Dermatology Practical & Conceptual

Serum Biomarkers IL-6 and HIF-1α in Rosacea: Assessing Their Significance in Disease Pathogenesis and Telangiectasia Formation

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Key words: Rosacea, IL-6, HIF-1α, Telangiectasia

Citation: Taş-Aygar G, Çevirgen Cemil B, Nuri Aydın F, Buran V, Pelin Kartal S. Serum Biomarkers IL-6 and HIF-1α in Rosacea: Assessing Their Significance in Disease Pathogenesis and Telangiectasia Formation. *Dermatol Pract Concept*. 2024;14(4):e2024267. DOI: https://doi.org/10.5826/dpc.1404a267

Accepted: July 26, 2024; **Published:** October 2024

Funding: Our study was supported by the Scientific Research Support Board of Dışkapı Yıldırım Beyazıt Training and Research Hospital.

Competing Interests: None.

Authorship: All authors have contributed significantly to this publication.

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ABSTRACT

Introduction: Rosacea, a chronic inflammatory disease primarily affecting the central area of the face, is a complex condition whose mechanisms are still not fully understood. However, research has indicated a positive correlation between two molecules: hypoxia-inducible factor-1α (HIF-1α) and interleukin-6 (IL-6). The levels of HIF-1α in rosacea patients have yet to be assessed.

Objectives: The aim of this study was to assess the levels of HIF-1α and IL-6 in patients with rosacea in relation to both the severity of the disease and the primary and secondary clinical manifestations of the condition.

Methods: The study included patients diagnosed with rosacea and sex-and age-matched healthy controls (N=40, N=40). Serum HIF-1α and IL-6 levels were quantified using enzyme-linked immunosorbent assay (ELISA).

Results: Compared to the control group, the patient group had significantly elevated serum levels of HIF-1α and IL-6. A positive correlation was found between the level of HIF-1α and the severity of the disease (r=0.374, *P*=0.017); furthermore, a significant association was observed between the presence of telangiectasia, one of the primary manifestations, and HIF-1α (z=2.401, *P*=0.016).

Conclusion: The significantly elevated levels of IL-6 and HIF-1α in patients with rosacea compared to the control group support the hypothesis that these molecules play a role in the pathogenesis of the disease. The correlation between HIF-1α and the severity of the disease, and its significant elevation in patients with telangiectasia, suggest its potential involvement in the pathogenesis of the disease, particularly in the formation of telangiectasia.

Introduction

Rosacea is a chronic inflammatory disease that predominantly affects the central area of the face, including the cheeks, chin, nose, forehead, and eyes [1]. Rosacea is seen in 5.46% of the adult population: it is found in both sexes, although it is more common in women over the age of 30, and its incidence increases with age, becoming more common at the ages of 45–60 years [2]. Despite ongoing research, the understanding of the etiology and pathophysiology of rosacea remains incomplete. However, recent findings indicate that a combination of genetic and environmental factors contributes to the onset and/or exacerbation of rosacea by influencing both the innate and the adaptive immune systems as well as neurovascular dysfunction [3,4]. Various trigger factors—such as sun exposure, temperature extremes (hot and cold), hot beverages, spicy foods, and physical activity—have been identified, although they can vary across individuals; these factors may worsen the disease, both through the release of mediators from cells and by directly activating the cutaneous nervous system [2,4].

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcription factor regulated by oxygen, and it comprises the subunits HIF-1α and HIF-1β [5]. In normal skin, HIF-1α is expressed in epidermal cells, and its expression has been demonstrated as higher in psoriatic lesions [6]. Selective upregulation of HIF-1α has been observed in the differentiation of T cells toward T helper (Th)-17 polarization; however, HIF-1α is necessary to inhibit regulatory T (T-reg) cell differentiation. The cell decision of polarization from precursor cells toward Th-17 and T-reg cells is critically dependent on interleukin (IL)-6. Under hypoxic conditions, IL-6 expression, like HIF-1α, increases. A positive relationship exists between HIF-1α and IL-6, wherein elevated IL-6 is accompanied by higher HIF-1α levels. This suggests a significant role for IL-6 signaling in HIF-1α induction [5,7].

Objectives

The purpose of our study was to investigate serum HIF-1α and IL- 6 levels in rosacea patients and their relationship with patients' demographic and clinical characteristics.

Methods

The study protocol was approved by the University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital ethics committee, and it followed the International Ethical Guidelines of the Declaration of Helsinki. Informed consent was obtained from all participants prior to initiation of the study.

Forty adult patients diagnosed with rosacea visiting the Department of Dermatology at the University of Health Sciences, Diskapi Yildirim Beyazit Training and Research Hospital from May 2022 to December 2022 were enrolled in the study. The inclusion criterion was having a diagnosis of rosacea according to the American National Rosacea Society Expert Committee (NRSEC) criteria [8]; exclusion criteria included patients younger than 18 or older than 65 years, pregnant or lactating patients, and patients with diabetes mellitus, chronic inflammatory disorders, immunological disorders, infections, obesity (BMI >30), or malignant cancer. The control group comprised healthy volunteers with no known diseases who sought routine physical examinations at our hospital; age and sex compatibility with the patient group was ensured. Neither the patients nor the control group members were subjected to any dietary restrictions. Blood samples were taken from all participants under the same conditions in our hospital laboratory.

All patients were asked for a detailed anamnesis, and physical examinations were performed. Patients were also asked about disease duration and treatments; rosacea type was determined using the NRSEC classification system for the categorization of rosacea cases, and severity scores were determined in accordance with this classification [8]. With this scoring system, patients' symptoms were scored as one, two, or three, with assessments of mild, moderate, or severe, respectively. Non-existent symptoms received a score of zero. In the final step, the type of disease was determined, and the severity score was again assessed by the physician in the same manner. After the overall severity of the disease was evaluated by the physician, the total obtained score provided the rosacea severity score.

Following an 8-hour fasting period, patients had their blood drawn; these non-stimulated blood samples were then

centrifuged at 1000 x g for 20 minutes at 4 °C. The serum was subsequently stored at –20 °C in the biochemistry department until analysis. Serum HIF-1α and IL-6 levels were measured through enzyme-linked immunosorbent assay (ELISA) using commercial kits (Sandwich-ELISA, Cloud-Clone Corp., SEA079Hu (HIF-1α), SEA798Hu (IL-6), USA) at Diskapi Yildirim Beyazit Training and Research Hospital Laboratories. HIF-1α and IL-6 concentration of the samples were interpolated from the standard curve. The detection ranges for the assay were 0.156–10 ng/ml for HIF-1α and 7.8–500 pg/ml for IL-6.

The obtained data were transferred to a computer environment and evaluated using the statistics program SPSS (v.15.0). The conformity of the data to normal distribution was evaluated using the Kolmogrov–Smirnov test: analysis of data inappropriate to normal distribution was conducted using the Mann–Whitney U, Kruskal–Wallis, and Spearman Correlation tests, while analysis of data appropriate to normal distribution was conducted using Student's t-test. Chi-square analysis was employed to assess categorical variables in the study.

Results

The study included 40 (30 female, 10 male) patients diagnosed with rosacea and 40 (27 female, 13 male) healthy controls. No significant differences were observed between the patient and control groups in terms of sex. The participants in the study group were aged 18–62 years, with a mean age of 39.97±10.26: participants in the patient group were aged 23–61 years, with a mean age of 40.25±10.86, while those in the control group were aged 18–62 years, with a mean age of 39.7±9.74. There was no statistically significant difference in the mean ages between the patient and control groups (P = 0.264).

Our study group consists of patients diagnosed with erythematotelangiectatic and papulopustular rosacea; there were no patients with ocular rosacea or phymatous rosacea. All patients had at least one triggering factor. The Fitzpatrick skin types included in the study were 2, 3, and 4; there were no patients with other skin types. Patient characteristics—including age at onset of disease, duration of disease, disease subtype, triggering factors, skin phototype, and family history—are presented in Table 1. The clinical findings used in calculating patients' severity scores, along with their distribution in terms of severity and number of patients, are detailed in Table 2.

A significant difference was observed between the patient and control groups in terms of HIF-1α and IL-6 levels (P < 0.001 for each) (Table 3). When patients were grouped according to severity score, we observed that eight (20%) patients had mild severity, while 32 (80%) patients had moderate severity. Although there were severe symptoms in some specific aspects, the overall severity scores placed our patients in the 'mild' and 'moderate' severity groups, and thus there were no patients classified as 'severe'. A positive correlation was found between the Rosacea Severity Score and serum HIF-1α (r=0.374, P = 0.017). No relationship was found between serum IL-6 and the severity score of the disease (z=0.032, P = 0.844). There was no observed correlation between serum HIF-1α and IL-6 (r=0.104, P = 0.523). In an examination of associations between the presence of primary and secondary manifestations of the disease and HIF-1α and IL-6 levels, it was determined that the level of HIF-1α was

Table 1. General Characteristics of Rosacea Patients.

Characteristics	n(%)
Age, years	40.25(±10.86)
Sex	
• Female	30 (75%)
• Male	10 (25%)
Age at disease onset (mean)	35.75 ± 10.82 (19.0-59.0)
Disease duration, months (mean)	51.73 ± 46.02 (1-180)
Rosacea subtype	
• ET	21 (52%)
• PP	19 (48%)
Aggravating factors	
• Sun exposure	38 (95%)
• Stress	28 (70%)
• Heat	38 (95%)
• Cold	21 (52.5%)
• Wind	20 (50%)
• Exercise	9 (22.5%)
• Spice	16 (40%)
Skin phototype	
• Phototype 1	
• Phototype 2	5 (12.5%)
• Phototype 3	26 (65.0%)
• Phototype 4	9 (22.5%)
• Phototype 5	
• Phototype 6	
Family history	
• Present	13 (32.5%)
• Absent	27 (67.5%)

Abbreviations: ET: erythematotelangiectatic; PP: papulopustular.

Table 2. Distribution of Rosacea Symptoms and Severity Scores Among Patients.

	Absent n(%)	Mild n(%)	Moderate n(%)	Severe n(%)
Primary features				
• Flushing	14 (35.0)	21 (52.5)	3 (7.5)	2 (5)
• Nontransient erythema	0	18 (45.09)	19 (47.5)	3 (7.5)
• Papules and pustules	18 (45.0)	10 (25.0)	7 (17.5)	5 (12.5)
• Telangiectasia	8 (20)	18 (45)	13 (32.5)	1 (2.5)
Secondary features				
• Burning	10 (25.0)	21 (52.5)	8 (20.0)	1 (2.5)
• Plaques	27(67.5)	11 (27.5)	2 (5)	0
• Dry appearance	6 (15.0)	27 (67.5)	6 (15.0)	1 (2.5)
• Edema	27 (67.5)	8 (20.0)	5 (12.5)	0
• Ocular manifestations	39 (97.5)	1 (2.5)	0	0
• Phymatous change	36 (90.0)	4 (10.0)	0	0
Physician ratings by subtype				
• ET		10 (25)	9 (22.5)	2 (5)
• PP		7 (17.5)	6 (15)	6 (15)
• Phymatous	0			
• Ocular	0			
Patient's global assessment		17 (42.5)	15 (37.5)	8 (20)

Abbreviations: ET: erythematotelangiectatic; PP: papulopustular.

Table 3. Evaluation of HIF-1α and IL-6 levels in Patient and Control Groups.

	Patient (mean±SD (min-max))	Control (mean±SD (min-max))	Z ; p-Value
HIF-1α	0.207±0.009 (0.195-0.242)	0.035±0.063 (0.03-0.317)	6.983: $P<0.01$
IL-6	0.359±0.184 (0.051-1.204)	0.072±0.109 (0.046-0.742)	6.639: $P<0.01$

Abbreviations: HIF: hypoxia-inducible factor; IL: interleukin; SD: standard deviation.

found to be statistically significantly higher in patients with telangiectasia (z=2.401, P = 0.016) (z=2.401, P = 0.016), although no correlation was found with the severity of telangiectasia. No significant association was found between HIF-1α and IL-6 levels and the primary clinical manifestations flushing, non-transient erythema, and papules and pustules. Additionally, no significant relationship was found between the secondary clinical manifestations of rosacea and HIF-1a or IL-6 levels ($P > 0.05$ for each).

Discussion

While greater IL-6 expression has been demonstrated multiple times in tissue studies, no consensus has been reached regarding serum levels. Specifically, in a study by Ertekin et al., IL-6 levels were found to be higher and associated with disease severity in rosacea patients [9–15]. Additionally, IL-6 has been reported to play a role in the pathogenesis of rhinophyma in conjunction with transforming growth factor-beta (TGF-β) [3]. Furthermore, timolol, a non-selective β-adrenergic receptor blocker, exhibits vasoconstrictive and anti-angiogenic effects by modulating the activity of matrix metalloproteinases (MMPs) and IL-6, and reducing VEGF expression. Moreover, metronidazole, an antimicrobial agent used in treatment for its anti-Demodex and anti-inflammatory properties, has been shown to impair the induction of IL-17 both directly and indirectly via the suppression of IL-6 and chemokine (C-X-C motif) ligand-8 (CXCL-8) [16]. To our knowledge, serum HIF-1α levels in rosacea patients have not been explored. Our findings revealed that, compared to those in the control group, patients with rosacea exhibited significantly higher concentrations of both serum HIF-1α and serum IL-6. We found a significant association between the HIF-1α level and the severity of the disease, but we did not find an association between the IL-6 level and the severity of the disease.

Significant activation of the immune system has been shown in all subtypes of rosacea: the increase in interferon (IFN)-γ and IL-17 cytokines leads to Th-1 and Th-17 polarization (12), and the deficiency in HIF-1α leads to a reduction in Th-17 development and an increase in the differentiation of T-reg cells. Earlier work has demonstrated that the HIF-1α-dependent glycolytic pathway serves as a metabolic control point that regulates the differentiation of both Th-17 and T-reg cells [7]. Furthermore, patients with rosacea exhibit an increase in the genes that provide TH-17 polarization in tissue, and the amount of IL-17 was also found to be increased in both tissue and serum samples [12,17]. It has been suggested that serum IL-17 levels are not associated with the severity of rosacea, although they may play a role in its pathogenesis [17]. The overexpression of HIF-1α has been demonstrated in various autoimmune conditions, including systemic lupus erythematosus, inflammatory bowel disease, rheumatoid arthritis, systemic sclerosis, multiple sclerosis, and psoriasis [18].

Investigation of chronic inflammatory processes and comorbidities constitutes an intriguing aspect of the pathogenesis of rosacea. In this context, numerous studies have been conducted to elucidate the involvement of oxidative stress, antioxidant levels, and levels of proinflammatory/inflammatory mediators and markers. The majority of such studies have demonstrated an increase in these markers compared to the control group; however, in most studies, these markers have not been found to be associated with disease severity but rather have been more closely linked to pathogenesis [14]. In our study, we identified an association between the level of HIF-1α and disease severity, which indicates both its role in pathogenesis and its potential use as an indicator of severity.

The participants in this study included patients with erythematotelangiectatic rosacea. Although no study has directly shown an increased expression of HIF-1α in the tissue of patients with rosacea, the presence of Th-17 polarization and the elevated expression of cytokines such as IL-6 and VEGF in these patients suggests a potential increase in HIF-1α levels. Earlier observed decreases in HIF-1α expression following the application of green tea extract further support his hypothesis [17,19,20].

In our study, we found an association between the level of HIF-1α and the presence of telangiectasia in patients. We hypothesize that HIF-1α may wield its effects in individuals with rosacea through the promotion of neovascularization via VEGF. Compared to participants in the control group, IL-6 levels were found to be significantly elevated in patients, although they could not be correlated with the severity of the disease or the presence of clinical symptoms and findings. The influence of IL-6 on VEGF has been demonstrated as occurring through HIF-1α in patients with psoriasis. However, the lack of association between the IL-6 level and both clinical manifestations—specifically telangiectasia—along with the absence of correlation of IL-6 levels with HIF-1α levels in rosacea patients suggest that there may be pathways other than IL-6 that are influencing HIF-1α levels in rosacea. Understanding this mechanism could lead to a deeper comprehension of rosacea pathophysiology and aid in identifying potential therapeutic targets. Accordingly, we anticipate that our study will contribute to the existing knowledge in this field.

Limitations

Our study is limited by the fact that it was conducted at a single center and with a limited patient group; furthermore, there were no patients with severe rosacea in our study. In addition, patients were not randomly selected; meeting the inclusion and exclusion criteria, they were included in the study.

Conclusions

Levels of IL-6 and HIF-1α are elevated in patients with rosacea compared to people without rosacea. In addition, a patient's HIF-1α level correlates with the disease severity. The higher level of HIF-1α—a known participant in the pathogenesis of vascular formation—in patients with telangiectasia suggests its potential role in the pathogenesis of telangiectasia formation in rosacea patients. These findings imply that drugs affecting HIF-1α could be considered for the treatment of rosacea. Nevertheless, further research with larger patient cohorts is needed to substantiate our findings.

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