

# Stress and Serum TNF- $\alpha$ Levels May Predict Disease Outcome in Patients With Pemphigus: A Preliminary Study

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*The aim of the current preliminary case-control study was to estimate the initial serum levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in case patients with pemphigus vulgaris (PV) and pemphigus foliaceus (PF) and correlate them with history of stress, body surface area (BSA) affected, disease severity, and disease outcome. Ten PV and 4 PF case patients as well as 7 healthy matched controls had their serum levels of TNF- $\alpha$  measured by an enzyme-linked immunosorbent assay. Case patients were treated and followed up for 2 months. A statistically significant elevation in serum levels of TNF- $\alpha$  in PV case patients compared with controls and in PV case patients compared with PF case patients was detected ( $P < .05$ ), with no significant difference between PF case patients and controls ( $P > .05$ ). No significant correlation was detected between the serum levels of TNF- $\alpha$  and the BSA affected ( $P > .05$ ). Four PV case patients had a bad disease outcome, of which 3 had severe emotional stress a month prior to the onset of the attack. All 4 showed significantly elevated initial serum levels of TNF- $\alpha$  compared with those who had a good disease outcome ( $P < .05$ ).*

*Emotional stress is a factor affecting prognosis of the disease. Pretreatment assessment of serum TNF- $\alpha$  levels in patients with*

*pemphigus may be a guide to the expected prognosis and selection of the proper treatment regimen.*

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Pemphigus vulgaris (PV) is a fatal immunobullous disease clinically characterized by formation of blisters that rupture, leaving painful mucosal and/or cutaneous erosions. On the other hand, pemphigus foliaceus (PF) shows no mucosal lesions and the skin presents with scaly crusted erosions that follow short-living superficial fragile blisters. Histologically a blister forms in a suprabasal location in PV and just beneath the stratum corneum in PF. Blisters are formed from loss of cell cohesion mediated by desmosomes,<sup>1</sup> which occurs due to autoantibodies directed against the extracellular portion of desmoglein (Dsg) (a cadherin-type cell adhesion molecule), leading to loss of function, acantholysis of keratinocytes, and eventually formation of a blister. Autoantibodies are directed against Dsg1 in PF, while in PV Dsg3 and/or Dsg1 are the targeted epitopes.<sup>1,2</sup>

A number of factors contribute to the production of acantholysis on antibody binding that initiates a cascade of events in the keratinocyte. These factors include structural changes in the desmosomes<sup>3</sup>; production of urokinase plasminogen activator (uPA) and expression of its receptor<sup>4</sup>; and production of a number of cytokines, most notably tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). Other interleukins—IL-1 $\alpha$ , IL-4, IL-5, IL-6, IL-8, and IL-10—as well as IFN- $\gamma$  also are expressed.<sup>5-7</sup> All of these processes are under T-cell control.<sup>8</sup>

Tumor necrosis factor  $\alpha$  and IL-1 $\alpha$  play a role in C3 activation<sup>9</sup> and uPA induction.<sup>10</sup> Activated C3 amplifies the blistering process<sup>11</sup> and uPA activates plasmin, which causes extracellular proteolysis and acantholysis.<sup>10</sup>

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The aim of our preliminary case-control study is to estimate the initial serum levels of TNF- $\alpha$  in case patients with PV and PF and correlate them with history of stress, body surface area (BSA) affected, disease severity, and disease outcome.

## Patients and Methods

**Patients**—This study included 14 pemphigus patients (inpatients) from the Department of Dermatology and Venereology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Patients were recruited from January 2005 to July 2005. Case patients did not receive any systemic medication for 4 weeks prior to the study and were free of diabetes mellitus and hypertension. Laboratory and radiologic investigations were done to exclude internal malignancy. Exclusion criteria included treatment for pemphigus in the last 4 weeks, diabetes mellitus, hypertension, malignancy, and association of other autoimmune diseases. Seven apparently healthy patients matching in age and sex with the case group served as controls.

**Methods**—A detailed history, with special consideration for systemic drug intake and emotional stress prior to the onset of the disease attack, was taken. Ten case patients were diagnosed with PV and 4 were diagnosed with PF. Diagnosis was made by clinical morphology of the lesions and confirmed by histopathologic examination and direct immunofluorescence. The BSA affected was calculated according to the rule of nines and the palm of the patient was considered to represent 1% BSA.

The general condition of the patients was classified as good (normal activity with mild pain, mild malaise), moderate (diminished activity, lassitude, malaise, moderate pain), and poor (bedridden patients with severe pain, severe malaise, depression symptoms, loss of appetite).

According to our inpatient protocol, case patients with PV received prednisone at a dosage of 2 mg/kg daily combined with azathioprine at a dosage of 3 mg/kg daily,<sup>12,13</sup> while PF case patients received prednisone 2 mg/kg daily without adjuvant therapy.<sup>13</sup> Patients were followed up for 2 months. Case patients who showed good response to treatment with healing of old lesions and absence of new lesions were considered to have good disease outcome, and those who were resistant to treatment or died during the 2-month follow-up were considered to have bad disease outcome. Each patient consented to the use of residual blood from a routine laboratory draw to measure serum TNF- $\alpha$  levels.

Venous blood (10 mL) was collected from case patients before treatment was initiated and from controls; it was divided for routine laboratory investigations and part was taken for TNF- $\alpha$  measurement.

Blood was left to clot for 15 minutes and centrifuged for 10 minutes at 5000 rpm; the supernatant serum was separated and stored at  $-70^{\circ}\text{C}$  until the time of analysis. Serum TNF- $\alpha$  levels were determined using an enzyme-linked immunosorbent assay.

**Statistical Analysis**—SPSS version 12 was used for analysis of data. The Student *t* test was used to determine the *P* value and the Pearson product moment correlation was used to determine the correlation coefficient (*r*). *P* < .05 was considered statistically significant.

## Results

**Clinical Results**—There were 8 females and 6 males with pemphigus included in the study (Table) ranging in age from 17 to 75 years (mean [SD], 46.8 [15.5] years). There were 3 PV male and 4 female controls, with ages ranging from 22 to 73 years (mean [SD], 44.7 [18.2] years). Results of histopathologic examination and direct immunofluorescence verified the clinical diagnosis of PV in 10 case patients and PF in 4 case patients.

None of the patients had a family history of an immunobullous disease. Four case patients had a history of similar attacks; 3 PV case patients (30%) (cases 1, 2, and 4) had a history of severe emotional stress within a month prior to the attack (2 women got divorced and 1 woman had a child who died). None of the patients had signs suggestive of internal malignancy at the level of our investigations.

Affected BSA ranged from 0.5% to 40% (mean [SD], 17.1% [12.9%]) (Table).

All PF case patients (*n* = 4) had good general condition. Five PV case patients had good general condition, 2 were moderate, and 3 were poor. After 2 months of treatment, 4 PV case patients showed bad disease outcome (cases 1, 2, 4, and 9)—3 case patients had a history of severe emotional stress—and 6 case patients showed good disease outcome (cases 3, 5, 6, 7, 8, and 10), while all PF case patients had a good disease outcome.

**Results of Assessment of Serum TNF- $\alpha$** —There was a statistically significant elevation in serum levels of TNF- $\alpha$  in all case patients (range, 7.6–50.0 ng/mL; mean [SD], 22.2 [16.7] ng/mL; median, 18.3 ng/mL) compared with controls (range, 0.2–13.5 ng/mL; mean [SD], 8.2 [4.2] ng/mL) (*P* < .05). Significantly higher serum levels of TNF- $\alpha$  were noted in PV case patients compared with controls and compared with PF (*P* < .05 for both). No statistically significant difference was found in serum levels of TNF- $\alpha$  in PF case patients compared with controls (*P* > .05) (Figure 1).

Although a higher mean serum level of TNF- $\alpha$  was detected among females (mean [SD],

## Clinical Data of Case Patients

Case No.	Sex	Age, y	Diagnosis	BSA	General Condition <sup>a</sup>	Attack No.	Serum TNF- $\alpha$ Level, ng/mL	Disease Outcome <sup>b</sup>
1	F	29	PV	0.5%	Good	1	50.0	Bad
2	F	50	PV	20%	Poor	1	23.0	Bad
3	M	28	PV	32%	Good	1	9.6	Good
4	F	75	PV	40%	Poor	1	43.0	Bad
5	M	67	PV	25%	Moderate	1	7.6	Good
6	F	43	PV	15%	Good	3	13.5	Good
7	F	43	PV	0.5%	Good	1	9.0	Good
8	F	17	PV	25%	Poor	1	11.0	Good
9	M	39	PV	25%	Moderate	4	45.0	Bad
10	F	46	PV	30%	Good	1	50.0	Good
11	M	56	PF	4%	Good	3	12.0	Good
12	M	57	PF	12%	Good	4	9.8	Good
13	F	53	PF	5%	Good	1	17.0	Good
14	M	53	PF	5%	Good	1	10.5	Good

Abbreviations: BSA, body surface area; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; F, female; PV, pemphigus vulgaris; M, male; PF, pemphigus foliaceus.

<sup>a</sup>Good=normal activity with mild pain, mild malaise; moderate=diminished activity, lassitude, malaise, moderate pain; poor=bedridden patients with severe pain, severe malaise, depression symptoms, loss of appetite.

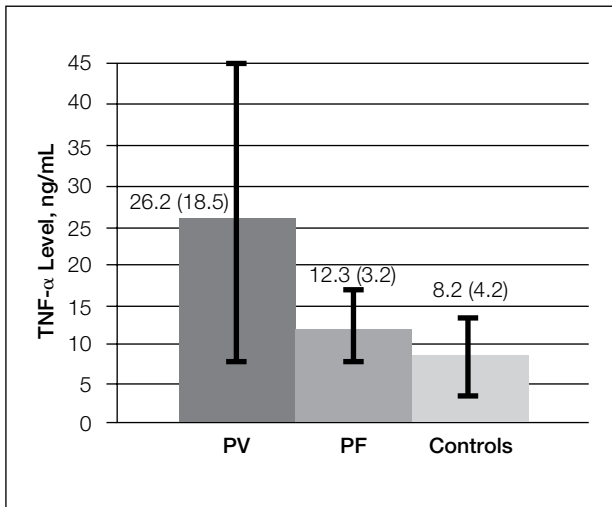
<sup>b</sup>Good disease outcome classified as case patients who showed good response to treatment with healing of old lesions and absence of new lesions; bad disease outcome, those who were resistant to treatment or died during the 2-month follow-up.

27.1 [17.6] ng/mL) compared with males (mean [SD], 15.8 [14.4] ng/mL), this difference was not statistically significant ( $P>.05$ ).

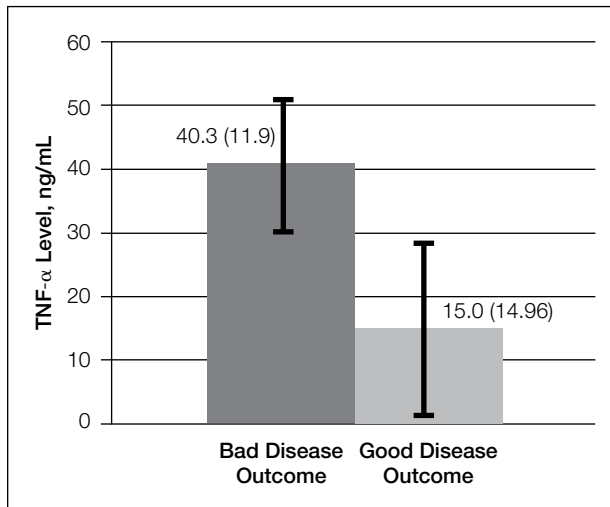
We found no significant correlation between serum levels of TNF- $\alpha$  and affected BSA, (Pearson  $r=0.28$ ;  $P>.05$ )(Figure 2). Also, there was no

significant correlation between serum TNF- $\alpha$  levels and age (PV and PF: Pearson  $r=-0.021$ ;  $P>.05$ ).

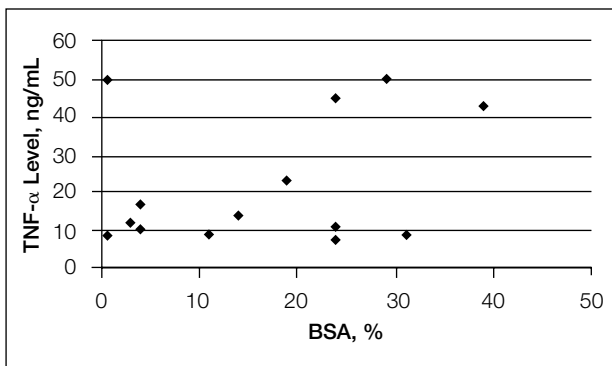
Slightly higher serum levels of TNF- $\alpha$  were observed among case patients with moderate and poor general condition compared with those with good general condition, but the difference was not statistically



**Figure 1.** Mean (standard deviation) serum levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) case patients as well as controls. A statistically significant difference was found between PV case patients and controls ( $P < .05$ ) as well as between PV and PF case patients ( $P < .05$ ). No statistically significant difference was found between PF case patients and controls ( $P > .05$ ). Error bars indicate standard deviation.



**Figure 3.** Comparison between mean (standard deviation) serum levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in pemphigus vulgaris case patients with bad disease outcome (resistant to treatment or died during the 2-month follow-up) and those with good disease outcome (showed good response to treatment with healing of old lesions and absence of new lesions). Significantly higher levels of serum TNF- $\alpha$  were observed among case patients with bad disease outcome compared with those with good disease outcome ( $P < .05$ ). Error bars indicate standard deviation.



**Figure 2.** Correlation between mean serum levels of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and affected body surface area (BSA). No statistically significant correlation was found between serum levels of TNF- $\alpha$  and affected BSA in pemphigus vulgaris and pemphigus foliaceus case patients (Pearson  $r = 0.28$ ;  $P > .05$ ).

significant ( $P > .05$ ). No statistically significant difference was found between case patients with good general condition and those with bad general condition regarding the disease outcome ( $P > .05$ ). Pemphigus vulgaris case patients who had a bad disease outcome showed significantly elevated initial serum levels of TNF- $\alpha$  compared with those who had a good disease outcome ( $P < .05$ ) (Figure 3).

**Comment**

Tumor necrosis factor  $\alpha$  is one of the cytokines that is thought to participate in the pathogenesis of pemphigus,<sup>6,7,9</sup> as well as many other autoimmune diseases such as psoriasis, bullous pemphigoid, and rheumatoid arthritis.<sup>14</sup> Results of the present study showed significantly higher serum levels of TNF- $\alpha$  in PV case patients compared with healthy controls ( $P < .05$ ). Similar results were reported by D’Auria et al<sup>5</sup> and Ameglio et al.<sup>14</sup> Tumor necrosis factor  $\alpha$  is produced by a number of cells including helper T cells subset 1 (T<sub>H1</sub>) and keratinocytes.<sup>15</sup> The explanation for the elevation of TNF- $\alpha$  levels in pemphigus is still controversial; it could be a nonspecific phenomenon resulting from the immune/inflammatory response,<sup>15</sup> as Alecu et al<sup>16</sup> found an elevation of TNF- $\alpha$  in serum and blister fluid levels in both PV and herpes zoster patients without a significant difference between both diseases ( $P > .05$ ). Elevation of TNF- $\alpha$  in pemphigus could, on the other hand, be of relevance to the pathogenesis of the disease, as TNF- $\alpha$  was found to have a role in factors that favor blister formation such as complement production and activation,<sup>9</sup> induction of uPA and uPA receptor,<sup>10,17</sup> enhancement of tannic acid-mediated acantholysis,<sup>18</sup> and enhancement of Langerhans cell migration to regional lymph nodes.<sup>19</sup>

In the current study, PV case patients had significantly higher levels of serum TNF- $\alpha$  compared with PF ( $P < .05$ ), which could be because PV is a much more severe disease compared with the milder PF.<sup>2</sup>

There was no significant correlation between serum levels of TNF- $\alpha$  and the gender or age of the case patients or controls, suggesting that pemphigus is the main factor affecting serum levels of TNF- $\alpha$  in the case group.

Surprisingly we found no significant correlation between serum TNF- $\alpha$  levels and BSA involved, while D'Auria et al<sup>5</sup> and Ameglio et al<sup>14</sup> detected a significant correlation between serum TNF- $\alpha$  and the number of lesions ( $P < .01$ ). The explanation of this finding is not clear because TNF- $\alpha$  is secreted by keratinocytes as well as other cells and the secreted amount correlates to the disease severity as measured by the number of lesions. In the present study, BSA was measured, which may be less sensitive than the number of lesions in reflecting disease severity. The absence of correlation in the current study also could be due to the action of other cytokines that were not measured such as IL-1 $\alpha$ , IL-5, IL-6, IL-8, and IFN- $\gamma$ , which have a role in augmenting the disease process,<sup>10,18,20-23</sup> or IL-4 and IL-10, which have a protective and inhibitory role.<sup>24,25</sup> Widely affected BSA usually is associated with poor general condition due to the complications of loss of barrier function on large areas of the skin including protein, fluid, and electrolyte loss, as well as infection.<sup>26</sup>

The group of PV case patients who responded well to treatment during the follow-up period and consequently had a good prognosis also had significantly lower levels of serum TNF- $\alpha$  compared with those who showed poor response to treatment ( $P < .05$ ). The correlation between the disease outcome and the level of serum TNF- $\alpha$  could be attributed to the systemic effects of TNF- $\alpha$  as well as TNF- $\alpha$  genetic polymorphism among patients. Tumor necrosis factor  $\alpha$  was reported to decrease glucocorticoid sensitivity,<sup>27</sup> which explains the slow response to treatment and bad disease outcome in the group with higher TNF- $\alpha$  serum levels. In addition, those who showed bad or slow response to treatment and an unfavorable prognosis could have carried the high-producer allele of the TNF- $\alpha$  gene.

Overproduction of TNF- $\alpha$  has been implicated in many autoimmune diseases.<sup>28</sup> Furthermore, an association between TNF- $\alpha$  -308 A allele, which is associated with a 6- to 7-fold higher level of TNF- $\alpha$  transcription, and pemphigus was observed.<sup>29</sup> Although this association was later denied,<sup>25</sup> we might postulate that the high-producer allele is not merely associated with the occurrence of pemphigus

but more probably with the unfavorable disease outcome. Pretreatment assessment of serum TNF- $\alpha$  levels may guide physicians to determine if an aggressive therapeutic regimen is needed from the start.

In our study, 30% of PV case patients reported severe emotional stress within a month prior to the onset of the disease. They had high initial levels of serum TNF- $\alpha$  and showed bad response to treatment, resulting in bad prognosis. By using 2 types of questionnaires, Morell-Dubois et al<sup>30</sup> have shown that stressful life events worsen or trigger pemphigus. Here we relied on history of severe emotional stress, with 2 women who experienced divorce and 1 woman whose child died shortly before the present attack, which further supports the possibility that psychoneural disorders can influence the onset and course of autoimmune disease.<sup>31,32</sup> Psychologic care is an important adjuvant to immunosuppressive treatment.

## Conclusion

Tumor necrosis factor  $\alpha$  plays a role in the pathogenesis of pemphigus. Stressful life events influence TNF- $\alpha$  production and consequently the disease outcome, especially in PV. The use of anti-TNF- $\alpha$  biologics as an adjuvant therapy in pemphigus should be studied on a wider scale.

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