## Original article

# The relation between oxidative stress and adhesion molecules in Egyptian children and adolescents with type 1 diabetes mellitus

***Background:*** *Antioxidant potential decreases while plasma lipid peroxidation products increase in type1 diabetes mellitus. The vascular endothelium is a major target of oxidative stress (OS). Reactive oxygen species signal events leading to impairment of endothelial function and promotion of leukocyte adhesion to the vascular endothelium..* ***Objective:*** *To explore the relation between OS and adhesion molecules in type1 diabetes and correlate it with the state of metabolic control, disease duration and microvascular complications (MVCs).* ***Design:*** *Thirty-eight type 1 diabetics were included: 22 patients with disease duration less than 5 years and 16 patients with duration of 5 years or more. Thirty healthy age and sex matched subjects served as controls. They were assessed clinically. Laboratory investigations included, random blood sugar (RBS), glycated hemoglobin ($HbA_1c$), fasting lipid profile and measurement of serum malondialdehyde (MDA) as a marker of lipid peroxidation and serum soluble P-selectin as a marker of endothelial/platelet activation.* ***Results:*** *Serum MDA and P-selectin were significantly elevated in type 1 diabetics compared to controls with the highest level in diabetics with disease duration of 5 years or more ($p < 0.0001$). Both MDA and P-selectin levels were significantly elevated in complicated compared to non complicated diabetics ($P<0.0001$) with strong relation to complication severity. Serum MDA level was positively correlated with serum P-selectin level in diabetics ($p <0.0001$). Serum MDA and P-selectin were positively and significantly correlated with disease duration ($p<0.0001$ ), RBS ($p<0.0001$,$p=0.001$ respectively), HbA1c ($p<0.0001$), diastolic blood pressure ($p=0.03$,$p=0.005$ respectively), total cholesterol ($p=0.04$,$p=0.02$, respectively), triglycerides ($p=0.006$, $p<0.0001$ respectively) and low density lipoproteins ($p=0.03$,$p=0.05$ respectively) but negatively correlated with high density lipoproteins($p=0.03$). On multiple regression analysis, HbA1c had the strongest effect on both MDA and P-selectin levels ($P<0.0001$). Cut off values for serum MDA and P-selectin equal to 8.035 nmoles/ml and 45.15ng/dl respectively for early detection of diabetic MVCs were defined.* ***Conclusion:*** *Levels of MAD and P-selectin are elevated in type1 diabetics with evident relation to disease duration, metabolic control and severity of MVCs. Hence both of them might act as good markers to identify diabetics who are more susceptible to develop vascular disease.*

**Key words**: Type1 diabetes, oxidative stress, P-selectin, adhesion molecules, microvascular complications.

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## INTRODUCTION

Type1 diabetic patients usually develop clinically evident microangiopathy later in adolescence although subclinical functional and structural abnormalities precede its development[1]. Microangiopathy in type1 diabetes mellitus (T1DM) is associated with platelet hyperactivity, endothelial dysfunction and low grade inflammation[2].

Endothelial dysfunction is known to be responsible for diabetic vascular complications[3]. Oxidative stress (OS) contributes significantly to this dysfunction by disturbing the balance between production of reactive oxygen species (ROS) and antioxidant defense in favor of the former, leading to tissue injury[4]. Malondialdehyde (MDA) is one of the end products of lipid hydroperoxide decomposition and it is the most important marker measured as an index of lipid peroxidation[3].

The vascular endothelium is the primary target of OS. Upon exposure to ROS, endothelial cells display a variety of adverse biological effects including production of inflammatory mediators**,** expression of adhesion molecules and increased cell permeability[5]. Leucocyte adhesion and migration to the sub-endothelium in response to chemo-attractants and other activating molecules are mediated by adhesion molecules expressed on endothelial cells including E-selectin, P-selectin, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1)[6].

The main role of selectin group is the tethering of leucocytes to the endothelium; they induce relatively weak and transient adhesion allowing the cells to roll along the vascular wall[7]. Thus, they play a part in the earliest stages of endothelial dysfunction and microangiopathy. One of the most important members of this group is P-selectin[8].

P-selectin is found in the storage granules of resting platelets as well as in the weibel–palade bodies of endothelial cells. After endothelial or platelet activation, P-selectin may also be deposited on the provisional matrix providing a surface for recruitment and subsequent migration of leucocytes including monocytes, mediating the initial steps of inflammation[9]. Elevated level of p-selectin has been shown to predict future vascular risk[10].

The aim of this study was to investigate whether OS and adhesion molecules derived from endothelial/platelet activation were interrelated in T1DM in order to clarify the effect of OS on the process of endothelial dysfunction and microangiopathy.

## METHODS

This study included 38 children and adolescents with T1DM recruited from the regular attendants of the Diabetes Clinic, Children's Hospital, Ain Shams University. They were 11 (29.0%) males and 27 (71.0%) females, their ages ranged between 6-17 years with a mean of 11.30 ± 3.10 years. They were subdivided into two groups according to their disease duration.

***Group I:*** included 22 patients with diabetes duration less than 5 years (range was 6 months to 4 years) with a mean of 2.36±1.28 years. Their ages ranged between 6-17 years with a mean of 10.98±3.41 years. They were 5 (22.7%) males and 17 (77.3%) females.

***Group II:*** included 16 patients with diabetes duration of 5 years or more (range was 5 to 10 years) with a mean of 6.75±2.12 years. Their ages ranged between 8-14.5 years with a mean of 12.19±1.89 years. They were 6 (37.5%) males and 10 (62.5%) females.

**Control group:** included 30 healthy ages and sex matched subjects. Their ages ranged between 6-17 years with a mean of 10.33±4.15 years. They were 14 (46.7%) males and 16 (53.3%) females.

All patients and controls were subjected to the following:

**I. Clinical history taking** stressing on; demographic data, disease duration and insulin therapy regarding its type, dose and frequency. The mean insulin dose was calculated. History suggestive of acute metabolic complications and numbers of hypoglycemic and diabetic ketoacidosis (DKA) attacks were recorded. The files were revised for the number of hospital admissions due to these attacks during the last one year prior to the study. Inquiry about chronic diabetic complications including nephropathy, peripheral neuropathy and retinopathy was made.

**II- Clinical examination:** with particular emphasis on:

1. Anthropometric measures; height, weight and body mass index standard deviation scores (SDS) were calculated[11]**.**
2. Full neurological examination to detect evidence of peripheral neuropathy.
3. Blood pressure measurement by conventional mercurial sphygmomanometer after 5 minutes rest, blood pressure was measured three times for every patient and the mean value of the three readings was calculated.
4. Fundus examination, by direct ophthalmoscopy to detect diabetic retinopathy.

**III- Laboratory investigations:** included

1. Random blood sugar (RBS); using glucocard II blood glucose monitoring system supplied by ARKRAY, Inc.
2. Glycosylated hemoglobin ($HbA_1c$); using quantitative calorimetric determination of glyco-hemoglobin in whole blood.
3. Quantitative determination of urinary microalbumin for diabetic nephropathy. Microalbuminuria was defined as albumin excretion rate of 30- 300 mg/gm urinary creatinine.
   Calculation of mean random blood glucose (MRBG) and mean $HbA_1c$ in the last one year prior to the study through revision the patients' files were done.
4. Fasting lipid profile assay using the enzymatic colorimetric method for measuring serum total cholesterol (TC), serum triglycerides (TG), low

density lipoproteins (LDL) and serum high density lipoproteins (HDL).

5. Assessment of lipid peroxidation by measuring serum malondialdehyde as a marker of OS, by colorimetric method (*Biodiagnostic, Comp* with catalog number MD 25- 28). In this method thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product was measured at 534 nm.
6. Measurement of serum soluble P-selectin (s P-selectin) by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (*Bender Med Systems, Inc*. with catalog number BMS 219/3).

**Statistical methods**

All data were processed using SPSS (version 11). The mean and standard deviation were calculated, the level $P \leq 0.05$ was considered the cut-off value for significance. Chi-Square test $X^2$ was used to test the association variables for categorical (descriptive) data. Fisher exact test was performed in table containing value less than 5. Student's t-test was used to assess the statistical significance of the difference between means of independent samples. Values not normally distributed were tested using Mann-Whitney test. One way ANOVA test was used to compare more than 2 quantitative groups. Correlation analysis was employed to assess the strength of association between two variables. Sensitivity, specificity positive predictive value (PPV) and negative predictive value (NPP) were all calculated. A receiver operating characteristic (ROC) curve was used to illustrate the diagnostic properties of a test on a numerical scale.

## RESULTS

The demographic, anthropometric measurements and mean values of blood pressure of all studied groups are presented in table (1). There were no significant differences among the three studied groups as regards their mean age, weight , height and BMI SDS ($p>0.05$). The three studied groups were comparable also with respect to their mean systolic blood pressure ($p>0.05$), although a significantly higher diastolic blood pressure was found in group II compared to other groups ($p= 0.04$).

Mean RBS and mean insulin dose, were higher in group II compared to group I although this did not reach statistical significance ($p>0.05$). On the other hand group II had significantly higher HbA1c compared to group I ($p=0.03$).

Number of DKA attacks in group II was significantly higher compared to group I ($p= 0.04$). A higher median number of hypoglycemic attacks was seen in group I compared to group II; however this did not reach statistical significance ($p = 0.34$).

**Table 1.** Comparison among the three studied groups with respect to their age, anthropometric and blood pressure values.

| Variables | Group I n=22 | Group II N=16 | Control n=30 | Test of significance | p value |
|---|---|---|---|---|---|
| | Mean±SD Range | Mean±SD Range | Mean±SD Range | | |
| **Age (years)** | 10.98±3.41 (6-17) | 12.19±1.89 (8-16.5) | 10.33±4.15 (6-17) | F=0.74 | 0.48 |
| **M/F** | 5/17 | 6/10 | 14/16 | $X^2 = 2.38$ | 0.30 |
| **Height SDS** | -0.48.±1.87 (-3.47-2.33) | -1.04±1.57 (-4.71-2.33) | -0.64±1.16 (-2.00-0.85) | F= 1.37 | 0.26 |
| **Weight SDS** | 0.46±1.67 (-1.81- 2.51) | 0.63±2.04 (-2.73-5.84) | 0.41±1.76 (-2.00-4.50) | F=0.13 | 0.88 |
| **BMI SDS** | 0.35±1.69 (-3.50-1.95) | 0.63±2.04 (-3.50-2.88) | 0.61±1.01 (-1.50-3.0) | F=0.64 | 0.53 |
| **Systolic blood Pressure (mmHg)** | 105.45±9.12 (90-121) | 111.25±9.91 (90-122) | 102.67±6.32 (90-120) | F=1.45 | 0.25 |
| **Diastolic blood Pressure (mmHg)** | 64.73±6.31 (60-80) | 73.75±7.44 (64-80) | 62.67±4.17 (50-80) | F=4.64 | 0.04 |

Group I: Patients with disease duration less than five years. F=ANOVA
Group II: Patients with disease duration five or more years. $X^2$ = chi-square
BMI=Body mass index. SDS=Standard deviation score
M/F=Male/female

Group II diabetics had significantly higher percentage of peripheral neuropathy (75%) and nephropathy (50%) compared to group I diabetics (9.1% and 0% respectively). Diabetic retinopathy occurred in 12.5% and 0% of group II and group I patients respectively (P = 0.27).

As regards the lipid profile; serum TC, TG and LDL were significantly higher in diabetics with disease duration of 5 years or more compared to other groups while HDL was significantly lower in group II compared to group I diabetics and controls table (3), Fig. (1).

**Table 2.** Metabolic control, acute and chronic diabetic complications in the studied patients.

| Variant | Group I N=22 | Group II n=16 | Test of significance | p value |
|---|---|---|---|---|
| **Random Blood Glucose (mg/dl)** | | | | |
| Mean ±SD | 191.59 ±46.71 | 214.63± 42.39 | t= 1.22 | 0.23 |
| Range | (110-280) | (180-280) | | |
| **HbA1c (%)** | | | | |
| Mean ±SD | 9.47 ±2.16 | 11.85± 3.33 | t=2.30 | 0.03 |
| Range | (5.6-13.2) | (7.7-16.3) | | |
| **Mean Insulin Dose(U/kg/d)** | | | | |
| Mean ±SD | 1.03±0.23 | 1.15±0.47 | t=1.38 | 0.18 |
| Range | (0.71-1.54) | (0.7-2) | | |
| **Acute Metabolic Complications** | | | | |
| **DKA** (n/year) | | | | |
| Median | 1.00 | 2.0 | Z=2.04 | 0.04 |
| Range | (1-2) | (1-6) | | |
| **Hypoglycemia** (n/year) | | | | 0.34 |
| Median | 3.50 | 2.0 | Z= 0.95 | |
| Range | (1-10) | (1-3) | | |
| **Chronic MVCs** | | | | |
| Peripheral neuropathy n (%) | 2(9.17) | 12(75) | * | 0.001 |
| Nephropathy n (%) | 0(0) | 8(50) | * | 0.003 |
| Retinopathy n (%) | 0(0) | 2(12.5) | * | 0.27 |

DKA: Diabetic ketoacidosis; MVCs: Microvascular complications, n: Number; HbA1c: glycosylated hemoglobin and
*: Fisher Exact test, Z: MannWhitney, t: Student t test

**Table 3.** Comparison between the three studied groups regarding their lipid profile, MDA and P-selectin levels.

| Variable | | Group I n=22 | Group II n=16 | Control n=30 | Test of significance | P value |
|---|---|---|---|---|---|---|
| **TC(mg/dl** | **Mean ±SD** | 175.05±29.1 | 199.38±37.15 | 159.53±9.01 | F=6.11 | 0.005 |
| | **Range** | (110-240) | (145-268) | (148-180) | | |
| **TG (mg/dl)** | **Mean ±SD** | 64.95±17.73 | 111.13±46.34 | 47.6±6.56 | F = 20.12 | < 0.0001 |
| | **Range** | (42-127) | (50-198) | (40-60) | | |
| **LDL (mg/dl)** | **Mean ±SD** | 100.14±17.66 | 127.25±32.12 | 81.27±8.16 | F = 15.88 | < 0.0001 |
| | **Range** | (72-134) | (89-191) | (69-98) | | |
| **HDL (mg/dl)** | **Mean ±SD** | 51.77±11.90 | 41.38±3.2 | 57.80±6.53 | F = 8.13 | 0.001 |
| | **Range** | (27-83) | (37-48) | (48-67) | | |
| **MDA** (nmol/ml) | **Mean ±SD** | 9.67±1.24 | 13.56±3.05 | 5.36±1.63 | F=58.59 | < 0.0001 |
| | **Range** | (7.83-12.11) | (9.64-17.9) | (3.8-8.5) | | |
| **P-selectin** (ng/dl) | **Mean ±SD** | 58.74±15.51 | 116.43±43.76 | 34.03±6.29 | F=39.33 | < 0.0001 |
| | **Range** | (28.1-89.7) | (61.7-189) | (24.1-44.1) | | |

NS=Non-significant (p>0.05). MDA= Malondialdehyde
S=Significant (p≤0.05). HDL = High density lipoprotein
HS=highly significant (p≤0.01). LDL = Low density lipoprotein
TG = Triglycerides T C= Total cholesterol

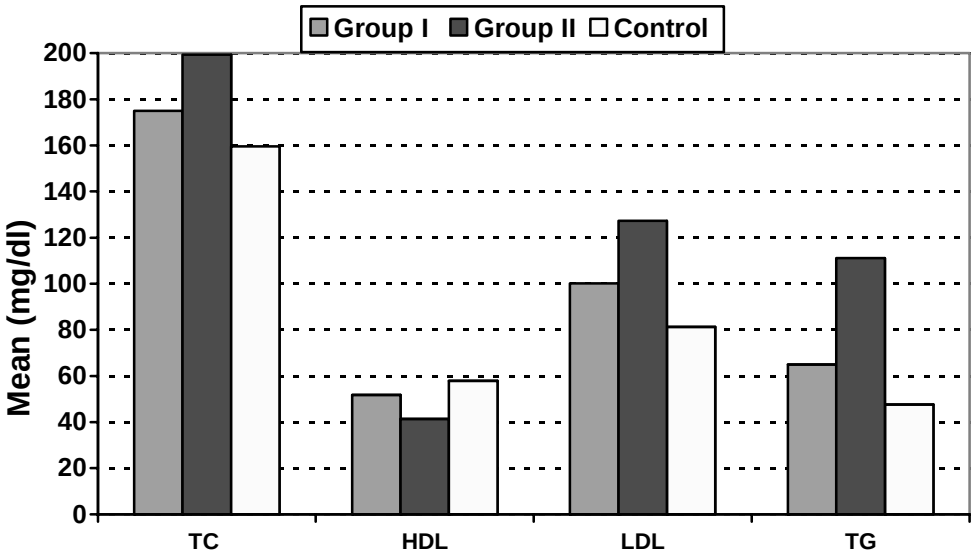


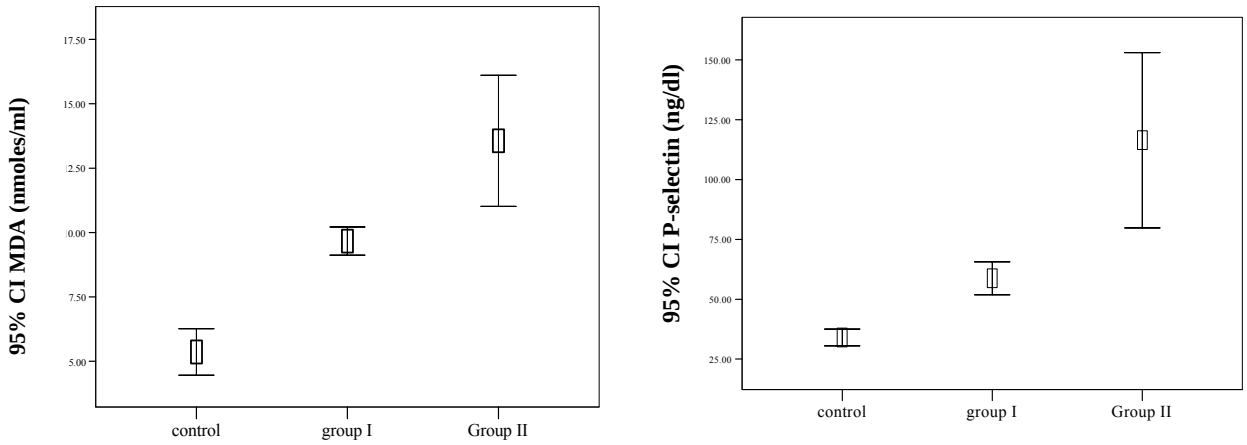

**Figure 1.** Mean values of lipid profile in the three studied groups.

Serum MDA and P-selectin were significantly higher in group II compared to group I diabetics and controls (p <0.0001) (Fig. 2a, b). Moreover, significantly higher mean MDA and P-selectin levels were found in patients with complicated compared to those with non complicated diabetes (p<0.0001) table (4).

**Table 4.** Mean values of P-selectin and MDA in complicated versus non-complicated course diabetics

| | **Non-complicated diabetics n =24** | **Complicated diabetics N =14** | **Test of significance** | **p value** |
|---|---|---|---|---|
| **P-selectin (ng/dl) Mean ±SD Range** | 58.23±14.91 (28.1-89.7) | 117.83±42.21 (66.6-189) | t= 4.98 | <0.0001 |
| **MDA (nmoles/ml) Mean ±SD Range** | 9.54±1.09 (7.83-11.95) | 13.92±2.62 (10.6-17.9) | t=4.99 | <0.0001 |

MDA=malondialdehyde




**(a)** **(b)**

**Figure 2.** Plots of mean and 95% confidence intervals of MDA (a) and P-selectin (b) showing no overlap between the three studied groups with the ability of having sharp cut off points (no false positive or false negative).

Serum P-selectin levels were related to severity of MVC in the studied diabetics as they were significantly higher in diabetics with more than one (mean of 149.50 ±35.97 ng/dl) compared to patients with one MVC (mean of 86.15 ±13.71 ng/dl, p=0.02), although no significant difference was found between them regarding their serum MDA (p>0.05) (Fig. 3a, b).

The association between OS (MDA level) and level of adhesion molecule (P-selectin) was examined and a significant positive correlation was found between P-selectin and MDA levels in the studied diabetics (r = 0.91, p < 0.0001) (Fig.4).

A significant positive correlation was found between MDA and P-selectin levels with disease duration, mean random blood glucose, $HbA_1c$, diastolic blood pressure, cholesterol, triglycerides and LDL levels in the studied diabetic patients. A significant negative correlation was found between both MDA and P-selectin with HDL levels in the studied diabetics (table 5).

A multiple regression analysis was done to identify the parameter that most significantly affects both MDA and P-selectin levels. Among all studied variables, $HbA_1c$ was the most significant variable positively associated with both MDA and P-selectin levels (p<0.0001) in the studied diabetics table (6).

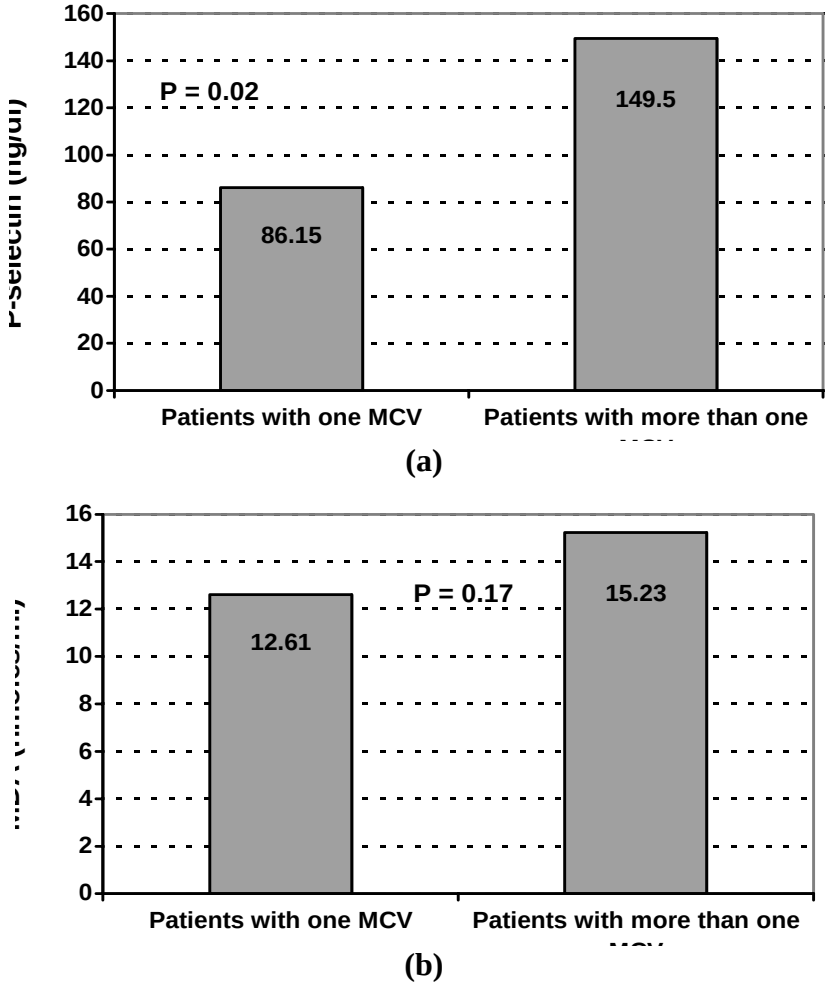



**Figure 3.** Mean P-selectin levels (a) and MDA levels (b) in patients with single microvascular complication (MVC) versus those with multiple MVCs.

**Table 5.** Correlation between MDA and P-selectin levels with variable parameters in the studied diabetics.

| | **MDA** | | **P-selectin** | |
|---|---|---|---|---|
| | ***R*** | **p** | ***R*** | **p** |
| **Age (year)** | 0.11 | 0.57 | 0.14 | 0.45 |
| **Disease duration (year)** | 0.75 | **<0.0001** | 0.85 | **<0.0001** |
| **BMI (kg/m²)** | -0.38 | 0.04 | -0.28 | 0.12 |
| **Systolic blood pressure (mmHg)** | 0.23 | 0.21 | 0.28 | 0.13 |
| **Diastolic blood pressure (mmHg)** | 0.40 | **0.03** | 0.50 | **0.005** |
| **MRBS (mg/dl)** | 0.63 | **<0.0001** | 0.57 | **0.001** |
| **$HbA_1c$ (%)** | 0.86 | **<0.0001** | 0.80 | **<0.0001** |
| **Mean insulin dose (U/kg/d)** | 0.01 | 0.95 | 0.09 | 0.63 |
| **Total cholesterol (mg/dl)** | 0.38 | **0.04** | 0.41 | **0.02** |
| **Triglyceride (mg/dl)** | 0.49 | **0.006** | 0.63 | **<0.0001** |
| **Low density lipoprotein (mg/dl)** | 0.39 | **0.03** | 0.37 | **0.05** |
| **High density lipoprotein (mg/dl)** | -0.39 | **0.03** | -0.39 | **0.03** |

MRBS= mean random blood sugar BMI=body mass index

**Table 6.** Multiple regression analysis for the relation of MDA and P-selectin levels to various studied parameters

| **Parameter** | **MDA (nmoles/ml)** | | **P-selectin ng/dl** | |
|---|---|---|---|---|
| | **Standardized Coefficients** | **Sig.** | **Standardized Coefficients** | **Sig.** |
| Age | -0.16 | 0.36 | -0.07 | 0.52 |
| Disease duration | 0.24 | 0.06 | **0.37** | **<0.0001** |
| BMI (kg/m²) | -0.07 | 0.60 | 0.02 | 0.83 |
| HbA1c% | **0.61** | **<0.0001** | **0.54** | **<0.0001** |
| Systolic blood pressure | 0.14 | 0.40 | 0.08 | 0.49 |
| Diastolic blood pressure | -0.01 | 0.95 | -0.04 | 0.73 |
| Total cholesterol | 0.03 | 0.82 | 0.00 | 0.96 |
| Triglycerides | 0.15 | 0.37 | **0.42** | **0.001** |
| HDL | -0.05 | 0.63 | -0.07 | 0.39 |
| LDL | 0.04 | 0.78 | -0.17 | 0.13 |

HDL: High density lipoprotein, LDL: Low density lipoprotein, BMI: Body mass index, MDA: Malondialdehyde and HbA1c: glycated hemoglobin.

Receiver Operating Characteristic curve was done to determine the cut off levels of MDA and P-selectin for prediction of microvascular affection in diabetics. A cut off value for serum MDA equal to 8.035 nmol/ ml with a sensitivity of 96.67% and specificity of 93.33% and for serum P-selectin equal to 45.15 ng/dl with a sensitivity of 90% and specificity of 100% were identified. Elevated levels above these cut off values are potentially early markers of susceptibility to diabetic microvascular complications; table 7 and figure 5.

**Table 7.** Sensitivity, specificity, PPV, NPV and cut-off values of MDA and P-selectin.

| | **Sensitivity%** | **Specificity%** | **PPV%** | **NPV%** | **Cut off value** | **AUC** | **SE** | **p value** |
|---|---|---|---|---|---|---|---|---|
| **MDA** (nmoles/ml) | 96.67 | 93.33 | 93.55 | 96.55 | **8.035** | **0.99 (95% CI 0.97 to 1.00)** | **0.001** | **<0.001** |
| **P-selectin** (ng/dl) | 90 | 100 | 100 | 90.91 | **45.15** | **0.95 (95%CI 0.89 to 1.00)** | **0.03** | **<0.001** |

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve; SE: Standard error and MDA: malondialdehyde.

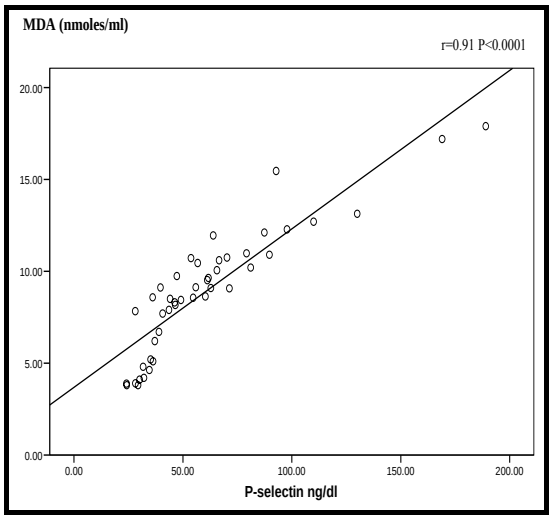



**Figure 4.** Scatter diagram showing a significant positive correlation between P-selectin and MDA levels in diabetics (strong association).

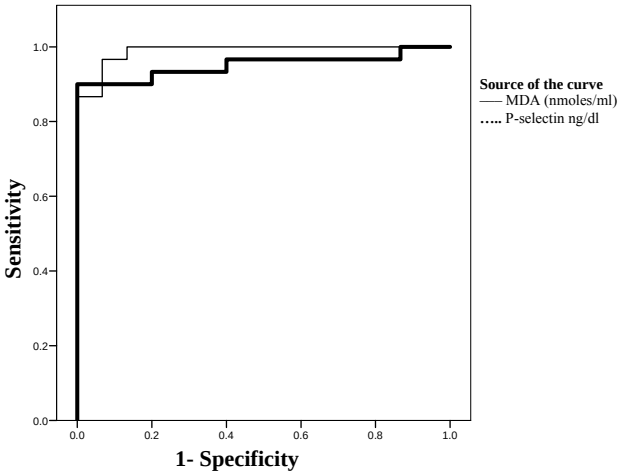



**Figure 5.** Receiver Operating Characteristic (ROC) curve for cut off levels of MDA and P-selectin.

## DISCUSSION

Diabetic patients with disease duration of 5 or more years had less glycemic control as evident by an increase in mean RBS and mean insulin dose with significantly higher HbA1c compared to patients with disease duration less than 5 years. Furthermore, they experienced more frequent episodes of DKA reflecting their less efficient control of their diabetic state.

Lachin et al.[12] reported that each 1% reduction in HbA1c levels corresponded to 40% reduced risk of developing microvascular disease and it was highly related to disease duration.

This same group of diabetics with higher HbA1c had higher percentage of peripheral neuropathy and nephropathy compared to patients with less than 5 years disease duration. Retinopathy was seen in 2/16 patients with long standing diabetes and not present in group1.

Studies documented that the increased risk of developing MVCs in type 1 diabetes was increased with progression of diabetes duration [13,14]. Recently Caroli et al.[15] reported that, diabetes duration is an established risk factor for retinopathy and nephropathy. Svensson and colleagues[16] reported that the prevalence of retinopathy increased with increasing disease duration, since one third of diabetics with duration between 10 and 12 years developed retinopathy.

Vincent and coworkers[17] demonstrated that the most common MVC in diabetes is peripheral neuropathy and its incidence increases with increased diabetes duration and is accelerated by poor glycemic control. They returned that to the increased effect of OS with increased disease duration, which is associated with the development of apoptosis in neurons and supporting glial cells and so could be the unifying mechanism that leads to nervous system damage in diabetes.

Hypoglycemia was more frequently encountered in patients with shorter disease duration. This could be explained by the fact that patients attempting to have a good glycemic control (as evident by lower HbA1c in group1) are likely to have a higher frequency of hypoglycemia and lower frequency of DKA attacks[18]. Golubnitschaja et al.[19] documented an inverse correlation between the frequency of hypoglycemic attacks and HbA1c level.

In the present study patients with long standing diabetes had higher levels of TC, TG and LDL with lower levels of HDL compared to group I diabetics and controls. This is contrary to what was reported by Erciyas et al.[20] who demonstrated that there was no significant difference in lipid profile between diabetics with long and those with short disease duration. On the other hand Jarvisalo et al.[21] reported that the protection afforded by HDL in the healthy controls was lost in the diabetic patients.

Hyperglycemia is the primary mediator of atherosclerosis in type1 DM and previous studies have shown that intensive insulin treatment had a significant effect on serum lipid levels[22]. Many studies have demonstrated serum lipid abnormalities in children with T1DM as well as an association between elevated HbA1c and dyslipidemia[23,24]. The importance of this association emerges from that pediatric dyslipidemia is

associated with atherosclerosis and requires preventive measures[25]. As part of these preventive measures, there is indirect evidence for the importance of glycemic control in improving lipid profile in children with T1DM[26]. Moreover glucose is known to increase both cholesterol synthesis and OS[27], and in the present study diabetics with longer diabetes duration had elevated blood glucose with elevated HbA1c values.

In the present study serum MDA was highest in diabetics with longer disease duration compared to other groups. Varvarovska et al.[28] studied the aspects of OS in type 1 diabetics and their first degree relatives. They confirmed increased OS in diabetics and their siblings. A one-year follow-up with supplementation of those patients with vitamins E and C led to improvement of diabetes control and reduced markers of OS compared with non-supplemented diabetics.

Karataş et al.[29] reported higher MDA level in diabetics with longer disease duration compared to newly diagnosed patients. As the disease progresses the free radicals production are increased and the capacity of antioxidant systems are reduced. Free radicals are produced as a result of glycosylation of several proteins including hemoglobin by non-enzymatic mechanisms. Subsequently, these free radicals change lipid/protein ratio of membranes by affecting poly-unsaturated fatty acids and lipid peroxidation causing functional irregularities of several cellular organelles[30]. Lipid peroxides are disintegrated quickly forming reactive carbon compounds. Among these, MDA is an important reactive carbon compound used commonly as an indicator of lipid peroxidation[31].

In this study there was a significant increase in P-selectin level in diabetics (even with disease duration <5 years), with the highest level in those with longer disease duration. This means that P-selectin is increased in diabetics even before the occurrence of MVC and this increase was parallel to the increase in disease duration. Karayanni et al.[32] demonstrated increased level of P-selectin as early as the first year of diabetes and this was in accordance with our results. On the other hand Glowinska et al.[33] found no change in the level of P-selectin in patients with atherosclerotic risk factors (obesity, diabetes and hypertension) compared to healthy children.

Pirot et al.[34] studied abnormal markers of endothelial cell activation and OS in type 1 diabetics with no clinical vascular disease. They demonstrated increased levels of these abnormal markers confirming the hypothesis that vascular disease starts early in the course of childhood diabetes. Recently, Suys et al.[30] presented a good evidence of early endothelial dysfunction in diabetic children.

Toivonen et al.[35] reported that soluble adhesion molecule disturbances started about 4 years before the clinical manifestations of diabetes, which were more obvious about 1.5 years before diagnosis. They proposed that adhesion molecules were associated with the initiation of destructive process of insulinitis with contribution in development of diabetes.

In the present study there was a positive correlation between MDA and P-selectin which means a strong relation between the degree of OS and the damage of the vascular endothelium leading to endothelial dysfunction and microangiopathy. Suys et al.[30] reported a parallelism between OS and abnormal markers of endothelial cell dysfunction in young patients with T1DM. Karayanni et al.[32] studied 45 type 1 diabetics and they found that P-selectin elevation was related to the imbalance in the oxidant/antioxidant status that accompanied diabetes suggesting the association between endothelial/platelet activation with OS even in the first years of the disease being more pronounced as the disease progresses.

Soriano et al.[36] reported that expression of P-selectin was among the adverse biological effects displayed by the endothelial cells upon exposure to ROS. They found that the vascular endothelium was the primary target of OS. At higher concentrations, ROS react rapidly with endothelial nitric oxide (NO) radicals, forming peroxynitrate anion and thus decreasing NO bioavailability, leading to protein nitration with subsequent vascular cell injury[37].

Booth et al.[38] reported that ROS may activate phospholipases which in turn generate a multitude of cellular messengers and cofactors that are critical in the regulation of actin binding proteins and adhesion molecules expressed on the endothelial cell membrane.

In the present study MDA level was positively correlated with disease duration. Changes in oxidant and antioxidant systems are related to duration of the disease and become more prominent as complications develop depending upon the level of glycemic control[39-41].

In a study done by Erciyas et al.[20] type 1 diabetics were evaluated in two groups in view of their mean $HbA_{1c}$ values, as metabolically well controlled ($HbA_{1c}$ ≤8%) and poorly controlled (HbA1c >8%) patients. They concluded increased level of MDA and dyslipidemia in poorly controlled patients with a positive correlation between MDA

level and the levels of TC, LDL, apolipoprotein B. These findings go with our study which found a positive correlation between glycemic control (RBS and HbA1c) and MDA.

Suys et al.[30] found a negative relationship between endogenous scavenger antioxidants and levels of HbA1c as well as positive one between levels of HbA1c with peroxidation product (MDA) in type 1 diabetics. This points out to the importance of glycemic control in antioxidant/oxidant balance in young diabetics.

Davi et al.[3] reported that enhanced lipid peroxidation in recently diagnosed diabetics was improved later on parallel to the improvement in glycemic control this could be explained by that some antioxidants are probably lost due to renal hyperfiltration in early phase of T1DM. In addition, hyperglycemia leads to redox imbalance and glycation of enzymes and can thus cause impairment in the recycling of some antioxidants molecules such as ascorbate[42]. Also, it leads to enhanced metabolism of glucose through the polyol (sorbitol) pathway, which results in increased intracellular osmolarity, depleted glutathione reserves and enhanced production of free radicals[43].

A positive correlation was evident between OS as presented by MDA levels and diastolic blood pressure. A possible explanation is that free radicals generated from OS had an important role in regulation of smooth muscle tone[44]. Furthermore it reacts with NO generating cytotoxic NO which alters function of biomolecules by protein nitration; potassium channels which regulate the vasorelaxation response are inhibited by this nitration process[45].

Also the present study revealed a significant positive correlation between dyslipidemia and MDA levels. Poor metabolic control is associated with increase in both OS (evident by high MDA levels) and dyslipidemia[25]. Previous studies correlated dyslipidemia in type1 diabetics to poor metabolic control whereas optimal glycemic control adjusted both dyslipidemia and decrease the level of OS[46].

In the present study serum levels of P-selectin showed a significant positive correlation with disease duration. This was in agreement with Karayanni et al.[32] who reported elevated level of P-selectin in diabetics especially those with long disease duration.

Moreover the present study found significant positive correlation between parameters of metabolic control (RBS and HbA1c) and P-selectin level. Brawnlee[1] reported that hyperglycemia could lead to endothelial damage through multiple mechanisms; enhanced polyol pathway, increased advanced glycation end products (AGEs), these in turn increase production of ROS, with subsequent increased expression of endothelial adhesion molecules especially P-selectin. Moreover hyperglycemia causes a rapid inflammatory response in the microcirculation. This response is triggered via up regulation of P- selectin on the endothelial cell surface probably due to reduced endothelial derived NO. This phenomenon might represent an important and early mechanism of the diabetic microangiopathy[31].

Jarvisalo et al.[21] had demonstrated that TG level has a positive while HDL level has a negative relationship with endothelial dysfunction and increased arterial intimae media thickness in children with T1DM.

In the present study HbA1c had the strongest effect on serum MDA and P-selectin. Varvaroska et al.[28] demonstrated a strong relation between OS and glycemic control. They achieved improvement of metabolic control in response to vitamin E supplementation over one year in a group of type 1 diabetics. Karayainni et al.[32] demonstrated that elevation of P-selectin in patients with T1DM is related to poor glycemic control.

Cipollone et al.[7] studied the contribution of soluble CD40 ligand (sCD40L) and soluble P-selectin to endothelial cell dysfunction and monocytes activation in diabetics (40 type 2 and 30 type 1 diabetics). They found up regulation of sCD40L (a pro-inflammatory agent released from activated platelets and associated with diabetic vascular complications) as a consequence of persistent hyperglycemia resulting in endothelial cells activation and monocytes recruitment to the arterial wall possibly, contributing to accelerated atherosclerosis. Elevated sCD40L in diabetics was associated with in vitro adhesion molecules release and significantly correlated with HbA1c levels. This means that poor glycemic control initiates an inflammatory response through, release of cell adhesion molecules, especially P-selectin with monocytes recruitment to the arterial wall contributing to vascular diabetic disease[47].

In the present study significant increase in MDA and P-selectin levels was found in complicated compared to non-complicated diabetics ($p<0.0001$). The importance of OS in the etiology of diabetes complications is based on the fact that the main biochemical pathways (glucose oxidation, polyol pathway, protein glycation, and prostanoid synthesis) that can increase free radical formation are tightly related to hyperglycemia[40]. Yngen et al.[48] demonstrated significant increase in serum level of

soluble P-selectin along with elevated sCD40L and C-reactive protein in type 1 diabetics with MVCs compared to non complicated and healthy subjects, confirming the hypothesis that microangiopathy is associated with platelet hyperactivity, endothelial dysfunction and low-grade inflammation[49]. Hu et al.[50] assessed platelet and leukocyte function and cross-talk between these cells in type 1 diabetics with and without MVCs. They demonstrated that thromboxane A2 analogue induced marked increase of platelet P-selectin expression and platelet/leukocyte aggregation in diabetics compared to healthy controls and this increase was more evident in complicated diabetics. This finding indicates that platelet and leukocyte dysfunction and enhanced leukocyte/platelet cross-talk are increased in diabetics with evident relation to MVCs.

In the present study significant increase in serum level of P-selectin was found in diabetics with more than one MVCs compared to those with one MVC ($p<0.05$). Meanwhile, P-selectin might act as a good index of microangiopathy severity. Recently elevated levels of adhesion molecules especially P-selectin in diabetics with MVCs was related to the severity of microcirculatory abnormality[51,52]. Similarly Devaraj et al.[5] found that increased level of adhesion molecules in complicated diabetics with evident relation to the severity of microcirculatory abnormality.

In conclusion; the present study highlights the role of OS and adhesion molecules in the development of diabetic MVCs and its correlation with parameters of metabolic control especially $HbA_1c$. It allows better understanding of the early steps in the pathophysiology of developing diabetic MVCs and so giving a future hope for novel therapeutic agents preventing the early crawling diabetic vascular disease. An important issue is that these findings appear even in patients with short non-complicated diabetes course and this provides a direct evidence for the beneficial and early effect of tight metabolic control and antioxidant therapy in prevention of MVCs.

Recommendations for achieving the appropriate metabolic control and management of dyslipidemia are the most beneficial in preventing the OS and improving endothelial function. Diet of diabetic patients should contain the recommended daily allowance of antioxidants. Starting antioxidant therapy as one of the corner stones of treatment early after diagnosis of type 1DM may give a future hope in preventing diabetic microvascular disease.

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