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Is enhanced platelet activation the missing link leading to increased cardiovascular risk in psoriasis?

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ABSTRACT

Background: Psoriasis is an immune mediated inflammatory skin disease associated with systemic inflammation resulting in increased risk for associated cardiovascular co-morbidities. The role of platelet activation in the pathophysiology of this condition has not been clearly studied. We undertook to study the platelet activation markers in psoriasis, as compared to controls and to identify its association with disease severity in psoriasis.
Methods: Sixty-two patients with psoriasis and 62 age and gender matched healthy controls were enrolled in this cross-sectional study. The severity of the disease was assessed using the psoriasis area severity index (PASI) scoring. The platelet indices [mean platelet volume (MPV) and platelet distribution width (PDW)] were estimated by an automated haematological laser optical analyzer. Plasma soluble P-selectin and platelet derived microparticle (PDMP) concentrations, serum high sensitivity C-reactive protein (hs-CRP) and interleukin (IL)-6 concentrations were estimated in all study participants. Platelet aggregation was assessed using adenosine diphosphate (ADP) as aggregating agent.
Results: We observed that there was significantly higher platelet indices (MPV and PDW) in patients with psoriasis, when compared to controls. Plasma soluble P-selectin concentrations, PDMP and platelet aggregation were significantly elevated in patients with psoriasis, as compared to controls. We also found significantly higher concentrations of hs-CRP and IL-6 in patients with psoriasis, as compared to controls. Platelet activation and systemic inflammation markers correlated positively with PASI, except PDW. We also observed significant positive correlation between platelet activation and systemic inflammation in psoriasis.
Conclusion: Significant platelet activation and systemic inflammation were observed in patients with psoriasis, especially when associated with severe disease. The increased platelet activation might be the missing link between the persistent inflammation and the development of atherosclerotic plaque leading onto cardiovascular co-morbidities seen associated with psoriasis.

1. Introduction

Psoriasis is a chronic inflammatory immune mediated skin disease. Though considered primarily a skin disease, recent research implicates its systemic nature, its association with diverse co-morbidities, cardiovascular morbidity being the most portentous one. The concept of psoriatic march describes how the persistent cutaneous inflammation seen in psoriasis leads onto systemic inflammation, resulting in insulin resistance, dyslipidemia, endothelial dysfunction, leading to coronary artery disease [1]. Recent studies explore the possible role of platelets in increasing inflammation in addition to their main role in hemostasis and thrombosis. Platelet activation has been implicated in the pathogenesis of a number of diseases, which include atherosclerosis, coronary vascular disease, and cerebrovascular disease [2]. Psoriasis patients have been shown to have increased incidence of occlusive vascular disease and platelet hyperaggregability [3,4].

Various platelet indices such as mean platelet volume (MPV) and platelet distribution width (PDW) are being used as markers of platelet activation. Patients with psoriasis are found to have higher MPV when compared to controls [5]. Larger platelets are found to be more reactive, release more serotonin and granule contents and express more GP1b and are also preferentially aggregated after addition of ADP, collagen and thrombin [6]. Psoriatic patients were also found to have increased platelet aggregation on in vitro addition of thrombin or adenosine diphosphate [8]. Activation of platelets have been shown to shed a part of its surface membrane as a microparticle and also release soluble P-Selectin into plasma [9,10]. Thus PDMP and soluble P-selectin

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concentrations are useful markers of estimating platelet activation in patients with psoriasis.

Platelet activation may be the missing link between the persistent inflammation and the development of atherosclerotic plaque leading onto the cardiovascular co-morbidities seen associated with psoriasis. However, the exact mechanisms of these associations remain unclear.

2. Methods

This hospital-based, cross sectional study was carried out in 62 patients diagnosed with psoriasis and 62 age and gender matched healthy controls. Ethical clearance was obtained from the Institute Ethics Committee (Human Studies) and written informed consent was obtained from all study subjects prior to participation in the study. This study was conducted in accordance with the guidelines of the Helsinki Declaration of 1975, as revised in 1983.

2.1. Sample size estimation

Sample size is estimated using the statistical formula for sample size for comparing two independent means with equal variance. The mean concentration of MPV among normal individuals in Indian population was reported as 9.20 fl with a S.D of 0.91 fl [11]. The minimum expected difference in the mean MPV value between the psoriasis patients and normal individuals was 0.46 fl with a S.D of 0.91 fl [5]. The sample size was estimated at 5% level of significance and 80% power in 1:1 ratio. The estimated sample size for this study was thus calculated as 62 in each group (62 subjects with psoriasis and 62 age and gender matched healthy controls).

2.2. Study population

Patients with psoriasis (International Psoriasis Council Consensus Classification of Psoriasis) [12] attending the dermatology outpatient department of our institute were recruited as cases in our study. Age- and gender-matched healthy volunteers were recruited as controls.

The exclusion criteria for cases and controls were the following: Patients on any medication for the last one month prior to inclusion, dyslipidemia, age < 18 y, pregnancy, malignancies, hepatic and renal disease, diabetes mellitus, morbid obesity (>30 kg/m^2), cardiovascular disease, infectious diseases and other inflammatory skin diseases.

2.3. Workup

In all patients with psoriasis, a detailed history with treatment details was taken followed by clinical examination and anthropometric measurements, all of which were recorded in a predesigned proforma. The disease severity was assessed using Psoriasis Area Severity Index (PASI) scoring [13] by 2 dermatologists independently and the mean PASI score was taken to assess disease severity. For controls, anthropometric measurements were recorded. Metabolic syndrome was evaluated by the National Cholesterol Education Program (NCEP-3) criteria-Waist circumference (WC), triglycerides (TG), High density lipoprotein (HDL), blood pressure (BP), fasting plasma glucose were recorded [14]. Presence of any three criteria indicates presence of metabolic syndrome. (WC > 102 cm in males/>88 cm in females, TG > 150 mg/dl, HDL < 40 mg/dl, BP > 130/85 mm Hg, fasting plasma glucose > 100 mg/dl).

3. Cardiovascular risk assessment

Framingham risk score predicts a person's chance of having a heart attack in the next 10 y. This tool is designed for adults aged ≥20 y who do not have heart disease or diabetes. This score has originally been designed for the Caucasian population and does not take into account the ethnicity of the individual. This is important in Indians, as the Indian ethnicity by itself confers a 2-fold increased cardiovascular risk [15]. Further, the cut off levels of the various factors used in the traditional Framingham risk score calculation may be way higher than that considered high risk for an Indian population [16]. Chow et al [17], in his study has shown that recalibration of Framingham risk score based on Indian national data also was irrelevant and stressed the importance of local data collection for each particular region in India and to recalibrate the Framingham score accordingly. But this has also not been validated yet. Hence, though not the ideal tool for calculating cardiovascular risk in Indians, in the absence of other well-established standard scoring systems for cardiovascular risk in the atherosclerosis-prone South Indian population, we used the Framingham risk score for calculating cardiovascular disease risk in the present study. This was a limitation of this study.

Framingham risk score for cardiovascular risk assessment was calculated in all study subjects as follows-Age, gender, total cholesterol, HDL-cholesterol, BP, presence or absence of diabetes mellitus, and smoking: Scoring <10% low risk, 10-20% intermediate risk, and >20% high risk for coronary event within 10 y [18].

3.1. Sample collection

After a fasting period of 12 h, 6 ml of venous blood was taken from the antecubital vein of all study subjects. It was divided into 3 aliquots of 2 ml each, in ethylene diamine tetraacetic acid (EDTA) containing tubes, citrate containing tubes and plastic tubes with no anti-coagulant. The initial 2 ml was collected in EDTA containing tubes for estimation of platelet number and platelet indices and for assay of blood glucose levels. The platelet indices were estimated by an automated haematological laser optical analyzer (Sysmex XT-1800i). Mean platelet volume (MPV) and platelet distribution width (PDW) were routinely measured. Mean platelet volume, measured in femtolitres (fl) was calculated by the following formula: MPV (fl) = [plateletcrit (%) / platelet count (10^9/L)] x 10^5. Platelet volume and PDW were derived from the platelet size distribution curve. The distribution width at the level of 20% was defined as PDW. The next aliquot of 2 ml was collected in plastic tubes with no anti-coagulant for estimation of lipid profile and for assay of hs-CRP and IL-6 in serum by using commercially available ELISA kits (Diagnostics Biochem and Diaclone Research, respectively).

To avoid artificial platelet activation during collection of samples, the remaining 2 ml of blood was transferred and mixed immediately, avoiding frothing into plastic tubes containing 3.8% sodium citrate (1:9 volume) and used for estimation of soluble P-selectin and PDMP concentrations in plasma by using commercially available ELISA kits (R & D Biosystems and MyBioSource Inc, respectively) and platelet aggregation by impedance method, using Model 700-2 Chronolog aggregometer. All measurements were done in duplicate as per standard procedure and mean values obtained. Platelet aggregation studies were performed, half an hour after collection of sample. Adenosine diphosphate (ADP) (Sigma Chemical Co) was used as the aggregating agent. Platelet aggregation was stimulated with 10 μmol ADP. Platelet aggregation was expressed as the change in electrical impedance and is expressed in ohms. Aggregation curves were recorded for 6 min and analyzed using AGGRO/LINK8 control software.

4. Statistical analysis

Both descriptive and inferential statistics were used to analyze the data. Baseline characteristics of the study subjects were analyzed by descriptive statistics. Categorical data are described using percentages and frequencies and compared by using χ^2 test or Fischer's exact test, as appropriate. The normality of continuous data was assessed by Kolmogrov–Smirnov test. The normally distributed continuous data are described as mean ± standard deviation and median and interquartile range is used for non-Gaussian data. Normally distributed continuous data were compared by Independent Student's t-test and Mann-Whitney "U" test was used for non-Gaussian data. The Bonferroni correction was used to reduce the chances of obtaining false-positive results (type I