Systemic Lupus Erythematous(SLE), and 24 cases of Celiac Disease (CD) by ELIZA technique of ages ranging from (10-65) years at the hospitals teaching in Mosul city/Iraq. The results indicated that Concentrations of interleukins were low in all patients. Then it has been done a comparison was made between males and females and also between age groups of patients for each of the three parameters in each disease by using statistical methods it was found Significant differences between males and females in measuring IL-1 For a CD and between ages in

measuring IL-6, TNF for a SLE. These results may suggest that IL1, 6, and TNF have a potential role in the development of Autoimmune diseases, however, more studies on a larger scale are needed.

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