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RESEARCH ARTICLE

Evaluation of Lipid peroxidation Marker (MDA), C - reactive protein, and leucocytes indices as Inflammatory Markers in Patients with Type 2 Diabetes Mellitus

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Abstract

This study compared the Malondialdehyde (MDA) as lipid peroxidation marker, high-sensitive C reactive protein (hs-CRP), and leucocytes indices as inflammatory markers between type 2 diabetic patients and healthy controls. This study indicates that hyperglycemic induced elevation in Lipidperoxidation marker (MDA) and inflammatory markers hs-CRP and leucocytes indices, in type 2 DM could supportive in prognosis of vascular complications in patients with diabetes.

This study was conducted on 30 Type 2 Diabetic patients and 30 age and sex matched healthy control subjects. Fasting blood glucose (FBG), Glycosylated hemoglobin (HbA_{1c}), hs-CRP level and leucocytes indices including white blood cells count (WBCs), Percentage and Number of Lymphocyte, Monocytes and Granulocytes were estimated and compared with normal subjects. The results were evaluated statistically. The study demonstrated that FBG, HbA1c, hs-CRP, MDA, WBCs and # of Granulocytes were significantly higher in the diabetic patients as compared to controls ($P< 0.05$). Positive correlation of FBG with HbA1c ($r = 0.512$, $P = 0.004$), FBG with hs-CRP ($r = 0.498$, $P = 0.005$) was found in diabetic patients.

The elevated levels of MDA and hs-CRP along with leucocytes indices are surrogate markers of inflammation, may be used in finding the degree of developing complications, caused by hyperglycemic induced oxidative stress and Lipid peroxidation in type 2 DM .

Introduction

Oxidative stress and Lipid peroxidation are major burden in patients with diabetes mellitus, along with endothelial dysfunction as an early sign of diabetic vascular complications that is related to the presence of a vascular low-grade inflammation (Davì and Falco, 2005). Alteration in balance of the endothelium is the key event in the initiation of such complications, via activation of leukocyte adhesion, which is linked to the presence of a vascular inflammation. Inflammatory molecules may be an index of endothelial activation or even a molecular marker of early vascular complications (Savoia Carmine and Schiffrin Ernesto, 2007; Tostes and Muscar´a, 2005; Meydani, 2003).

Hyperglycemia can elicit endothelial damage via augmented oxidative stress (Dav`I, 2005) moreover; type 2 DM is related with increased risk for complications by inducing inflammatory vascular dysfunction (Ergul et al, 2005). The lipid peroxide in the blood provides valuable information for the prediction of diabetes whose late complications are often lethal (Tappel, 1973).

Free radicals may play an important role in the causation and complications of diabetes mellitus. In diabetes mellitus, changing in the endogenous free radical scavenging defense system may cause failure in scavenging of reactive oxygen species that result in tissue damage (Moussa, 2008). If not finished rapidly, there will be harm to the

cell membrane, which consists mainly of lipids. Additionally, end-products of lipid peroxidation perhaps mutagenic and carcinogenic.(Laura, 2003).

Therefore, this study aimed at measuring the levels of glycemic control, the lipid peroxidation product malondialdehyde (MDA), and acute-phase reactant high-sensitivity C - reactive protein (hs-CRP) and leucocytes indices in type 2 DM patients in comparison with healthy control subjects. Evaluation of these inflammatory markers may be used as an index of inflammatory endothelial activation and for the assessment of the conventional diabetic risk factors. Furthermore, the current work mainly aimed to study the connections between type 2 DM and vulnerability of lipoproteins to oxidation, in addition to their involvement in inflammation. In view of the fact that type 2 DM subjects have multiple risk factors that potentiate each other.

MATERIAL AND METHODS:

The study was conducted on 30 patients with type 2 diabetes both males and females (17 males & 13 females) between the age group 35-65 years who have registered at Baqai Institute of Diabetology and Endocrinology, Karachi, Pakistan. Thirty (12 males and 18 females) age and sex matched control subjects were also selected from the general population at random for comparison. Ethical approval was obtained from the institutional review board (IERB) of BIDE prior to the commencement of the study. Informed consent was taken from each individual at the time of recruitment in the study. All the patients who were diagnosed with type 2 diabetes using the ADA criteria i.e. fasting blood glucose (FBG) of $\geq$ 126 mg/dl were included in the study. The patients who had any recent clinical evidence of heart, kidney or liver disease and any hemoglobinopathy were excluded from the study. BMI was calculated as an index of the weight in kilograms divided by the square of the height in meters. Blood samples were taken in tubes with EDTA as anticoagulant and analyzed within 2 hours of venepuncture for leukocyte indices and HbA_{1c}. Plasma was also separated and analyzed for other Biochemical parameters such as fasting blood glucose (FBG),hs-CRP and MDA levels. leucocytes indices including white blood cells count (WBCs), Percentage of Lymphocyte (% Lym), Percentage of monocytes (% Mon), Percentage of Granulocytes (% Gra), Number of Lymphocyte (# Lym), Number of monocytes (# Mon), Number of Granulocytes (# Gra)were analyzed by using hematological analyzer ABX micros 60 fully automated analyzer HORIBA (France). Fasting blood glucose was estimated by following glucose oxidase method on UV- visible spectrophotometer Jenway 6305.The HbA_{1c} was analyzed by automatic analyzer (D-10 analyzer). hs-CRP level was determined by quantitative turbidimetric method using Spinreact test kit. Determination of Total Malondialdehyde (MDA) in Plasma was done by Bioxytech MDA –586^{tm} Kit method)

All statistical analyses were performed using SPSS version 20 software package (SPSS Inc, Chicago, IL, USA). Data are presented as mean ± SEM. Correlations between FBG, hs-CRP, MDA and leucocytes indices were evaluated by Pearson's correlation. Multiple linear regressions were used to evaluate whether poor glycemic control in type 2DM and inflammatory risk factors independently predicted the levels of vascular complication Type 2 DM patients. P-values and 95% confidence intervals (CI) were also calculated. The level of statistical significance was set at $P \leq .05$.

RESULTS: The study was based 30 patients with diabetes and 30 healthy control subjects. The baseline characteristics of the studied participants are represented in Table I. The groups significantly differ in relation to Age and weight but not significantly differ in height and BMI. The results of Biochemical parameters (FBG, HbA1c, MDA and hs-CRP) and leucocytes indices reflects significant increase in FBG, HbA1c, MDA, hs-CRP, %Lymphocyte, # Granulocytes in diabetic patients as compared to healthy control subjects **(Table -1)**. The Variations of FBG, HbA1c, hs-CRP, MDA levels and leucocytes indices in male and female control and Diabetic subjects are shown in **(Table-II)** indicating significant increase in FBG, HbA1c, hs-CRP, MDA and %Lymphocyte in diabetic male and female patients where as diabetic male showed significant increase in % Granulocyte whereas diabetic females showed significant increase in # Granulocytes in comparison with their respective controls.

Pearson correlation was used to find the correlation between Biochemical parameters as well as leucocytes indices in diabetic patients **(Table- III and IV)** indicated that insignificant positive correlation was found in FBG with WBCs, %LYM, #GRA and negative correlation was found with %MON, % GRA, #LYM, #MON. HbA1c also showed insignificant positive correlation with WBCs, %LYM, , #LYM and #GRA and negative correlation with %MON, % GRA and #LYM. hs-CRP found to be insignificantly positively correlated WBCs, % and #LYM, % and #MON and negatively correlated with % and # GRA.MDA showed insignificant negative correlation with WBCs, %

and #LYM ,#GRA and insignificant positive correlation with % and #MON ,%GRA. (Table -3).Correlation studies also revealed significant Positive correlations between FBG with HbA_{1c}, hs-CRP and MDA **(Figure 1-3)**.

TABLE –I Comparison between Control and Diabetic Subjects with respect to the Physical, Biochemical and Hematological Parameters.

Parameters	Control (n = 30)	Diabetic Patients (n = 30)	P- Value
Physical Parameters:			
Age (years)	45.33±1.52	49.90±1.67	P = 0.0476*
Height (Cm)	165.44±1.86	167.93±1.70	P = 0.3272
Weight (Kg)	61.20±1.90	68.23±1.84	P = 0.0101*
BMI (Kg/m^2)	22.67±0.59	24.23±0.63	P = 0.0759
Biochemical and Hematological Parameters:			
FBG (mg/dl)	88.62±2.01	166.26±8.30	P = 0.0001*
HbA1c (%)	4.62±0.05	7.44±0.32	P = 0.0001*
hs-CRP (mg/L)	0.90±0.26	4.54±0.49	P = 0.0001*
MDA (µM)	8.60±0.33	13.18±0.88	P = 0.0001*
WBCs ($10^3/mm^3$)	7.52±0.38	9.31±0.86	P = 0.0631
%LYM (%)	32.18±1.36	26.29±1.76	P = 0.0104
%MON (%)	5.77±0.24	7.02±1.13	P = 0.2837
%GRA (%)	62.04±1.51	65.78±2.12	P = 0.1561
#LYM ($10^3/mm^3$)	5.70±3.25	2.71±0.39	P = 0.3648
#MON ($10^3/mm^3$)	0.40±0.02	0.55±0.07	P = 0.0911
#GRA ($10^3/mm^3$)	4.73±0.24	6.12±0.49	P = 0.0152

Fasting blood glucose (FBG), Glycosylated hemoglobin (HbA_1c), high sensitivity C - reactive protein (hs-CRP) leucocytes indices including white blood cells count (WBCs), Percentage of Lymphocyte (% Lym), Percentage of monocytes (% Mon), Percentage of Granulocytes (% Gra), Number of Lymphocyte (# Lym), Number of monocytes (# Mon), Number of Granulocytes (# Gra). n = no of subjects, values are represented as mean ± SEM (Standard error of mean).* $P < 0.05$ is considered to be statistically Significant.

Table-II Variations of FBG, HbA1c, hs-CRP, MDA and Leukocyte Indices in Control and Diabetic Male and Female Subjects

Variables	Control (n=30)		Diabetic Patients (n=30)	
	Male (n= 12)	Female (n=18)	Male (n= 17)	Female (n=13)
FBG (mg/dl)	81.96±1.89	93.07±2.66	*167.17±11.5	*165.07±12.4
HbA1c (%)	4.45±0.05	4.73±0.08	*7.92±0.48	*6.8±0.35
hs-CRP (mg/L)	0.68 ±0.33	1.05±0.38	*5.42±0.61	*3.38±0.73
MDA (µM)	8.20±0.39	8.86±0.49	*13.83±1.30	*12.35±1.1
WBCs ($10^3/mm^3$)	7.40±0.73	7.60±0.42	9.37±1.47	*9.22±0.61
%LYM (%)	33.96±1.62	30.99±1.98	*26.51±2.29	*26±2.85
%MON (%)	5.68±0.36	5.83±0.32	6.02±0.46	8.33±2.56
%GRA (%)	60.35±1.94	63.16±2.17	*67.45±2.39	63.59±3.8
#LYM ($10^3/mm^3$)	2.51±0.31	7.83±5.42	2.88±0.66	2.49±0.28
#MON ($10^3/mm^3$)	0.40±0.059	0.40±0.03	0.48±0.06	0.63±0.15
#GRA ($10^3/mm^3$)	4.50±0.40	4.88±0.31	6.19±0.79	*6.03±0.52

Fasting blood glucose (FBG), Glycosylated hemoglobin (HbA_1c), high sensitivity C - reactive protein (hs-CRP),Malondialdehyde (MDA) **,**White blood cell count (WBCs), Percent Lymphocyte (%LYM), Percent Monocyte (%MON), Percent Granulocyte (%GRA), Number of Lymphocyte (#LYM), Number of Monocytes (#MON), Number of Granulocyte (#GRA),n = no: of subjects, values are represented as mean ± S.E.M (Standard error of mean).* Statistically significant as compared to control.

Table-III Correlation between Biochemical Variables

		Glucose	HbA1c	hsCRP	MDA
Glucose	Pearson Correlation	1	.512**	.498**	.564**
	Sig. (2-tailed)		.004	.005	.001
	N	30	30	30	30
HbA1c	Pearson Correlation	.512**	1	.552**	.124
	Sig. (2-tailed)	.004		.002	.513
	N	30	30	30	30
hs-CRP	Pearson Correlation	.498**	.552**	1	.271
	Sig. (2-tailed)	.005	.002		.148
	N	30	30	30	30
MDA	Pearson Correlation	.564**	.124	.271	1
	Sig. (2-tailed)	.001	.513	.148	
	N	30	30	30	30

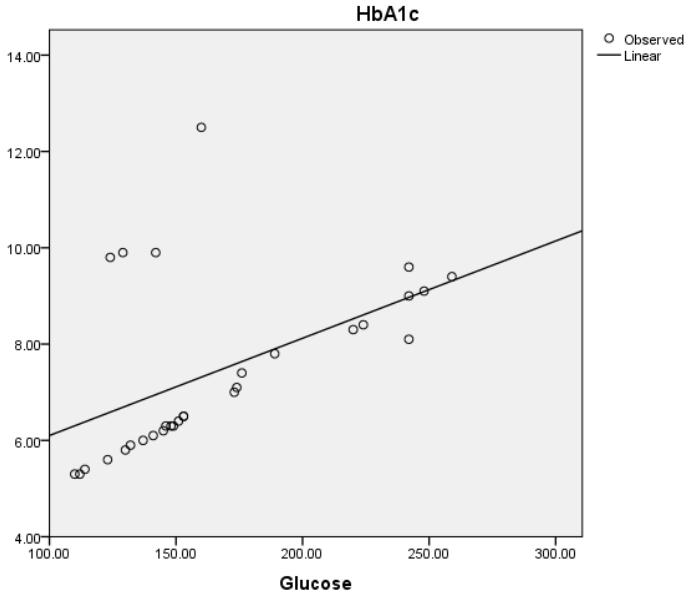
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table-IV Correlation between Biochemical Variables and Leukocyte Indices

		WBCS	%LYM	%MON	%GRA	#LYM	#MON	#GRA
Glucose	Pearson Correlation	.021	.104	-.072	-.024	-.020	-.064	.058
	Sig. (2-tailed)	.913	.585	.706	.898	.918	.739	.759
	N	30	30	30	30	30	30	30
HbA1c	Pearson Correlation	.206	.187	-.139	-.038	.264	-.053	.197
	Sig. (2-tailed)	.275	.323	.463	.840	.158	.779	.297
	N	30	30	30	30	30	30	30
hsCRP	Pearson Correlation	.012	.254	.166	-.242	.167	.145	-.089
	Sig. (2-tailed)	.950	.176	.381	.197	.378	.446	.639
	N	30	30	30	30	30	30	30
MDA	Pearson Correlation	-.143	-.126	.228	.022	-.226	.157	-.111
	Sig. (2-tailed)	.449	.507	.226	.910	.230	.407	.561
	N	30	30	30	30	30	30	30

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Fig-1


HbA1c
Observed
Linear
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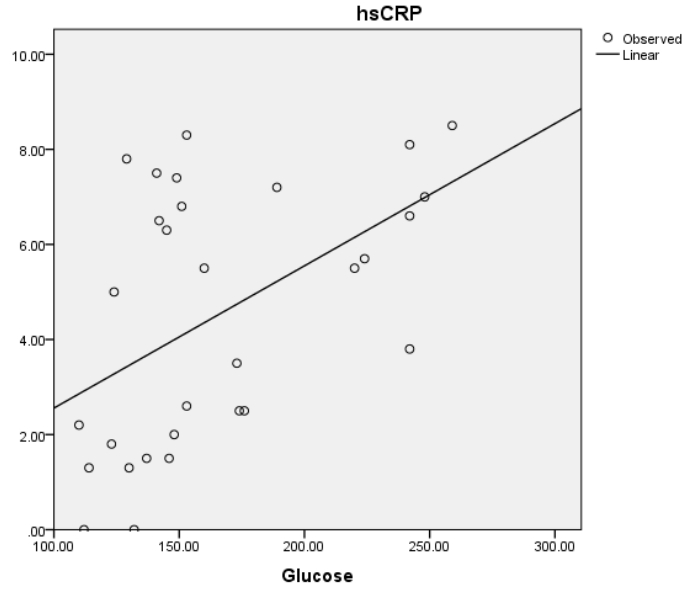
8.00
6.00
4.00
100.00
150.00
200.00
250.00
300.00
Glucose

Fig-2


hsCRP
Observed
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150.00
200.00
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Glucose

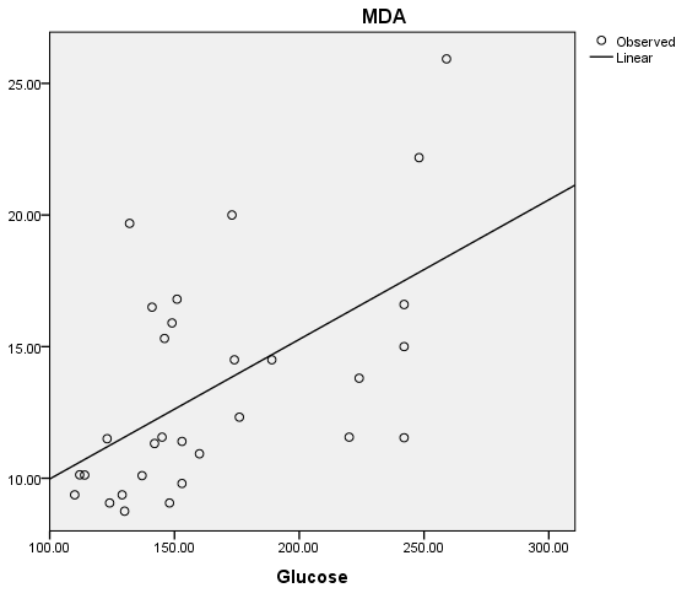

Fig-3

Fig-1-3: Scatter plot showing positive correlation between Fasting Blood Glucose (FBG) level with glycosylated hemoglobin (HbA_{1c}), high sensitivity C reactive Protein (hs-CRP) and Malondialdehyde (MDA).

DISCUSSION:

Peroxidation of lipid membrane has been related to the pathogenesis of many degenerative diseases, such as atherosclerosis, oxidative damage to DNA, aging. Carcinogenesis, sickle cell disease and Diabetes Mellitus etc (Chattergee, 1988).Thus, the lipid peroxide in the blood provides useful information for the prognosis of diabetes in which secondary disorders are often fatal. Various diagnostic tests are available for quantitative analysis of the end-products of lipid peroxidation especially, malondialdehyde (MDA) (Janero, 1990).

The main objective was to Evaluate Lipid peroxidation Marker (MDA), C - reactive protein, and leucocytes indices as Inflammatory Markers in Type 2 Diabetes Mellitus Patients. Inadequate glycemic control and impairment in the oxidant/antioxidant equilibrium induce oxidative stress and lipid peroxidation that cause considerable change in the cell membrane that lead to endothelial injury and vascular complications in diabetics (Woodman et al, 2002).

Our study reveals the significant increase in malondialdehyde (MDA), high sensitivity C reactive protein (hs-CRP) and some leukocyte indices with poor glycemic control **(Table -I)**. In the present study it was found that various parameters such as FBG, HbA1c, hs-CRP, MDA and leukocyte indices were high in male and female patients as compared to control subjects **(Table -II)**, suggesting these patients at high risk of developing vascular complications in future. Several studies revealed that elevated levels of hs-CRP may point to the existence of inflammation in blood vessels and be an early indicator for heart disease (Ridker, 2008). Insulin affects many sites of mammalian lipid metabolism. From this point of view the assessment of various lipid fractions and lipid peroxide in the cases of Diabetes Mellitus may be of some help in the prognosis of patients and in preventing the possibilities of complications or secondary disorders (Suryawanshi et al, 2006). By measuring the MDA level along with leukocyte indices and hs-CRP and the administration of selective antioxidants along with essential trace elements and minerals might be effective in reducing deleterious effects caused by hyperglycemic induced oxidative stress and Lipid peroxidation in diabetics.

CONCLUSION:

Our study shows that MDA, hs-CRP and leucocytes indices as surrogate markers of inflammation are elevated in diabetic patients. The data support the opinion that diabetic patients present a high risk for developing vascular complications and need early aggressive intervention. Increased oxidative stress, Lipid peroxidation along with

inflammation in type 2 DM could be to a certain extent overcome by antioxidants supplementation accordingly, the physician possibly will manage the severity of vascular complications in diabetic patients.

Acknowledgement:

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