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Original Article

Expression level of hypoxia inducible factor 1α gene in psoriatic patients in Suez Canal region

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Abstract

Objective To compare the expression of the HIF-1α gene in patients with psoriasis to that of healthy controls to assess the role of the HIF-1α gene in the initiation, progression and severity of psoriasis.

Methods A case–control study with 36 patients with psoriasis and 36 healthy controls was conducted. Blood samples were taken from both the patients and controls. For both groups, the relative expression of the HIF-1α gene will be evaluated using the polymerase chain reaction (PCR) technique.

Results PASI scores ranged from 2–34. Eighteen patients (50%) had mild psoriasis, 9 patients (25%) had moderate psoriasis and 9 patients (25%) had severe psoriasis. Only 6 patients (16.7%) had a family history of psoriasis. The mean BMI for population with psoriasis was 24.06±3.18. The control group matched the psoriasis group regarding age, gender. The mean relative HIF-1α expression levels of the psoriatic and control groups were 3.38±1.87 and 1.0±0.0, respectively. The main findings were that there was a significant increase in the HIF-1α gene expression in patients with psoriasis compared with controls, and that expression level significantly increased with psoriasis severity.

Conclusion HIF-1α expression level was significantly higher in patients with psoriasis compared to healthy controls, implying its role throughout psoriasis pathogenesis.

Key words
Psoriasis, hypoxia inducible factor-1α gene, hypoxia.

Introduction

Psoriasis is now recognized as a common serious systemic inflammatory condition, with females under the age of 20 having a higher frequency than males.[1,2] Its chronicity, consequences, treatment burden has effect on quality of life and community.[3] It is a multifactorial disease triggered by environmental factors in a genetically susceptible person, with first-degree family member affection in 30% of them.[4]

Psoriasis is linked to more than 40 regions of the genome, including signal transduction and transcription activation of 3C (STAT3C), late cornified envelope 3A (LCE3A), zinc finger protein 816A (ZNF816A), endoplasmic reticulum aminopeptidase 1 (ERAP1), psoriasis susceptibility 1 candidate 3 (PSORS1C3) and intracellular adhesion molecule 1 (ICAM1).[5]

In psoriasis, keratinocyte proliferation, angiogenesis, and surveilling immune cells intensify skin hypoxia, increasing hypoxia

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inducible factor-1α (HIF1α) levels in the skin and serum of patients with psoriasis.[6,7] HIF1α gene is located on chromosome 14q23.2 (National Centre for Biotechnology Information, NCBI). HIF-1α regulates the gene expression of over 100 genes region that are involved in major cellular functions such as cell proliferation, erythropoiesis, angiogenesis, apoptosis, iron metabolism and glucose metabolism. Hypoxia, inflammation and leukocyte activation, vascular endothelial growth factor (VEGF), and tumour necrosis factor–α trigger HIF1α.[8,9] Cells undergoing hypoxic stress exposed to genetic instability through HIF1α that acts as a transcriptional repressor of the Mut S homolog 6 (MSH6) and Mut S homolog 2 (MSH2) genes by blocking mismatch recognition and DNA repair.[10] The HIF1α gene is involved in innate immunity, (TCR) T cell receptor integration, cytokine receptor–mediated signals of CD4+ helper T cells, and Th17 development.[11,12]

Methods

This case-control study recruited patients from the Dermatology clinic, Suez Canal University hospital. Molecular analysis was performed at the Oncology Diagnostic and Research Unit, Faculty of Medicine, Suez Canal University. The study population included 36 patients diagnosed with psoriasis compared to 36 healthy controls. Following approval from the Suez Canal University's Faculty of Medicine's Institutional Research Review Board Ethical Committee, the study was conducted in accordance with the Helsinki Declaration guidelines. All participants signed written informed consent forms.

Selection criteria

Group I: cases inclusion criteria Egyptian patient with psoriasis ≥18 years old, and both genders.

Exclusion criteria Chronic dermatological diseases, autoimmune diseases, malignancies, ischaemic heart or lung disease, patients receiving topical or systemic therapy for psoriasis in the last three months, and those who refused to take part in the research.

Group II: controls inclusion criteria Egyptian normal healthy volunteers, both genders, and any age.

Exclusion Criteria Refusal to take part in the research.

Sample size

$$n = \left[\frac{Z_{\alpha/2} + Z_{\beta}}{P_1 - P_2}\right]^2 (p_1 q_1 + p_2 q_2)$$

The aforementioned formula was used to calculate the sample size where n=sample size Z α/2=1.96 (The critical value that separates the central 95% of the Z distribution from the tail's 5%). Zβ=0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%). p1=18.2% of the study groups relative expression.[13] p2=proportion of gene expression in the control group=0% q=1-P. As a result, the sample size (n) is calculated to be 36 patients per group.

Participants were subjected to personal history, general and clinical examination.[14] The extension and severity of psoriasis were evaluated with PASI scores. PASI is a composite score based on four body areas: the head (10% of body skin), the arms (20% of body skin), the trunk (30% of body skin), and the legs (40%). In addition, the degree of erythema (redness), induration (thickness), and desquamation were evaluated for each region of the body as plaque clinical signs (scaling). The clinical sign scores were added up in each region of the body, then weighted based on the region's proportion of the body before being converted to

the final score, which ranged from zero to a theoretical maximum of 72.[15]

Laboratory investigation for both groups

Blood sampling 2 mL blood sample was collected in EDTA vacutainers from peripheral veins of each participant in this study. Samples were centrifuged for 15 min at a speed of 4000 rpm. Samples were processed immediately for molecular analysis within 24 h.

Workplace preparation The laminar flow was swabbed with ethyl alcohol (70%), and then UV lamp of the laminar flow was turned on for 15 min for sterilization. All chemicals and bottles, micropipette and tube rack were swabbed with ethyl alcohol (70%). All used micropipettes, and tips, and Eppendorf sterilized by auto clave device.[16]

HIF1 gene real-time PCR expression analysis

Total RNA was extracted from whole blood using RNeasy Mini Kit (GeneJET Whole Blood RNA Purification Mini Kit, Catalogue no. K0761, Thermo Fisher Scientific, UK) following the manufacture protocol. The Nanodrop (ND1000) spectrophotometer was used to determine the total RNA concentration and purity at 260/280 nm absorbance ratio (Nanodrop Tech. Inc., Wilmington, DE, USA).

Furthermore, we tested its integrity using 1 percent agarose gel electrophoresis. The purified RNA was used immediately in downstream applications or stored at (-80 ^{0}C) until assessing expression of desired genes. The Applied Biosystems^{TM} High-Capacity cDNA Reverse Transcription kit manufacturer's instructions were followed for cDNA synthesis (catalogue no. 4368814, Thermo Fisher Scientific, UK). Relative expression of HIF-1α was determined using real-time polymerase chain reaction (PCR) technique, which was conducted in 25µL reaction volume containing 12.5 SYBR Green PCR master mix (Powerup SYPER Green Master Mix, catalogue no A25779, Thermo Fisher Scientific, UK), 1µl (10 µM) for upper and lower primer listed in **Table 1**, 2.5 µL of 25 ng cDNA template and 8µl of PCR nuclease-free water. The following PCR conditions were used with a step one Real-Time PCR system (Applied Biosystems, Foster City, CA, USA): after 5 min of denaturing at 95°, PCR amplification was performed for 40 cycles of 15 seconds at 95°, 1 min at 58°, and 1 min at 72°.

The mRNA expression levels of the mentioned gene were normalized with average expression of housekeeping gene (GPDH). Differences in expression were observed by comparing psoriasis samples with control samples. The 2-ΔΔCT method was used for quantification (Livak and schmittgen, 2001).

Statistical data analysis

Data was fed into the computer and analyzed with the IBM SPSS software package version 20.0. (IBM Corporation, Armonk, New York) Numbers and percentages were used to describe qualitative data. The Kolmogorov-Smirnov test was used to confirm the distribution's normality. Range (minimum and maximum), mean, median, and standard deviation were used to describe quantitative data.

Table 1 The sequences of the primers used for PCR amplification.

Gene	*Sequence*
HIF1α	Upper 5′-GATGGAAGCACTAGACAAAGTTCA-3′ Lower 5′-ATCAGTGGTGGCAGTGGTAGTG-3
GPDH	Upper 5′- CGGAGTCAACGGATTTGGTCGTAT-3′ Lower 5′-AGCCTTCTCCATGGTGGT-3′

Table 2 Demographic data of psoriatic group [36].

	N (%)
Gender	
Male	28 (77.8)
Female	8 (22.2)
Age (Years)	
Adolescents and young youth (13 – <25)	0 (0.0)
Adulthood (25–<40)	18 (50.0)
Middle age (40–<60)	18 (50.0)
Old age (≥60)	0 (0.0)
Min.–Max.	28.0–55.0
Mean±SD.	39.94±8.26
BMI (kg/m^2)	
Underweight (< 18.5)	0 (0.0)
Ideal weight (18.5 – < 24.0)	24 (66.7)
Overweight (24.0 – < 27.0)	6 (16.7)
Obese (≥ 27.0)	6 (16.7)
Min.–Max.	18.78–31.95
Mean ± SD.	24.06±3.18
Grade of severity (PASI score)	
Mild	18 (50.0)
Moderate	9 (25.0)
Severe	9 (25.0)
Min.–Max.	2.0 – 34.0
Mean±SD.	14.30±10.64
Family history	
Positive	6 (16.7)
Negative	30 (83.3)

The significance of the obtained results was determined at the 5% level.

The chi-square test was used to compare different groups of categorical variables. Monte Carlo correction or Fisher's Exact; chi-square correction when more than 20% of the cells have an expected count of less than 5. The Student t-test is used to compare two groups of normally distributed quantitative variables. Mann–Whitney test: used to compare two groups with abnormally distributed quantitative variables. Kruskal Wallis test; used to compare more than two groups being studied with abnormally distributed quantitative variables. Spearman coefficient; to correlate with two quantitative variables that are abnormally distributed. The odds ratio (OR) is the ratio of the odds and 95% CI of an event occurring in one risk group to the odds of it occurring in the non-risk group.

Results

Table 2 revealed that the level of HIF-1α gene expression in the psoriasis group was statistically significantly higher than in the control group (p<0.001).

In the psoriatic group, there was no statistically significant difference in HIF1α relative expression between groups based on age, gender, or BMI, according to **Table 3**. Furthermore, the level of HIF 1α gene expression increased with increasing severity of psoriasis, and this difference was highly statistically significant (p≤0.001). There was no statistically significant difference in HIF1 relative expression based on family history in the psoriatic group.

Table 4 revealed a negative correlation between relative HIF1α expression level and disease duration, as well as a positive correlation between relative HIF1α expression level and age, but both were statistically insignificant (P>0.05). There was also a statistically significant (P<0.05) positive correlation between relative HIF1α expression level and PASI scores.

Table 3 Comparison between the two studied groups according to HIF1α relative expression.

HIF1α relative expression	*Psoriasis group (n = 36)*	*Control group (n = 36)*	*U*	*P*
Min. – Max.	0.40–7.50	1.0–1.0		
Mean ± SD.	3.38±1.87	1.0±0.0	36.0*	<0.001*
Median (IQR)	3.30 (1.54 – 4.60)	1.0		

U: Mann Whitney test p: p value for comparing between the studied groups

*: Statistically significant at p ≤ 0.05

Table 4 Relation between HIF1α relative expression and demographic data, family history & grade of severity (PASI) in psoriasis group (n= 36).

	N	*HIF1α relative expression*		*IQR (25th – 75th)*	*Test of sig.*	*P value*
		Mean ± SD.	*Median*			
Age (years)						
Adulthood	18	3.43 ± 2.15	2.90	1.54 – 4.80	U=156.500	0.861
Middle age	18	3.33 ± 1.60	3.75	1.59 – 4.40		
Gender						
Male	28	3.56 ± 1.90	3.75	1.54 – 4.70	U=92.0	0.445
Female	8	2.76 ± 1.74	2.25	1.52 – 4.50		
BMI						
Ideal weight 18.5 ≤ < 24.0	24	3.25 ± 2.06	2.65	1.54 – 4.60		
Overweight (24.0< 27.0)	6	3.39 ± 1.65	3.45	1.80 – 4.60	H=0.895	0.639
Obese ≥ 27.0	6	3.89 ± 1.39	4.40	3.30 – 4.80		
Family history						
Positive	6	4.92 ± 2.35	5.50	1.54 – 4.40	U=48.500	0.077
Negative	30	3.07 ± 1.64	2.90	3.30 – 6.50		
Grade severity PASI						
Mild	18	1.75 ± 0.71	1.54	1.35 – 2.0		
Moderate	9	4.57 ± 0.81	4.40	4.40 – 4.60	H=26.979*	<0.001
Severe	9	5.44 ± 1.13	5.10	4.60 – 6.40		

U: Mann Whitney test H: H for Kruskal Wallis test
p: p value for association between HIF relative expression and different parameters
*: Statistically significant at p ≤ 0.05

Table 5 Correlation between HIF1α relative expression and different parameters in psoriasis group (n= 36).

	HIF1α relative expression	
	rs	*P*
Age (years)	0.041	0.814
PASI score	0.806*	<0.001*
Duration of disease	-0.069	0.689

rs: Spearman coefficient

Table 5 revealed that there was a statistically significant (P<0.05) difference in relative HIF1α expression in the psoriasis group based on PASI grade, but no difference based on family history.

Discussion

HIF-1α is a heterodimeric transcriptional complex encompasses HIF-1α and HIF-1β subunits that have an imperative role in the maintenance of oxygen, energy homeostasis and, angiogenesis developing in psoriatic skin.[18]

The aim of this analysis was to use the real-time polymerase chain reaction (PCR) technique to determine the relative expression of the HIF-1α gene in blood samples of patients with psoriasis. It included 72 participants who were subdivided into 2 groups, 36 patients with psoriasis and 36 apparently healthy individuals as control group. The mean age 39.6±8.5 (22 to 55 years), 77.8% of them were males. PASI scores ranged from 2-34. Eighteen patients (50%) had mild psoriasis, 9 patients (25%) had moderate psoriasis and 9 patients (25%) had severe psoriasis. Only 6 patients (16.7%) had family history of psoriasis. The mean of BMI for psoriasis group was 24.06±3.18. The mean relative HIF1α expression level of the psoriatic and control group was 3.38±1.87 and 1.0±0.0 respectively.

The study showed significant increase of HIF-1α gene expression in psoriatic patients in comparison to controls; these results agreed with that reported by Yongjian *et al*.[19] significant expression of HIF-1α in psoriatic lesions of 32 Chinese patients versus 20 healthy controls (p<0.05). Rosenberger *et al*.[7] discovered that HIF-1α expression is reduced in normal skin in animal and human models, but elevated in cell

Table 6 Relation between HIF1α relative expression and different parameters in psoriasis group (n= 36).

	HIF1α relative expression				*X2*	*P*	*OR*	*95% C. I*
	Below median (≤ 3.30) (n= 19)		*Above median (>3.30) (n= 17)*					
	No.	*%*	*No.*	*%*				
Grade severity PASI								
Mild	18	94.7	0	0.0	36.757	MCp	–	–
Moderate	1	5.3	8	47.1		<0.001*	16.0	1.72 – 148.44
Severe	0	0.0	9	52.9			–	–
Family history								
No	17	89.5	13	76.5	1.092	FEp=	0.382	0.06 – 2.42
Yes	2	10.5	4	23.5		0.391	2.615	0.41 – 16.54

χ^2: Chi square test MC: Monte Carlo FE: Fisher Exact
p: p value for association between HIF1α relative expression and different parameters
OR: Odds ratio, CI: Confidence interval

types that express key angiogenic factors (psoriatic skin). The vasculature of adult skin remains normally dormant due to the predominant control of intrinsic angiogenesis inhibitors over angiogenic stimulation, according to Detmar,[20] but skin possesses the potential for rapid angiogenesis induction. In the growth process of hair follicles, cyclic vascular growth caused by keratinocyte-derived VEGF occurs. HIF-1α mRNA is translated into protein by VEGF via Akt and phosphoinositol-3 kinase. According to Xia and colleagues,[21] genetically modified VEGF delivery to mouse skin can induce the full psoriatic phenotype. After 6 months, these mice developed psoriatic plaques on their own, which could be prevented by inhibiting VEGF. Short-term VEGF administration to the skin, on the other hand, did not result in psoriatic lesions.[22] Such findings imply that long-term dermal VEGF activation is both required and adequate to cause psoriasis. Rosenberger and colleagues[7] suggested that HIFs and Akt are critical elements in psoriatic angiogenesis, and that they fit in to established scene of inflammatory response and epithelial proliferation. Given that HIF-1α is involved in T-cell sustainability and activity, HIF-1α stimulation in T cells relates to psoriatic pathophysiology.[23] Furthermore, our findings were consistent with those of Ioannou and colleagues,[18] who found that HIF-1α immunostaining was distinguishable in psoriasis. They also found that HIF-1α immunoreactivity 'final' scores were significantly higher on average of psoriasis or psoriasiform dermatitis rather than the samples of normal skin. Results showed a statistically significant difference in HIF-1α immunoreactivity scores among psoriasis and psoriasiform dermatitis. The factor(s) underlying the increased HIF-1α immunoreactivity in psoriasis vary from those able to operate in hypoxia, and they require pro-inflammatory cytokine activation.[18]

At least 8 widely accepted cytokines can cause HIF-1α accumulation.[24] Most these cytokines are identified to be active in psoriasis and involved in the expanding HIF-1α immunoreactivity in psoriatic keratinocytes.[25] Eventually, Ioannou *et al*.[18] discovered that the enhanced HIF-1α staining had a particular localization, with favourable immunostaining in the keratinocytes of the suprapapillary and peripapillary epidermis overlying the inflamed, elongated dermal papillae that housed the characteristic tortuous blood vessels. Vasillopolus *et al.*[26] discovered a significantly higher HIF-1α protein expression in patients with psoriasis compared to healthy controls. Moreover, several other cytokines engaged either in promoting (IL-2, IL-12) or sustaining

(IL-6, TNF, IFN) inflammation were significantly higher in patients with psoriasis compared to healthy controls. Even so, the association of HIF-1α and IL-6 in psoriasis indicated a strong bond between HIF-1α and IL-6 in the immune microenvironment of hypoxia-driven angiogenesis. These findings corroborated our previous findings on HIF-1α overexpression in patients with psoriasis. Inordinate keratinocyte proliferation in psoriasis causes local environmental hypoxia, which indicates an increase in HIF-1α expression.[27] Microenvironment hypoxia speeds up vascular overall growth rate to supply the oxygen necessary for cell growth, as well as increased VEGFA expression during this step. Furthermore, HIF-1α and VEGFA have been identified as critical regulators during the immunopathogenesis of psoriasis.

Rosenberg *et al.*[7] (on 23 psoriatic specimens) and Tao *et al.*[28] (on 30 psoriatic specimens) found that HIF-1α expression was weak and central focus in healthy epidermis compared to fierce and diffuse expression in psoriatic epidermis. HIF-1α expression was also found in hair follicles, sebaceous glands and sweat glands. The presence of high HIF-1α levels in both involved and uninvolved psoriatic epidermis was also observed in Li *et al.*[29] research, which used the western blot technique on 70 lesional and non-lesional specimens. Pathological changes in unassociated skin in patients with psoriasis explains its susceptibility to Koebnerization. All results concurred with our findings on HIF-1α at the molecular level.

The master transcriptional regulator of the dynamic response to hypoxia is defined as HIF-1α. HIF-1α is an intracellular in an inactive form throughout normoxic conditions; under hypoxic conditions, HIF-1α is over-expressed and transferred to the nucleus. It needs to interact with HIF-1β in the nucleus to promote transcription of hypoxia-related target genes, which can govern a variety of processes including apoptosis, angiogenesis, proliferation, energy metabolism and erythropoiesis.

Conclusion

This study shed light on the potential role of the HIF1α gene in the pathogenesis of psoriasis by demonstrating a statistically significant higher HIF1α gene expression in patients with psoriasis than in healthy controls. More research is needed to confirm the mechanism by which the HIF1α gene contributes to psoriasis pathogenesis.

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