Measurement of Serum Nitric Oxide in Different Types of Psoriasis

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Abstract

Background: Psoriasis is a chronic, inflammatory, and hyperproliferative disease. Recently there have been studies regarding increases in the levels of NO in inflammatory dermatoses including psoriasis.

Objective: The aim of this study was to measure serum nitric oxide (NO) levels in patients with active psoriasis, to correlate these levels with duration and severity of the disease and compare them with those in normal individuals.

Patients and Methods: In this study, 40 patients with psoriasis were scored with PASI score and the levels of serum nitric oxide were detected by Greiss method. The results were compared with forty (40) healthy volunteers. The relation of the results with the clinical severity, duration of the disease as well as duration of the current episode were also evaluated.

Results: Out of the 40 patients with psoriasis 30 (75%) presented with chronic plaque psoriasis, 7 (17.5%) presented with acute attack of guttate psoriasis, 1 (2.5%) patient with erythrodermic psoriasis, 1 (2.5%) with pustular psoriasis and 1 (2.5%) with scalp psoriasis. The duration of the disease ranged between 6 months to 25 years while the PASI score ranged between (1.60- 49.50). The mean NO level in psoriatic group was 125.0 µmol/L with SD 38.77, while in the control group it was 42.37 µmol/L with SD 23.57. The difference was statistically significant (\( p < 0.001 \)). A positive correlation was observed between sNO levels and each of the following: Duration of the disease, PASI score, age of patients and age of disease onset.

Conclusion: The data of this study confirmed the vital role played by NO in the pathogenesis of psoriasis. The NO concentration in the blood serum considerably increased in patients with severe symptoms of disease. Measuring of serum NO may permit an objective estimation of the intensity of inflammatory response, as well as an opportunity to propose the prognosis of psoriasis.

Key Words: Psoriasis – Nitric oxide – Constitutive NO synthase – Inducible NO synthase – Inflammation.

Introduction

PSORIASIS is a systemic chronic, relapsing inflammatory skin disorder, with worldwide distribution, affects 1-3% of the world population, prevalence varies according to race, geographic location, and environmental factors [1]. The causes of this disease are unknown, though genetic, metabolic, immune and environmental factors have been proposed [2].

As a potent regulator of keratinocyte growth and differentiation, the multifunctional signaling molecule nitric oxide (NO) has been considered to be a strong candidate in the pathogenesis of psoriasis [3]. This heat-labile and unstable compound is synthesized in endothelial cells as well as neurons by constitutive NO synthase (cNOS), while inducible NO synthase (iNOS) is found in leucocytes, macrophages, and mesengial cells. A small amount of NO produced by cNOS in endothelium is responsible for the relaxation of adjacent smooth muscles and prevents adhesion of platelets and leucocytes to the endothelium. This is the anti-inflammatory effect of NO [4]. However, when produced in large amounts NO can destroy tissues and impair immune response [5].

Although studies showed the elevated NO levels in psoriatic tissue samples, as far as we know there are very few studies exploring serum NO levels in psoriatic patients [6].

Serum nitric oxide (NO), acts as one of the universal regulators of physiological and biochemical processes and has, therefore, attracted the special attention of researchers, over recent years [7]. In the present study, we measured serum nitric oxide (NO) levels in patients with active psoriasis, to correlate these levels with duration and severity of the disease and compare them with those in normal individuals.

Patients and Methods

A total of 40 patients with active psoriasis and 40 healthy controls were enrolled in this study.
They were selected from the Outpatient Clinic of Dermatology and Andrology in Benha University Hospital from August, 2012 to September, 2013.

Patients were assessed by a dermatologist and the diagnosis of psoriasis was based on clinical findings. The exclusion criteria were coexisting inflammatory skin disease, topical therapy within 4 weeks, systemic therapy or phototherapy within 3 months. Pregnant or lactating women and patients with systemic disease were also excluded. A formal consent was taken from each patient prior to their inclusion in this study. The approval of the ethical Committee of Human Research in Benha University was also obtained.

A full history was taken from each participant according to a written questionnaire regarding personal history (name, sex and age). A detailed history was also taken from each patient including duration of disease as well as duration of current episode. General investigations like C.B.C. (included hemoglobin level, white blood cell count and platelet count), liver function tests (SGPT and SGOT), renal function tests (serum urea and creatinine). The assessment of the severity and extent of disease was done by PASI score. The psoriasis area-and-severity index combines assessments of psoriasis-induced erythema, scaling, and skin thickness, each weighted according to the size of the affected area [8].

Venous blood samples (5ml) were taken into vacutainer tubes under sterile conditions from patients and controls. Blood samples were rapidly centrifuged at 1000xg. serum for 15 minutes and then serum was collected and stored at ≤-20°C until analysis. On the days of blood collection, clinical severity of the disease was evaluated by PASI score as mild (PASI <10), moderate (PASI=10-20) and severe (PASI >20) [9].

As NO is an unstable molecule, it is rapidly converted to nitrates and nitrites in the body, hence their concentration is parallel to NO levels [10]. So, NO level was evaluated as the sum of the stable metabolites of nitrates and nitrites (NO2 and NO3) using the technique which was used by Golikov et al., [11].

Statistical analysis:
Statistical analysis was done using IBM SPSS software package version 16. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using minimum and maximum. Comparison between two independent population were done using independent t-test. Person correlation coefficient® test was used correlating different parameters to test relationships. The p-values less than 0.05 were considered significant.

Results
Forty patients with psoriasis; 28 males (70%) and 12 females (30%), their ages varied between 19-85 years with a mean of (51.05 ±SD 18.36) were enrolled in this study. Out of the 40 patients with psoriasis 30 (75%) presented with chronic plaque psoriasis, 7 (17.5%) presented with acute attack of guttate psoriasis, 1 (2.5%) patient with erythrodermic psoriasis, 1 (2.5%) with pustular psoriasis and 1 (2.5%) with scalp psoriasis. The duration of the disease ranged between 6 months to 25 years while the PASI score ranged between (1.60- 49.50). Regarding PASI score, 8 (20%) patients had PASI score <10, 21 (52.5%) had PASI score between 10-20, and 11 (27.5%) patients had PASI score above 20. Forty healthy normal volunteers were also included; 22 males (55%) and 18 females (45%), their ages varied between 19-65 years with a mean of 36.15±SD 12.39 years as a control group.

Fig. (1) shows that the mean NO level in psoriatic group was 125 µmol/L with SD 38.77, while in the control group it was 42.37 µmol/L with SD 23.57 µmol/L with a high statistically significant difference (t=11.53, p<0.001). A high positive significant correlation (r=+0.0638, p=0.001) was observed when we compared the relation between sNO levels and duration of the disease. As the duration of psoriasis increased, NO level also increased (Table 1 & Fig. 2). We found a non-significant positive correlation (r=+0.292) between the levels of NO and PASI score (Table 2). Thus, the above findings suggest that NO levels were increased in psoriatic patients and, they were also related to the severity and duration of the disease.

Table (3) shows that patients with pustular psoriasis with a mean of 145.12 µmol/L had the highest levels of sNO, while those with guttate and erythrodermic psoriasis with mean values of 134.88 and 127.91 µmol/L respectively came in second and third places. However, as the total number of erythrodermic, pustular and scalp psoriasis was low, it was difficult to draw any definite conclusions (p>0.05).

Furthermore, we compared the mean values of sNO in both groups regarding sex, but there was
Table (1): Relation between sNO levels (µmol/L) and duration of disease.

<table>
<thead>
<tr>
<th>Duration (in years)</th>
<th>Mean±SD</th>
<th>Range</th>
<th>F-test</th>
<th>Pearson correlation @</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1y (n=3)</td>
<td>101.1±26.29</td>
<td>82.53-131.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4y (n=14)</td>
<td>110.99±30.49</td>
<td>82.25-184.79</td>
<td>8.22</td>
<td>0.638</td>
<td>0.001 HS</td>
</tr>
<tr>
<td>5-9y (n=12)</td>
<td>111.41±27.77</td>
<td>80.43-180.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥10y (n=11)</td>
<td>164.5±35.83</td>
<td>86.46-224.15</td>
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</tr>
</tbody>
</table>

Table (2): Relation between sNO levels (µmol/L) and PASI score.

<table>
<thead>
<tr>
<th>PASI score</th>
<th>Mean±SD</th>
<th>Range</th>
<th>F-test</th>
<th>Pearson correlation @</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 (n=8)</td>
<td>115.74±46.2</td>
<td>82.53-224.15</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10-20 (n=21)</td>
<td>126.39±38.42</td>
<td>80.43-194.2</td>
<td>0.301</td>
<td>0.292</td>
<td>0.742 NS</td>
</tr>
<tr>
<td>&gt;20 (n=11)</td>
<td>129.41±36.36</td>
<td>86.51-184.79</td>
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</table>

Table (3): Relation between mean values of sNO levels (µmol/L) and different types of psoriasis.

<table>
<thead>
<tr>
<th>Types of psoriasis</th>
<th>Serum NO (µmol/L)</th>
<th>Mean±SD</th>
<th>Range</th>
<th>F-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic plaque ps. (n=30)</td>
<td>123.47±40.68</td>
<td>80.43-224.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guttate psoriasis (n=7)</td>
<td>134.28±36.68</td>
<td>97.77-184.79</td>
<td></td>
<td></td>
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<tr>
<td>Erythrodermic ps. (n=1)</td>
<td>127.91±44.32</td>
<td>127.91-127.91</td>
<td>0.401</td>
<td>0.807 NS</td>
<td></td>
</tr>
<tr>
<td>Pustular ps. (n=1)</td>
<td>145.12±38.42</td>
<td>145.12-145.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scalp psoriasis (n=1)</td>
<td>86.51±36.36</td>
<td>86.51-86.51</td>
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</table>

Table (4): Comparing between mean values of sNO levels (µmol/L) regarding to sex in both groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
<th>Student t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient group:</td>
<td></td>
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<tr>
<td>Male (n=28)</td>
<td>128.69±42.18</td>
<td>0.894</td>
<td>0.377 NS</td>
</tr>
<tr>
<td>Female (n=12)</td>
<td>116.7±29.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=22)</td>
<td>47.82±25.16</td>
<td>1.65</td>
<td>0.107 NS</td>
</tr>
<tr>
<td>Female (n=18)</td>
<td>35.71±20.18</td>
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Significantly positive correlation (p=0.015, r=0.381) between NO values and age of patients and a non-significant positive correlation with age of disease onset (p=0.27, r=0.179).
Discussion

Psoriasis is a chronic inflammation of the skin. Several types have been defined based on clinical grounds. They include guttate, plaque, inverse, pustular, erythrodermic, plano-planter, scalp, and nail [12]. Recent genetic and immunological advances have greatly increased our understanding of the pathogenesis of psoriasis [13]. It is widely accepted that genetic predisposition and environmental factors have a profound effect on the immune system and play a crucial role in triggering psoriatic lesion development [14]. With the understanding of the immune mechanisms of psoriasis, the role of keratinocytes in psoriasis has changed from a passive bystander to an active participant, which is supported by the ability of these cells to synthesize numerous proinflammatory cytokines, chemokines, and growth factors, as well as prostaglandin upon stimuli [15].

The regulatory factors involved in the psoriatic microcirculation are not known but nitric oxide (NO) is of general physiological importance in local paracrine control of blood flow [16]. This signal molecule is produced by NO synthetase when converting L-arginine to L-citrulline. Nitric oxide is an important endogenous regulator of both cutaneous microcirculation and cell proliferation [17].

The labile nature of NO makes it impossible to analyze its serum level, so the metabolites of NO; nitrite and nitrate are helpful to measure the level of NO. In Griess method (which was used in this research) nitrate (NO$_3$) was first reduced to nitrite (NO$_2$) [18], the nitrite levels are considered the marker of NO in body fluids including serum [19].

Nitric oxide (NO), a free radical with a wide range of actions; it is synthesized by all cell types in the skin. NO is synthesized from arginine in low concentrations by the constitutive NO synthases (cNOS), and at higher concentrations by inducible NOS (iNOS). Although often associated with tissue inflammation, iNOS may also be anti-inflammatory, as shown by iNOS derived NO protection from ischaemia-reperfusion injury, or enhancement of inflammation by NOS inhibitors [20].

In our study the mean NO level in the psoriatic group was 125.08 µmol/L while in the control group it was 42.37 µmol/L, with a high statistically significant difference between both groups ($p<0.001$). This was in the same range as Sikar et al., study [21] which revealed 135.8 µmol/L and 33.6 µmol/L in patients and control groups respectively. Moreover, Tekin et al., [22] compared serum levels of NO in normal controls, psoriatic patients prior to and after treatment with methotrexate. They observed that NO levels in patients prior to treatment were higher than that in controls and patients after treatment and based on their results the authors suggested that NO is possibly a mediator in the pathogenesis of psoriasis.

On the other hand, Kilinc et al., [23] stated that there was no statistically significant difference in NO levels between psoriatic patients and healthy volunteers. They also observed that the total nitrite levels in samples obtained during and at the end of therapy (after 6-10 exposures to BB UVB phototherapy) were significantly higher than basal levels ($p=0.033$ and $p=0.005$, respectively). This difference could be explained by the fact that they used BB UVB phototherapy which causes a significant increase in NO levels.
Abeyakirithi et al., [20] tried to determine whether restoration of NO levels might help resolve psoriasis, they conducted a small, singly blinded pilot study on the topical application of NO to psoriatic lesions in stable plaque psoriasis, by means of an aqueous NO donor gel system. The improvement in size and appearance of actively treated plaques provides further evidence that a relative deficiency in NO underlies much of the psoriatic phenotype.

Coto-Segura et al., [24] declared that the potential role of the NO in the pathogenesis of psoriasis is still unclear due to conflicting results. On one hand, an increased expression of NOS has been found in psoriatic plaques and this could lead to high local concentrations of NO [28]. On the other hand, over expression of arginase 1 and the calcitonin gene-related peptide in the psoriatic skin could act in a compensatory manner, leading to a significant consumption of arginine and a decrease in NO production in the psoriatic plaque [20,26]. Some authors [20], have reported improvement of the psoriasis plaque with the topical application of NO-donors, while others found the opposite effect [27].

In our study we also measured the serum NO levels in patients with different types of psoriasis (plaque, guttate, erythrodermic, pustular and scalp), those with pustular psoriasis had the highest with a mean of 145.12 µmol/L, while those with guttate and erythrodermic psoriasis with mean values of 134.88 µmol/L and 127.91 µmol/L respectively came in second and third places. In Gokhale et al., [10] study, out of 36 patients, 30 had chronic plaque psoriasis (mean NO= 157.5 µmol/L), 4 had erythroderma (mean NO=120.2 µmol/L) and 2 had generalized pustular psoriasis (mean NO= 144.3 µmol/L). However, as the total number of erythrodermic, pustular and scalp psoriasis was low, it was difficult to draw any definite conclusions (p>0.05).

A high positive significant correlation (r=+0.0638, p=0.001) was observed when we compared between duration of the disease and sNO levels. As the duration of psoriasis increased, NO level also increased. Like minded, Orem et al., [28] studied NO levels in 17 patients of psoriasis. They observed a positive correlation between NO levels and the activity of disease. On the contrary, Kilinc et al., [23] showed no statistically significant correlation between disease duration, disease severity, and serum levels of nitrite-nitrate in psoriatic patients. Nevertheless, we found a positive correlation (r=+0.292) between the levels of NO and PASI score with no statistically significant difference (p<0.05). This was in agreement with some studies [10,21] and opposed by others [22]. Tekin et al., [22] clarified that the elevation of the serum NO levels in psoriasis only indicates the systemic inflammation and not a biomarker of psoriasis severity.

Gaber and Al-Ghadir [29] stated that knowing that NO is synthesized from arginine in low concentrations by the constitutive NO synthases (cNOS), and at higher concentrations by inducible NOS (iNOS) and the overexpression of iNOS mRNA in psoriatic lesions is the main reason for increase NO level in patients with psoriasis. Bachmann and Mundel [30] also explained the elevation of serum level in psoriatic patients was due to increase release of cytokines such as IFN γ, TNF α, IL-1, in psoriatic skin which stimulate iNOS activity. Tekin et al., [22] also believed that NO may trigger the psoriatic disease process at least partly through increment of the release and actions of calcitonin gene-related peptide and substance P, which are considered to play important roles in the pathomechanism of psoriasis by inducing the production of adhesion molecules, keratinocyte hyperproliferation, mast cell degranulation, vasodilation, and chemotaxis of neutrophils. Cals-Giersen and Ormerod [26] have suggested that NO stimulates epithelial cells to release chemokines and growth mediators which appear to be important for keratinocyte proliferation and angiogenesis. Bruch-Gerharz et al., [31] found out that a combination of IL-8 and interferon gamma induces the expression of iNOS-specific mRNA in cultured human keratinocytes. These results suggest an important role for iNOS in concert with IL-8 and its receptor early during the formation of psoriatic lesions. Nitric oxide is also involved in the regulation of the release of hormones which have been shown to control inflammatory process centrally. For example, NO plays a marked inhibitory role in the CRH-induced ACTH secretion and inhibits corticosterone secretion [32].

Our results revealed a positive significant correlation (p=0.015, r=0.381) between NO values and age of patients while Sikar et al., [21] found a significant negative correlation was between the plasma NO levels and age.

We can suggest that immunological and inflammatory mechanisms are important in the etiopathogenesis of psoriasis and that NO plays a role in both mechanisms. The results in our study are comparable with the studies investigating the local psoriatic tissue iNOS and NO levels,
This relationship between psoriasis and NO may pave the way for novel therapeutic approaches such as selective iNOS inhibitors in the management of this difficult disease [22], or perhaps antimicrobial agents such as gentamycin, tobramycin, azathiprim and isoniazide were shown to suppress physiological levels of NO and in doing so possibly infectious agents that provoke the disease would be eliminated [33].

Conclusion:
The data of this study confirmed the vital role played by NO in the pathogenesis of psoriasis. The NO concentration in the blood serum considerably increased in patients with severe symptoms of psoriasis.

The NO level, being a key position in the pathogenesis of psoriasis, may be considered one of the current high-sensitive criteria of inflammation. Its definition permits an objective estimation of the intensity of inflammatory response, activity of the pathological process, success of the therapy, as well as an opportunity to propose the prognosis of psoriasis. This relationship between psoriasis and NO may lead to new therapeutic approaches in the management of this difficult disease.

References


