Alterations in the serum levels of soluble L, P and E-selectin 20 years after sulfur mustard exposure: Sardasht-Iran Cohort Study

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ABSTRACT

The selectins (L, P and E) are carbohydrate-binding membrane glycoproteins acting as adhesion molecules involved in the development of different inflammatory reactions. Various eye, skin and lung diseases are associated with induction of soluble selectins. In this study serum levels of soluble forms of selectins (sL-selectin, sP-selectin and sE-selectin) were evaluated in the sulfur mustard (SM) exposed and the control groups using ELISA method. sL-selectin was significantly lower in the SM exposed group compared to the control group (1131.5 ± 16.3 and 1205.7 ± 26.9 pg/ml respectively; $p = 0.021$). The serum levels of sP-Selectin was significantly reduced in the SM exposed group in comparison to the control group (149.35 ± 2.61 and 170.25 ± 5.16 pg/ml respectively; $p < 0.001$). sE-selectin was significantly increased in sera of the exposed group compared to the control group (29.64 ± 0.902 and 24.61 ± 1.26 pg/ml respectively; $p = 0.003$). sL-selectin positively correlated with the percentage of polymorphonuclear cells and negatively with the percentage of lymphocytes. There was a significant correlation between the count of platelets and sP-selectin in both the control and exposed groups. The change in the pattern of selectins in the SM exposed group in comparison to the control group may indicate suppressed acute inflammatory condition in which new remodeling of cytokine expression play a more crucial role in the immune-regulation.

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1. Introduction

The selectins (L, P and E) are carbohydrate-binding type 1 membrane glycoproteins acting as adhesion molecules involved in the development of different inflammatory reactions. L-selectin is expressed by most of the leukocytes and mediates the interaction of circulating leukocytes with vascular endothelium [1–3]. P-selectin is constitutively produced and packaged in the α-granules of the platelets and Weibel–Palade bodies of endothelial cells. Following activation P-selectin rapidly translocates to the plasma membrane of activated platelets and endothelial cells. E-selectin is not expressed on resting endothelial cells. Up-regulation of this adhesion molecule on activated endothelial cells requires transcription of the responsible gene [4,5]. When leukocytes are activated L-selectin expression is transiently increased on the cell surface and followed by its rapid endoproteolytic cleavage and generation of soluble form (sL-selectin). sL-selectin retains its functional activity and at high concentrations can decrease the inflammatory responses by competing with cell-surface L-selectin [2]. Higher circulating levels of soluble forms of P- and E-selectin have been reported in serum of patients with inflammatory diseases such as cardiovascular disorders. Chronic induction of soluble form of selectins has been stated in various inflammatory disorders such as atherosclerosis, rheumatoid arthritis (RA), diabetes, systemic lupus erythematosus (SLE) and vasculitic diseases [4–6]. Various eye, skin and lung diseases are also associated with induction of soluble selectins [7–11]. Due to their involvement in the pathogenesis of several systemic and local inflammatory disorders, selectins are now considered as potential diagnostic and therapeutic tools [12–15].

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The cellular and molecular mechanisms leading to various long term pathological conditions which are observed in the sulfur mustard (SM) intoxicated patients have not been thoroughly elucidated yet. These patients suffer from chronic eye, skin and especially pulmonary problems [16]. Some researchers have been reported previously that long term clinical manifestations of SM exposed subjects indicate the possible involvement of immune and inflammatory processes [17–20].

As previously reported the Sardasht-Iran Cohort Study (SICS) was designed to examine the long term effects of SM 20 years after exposure [21]. The history of the exposure was also reported previously [21–23]. In the present study serum levels of soluble forms of selectins (L, P and E) were investigated to assess the potential role of these adhesion molecules in the long term clinical complications after SM exposure. The control population was from a regional group who were not exposed to sulfur mustard as previously described [23].

2. Materials and methods

2.1. Study design and participants

This study was approved by the Ministry of Health of Iran, Shahed University and the Board of Research Ethics of Janbazan Medical and Engineering Research Center. A written informed consent was obtained from all the subjects in the study. The details of SICS study were reported previously [23,24]. Briefly, the exposed group was selected from individuals who was exposed to SM 20 years ago ($n = 372$) and the control group ($n = 128$) was selected from the unexposed individuals [25].

2.2. Serum preparation

Peripheral blood was drawn using Vacutainer tubes (BD Biosciences). The sera were separated by 20 minute centrifugation at $2000 \times g$ in 4 °C, aliquated and stored at -80 °C until test.

2.3. Selectin measurement

Human sL-selectin/CD62L, sE-selectin/CD62E and sP-selectin/CD62P Quantikine® ELISA kits (R&D Systems) were used to measure the selectin levels in the sera. A mouse monoclonal antibody specific for sL-selectin, sE-selectin and sP-selectin had been precoated onto microplates. sL-selectin an sP-selectin conjugates were sheep polyclonal antibody to recombinant human sL-selectin and sP-selectin conjugated to horseradish peroxidase. Wash buffer concentrate was 21 ml/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. Stop Solution was 2 N sulfuric acid. The detection limit of kits was between 1 and 2 pg/ml. ELISA reader and washer were Stat-Fax 2100 and Stat-Fax 2600 (USA) respectively.

2.4. Hematological methods

The method of Shams et al. was utilized in this article [25]. Briefly, the blood count data was obtained from both the control and exposed groups within 1 h after collection of the blood samples. White blood cell (WBC) and differential (Diff) were assessed for each sample by an automatic cell counter (Sysmex, KX21N, Japan). Diff is done with K1000. Hematological related data was presented as mean ± standard deviation.

2.5. Statistical analysis

Data was presented by mean ± standard error of mean and the control and exposed groups were compared together by student *t*-test. Correlations of sL, sP and sE-selectins with each other and with hematological factors were measured by using Pearson correlation coefficient. *P*-value less than 0.05 was considered as statistically significant. Data analysis was performed by SPSS, version 13 (SPSS Inc, Chicago, USA).

3. Results

3.1. Serum levels of soluble selectins

The results of serum level of selectins (sL, sP and sE) are presented by mean ± standard error of mean in the SM exposed ($n = 370$) and the control ($n = 126$) groups are shown in Table 1. As seen in this table the serum levels of sL-selectin and sP-selectin were significantly lower in the SM exposed group in comparison to the control group (1131.5 vs. 1205.7, $p = 0.021$ for sL-selectin and 149.35 vs. 170.25, $p < 0.001$ for sP-selectin). In contrast, the serum levels of sE-selectin was significantly increased in the SM exposed group compared with the control group (29.64 vs. 24.61, $p = 0.003$).

3.2. Correlations between soluble selectins and inflammatory cytokines

According to Table 2 Spearman's rho Correlations between soluble selectins in the control group revealed only a significant positive correlation between sL-selectin and sP-selectin ($r = 0.308$ and $p = 0.00$). There was no correlation between sE-selectin with sP-selectin as well as sL-selectin. However in the exposed group all selectins showed significant positive correlations with each other (Table 2).

As seen in Table 3 there are significant positive correlations between sE-selectin and all inflammatory cytokines in the exposed group. sL-selectin has positive significant correlation with IL-1α and IL-1 Receptor antagonist (IL-1Ra) in the exposed group and sP-selectin has negative significant correlation only with IL-1β in the exposed group.

3.3. Correlations between soluble selectins and blood cells

There was a significant correlation between the platelet counts and sP-selectin in both the control and exposed groups. While sL-selectin significantly correlated with the platelet counts only in the control group, sE-selectin showed the same pattern only in the exposed group (Table 4).

In addition, we examined any possible statistical correlations between the serum levels of selectins and WBC. In the control group there was no significant correlation between the serum levels of selectins and the total count of WBC or percent of each of the blood cells. However in the exposed group a significant positive correlation was observed between the count of WBC with E- and P-selectins but not with L-selectin (Table 5). sL-selectin positively correlated with percent of polymorphonuclear cells and negatively with the percent of lymphocytes. In addition, there was significant positive correlation between sP-selectin and the percentage of eosinophils and negative correlation with the percentage of lymphocytes.

Table 1
Comparison of the serum level of selectins (pg/ml) between control ($N = 126$) and exposed ($N = 370$) groups.

Study groups	Control		Exposed		*p*-value
	Mean	SEM	Mean	SEM	
sL-selectin	1205.7	26.9	1131.5	16.3	**0.021**
sP-selectin	170.25	5.16	149.35	2.61	**<0.001**
sE-selectin	24.61	1.26	29.64	0.90	**0.003**

The serum levels of selectins were assessed by the ELISA method and a comparison of selectin levels was undertaken between the control and exposed group data presented by median ± SEM.
p-value: comparison of the exposed group with Control (*t*-test).