

Serum biomarkers for diagnosis and assessment of severity in psoriasis

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Abstract

Introduction: Psoriasis is a common inflammatory and proliferative disease of the skin. The current gold standard for assessment of extensive psoriasis has been Psoriasis Area and Severity Index (PASI). However the limitations of using PASI score in clinical practice and research necessitates for search of biomarkers.

Aims & Objectives: To determine the role of serum high sensitivity C-Reactive Protein (hs-CRP), Ceruloplasmin and Malondialdehyde (MDA) as biomarkers in psoriasis and their significance in assessment of disease severity in patients with mild, moderate and severe psoriasis.

Materials & Methods: The study group consisted of 120 subjects of age group 20-50 years and of both sexes. Of these 40 patients had active psoriasis with PASI score <10 (Group 2), 30 patients had active psoriasis with PASI score > 10 (Group 3) and 50 were healthy age and sex matched controls (Group 1).

Results & Observations: The serum levels of CRP, ceruloplasmin and MDA are elevated in both the groups of psoriasis patients when compared to controls. The increase is statistically highly significant ($p < 0.001$). Very large positive correlation was obtained for hs-CRP and MDA values with PASI score in both the groups, while moderate correlation was obtained for Ceruloplasmin with PASI score.

Conclusion: Finally, it can be concluded that hs-CRP, MDA and Ceruloplasmin levels can be used as biomarkers and also to assess the disease severity in psoriasis.

Keywords: PASI score, hs-CRP, Ceruloplasmin, MDA.

1. Introduction

Psoriasis is a *chronic inflammatory skin disease* (CISD) affecting approximately 0.44 to 2.8% of people in India [1]. The disease appears in different clinical variants i.e., Chronic plaque psoriasis (accounts for 90% of all cases of psoriasis), inverse psoriasis, generalized pustular psoriasis, palmo plantar psoriasis and guttate psoriasis [2].

Psoriasis is assumed as an immune mediated inflammatory disease. The development of psoriasis is associated with genetic predisposition which has a basis of T cell activation secondary to dermal inflammation and abnormal keratinocyte proliferation. T cells together with their cytokines and chemokines are shown to induce a sustaining serious inflammation by Th-1 and Th-17 driven immune response including tumor necrotic factor-alpha, interferon-gamma, IL-8, IL-12, IL-20, IL-22. The constant inflammatory cell chemotaxis and cytokine release causes chronic clinical course with recurrent lesions.

The basic characteristics of psoriasis lesions-redness, thickness and scaliness, provide a means of assessing

the severity of psoriasis. The current gold standard for assessment of psoriasis, the Psoriasis Area and Severity Index (PASI) score, is calculated based on these characteristics. Patients with PASI score < 10 have Mild psoriasis. Patients with PASI score > 10 have Moderate to Severe psoriasis. However, the limitations of using PASI score in clinical practice and research are related to low reproducibility and high variability between dermatologists (Subjective marker)[3]. Also, several studies have proven that patients graded as mild psoriasis by PASI score showed increased inflammatory activity when compared to patients graded as Moderate to severe Psoriasis [4].

Hence there must be an Objective way to assess the severity of psoriasis based on serum inflammatory markers. CRP is one such inflammatory biomarker [5]. The worsening of psoriasis is also linked with oxidative stress. Malondialdehyde (MDA) is a marker of oxidative stress [5]. Ceruloplasmin is an acute phase reactant that scavenges oxygen derived free radicals [6]. Thus serum MDA and

ceruloplasmin levels may also be used to assess severity of psoriasis.

C-reactive protein (CRP) is a hepatic acute phase reactant. Its synthesis is mainly controlled by IL-6, but IL-1 and TNF- α may also influence CRP levels. Hence serum CRP is an indirect marker of proinflammatory cytokine activity [2]. Ceruloplasmin is a copper containing α_2 globulin, which is also an acute phase reactant synthesized by liver. The known functions of ceruloplasmin include copper transport, iron metabolism, antioxidant defense, involvement in angiogenesis and coagulation [6][7]. Malondialdehyde is a marker of plasma lipid peroxidation and also a marker of oxidative stress. Increased oxidative stress in psoriasis arises due to increased inflammatory activity [2].

1.2 Aims & Objectives

The aim of this study is to know whether inflammatory marker - hs-CRP, oxidative stress marker - MDA and endogenous anti-oxidant - Ceruloplasmin levels can be used as biomarkers in psoriasis and whether they can be used to assess severity of psoriasis.

The objective of the present study is to -

- Estimate the levels of CRP, MDA and Ceruloplasmin in patients with Mild Psoriasis, Moderate to Severe Psoriasis and healthy controls.
- Correlate them with PASI score, and demonstrate their utility as a valuable tool in assessing the severity of psoriasis.

2. Materials and Methods

3.1. Study centre & Period

This research was conducted at clinical laboratory, Department of Biochemistry, Andhra Medical College between October 2013 and May 2015.

3.2. Subjects Selection:

Patient selection was done by simple random sampling of individuals presenting to the outpatient clinic of Department of Dermatology, King George Hospital attached to Andhra Medical College, Visakhapatnam. An informed consent was taken from the psoriasis patients and controls before the collection of blood sample. The subjects were selected based on following inclusion and exclusion criteria.

3.3. Inclusion Criteria

- Clinically diagnosed new cases of psoriasis (who are not on medication/PUVA therapy) in the age group of 25-50 years.
- Healthy controls from general population in age group 25-50 years.

3.4. Exclusion Criteria

- Patients with active psoriasis not on medication, PUVA therapy.
- Patients or controls with bacterial or viral infections.

- Patients with sore throat or urinary tract infections.
- Patients with history of acute or chronic liver diseases and renal disease.
- Women on oral contraceptive pills.
- Patients having chronic inflammatory disorders like Rheumatoid Arthritis, Tuberculosis, Osteoarthritis, Inflammatory Bowel Disease, eczematous dermatitis.
- Use of antioxidants, anti-inflammatory drugs, hypolipidemic drugs etc;

3.5. Study Pattern

After obtaining consent, the study subjects were subjected to detailed history taking including demographic data, drug history, personal history, family history present and past medical history and drug intake. The available case records were scrutinized to collect any valid data. The severity of psoriasis has been graded using Psoriasis Area and Severity Index (PASI) score.

Group 1: Controls - 50 age matched controls of both sex, who came for routine health check-up / healthy volunteer.

Group 2: Cases - 40 patients with active psoriasis, PASI score <10

Group 3: Cases - 30 patients with active psoriasis, PASI score >10

3.6. Specimen Collection

Venous blood (5ml) was obtained from each of the subjects by vein puncture of the ante cubital vein using a sterile needle and syringe. The blood samples were then transferred into clean sterile centrifuge tubes and allowed to clot. Each clotted sample was centrifuged at 3000 rpm for 3 min to obtain the serum. The serum was removed using a micropipette and transferred to Eppendorf tubes. The biochemical assay was carried out within 24hrs of collection.

3.7. Assay of Markers

CRP in serum was measured by turbidimetric immunoassay [8] and carried out in a semi-automated analyzer. Ceruloplasmin in serum was measured by Houchin method [9] using Para Phenylene Diamine (PPD), Acetate buffer and sodium azide. MDA in serum was measured by Mahalouz method [10] using 20% TCA & 0.67% TBA.

3.8. Statistical Analysis

The data obtained were analyzed using Student's t-test where $p < 0.001$ was considered as highly significant. All results were expressed as Mean \pm S.D. Correlation with PASI score was done by Pearson correlation coefficient.

4. Results & Observations

The results are shown in Table 1 (Mean \pm SD), Table 2 (p values between the groups) & Table 3 (correlation of biomarkers with PASI score).

Table 1: Correlations of Biomarkers (Mean \pm SD)

Analyte	Group 1 (N=50)	Group 2 (N=40)	Group 3 (N=30)	Group 2 + Group 3
hs-CRP (mg/L)	1.57 \pm 0.64	3.17 \pm 1.53	7.42 \pm 2.35	5.03 \pm 2.79
CP (mg/dl)	27.77 \pm 5.24	43.2 \pm 8.06	45.5 \pm 7.75	45.2 \pm 7.85
MDA (nmol/ml)	2.27 \pm 0.59	4.01 \pm 0.84	6.05 \pm 1.25	4.91 \pm 1.38

From the above table it was observed that, Mean \pm SD of all the biomarkers are increased in cases when compared to the controls (Group 2+3 Vs Group 1). The increase is much higher in Group 3 patients than Group 2 patients.

Table 2: Correlations of Biomarkers (p value)

	p value (Group2/ Group 1)	p value (Group3/ Group 1)	p value (Group3/ Group2)	p value (Group2+3/ Group 1)
hs-CRP	<0.001	<0.001	<0.001	<0.001
CP	<0.001	<0.001	0.316	<0.001
MDA	<0.001	<0.001	<0.001	<0.001

p value <0.001 is considered statistically highly significant.

From the above table it was observed that the increase in biomarkers were statistically significant in cases when compared to controls. (Group 2+3 Vs Group 1). Also the increase in Group 3 patients was significantly higher than in Group 2 patients (with exception of ceruloplasmin).

Table 3: Correlations of Biomarkers

Category	PASI Vs CRP	PASI Vs CP	PASI Vs MDA
Group 2	r = 0.840	r = 0.449	r = 0.775
Group 3	r = 0.775	r = 0.384	r = 0.733
Group 2 + Group 3	r = 0.833	r = 0.328	r = 0.831

From the above table it was observed that there was very large positive correlation (0.7-0.9) of serum hs-CRP and serum MDA levels with PASI score in both Group 2 and Group 3 patients and also in Group 2 + Group 3 patients.

Moderate positive correlation (0.3-0.5) was shown by serum Ceruloplasmin with PASI score in above groups.

4.4. Scatter plots showing the distribution of -

4.4.1. Serum CRP levels in Group 1, Group 2 & Group 3.

4.4.2. Serum MDA levels in Group 1, Group 2 & Group 3.

Figure 1: Distribution of hs-CRP Levels in Group 1, Group 2, Group 3

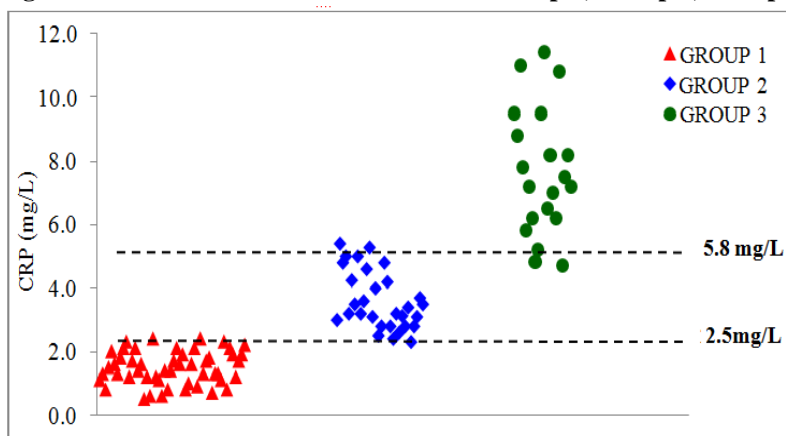
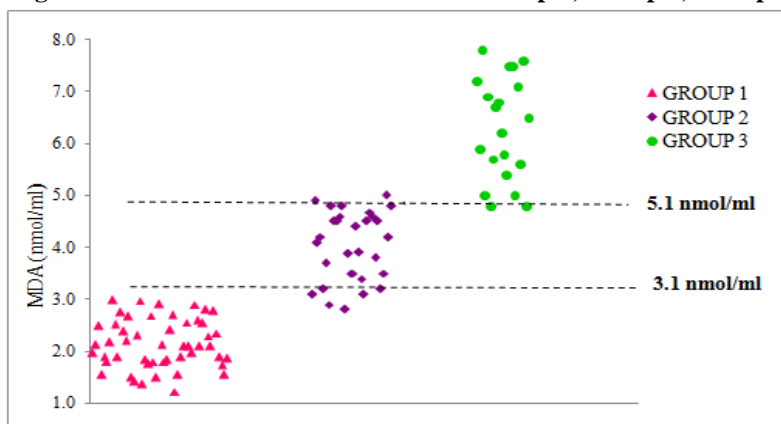


Figure 2: Distribution of MDA Levels in Group 1, Group 2, Group 3



5. Discussion

Psoriasis is a paradigm of a chronic and relapsing inflammatory skin disease, characterized by marked inflammatory changes in the epidermis and dermis. Psoriasis patients demonstrate increased inflammatory activity; the increase in inflammatory activity is proportional to severity of disease. Inflammation, as described previously plays an important role in pathogenesis of psoriasis.

Considering the indispensable role of inflammation in development of psoriasis and of studies reporting increased levels of inflammatory markers in psoriasis, present study was undertaken to study role of hs-CRP, Ceruloplasmin as biomarkers of severity in psoriasis, and also to study degree of oxidative stress by evaluating MDA in psoriasis patients with differing severity.

5.1. Increased C - reactive protein levels

The inflammatory state in psoriasis releases pro inflammatory cytokines, which stimulate liver to produce acute phase reactants. CRP is one such acute phase reactant. Elevated CRP levels result from the interaction between pro-inflammatory cytokines, namely IL-6, TNF- α and IL-1, their receptors and inhibitory factors. TNF- α induces secretion of IL-6, which stimulates hepatic production of CRP, an effect that can be enhanced by IL-1 β [11].

C-Reactive Protein concentrations in serum increase with increasing severity of psoriasis and show positive correlation with PASI. When aggregated or bound to macromolecular ligands (autologous and extrinsic ligands), human CRP is recognized by C1q and potently activates the classical complement pathway, engaging C3, the main adhesion molecule of the complement system, and the terminal membrane attack complex, C5–C9[12]-[14], which cause destruction of tissue, thereby playing a role in development of clinical features of psoriasis.

The findings of present study correlate well with findings of previous studies - Pereira *et al.* (2004)[5], Coimbra *et al.* (2009)[15], Yiu *et al.* (2011)[16] and Lucy Piper *et al.* (2012)[4].

5.2. Increased Ceruloplasmin levels

The increased inflammatory activity in psoriasis results in increased neutrophil activation resulting in degranulation and generation of superoxide radicals that result in development of oxidative stress in psoriasis.

Increased oxidative stress results in increased production of ceruloplasmin, an acute phase reactant, which also acts as a circulating antioxidant, scavenging superoxide free radicals and by linking iron avoid the development of the Fenton reaction. This reaction appears to be an important mechanism for generation of the hydroxyl radical, the more deleterious oxygen metabolite, from hydrogen peroxide in the presence of free iron and reducing agents, namely superoxide, ascorbate and lactate[5].

Serum ceruloplasmin level may be a complementary factor associated with inflammatory conditions and its levels

are raised in psoriasis. However, raised levels of ceruloplasmin show only moderate correlation with severity of psoriasis.

The findings of present study correlate well with findings of previous studies – Pereira *et al.* (2004)[5] and Manjula *et al.* (2013)[6].

5.3. Increased MDA levels

Serum malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation. MDA is able to impair several physiological mechanisms of the human body through its ability to react with molecules such as DNA and proteins.

Generation of ROS from neutrophils, keratinocytes and fibroblasts can contribute to neutrophil activation which may play an important role in psoriatic process. ROS can act as second messengers in the induction of several biological responses such as the activation of NF-kB or AP-1, the generation of cytokines, the modulation of signaling pathways and the activation of peroxisome proliferator-activated receptors[17][18]. Increased generation of ROS increases peroxidation of membrane unsaturated fatty acids generating malondialdehyde (MDA), whose serum levels are increased in psoriasis.

The findings of present study correlate well with findings of previous studies –Pereira *et al.* (2004)[5], Jyothi *et al.* (2011)[18], Mahmoud *et al.* (2012)[18].

6. Conclusion

The results of the present research provide valuable information and association between the measured biomarkers and severity of psoriasis. Serum hs-CRP, Ceruloplasmin and MDA levels were significantly elevated in psoriasis cases when compared to the control group. Positive correlation was also established between the serum C-RP levels, serum MDA levels and serum Ceruloplasmin levels with PASI score in patients with psoriasis.

Hence, we suggest that combined estimation of Serum hs-CRP, MDA and Ceruloplasmin may be used as biomarkers for diagnosis and also for assessment of severity in psoriasis.

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