

Study of serum carbonic anhydrase activity, uric acid, C-reactive protein levels and lipid parameters in patients with Psoriasis

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Abstract

Introduction: Psoriasis is a commonly occurring chronic skin disease which is characterized by recurrent erythematous skin plaques exhibiting epidermal hyperplasia accompanied by infiltration of varied inflammatory cells leading to abnormalities of the papillary dermal vasculature. Earlier studies postulated that the rise in serum uric acid levels which is observed in approximately 30 to 40 per cent of patients with psoriasis was due to the increased turn over in the skin.

Objectives: Since there is scanty literature reports based on carbonic anhydrase activity in psoriatic skin disorder patients, the aim of our study to evaluate serum carbonic anhydrase activities, uric acid, and C - reactive protein levels and lipid parameters in various stage of psoriatic patients with the objectives to correlate the possible changes with the different clinical and biochemical parameters.

Material & Methods: Forty-four psoriatic patients were enrolled for the present study for a period of one year and one month. The patients who attended skin OPD with psoriasis, confirmed with supportive clinical and histopathological findings, were included in the study. Serum uric acid levels and carbonic anhydrase activity were measured using standard procedure. All chemicals were procured from Sigma Chemicals, USA.

Results And Conclusion: Serum Uric acid, Carbonic anhydrase activity and C-reactive proteins and lipid parameters were elevated in patients compared to controls. Of these the uric acid levels and carbonic anhydrase activities, serum triglyceride and LDL/HDL ratio were significantly elevated in patients. The study concludes all patients diagnosed of psoriasis must be equally predicted for future risk of cardiovascular diseases.

Keywords: Psoriasis, Uric Acid, Carbonic anhydrase, C - reactive protein,

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Original Article**Introduction:**

Psoriasis is a commonly occurring chronic skin disease which is characterized by recurrent erythematous skin plaques exhibiting epidermal hyperplasia accompanied by infiltration of varied inflammatory cells leading to abnormalities of the papillary dermal vasculature.¹⁻⁴ The micro vessels in the papillary dermis of psoriatic plaques are usually elongated, dilated and hyperpermeable^{5, 6} which closely resembles like post capillary venules than the capillary loops of normal skin.^{7, 8} The changes in vascular morphology may precede inflammatory cell infiltration in developing psoriatic plaques.⁹⁻¹¹ Reports from the study suggests epidermal alterations precede capillary leakiness and vascular anomalies in the development of psoriatic skin lesions.¹² Usually hyperuricemia is observed in approximately 30 to 40 per cent of patients with psoriasis.¹⁻⁵ Rather little attention was given to link the possible relation to the psoriatic process with hyperuricemia. Earlier studies postulated¹³ that the rise in serum uric acid levels in patients with psoriasis was due to the increased turn over in the skin. In psoriasis, there is hyperplasia of the epidermis causing the skin lesions, due to accelerated cellular reproduction to that extent that the maturation of the epidermal cells overwhelms the keratinization process. Analogous with the hyperuricemia observed in myeloproliferative diseases, it could be possible that the hyperuricemia of psoriasis may reflect an increased nucleic acid turnover resulting from the marked acceleration of epidermal proliferation that occurs in this disease.

Earlier studies⁹⁻¹¹ established psoriasis as an inflammatory process. Inflammatory markers like vanin-1 and vanin-3 are expressed in epidermal cells in psoriatic skins, which are a part of the inflammatory-regenerative differentiation program of human epidermis and proved to be beneficial response to supply the skin with antioxidant activity and sufficient pantothenic acid to restore skin barrier function in psoriatic skin. Microarray studies on the epidermal transcriptome in psoriasis and atopic dermatitis (AD) have revealed genes expressions in keratinocytes of lesional epidermis

are disease-specific. These genes are possible candidates for disease-specific pathogenetic changes. As psoriasis is an inflammatory condition, C-reactive protein (CRP) levels in serum is also reported to be elevated. Normally vascular permeability factor (VPF) a glycosylated protein that is over expressed by many human and animal tumors¹⁴⁻¹⁷ and also by the epidermis of healing wounds.¹⁸ The previous study reported that the angiogenic nature of psoriatic skin are rather associated with the epidermis and not with the dermis.¹⁹ In psoriasis there is enhanced microvascular permeability and angiogenesis along with the over expression of this VPF protein. Study reported that the extracellular carbonic anhydrase mediates hemorrhagic retinal and cerebral vascular permeability through prekallikrein activation. Expression of this protein is associated with increase in carbonic anhydrase activity. The carbonic anhydrase is well-characterized and widely distributed and expressed in a variety of tissues, including kidney, erythrocytes, sweat glands, salivary glands, and skin^{20,21,22} and the enzyme is involved in the maintenance of cellular pH, water transport, and ion homeostasis in all living organisms. The changes in enzymatic activities are associated with various diseases. Carbonic anhydrase is highly induced in epidermis in all forms of eczema and other skin disorders. Carbonic anhydrase belongs to the family of metalloenzymes. Of date fourteen different isoforms are known so far and they all show distinct distribution patterns.^{22, 23} Carbonic anhydrase (CA) levels in serum has recently been reported to be elevated in psoriatics. There is a strong correlation between capillary fenestrations (abundant in psoriatic skin) and demonstration of CA on histochemical study.²⁴ It is also seen that pustular psoriasis has been precipitated by acetazolamide, a CA inhibitor, when used for glaucoma in some psoriatics.²⁵ Recently it has been suggested that increased reactive oxygen species (ROS) production and deficient function of antioxidant systems activities may be involved in the pathogenesis of the disease.²⁶ The carbonic anhydrase enzyme has recently been reported to have an anti-oxidant activity, which is glutathione-

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mediated.²⁷ Our earlier study demonstrated that the carbonic anhydrase activity had a strong correlation with lipid parameters.²⁸

Aim and Objective:

Since there is scanty literature reports based on carbonic anhydrase activity in psoriatic skin disorder patients, the aim of our study to evaluate serum carbonic anhydrase activities, uric acid, and C-reactive protein levels and lipid parameters in various stage of psoriatic patients with the objectives to correlate the possible changes with the different clinical and biochemical parameters.

Materials and Methods:

Forty-four psoriatic patients (25 males; 19 females) were enrolled for the present study with ages ranging from 9 to 85 years for a period of one year and one month from January 2005 to January 2006. The study was pre-approved by the Ethical Committee of this Institution Review Board.

Inclusion Criteria: Patients who attended skin OPD with psoriasis confirmed with supportive clinical and histopathological findings were included in the study.

Exclusion Criteria: Patients with hypertension, hyperuricemia, diabetes mellitus, and other endocrine disorders like hypo/hyperthyroidism, lipid disorders and pregnancy were excluded from the study. Patients on lipid lowering drugs and antioxidant vitamin supplements were also excluded.

Sample Collection:

The patients who attended in Skin OPD clinic at Nilratan Sarkar Medical College where physically examined for the psoriatic pattern in them and the extent of skin involvement in those patients were assessed by the rule of nine's²⁹ in which the surface of the body is divided into 11 areas assumed to be 9% of the total body area, the genitals being assessed as the remaining 1%. The selected patients were further evaluated for biochemical tests for which twelve hours fasting blood samples were collected from them.

Informed consent had been taken before collecting the sample. 42 age-sex matched healthy volunteers were selected as controls. Five ml of blood samples was collected, in a sterile test tube from the participants, allowed to clot and then carefully centrifuged at 3000 r.p.m for 10 minutes. Clear serum were collected and kept in - 4°C until tests were performed. Serum samples obtained were used for analysis of biochemical parameters.

Serum Uric Acid Levels: Serum uric acid levels were assayed with caraway's method³⁰.

Assay of Serum Carbonic Anhydrase activity by Racker's method³¹: The assay system consisted of 100 μ L of sample (serum) containing 1.4 mL of 0.05 M Tris-SO₄ buffer (pH 7.4) and 1.5 mL of 3mM p-nitrophenyl acetate. The change in absorbance at 348 nm was measured over a period of 3 min., before and after adding the sample. One unit of enzyme activity was expressed as 1 μ mol of released p-nitrophenol per minute at room temperature. All chemicals were procured from Sigma Chemicals, USA.

Serum CRP levels: The serum hsCRP levels were determined using ELISA method.

Lipid Profile: TC, TG and HDL-cholesterol were analyzed enzymatically by using a kit which was obtained from Randox Laboratories Limited, Crumlin, UK. Plasma LDL-cholesterol was determined from the values of total cholesterol and HDLcholesterol by using the following formula:

$$\text{LDL-cholesterol} = \frac{\text{TC} - \text{TG} - \text{HDL-cholesterol}}{5} \text{ (mg/dl)}$$

Statistical analysis: The data from the patients and controls were compared by using the student's *t*-test. The values were expressed as mean \pm standard deviation (SD). Microsoft Excel for Windows 2003 was used for statistical analysis. *P*-values <0.05 were considered to indicate statistical significance.

Results:

The finding of the current study is tabulated in

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Table 1. It was noticed that serum uric acid, carbonic anhydrase activity and C-reactive proteins were elevated in patients compared to

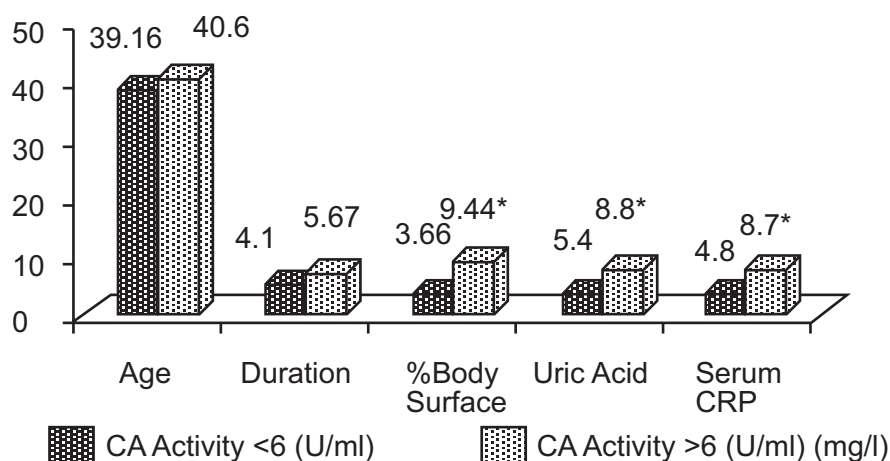
controls. Of these the uric acid levels and carbonic anhydrase activities were significantly elevated in patients.

Table 1. Baseline Variables in Psoriatic patients and controls

Parameters	Patients (n=44)	Healthy Controls (n=42)	p value
Age (Y)	39.7 ± 7.3	41.5 ± 18.5	NS
Sex (M/F)	25/19	27/15	NS
Serum Uric Acid (mg/dl)	8.05 ± 2.01	4.1 ± 1.5	<0.01
Serum Carbonic Anhydrase Activity (U/ml)	7.89 ± 2.2	3.2 ± 1.4	<0.05
CRP (µg/l)	5.1 ± 2.2	3.4 ± 1.8	NS
Duration of Illness (Y)	5.38 ± 4.85	-	
Body surface area involved (M ²)	8.15 ± 6.92	-	
Family History (P/N)	4/35	-	
Cholesterol (Total)	204.08 ± 47.02	146.46±23.61	NS
Triglyceride(TG)	219.18±27.48	135.2±15.62	<0.05
HDL	44.87±4.42	45.4±3.84	NS
LDL	115.36±29.31	74.16±14.8	NS
VLDL	43.83±13.49	27.04±5.1	NS
LDL/HDL	2.85	1.63	<0.05

NS indicates not significant, P=0.05 considered significant by unpaired t test. Among lipid parameters, though the total cholesterol, LDL and VLDL levels were increased, the serum triglyceride levels and LDL:HDL ratio were significantly raised in the patients compared to the healthy individuals. The data analysis based on the carbonic anhydrase activity is given in Figure 1.

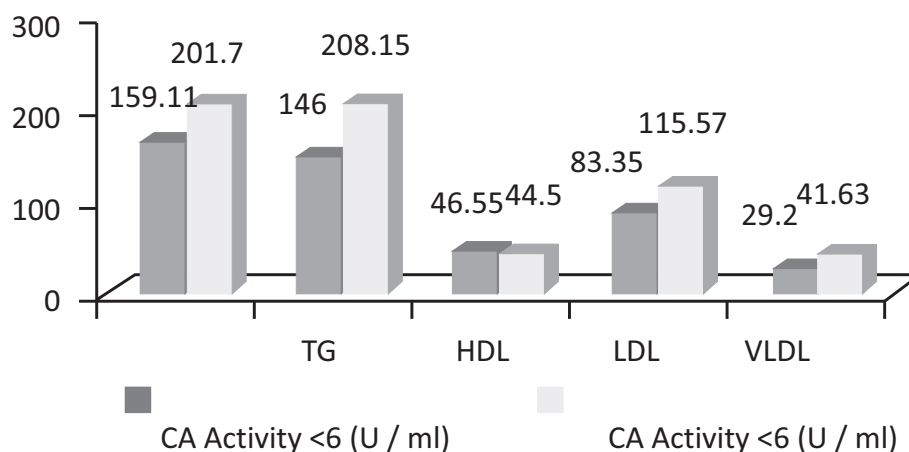
Figure 1. Analysis based on the carbonic anhydrase activities in patients with psoriasis



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Values for age and duration are in years, % body surface in M², Uric acid in mg/dl and CRP levels in mg/l.

Figure 2. Carbonic anhydrase activities and Lipid parameters in patients with psoriasis

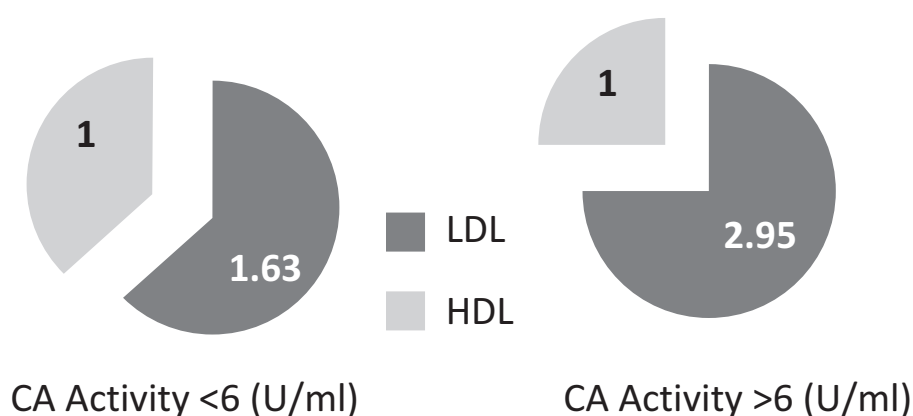


It was observed that the duration of illness, percentage of body surface involvement, serum uric acid levels and CRP levels influenced the carbonic anhydrase activity.

Cholesterol: Total Cholesterol, TG: Triglyceride, High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein. Values are in mg/dl.

The serum lipid parameters were also related to the serum carbonic anhydrase activity as shown in Figure 2 and Figure 3,

Figure 3: Carbonic Anhydrase Activity and LDL:HDL ratio in Psoriasis



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The current study observed a significant elevation of serum uric acid levels in the patients. Earlier study in this direction observed that hyperuricemia is not a demarcating feature with all psoriatic patients as of the fifty patients confirmed of psoriasis only seven had elevated serum uric acid levels and no correlation was observed with the elevation of uric acid with the extent of skin involvement.³² Similar observation was made in another study, where it involved four hundred seventy two psoriatic patients of which only forty had psoriatic arthritis and among them only 18 (45%) had above normal levels of uric acid.³³ Another study focused on antioxidant status in psoriatic patients observed, that even though the serum urate levels are increased in psoriatic patients, their total antioxidants remains unchanged.³⁴

Elevated uric acid levels are a frequent finding in psoriasis and despite of some reports to the contrary, it is generally believed that an association does exist between hyperuricemia and psoriasis. It seems a convincing idea that the rapid epidermal turnovers in psoriasis might lead to an increased purine breakdown and may this influence the uric acid serum levels. Consequently, a relationship might well be expected between hyperuricemia and the extent of psoriatic involvement.³⁵ Studies have demonstrated that carbonic anhydrase activity could act as a marker for fenestrated capillaries in psoriasis.

Considering the carbonic anhydrase activity in psoriatic patients we observed drastic changes in its activity, showing significantly elevated levels in patients when compared to normal healthy controls. As such literature reports in this area is scanty but few works have been reported where it was suggested that a generalized over expression of host defense genes of carbonic anhydrase occurs in psoriasis.³⁶ Quantification of carbonic anhydrase mRNA by real time PCR (qPCR) revealed an increase in carbonic anhydrase enzyme expression in epidermal sheets of lesional skin of psoriasis patients compared to the healthy

controls. In our study we also observed an elevated CRP levels in patients but the elevations were not significant compared to normal healthy controls. Earlier studies have also focused the clinical usefulness of CRP assay in psoriasis patients. Several studies have observed the elevation of CRP levels in psoriasis.³⁷ Even some studies have documented risk of myocardial infarction in patients of psoriasis.³⁸

Considering the observations of the current study, it could be suggested that every patients with psoriasis is prone for future risk prediction of cardiovascular events as justified by the elevation of inflammatory markers. Even though uric acid is elevated in these patients due to increased turnover of the epidermal cells, but overall beneficial effect is not noticed as the total antioxidants status remains unchanged taking into consideration that uric acid is an endogenous antioxidant. The target approach for patients with psoriasis diagnosed in skin OPD must be advised to change their dietary habits and decrease their lipids intake as this could aggravate the risk of cardiovascular diseases. Possibly the role of carbonic anhydrase needs to be identified and each patients should be assessed with CA activity as this could foresee the future problems which could be well managed.

Conclusion: All patients diagnosed of psoriasis must be equally predicted for future risk of cardiovascular diseases.

Limitations of the Study: Limited sample size

Future directions of the study: All patients diagnosed of psoriasis must be evaluated for serum carbonic anhydrase activity, uric acid and C-reactive protein levels to establish the findings from the current study

What this study contributes: Psoriasis is accompanied by increase in carbonic anhydrase activity and hence assessment of carbonic anhydrase activity is suggested in all diagnosed cases of psoriasis.

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