

THE EQUILIBRIUM BETWEEN ENDOTHELIN-1/ NITRIC OXIDE IN ACNE VULGARIS

AKNE VULGARİSTE ENDOTELİN-1/NİTRİK OKSİT DENGESİ

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ABSTRACT

Objective: To investigate plasma ET-1 and NOx (nitrate/ nitrite- two end products of nitric oxide metabolism) in acne vulgaris and to evaluate if there is any relationship between these parameters and disease severity.

Materials and methods: Plasma samples of 30 patients with acne vulgaris and 20 healthy controls were investigated by means ET-1 and NOx values.

Results: Plasma ET-1 and NOx in patients with acne vulgaris were significantly increased ($p < 0.01$) in comparison with controls. There was a weak, but significant negative correlation between ET-1 and NOx in control subjects ($r = -0.456$, $p < 0.05$). There was no correlation between ET-1 and NOx in the study group ($r = -0.195$, $p = 0.302$). Among the study group there was a significant correlation between ET-1, NOx and duration of disease ($r = 0.412$, $p < 0.05$ and $r = 0.570$, $p < 0.01$, respectively). There was no correlation between degree of acne lesions and ET-1 or NOx levels in study group.

Conclusion: Increased plasma ET-1 and NOx concentrations in acne vulgaris are probably result of and/or reason for the accentuated ductal hypercornification, hyperkeratinization and sebum accumulation. The increased production of ET-1 and NO by keratinocytes may function as growth and cytotoxic factors and potential mitogens. The lack of correlation between ET-1 and NOx in patients with acne vulgaris suggests that the ET-1/NOx equilibrium may be disturbed, leading subsequently to formation of acne lesions.

Key words: Acne vulgaris, endothelin-1, nitric oxide

ÖZET

Amaç: Akne vulgaris'te plazma ET-1 ve NOx (nitrik oksidin metabolitleri olan nitrat/nitrit) konsantrasyon düzeylerini tayin etmek, ayrıca bu iki parametre ve hastalığın derecesi arasında bir ilişki olup olmadığını incelemek.

Gereç ve yöntem: Akne vulgaris tanısı konmuş 30 hasta ile 20 sağlıklı kişinin plazma örneklerinde ET-1 ve NOx ölçümlendi.

Bulgular: Akne vulgarisli hastalarda kontrol grubuna göre plazma ET-1 ve NOx düzeyleri anlamlı ($p < 0,01$) bir artış gösterdi. Kontrol grubunda ET-1 ve NOx arasında zayıf, fakat anlamlı bir negatif korelasyon ($r = -0,456$, $p < 0,05$) bulundu. Çalışma grubunda ise ET-1 ve NOx düzeyleri arasında bir korelasyon bulunmadı ($r = -0,195$, $p = 0,302$). Ayrıca, çalışma grubunda ET-1, NOx düzeyleri ve hastalık süresi arasında anlamlı korelasyonlar ($r = 0,412$, $p < 0,05$ ve $r = 0,570$, $p < 0,01$) olduğu gözlemlendi. Akne lezyonlarının derecesi ve ET-1, NOx düzeyleri arasında bir korelasyon bulunmadı.

Sonuç: Akne vulgariste görülen plazma ET-1 ve NOx düzeylerindeki yükseliş muhtemelen artmış duktal hiperkeratinizasyon, hiperkeratinizasyon ve sebum birikiminin sebebi ve/veya sonucu olabilir. Artmış olan ET-1 ve NOx üretimi keratinositlerde büyük olasılıkla büyüme, sitotoksik ve mitojenik factor gibi davranıyor olabilir. Çalışma grubunda ET-1 ve NOx arasında korelasyonun bulunmaması, akne lezyonlarının oluşmasına yol açan bu iki parametre arasındaki denge bozulduğunu yansıtmaktadır.

Anahtar kelimeler: Akne vulgaris, endotelin-1, nitrik oksit

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INTRODUCTION

Acne vulgaris is a most common cutaneous disorder, and is related with a chronic inflammatory disease of the pilosebaceous unit. Pathogenesis of inflammatory acne is not fully understood. Several pathogenic factors such as abnormal cornification of the pilosebaceous duct, increased keratin and sebum production, induction of inflammation and Propionibacterium acnes colonization contribute to the etiology of this multifactorial disease (15,16). Little is known about the mechanism(s) by which hypercornification (hyperkeratinisation) and sebum accumulation are accentuated. Recent studies have suggested the importance of the paracrine linkage of some cytokines (such as TNF α , IL-8, IL-1 α , IL-1 β) among epidermal cells (2,9,23), endothelin-1 (ET-1) and nitric oxide (NO) – two important mediators active in many tissues of the body (5,8).

ET-1 – an oligopeptide consisted of 21 aminoacids, is synthesized by various cell types, such as neurons, astrocytes, keratinocytes and fibroblasts (6,33). Elevated serum ET-1 levels in certain inflammatory, hyperproliferative or immune-mediated skin disease, such as psoriasis (4), Behçet's disease (28) and actinic keratosis (29) has been reported. NO is a multifunctional signaling molecule synthesized by various cell types that reside in the skin, and increasing data indicate that this simple inorganic molecule serves as a paracrine mediator in diverse and complex regulatory processes throughout the skin (1,22,31). Numerous studies indicate the existence of various isoforms of nitric oxide synthase (NOS) – an enzyme responsible for NO synthesis in most cells of the skin, including keratinocytes, fibroblasts, Langerhans and vascular endothelial cells (6,19,30). Increased serum nitrate/nitrite levels in psoriasis and correlation with the severity of disease have been reported (19,21). Moreover, it was stated that the expression of iNOS (inducible NOS) in cells of the skin is predominantly associated with inflammatory processes early in cutaneous immune responses (19).

The equilibrium between ET-1 and NO is important in the regulatory and homeostatic functions of the skin. The disturbance of this balance may result in pathophysiologic mechanisms underlying many skin disease including acne. The aim of this study was to evaluate are altered plasma ET-1 and NOx concentrations in Acne vulgaris, and is there any relationship between these parameters and disease severity.

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MATERIALS and METHODS

The study was approved by the Institutional Review Board at the Istanbul Medical Faculty and informed consent was obtained from each subject. Thirty patients (24 women, 6 men, age range 18-30) with acne vulgaris, in the care of the Dermatology Department of Istanbul Medical Faculty, were entered into the study. Acne duration was 2 months – 10 years, mean 58 ± 35 months, 20 patients had second degree, 10 patients – third degree of acne lesions. Patients were excluded if they had used any topical or systemic acne treatment in the past 30 days or had ever received systemic retinoids. The control group consisted of 20 healthy volunteers (17 women, 3 men), aged between 20-40 years. All participants were nonsmokers and were free from cardiovascular, hepatic, renal, endocrine and metabolic disorders. Peripheral venous blood samples were collected in EDTA. K₃ tubes. Subjects were prescribed with low nitrite/ nitrate diets (no spinach, beets or cured meats, the most ample sources of alimentary nitrite and nitrate for 24 h) and fasted overnight before the collection of plasma samples. Plasma samples were placed in 0.5- 1 ml portions into eppendorf tubes and kept at -40°C until used. The Gris-ham's method was used for the measurement of plasma nitrite (NO₂⁻) and nitrate (NO₃⁻) concentrations (two end products of nitric oxide metabolism (10)). Plasma samples were thawed on ice. Plasma (100 μl) was added to 50 μl (0.5M) N-2-hydroxiethylpiperazine-N-2-ethanesulfonic acid (HEPES), 10 μl (5mM) of the reduced form of nicotinamide adenine dinucleotidephosphate (NADPH), and 316 μl distilled water. Nitrate reductase (15 μl , 10U/ml) was pipetted to the reaction mixture, followed by incubation at 37°C for 60 min. Then, 10 μl (750U/ml) lactate dehydrogenase (LDH), 50 μl (10mM) pyruvic acid, and 1000 μl Griess reagent were added. After 10min of incubation at room temperature, the absorbance was read at 543nm against the blanc. Sodium nitrate (200 μM) was used as a standard. The results were expressed in mmol/L. Plasma endothelin-1 concentrations were assigned by Endothelin (¹²⁵I)(Euro. Diagnostica) kit after extraction with sep-pac C18 columns. It was suggested that radioimmunoassay (RIA) methodologies are more sensitive than enzyme-linked immuno-

Table 1. Plasma ET-1 and nitrate/ nitrite levels [median (range)] in controls and patients with acne vulgaris.

	Control (n=20)	Acne vulgaris (n=30)
ET-1 (pmol/L)	11.7 (9.1 – 15.0)	15.8 (6.1 – 25.0)
		p<0.01
Nitrate/ Nitrite (NOx) ($\mu\text{mol/L}$)	25.9 (10.9 – 40.7)	34.1 (17.4 – 73.8)
		p<0.01

p: Mann-Whitney U test

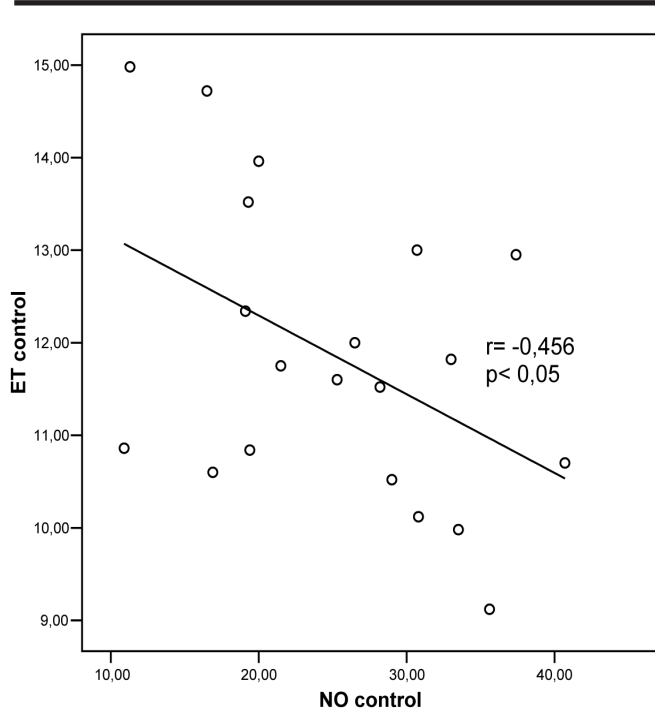


Figure 1. Correlation between plasma ET-1 and NOx in control subjects

sorbent assay (ELISA) techniques for measuring ET-1 concentrations (20). The measuring range of the assay was 0 – 125 pmol (0 – 250 pg/ml) and its sensitivity was 0,4 pmol/L. Data were given as median (range), Mann-Whitney U and Spearman correlation tests were used for statistical evaluation.

RESULTS

Plasma ET-1 and NOx values in controls and patients with acne are shown in Table 1. Plasma ET-1 (15.8 pmol/L) and NOx (34.1 μ mol/L) in patients with acne were significantly increased ($p < 0,01$) in comparison with controls (11.7 pmol/L and 25.9 μ mol/L, respectively). There was a weak but significant negative correlation (Table 1) between ET-1 and NOx concentrations in control subjects ($r = -0.456$, $p < 0.05$), (Figure 1). There was no correlation between ET-1 and NOx in the study group ($r = -0.195$, $p = 0.302$). Among the study group, there was a significant correlation between ET-1, NOx levels and duration of disease ($r = 0.412$, $p < 0.05$ and $r = 0.570$, $p < 0.01$, respectively). There was no differences in mean of ET-1 and NOx values between patients with second ($n = 20$) and third ($n = 10$) degree of acne lesions in study group (data was not shown). There was no correlation between degree of acne lesions and ET-1 or NOx levels.

DISCUSSION

It is seen from the results, that there is a significant increase in plasma ET-1 and NOx levels in patients with acne in comparison with healthy controls. Is there any relationship between increased ET-1 and NOx concentrations and accentuated follicular hyperkeratinization, abnormal

keratine and sebum accumulation seen in acne? ET-1 and NO are important mediators synthesized in various cell type, such as keratinocytes, mastocytes, fibroblasts, Langerhans and vascular endothelial cells (5,19). It has been demonstrated that increased serum and lesional skin ET-1 levels are observed in certain hyperproliferative, inflammatory or immune-mediated skin disease, such as psoriasis (4), Behcet's disease (28) and actinic keratosis (29). In addition, increased serum/plasma nitrate/nitrite levels in psoriasis and correlation with the severity and extend of the disease have been reported (19,21). The study of Sirsjo et al. (24) has demonstrated that messenger RNA (mRNA) for the inducible form of nitric oxide synthase (iNOS) is overexpressed in psoriatic lesions, and that some cytokines, such as interleukin -1 β (IL-1 β) and tumor necrosis factor α (TNF α) stimulate iNOS expression by epidermal keratinocytes in vitro. Moreover, the presence of correlation between the serum and lesional skin ET-1 and NOx concentrations, and between ET-1, NOx and disease severity suggest a direct relationship between the local and blood concentration of ET-1 and NOx in psoriasis (4). Therefore, increased plasma ET-1 and NOx levels in Acne vulgaris probably reflect increased lesional ET-1 and NOx concentrations. Many studies clearly indicate that ET-1 acts as mitogenic factor for the keratinocytes (13,14,26,32). In particular, the inhibition of the basal growth of keratinocytes in the presence of a specific antagonist for the ET receptors demonstrates that ET-1 is one of the most important factors involved in keratinocyte proliferation, which, in turn, could lead to abnormal proliferation and turnover of keratinocytes in acne vulgaris (32). The probable sources of elevated plasma ET-1 and NOx levels besides keratinocytes, are mastocytes, Langerhans cells, fibroblasts and vascular endothelial cells. Increased plasma ET-1 and NOx concentrations in acne vulgaris are probably related with accentuated ductal hypercornification, hyperkeratinization, keratinocyte proliferation and keratin/sebum accumulation.

What is the relationship between ET-1 and NO? Our results obtained from our controls showed that there is a weak, but significant negative correlation between plasma ET-1 and NOx values. This suggests that there is a balance between these two substances in healthy subjects. The lack of correlation between ET-1 and NOx in patients with acne vulgaris suggests that the ET-1/NOx equilibrium may be disturbed, leading subsequently to formation of acne lesions. The mechanism by which ET-1 and NO influence the pathogenesis of acne Vulgaris was not elucidated. The study of Jeremy et al. (15) provided evidence for the involvement of inflammatory events in early stages of acne lesion development. Activated monocytes and Langerhans cells produce many pro-inflammatory cytokines such as IL-1 α , IL-1 β , TNF α and IL-8, which in turn induce NO synthesis (3,17). NO stimulates guanylate cyclase in keratinocytes and mastocytes, therefore promotes the synthesis of the cyclic guanosine monophosphate (cGMP) – nucleotide reported to be a potent mitogen for kerati-

nocytes (11). On the other hand, proinflammatory cytokines promote synthesis of ET-1 in keratinocytes (13,14). It has been reported that ET-1 stimulates the proliferation of fibroblasts (26), mitogenesis and melanogenesis in human melanocytes (32). Increased production of ET-1, probably via ETB receptors, stimulates increased NO synthesis (27).

Although for many years sunlight was used in acne treatment, the role of UV radiation on acne lesions is controversial. While in some patients UV treatment decreases the number and degree of lesions, in another group aggravates the disease (18). It was reported that UV-mediated increase of acne lesions in clinically stationary acne patients is related with increase of cytokine production such as IL-1 α , IL-1 β , IL-8 and TNF α , which are potent synergistic inducers of ET-1 and NO synthesis in human keratinocytes (14,25,28). The elevation of ET-1 and NO in keratinocytes may result in the hypersecretion of certain growth factors, such as basic fibroblast growth factor (bFGF), stem cell factor (SCF), and granulocyte-macrophage colony stimulating factor (GM-CSF)(12).

It is seen from the results, that there is a weak, but significant negative correlation between plasma ET-1/NOx in control subjects, which suggests that there is a balance between these two mediators in normal healthy state. The lack of correlation between ET-1/NOx in acne vulgaris and increased production or secretion of these compounds, probably are causative factors for development of hyperkeratinisation, hypercornification and abnormal keratine and sebum accumulation seen in this common dermatologic problem. On the other hand, increased ET-1, NOx and disequilibrium between these compounds, are also probably the result of above mentioned pathological changes seen in acne vulgaris.

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