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Association of Serum Bilirubin, Serum Malondialdehyde and Glycemic Control with Retinopathy in Type 2 Diabetic Subjects

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Abstract: Objective: Determine the serum bilirubin, serum malondialdehyde (MDA) and glycemic control with retinopathy in type 2 Diabetes mellitus. Subjects and Methods: The present case control study was conducted at the Department of Medicine, Liaquat University of Medical and Health Sciences Jamshoro/Hyderabad from January 2014 to February 2015. 50 type 2 DM (controls group 1) without DR and 50 type 2 DM with DR (cases group 2) were selected. Inclusion and exclusion criteria were followed strictly. RBG, FBG, fasting insulin, HbA1c, blood lipids, serum creatinine and bilirubin were detected and HOMA model was calculated. MDA was estimated by assay kit. Data was analyzed on *Statistix 8.1* (USA) software ($P \leq 0.05$). Results: Blood glucose, Glycated HbA1, serum creatinine, serum bilirubin and serum malondialdehyde (MDA) showed statistically significant difference ($p<0.05$). Serum bilirubin was noted as 1.09±0.25 and 0.96±0.21 mg/dl in controls and cases ($p=0.005$). Serum MDA was significantly elevated in DR cases 5.94±2.02 µmol/dl vs. 3.88±2.24 µmol/dl in controls. Serum bilirubin showed negative correlation with blood glucose, HbA1c and MDA (r-value noted as -0.359, -0.306 and -0.302 respectively). Serum MDA showed positive correlation with blood glucose ($r = 0.478$) and HbA1c ($r = 0.507$). Conclusion: The present study reports low serum bilirubin and high serum malondialdehyde levels in diabetic retinopathy.

Keywords: Malondialdehyde, Bilirubin, Glycemic Control, Diabetes Mellitus

1. Introduction

Prevalence of diabetes mellitus (DM) is now touching a serious peak in the Asian countries including Pakistan. Asian countries are forecasted to be future "diabetes capital" by the year 2030 [1]. Most common type of DM is the non-insulin dependent termed as type 2 DM (T2DM). T2DM accounts for >90% of cases. Prolonged hyperglycemia of DM causes non-enzymatic glycation of cell proteins and stimulates the polyols pathway. Non-enzymatic glycation and polyols hyper accumulation are major mechanisms of cell and tissue injury in T2DM subjects. Non-enzymatic glycation of proteins and polyol formation and accumulation cause tissue damage due to chronic hyperglycemia [2]. Oxidative stress is a characteristic feature of DM due to hyperglycemia, which is noted in extra- and intra cellular compartments [3]. Free radical formation is indigenous to the DM produced by oxidation of glucose, glycation of collagen, glycation of proteins, lipid peroxidation, etc [4]. Diabetic retinopathy (DR) is one of the micro vascular complications of the DM which may culminate in blindness [5]. Chronic hyperglycemia, hyperlipidemia and dyslipidemia, high blood pressure and long duration of DM initiate and aggravate the DR [6], but the oxidative stress, free radical formation, lipid peroxides, and reduced anti oxidants contribute to the DR also. Lipid peroxidation occurs due to chronic hyperglycemia, and free radical formation. Hyperglycemia and dyslipidemia induce the glucolipotoxicity, both of which cause the vascular endothelial dysfunction [7-9]. Bilirubin is a natural anti oxidant and cytoprotectant with anti inflammatory potential hence protects against the vascular complications [9,10]. Endogenous reactive oxygen species (ROS) destroy the anti oxidants of body resulting in oxidative stress [11,12]. A previous study [13] showed bilirubin accelerated the wound

healing in diabetic rat model due to the its anti oxidant property. Normal bilirubin levels are protective against the diabetic complications [14]. ROS react with lipids called peroxidation, and generate lipid peroxides increase the peroxidative load. The malondialdehyde (MDA) is a surrogate marker of lipid peroxidation which reacts with cell membrane phospholipids [15]. Elevated blood level of MDA is a good marker of diabetic oxidative stress. A few studies are available in the literature showing association of MDA and bilirubin in DR [16-18]. The present study hypothesized that there is no correlation of MDA and bilirubin with DR in type 2 DM. This is the first study on the Pakistani population showing the association of MDA and serum bilirubin with DR. The study will help better management of DR in type 2 DM subjects.

2. Subjects and Methods

Ethical approval was taken from the institutional ethics committee for the present case control study. The study covered duration of January 2014 to February 2015. Type 2 DM subjects attending the Diabetic clinic, Department of Medicine, Liaquat University of Medical and Health Sciences Jamshoro/Hyderabad, were selected according to inclusion and exclusion criteria. Type 2 DM subjects were approached for their willingness. For gaining confidence of patients they were interviewed of any visual problem if they are having. The problem of blindness in DM was discussed with them in detail. Once the patients understood the problem of DR and blindness, they were informed about the purpose of study. They were told benefits of study that there will be no harm, instead study will be helpful for future therapy of preventing blindness. These subjects were asked either they are willing for participation or not. They were informed that if they don't participate, this will not affect their treatment. They were further informed that they have right to withdraw from study protocol at any time without telling any reason even after they signed the consent form. Willing volunteers were asked that signing the consent form is mandatory for study protocol. Diabetic subjects were examined by medical officer followed by consultant physician and were referred to ophthalmology department for the retinopathy.

2.1. Study Groups and Sampling Design

A sample of 100 type 2 diabetic subjects was chosen and divided into 2 groups; Controls – included type 2 DM without retinopathy and Cases– included type 2 DM with retinopathy. Diabetic's subjects were selected by non-probability purposive sampling and study observed strict inclusion and exclusion criteria.

2.2. Inclusion and Exclusion Criteria

Duration of DM ≥5 years of age 40 -60 years, taking oral anti diabetic therapy was included. Diabetics suffering from severe uncontrolled systemic hypertension, uncontrolled DM taking insulin, cardiac disease, chronic lung disorders, chronic liver disease, smoker and alcoholics were exclusion criteria in the present study. Diabetics taking vitamin pills, mineral supplements, antioxidant formulations, and cholesterol lowering agents were also criteria of exclusion. Patient's data was maintained in a pre-structured Performa. Confidentiality was strictly maintained. Only concerned physician could watch the patient's record. A Performa was designed for data collection including demographics.

2.3. Blood Sampling and Biochemical Estimation

Blood sampling was performed from a prominent peripheral vein; most often used was the antecubital vein. Before venesection, the area was sterilized with alcohol swab. Ten ml of blood was drawn into gel tube by disposable syringe (BD, USA). Blood samples were centrifuged for 10 minutes at 4000 rpm, sera were stored at -20°C. Blood glucose- fasting and random, fasting insulin, glycated HbA1, blood lipids, serum creatinine and bilirubin were analyzed by standard laboratory techniques. HOMA model was calculated as; HOMA = fasting insulin × fasting glucose/22.5 [19]. Malondialdehyde (MDA) was estimated by assay kit (Cayman Assay kit). Cobas e 411 chemical analyzer of Roche Diagnosis GmbH, Mannheim, Germany was used for biochemical testing.

2.4. Data Analysis

Student's t test and Chi square test were used on *Statistix 8.1* (USA) and Graph Pad Prism software for analysis of continuous and categorical variables respectively at 95% confidence interval ($P \leq 0.05$).

3. Results

Age and gender matched controls and diabetic retinopathy (DR) subjects were studied. Age, gender, weight, height and BMI are shown in Table 1. Systolic and Diastolic BP showed statistically significant differences ($p<0.05$). Blood glucose, Glycated HbA1, serum creatinine, serum bilirubin and serum malondialdehyde (MDA) showed statistically significant differences between controls and cases. HOMA-IR model was used for estimation of insulin resistance in diabetics. Hyperlipidemia was noted in both groups but more in DR (Table 1). Serum bilirubin was noted as 1.09±0.25 and 0.96±0.21 mg/dl in controls and cases. Serum bilirubin was low in cases with significant p-value (p=0.005). Serum MDA was significantly elevated in DR cases. MDA in controls and cases was noted as 3.88±2.24 and 5.94±2.02 µmol/dl respectively. Serum bilirubin showed negative correlation with blood glucose, HbA1c and MDA. Correlation co-efficient (r-value) of blood glucose, HbA1c and MDA were noted as -0.359, -0.306 and -0.302 respectively. Serum MDA showed positive association with blood glucose (r = 0.478) and HbA1c (r = 0.507) respectively as shown in Table 2. Figures 1-3 show the correlation of HbA1c, serum bilirubin and malondialdehyde.

Table 1. *Demographic characteristics and Laboratory findings of study subjects.*

	Controls (n=50)	Cases (n=55)	p-value
Age (years)	43.5±8.04	45.16±7.5	0.056
Male	33 (66%)	27 (49.09%)	0.06
Female	17 (34%)	28 (50.9%)	0.06
Weight (kg)	71.0±9.14	82.1±12.14	0.07
Height (cm)	162.5 ±4.6	161.3 ±5.5	0.81
BMI (kg/m^2)	28.1±5.1	29.5± 5.3	0.01
Systolic BP (mmHg)	131.8±8.4	139.6±15.3	0.04
Diastolic BP (mmHg)	68.8±4.1	72.5±9.3	0.011
Blood glucose (R) (mg/dl)	134.8±8.3	266.0±49.5	0.0001
Blood glucose (F) (mg/dl)	153.4±40.7	145.9±19.7	0.049
Serum Insulin (F) (mg/dl)	16.3±4.2	15.9±6.5	0.067
Glycated HbA1 (%)	6.03±0.59	7.4±3.5	0.0001
HOMA-IR (%)	7.47	7.95	0.54
Total cholesterol (TC) (mg/dl)	206.1±80.5	233.3±72.4	0.011
Triglycerides (TAG) (mg/dl)	272.9±15.5	321.3±114.1	0.0001
LDLc (mg/dl)	144.5±41.5	162.5±41.0	0.0001
HDLc (mg/dl)	37.5±9.5	35.5±5.3	0.034
Serum creatinine (mg/dl)	0.93±0.18	1.10±0.25	0.0001
Serum Bilirubin (mg/dl)	1.09±0.25	0.96±0.21	0.005
Malondialdehyde (µmol/dl)	3.88±2.24	5.94±2.02	0.0001

Table 2. *Pearson's correlation of different variables.*

		HbA1c	MDA	S. Creatinine	S. Bilirubin
Blood glucose (R) (mg/dl)	r-value	0.478**	0.513**	0.366**	- 0.359**
	p-value	0.0001	0.0001	0.0001	0.0001
Glycated HbA1 (%)	r-value	-	0.507**	0.425**	-0.306**
	p-value		0.0001	0.0001	0.001
Serum Malondialdehyde (MDA)(µmol/dl)	r-value	0.507**	-	0.297**	-0.302**
	p-value	0.0001		0.002	0.002

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

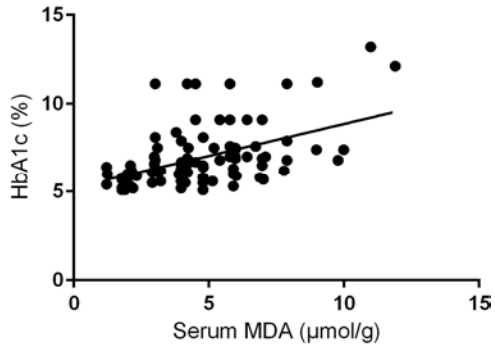

Figure 1. *Scatter graph showing correlation of Malondialdehyde and Glycated HbA1.*

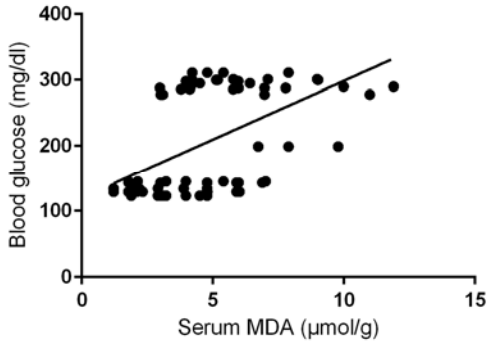

Figure 2. *Scatter graph showing correlation of Blood glucose and Serum MDA.*

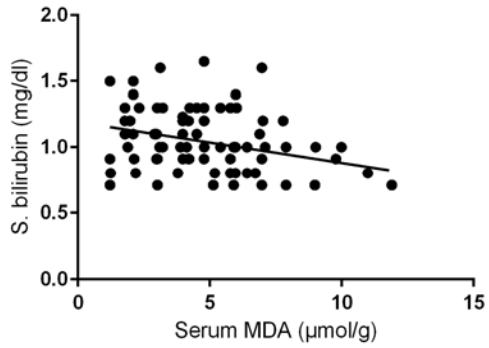

Figure 3. *Scatter graph showing negative correlation of serum MDA and Serum bilirubin.*

4. Discussion

The present study is the first research conducted at our tertiary care hospital which caters hundreds of diabetic patients a month. The present case control study showed low serum bilirubin and high malondialdehyde in diabetic retinopathy. Blood glucose, Glycated HbA1, serum creatinine, serum bilirubin and serum malondialdehyde (MDA) showed statistically significant differences between controls and cases. Systolic and Diastolic BP showed statistically significant differences ($p<0.05$). HOMA-IR model was used for estimation of insulin resistance in diabetics. It has been suggested the main culprit of

oxidative and peroxidative load in T2DM subjects is the chronic hyperglycemia [20], similar diabetic milieu of chronic hyperglycemia is noted in our diabetics, majority of whom showed bad glycemic control as revealed by HbA1. Previous studies [16-18] have shown association of serum bilirubin and micro vascular complications- diabetic retinopathy in type 2 DM. Our findings of low serum bilirubin in DR patients are in keeping with above studies. Bilirubin is a strong anti antioxidant and anti-inflammatory being consumed in neutralizing the increased oxidative load when it is increased. Low bilirubin of present study reveals high oxidative load in diabetic retinopathy patients. The findings are in agreement with previous studies [9, 10]. Retina is damaged by the free radical and peroxides resulting in DR, the finding are in agreement with previous study [21]. Previous studies have shown the protective role of serum bilirubin against the DR [22, 23]. In the present study, controls were matched for age and gender matched because oxidant and antioxidant levels may change with increasing age and gender. Similarly, we have chosen patients of nearly equal duration of DM to overcome any confounding effect. In the present study, serum bilirubin showed negative correlation with blood glucose, HbA1c and MDA as shown in Table 2 and Figure 2. Our findings are in agreement with previous studies [17, 18, 22]. Najam *et al* [23] reported that the serum bilirubin levels tend to be inversely correlated with DR, these findings support the findings of present study. Another important variable researched in our study is the lipid peroxidation marker known as the serum MDA. Serum MDA was significantly elevated in DR cases. MDA in controls and cases was noted as 3.88±2.24 and 5.94±2.02 µmol/dl respectively. Serum MDA showed positive association with blood glucose (r = 0.478) and HbA1c (r = 0.507). Our findings are consistent with previous studies reported from outside the country [16, 18, 20]. Elevated serum MDA in DR in type 2 diabetics indicates increase peroxidative load, and MDA in control diabetics was also found higher than normal range. Hence it may be used as biomarker for prediction of DR. Serum MDA may vary in smokers and alcoholics [20] which were excluded in the present study. Serum MDA levels of present study are supported by previous work [20, 24]. Turk et al [24] compared ischemia modified albumin (IMA) with MDA and concluded both MDA and IMA are predictors of DR, the finding of MDA supports our present study. Our results also support previous studies [25- 27] which reported that the MDA was predictor of DR severity. Main limitation of the present study is we could not study the malondialdehyde and bilirubin according to different grading of diabetic retinopathy due to lack of facilities. The strength of study lies in its prospective designs of case controls, however, cause effect relationship cannot be ascertained because of cross sectional design of study. We conducted study on chronic diabetics of local population hence our findings cannot be generalized to other setting, ethnicity, and racial groups.

5. Conclusion

The present study reports increased oxidant and peroxidant load in Diabetic retinopathy as revealed by low serum bilirubin and high serum malondialdehyde levels. It is concluded that the serum bilirubin and malondialdehyde may be used for the prediction of diabetic retinopathy and may be evaluated in diabetics as screening tests in clinical practice.

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