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Correlation of TNF-a with selective Anti Neutrophil cytoplasmic antigens (Anti PR3&Anti MPO) in patients with Behçet's disease in Kerbala province

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Abstract

Behçet's disease (BD) is a disorder characterized by inflammation of many parts of the human body and have many symptoms like sores of genital and moth ,inflammation of eyes and also arthritis. Tumor necrosis factor (TNF)-α is an important cytokine play a role in inflammation and immunity of different types of disease. Anti-neutrophil cytoplasmic antibodies (ANCA) have many types called elastase, lysozyme, cathepsin G, lactoferrin and also BPI)these may be detected in patients with Behçet's diease This study aimed to demonstrated if there is any relation between TNF-a and different types of ANCA and also it levels in Iraqi population /Kerbala province. Samples of peripheral blood were collected from 20patients with Behçet's disease and 20 apparently healthy controls. The TNF-a and ANCA were detected by using ELIZA assay .The TNF-a give statically significant difference in BD when camper to control group ,while the MPO and PR3 not give statistically significant difference. There is a relation between increase levels of TNF-a and PR3 but not with MPO in patients with BD.

Keywords: *Behcet disease , TNF-a, ANCA*

INTRODUCTION

Behcets disease a disorder characterized by inflammation of many parts of the human body and have many symptoms like sores of genital and moth ,inflammation of eyes and also arthritis. [1] Which consider as one of vasculitis disease ,and one of rare disease occur the silk road (3).also consider as disease effect different type of body system ,have mean symptoms oral aphthous ulcer with recurrent episode ,lesion of skin and eyes ,genital ulcer ,joint arthritis with no deformity ,lesion of gastrointestinal tract ,epididymitis ,vascular and nervous system lesion.(4).The major inflammatory cytokine produce in BD and play central role in its pathogenesis are Tumor necrosis factor –a(TNF-a) . Tumor necrosis factor (TNF)-α is an important cytokine play a role in inflammation and immunity of different types of disease.[2]The products of neutrophils and its self may be considered basic players in development in response to autoimmune and tissue destruction of vasculitis and also granulomatous inflammation[5].Anti-neutrophil cytoplasmic autoantibodies may be consider as principle cause of vasculitis that due to necrotizing granulomatosis [6]. The classification of ANCA-Associated Vasculitis involved both the the clinic pathological phenotype and specificity of ANCA antigen, for example MPO-ANCA and PR3-ANCA [7]. ANCA are markers with many sub-sate of patients with primary systemic vasculitis. The patients having vasculitis, there are two major antigens of ANCA present in the primary granules of neutrophils: serine proteinase 3 (PR3) and myeloperoxidase (MPO) . At least the human neutrophils contain at least three types of granules, each of which contains a variety of constituent proteins: azurophilic granules [PR3, MPO, bactericidal permeability increasing protein (BPI), elastase (Elast), cathepsin G (Cath G)]; secondary granules [lactoferrin (LF), lysozyme]; and tertiary granules (gelatinase) . Antigens within any of these granules are potential targets for an ANCA response .(8,9)

MATERIALS AND METHODS

Subjects and study design

A total of 20 cases of behcets disease were included in this study, their mean age (17 - 35years old) including 12 male and 8 female**.** The patients were complained of a skin lesion in different part of the body mostly in the mouth, genital, and eyes . Cases diagnosed clinically by a special dermatologist, immunologist and confirmed as behcets disease patients based on clinical symptoms and laboratory diagnosis. Control group included 20 apparently healthy individuals were used in this study, their mean age (18-30years old) including 12 males and 8 females.

Sample collection and assay procedure

Blood sample (5ml) was collected left at room temperature and then centrifuge for 15 min. at (3000 RPM). Serum was then separated and freezed until time of analysis. Estimation of TNFα ELISA kit (Cusabio/China),the ANCA estimated by ELISA assay(Euro immune) and in serum using commercially available and performed as recommended in leaflet with kits

Statistical analysis:- The result was analyzed by using the statistical software package SPSS 23. The probability of ($P \leq 0.05$) was considered in statistically significant. [16].

RESULTS

In total, 20 BD cases and 20 controls were diagnosed in this study and determined the clinical symptoms of BD patients in association to determine the ANCA tests for all patients and study the relation between these symptom and ANCA illustrated in table(1)

In table number two the patients divided to three groups according to the age ,the highly percent of patients occur at age group range between 20-29 years and the positive result for PR3and MPO also represented highly percent in these group.

Table number three show levels of Tumor Necrosis Factor–a give highly significant difference between BD and healthy cases ,While there are non-significant difference for PR3 and MPO between these group.

Table 1: The Clinical manifestation of patients with BD.

	+ANCA N(%)	- ANCA N(%)	P-Value
Oral aphthous ulcer	8(40)	12(60)	<0.01
Lesion of skin	6(30)	1(5)	<0.01
Lesion of eyes	5(2.5)	2(10)	<0.01
Genital ulcer	9(45)	3(15)	<0.01
Arthritis	4(20)	4(20)	>0.05
Nervous ulcer	7(35)	0(0)	<0.01

Table 2: Distribution of patients and healthy cases according to the age and tests for PR3&MPO.

Patients age (years)	Number	Percent	MPO		PR3	
			+Ve N(%)	-Ve N(%)	+Ve N (%)	-Ve N (%)
20-29	9	45%	1(5)	9(100)	1(5)	9(100)
30-39	7	35%	0 (0)	9(100)	1(5)	9(100)
40-49	4	20%	0(0)	9(100)	0 (0)	9(100)

Table 3: The comparison of mean levels of three selected parameters (TNF-a ,PR3&MPO) between BD and healthy group.

	Healthy group (Mean± SD)	BD group (Mean ±SD)	P-Value
TNF-a (Pg/ml)	44.5±11.6	185.0±50.30	0.01
Anti-PR3 (IU/ml)	0.18±0.11	0.43±0.21	0.08
Anti-MPO (IU/ml)	0.21±0.12	0.67±0.33	0.9

Table 4: The mean levels of PR3 ,MPO in patients with normal and high levels of TNF-a

	BD with Normal levels of TNF-a (Mean±SD)	BD with High levels of TNF-a (Mean±SD)	P-Value
Anti-PR3(IU\ml)	1.10±0.22	0.23±0.43	0.04
Anti-MPO (IU/ml)	1.6±0.110	0.31±0.14	0.06

In table number four the mean levels of anti-PR3give significant difference between patents with high levels of TNF-a and Patients with normal levels ,but the MPO not give any significant between these groups .

DISCUSSION

In this study ,we analyzed the mean levels of TNF-a and different types of ANCA in serum of patients with behcets disease, ANCA are an autoantibodies especially IgG play role against different antigens inside the cytoplasm of neutrophil granulocyte , these Ags can detected in blood of a number of autoimmune disease [10], generation of these autoantibodies usually obscure ,but the possibility may be due to immunological cross reactivity due to microbial super antigens these process called molecular mimicry by microorganisms have ability to stimulate a high immune response [11]. The role of ANCA in pathogenesis may be still controversial also the ideas represented these antibodies play a direct pathological role to formation of these inflammation. MPO(myeloperoxidase) and proteinase-3 PR3 have ability to activate the neutrophils ,these activation increased by many cytokines which stimulate the neutrophils to express MPO and PR3 on its surface. The activated neutrophil have ability to adhere to endothelial cells and then release free oxygen radicals and also lytic enzymes and finally causes damage of tissue by activation of necrosis and apoptosis.[12]. In this the TNF-a give statistically significant difference in BD when camper with healthy cases and the PR3 give association with increased levels of TNF-a, that explain the role of TNF-a in pathogenesis of disease the neutrophil activation induced by ANCA is highly enhanced by TNF-a ,and that lead to an increase production and release of oxygen radicals and granules with toxic constituents. [13]There are uncertain mechanism for this effect but many study recorded the incubation of TNF-a with neutrophil caused increase presence PR3 and or MPO on the outer membrane of neutrophil.[14] that causes increase of Ags of ANCA for binding with autoantibodies .More over the TNF-a have ability to causes up-regulation of different types of molecules that play a role in adhesion of neutrophil to endothelium. In *vitro*, TNF-a make endothelial cells after pretreatment with it more susceptible to distraction. Finally the mechanism of TNF-a and ANCA to gather causes activation of neutrophils ,attachment to endothelium ,release of oxygen radical and toxic granule , all that causes vascular damage.[15]

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