

Letter to the Editor

Vascular endothelial growth factor levels in patients with chronic urticaria

Dear Editor

Chronic urticaria (CU) is a debilitating disease characterized by the occurrence of wheals lasting for >6 weeks. Pathomechanisms of CU have not been well understood till date, albeit several mechanisms have been implicated but the exact pathogenesis has not been conclusively elucidated as yet. However, activation of skin mast cells plays a key role in inflammation associated with the disease. Only few patients with CU have autoimmune origin, suggesting that other factors may contribute to its pathogenesis. Recent studies have suggested the role of various cytokines and inflammatory mediators in CU. We evaluated the possible involvement of VEGF, one of the major mediators of vascular permeability, in CU.

Vascular endothelial growth factor (VEGF) family and its receptors are essential regulators of angiogenesis and vascular permeability [1]. VEGF acts as a pro-inflammatory cytokine by increasing the permeability of endothelial cells, inducing the expression of endothelial adhesion molecules and via its ability to act as a monocyte chemoattractant. VEGF is strongly expressed by epidermal keratinocytes in wound healing and psoriasis, conditions that are characterized by increased microvascular permeability and angiogenesis [1].

In addition, VEGF overexpression has been found in patients with eosinophil-related disorders including asthma, acute eosinophilic pneumonia and angioedema with eosinophilia [2]. Amongst the main cells producing VEGF are mast cells and eosinophils, two cells which are known to play a role in allergic conditions as well as in CU [3,4]. Moreover, VEGF has vasopermeability and vasodilatory effects which suggest a possible involvement in the pathophysiology of CU. Hence we undertook this study to assess the plasma VEGF levels in patients with CU when compared to healthy controls and to evaluate its association with the disease severity.

Forty-five patients with CU and 45 age and gender matched healthy controls were included in this hospital-based, cross sectional study, after obtaining clearance from the Institute Ethics Committee (Human Studies) (4). A written informed consent was obtained from all study subjects, prior to participation in the study. Cases included patients with CU, characterized by the presence of continuous or recurrent wheals with or without angioedema for greater than 6 weeks duration of age ranging between 18 to 40 y, attending the dermatology outpatient department of our institute. Controls were age and gender matched healthy volunteers.

Cases and controls were excluded based on the following criteria: acute urticaria, age <18 y and >40 y, on any medication for the past 3 months, other than anti-histamines which had to be stopped for 1 week prior, physical urticaria, urticarial vasculitis, pregnant women, obesity (BMI > 25 kg/m²), dyslipidemia, diabetes, pre-existing cardiovascular disease, hypertension, smokers, deep vein thrombosis, cerebro-vascular accidents, malignancies, hepatic and renal disease,

inflammatory cutaneous disorders, vitiligo and infections. All patients with CU were evaluated by a detailed history and clinical examination. The disease severity was assessed using the urticaria severity score (USS) [5]. Haemogram, urinalysis, filarial serology, stool parasites, blood glucose (fasting and post prandial), serum lipids, serum creatinine, liver function tests and thyroid stimulating hormone levels were done routinely for all patients with CU. Autologous plasma skin test (APST) was done in all patients with CU to assess the autoimmune status. This intradermal skin test using 0.05 ml of citrated autologous plasma was done as described by Asero et al. [6], after stopping anti-histamines for one week. Those who were positive for APST were diagnosed to have autoimmune urticaria.

Blood sampling for estimation of VEGF from cases was done during active urticarial episodes. Five milliliters of venous blood was drawn in EDTA containing tubes, from the antecubital vein of all study subjects and the plasma was separated and stored at –80 °C until VEGF assay. Plasma VEGF concentration was measured by an enzyme immunoassay (R&D Systems, Inc., Minneapolis, MN) according to manufacturer's instructions. The assay was done in duplicate and the mean was taken to increase the reliability of the measurements. Statistical analysis was performed using IBM SPSS statistics ver 20 for Windows. Baseline characteristics of cases and controls were analyzed using descriptive statistics. The normality of continuous data was assessed by Kolmogorov–Smirnov test. The normally distributed data were described as mean ± standard deviation and compared by independent student's *t*-test. The VEGF level was correlated with USS, using Spearman rank correlation test. The VEGF level was correlated with absolute eosinophil count using Pearson correlation. Analysis was carried out at 5% level of significance and *p* < 0.05 was considered as statistically significant.

Baseline characteristics were comparable between the 2 groups as the controls were age and gender matched. The mean plasma VEGF level was significantly higher in CU patients (222.24 ± 25.23 pg/ml), when compared to healthy controls (139.84 ± 11.80 pg/ml) (*p* < 0.001, Fig. 1A). Mean USS in patients with CU was 4.27 ± 1.72. Plasma VEGF levels showed a significant positive with the severity of urticaria with a Spearman correlation coefficient of 0.950 (*p* < 0.001) and with the absolute eosinophil count with Pearson correlation coefficient of 0.746 (*p* = 0.007).

The mean duration of CU was 24.69 ± 27 months with the mean time since last attack of wheals being 2.29 ± 3.23 days. There was no significant correlation between the VEGF levels and the duration of urticaria or the time since last attack.

Amongst the 45 patients with CU, APST performed was positive in 27 patients (60%), indicating autoimmunity and negative in 18 patients (40%). Among the patients with CU, APST-positive patients had significantly higher plasma VEGF levels (236.34 ± 22.76 pg/ml), when compared to the APST-negative patients, (201.10 ± 8.17 pg/ml). (*p* < 0.001, Fig. 1B). USS was significantly higher in APST-positive group (5.55 ± 0.75), compared to APST-negative group (2.33 ± 0.49). (*p* < 0.001).

Activation of skin mast cells plays a key role in the pathogenesis of CU.¹ However, the factors responsible for this mast cell activation in CU

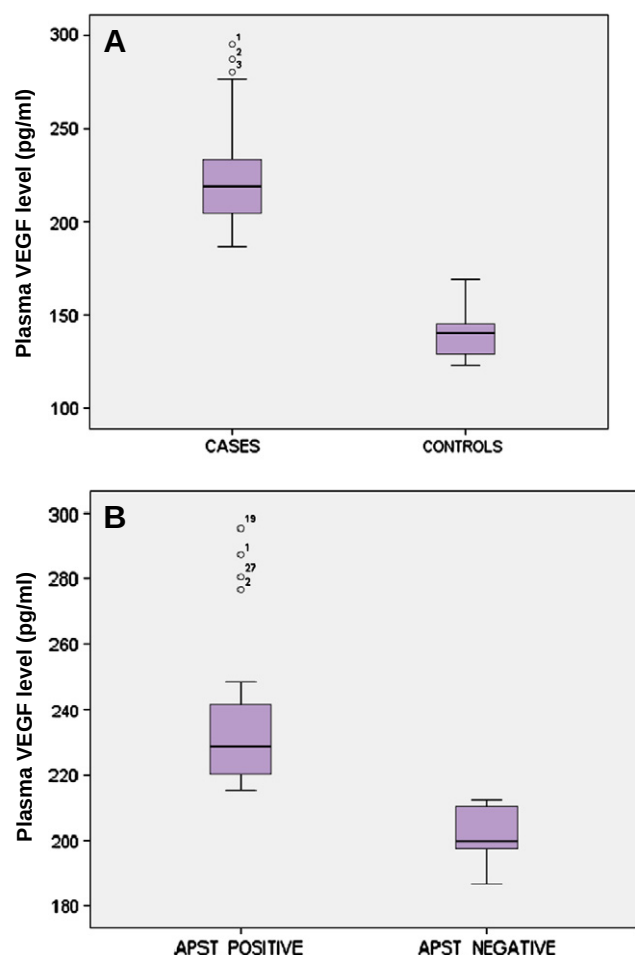


Fig. 1. A: Comparison of plasma VEGF levels between cases and controls. B: Comparison of plasma VEGF levels between APST-positive and APST-negative patients with CU.

in inflammatory reactions, and VEGF itself promotes inflammatory processes by mobilizing leukocytes [13,14].

In the current study, we observed plasma levels of VEGF to be significantly higher in CU patients when compared to healthy controls and in APST-positive patients when compared to APST-negative patients. In addition, the VEGF levels correlated with the disease severity. These observations were in concordance to the observations made by Tedeschi et al. [2] who reported that plasma VEGF levels are increased in CU patients possibly as a consequence of an overexpression by eosinophils in lesional skin based on the co-localization of VEGF and eosinophilic cationic protein. In our study, we observed that the absolute eosinophil counts tend to positively correlate with the plasma VEGF levels, indicating that eosinophils could have been the major source of VEGF.

Many possible mechanisms or factors may be responsible for the increased plasma VEGF concentrations found in this study. Firstly, reactive oxygen species, which has been shown to be increased in CU, is released by activated neutrophils during inflammation and causes VEGF production by macrophages. Second, eosinophils also secrete VEGF and increase in absolute eosinophil count contributes to elevated VEGF secretion. Finally, pro-inflammatory cytokines (IL-1, IL-6, and TNF- α) themselves stimulate VEGF gene expression [13–15]. We have also found that pro-inflammatory cytokines such as IL-6 were increased in patients with CU, especially in active disease (Manuscript under review for publication currently). Therefore, this cytokine may be involved in the up-regulation of VEGF production. A limitation of our study was a small sample size involving 90 study subjects. Further studies need to be done on a larger sample size to validate our findings.

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