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Hanaa Rasmy
19 Ahmed Fahim,
7th District, Nasr City,
Cairo, Egypt

Tel: +202-4037491

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Soluble P-selection in Systemic Lupus Erythematosus

[1]H. Raslan and [2]Hanaa Rasmy

To investigate the level of soluble P-Selection in Systemic Lupus Erythematosus (SLE) patients and to correlate it with disease activity and any of the laboratory data currently available. Serum levels of soluble P-selection were determined in 27 SLE patients and 10 healthy subjects, matched for age and sex, as control group. The relations between soluble P-selection and clinical and laboratory data were analysed. Mean serum concentrations of soluble P-selection were higher in SLE patients(182.34±54.64) compared to controls (103.47±32.75) with a significant positive correlation between P-selection and homocysteine. No significant correlations were found between the levels of soluble P-selection and other laboratory data and SLE Disease Activity Index (SLEDAI). Elevated serum soluble P-selection in SLE patients could indicate the presence of a continuous inflammatory stimulus in SLE and that activated platelets participate in the pathogenesis of SLE and this is important to note as it may have potential therapeutic implications with respect to use antiplatelet agents in these patients.

Key words: System lupus erythematosus, P-selection, Atherosclerosis

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[1]Department of Internal Medicine,
[2]Department of Clinical and Chemical Pathology,
National Research Center, Cairo, Egypt

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a debilitating systemic autoimmune disease in which tissues throughout the body, particularly the kidney, skin, joints and brain are the target of an ongoing inflammatory attack. Much of this aberrant inflammatory response is directed against the vasculature (vasculitis) (Hickey *et al*., 2002).

The process of leucocyte recruitment to sites of inflammation requires that leucocytes undergo a well defined sequence of interactions with endothelial cells lining the microvasculature (Harari *et al*.,1999).

Evidence from many *in vitro* and *in vivo* studies indicates that the tethering and rolling of leucocytes along the endothelium are predominantly mediated by the selection family of adhesion molecules (Harari *et al*.,1999)

The glycoprotein P-selection is a cell adhesion molecule of stimulated platelets and endothelial cells (Palabrica *et al*., 1992). It is colocalised with von Willebrand factor (vWF) in the Weibel Palade bodies and the alpha granules. Increased concentrations of soluble P-selection (sP-selection) is reported in disorders accompanied by endothelial cell activation (Fruns *et al*., 2001).

The physiologic role of P-selection might be the mediation of initial leucocyte adhesion to activated endothelium during acute inflammation. It may work in concert with E-selection to direct early, regionally specific adherence of neutrophils and monocytes at sites of acute inflammation. Soluble P-selection is a potentially important molecule to provide more detailed insight into pathological situations. Excessive accumulation of neutrophils on the endothelial surface accompanied by high exposure of P-selection has been implicated in a number of inflammatory disorders, including Adult Respiratory Distress Syndrome (ARDS), acute lung injury, ischaemia-reperfusion injury, gram negative septic shock, thrombotic diseases and rheumatoid arthritis (Wu and Ruan, 1993).

The aim of the present study is to investigate the level of soluble P-selection in SLE patients and to correlate it with disease activity and any of the laboratory data currently available.

MATERIALS AND METHODS

The study was conducted at the Outpatients Clinic and the Laboratories of Clinical Medical Services Unit of the National Research Center (from November 2004 to July 2005). Twenty seven SLE patients were selected. They were 26 female and 1 male with an age range between 19 to 30 years (mean±SD:26.5±9.3 years), All patients fulfilled at least four criteria of the American College of Rheumatology (ACR) for the diagnosis of SLE (Tan *et al*., 1982). Ten healthy subjects matched for age and sex were enrolled in the study as a control group. All patients were subjected to full history taking with particular attention to the time of appearance of first symptom, history of thrombotic events, type of medication. Also a thorough clinical examination was performed to all patients including mucocutaneous, articular and neurological examination.

Mean duration of disease was 4.29±4.09 years. The duration of the disease was calculated from the time of onset of the first clinical event. The disease activity index was assessed using SLE disease activity index (SLEDAI). Theoretically the maximum score of SLEDAI is 105 (Bombardier *et al*., 1992). Patients scoring 6 or more were considered to have active disease. Hypertension was defined as systolic blood pressure≥160 mmHg and/or diastolic≥90 mmHg. Lupus nephritis was diagnosed if patient fulfilled the ACR criteria for renal involvement i.e., persistent proteinuria >0.5 g/24 h or cellular casts in the absence of infection (Tan *et al*., 1982).

The Routine laboratory investigations (erythrocyte sedimentation rate, ESR; complete blood count; serum creatinine; fasting blood sugar and lipid profile); antinuclear antibody, ANA; antidouble stranded DNA; complement C3 and C4; serum homocysteine and serum soluble P-selection investigations were done.

LABORATORY METHODS

Determination of soluble P-selection: Soluble P-selection was measured using the Parameter® human sP-selection Immunoassay supplied by R&D systems Inc. (614 McKinley Place NE Minneapolis, MN 55413 USA).

This assay employs the quantitative sandwich immunoassay technique.

Determination of serum homocysteine: Homocysteine was determined by an enzyme immunoassay (Frantzen *et al*., 1998). The kit was supplied by Axis-Shield (Bickbeerngrund 4 D-29614 Soltau, Germany).

Determination of serum C3 and C4: C3 and C4 were determined quantitatively by radio immunodiffusion (RID) plates using Biocientifica S.A. kits (Fries and Frank, 1988).

ANA and Anti-DNA demonstration: These parameters were detected by means of indirect immunofluorescence technique using air dried cryostat sections from mouse kidney stomach and crythedia luciliae as substrates,

respectively. They were supplied from IMMCO Diagnostics, Inc. (60 pineview Drive-Buffalo, NY 14228-2120 USA).

Fasting blood chemistry profile were measured by usual chemical methods according to Trinder (1969) and Barr *et al.* (1951).

Statistical methods: Results were tabulated and statistically analysed using the arithmetic mean and standard deviation. The Mann-Whitney test was used to determine differences in mean values. Pearson correlation coefficients were calculated to study the associations between different variables. A $p<0.05$ was considered statistically significant.

RESULTS

Fourteen patients (51.8%) had active disease at the time of the study. Four patients (14.8%) had history of thrombosis; one patient (3.7%) had venous thrombosis, one patient (3.7%) had arterial thrombosis and two patients (7.4%) had both arterial and venous thrombosis. Nine patients (33.3%) were hypertensive. Photosensitivity was present in five patients (18.5%), mucocutaneous lesions were present in 15 patients (55.5%), arthritis in eight patients (29.6%), serositis in six patients (22.2%), hematological abnormalities in three patients (11.1%), nephritis in nine patients (33.3%) and neurological manifestations in five patients (18.5%).

SLE patients had significant higher mean serum concentrations of soluble P-selection (182.34±54.64) compared to controls (103.47±32.75) p = 0.02. (Table 1).

There were no significant difference in mean serum concentrations of soluble P-selection between SLE patients with nephritis (mean: 210.53±85.37) and those without nephritis (mean: 168.62±49.628) (Table 2).

Hypertensive had higher mean serum concentrations of soluble P-selection (mean: 200.58±76.6) than non hypertensive (mean: 173.6±59) but this is statistically insignificant (Table 3).

There was a significant positive correlation between soluble P-selection and homocysteine r = 0.4438 (Fig. 1). No other significant correlation was found between soluble P-selection and any of the clinical nor laboratory data available (Table 4).

Table 1: Mean serum soluble P-selection concentrations in SLE patients and controls

	SLE patients	Controls	p-value
P-selection (ng mL^{-1})	182.34±54.64	103.47±32.75	0.02*

Values are in mean ±SD, *p is significant

Table 2: Mean serum concentrations of soluble P-selection in patients with a history of lupus nephritis and in those without nephritis

	SLE patients with nephritis (n = 9)	SLE patients without nephritis (n = 18)	p-value
Soluble P-selection (ng mL^{-1})	210.53±85.37	168.62±49.63	Non Significant

Values are in mean±SD

Table 3: Mean serum concentrations of soluble P-selection in patients with hypertension and in those without hypertension

	SLE patients with hypertension (n = 9)	SLE patients without hypertension n = 17)	p-value
Soluble P-selection	200.58±76.6	173.6±59	Non significant

Table 4: Correlation between soluble P-selection and other studied parameters

Variables	R	p-value
Age	0.1	0.5
Duration of disease	-0.01	0.9
Total cholesterol	0.4324	0.024*
Triglycerides	0.2	0.3
Creatinine	0.1	0.6
C3	0.01	0.9
C4	0.18	0.39
SLEDAI	-0.08	0.68
Homocysteine	0.44	0.026*

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index
C: Complement, *p is significant

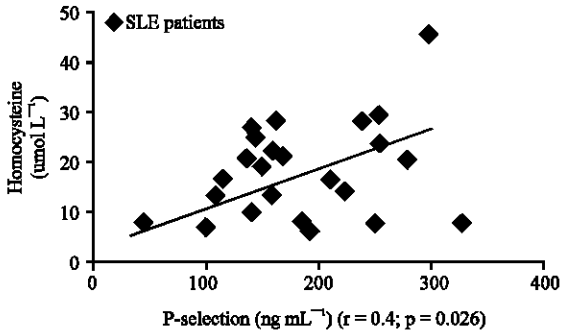

Fig. 1: Correlation between soluble P-selection and homocysteine in SLE patients

DISCUSSION

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease. It is an inflammatory disorder with a high atherothrombotic tendency. Cerebrovascular, coronary and peripheral vascular thrombotic events are major causes of morbidity and mortality (Petri *et al.*, 1996). Immunologically mediated vascular endothelial cell activation is considered a pathogenetic factor in the disruption of normal organ function in SLE (Fruns *et al.*, 2001).

P-selection is a glycoprotein contained in the platelet α granules and in the Weibel Palade bodies of endothelial cells, from where it is mobilized to the cell surface after activation. There is accumulating evidence

that P-selection mediates leucocyte adhesion to platelets and endothelial cells during inflammation, thrombosis and atherosclerosis (Davi *et al*., 1998).

In the present study we found increased mean serum concentrations of soluble P-selection in SLE patients compared to controls. Several previous studies demonstrated similar results (Aleksandrova *et al*., 2002 ; Joseph *et al*., 2001; Fruns *et al*., 2001; Takeda *et al*., 1994)

Recently P-selection revealed to be a key molecule in hemostasis and thrombosis, mediating platelet rolling, generating procoagulant microparticles containing active tissue factor and enhancing fibrin deposition (Cambien and Wagner, 2004). P-selection can respond quickly to proinflammatory stimuli because it is normally stored in α-granules of platelets and Weibel-Palade bodies of endothelial cells. Following stimulation with proinflammmatory stimuli, P-selection can translocate to the cell surface rapidly to interact with its ligand on leucocytes (Tan, 2002).

Measurement of soluble P-selection, which originates from both platelets and endothelial cells may be proposed as a marker of increased membrane bound P-selection expression attributable to vascular dysfunction and/or platelet activation and may provide comprehensive information on dynamic *in vivo* interactions among vascular and circulating cells (Celi *et al*.,1994).

Elevated levels of soluble P-selection are indicative of thrombotic disorders and predictive of future cardiovascular events (Cambien and Wagner, 2004). Raised levels of soluble P-selection in the plasma has been described in diabetes mellitus and also in both atherosclerosis and hypertension (Ross, 1999).

Since hypertension has been reported to influence endothelial cell function, its presence in a large proportion of patients with SLE could confound elevated plasma levels of P-selection. However in our study no statistical significant difference was found between hypertensive and non hypertensive.

In the response to injury hypothesis of atherosclerosis, endothelial cell dysfunction can result from various sources of injury including shear stress, immune complexes and other toxins such as homocysteine, all of which are also relevant to SLE. Such injury results in the up regulation of adhesion molecules on the endothelial surface, increased permeability and subsequent trapping of inflammatory cells at the site of activation. Factors, commonly seen in SLE, may contribute to endothelial injury and recruitment of inflammatory cells (Ross, 1999).

The present study demonstrated a significant positive correlation between homocysteine and P-selection in SLE patients.

Spencer *et al*. (2004), found in his study a significant positive correlation between plasma homocysteine and P-selection in hypertensive patients.

An observational study indicates that both platelet activation and increased oxidative stress are associated with the presence of hypertension-related microvascular changes. In this setting, platelet activation is related to increased oxidative stress and is also related to increased plasma fibrinogen, as well as to subtle changes in homocysteine metabolism (Minuz *et al*., 2004).

The effects of homocysteine on platelets kinetics and functions are contentions. Study on platelet kinetics in four homocystinuric patients and in a baboon model suggested an increased turnover of platelets (Harker *et al*., 1976).,but this was not confirmed in subsequent study (Hill-Zobel *et al*., 1982).

Homocysteine influences several vascular responses, including coagulation, fibrinolysis, platelet function, vascular smooth muscle cell proliferation and endothelial function. Increased platelet activation as reflected by soluble P-selection may be one mechanism by which hyperhomocysteinaemia confers an increased thrombotic risk (Spencer *et al*., 2004).

Acute hyperhomocysteinemia induces endothelial dysfunction characterized by a loss of endothelium derived nitric oxide, leading to an inflammatory state. This state results in increased leucocyte rolling, adherence and transmigration by up regulation of cell adhesion molecules (Pruefer *et al*., 1999).

There was no significant difference in the mean concentration of soluble P-selection between SLE patients with nephritis and those without nephritis suggesting that it is not the renal component of SLE that is responsible for the increased release of P-selection.

The absence of a significant correlation between P-selection and SLEDAI suggests a state of permanent activation of endothelial cells in SLE.

Present results cannot distinguish with certainty between endothelial cell activation and damage, however elevated levels of soluble P-selection indicates that endothelal cells are not damaged to such an extent that they have become incapable of protein synthesis.

In conclusion, this study shows that soluble P-selection concentrations are increased among patients with SLE and correlate with homocysteine concentrations. These findings suggest the possibility that activated platelets participate in the pathogenesis of SLE and this is important to note as it may have potential therapeutic implications with respect to use of antiplatelet agents in these patients.

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