

A study of serum nitric oxide levels in psoriasis

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ABSTRACT

Background: Many inter and intracellular mediators have been implicated in the pathogenesis of psoriasis. Nitric oxide has been shown to play an important role in many diseases. Previous studies have demonstrated raised levels of nitric oxide in psoriatic plaques which may be attributed to its effect on keratinocytes, on local cGMP levels or its ability to induce angiogenesis. **Aims:** To detect serum nitric oxide (NO) levels in patients with active psoriasis, to correlate these levels with severity of disease and compare them with those in normal individuals. **Methods:** Thirty six patients with active psoriasis were selected after written consent. All patients on topical or systemic treatment for fifteen days prior to the study were excluded. Disease severity was assessed by PASI score and serum nitric oxide levels were detected by Greiss method and compared with age and sex matched controls. Statistical analysis of all data was done by unpaired t test. **Results:** Out of 36 patients, 30 had chronic plaque psoriasis (mean NO 157.5), 4 had erythroderma (mean NO 120.2) and 2 had generalized pustular psoriasis (mean NO 144.3). The mean NO level in the psoriatic group was 157.7 with SD 50.4 while in the control group it was 32.8 with SD 4.03. The difference was statistically significant ($t=13.8$, $P<0.001$). In the chronic plaque group, as the duration of disease increased, the NO levels increased significantly. **Conclusions:** Nitric oxide levels were significantly increased in patients with psoriasis and these levels showed a positive correlation with severity and duration in the chronic plaque type group.

KEY WORDS: Psoriasis, Nitric oxide

INTRODUCTION

Nitric oxide (NO) is increasingly recognized as an important intra and intercellular messenger. 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 mesangial cells. It is expressed by various inflammatory factors like IL1, TNF alpha etc. Once expressed it generates a sustained amount of NO. A small amount of NO

produced by constitutive NOS in the 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.^[1] However, when produced in large amounts, NO can destroy tissues and impair immune response. Such high levels are demonstrated in immunological disorders like systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA). Hence, inhibition of iNOS is an effective modality of treatment in these conditions.^[2]

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The exact pathogenesis of psoriasis is still not known but along with genetic and environmental factors, an immune-mediated process involving many mediators has been implicated. One of the mediators responsible for psoriasis pathogenesis is NO. Keratinocytes are known to express NOS2 message and protein following exposure to inflammatory cytokines. NOS2 expression is found in a number of inflammatory conditions like psoriasis, atopic eczema and contact dermatitis.^[3,4]

METHODS

Thirty-six patients of psoriasis, more than 18 years of age, attending dermatology OPD were selected after obtaining a written informed consent. Detailed history including duration of disease as well as duration of current episode was taken. Clinical examination and investigations like hemogram, liver function tests, renal function tests, platelet count were carried out. The assessment of the severity and extent of disease was done by PASI score. All patients who were on either topical or systemic treatment for 15 days prior to blood collection were excluded from the study. Other systemic illnesses like SLE, RA and malignancy were ruled out.

Five ml of blood was collected and allowed to clot. Serum was separated by centrifugation. 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. Nitrate and nitrite concentrations were then estimated by the Griess method. In this method nitrate is first reduced to nitrite which is treated with sulfanilamide and N-1-naphthyl-ethylene diamine. A red colored compound is formed after which its characteristic absorption spectrum is determined on spectrophotometry. The concentration of nitrite is determined by regression analysis.

Another group of age and sex-matched normal individuals was selected and their NO levels were also measured by the Griess method. Statistical analysis of all results was done by unpaired t test.

RESULTS

Out of 36 patients selected, 28 were males and 8 were females. Out of 34 patients with chronic plaque type

psoriasis, 4 presented with erythroderma and 3 with acute attack of guttate lesions. Two were suffering from de novo generalized pustular psoriasis. Positive family history was noted in 4 patients and severe joint involvement was seen in 1 patient. Sixteen patients had PASI score of <10, 12 had PASI between 10-20, 5 had PASI between 20-30, 1 had PASI between 30-40 and 2 had PASI above 40.

The mean NO level in patients was 152.7 micromole per liter with SD of 15.4, while in controls the mean NO level was 32.8 micromole per liter with SD 4.03. The t value was 13.8 and p was <0.001, i.e. observed difference between these two values was highly significant. In the chronic plaque group, the mean NO value was 150.4, in the generalized pustular type it was 144.3, in erythroderma it was 120.2 while in the group having chronic plaque psoriasis with guttate lesions it was 157.5.

Table 1 shows the relation between PASI score and nitrite levels. Most of the patients with PASI score 0-20 were suffering from chronic plaque type psoriasis and as PASI score increased, NO levels also increased in this group. Although patients of erythroderma and pustular psoriasis had PASI score >20, the NO levels were low compared to those found in the patients with chronic plaque type psoriasis. However, as the total number of patients of erythroderma and pustular psoriasis was low, it was difficult to draw any definitive conclusions.

Table 2 shows the association of the duration of disease in chronic plaque type psoriasis and NO level. As the duration increases, NO levels also increase. We could not find any significant association between the duration of current episode and levels of NO.

Thus the above findings suggest that NO levels are increased in psoriatic patients and in chronic plaque

Table 1: Relation between PASI score and nitric oxide levels

PASI score	Number of patients	NO levels (mmol/lit)
0-10	16	133.2
10-20	12	190.5
20-30	5	96.8
30-40	1	122.8
>40	2	101.1

Table 2: Relation between NO levels and duration of disease in chronic plaque type psoriasis

Duration of disease (years)	Number of patients	NO levels (mmol/lit)
> 10	3	198.7
5-10	10	175.0
1-5	17	133.4
<5	4	113.1

type, they are related to the severity and duration of the lesions.

DISCUSSION

The aim of this study was to find the levels of NO in different types of psoriasis, compare them with normal individuals and correlate them with disease activity. Orem et al studied NO levels in 17 patients of psoriasis during active and inactive phases.^[5] They observed increased NO production in patients with a mean PASI of 16.5 and above. They also demonstrated a positive correlation between NO levels and the activity of disease. During the inactive phase (mean PASI 1.7), the NO levels were low. In our study, we found significantly high NO levels in patients with active disease as compared to normal individuals. There was significant positive correlation between the severity and duration of disease and NO levels in patients with chronic plaque type psoriasis. Studies on the effect of NO on various cell functions provide insights into the mechanisms by which NO could contribute to the pathogenesis of psoriasis.^[1,2] Evidence suggests that NO promotes vasodilatation and vascular permeability, activates cyclo-oxygenase and stimulates TNF alpha production.^[1,2,6] Whilst high concentrations of NO have cytotoxic and pathophysiological functions, low concentrations as produced by vascular endothelium are believed to play a protective role in the microvasculature.

Kolb-Bachofen et al who demonstrated increased expression of iNOS in psoriatic plaque concluded that iNOS expression is involved in the pathogenesis of cutaneous inflammation of psoriasis.^[7]

Cals –Gierson and Ormerod have stated that NO is also known to stimulate epithelial cells to produce and release chemokines and other growth mediators such

as vascular endothelial growth factor which appear to be important for keratinocyte proliferation and angiogenesis.^[3] They have also mentioned that the increased NOS2 expression observed in various inflammatory conditions like dermatitis may be responsible for the impaired barrier function found in these situations. The events leading to this are the elevated levels of NO and peroxy-nitrite formation which in turn cause increased activation of poly (ADP ribose) polymerase which further translates into inhibition of keratinocyte differentiation. This could be one of the postulated mechanisms of psoriasis pathogenesis.^[3] NO is also found to increase the level of cGMP, which may act as a secondary mediator and bring about proliferation of keratinocytes. Ormerod et al showed that application of an NO-releasing cream to normal skin produced an increase in markers for T lymphocytes and endothelial cells both of which are features of psoriasis.^[8] He also demonstrated decreased NO production in psoriatic plaque after application of iNOS inhibitor-NG monomethyl L arginine (L-NMMA).^[9] On the other hand, Morhenn demonstrated deterioration of psoriatic plaque after application of NO donor i.e. nitroglycerine.^[10]

Clancy et al in their review on NO have stated that this molecule plays an important role in autoimmunity and inflammation.^[2] Excessive NO is produced during the course of a variety of rheumatic diseases like SLE, RA etc. The pro and anti-inflammatory properties may vary according to NO concentration, the potential for formation of toxic derivatives, site of pathologic process and adaptive responses of the target cells. All the above studies support the theory that in psoriasis, NO may be the mediator of inflammation and the driving force behind the pathogenesis. Our findings are consistent with those reported by earlier authors.^[6] On the other hand, Cals-Gierson et al have quoted a second school of thought according to which over-expression of NOS2 is associated with an increased, perhaps compensatory, arginase 1 activity which may reduce available substrate for NO production. This leads to the interpretation that NO has a dual effect on keratinocytes depending upon its concentration. It encourages keratinocyte proliferation at low concentration or differentiation at high concentration.

Thus in psoriasis there may be inadequate response of keratinocytes to observed increased levels of NO.^[3]

However, as the total number of patients in our study was less, we suggest that more studies involving a greater number of patients are warranted. In addition, studies to detect local levels of NO in psoriatic plaques may be helpful to confirm its role in the pathogenesis of this disease. This may pave the way for novel therapeutic approaches such as selective iNOS inhibitors in the management of this difficult disease. To support this view, Namazi has demonstrated that statins which are known to block expression of iNOS and other pro-inflammatory cytokines may be effective in conditions like psoriasis.^[11]

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