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Original Article

Dermatology Section

Association between Serum Sialic Acid Levels and Disease Severity in Psoriasis: A Case-control Study

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ABSTRACT

Introduction: Psoriasis is a chronic inflammatory disease distributed worldwide with varying prevalence among different geographical areas and ethnic groups. It has been recently found that oxidative stress is one of the important factors in the pathogenesis of psoriasis. The varied effects of oxidative stress include changes in cellular uptake, altered enzymatic activity of proteins, increased predisposition to aggregation and proteolysis, which subsequently alter their immunogenicity. Sialic Acid (SA) is an acetylated derivative of neuramic acid. It is a marker for acute phase inflammatory response, with increased levels observed in inflammatory diseases.

Aim: To estimate the levels of SA in psoriasis patients and to correlate with the severity of the disease.

Materials and Methods: This case-control study was conducted among 50 patients, diagnosed with psoriasis, and 50 age and sex-matched subjects without psoriasis. General, systemic and dermatological examination was carried out. The severity of psoriasis was assessed according to Psoriasis Area Severity Index (PASI). The serum was treated with ethanol and centrifugation to precipitate proteins. The SA of both the precipitate and supernatant was estimated based on the reaction of SA with the ninhydrin reagent. Calculation of sialic acid was done by the formula: OD of test/OD of standard×concentration of the standard. To establish a correlation student's t-test was used.

Results: There were 39 males and 11 females in each case and control group. The mean±SD age of cases and controls was 44.04±10.9 years and 44.10±9.996, respectively. The mean PASI value was 23.1666±18.47. Mean SA in cases and controls were 35.792±2.124 and 28.556±3.854 (p-values <0.001). A positive correlation was observed between the free SA and psoriasis severity (p-value <0.001, r=0.460).

Conclusion: Higher levels of free serum SA were significantly associated with more severe forms of psoriasis.

Keywords: Inflammatory disease, Immunogenicity, Psoriasis area severity index

INTRODUCTION

Psoriasis is a chronic, inflammatory, multisystem disease with varying clinical phenotypes of which chronic plaque psoriasis is most common type. It is influenced by various genetic and environmental factors in combination with skin barrier disruption and immune dysregulation [1,2]. Psoriasis is globally distributed disease with a worldwide prevalence of 2-3%. Its prevalence varies among different geographical locations and ethnic groups [3].

Cells and inflammatory markers involved in the pathogenesis of psoriasis include T-cells, Antigen-Presenting Cells (APC), keratinocytes, Langerhans cells, macrophages, natural killer cells, TH1 cytokines and Vascular Endothelial Growth Factor (VEGF). Oxidative stress is one of the important factors in the pathogenesis of psoriasis [4]. It has been suggested that increased production of Reactive Oxygen Species (ROS) may be involved in the pathogenesis of the disease. Higher concentration of ROS leads to the development of oxidative stress which has detrimental effects on various cells and organelles made up of lipids, proteins and nucleic acids by biochemical processes like lipid peroxidation, breakdown of DNA strands, proteolysis etc., [4,5].

The SA are cytoprotectors found widely distributed in mammalian tissue and bacteria. SA is an acetylated derivative of neuramic acid which occurs as a terminal part of glycoconjugates (glycoproteins, glycolipids) and carbohydrate chains. Free levels of SA form a very small proportion of SA levels. It has been reported as a marker for acute phase response; increased SA concentration has been observed in myocardial infarction, diabetes, tumour's, psoriasis, and alcoholism. Serum SA levels are found to be elevated during inflammation due to increased levels of richly sialylated acute phase-glycoprotein which serves as a marker of inflammation [5,6].

A study on serum SA in inactive and primary rheumatoid arthritis hypothesised that primary osteoarthritis may be an inflammatory disease and it was found that their levels were increased in patients with arthritis [6]. In serum/plasma, the normal range of Total Sialic Acid (TSA) levels is 1.58-2.22 mmol/L, the free form of SA only amount to 0.5-3 mmol/L, and the Lipid bound Sialic Acid (LSA) forms 10-50 mmol/L [7]. A study was done on free serum SA also concluded that it can be a useful marker of inflammation in psoriasis [8]. Recent data revealed the role of raised serum levels of free SA in inflammatory diseases like rheumatoid arthritis, psoriasis, systemic lupus erythematosus, and inflammatory bowel disease [9]. Psoriasis is a chronic inflammatory disease. Rajappa M et al., reported a significant increase in SA in psoriasis patients and SA levels were positively correlated with disease activity [10]. SA is a more stable inflammatory marker with less intraindividual variability providing a more accurate reflection of an individual's inflammatory status [11].

The present study aimed to evaluate the usefulness of the determination of serum levels of SA assessment of disease activity and severity, and for prediction of the outcome of a therapeutic intervention for psoriasis.

MATERIALS AND METHODS

This case-control study was conducted in the Outpatient Department of Dermatology, Venereology, and Leprosy in the Yenepoya Medical College and Hospital, Mangalore, Karnataka, India from October 2012 to May 2014.The study was conducted after approval from the Institutional Ethical Committee (IEC) dated 9/01/2013, and also after obtaining consent from all the participants.

The study was conducted among all the patients diagnosed to have psoriasis above the age of 18 years who attended OPD during the

study period. Age and sex-matched subjects like patient bystanders and people without psoriasis coming for routine health check-up willing to participate in the study were considered as controls and included in the study. The study included 50 cases and 50 age and sex-matched controls.

Inclusion criteria:

- Patients above the age of 18 years attending the OPD of Dermatology with a clinical diagnosis of psoriasis having erythematous scaly plaques, showing positive Auspitz sign (both new and old) were included in the study.
- Age and sex-matched subjects without psoriasis and willing to participate in the study were recruited controls.

Exclusion criteria:

- Patients with acute febrile illness, active systemic diseases, or events such as arthritis, hepatic disease, renal disease, malignancy, and pregnancy.
- Patients on systemic therapy or phototherapy for psoriasis for the past month.
- Participants who refused to participate in the study were excluded.

Clinical Methods

Clinical examination including general, systemic and dermatological examination was carried out. The severity of psoriasis was assessed according to PASI. The PASI is a useful tool for monitoring the response of psoriasis to any therapeutic regimen. A score can vary between 0-72. Body surface area was measured using the 'rule of 9' [12]

The study estimated free serum sialic acid among cases and control. To estimate the serum SA, the method proposed by Yao K et al., was used [13]. In this method, precipitation of proteins was obtained by treatment of serum with ethanol. The estimation of SA of both the precipitate and supernatant was estimated based on the reaction of SA with ninhydrin reagent. This was compared with N-acetyl neuraminic acid standards ranging from 20-100 mg/mL. SA concentration obtained from the precipitate was protein-bound SA and that obtained from the supernatant was free SA which was read at 470 nm by spectrophotometer.

Reagents used were:

a) 95% ethanol

b) Sialic acid standard (10 mg/100 mL distilled water)

c) Ninhydrin reagent: Mix 6 mL acetic acid, 4 mL of concentrated hydrochloric acid, and 250 mg ninhydrin, vortex for 20-30 minutes

d) Glacial acetic acid

e) 0.1 N sodium hydroxide

Study Procedure

A 100 µL of serum were precipitated using 5 mL ethanol and centrifuged at 5000 rpm for 5 minutes. A precipitate was formed and the precipitate was dissolved in 1 mL of 0.1 N sodium hydroxide. In the standard test tube, 100 L of a SA standard of which 1 mL of glacial acetic acid and 1 mL of ninhydrin reagent was added to all the test tube and kept in a boiling water bath for 10 minutes and cooled under tap water and absorbance was taken at 470 nm in a spectrophotometer against blank set at zero.

Calculation of SA was done by the formula: OD of test/OD of standard×concentration of the standard [14].

STATISTICAL ANALYSIS

Software Statistical Package for the Social Sciences (SPSS) version 17.0 was used to perform statistical analysis. Statistical significance was evaluated between cases and controls using Student's unpaired t-test and Pearson's correlation coefficeient. SA levels were correlated with PASI, using Pearson's correlation coefficient. All values were expressed as Mean±Standard Deviation (SD). The level of significance was set at 5%.

RESULTS

The current study included 50 cases and control with a mean±SD age of cases and controls was 44.04±10.9 years and 44.10±9.996, respectively. Demographic characteristics are shown in [Table/Fig-1].

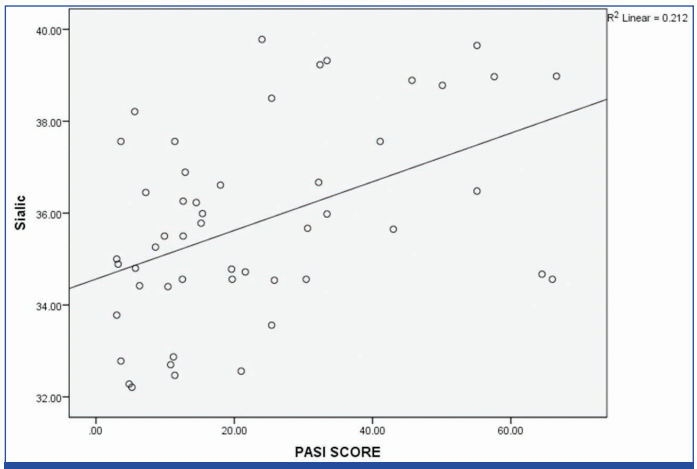
Demographic details	Cases (N=50)	Controls (N=50)	p-value	Z-score
Age (mean±SD) years	44.040±10.928	44.100±9.996	0.977	-
Male, n (%)	39 (78)	39 (78)	1	-
Female, n (%)	11 (22)	11 (22)	1	-
Disease details				
Duration of psoriasis (mean±SD) years	5.8750±5.478	-	-	-
<6 months n (%)	5 (10)	-	-	
6 months-1-year n (%)	5 (10)	-	-	
More than 1-year n (%)	40 (80)	-	-	
Psoriasis Area Severity Index (PASI)				
Mild	2 (4)	-	-	
Moderate	11 (22)	-	-	
Severe	37 (74)	-	-	
Mean serum free SA	35.792±2.124	28.556±3.854	<0.001	11.623

[Table/Fig-1]: Demographic details and correlation between sialic acid levels of cases and controls.

The mean PASI was 23.16. There was a positive correlation between serum sialic acid values in cases with the PASI score. The p-value was <0.001 and the coefficient of correlation was 0.460 [Table/Fig-2,3].

Variables	Values (mean±SD)
PASI	23.1666±18.471
Free Serum SA	35.792±2.124

[Table/Fig-2]: Mean PASI and free serum sialic acid levels.



[Table/Fig-3]: Correlation between the PASI score and sialic acid.

DISCUSSION

Psoriasis is a chronic inflammatory disease characterised clinically by erythematous scaly plaques with induration predominantly involving the scalp, extensors of the upper limbs, lower limbs, and lumbosacral region. In addition to keratinocytes, dendritic cells, and T-cells which are the three main cells involved in the pathogenesis of psoriasis, other cells such as Natural killer (NK) cells, NK-T cells, and neutrophils also play a role. Psoriasis lesions contain increased numbers of T cells of different subsets cells that produce Interleukins (IL)IL-17 and IL-23 indicating a Th 17 response. Th1 cell polarisation is also seen with a production of Interferon-γ and Tumour necrosing factor -α was recently described to play an important role in

maintaining chronic inflammation [15,16]. Psoriasis patients have increased levels of inflammatory markers, ROS, a compromised function of the antioxidant system, and lipid peroxidation. This suggests a link between psoriasis, inflammation, and oxidative damage [17]. In the present study, levels of free SA were found to be elevated, which may be a result of inflammation.

The present study observed a significant increase in the free SA levels in patients with psoriasis compared to controls. Significant increase in free serum SA levels parallels oxidative stress in psoriasis patients. These findings were consistent with an Indian study by Rajappa M et al., which found TSA levels (181.44±67.60 vs 33.09±7.72, p-value <0.0001) and protein-bound sialic acid (PBSA) (56.06±17.96 vs 13.10±2.77 p-value <0.0001) higher in psoriasis patients. In their study, patients with arthritis were included and Serum TSA and PBSA levels were also assayed by modified Aminoff's method. while in the present study, patients with arthritis were excluded and free serum SA measured by a method proposed by Yao K et al., They demonstrated that there was a significant association of oxidative stress and inflammation with psoriasis and therapy with Methotrexate significantly reduces inflammatory and oxidative stress parameters [10,13].

The findings of the present study are also consistent with a study by Shenoy C et al., in which Lipid-Bound Sialic Acid (LBSA) levels were measured. This is a more accurate measure since only small amount of SA is found free in the blood and it is usually bound to glycoproteins, glycolipids, oligosaccharides, and polysaccharides [18] .

There was a significant correlation between the free serum SA levels with the PASI scoring found in this study with the p-value of 0.001 and the coefficient of correlation was 0.460. Shenoy C et al., reported a non significant negative correlation between LBSA levels and PASI (r=-0.1533, p-value=0.464). This was probably because they studied LBSA, which acts not only as marker of inflammation but also as free radical scavenger [18]. The present study suggests the potential utility of serum free SA as a biomarker of multiple parameters like hyperproliferation, inflammation, and oxidative stress in psoriasis. Oxidative stress can be used as a biomarker for the assessment of the severity and therapeutic response in psoriasis. One of the pivotal factors in cardiovascular morbidity is oxidative stress. It is hypothesised that oxidative stress and systemic inflammation occurring in psoriasis could serve as a missing link in the causal relationship between psoriasis and co-morbidities, thus warranting early intervention measures.

Limitation(s)

The LBSA value was not measured which is more accurate.

CONCLUSION(S)

The role of oxidative stress and its implication as a biomarker for assessing disease severity and treatment response in psoriasis has been previously studied. This present study is, nevertheless, an important addition to support the role of free serum SA as a marker of inflammation in psoriasis. The severity of psoriasis as estimated by PASI scoring was associated with higher levels of free serum SA. There is a scope for future research in this field with a larger sample size to develop concrete evidence regarding the role of SA. This may translate into a potential target for pharmacotherapeutics in this ever-evolving field of psoriasis.

REFERENCES

[1] Oliveira A, Simoes M, Simoes R, Malachias M, Rezende B. Cardiovascular risk in psoriasis patients: Clinical, functional and morphological parameters. Arq Bras Cardiol. 2019;113(2):242-49.
[2] Parisi R, Symmons DP, Griffiths CE, Ashcroft DM. Identification and Management of Psoriasis and Associated Comorbidity (IMPACT) project team. Global epidemiology of psoriasis: A systematic review of incidence and prevalence. J Invest Dermatol. 2013;133(2):377-85.
[3] Pariser DM, Bagel J, Gelfand JM, Korman NJ, Ritchlin CT, Strober BE, et al. National Psoriasis Foundation. National psoriasis foundation clinical consensus on disease severity. Arch Dermatol. 2007;143(2):239-42.
[4] Baz K, Cimen MY, Kokturk A, Yazici AC, Eskandari G, Ikizoglu G, et al. Oxidant/antioxidant status in patients with psoriasis. Yonsei Med J. 2003;44(6):987-90.
[5] Schauer R, Kamerling JP. Exploration of the sialic acid world. Adv Carbohydr Chem Biochem. 2018;75:01-213.
[6] Alturfan AA, Uslu E, Alturfan EE, Hatemi G, Fresko I, Kokoglu E, et al. Increased serum sialic acid levels in primary osteoarthritis and inactive rheumatoid arthritis. Tohoku J Exp Med. 2007;213(3):241-48.
[7] Guruaribam VD, Sarumathi T. Relevance of serum and salivary sialic acid in oral cancer diagnostics. J Cancer Res Ther. 2020;16(3):401-04.
[8] Devatdarova MM. Sialic acid levels in the blood serum of healthy persons and patients with psoriasis. Vestn Dermatol Venerol. 1968;42(8):43-46.
[9] Reily C, Stewart TJ, Renfrow MB, Novak J. Glycosylation in health and disease. Nat Rev Nephrol. 2019;15(6):346-66.
[10] Rajappa M, Shanmugam R, Munisamy M, Chandrashekar L, Rajendiran KS, Thappa DM, et al. Effect of antipsoriatic therapy on oxidative stress index and sialic acid levels in patients with psoriasis. Int J Dermatol. 2016;55(8):e422-30.
[11] Pulimamidi VK, Murugesan V, Rajappa M, Satheesh S, Harichandrakumar KT. Increased levels of markers of oxidative stress and inflammation in patients with rheumatic mitral stenosis predispose to left atrial thrombus formation. J Clin Diagn Res. 2013;7(11):2445-48.
[12] Bhor U, Pande S. Scoring systems in dermatology. Indian J Dermatol Venereol Leprol. 2006;72(4):315-21.
[13] Yao K, Ubuka T, Masuoka N, Kinuta M, Ikeda T. Direct determination of bound sialic acids in sialoglycoproteins by acidic ninhydrin reaction. Anal Biochem. 1989;179(2):332-35.
[14] Witko-Sarsat V, Friedlander M, Nguyen Khoa T, Capeillère-Blandin C, Nguyen AT, Canteloup S, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J Immunol. 1998;161(5):2524-32.
[15] Kim GK, Del Rosso JQ. Drug-provoked psoriasis: Is it drug induced or drug aggravated? Understanding pathophysiology and clinical relevance. J Clin Aesthet Dermatol. 2010;3(1):32-38.
[16] Li K, Huang CC, Randazzo B, Li S, Szapary P, Curran M, et al. HLA-C*06:02 Allele and response to IL-12/23 inhibition: Results from the Ustekinumab Phase 3 Psoriasis Program. J Invest Dermatol. 2016;136(12):2364-71.
[17] Kadam DP, Suryakar AN, Ankush RD, Kadam CY, Deshpande KH. Role of oxidative stress in various stages of psoriasis. Indian Journal of Clinical Biochemistry. 2010;25(4):388-92.
[18] Shenoy C, Shantaram M, Sharanya K, Shenoy MM. Lipid-bound sialic acid in psoriasis and its correlation with disease severity. Saudi J Health Sci. 2015;4:56-58.

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