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. Ocular manifestation is associated with a severe 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.

Despite the diverse inflections in different organ systems, vasculitis is perceived as the common basic pathological process in BD. The exact cause is unclear but viral, genetic, immunological and environmental factors have been implicated in the pathogenesis of BD.

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. Cytokines are proteins which are produced by various cell types, are important mediators of immunoinflammatory reactions. One such regulating cytokine is tumor 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 NO₂ and NO₃.
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. 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.

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).

![Table (1): Clinical characteristics of the controls and BD group](image)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Number</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>- Sex (M/F)</td>
<td>10/6</td>
<td>15/12</td>
</tr>
<tr>
<td>- Age (years)</td>
<td>37.6 ± 9</td>
<td>37.4 ± 10</td>
</tr>
<tr>
<td>- Duration of disease (years)</td>
<td>-</td>
<td>7.6 ± 6.4</td>
</tr>
</tbody>
</table>

3
Table (2): Mean ± SD of TNF-α, NO and MDA levels in serum of controls and BD patients

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>BD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>- TNF-α (pg/ml)</td>
<td>12.9 ± 2.9</td>
<td>31.5 ± 6.7</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>- NO (µmol/L)</td>
<td>23.3 ± 2.5</td>
<td>30 ± 3.1</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>- MDA (µmol/L)</td>
<td>5.04 ± 1.3</td>
<td>15.6 ± 2.5</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

**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.

**REFERENCES**


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مستوى عامل نخر الورم والاكتسيد النيتريك والمالون الثنائي الاندهاد في مصل الدم لمرضى بهجت

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يهدف هذا البحث دراسة مستوى عامل نخر الورم والاكتسيد النيتريك والمالون الثنائي الاندهاد في مصل الدم لمرضى بهجت وكذلك تقييم العلاقة بين الدلالات المدروسة. وقد تم اختيار سبع وعشرين مريضاً بمرض بهجت ومقارنتهم بسبعية عشرة شخصاً من الأصحاء كمجموعة ضابطة. وتم عمل فحص رمدي شامل وسحب عينات الدم وفحصها وقياس كل مستوى عامل نخر الورم والاكسيد النيتريك والمالون الثنائي الاندهاد. وقد وجد ارتفاع دلاة إحصائية في مستوى كل من عامل نخر الورم والاكسيد النيتريك والمالون الثنائي الاندهاد في مصل الدم لمرضى بهجت مقارنة بالمجموعة الضابطة. وكذلك وجدت علاقة ارتباط ايجابية بين اكسيد النيتريك والمالون الثنائي الاندهاد لمرضى بهجت ومن هذه النتائج يمكن استنتاج أن ارتفاع هذه الدلالات من اهم الاسباب المؤدية لحدث اعراض مرض بهجت.