ORIGINAL ARTICLE

ASSOCIATION OF SERUM TOTAL BILIRUBIN LEVEL WITH DIABETIC RETINOPATHY IN TYPE 2 DIABETES MELLITUS

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Background: Serum bilirubin has anti-inflammatory, antioxidant and immunological properties. It is considered a protective substance against atherosclerotic and microvascular complications of diabetes mellitus (DM). This study was designed to find the association between total serum bilirubin concentration and diabetic retinopathy (DR). **Methods:** This case control study was conducted in the Department of Endocrinology, Diabetes and Metabolic Diseases, Hayatabad Medical Complex, Peshawar. Type-2 DM patients more than 18 years of age of either gender with duration of T2DM more than 6 months were included and sub categorized in two groups. Cases (DM with DR) and Controls (DM without DR) while patients with acute and chronic liver diseases, haemolytic anaemia, history of chronic alcohol consumption, use of hepatotoxic drugs (anti-tuberculous, anti-epileptic), women on oral contraceptive pills were excluded. All participants underwent ophthalmic examination at diabetic retinopathy screening clinic followed by pre designed set of investigations. **Results:** A total of 152 patients, 76 cases and 76 controls were included. Serum bilirubin concentration was found inversely and independently (*p* 0.000) associated and inversely co related (r -0.345and *p* 0.000) with prevalence of DR. Cases were concentrated in the lower quartiles of serum bilirubin concentration and vice versa. Low haemoglobin (*p* 0.00) and longer duration of DM (0.003) were independently and directly associated with prevalence of DR. **Conclusion:** Serum bilirubin concentration is inversely and independently associated and inversely correlated with the prevalence of DR and may predict progression of DR over time.

Keywords: Serum bilirubin; Diabetic retinopathy; Diabetes mellitus

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INTRODUCTION

Diabetes Mellitus (DM) claimed the lives of 4.9 million people around the globe as per International Diabetes Federation (IDF) report 2014. In 2014, 375 million people were living with DM and another 316 million with pre-diabetes. If bordered together all diabetics of the world can constitute the third most populated country of the world after China and India, a disease of such massive magnitude. One billion persons on earth will either suffer from DM or will have a high risk of developing DM by year 2035. Almost, 80% of the diabetic population lives in low to middle socio economic countries, having sub optimum health care facilities.[1]

Pakistan is currently ranked 7th among the countries with most diabetic patients by IDF, by the year 2025 it will take fourth place.[2,3] The rise in diabetic population in Pakistan to almost 14.5 million by 2025 is predicted.[2] About 10% of the adult population in Pakistan have DM while the other 10% have pre-diabetes.[4,5] DM is associated with 10–30% decrease in life expectancy and mainly due to the complications of DM, they die at an earlier age compared to non-diabetic population.[6,7]

Diabetic Retinopathy (DR) is the leading cause of blindness and visual impairment in the economically productive sub class of population in the developed world.[8] Prevalence of DR varies a lot among different parts of the world from as low as: 4.8% in Netherlands, 4% in Finland, 5% in Denmark, and rural France[9] to as high as: 17.6% in Chennai India[10] and 33.2% in certain areas of the United States[11]. Population based studies suggest that after 20 years, 70% DM patients will have some form of DR.[12]

Bilirubin once considered a potentially toxic by product of heme metabolism has been recognized as potentially beneficial substance over the past decades. Its effects are widely studied on the prevalence of DM as well as its effects on microvascular and macrovascular complications. The US National Health and Nutrition Examination Survey (NHANES) conducted on almost 16000 subjects showed that those with higher bilirubin levels had 20% less chance of developing DM. A large Korean study found serum bilirubin level was negatively co-related with DM prevalence and most components of metabolic syndrome. The markers of insulin resistance (HOMA-IR) and inflammation (CRP) were low in individuals with higher serum bilirubin level.[13] Another Korean study found that high bilirubin level have protective effects on the

prevalence of vascular complications in patients with Gilbert syndrome and DM.[14]

Total serum bilirubin concentration was found to be negatively and independently correlated with diabetic nephropathy (DN) and its progression in type-2 DM (T2DM) patients in Japan. It was also found that graded decrease in eGFR was associated with decrease in serum bilirubin levels.[15] The association was found to be independent and as strong as the other known factors of eGFR determination.[15] Higher serum bilirubin level was found to be an important predictor of cardiovascular disease in type-2 DM patients.[16]

Bilirubin level and its effects on DR are published recently. Serum bilirubin levels may have a protective role in patients with DR, independent of known risk factors for DR.[23] A study conducted in China showed that diabetic patients having higher serum bilirubin level have low prevalence of DR compared to those with lower levels.[17]

We hypothesized that the same association exists in our population as well. This study was designed to find association between total serum bilirubin concentration and DR, to identify serum bilirubin levels as an independent risk factor for DR in patients with T2DM and correlate the severity of bilirubin with the severity of DR.

MATERIAL AND MEHTODS

This case control study was conducted at the Department of Endocrinology, Diabetes, and Metabolic Diseases at Hayatabad Medical Complex, Peshawar from 1st March 2015 to 29, February 2016**.** Ethical Clarence was obtained from hospital ethical committee and participants were enrolled after their informed written consent. T2DM patients more than 18 years of age of either gender attending our out-patient department with duration of DM more than 6 months were included.

Patients were divided into two groups: Cases (DM with DR) and Controls (DM with no evidence of DR). DR was diagnosed on the basis of stereoscopic fundus photographic imaging by Japanese Canon digital retinal camera model K-1. DR was classified according to the International Clinical Diabetic Retinopathy and Diabetic Macular Oedema Disease Severity Scale into non-proliferative diabetic retinopathy (NPDR), which was further sub-categorized into mild, moderate and severe sub types and proliferative diabetic retinopathy (PDR). Consecutive patients were included. Those with liver disease (acute or chronic hepatitis, cirrhosis), haemolytic anaemia, history of chronic alcohol consumption, use of hepato-toxic drugs (anti-tuberculous, anti epileptic) and women on oral contraceptive pills were excluded.

All the patients underwent ophthalmic examination at Diabetic Retinopathy Screening Clinic (Endocrinology Department), by well trained and experienced optometrist under the direct supervision of researchers and finding were reconfirmed by consultant ophthalmologist. After pupil dilation with 1% Tropicamide (Mydriacyl) eye drop, screening for DR was done by direct ophthalmoscopy.

Blood was drawn for complete blood profile, serum total bilirubin level, complete blood count, random blood glucose level, serum creatinine level and HbA1c. Serum bilirubin level was measured using Hitachi 902 analyser and HbA1c was performed using standard DCCT certified and NGSP approved technique.

Data were analyzed using SPSS Version 20.0 and baseline characteristics of the participants like age and duration of DM were described as mean±SD. Frequencies and percentages were used to describe categorical variable like gender, types of DR. Student's *t*-test was applied to compare data among the two groups for numerical variables. The variables found statistically significant during univariate analysis were subjected to multiple logistic regression analysis to find the independent associations between the statistically significant variables. Spearmen's correlation coefficient was calculated to determine the bivariate relationship between total serum bilirubin concentration and progressive categories of NPDR and PDR. Participants were categorized to quartiles based on total serum bilirubin concentration (Quartile - Q1≤0.30; Q20.31–0.59; Q3 0.60–0.89; Q4 ≥0.9). Chi square test was used to see the comparison between the proportions. Kendall's tau- b test was applied to cross tabulate the sets of ordinal data. The p value <0.05 was considered statistically significant during all kind of statistical analysis.

RESULTS

A total of 152 participants, **cases** (n=76) and **controls** (n=76) were compared and analyzed. Demographic data and other data of both groups were compared (Table-1).

We found that duration of DM was longer in patients with DR (p 0.001) while their HLC-C and haemoglobin levels were significantly lower (p 0.003 and 0.000 respectively) compared to those without DR. Serum total bilirubin level was significantly lower in cases compared to controls (p 0.002) proving the inverse association among increasing bilirubin level and presence of DR in T2DM patients in univarite analysis. To analyse the independent association among these key risk factors and the DR, a multiple logistic regression analysis was carried out. Low haemoglobin level, longer duration of DM

and total serum bilirubin concentration were found significantly and independently associated with DR in T2DM. (Table-2)

Among the cases 70 patients had NPDR, 28 (%) mild, 20 (%) moderate, 22 (%) severe NPDR. Serum bilirubin level was inversely co related with DR with spearmen's correlation coefficient of r= -0.345 with a p value of 0.000 (NPDR) and r= -0.151 with a p value of 0.109 (PDR) in all 152 subjects.

Most of the controls (89.5%) were concentrated in 3rd quartile and highest quartile contained more control than cases. On the contrary cases were more concentrated in 2nd (31.6 %) and 3rd (63.2 %) quartiles and in the lowest quartile only had patients with DR (Table-3).

Table-1: Baseline characteristic of cases and controls (n=152)

		Case (n=76)	Control (n=76)	*p*-valve
Age		52.81±11.12	50.00±11.50	0.211
Gender	**Male**	34	32	
	Female	42	44	0.790
Duration of DM		13.50±7.42	8.11±9.44	0.001
HbA1c		10.53±2.08	10.00±3.15	0.288
Creatinine		1.17±0.61	1.34±1.38	0.373
SBP		133.37±18.95	131.05±15.11	0.513
DBP		84.34±10.27	81.57±7.54	0.144
LDL-C		111.42±27.26	102.53±31.56	0.122
HDL-C		31.76±10.49	38.78±13.19	0.003
Triglyceride		188.82±74.06	204.79±62.20	0.256
Total Cholesterol		176.42±45.40	178.63±40.41	0.800
Haemoglobin		11.13±197	12.99±1.80	0.000
Bilirubin		0.610±0.16	0.73±0.22	0.002
BMI		19.11±0.40	23.08±1.11	0.007
Hypertension		(42) 36.8%	(44) 19.3%	0.790
Cataract Surgery		8 (7.0%)	0 (0%)	0.038

Table-2: Association of total serum bilirubin with DR in T2DM (N=152) (Multivariate logistic regression analysis).

Parameter	Odd ratio	Confidence Interval	*p*-valve
Duration of DM	1.120	1.038-1.208	0.003
Serum bilirubin level	0.004	0.00-0.058	0.000
Haemoglobin	0.607	0.479-0.770	0.000
HDL-C	0.94	0.920-1.010	0.120

Table-3: Prevalence of diabetic retinopathy by quartiles of serum concentration of bilirubin.

	Quartiles of serum bilirubin				Total	*p*-valve
	< 0.30mg/dl	0.3-0.59mg/dl	0.60-0.89mg/dl	≥0.90mg/dl		
Cases (DM with DR)	2	24	48	2	76	
Control(DM without DR)	0	4	68	4	76	0.000
No NPDR	2	6	70	4	82	
Mild NPDR	0	4	24	0	28	
Moderate NPDR	0	2	16	2	20	
Severe NPDR	0	16	6	0	22	0.000
No PDR	0	26	114	6	146	
PDR	2	2	2	0	6	0.000

DISCUSSION

Chronic hyperglycaemia (glucotoxicity) is the core pathophysiological process involved in the initial development and progression of vascular complications in DM. Several mechanisms have been postulated for vascular endothelial damage including increased polyol pathway flux theory, advanced glycosylation end products (AGE), activation of protein kinase pathway (PKC) and widely studied oxidative stress damage theory.[18]

Bilirubin is considered a potent anti-oxidant generated as a result of heme catabolism. Heme oxygenase (HMOX) enzyme converts heme to biliverdin and then it is converted to bilirubin by enzyme biliverdin reductase. Bilirubin is conjugated with albumin in blood and taken up by liver and secreted in the bile in conjugation with glucoronic acid. Increased bilirubin production and/or decreased metabolism beyond physiologically considered normal upper limits and its harmful effects has been well recognized especially kernicterus. However, beneficial effects of higher but physiologically normal serum bilirubin levels are an area of ongoing research. Data show its beneficial role as an antioxidant and anti-inflammatory agent.[19] Its antioxidant properties were published in early 1950s.[20] Bilirubin is more potent than vitamin E

analogues and glutathione in preventing LDL and lipid oxidation respectively.[21,22] TNF mediated up-regulation of adhesion molecules and its effects are inhibited by bilirubin and is negatively correlated with CRP levels.[19,23] Recently the effects of bilirubin on regulatory T cell function has been explored and hence it may have a role in autoimmune conditions.[24]

In our study, the inverse relationship between serum total bilirubin concentration and DR, cases had lower serum bilirubin concentration compared to controls and this finding was statistically significant. After correcting for confounding factors in multivariate logistic regression analysis, lower serum bilirubin concentration was confirmed to be an independent risk factor for development of DR. Yasuda *et al*[17], reported inverse correlation between DR and serum bilirubin, similar to our findings. Cho *et al*[25] and Najam *et al*[26] also concluded that serum total bilirubin level was inversely and independently associated to DR.

Longer duration of DM, low haemoglobin levels, low HDL-C levels were also found significantly different among cases and controls, and thus predictors of DR in diabetic patients. In multivariate logistic regression analysis after adjusting for confounding variables duration of DM, low haemoglobin level and serum total bilirubin concentration found out to be independent risk factor of progression of DR. DM for longer duration and poorly controlled blood glucose level are well known independent risk factor for progression of DR.[25,8] Association of increasing age with DR prevalence revealed controversial results. We didn't find direct association of increasing age with progression of DR unlike others.[27] Low haemoglobin concentration has been studied and considered as independent risk factor for development of DR.[28]

We found that the prevalence of DR rises with increasing serum total bilirubin level and when correlated with progressive NPDR and PDR we found the inverse correlation of serum bilirubin concentration with DR. Patients without DR (controls) were concentrated in higher serum bilirubin quartiles (Q 3 and Q4) compared to patients with DR (cases), majority populated in lower quartiles (Q1 and Q2). Yasuda M *et al*[17] and Dave A *et al*[29] also suggested that higher total serum bilirubin level is inversely co related with DR in T2DM patients.

We found that in patient who progressed to severe cases of NPDR, serum bilirubin concentration was low and we hypothesize that serum bilirubin level may predict progression of NPDR over stages and progression to PDR.

Several limitations in our study may be discussed. Our study suggest association between DR and serum bilirubin level, similar to all other studies on this association which does not proves or suggests causal relationship. Similarly our study is based on single serum bilirubin measurement and fundi examination, an individual may have different levels of bilirubin though within normal limits over time, and a participant presently in highest quartile may reside in lowest quartile over time. Finally, limited sample size may be considered a limitation too though the rigour applied in conducting the study may be given due weightage while interpreting the results.

CONCLUSION

We conclude that higher total serum bilirubin level is inversely and independently associated with DR, and may predict the progression of DR over different stages of DR. This may not be causal association, and further long term studies are required before lowering serum bilirubin concentration can be considered as potential target to prevent development and/or delay progression of DR. We also conclude that low serum haemoglobin and longer duration of DM are directly and independently associated with prevalence of DR.

AUTHOR'S CONTRIBUTION

All the authors contributed equally.

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