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E-SELECTIN AS A BIOMARKER IN FEMALE PATIENTS WITH B-THALASSEMIA IN AL- NAJAF PROVENCE, IRAQ

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ABSTRACT

E-selectin, as identified (CD62E), is expressed on endothelial cells after stimulation with inflammation cytokines. β-Thalassemia diseases (βT) and early diagnosis are of utmost significance in the entire world population. This study was performed in the Thalassemia Center of the Al-Zahraa Educational Hospital in Al-Najaf Province, Iraq, on sixty-nine with β-thalassemia (54 βT major and 15 βT Intermedia) aged 8-40 years who transfused blood. Compared to 20 healthy volunteers as a control group. In both βT patients and healthy groups were assessed serum E-selectin levels. It was investigated the relationship with RBC, Hb, PCV, WBC, PLT, BMI, splenic status, iron, and ferritin levels. The results revealed a significant ($P<0.05$) decreased values of HB, RBC, P.C.V, and BMI. In contrast, values of WBC, PLT, Iron, and Ferritin were significantly increased in βT patients as compared to the healthy control groups. A significant ($P<0.05$) increase in serum E- Selectin level in βT patients (20.55±0.47) ng/ml to compare with the healthy group (9.16±0.50) ng/ml. Furthermore, it was a significant decrease in groups of βT major (19.87±0.42) ng/ml more than in βT intermedia (23±1.42) ng/ml. E-Selectin revealed a significant increase ($P<0.05$) in progress age and associated with splenectomies and underweight groups compared to splenectomies and the normal weight groups, respectively. Also, E-Selectin levels significantly positively correlated with WBC, PLT value, iron, and Ferritin levels. However, it was no significant with RBC, PCV, Hb. As a conclusion from this study, E- Selectin is an important biomarker in β-thalassemia patients can be identified as the complications associated with iron overload, inflammatory process, and endothelial dysfunction in βT disease.

Keywords: *E- Selectin, Inflammation cytokines, Iron overload, and endothelial dysfunction*

1. INTRODUCTION

Thalassemia is one of the world's main hemoglobinopathies in the population. In human β-thalassemia (βT), a single gene inherited hemoglobin disease, an autosomal recessive disorder caused by diminished or absent development of β globin chains. (Lettre, 2012; Bernard *et al*., 2013).

Anemia in thalassemic patients has a significant effect on increasing damage to the cytoskeleton proteins of the RBC membranes, leading to intravascular hemolysis, particularly in the spleen, and thus to the regular intramedullary destruction of red cells. (Thein, 2013).

A previous study has shown that hypoxemia, activation of leukocytes, and platelets may partly contribute to anemia that may influence endothelial function and raise levels of cell and endothelial adhesion markers. (Ibrahim *et al*., 2013b).

E-selectin (CD62E) is a cell adhesion molecule for membrane glycoproteins. It is one of three selectin-endothelial forms (E-, P-, and L-selectins) (McEver and Zhu, 2010). After induction by inflammatory cytokines, it is expressed on endothelial cells, and the slow rolling of leukocyte adhesion to the vascular endothelium mediates efficiency. E-selectin represented endothelial dysfunction resulting from oxidative stress in many inflammatory diseases (Chase *et al*., 2012; Ibrahim *et al*., 2013a).

This study aimed to investigate serum E-selectin levels and their relationship with hematological and biochemical parameters associated with iron overload in β-thalassemia diseases.

2. MATERIALS AND METHODS

2.1. Study design

This study was supported by the approval of the ethical committee regulations of Kufa University. The study included sixty-nine females with β-thalassemia (54) β-thalassemia major and (15) intermedia β- β-thalassemia, the aged range from (8–40) year. They were visited at The Thalassemia Center in Al–Zahraa Educational Hospital in Al–Najaf province, Iraq. They were presently transfused and treated every 2-3 weeks for the clinical signs and manifestations of the disease that involved blood transfusion, which was about (10 ml of blood for each kilogram of body weight, to keep of Hb level leastwise 10 g/dl), and regulation iron-chelating therapy, where were considered as a target for the present study. Also, (20) healthy females who did not suffer from any disease served as a control group; the patient and controls were age-matched. This study was carried out from July 2014 to March 2015. Divided into β-thalassemia patients according to

2.1.1. Types of β-thalassemia divided into two groups

β-Thalassemia Major n=54, and β-Thalassemia Intermedia n =15

2.1.2. The aged of β-thalassemia divided into three groups:

Group 1 [(8 –18 year), n =51], Group 2 [(19 –29 year), n =12], and Group 3 [(30 – 40 year), n =6].

2.1.3. Splenic situation divided into two groups:

Patients with Splenectomized n=29 and Unsplenectomized patients n=40.

2.1.4. Body Mass Index (BMI) divided into two groups:

Patients with normal weight n=46 and Underweight patients n=29. The normal range (5th – 85th) % for (≤ 20) years of age, and (18.5 – 25) kg/m2 for (>20) years of age. (WHO, 2006)

2.2. Methods

2.2.1. BMI (Body Mass Index)

An electronic balance and height unit carried out the BMI measurement to measure weight divided on the height square and Equation 1 was applied (WHO, 2006).

$$BMI = \text{Weight (kg)} / \text{Height (m}^2\text{)} \quad \text{(Eq. 1)}$$

In children, BMI is compared against the percentile for children of the same gender and age, the standard values in adults and children (CDC, 2014).

2.2.2. Hematological Assessments

At Hematology Laboratory of Al-Zahraa Educational Hospital, both patients and healthy were withdraw 5 ml of venue blood. Hematological criteria were conducted on EDTA anticoagulated blood by using a completely automated hematology analyzer Mythic 18 (RINGELSAN CO., Turkey) for estimated complete blood count (CBC) (Wasmuth, 2010).

2.2.3. Estimation of Serum Iron concentration

Serum iron concentration was measured by iron (using the chromogen ferrozine method) kit (bt 35i, Turkey). The principle transferrin-bound iron is released at acid pH and reduced from ferric Fe^{3+} to ferrous ions Fe^{2+} iron. These ions react with ferrozine to form a violet-colored complex, which is measured spectrophotometrically at 560 nm. This absorbance is proportional to serum iron concentration in the sample, and Equation 2 was applied (Persijn *et al*., 1971).

$$\text{Total iron (µg/dl)} = [(\text{Abs. 2 Sample} - \text{Abs.1Sample}) / (\text{Abs. 2 Std.} - \text{Abs. 1 Std})] \times \text{Conc. Std.} \quad \text{(Eq .2)}$$

Abs. = Absorbance, Std. = Standard

2.2.4. Estimation of Serum Ferritin

This methodology was created to measure ferritin serum levels using the enzyme-linked immunosorbent assay (ELISA) immunoenzymatic technique using the bioelisa ELx 80000 reader (biokit, U.S.A). According to the Manufacturing Firm, the Human Accu Bind Ferritin ELISA Kit (Monobind Inc., U.S.A, code number 2825-300). (Anderson and Kelly,1981).

2.2.5. Estimation of serum E-selectin level

Evaluating of Human E-Selectin ELISA Kit was executed as mentioned by the manufacturing company (CUSABIO BIOTECH Co., Ltd., P.R.C, code number CSB-E04540h) that depended on the technique of the quantitative sandwich enzyme immunoassay (Koch *et al*., 1995).

2.4. Statistical analysis

The data were evaluated statistically

through the SPSS bundle (SPSS, Version 17). Descriptive analyses between the patients and control groups, also among patients. Data represented at mean± standard error of the mean. The distinction between subdivided classes in the calculated parameters, Pearson correlation, and multivariate ANOVA was used, while the figures constructed using the Microsoft Office 2013 EXELL software were used. All of these were evaluated statistically at a significant $p<0.05$ level.

3. RESULTS AND DISCUSSION:

3.1. General criteria of the study subjects

The general characteristics of the studied groups were shown in Table 1. Each category of β-thalassemia patients can be identified approximately by hematological and biochemical parameters. The results revealed a significant difference ($p<0.05$), decreased levels of HB, RBC, and P.C.V, were found in β-thalassemia patients (7.7±0.14) g/dl, (3.13±0.07) 10^6/ml, and (23±0.41) % as compared to the healthy control groups (12.01±0.19) g/dl, (4.36±0.07) 10^6/ml, and (36.34±0.52)% respectively. WBC, PLT count values in the patients of β-thalassemia (11.05±0.26) 10^3/ml, (361.86±13.92) 10^3/ml exhibited highly significant ($p<0.05$) as compared to the healthy group (7.59±0.33)10^3/ml, (244±14.23) 10^3/ml. The results revealed a significant decrease ($p<0.05$) in BMI of β-thalassemia patients (16.59±0.43) kg/m^2 comparison to (22.15±0.36) kg/m^2 in the healthy control groups.

Iron and ferritin were statistically significant increase noted in β-thalassemia patients (172.2 ± 4.4) µg/ml, and (5156.5±438.7) ng/ml as compared to the healthy control groups (29.86±2.32) µg/ml, and (99.15±8.95) ng /ml respectively. The hematological parameter (Table 1) in the present study showed a substantial decrease in Hb, PCV, and RBC levels, in addition to a significant increase in WBC and PLT in the number of female patients with β-thalassemia. Current findings agree with Arshad *et al.* (2014), who reported that, due to the reduction of erythrocyte numbers and reduced RBC index values, Thalassemia patients might have anomalies associated with lower Hb level (MCV, MCH, MCHC, HCT). Thus, these patients suffer from anemia, which results in reduced blood oxygen content. Some research has shown that reduced amounts of Hb, PCV, and RBC counts detected in patients with β-thalassemia are attributed to their early deterioration and persistent erythrocyte dissolution due to an irregular globin molecule contributing to erythrocyte breakup before maturation (Shanthi *et al.,* 2013). In patients with β-thalassemia, a reduced erythrocyte lifetime and premature cell death are essential theories of accrual of free extra globin chains within the RBC membrane surface, inducing oxidative stress to create a free radical that causes damage to the RBC membrane (Ibrahim *et al*., 2013b). The findings of this analysis are acceptable, with recent research referring to results related to RBC mass, WBC, and a significant difference in PLT count in patients with β-thalassemia. This is due to continued extreme anemia followed by hypercellular (thrombocytosis and leukocytosis) triggered by activation of the erythropoietin hormone that works on the bone marrow to enhance the distribution of blood cells or to stimulate the immune system by receiving blood from different donors (Yassin *et al*., 2013; Arshad *et al.,* 2014). Present findings of this study showed that patients with β-thalassemia had a substantial drop in BMI, and almost two-thirds (66.7 %) were underweight and (33.3 %) average weight relative to stable control (Table 1). These findings corresponded to those of (Eissa and El-Gamal, 2014). They reported that BMI and iron overload resulted in ferritin being proposed as low BMI markers for patients with β-thalassemia. Many studies have suggested that the pathogenesis of β-thalassemia growth deficiency is multifactorial such as systemic diseases, endocrinopathies secondary to iron overload, and Deficiency in essential dietary elements resulted from iron overload, which Important contributing factors to the development of underweight patients (Skordis, 2011; Manali *et al*., 2015).

3.2. Serum E-selectin level and correlated with the hematological and biochemical parameters

The results were showed in (Figure 1) indicated that a significant ($p<0.05$) increase in serum level of E-Selectin patients with β-thalassemia (20.55±0.47) ng/ml, in comparison with that of control groups (9.16±0.50) ng/ml. These were accepted with (Kanawaki *et al*., 2012), While it was a significant decrease in significant β-thalassemia (19.87±0.42) ng/ml, to compare with that of intermedia β-thalassemia (23.00±1.42) ng/ml, (Figure 2). Therefore, Serum E-selectin showed a significant decrease ($P< 0.05$) in the age group (8-18) about (19.16±0.40) ng/ml in comparison with the age group (19-29) and (30-40) about (22.11±0.59) ng/ml and (29.2±0.58) ng/ml, respectively. Moreover, it presented the same significant decrease when it compared of the age group (19-29) about (22.1±0.59) ng/ml, less

than the age group (30-40) about (29.2±0.58) ng/ml, (Figure 3). The results indicate a significant increase ($p< 0.05$) in E-Selectin level in β-thalassemia patients with splenectomized (22.13±0.63) ng/ml, in comparison with unsplenectomized (19.4±0.61) ng/ml (Figure 4). Moreover, it was a significant decrease ($p<0.05$) in patients with underweight (19.30±0.42) ng/ml to compare with normal-weight patients (23.04±0.95) ng/ml (Figure 5).

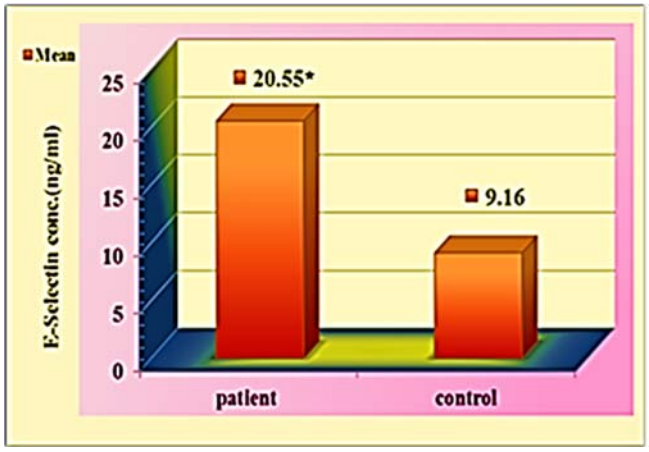

Figure 1. *serum E- selectin level in βT patients compare with the heathy groups as control.* * statistically significant at $p< 0.05$

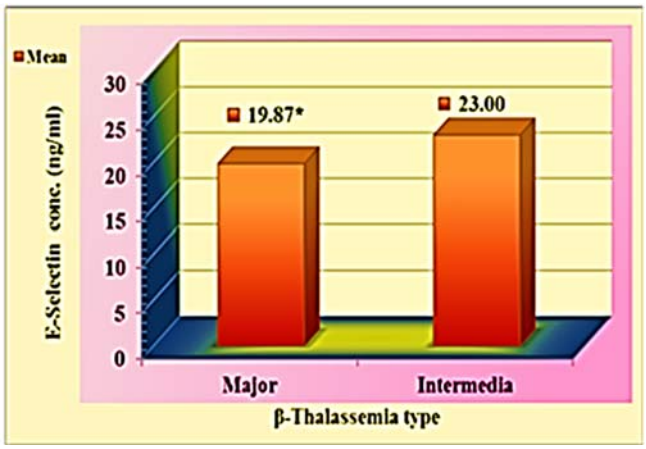

Figure 2. *Serum E- selectin level in βT major compare with βT- intermedia groups.* * statistically significant at $p< 0.05$

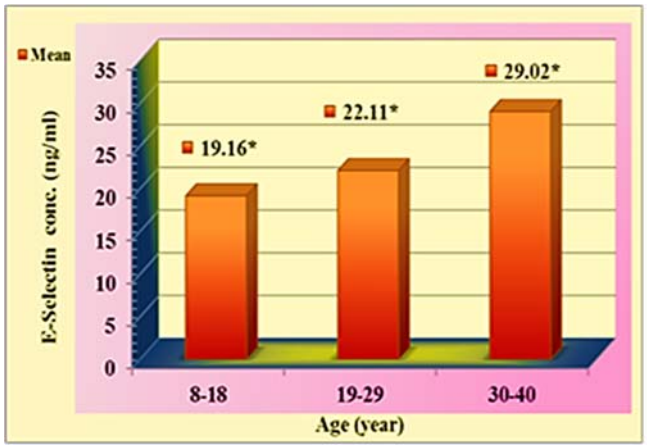

Figure 3. *serum E- selectin level in different age groups of βT patients.* * statistically significant at $p< 0.05$

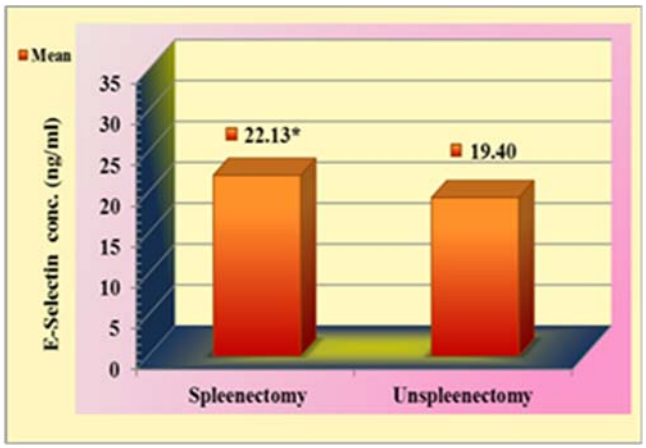

Figure 4. *Serum E- selectin level in βT patients with splenectomy and unsplenectomy groups.* * statistically significant at $p< 0.05$

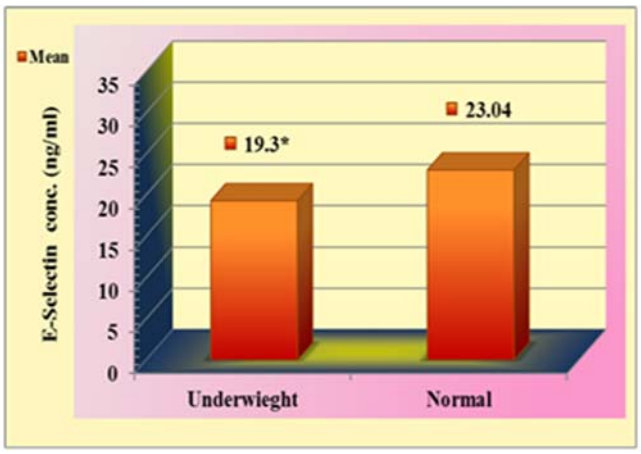

Figure 5. *Serum E- selectin level in βT patients with underweight and normal-weight groups.* * statistically significant at $p< 0.05$

The results of correlated showed that serum E- selectin level no significant correlated with RBC ($R2 =0.007$), PCV ($R2 =0.006$), and HB ($R2 = 0.002$), (Figure 6 a, b, and c). respectively.

In addition, it was a positively significantly correlated with WBC (R2 =0.203) and, PLT (R2 =0.117), Iron (R2 =0.091) and Ferritin (R2 = 0.168), (Figure 6 d, e, f, and g), respectively.

E selectin is exclusively expressed on the surface of endothelial cells in a highly inductive manner in response to inflammatory cytokines, endothelial E-selectin assists in the initial adhesion of circulatory leukocytes to the surface of vascular endothelium. Some studies were showed that chronic inflammatory is more prevalent in thalassemia patients; because the oxidative stress resulting from iron overload plays an essential role in endothelial vascular activation of adhesion molecules and induction of monocyte and neutrophil in the inflamed site (Taher *et al.,* 2011; Vinchi *et al*., 2013),

Endothelial dysfunction is due to intravascular hemolysis, leading to proliferative vasculopathy. Increased adhesion molecules such as E-selectin on the vascular endothelium, high levels of inflammatory cytokines and stimulated leukocytes, significant amounts of ROS, and promote thrombus (Stoyanova *et al.,* 2012). The present study (Figures 2 and 4) showed a significant increase of serum E-selectin level in intermedia and splenectomized groups compared with thalassemia major and non-splenectomized groups, respectively. These were in agreement with (Atichartakarn *et al.*, 2014). Who found that splenectomized patients had a more severe hemolytic disease and higher levels of E- P-selectins. which suggested that intravascular hemolysis (IVH) and vascular endothelial cell (EC) activation were occurred by chronic iron overload and activation of the chronic inflammation (Taher *et al*., 2011) has been explained that among the medical complications of thalassemia intermedia that found to occur at higher rates. Even more frequently than in patients with thalassemia major. Splenectomized and transfusion were significant risk factors for thromboembolism events, which increased in patients with thalassemia intermedia. An increase of serum E-selectin level with increasing age, resulting from repeated blood transfusion increase with age progress, leads to increases free radical production and oxidative stress of tissues, leading to elevated release endothelial molecules which included E-selectin. This finding agrees with the study of (Sena *et al*., 2013), showing that aging is associated with increased oxidative stress and a proinflammatory endothelial cell phenotype, and excessive or prolonged endothelium activation due to the action of proinflammatory cytokines underlies endothelium dysfunction (Zinovkin *et al.,* 2014) Study of Hisham *et al*., (2013) illustrated that high levels of E-selectin in the serum following a high level of leptin, and other study showed that serum levels of leptin in major beta-thalassemia reduce regardless of age and body mass. Also, there was a positive relationship between serum leptin level and BMI in patients with significant beta-thalassemia (Shahramian *et al.,* 2013). Previous studies have shown that serum E-selectin levels are a high expression in obesity and decline with reducing weight. They found an increased layer of visceral adiposity maybe a companion with E-selectin, then related to BMI (Pontiroli *et al.,*2009; Zanni *et al.,* 2011).

4. CONCLUSIONS:

E-selectin can be released into the circulation and quantified in plasma and serum, reflecting by these inflammatory processes within vascular cell walls and marker of endothelial dysfunction, also plays an important role in the recruitment of WBC, RBCs, PLT, and promote thrombosis at vascular inflammation sites (McEver and Zhu, 2010; Taher *et al*., 2011; Stoyanova *et al*., 2012). Previously study illustrated that heme from RBC lyses circulated in the blood and led to the expression of endothelial adhesion molecules, causing increased adhesion of leukocytes and reticulocytes endothelium in hemoglobinopathy (Wagener *et al.,* 2001).

The current study concluded that elevated serum E-selectin level in β-thalassemia patients, especially in patients with splenectomized and underweight, and development with progressed age associated with the inflammatory process and iron overload. This study suggested serum E-selectin as a potential biomarker for the early diagnosis of important complications related to endothelial dysfunction.

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Table 1. *The clinical characteristics in female patients with β-thalassemia Compare to the heathy groups*

Clinical characteristics	βT Patients n= 69	Control n= 20	P-Value
Age (year)	16.62 ± 0.96	20.75 ± 1.86	0.612
RBCs (106/mm3)	3.13 ± 0.07 *	4.36 ± 0.07	0.038
Hb (g/dl)	7.7 ± 0.14 *	12.01 ± 0.19	0.027
P.C.V %	23 ± 0.41 *	36.34± 0.52	0.036
WBCs (10^3/mm^3)	11.05 ± 0.26 *	7.59 ± 0.33	0.036
PLTs (10^3/ mm^3)	361.86 ± 13.92 *	244 ± 14.23	0.034
BMI (kg/m2)	16.59 ± 0.43 *	22.15 ± 0.36	0.001
Underweight	13.09±0.52	0	
Normal weight	23.7±2.04	0	
IRON (µg/ml)	172.2 ± 4.4 *	29.86 ± 2.32	0.0001
FERRITIN (ng /ml)	5156.5 ± 438.7 *	99.15 ± 8.95	0.0001

* P< 0.05 statistically significant with the control group. Red Blood Corpuscular - RBCs. Hemoglobin – Hb. Pact Corpuscular Volume - P.C.V. White Blood Cells – WBCs. Body Max Index – BMI. Platelets – PLTs. Data represented as mean± standard error of mean.

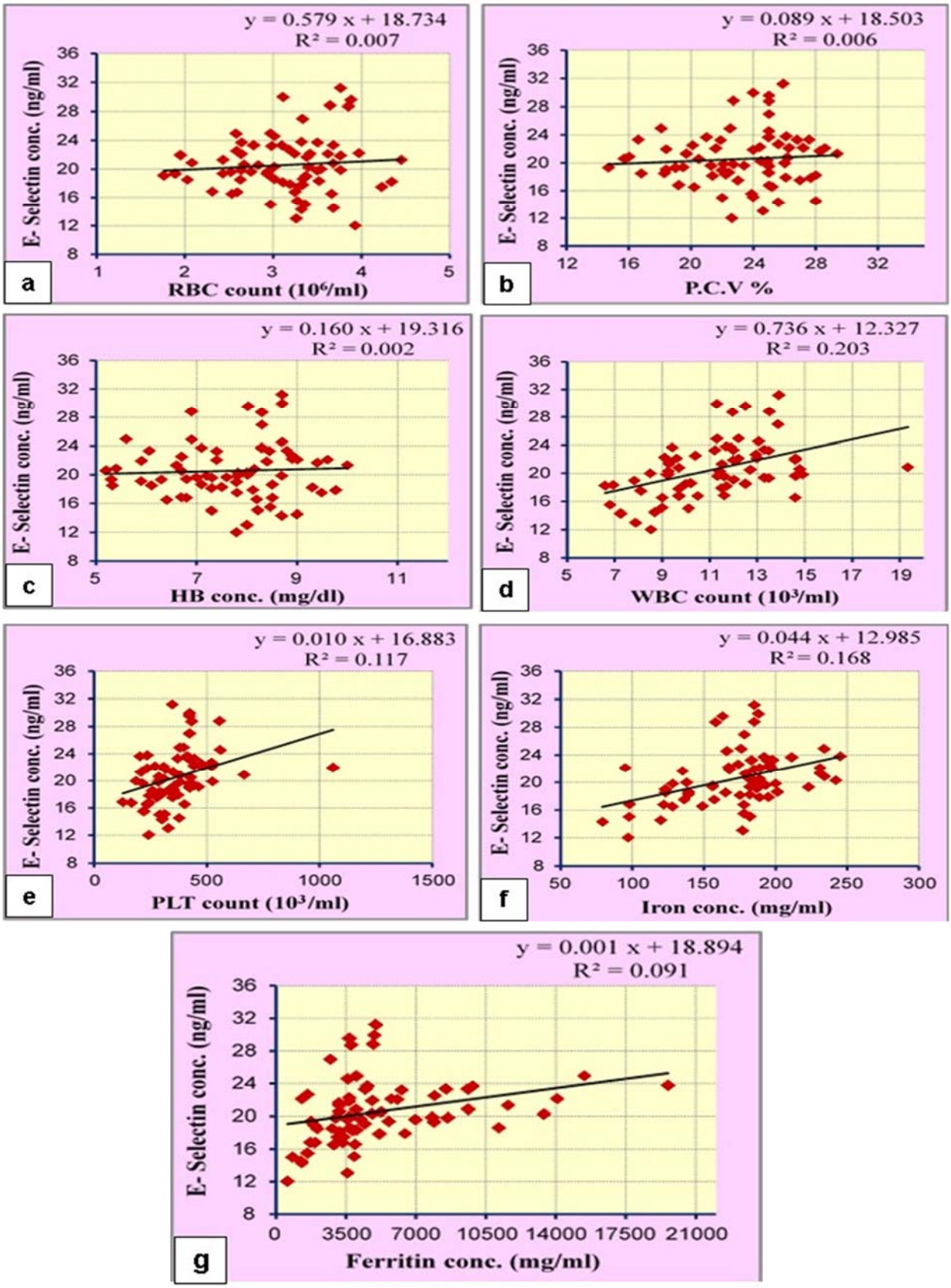

Figure 6. *Scatter pots of serum E- selectin level correlated. No significant with RBCs (a), P.C.V. (b), and Hb (c). A positive significance with values of WBCs (d), PLTs (e), iron (f), ferritin (g)*