Serum Levels of Tumor Necrosis Factor-alpha, Nitric oxide and Malondialdehyde in Patients with Behcet's Disease

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ABSTRACT

Objective: *To determine the serum levels of tumour necrosis factor-alpha (TNF α), nitric oxide (NO) and Malondialdehyde (MDA) in patients with Behcet's disease (BD). This study included 27 patients with Behcet's disease and 16 healthy control subjects. Serum (TNF-α) was measured by an enzyme linked immunosorbent assay (ELISA) while serum NO oxide levels were determined by Griess reaction. The MDA levels were detected by thiobarbituric acid reaction. There was a significant increase in the levels of TNF-α , NO and MDA in Behcet's disease patients compared to controls. No significant correlation was detected between TNF-α and NO or MDA levels in patients or controls. A significant positive correlation was detected between serum levels of NO and MDA in BD patients. This suggests that elevated levels of TNF-α , NO and MDA may be related to the pathogenesis of Behcet's disease.*

INTRODUCTION

Behcet's disease (BD) is characterized by oral aphthous lesions, genital ulceration and eye inflammation[1] Ocular manifestation is associated with a sever prognosis in BD and it leads to blindness in 15-25% of patients with ocular disease.The principal cause of visual loss being consecutive inflammatory ischaemic retinal vein occlusions and macular edema. Behcet's disease is a systemic inflammatory vasculitis of young adults with unknown etiology, characterized by endothelial dysfunction and occlusion in both deep venous and retinal circulation. Ocular involvement occurs in 70% of cases and is characterized by periphlebitis, periarteritis, vascular occlusion, and thrombosis leading to blindness despite vigorous treatment[2].

Despite the diverse inflictions in different organ systems, vasculitis is perceived as the common basic pathological process in BD [3].

The exact cause is unclear but viral, genetic, immunological and environmental factors have been implicated in the pathogenesis of BD[4].

Behcet's disease is considered as an autoimmune disease, since the activation of immune system, pro-inflammatory cytokines and mediators may affect the course of the disease [5]. Cytokines are proteins which are produced by various cell types, are important mediators of immuno-inflammatory reactions[6]. One such regulating cytokine is tumour necrosis factor (TNF)-alpha, which exerts

multiple stimulatory effects on T cells by binding to specific receptors and increase the expression of human leukocyte antigens[7].

Nitric oxide (NO) is an organic-free radical gas produced in the vascular endothelium by nitric oxide synthase (NOS) isoenzyme using L arginine as substrate[8]. Two isoforms of NOS have been clearly described. The first one is inducible NOS (iNOS); it is induced in macrophages and liver cells by endotoxin and cytokines[9]. The second form is constitutive NOS (cNOS), which is dependant on calcium and calmodulin. cNOS releases NO physiologically in the regulation of many cell functions and communication[10]. The (i NOS) synthesizes NO in greater amounts and it is implicated in the pathogenesis of numerous inflammatory and autoimmune diseases[11]. The origin of Behcet's disease (BD) is unclear. One of the prominent features of BD is vasculitis and thrombosis as a result of endothelial dysfunction[12]. Thrombosis is frequently seen in BD. The Major factor responsible for increased frequency of thrombosis is thought to be endothelial dysfunction[13]. Releasing NO by the endothelium promotes vasodilatation and inhibits inflammation, thrombosis, and vascular smooth muscle proliferation[14].

Malondialdehyde (MDA), one of end products of lipid peroxidation, is induced by reactive oxygen species, and is a marker of oxidative stress and T cell activation[15].

The aim of this study was to determine the serum TNF-alpha, NO and MDA levels, as well as their correlations with each other in patients with BD.

MATERIALS & METHODS

A total of 27 patients with ocular BD attended Behcet's disease clinic and 16 age and sex matched healthy control subjects were included in the present study. All BD patients fulfilled the criteria of the International Study Group for Behcet's Disease. Patients' history was obtained from case notes and ocular examinations were performed. In particular, a history of systemic thrombosis and evidence for retinal vascular occlusion was examined. Where the posterior segment could not be visualized, patients with an end stage ocular disease were assumed to have suffered vaso-occlusive disease of the retina.

Blood samples: Fasting blood samples (totally 10 ml) were drawn using a 25 gauge needle from a peripheral vein, avoiding haemolysis, into plain tubes. None of the patients and controls had received any topical or systemic medication at least two weeks before blood collection. Following an immediate centrifugation of the blood samples for 10 minutes at 4°C, serum was collected and kept at −70°C until use.

Determination of TNF-α**:** by an enzyme linked immunosorbent assay (ELISA)[16].

Determination of nitric oxide levels by spectrophotometric method. Total nitrite (nitrite NO_2 + reduced nitrate NO_3) analysis by Griess reagents for use in the determination of nitrite (NO_2) as an indicator of NO production in plasma. NO has brief

half life and is rapidly converted to the stable end products NO_2 and NO_3. Nitrate was measured as nitrite after enzymatic conversion by nitrate reductase. Briefly samples were mixed with 1 gm/100 ml sulfanilamide in 2.5% phosphoric acid and 0.5 gm/100 ml naphthyl–ethylenediamine in 2.5% phosphoric acid which was allowed to react at room temperature for 10 minutes. The concentration was determined by measuring absorbance at 530 nm in comparison with standard solutions of sodium nitrite at concentrations of 3.12, 6.25, 12.5, 25, 50 and 100 µmol/L with Griess reagent [17].

Determination of MDA levels by a method based on the reaction with thiobarbituric acid (TBA) at 90-100 C. In TBA test reaction, MDA or MDA like substances and TBA will react together to produce a pink pigment having an absorption maximum at 532 nm [18].

Statistical analysis:

Data was expressed as mean ± SD. The two groups were compared using the Anova; single factor test .The degree of association between the variables was assessed using Pearson's correlation coefficient (r), where values of $p < 0.05$ were considered significant.

RESULTS

Clinical data of the controls and BD subjects were summarized in table (I).

Table (II) shows mean ± SD of TNF-alpha, NO and MDA in controls and BD patients.

TNF levels in the serum of BD patients was (31.5±6.7 pg/ml) significantly higher than controls (12.9±2.9 pg/ml) ($p < 0.001$). Also, the mean serum concentration of NO was significantly elevated in patients with BD compared to the corresponding level in controls (23.3±2.5 µmol/L) and (30±3.1 µmol/L) respectively ($p < 0.05$).

The mean serum level of MDA in BD was 15.6±2.5 µmol/L which is significantly higher than controls 5.04±1.3 µmol/L ($p < 0.001$).

No significant positive correlation between TNF-alpha and NO levels and MDA levels $r = 0.2$ ($p > 0.05$). Meanwhile, there was a significant correlation between MDA and NO $r = 0.47$ ($p < 0.05$). Fig. 1

Table (1): **Clinical characteristics of the controls and BD group**

	Controls	***BD***
- Number	16	27
- Sex (M/F)	10/6	15/12
- Age (years)	37.6 ± 9	37.4 ± 10
- Duration of disease (years)	-	7.6 ± 6.4

Table (2): **Mean ± SD of TNF-α, NO and MDA levels in serum of controls and BD patients**

	Controls	*BD*	*p value*
- **TNF-α** (pg/ml)	12.9 ± 2.9	31.5 ± 6.7	p < 0.001
- **NO** (μmol/L)	23.3 ± 2.5	30 ± 3.1	p < 0.05
- **MDA** (μmol/L)	5.04± 1.3	15.6 ± 2.5	p < 0.001

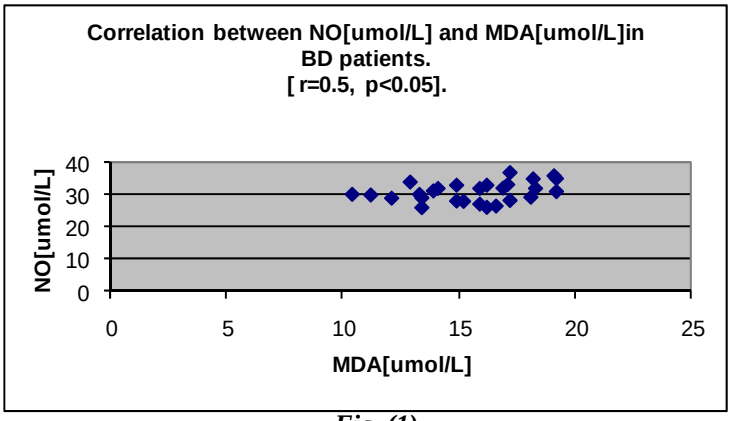

Fig. (1)

DISCUSSION

Behcet's disease is a chronic multisystemic disorder characterized by relapsing inflammatory activation.[19] Although the aetiopathogenesis of the disease has not yet been clarified, several mechanisms such as genetics, infection and autoimmunity have been suggested. The visual prognosis in patients with Behcet's disease is poor, the principal cause of visual loss being consecutive inflammatory ischaemic retinal vein occlusions and macular oedema.[20]

The present study showed that TNF-α levels in patients with BD were significantly higher compared to the controls. It is possible that the disease is associated with secretions of pro-inflammatory mediators by direct activation of circulating monocytes.[21] Previous studies have also reported increased serum TNF-α in BD patients. [22, 23] The results of the present study confirm these findings and suggest involvement of the immune system in BD. This activation could be related to the pathogenesis of the disease and takes part in tissue damage.

This study demonstrated increased serum NO levels in BD patients. In previous studies, NO was also found to increase in diseases such as ocular inflammation, [24] and systemic lupus erythematosus[25]. Increased NO production is believed to be associated with inflammatory

processes. Hence, increased NO production is expected in patients with BD during exacerbations as in inflammatory dermatosis. Previous studies have reported increased NO production in BD, similar to this study [21, 26]. However, in another study, decreased NO levels in patients with BD were demonstrated[27]. They postulated that decreased NO production might have critical biological activities relevant to pathological events during disease activity. On the other side, Aydin et al[28] could not demonstrate any change in NO levels between patients with BD and controls. They hypothesized that other types of nitric oxide synthases (NOs), the inducible or neuronal NOS may affect the plasma NO level rather than endothelial NO synthase.

MDA levels in patients with BD were significantly higher in comparison to the normal control group. These findings were consistent with other previous reports [3, 28].The imbalance between oxidant/antioxidants that are produced by the neutrophils and in the plasma gives rise to lipid peroxidation caused by oxygen free radicals (OFR) which in turn results in the elevation of MDA in BD. OFR interact with membrane lipids of the cells and generate MDA as a result of peroxidation[28].

In this study TNF-α, NO and MDA levels are found to be increased. However, the lack of correlation between TNF-α and NO suggests that activation of NOS and cytokine production could be by different mechanisms resulting in various clinical manifestations of the disease. A positive correlation was detected between MDA and NO serum levels in BD patients. This agreed with the finding of Aydin et al[28], they postulated that the endothelium can be damaged by excessive NO production due to direct toxicity of the molecule or due to peroxynitrite formation. Oxidative damage of polyunsaturated fatty acids initiates lipid peroxidation, which in turn elevates MDA.

Recommendation:

Amelioration of clinical manifestations would be envisaged by targeting cytokines, chemokines and lipid peroxidation with pharmacological agents. Currently, there is considerable interest in the potential role of anti-tumour necrosis factor (TNF) antibody therapy, which are potent anti-TNF medications, effective in certain forms of the disease, particularly mucosal ulceration. Early results with the monoclonal antibody against TNF have shown benefit in ocular, orogenital, and gastrointestinal Behcet's disease, but long term efficacy is unknown. [29]

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مستوى عامل نخر الورم و اكسيد النيتريك والمالون الثنائى الالدهيد في مصل الدم لمرضى بهجت

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يهدف هذا البحث دراسة مستوى عامل نخر الورم و اكسيد النيتريك والمالون الثنائى الالدهيد في مصل الدم لمرضى بهجت.وكذلك تقييم العلاقة بين الدلالات المدروسة.

وقد تم اختيار سبع وعشرين مريضاً بمرض بهجت ومقارنتهم بسبعةعشرة شخصا من الاصحاء كمجموعة ضابطة وتم عمل فحص رمدى شامل وسحب عيتات الدم وفصلها وقياس كل من مستوى عامل نخر الورم و اكسيد النيتريك والمالون الثنائى الالدهيد.

وقد وجد ارتفاع ذو دلالة إحصائية فى مستوى كل من عامل نخر الورم و اكسيد النيتريك والمالون الثنائى الالدهيد فى مصل الدم لمرضى بهجت مقارنة بالمجموعة الضابطة.

وكذلك وجدت علاقة ارتباط ايجابية بين اكسيد النيتريك والمالون الثنائى الالدهيد لمرضى بهجت ومن هذه النتائج يمكن استنتاج أن ارتفاع هذة الدلالات من اهم الاسباب المؤدية لحدوث اعراض مرض بهجت.