Dermatol Ther (Heidelb) (2023) 13:1549–1560
https://doi.org/10.1007/s13555-023-00952-w

ORIGINAL RESEARCH

Hypoxia-Inducible Factor-1α (HIF-1α) as a Biomarker for Changes in Microcirculation in Individuals with Systemic Sclerosis

Magdalena Maciejewska · Mariusz Sikora · Albert Stec · Michał Zaremba · Cezary Maciejewski · Katarzyna Pawlik · Lidia Rudnicka

Received: April 17, 2023 / Accepted: May 30, 2023 / Published online: June 14, 2023
© The Author(s) 2023

ABSTRACT

Introduction: Systemic sclerosis is an autoimmune disease characterized by tissue fibrosis and microangiopathy. Vascular changes such as a decrease in capillary density diminish blood flow and impair tissue oxygenation. Reliable ways to monitor disease activity and predict disease progression are desired in the process of patient selection for clinical trials and to optimize individual patient outcomes. Hypoxia-inducible factor-1 (HIF-1) is a dimeric protein complex that plays an integral role in the body's response to hypoxia. Our study aimed to investigate the potential abnormalities of HIF-1α plasma concentration and its possible association with disease activity and vascular abnormalities in patients with systemic sclerosis.

Methods: Blood plasma levels of HIF-1α were measured in patients with systemic sclerosis ($n = 50$) and in healthy individuals ($n = 30$) using commercially available ELISA test kits.

Results: The results showed a marked increase in HIF-1α levels in patients with systemic sclerosis (3.042 ng/ml [2.295–7.749]) compared to the control group (1.969 ng/ml [1.531–2.903] $p < 0.01$). Patients with diffuse cutaneous SSc (2.803 ng/ml, IQR 2.221–8.799) and limited cutaneous SSc (3.231 ng/ml, IQR 2.566–5.502) exhibited elevated serum HIF-1α levels compared to the control group ($p < 0.01$). We found a notable increase in HIF-1α plasma concentration in patients with an "active" pattern (6.625 ng/ml, IQR 2.488–11.480) compared to those with either an "early" pattern (2.739, IQR 2.165–3.282, $p < 0.05$) or a "late" pattern (2.983 ng/ml, IQR 2.229–3.386, $p < 0.05$). Patients with no history of digital ulcers had significantly higher levels of HIF-1α (4.367 ng/ml, IQR 2.488–9.462) compared to patients with either active digital ulcers (2.832 ng/ml, IQR 2.630–3.094, $p < 0.05$) or healed digital ulcers (2.668 ng/ml, IQR 2.074–2.983, $p < 0.05$).

Conclusions: Our results indicate that HIF-1α may serve as a biomarker in assessing microcirculatory changes in individuals with systemic sclerosis.

M. Maciejewska
Department of Dermatology, Doctoral School of Medical University of Warsaw, Warsaw, Poland

M. Sikora
National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland

M. Maciejewska (✉) · A. Stec · M. Zaremba · K. Pawlik · L. Rudnicka
Department of Dermatology, Medical University of Warsaw, Koszykowa 82a, 02-008 Warsaw, Poland
e-mail: magdalena.maciejewska@wum.edu.pl

C. Maciejewski
1St Chair and Department of Cardiology, Medical University of Warsaw, Warsaw, Poland

Keywords: Biomarker; Digital ulcers; HIF-1α; Hypoxia-inducible factor-1α; Microangiopathy; Systemic sclerosis

Key Summary Points

Why carry out this study?

The study investigated the potential use of hypoxia-inducible factor-1α (HIF-1α) as a biomarker in assessing microcirculatory changes in individuals with systemic sclerosis (SSc). There is an unmet need to identify reliable ways to monitor disease activity and predict disease progression to optimize individual patient outcomes and to select patients for clinical trials

The study explored the association between HIF-1α plasma concentration and disease activity and vascular abnormalities in patients with SSc

What was learned from the study?

HIF-1α plasma concentration was significantly increased in patients with SSc compared to healthy individuals, irrespective of disease subtype

The study suggests a potential role of HIF-1α as a valuable biomarker for assessing microcirculatory changes

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease defined by vascular and immune dysfunction, manifesting primarily as fibrosis of the skin and internal organs [1]. Two main subsets of SSc are described according to the extent of skin involvement: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc) [2]. Microangiopathy and Raynaud's phenomenon are the clinical hallmarks and can be found in almost all patients from the earliest stages of the disease [3, 4]. The gradual reduction in the quantity and diameter of vessels afflicted by SSc leads to chronic hypoxia in both the skin and organs of affected individuals [5]. The vascular aberrations observed in SSc are marked by capillary loss and structural alterations. Alterations in the capillary network can be seen early in the course of SSc using nailfold capillaroscopy (NFC) [6]. Proximal nailfold capillary abnormalities have been associated with the development of digital ulcers (DU) [7]. Ulcers on the fingertips pose a significant clinical obstacle because of the accompanying pain, risk of infection, and eventual necrosis [8, 9].

During the last 15 years, attempts have been directed toward identifying SSc at an early stage. Previously suggested criteria, known as VEDOSS, aim to detect early signs and symptoms of SSc in patients with Raynaud's phenomenon [10]. Patients with all signs or symptoms of the VEDOSS criteria already fulfill the 2013 American College of Rheumatology–European League Against Rheumatism (ACR–EULAR) classification criteria for SSc [11]. Patients who satisfy the criteria for early diagnosis of SSc often already exhibit digital ulcers [10].

Inflammation and microvascular dysfunction appear to be the primary events that progressively trigger the fibrotic process [12, 13]. The precise etiology of fibrotic changes remains only partially understood but may include impaired communication between endothelial cells, epithelial cells, and fibroblasts, as well as lymphocyte activation, autoantibody production, inflammation, and tissue fibrosis [14]. Angiogenesis, the formation of newly formed capillaries from preexisting vessels via a well-programmed cascade of events, is dysregulated in SSc and cannot ensure an efficient vascular recovery. Vascular injury induces hypoxia and tissual ischemia which are the primary triggers for angiogenesis [15].

Markers of endogenous hypoxia as well as molecular responses to hypoxia have been thoroughly detailed over the past two decades.

A growing body of evidence indicates that hypoxia-inducible factor-1α (HIF-1α), a key transcriptional factor involved in the response to chronic hypoxia, may be implicated in the pathogenesis of fibrotic diseases such as SSc [16]. HIF-1 is a transcription factor that

responds to changes in oxygen levels and enables organisms to adapt to low-oxygen environments. HIF-1 is composed of two different subunits: one α-subunit that is regulated by oxygen levels, and one β-subunit that is expressed continuously regardless of oxygen levels [17]. Each subunit of HIF-1 contains basic helix-loop-helix-PAS (bHLH-PAS) domains that facilitate the binding of the two subunits together and to DNA hypoxia response elements (HREs) [18, 19]. Since there excess HIF-1β is present in vivo, the transcriptional activity of HIF-1 is mainly determined by the levels of the HIF-1α subunit [20]. In addition to HIF-1, there are two other isotypes in the hypoxia-inducible factor family, known as HIF-2 and HIF-3, that also play a role in transcriptional responses to hypoxia, immunity, neovascularization, and other stimulators [21, 22]. However, HIF-1 is considered one of the most important hypoxia-inducible factors involved in cellular metabolism, tissue repair, and inflammation [22, 23]. The suspected functions of HIF-1 include stimulation of excessive extracellular matrix, vascular remodeling, and futile angiogenesis with further exacerbation of chronic hypoxia [22]. HIF-1α signaling is associated with cardiovascular, inflammatory, infectious, and metabolic diseases [24, 25]. The rs12434438 polymorphism of the HIF-1 gene has been linked with a predisposition to developing SSc [26].

Takagi et al. [27] demonstrated that the *HIF1A* gene is a risk factor for developing pulmonary arterial hypertension (PAH) in patients with SSc. The authors found that the AA genotype at rs12434438 was associated with a subset of patients with SSc and severe PAH, suggesting that the rs12434438 single nucleotide polymorphism (SNP) may contribute to the development of PAH with SSc [27].

Results of the study conducted by Mao et al. [28] suggest that the HIF-1α/vascular endothelial growth factor (VEGF) signaling pathway may have a critical role in mediating the hypoxia-induced endothelial to mesenchymal transition (EndMT) seen in the cutaneous microcirculation of patients with SSc [28]. It has been suggested that endothelial cell damage is a key event that triggers vascular remodeling, intimal arteriole growth, capillary collapse, and ultimately blood vessel occlusion [29]. The importance of EndMT in the pathophysiology of tissue fibrosis and fibroproliferative vasculopathy, observed in various fibrotic diseases, has been firmly established [30]. Thus, the role endothelial cells (ECs) play in the vascular alterations associated with SSc, as well as the identification of associated biomarkers, are common subjects of ongoing research [31, 32].

A remarkable breakthrough regarding early diagnosis of the disease was made a few years prior with the introduction of the new guidelines enabling the diagnosis of SSc before the onset of overt fibrotic symptoms [11]. Appropriate early augmentation of treatment can prevent pathological vascular remodeling and therefore abate the process of fibrosis [33, 34]. With the advent of advanced immunodiagnostic techniques, many autoantibodies specific to SSc have been described. Many of these autoantibodies help predict clinical manifestations such as internal organ dysfunction and the extent of skin involvement [35]. However, identifying patients who are at risk of developing digital ulcers and determining which patients are responding to vasoactive therapy remains a challenge. For these reasons, the aim of our study was to investigate the differences between plasma concentration of HIF-1α, disease activity, and vascular abnormalities in patients with SSc [36, 37].

METHODS

The study was cross-sectional and prospective in design.

Patients

A total of 50 patients who were diagnosed with SSc in accordance with the 2013 ACR/EULAR classification criteria [11] were recruited.

Exclusion criteria included respiratory diseases (PAH combined with interstitial lung disease, asthma, tuberculosis, pneumonia, bronchial pneumonia, lung cancer, and other pulmonary organic diseases), cardiovascular diseases (history of heart failure or cardiomyopathy; coronary heart disease); chronic kidney

disease (stage 3b–5); liver fibrosis; hemolytic disease; active neoplastic disease or neoplastic disease whose treatment has been completed in the last 5 years (except for basal cell carcinoma), pancytopenia or anemia, pregnancy, breast-feeding, and acute infection or inflammation.

The control group consisted of individuals matched for age, sex, and body mass index (BMI). The presence of primary Raynaud's phenomenon was considered an additional exclusion criterion in the control group.

All study participants underwent a comprehensive physical examination, and their demographic data was collected via a questionnaire. A detailed medical history was recorded, establishing the absence or presence of pulmonary disorders such as pulmonary hypertension and/or pulmonary fibrosis, esophageal motility disorders (which were documented through imaging or endoscopic examinations), and the duration of the disease. In addition, medication use and cardiovascular risk factors (such as smoking and hypertension) were also documented.

Clinical Assessment

The clinical assessments included quantification of various laboratory parameters such as complete blood count, erythrocyte sedimentation rate (ESR), concentration of C-reactive protein (CRP), lipid profile, as well as liver and kidney function tests. Additionally, the level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) was also measured from the peripheral blood during routine checkups. Indirect immunofluorescence and immunoblot analysis were performed on HEp-2 cells to evaluate the presence of antinuclear antibodies. Laboratory and immunological parameters were extracted from the patient's medical records.

Microvasculature Assessment

The same investigator (MM) conducted a nailfold video-capillaroscopy (NVC) examination on all patients with SSc using a Dino-Lite CapillaryScope 200 MEDL4HMA USB digital microscope (AnMo Electronics Corporation, Taiwan).

The capillaroscopic examination involved acclimatizing the patients to room temperature for 15–20 min (approximately 25 °C), followed by examining eight fingers (II to V bilaterally). To aid in imaging, a drop of paraffin oil was applied to the nailfold area, and four images were captured for each examined finger.

The microvascular abnormalities observed were categorized as either early, active, or late scleroderma patterns based on the criteria set forth by Cutolo et al. [10].

Measurement of Serum HIF-1α Concentration

Blood samples were collected once after an overnight fast. The blood samples were then centrifuged at 4000 rpm ($1500\times g$) for 10 min within 15 min of their collection. The plasma was subsequently collected and frozen at − 80 °C to be analyzed later.

HIF-1α concentration was evaluated using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human HIF-1A ELISA Kit, Bioassay Technology Laboratory, Shanghai, China), following the manufacturer's instructions. Each sample was subjected to duplicate testing using identical equipment and procedures in a single laboratory. The analytical sensitivity was 0.01 ng/ml. For the ELISA analyses mentioned above, intra-assay coefficients of variation were below 4.9% and inter-assay coefficients of variation were below 10%.

Statistical Analysis

Statistical analysis was performed using Statistica software v.13.3 (TIBCO, Palo Alto, CA, USA). The normal distribution of data was checked using the Shapiro–Wilk test. Descriptive statistics for continuous variables were presented as either mean and standard deviation (SD) or median and interquartile range (IQR), depending on the distribution of data. Categorical variables were expressed as numbers and percentages. Non-parametric tests were used because of the non-normal distribution of data. The Mann–Whitney *U* test was used for two independent samples, the Kruskal–Wallis

test with post hoc Dunn analysis was used for multiple independent comparisons, and the Wilcoxon rank test was used for two paired samples. Correlations between continuous variables were analyzed using Spearman's rank-sum coefficient. Statistical significance was considered at p values less than 0.05.

Ethical Statement

All participants gave their written informed consent before entering the study. The study was performed in accordance with the Helsinki Declaration and the ethics committee's authorized protocol (KB/90/2018 of 21 May 2018; Bioethics Committee at the Medical University of Warsaw, Warsaw, Poland).

Between September 2019 and May 2022, a cohort of individuals who had been diagnosed with SSc were recruited and subsequently attended outpatient or inpatient services.

RESULTS

Patient Characteristics

Table 1 depicts the anthropometric characteristics of the study population. A total of 50 Caucasian patients with SSc (42 women, 8 men), with a mean age of 56.4 years, along with 30 control individuals matched in age and sex (25 women, 5 men; mean age 52.1 years), were included in the study.

HIF-1α Plasma Concentration in Systemic Sclerosis

The HIF-1α plasma concentration in patients with SSc was 3.042 ng/ml (IQR 2.295–7.749), which was significantly higher compared to the control group (median 1.969 ng/ml, IQR 1.531–2.903, $p < 0.01$; Fig. 1).

In addition, we investigated whether there was a difference between HIF-1α plasma concentration and different clinical phenotypes of SSc. With regards to cutaneous subsets of SSc, patients with dcSSc (2.803 ng/ml, IQR 2.221–8.799) and those with lcSSc (3.231 ng/ml, IQR 2.566–5.502) had a higher HIF-1α plasma concentration compared to the control group ($p < 0.01$). However, there was no significant difference in HIF-1α plasma concentration between the lcSSc and the dcSSc group (Fig. 1).

We did not observe any significant differences between HIF-1α plasma concentration and age, BMI, Raynaud's phenomenon, or disease duration.

When the patients were grouped according to autoantibody production, no significant difference was observed between patients positive for anti-RNA polymerase III (anti-RNAP) and patients with other antibody types such as anticentromere autoantibodies (ACA) or anti-topoisomerase I autoantibodies (ATA). After analyzing the HIF-1α plasma concentration in patients with SSc, there were no observable differences in the extent of skin fibrosis when assessed by the modified Rodnan skin score (mRSS) and pulmonary hypertension or esophageal dysmotility. We did not observe differences in serum HIF-1α concentration between patients with and those without interstitial lung disease nor did we find a correlation between HIF-1α concentration and the carbon monoxide diffusion capacity (DLCO).

Association Between HIF-1α Concentration and Microvascular Dysfunction in Systemic Sclerosis

After comparing HIF-1α plasma concentration in patients with SSc and different nailfold video-capillaroscopy patterns, we observed a significant increase in HIF-1α plasma concentrations in patients with the "active" pattern of the disease (6.625 ng/ml, IQR 2.488–11.480) compared to those with the "early" pattern (2.739, IQR 2.165–3.282, $p < 0.05$) and the "late" pattern (2.983 ng/ml, IQR 2.229–3.386, $p < 0.05$, Fig. 2).

Regarding digital ulcers in the last 12 months, in patients with SSc, those without DUs had significantly higher plasma concentrations of HIF-1α (4.367 ng/ml, IQR 2.488–9.462) compared to patients with either active DUs (2.832 ng/ml, IQR 2.630–3.094,

Table 1 Characteristics of the individuals with systemic sclerosis and control group

	Systemic sclerosis (n = 50)	Control (n = 30)	p value
General characteristics			
Age, years, mean (SD)	56.4 (11.8)	52.1 (11.7)	0.12
Sex, women, n (%)	42 (84.00%)	25 (83.33%)	0.94
Body mass index, kg/m^2	23.56 (21.31–27.44)	25.29 (22.35–26.25)	0.67
Disease subset, n (%)			
Limited cutaneous systemic sclerosis	27 (54%)	–	–
Diffuse cutaneous systemic sclerosis	23 (46%)	–	–
Systemic sclerosis duration since the diagnosis, years	6 (4–13)	–	–
Autoantibody positivity, n (%)			
Antinuclear (ANA)	50 (100%)	–	–
Anticentromere (ACA), n (%)	23 (46%)		–
Antitopoisomerase I (Topo I), n (%)	19 (38%)	–	–
Anti-RNA polymerase III, n (%)	5 (10%)	–	–
Interstitial lung disease, n (%)	29 (58%)	–	–
Esophageal dysmotility, n (%)	27 (54%)	–	–
Modified Rodnan skin score	4 (IQR 2–9)	–	–
Raynaud's phenomenon, n (%)	50 (100%)	–	–
Digital ulcers, n (%)	18 (36%)	–	–
Nailfold capillaroscopy pattern, n (%)			
"Early"	12 (24%)	–	–
"Active"	24 (48%)	–	–
"Late"	14 (28%)	–	–
Immunosuppressive therapy, n (%)			
Cyclophosphamide	3 (6%)	–	–
Methotrexate	12 (24%)	–	–
Mycophenolate mofetil	14 (28%)	–	–
Vasoactive therapy, n (%)			
Alprostadil	45 (90%)	–	–
Bosentan	0 (0%)	–	–
Calcium channel antagonist	9 (18%)	–	–
Phosphodiesterase 5 inhibitors	27 (54%)	–	–
Sulodexide	29 (58%)	–	–

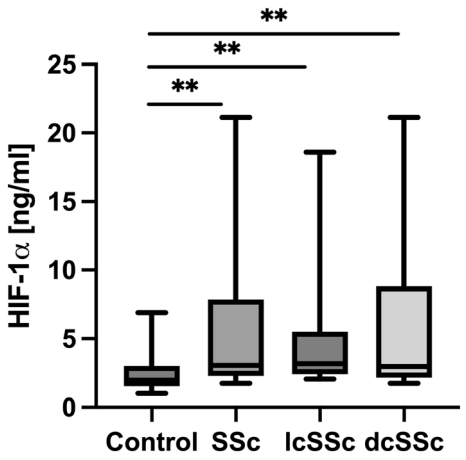

Fig. 1 Comparison of the hypoxia-inducible factor-1α (HIF-1α) plasma concentration [ng/ml] in control group, all individuals with systemic sclerosis (SSc), patients with limited cutaneous SSc (lcSSc), and patients suffering from diffuse cutaneous SSc (dcSSc)

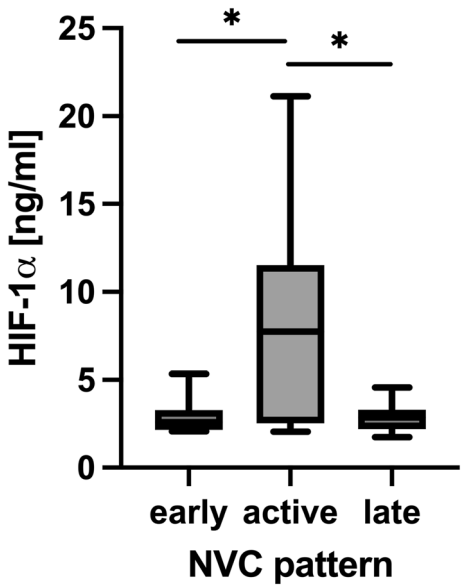

Fig. 2 Comparison of the hypoxia-inducible factor-1α (HIF-1α) plasma concentration [ng/ml] in patients with systemic sclerosis (SSc) and different nailfold capillaroscopy (NFC) patterns: "early", "active", and "late"

$p < 0.05$) or healed DUs (2.668 ng/ml, IQR 2.074–2.983, $p < 0.05$) (Fig. 3).

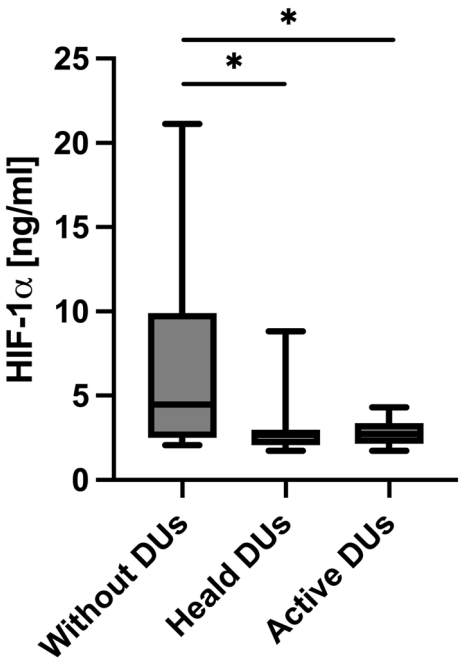

Fig. 3 Comparison of the hypoxia-inducible factor-1α (HIF-1α) plasma concentration [ng/ml] in patients with systemic sclerosis without digital ulcers (DUs), with healed DUs, and with active DUs

DISCUSSION

We observed a significant elevation in the HIF-1α plasma concentration among patients with SSc compared to the control group. This finding held true for both the subset of patients with diffuse cutaneous systemic sclerosis and limited cutaneous systemic sclerosis.

The findings of our research are consistent with those reported by Heger et al. [38], which showed that patients with SSc and secondary Raynaud's syndrome have higher serum levels of HIF-1α protein and messenger ribonucleic acid (mRNA) expression in monocytes than healthy control subjects. Heger's study included patients with both diffuse and limited SS subtypes, with approximately 40% of them having digital ulcers at the time of inclusion [38]. Mao et al. [28] reported increased expression of HIF-1α in skin biopsies obtained from patients with SSc compared to healthy tissue samples, which is consistent with our findings. However, the study was limited by its small sample size,

including only eight healthy controls and eight patients with diffuse cutaneous SSc [28].

The aforementioned studies lacked data regarding the potential correlation between HIF-1α levels and specific subtypes of SSc, as well as the evaluation of microcirculation abnormalities observed in capillaroscopy. Thus, our study serves as a significant complement to these prior investigations, enhancing knowledge on associations of HIF-1α with disease activity. We did not find a significant difference in HIF-1α concentration between the limited and diffuse cutaneous SSc groups. However, our findings revealed a significant correlation between elevated HIF-1α levels and microcirculatory dysfunction in patients with SSc, as evidenced by the abnormalities observed in nailfold capillaroscopy. Specifically, patients with the "active" pattern of the disease exhibited significantly higher levels of HIF-1α compared to those with the "early" or "late" pattern.

Vascular damage, which can be observed in capillaroscopy, is an inherent and prominent characteristic in the clinical presentation of SSc [39]. The "early" pattern of SSc is defined by the presence of a limited number of giant capillaries and microhemorrhages, the absence of avascular regions, and a relatively well-preserved capillary distribution. At this stage, loss of capillaries, vascular architectural disorganization, and ramified capillaries are uncommon. The "active" pattern exhibits a significant increase in nailfold capillary aberrations in comparison to the "early" pattern and is characterized by numerous giant capillaries and microhemorrhages, moderate capillary loss (20–30%), and a slightly disorganized capillary architecture with very few branched capillaries. In the "late" pattern a significant absence of giant capillaries and microhemorrhages, extensive avascular regions (with a 50–70% capillary loss), a large number of branched and ramified bushy capillaries (indicative of neoangiogenesis), and complete disarray of the capillary arrangement are seen serving as a hallmark of advanced microcirculation disease [40]. HIF-1α might promote dysregulation of angiogenesis and vasculogenesis, resulting in the abnormalities that can be observed in nailfold capillaries [41].

In contrast to our results, in a study by Ioannou et al. [16], the expression of oxygen-regulated subunit of HIF-1 and VEGF in the skin biopsies in patients with SSc were increased, but there were no statistically significant differences in the expression of HIF-1α and VEGF between patients at different stages of progression (early, active, or late, classified according to capillaroscopic and clinical criteria). In our study, we specifically analyzed blood-circulating HIF-1α levels. The dynamics and interrelation of skin and blood-circulating HIF-1α are still not well defined and require further investigation.

Previous studies examining HIF-1α levels did not analyze the relationship between HIF-1α levels and the existence of ulcers [28, 38]. According to our results, patients with SSc without digital ulcers had significantly higher levels of HIF-1α compared to patients with SSc and either active or healed digital ulcers. This observation, coupled with the finding of elevated HIF-1α levels in patients with SSc and "active" but not "early" or "late" capillaroscopic patterns could indicate the role of this biomarker in identification of patients without the history of ulcers but during acute acceleration in the process of ischemia. We consider this observation to be hypothesis-generating and believe it warrants prospective validation in future studies. The objective would be to identify a subgroup of patients early in the disease stage who have never experienced ulcers but may be at a higher risk of developing them.

Biomarkers related to angiogenesis have been thoroughly investigated in patients with SSc and further explored as potential indicators of organ involvement. Among these biomarkers, VEGF has been the most extensively researched angiogenic mediator. It is a powerful angiogenic factor that encourages the migration, proliferation, and survival of ECs as well as endothelial precursor cells. Distler et al. discovered higher levels of VEGF in patients with SSc who did not have fingertip ulcers, which further suggests a potential protective effect of VEGF [42]. The generation of new blood vessels is crucial in the process of repairing damaged tissues. *VEGF*, a target gene of HIF-α, was considered the most potent endothelial-specific mitogen among those that mediate the process

of vascular remodeling [43]. However, studies have shown that microvascular ECs isolated from patients with SSc have impaired responses to growth factors, including VEGF, which can result in insufficient angiogenesis [44]. VEGF is well known for its role in promoting angiogenesis, and its association with HIF-1α is well established [45]. The hypoxia-inducible factor is a pivotal transcription factor induced under hypoxia which transactivates target genes such as *VEGF* [46]. *VEGF* is a major transcriptional target for HIF-1. Signaling through VEGF receptors has been reviewed [47, 48]. HIF-1 stimulates the production of extracellular matrix fibers, inducing vascular remodeling which leads to abnormal angiogenesis, ultimately resulting in the exacerbation of hypoxia [49].

The serum concentration of HIF-1α seems to be related to the current state of microcirculatory damage. Heger et al. [38] investigated the impact of different treatments on serum HIF-1α levels in patients. The authors conducted a randomized, single-center study to compare the therapeutic outcomes of prostaglandin E_1 (PGE_1) monotherapy versus the co-administration of PGE_1 and an endothelin-1 blocker, bosentan. The authors also found that patients receiving single-drug therapy demonstrated an increase in HIF-1α expression, while patients undergoing combined therapy showed no significant differences in HIF-1α expression with a tendency toward lower expression. An increase in HIF-1α expression in patients treated with only a single drug may be due to disease progression and further deterioration of microcirculation which was prevented in the dual-therapy group [38]. Once again, these findings suggest the potential usefulness of HIF-1α as a biomarker for disease diagnosis and monitoring.

It is important to acknowledge the limitations of our study and interpret the results accordingly. The small number of participants is due to the rarity of the disease and the strict inclusion criteria. However, these criteria were necessary to minimize potential biases by controlling factors that could influence baseline levels of HIF-1α. Replicating these findings in larger patient cohorts is warranted, and further prospective evaluation of HIF-1α in patients at the early stages of the disease would contribute to expanding our knowledge in this field.

CONCLUSION

Vascular abnormalities are important clinical causes of morbidity and mortality in patients with SSc. Our study demonstrates that HIF-1α plasma concentration is significantly elevated in patients with SSc and was able to differentiate between those with "active" from "early" and "late" patterns of vascular damage in capillaroscopy. These findings emphasize the potential clinical utility of HIF-1α in evaluating microcirculatory changes and vascular abnormalities in patients with SSc. This holds promise in aiding the identification of individuals who are still in the early stages of the disease but at risk of disease progression.

ACKNOWLEDGEMENTS

We thank the participants of the study.

Funding. This research was funded by the Medical University of Warsaw, grant number 1M4/1/MBM/N/21. No funding or sponsorship was received for this study or publication of this article.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Medical Writing and Editorial Assistance. The authors did not receive any medical writing support or editorial assistance for this paper.

Author Contributions. Conceptualization (Mariusz Sikora and Lidia Rudnicka); Methodology (Magdalena Maciejewska, Mariusz Sikora); investigation (Magdalena Maciejewska, Mariusz Sikora, Albert Stec, Michal Zaremba); writing—original draft preparation (Magdalena

Maciejewska, Mariusz Sikora); writing—review and editing, (Magdalena Maciejewska, Mariusz Sikora, Cezary Maciejewski, Katarzyna Pawlik, Lidia Rudnicka); supervision (Mariusz Sikora and Lidia Rudnicka).

Disclosures. Magdalena Maciejewska, Mariusz Sikora, Albert Stec, Cezary Maciejewski, Katarzyna Pawlik, Michał Zaremba and Lidia Rudnicka have nothing to disclose.

Compliance with Ethics Guidelines. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Bioethical Committee of the Medical University of Warsaw (KB/90/2018 of 21 May 2018). Informed consent was obtained from all subjects involved in the study.

Data Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Open Access. This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc/4.0/.

REFERENCES

1. Denton CP, Khanna D. Systemic sclerosis. Lancet. 2017;390(10103):1685–99.
2. LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol. 2001;28(7):1573–6.
3. Saygin D, Highland KB, Tonelli AR. Microvascular involvement in systemic sclerosis and systemic lupus erythematosus. Microcirculation. 2019;26(3):e12440.
4. Miziołek B, Bergler-Czop B, Kucharz E, et al. Significance of the angiotensin I/angiotensin II/angiotensin-(1–7) axis in the pathogenesis of systemic sclerosis. J Eur Acad Dermatol Venereol. 2020;34(3):558–64.
5. LeRoy EC. Systemic sclerosis. A vascular perspective. Rheum Dis Clin N Am. 1996;22(4):675–94.
6. Cutolo M, Pizzorni C, Sulli A. Nailfold video-capillaroscopy in systemic sclerosis. Z Rheumatol. 2004;63(6):457–62.
7. Hughes M, Herrick AL. Digital ulcers in systemic sclerosis. Rheumatology (Oxford). 2017;56(1):14–25.
8. Hughes M, Allanore Y, Chung L, Pauling JD, Denton CP, Matucci-Cerinic M. Raynaud phenomenon and digital ulcers in systemic sclerosis. Nat Rev Rheumatol. 2020;16(4):208–21.
9. Maciejewska M, Sikora M, Maciejewski C, Alda-Malicka R, Czuwara J, Rudnicka L. Raynaud's phenomenon with focus on systemic sclerosis. J Clin Med. 2022;11(9):2490.
10. Minier T, Guiducci S, Bellando-Randone S, et al. Preliminary analysis of the very early diagnosis of systemic sclerosis (VEDOSS) EUSTAR multicentre study: evidence for puffy fingers as a pivotal sign for suspicion of systemic sclerosis. Ann Rheum Dis. 2014;73(12):2087–93.
11. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2013;65(11):2737–47.
12. Sakkas LI, Chikanza IC, Platsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. Nat Clin Pract Rheumatol. 2006;2(12):679–85.
13. Tsou PS, Varga J, O'Reilly S. Advances in epigenetics in systemic sclerosis: molecular mechanisms and

therapeutic potential. Nat Rev Rheumatol. 2021;17(10):596–607.

14. Abraham D, Distler O. How does endothelial cell injury start? The role of endothelin in systemic sclerosis. Arthritis Res Ther. 2007;9(Suppl 2):S2.

15. Cantatore FP, Maruotti N, Corrado A, Ribatti D. Angiogenesis dysregulation in the pathogenesis of systemic sclerosis. Biomed Res Int. 2017;2017: 5345673.

16. Ioannou M, Pyrpasopoulou A, Simos G, et al. Upregulation of VEGF expression is associated with accumulation of HIF-1α in the skin of naïve scleroderma patients. Mod Rheumatol. 2013;23(6): 1245–8.

17. Robinson A, Keely S, Karhausen J, Gerich ME, Furuta GT, Colgan SP. Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. Gastroenterology. 2008;134(1):145–55.

18. Yuan G, Nanduri J, Bhasker CR, Semenza GL, Prabhakar NR. Ca2+/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. J Biol Chem. 2005;280(6):4321–8.

19. Shankar J, Thippegowda PB, Kanum SA. Inhibition of HIF-1alpha activity by BP-1 ameliorates adjuvant induced arthritis in rats. Biochem Biophys Res Commun. 2009;387(2):223–8.

20. Li G, Zhang Y, Qian Y, et al. Interleukin-17A promotes rheumatoid arthritis synoviocytes migration and invasion under hypoxia by increasing MMP2 and MMP9 expression through NF-κB/HIF-1α pathway. Mol Immunol. 2013;53(3):227–36.

21. Sun X, Jiao X, Ma Y, et al. Trimethylamine N-oxide induces inflammation and endothelial dysfunction in human umbilical vein endothelial cells via activating ROS-TXNIP-NLRP3 inflammasome. Biochem Biophys Res Commun. 2016;481(1–2):63–70.

22. Deng W, Feng X, Li X, Wang D, Sun L. Hypoxia-inducible factor 1 in autoimmune diseases. Cell Immunol. 2016;303:7–15.

23. Cukrowska B, Sowińska A, Bierła JB, Czarnowska E, Rybak A, Grzybowska-Chlebowczyk U. Intestinal epithelium, intraepithelial lymphocytes and the gut microbiota—key players in the pathogenesis of celiac disease. World J Gastroenterol. 2017;23(42): 7505–18.

24. Smith V, Herrick AL, Ingegnoli F, et al. Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. Autoimmun Rev. 2020;19(3): 102458.

25. Luo Z, Tian M, Yang G, et al. Hypoxia signaling in human health and diseases: implications and prospects for therapeutics. Signal Transduct Target Ther. 2022;7(1):218.

26. Wipff J, Dieude P, Avouac J, et al. Association of hypoxia-inducible factor 1A (HIF1A) gene polymorphisms with systemic sclerosis in a French European Caucasian population. Scand J Rheumatol. 2009;38(4):291–4.

27. Takagi K, Kawamoto M, Higuchi T, Tochimoto A, Harigai M, Kawaguchi Y. Single nucleotide polymorphisms of the HIF1A gene are associated with susceptibility to pulmonary arterial hypertension in systemic sclerosis and contribute to SSc-PAH disease severity. Int J Rheum Dis. 2020;23(5):674–80.

28. Mao J, Liu J, Zhou M, Wang G, Xiong X, Deng Y. Hypoxia-induced interstitial transformation of microvascular endothelial cells by mediating HIF-1α/VEGF signaling in systemic sclerosis. PLoS ONE. 2022;17(3):e0263369.

29. Cabral-Marques O, Riemekasten G. Vascular hypothesis revisited: role of stimulating antibodies against angiotensin and endothelin receptors in the pathogenesis of systemic sclerosis. Autoimmun Rev. 2016;15(7):690–4.

30. Nicolosi PA, Tombetti E, Maugeri N, Rovere-Querini P, Brunelli S, Manfredi AA. Vascular remodelling and mesenchymal transition in systemic sclerosis. Stem Cells Int. 2016;2016:4636859.

31. Mostmans Y, Cutolo M, Giddelo C, et al. The role of endothelial cells in the vasculopathy of systemic sclerosis: a systematic review. Autoimmun Rev. 2017;16(8):774–86.

32. Michalska-Jakubus MM, Rusek M, Kowal M, Czop M, Kocki J, Krasowska D. Anti-endothelial cell antibodies are associated with apoptotic endothelial microparticles, endothelial sloughing and decrease in angiogenic progenitors in systemic sclerosis. Postepy Dermatol Alergol. 2020;37(5): 725–35.

33. Chrabaszcz M, Małyszko J, Sikora M, et al. Renal involvement in systemic sclerosis: an update. Kidney Blood Press Res. 2020;45(4):532–48.

34. Sakkas LI, Simopoulou T, Katsiari C, Bogdanos D, Chikanza IC. Early systemic sclerosis-opportunities for treatment. Clin Rheumatol. 2015;34(8): 1327–31.

35. Graf SW, Hakendorf P, Lester S, et al. South Australian Scleroderma Register: autoantibodies as predictive biomarkers of phenotype and outcome. Int J Rheum Dis. 2012;15(1):102–9.

36. Jog NR, James JA. Biomarkers in connective tissue diseases. J Allergy Clin Immunol. 2017;140(6): 1473–83.

37. Miziołek B, Sieńczyk M, Grzywa R, et al. The prevalence and role of functional autoantibodies to angiotensin-converting-enzyme-2 in patients with systemic sclerosis. Autoimmunity. 2021;54(4): 181–6.

38. Heger LA, Kerber M, Hortmann M, et al. Expression of the oxygen-sensitive transcription factor subunit HIF-1α in patients suffering from secondary Raynaud syndrome. Acta Pharmacol Sin. 2019;40(4): 500–6.

39. Furue M, Mitoma C, Mitoma H, et al. Pathogenesis of systemic sclerosis-current concept and emerging treatments. Immunol Res. 2017;65(4):790–7.

40. Cutolo M, Pizzorni C, Secchi ME, Sulli A. Capillaroscopy. Best Pract Res Clin Rheumatol. 2008;22(6):1093–108.

41. Cutolo M, Sulli A, Secchi ME, Olivieri M, Pizzorni C. The contribution of capillaroscopy to the differential diagnosis of connective autoimmune diseases. Best Pract Res Clin Rheumatol. 2007;21(6): 1093–108.

42. Distler JH, Strapatsas T, Huscher D, et al. Dysbalance of angiogenic and angiostatic mediators in patients with mixed connective tissue disease. Ann Rheum Dis. 2011;70(7):1197–202.

43. Zhang D, Lv FL, Wang GH. Effects of HIF-1α on diabetic retinopathy angiogenesis and VEGF expression. Eur Rev Med Pharmacol Sci. 2018;22(16):5071–6.

44. Müller-Ladner U, Distler O, Ibba-Manneschi L, Neumann E, Gay S. Mechanisms of vascular damage in systemic sclerosis. Autoimmunity. 2009;42(7): 587–95.

45. Basic J, Stojkovic S, Assadian A, et al. The relevance of vascular endothelial growth factor, hypoxia inducible factor-1 alpha, and clusterin in carotid plaque instability. J Stroke Cerebrovasc Dis. 2019;28(6):1540–5.

46. Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. J Neuroimmune Pharmacol. 2014;9(2):142–60.

47. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. Genes Cancer. 2011;2(12): 1097–105.

48. Jeltsch M, Leppänen VM, Saharinen P, Alitalo K. Receptor tyrosine kinase-mediated angiogenesis. Cold Spring Harb Perspect Biol. 2013;5(9):a009183.

49. Guan SY, Leng RX, Tao JH, et al. Hypoxia-inducible factor-1α: a promising therapeutic target for autoimmune diseases. Expert Opin Ther Targets. 2017;21(7):715–23.