ESTIMATION OF NITRIC OXIDE SYNTHASE EXPRESSION LEVEL IN DISTINGUISHING PSORIASIS FROM ATOPIC DERMATITIS AND CORRELATING IT WITH DISEASE SEVERITY

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Skin disorders, the fourth most prevalent cause of disease in humans, affect approximately one-third of the world's population. Nevertheless, their significance is typically overestimated (Karimkhani *et al*., 2017 and Flohr and Hay, 2021). The high incidence of skin conditions and the long-term morbidity they cause, such as atopic dermatitis and psoriasis, constitute the burden of skin disorders. As the average life expectancy of the global population rises, this is expected to get worse (Hay *et al*., 2015). Clinically distinct chronic inflammatory skin illnesses include psoriasis (PS) and atopic dermatitis (AD). Their prevalence ranges from 3% to 10% worldwide, and there is a yearly upward tendency. PS, however, is frequently confused with AD in the younger population (Chen *et al*., 2022). Even though the two disorders are distinct, they have several similarities, including increased expression of specific cytokines that cause inflammation, genetic variables, environmental factors, and altered barrier functions (Guttman-Yassky *et al*., 2018). Although psoriasis and AD are T-helper (Th)-mediated diseases, AD is seen as a polar Th2 disease, whereas psoriasis is driven by Th1/Th17 (Napolitano *et al*., 2019). According to the theory that links the Th1 and Th2 pathways, reducing or inhibiting the Th1 response will increase the Th2 balance and vice versa. As a result, AD and psoriasis have to oppose immune-mediated pathways (Napolitano *et al*., 2019 and Megna and Fabbrocini, 2020).

Skin produces nitric oxide (NO), a free radical molecule, along with nearly all other tissues. Physiological levels of NO are necessary to sustain normal cellular activity, even though that high levels of NO can cause oxidative stress and other systemic disorders. The three NO synthase enzyme isoforms are neuronal NOS (nNOS), constitutive endothelial NOS (eNOS), and inducible NOS (iNOS/NOS2). In human skin, all of these

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isoforms have been detected (Vaccaro *et al*., 2017). Unlike eczema, where it was shown to be expressed at lower levels or lacking at both an mRNA and protein level, NOS2 is absent in normal skin and significantly increased in psoriatic lesional skin (Nomura *et al*., 2003 and Natalie and Kilian, 2015). Low NO levels are the only levels that stimulate keratinocyte proliferation, a characteristic of psoriasis, in contrast to high NO levels, which stop cell proliferation and begin differentiation. Therefore, NO production in psoriasis is thought to be significantly reduced despite NOS2 overexpression (Natalie and Kilian 2015 and Abeyakirthi *et al*., 2010).

Therefore, our goal was to examine NOS2 expression to differentiate between atopic dermatitis and psoriasis and to establish its relationship with the severity and activity of the disease.

PATIENTS AND METHODS

Design and Population

This study was designed to investigate a molecular marker used to differentiate between psoriasis and eczema. Patients provided informed written consent about the research. The faculty of Medicine, Menoufia University's ethics approval committee accepted the current study in its monthly meeting. Patients were divided into three groups as follows: group 1 consisted of 50 patients with psoriasis who had been clinically diagnosed and evaluated; group 2 comprised of 50 patients with atopic dermatitis; and group 3 consisted of 50 healthy volunteers who were matched for age and sex and served as the control group. From the outpatient clinic of Menoufia University, clinically diagnosed patients with either atopic dermatitis or psoriasis were included in this study. The patients' diagnoses were made for the first time without any medical history. Gene expression analysis was performed at the Molecular Diagnostics Therapeutics and Genomics lab - Department of Molecular Diagnostics and Therapeutics at the Genetic Engineering and Biotechnology Research Institute - University of Sadat City. Patients received a thorough general, local examination and an in-depth review of their personal, recent, and family histories. Clinical criteria previously published were used to diagnose (Eyerich *et al*., 2011). Using the PASI (Psoriasis Area and Severity Index), EASI (Eczema Area and Severity Index), and SCORing Atopic Dermatitis scores (SCORAD), the patient's clinical condition was evaluated.

Inclusion criteria

Adult patients (ages ranging from 18 to 60 years) with newly diagnosed psoriasis and eczema who are not receiving any systemic treatment were the cases selected and included in the study.

Exclusion criteria

Patients with any dermatological disorder other than psoriasis and eczema, as well as those with any systemic, autoimmune, endocrine, or multiple sclerosis

diseases, as well as those who were taking systemic steroids, methotrexate, biological therapy, or any other systemic medications, were excluded from this study.

Laboratory investigations

Through sterile venipuncture, five milliliters of venous blood samples were taken. Each sample was divided into two portions. The first portion was placed into sterile vacutainer tubes containing EDTA to extract total RNA and perform a complete blood count (CBC) using an automated hematology analyzer (Pentra 80). The second portion was centrifuged at 1500 rpm for 10 minutes to separate serum samples for liver and kidney functions using the Cobas 6000 analyzer (c 501 modules).

Isolation of total RNA

Blood samples were stored at -80ºC until total RNA was isolated using the MagNA Pure Compact Nucleic Acid Isolation Kit I, following the manufacturer's instructions (Cat. No. 03 730 964 001). A NanoDrop ND-1000 spectrophotometer was used to measure the quantity and quality of RNA (Thermo Fisher Scientific, Inc.). RNA purity was determined by comparing the absorbance ratios of 260/280 nm and 260/230 nm (Farhat, 2012; Lehninger 1975 and Kilby 1976).

Quantification of NOS2 expression level

Using TaqMan Low-Density Array cards from Thermo Fisher, quantitative RT-PCR was used to synthesize complementary DNA (cDNA). A mixture of 1 µl of reverse transcriptase enzyme, 4 µl of 5x TransAmp buffer, and 5 µl of RNase-free water was added to 10 µl of RNA extract. The reverse transcriptase enzyme was stopped using an Applied Biosystems 2720 Thermal Cycler, ALT, USA (Bioline, USA) for a single cycle that lasted 10 minutes at 85ºC, 15 minutes at 42ºC., and finally 5 minutes at 85ºC. For the targeted amplification of the NOS2 gene and the internal control GAPDH gene, primers and hydrolysis probes were designed using NCBI's Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer blast/). The primer and probe sequences are summarized in Table (1). The reactions went through 40 cycles of denaturation for 15 s at 95ºC, annealing for 30 s at 58ºC, and extension for 30 s at 72ºC, with a final step of extension for 3 minute at 72ºC. The cycle threshold in real-time PCR refers to the number of cycles required for the concentration of the amplicon to pass the threshold (CT). The relative expression levels of NOS2 over GAPDH (housekeeping reference gene) were determined by using the equation $2^{(-\Delta\Delta CT)}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

Utilizing SPSS (Statistical Package for Social Sciences) version 28 for Windows®, the gathered data was coded, processed, and analyzed (IBM SPSS Inc, Chicago, IL, USA). Frequency distributions and relative percentages were used

to display qualitative data. The mean SD format was used to express quantitative data (Standard deviation). Statistical significance was determined using two-tailed P values ($p<0.05$). To differentiate between psoriasis and eczema, the Receiver Operating Curve (ROC) test was used to calculate the diagnostic indices (sensitivity, specificity, positive and negative predictive values, and accuracy) for NOS2.

RESULTS AND DISCUSSION

Patient's baseline characteristics

The demographic and medical characteristics of the study population are shown in table (2). This study included 20 males and 30 females diagnosed with psoriasis, with a mean age of 39.6 ± 15.4 years, and 37 males and 13 females diagnosed with eczema, with a mean age of 31.0 ± 13.8 years. Different parameters, including age, gender, smoking status, and the biochemical characteristics of the liver, kidneys, and complete blood count, did not differ significantly across the study groups with no statistically significant differences ($P>0.05$), despite the distribution of disease family history. The disease duration and all disease scoring systems of psoriasis and eczema showed a highly significant variation among the studied groups ($P < 0.0001$).

The demographic and clinical characteristics of study participants were reported in the current study as exposure to psoriasis and atopic dermatitis. All studied patients' ages ranged from 18 to 60 years, with a mean of 36.6±14.9 years. The mean age at presentation of psoriasis was 39.6 ± 15.4 years, while the mean age for atopic dermatitis was 31.0 ± 13.8 years. Even though there was a statistically non-significant difference between all the studied groups, psoriasis patients were older than eczema patients. According to this study's findings, which align with those of earlier studies, psoriasis and eczema can affect people of all ages, but eczema typically manifests later in life (Abo-Zaid *et al*., 2018 and Bozek *et al*., 2020). Due to differences in the physiology and structure of the skin between the sexes, some skin problems may present in men and women differently (Cassano *et al.*, 2014). In the current study, the prevalence of psoriasis was higher in females than males, 30/20 with a ratio of 1.5:1. This result was consistent with other research that showed that females had a higher prevalence of psoriasis (Egeberg *et al*., 2017 and Guillet *et al*., 2022). However, in other studies, there was no difference in the prevalence of psoriasis between the sexes (Tsai *et al.*, 2011 and Armstrong *et al.*, 2021). In contrast, males were more likely than females to have eczema 37/13 with a ratio of 2.85:1. This result was consistent with a prior study that indicated a higher prevalence of eczema in males (de Lusignan *et al*., 2021), while another study revealed the opposite, with a higher frequency of eczema in females (Arnedo-Pena *et al*., 2020). The gender difference in disease prevalence was not statistically significant ($P>0.05$). The majority of participants in this study (81.34%) were non-smokers. Between "smokers" and "non-smokers", there were

no significant variations in the prevalence of psoriasis and eczema. The smoking prevalence among psoriasis and eczema patients was 34% and 12%, respectively. According to previous studies, smoking increases the severity of psoriasis and eczema without having a major impact on the prevalence of the diseases (Patruno *et al.*, 2014 and Wei *et al.*, 2022).

Blood relative expression of NOS2 and distinguishing between psoriasis and eczema

Using RT-qPCR analysis, the NOS2 expression levels in the blood of all examined groups were evaluated. Patients with psoriasis had the highest levels of NOS2 expression, and there was a significant difference between expression levels between psoriasis and eczema. NOS2 expression levels are shown in Table (3). The severity of psoriasis and eczema increased significantly as the disease progressed. As shown in Table (4), statistical analysis of the mean NOS2 expression level in psoriasis patients versus the mean PASI score revealed a statistically significant increase in the NOS2 level as the PASI score increased. Conversely, NOS2 expression levels in eczema patients versus the mean SCORAD score, the NOS2 level revealed a statistically significant decline as the SCORAD score increased.

It is particularly important to distinguish between eczema and psoriasis; NOS2 expression, seen in various inflammatory conditions like dermatitis and psoriasis, may cause compromised barrier function. In this study, psoriatic patients had statistically significantly higher levels of NOS2 expression (3.99±0.67) than eczema and control group participants (1.64±0.40, 0.24±0.12, respectively) ($P<0.00001$). The results of this study were consistent with those of Quaranta *et al*., (2014), who reported that NOS2 expression was upregulated in psoriasis and associated with metabolic processes as well as Th1 and Th17 responses. NOS2 was thought to play a significant role in the inflammatory process in psoriasis due to the positive correlation between PASI and NOS2 expression level. In related studies (Kadam *et al*., 2010 and Mahmoud *et al*., 2013**)**, NOS2 levels were significantly higher in patients with active psoriasis compared to healthy controls and was significantly correlated with the severity of the disease. As SCORAD and the level of NOS2 expression correlated negatively, researchers believed that NOS2 was a key factor in distinguishing psoriasis from eczema.

Receiver Operating Characteristic (ROC) of the NOS2

Receiver operator characteristic (ROC) curve analysis was used to determine the cut-off value for all NOS2 expressions to distinguish between psoriasis and eczema (Fig. 1). Outcomes of the ROC curve indicated that psoriasis patients had the highest area under the curve (AUC) value (0.850). At a cut-off value of 5.45, NOS2 showed 83.3% sensitivity and 100% specificity. The ability to differentiate between the two disease types (psoriasis and eczema) could give doctors

a helpful tool for making therapeutic decisions and could be used to provide patients suffering from atopic dermatitis and psoriasis with individualized care.

Psoriasis and eczema diagnosis is primarily based on histological analysis and subjective visual examination. Up to 50% of cases are misdiagnosed due to phenotypic overlaps between the two disorders. Recently, a gene expression-based classifier utilizing different marker genes has been developed to overcome this critical diagnostic gap (Bentz *et al.,* 2021 and Fan and Cohen *et al*., 2022). The objective of this study was to estimate the level of NOS2 expression in psoriasis and eczema patients, compare them to healthy controls to confirm NOS2 expression level as a marker to distinguish between psoriasis and eczema, and correlate it with the severity of the disease.

Psoriasis and eczema prevalence in this study was comparable in participants with and without a family history of the diseases. There was no significant difference between the effects of the diseases in the past. This result was in contrast to prior studies, which found that psoriasis in the family was associated with earlier onset and enthesitis (Solmaz *et al*., 2020 and Saunes *et al*., 2011**)** and that eczema in the index child was strongly linked to eczema in both mothers and fathers. The current study demonstrated that eczema takes longer to heal than psoriasis. This outcome supported a prior study that indicated eczema to have a longer duration than psoriasis (Yamamah *et al*., 2012). Psoriasis, atopic dermatitis, clinical severity, and treatment effectiveness were evaluated using the recognized PASI, EASI, and SCORAD index scoring systems respectively (Bang *et al*., 2021 and Aalemi *et al*., 2022). Since the scoring systems frequently evaluate the same aspects of the disease, one method might be used in everyday clinical work and serve as the basis for disease quantification in dermatology. This study compared the PSAI, ESAI, and SCORAD scoring systems to determine the disease severity in psoriasis and atopic dermatitis patients. The mean values of all studied scoring tools were (8.7±1.2, 12·8± 9·82, and 45.6±14.05), respectively. There was a highly significant variation between the mean scores for all groups ($P < 0.0001$). The current study's findings showed no significant correlation between all of the biochemical studies included and either psoriasis or eczema (liver functions, kidney functions, and complete blood count parameters). The parameter values remained within the normal range.

Our molecular test's superior sensitivity and specificity are based on NOS2, which reflects the complicated disease profile of psoriasis and eczema (Felix *et al.,* 2022). In this investigation, the NOS2 differentiated psoriasis from eczema with an AUC of 0.85, 100% specificity, and 83.3% sensitivity for psoriasis. The two disease states could be distinguished using the NOS2 ($P<0.008$). The ability to differentiate between the two disease types (psoriasis and eczema) could be a crucial tool in the hands of physicians for

clinical decision-making and the treatment of eczema and psoriasis. This finding was comparable to that of (Garzorz-Stark *et al*., 2016), who noted that NOS2 had an AUC of 0.97 with specificity and sensitivity of 100% and 92%, respectively.

CONCLUSION

NOS2 may play a role in the distinction between psoriasis and eczema. NOS2 expression was upregulated in psoriasis compared to eczema, which means that psoriasis could be routinely differentially diagnosed with high sensitivity and specificity by measuring NOS2 expression level.

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Table (1): Primer and probe sequences.

Genes	Forward primer	Reverse primer	Probe
NOS2	CCTCGGCTCCAG-CATGTAC	TGG-GACAGCTTCTGATCAATG	TCGGTTCTGCGCCTTTGCTCAT
GAPDH	CTGGAGAAA-GCTGCCAAA	TGTT-GAAGTCACAGGA-GA	AGAAGGTAG-TGAAGCAG

Table (2): Demographic and clinical data among all studied groups.

Variables		Control (N=50)	Patients with psoriasis (N=50)	Patients with eczema (N= 50)	P-value
Demographic data					
Age (years)		37.3 ± 15.7	39.6 ± 15.4	31.0 ± 13.8	NS
Gender (F/M)		23/27	30/20	13/37	NS
Smoking status (smoker/non-smoker)		5/45	17/33	6/44	NS
Disease family history (Yes/No)		6/44	24/26	25/25	NS
Disease duration		--	15.5 ± 16.3	106.6 ± 13.8	(P < 0.0001)
Disease scoring					
PSAI		--	18.68 ± 9.92	--	(P < 0.0001)
EASI		--	--	22·8± 9·82	(P < 0.0001)
SCORAD		--	--	32.9±14.05	(P < 0.0001)
Laboratory parameters					
ALT (IU/L)		18.39±7.42	28.8± 7.2	30.4± 6.9	NS
AST (IU/L)		20.29±8.24	29.3 ± 7.9	32.6± 7.7	NS
Bilirubin (mg/dL)	Total	0.7±0.2	1.25±0.4	1.14±0.5	NS
	Direct	0.1±0.03	0.5±0.02	0.3±0.04	NS
Urea		21.5±7.88	23.3±11.66	21.2±8.88	NS
Creatinine (mg/dL)		0.87±0.18	0.89±0.21	0.84±0.15	NS
CBC	HB	11.6±2.3	11.2±2.6	10.8±3.1	NS
	TLC	6.6±3.9	6.9±3.8	7.1±4.1	NS
	PLT	223±52.9	214±54	205±49	NS

Data presented as means ± standard deviations; N, number; F, female; M, male; NS, not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HB, hemoglobin; TLC, total leucocyte count; PLT, platelets.

Table (3): The mean expression level of NOS2 in all studied groups.

Study groups	NOS2 expression level	P value
Control (n=50)	0.24±0.12	
Patients with psoriasis (n=50)	3.99±0.67	P<0.00001
Patients with eczema (n = 50)	1.64±0.40	

P<0.05 is significant

Table (4): Association between NOS2 expression level and disease severity.

Disease severity scoring		NOS2 expression level	P-value
Psoriasis/PSAI	Mild	2.67± 0.59	
	Moderate	3.89±0.64	
	Severe	5.41±0.81	P<0.001
Eczema/ SCORAD	Mild	2.04± 0.42	
	Moderate	1.71±0.49	
	Severe	1.22±0.38	

P<0.05 is significant

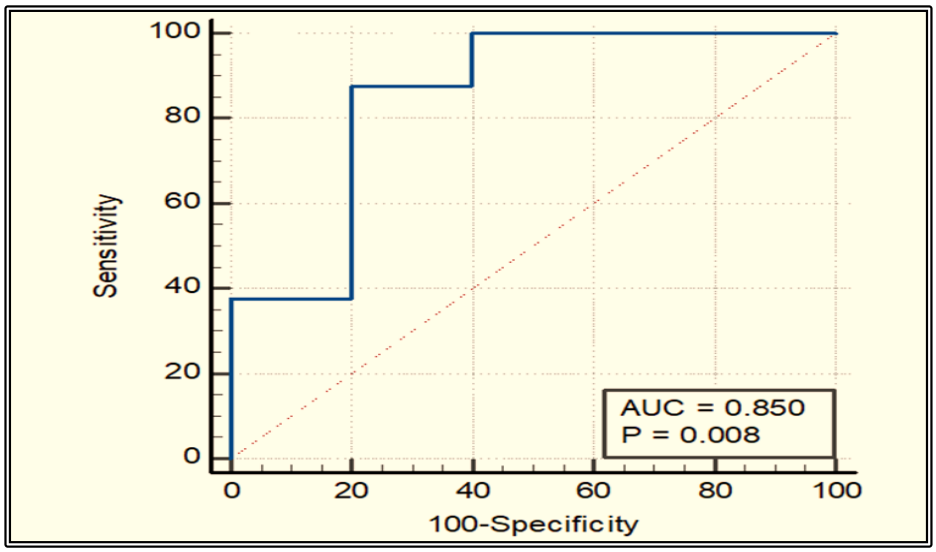

Fig. (1): Area under curve of the receiver operating characteristic (ROC) of the NOS2 to distinguish between psoriasis and eczema.