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ROLE OF TOTAL SERUM BILIRUBIN AS PROTECTIVE FACTOR FOR DIABETIC RETINOPATHY

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ABSTRACT **Aim:** To determine the association of total serum bilirubin levels with diabetic retinopathy.
Methods and Material: This was a hospital-based case-control observational study conducted in outpatient department of tertiary care ophthalmic centre in North India. Detailed history and examination, demographic data and risk factors of two hundred enrolled type 2 diabetic patients were recorded. Patients were divided into two groups: Cases having diabetic retinopathy (DR) and Controls with no diabetic retinopathy (No DR). Total serum bilirubin levels were estimated and divided into quartiles. Data documented was analyzed using Chi-square test and independent student t-tests for equal or unequal variances, as appropriate.
Results: Diabetic retinopathy had significant association with lower total serum bilirubin.
Conclusions: Bilirubin is a protective factor of diabetic retinopathy.

KEYWORDS :: case-control study, diabetic retinopathy, bilirubin, protective factor, north india

INTRODUCTION

With nearly 72.9 million diabetics found in India, it presently ranks second globally after China. It is expected that it might cross 134.3 million mark by year 2045 which will make India the Diabetic capital of the world.[1] Diabetic retinopathy (DR) is commonest ocular complication of diabetes responsible for visual morbidity. In one of the centre based studies, Rema M et al found diabetic retinopathy to be prevalent in 34.1% of Type 2 diabetic subjects, out of which 30.8% subjects had Non Proliferative Diabetic Retinopathy (NPDR) and 3.4% had Proliferative diabetic retinopathy (PDR).[2]

In the past, bilirubin was regarded as a cytotoxic waste product that was excreted in bile. But, later it was found that in micromolar concentrations, bilirubin scavenges peroxyl radicals generated chemically in liposomes. With its antioxidant property, it acts as a cytoprotectant for the microvasculature and prevents microangiopathy.[3]

In addition to this, bilirubin also exerts anti-inflammatory effect by inhibiting inflammatory cytokine-induced endothelial cell expression of vascular cell adhesion molecules[4], prevention of the oxidation of low density lipoprotein[5] and inhibition of inflammation and dysfunction of endothelial cells[6].

The present study was conducted to find out any significant association between the levels of total serum bilirubin and the presence of Diabetic Retinopathy and to find if serum bilirubin can be regarded as an independent novel biomarker for diabetic retinopathy.

MATERIAL AND METHOD

This was a hospital-based, case-control study conducted at tertiary ophthalmic centre in North India, over a period of 24 months, from December 2016 to November 2018, after obtaining permission from the Institutional Ethics Committee. Two hundred, randomly-selected, Type 2 diabetic patients from the out-patient of department were recruited in the study after a written informed consent in accordance with the Declaration of Helsinki. Detailed history and demographic data of patient including age, gender, history of smoking, alcohol consumption, hepato-biliary disorders, duration of diabetes mellitus, hypertension or cardiovascular diseases were recorded in the proforma.

All patients underwent baseline ocular examination and fundus examination after dilating pupils with Tropicamide 0.8% w/v and using direct ophthalmoscope/ binocular indirect ophthalmoscope/ 90-D lens on slit- lamp biomicroscope. Based on presence or absence of retinopathy, patients were divided into 2 groups:

A. Cases (Diabetes Mellitus with Diabetic Retinopathy) (100 patients), B. Controls (Diabetes Mellitus without Diabetic Retinopathy) (100 patients).

All these participants were investigated for glycosylated hemoglobin (HbA1c), fasting blood sugar (FBS), Blood Urea, serum creatinine, aspartate transaminase (AST) and alanine transaminase (ALT). Total serum bilirubin (TSB) levels were determined in these patients by quantitative DCA (Dichloroaniline) method in which diagnostic kits, based on principle of diazo reaction, and a semi-automated analyzer was used.

Participants were also categorized into quartiles(Q) based on total serum bilirubin concentration into: (a) Q1: < 0.30mg/dL, (b) Q2: 0.31-0.59mg/dL, (c) Q3: 0.60-0.89mg/dL, (d) Q4: >0.9mg/dL.

Inclusion Criteria: Patients having Type 2 Diabetes Mellitus, 18 years of age and above irrespective of their sex.

Exclusion Criteria: Patients who had a history of chronic alcohol drinking, smoking, hepato-biliary abnormalities, chronic renal failure or End stage renal disease, having blood levels of Aspartate transaminase (AST) or Alanine transaminase (ALT) greater than three times the normal level, hypertensive patients with systolic blood Pressure >160Hg and diastolic >100mm Hg, pregnancy, hemolytic anemia, pathological myopia (axial length > 26.5 mm), drug history of oral-contraceptive pills, anti-tuberculous or anti-epileptic drugs.

STATISTICAL ANALYSIS

All parameters were tabulated and analysis was performed using Data analysis tool pack and XL Statistics add-in in Microsoft Excel 2007. Chi- square test was used to assess if the differences existed between the categorical variables between groups. Independent student t- test was used whenever parametric variables were compared between two groups. Significance was defined as p-value <0.05.

Table – 1
Demographic Profile And Risk Factors Among The Two Groups

Mean ± S.D.	Cases (DR) (n=100)	Controls (No DR) (n=100)	'p' value
Age (years)	57.39 ±8.82	55.23 ±10.52	0.117*
Male/ Female (M:F)	56/39 (1.27:1)	39/61 (2:3)	-
Duration (years)	10.37 ±6.00	5.71 ±4.71	<0.001†
HbA1c	7.67±1.53	7.54± 1.31	0.522*
TSB	0.53±0.26	0.78±0.24	<0.001†
FBS	157.97 ±55.06	143.98 ±39.93	0.041†
Blood Urea	32.61 ±11.36	31.11 ±8.56	0.292*

Serum Creatinine	1.02±0.31	0.97±0.26	0.21*
Systolic Blood Pressure	135.52 ±12.52	131.54 ±15.18	0.04†
Diastolic Blood Pressure	79.30 ±7.97	78.00 ±7.16	0.23*
Serum Cholesterol	192.57 ±40.29	193.12 ±36.54	0.92*
Serum Triglyceride	197.28 ±83.1	199.27 ±88.05	0.87*
Serum HDL	43.63 ±6.43	44.33 ±6.22	0.43*
Serum LDL	109.48 ±32.25	108.94 ±32.28	0.9*

* p>0.05; Statistically Non Significant using student t test
† (p<0.05; Statistically Significant using student t test
Abbreviations: DR-Diabetic retinopathy, FBS- Fasting Blood Sugar, HbA1C-Glycated Hemoglobin, HDL- High Densuty Lipoprotein, LDL- Low Density Lipoprotein, M:F- Male: Female ratio, NPDR-Non Proliferative Diabetic retinopathy, PDR- Proliferative Diabetic Retinopathy, S.D.-Standard Deviation, TSB- Total Serum Bilirubin.

Table – 2
Distribution Of Cases And Controls In Quartiles Of Bilirubin Concentration

Quartiles of total serum bilirubin	Cases (DR) (n=100)	Controls (No DR) (n=100)
<0.30	25	1
0.31-0.59	31	16
0.60-0.89	37	60
>0.9	7	23

p <0.001, Statistically significant using chi-square test

RESULTS

Two hundred patients, who fulfilled the eligibility criteria, were recruited in the study. The status of worse eye was taken into account for data analysis.

There were 100 cases- patients with diabetic retinopathy (DR) and age matched 100 controls- patients with no diabetic retinopathy (No DR). DR group had more males and No DR group had more females. There was no significant difference between the groups with respect to glycated hemoglobin (HbA1c), blood urea, serum creatinine, diastolic blood pressure, serum cholesterol, serum triglyceride, serum high density lipoprotein (HDL) and Serum low density lipoprotein (LDL). However, significant difference was found for duration of diabetes, fasting blood sugar (FBS), systolic blood pressure, and total serum bilirubin levels between the groups. [Table 1]

In our study, on dividing total serum bilirubin levels into quartiles, cases with diabetic retinopathy were more clustered in lower quartiles whereas higher quartiles had more patients with no diabetic retinopathy. [Table 2]

DISCUSSION

Vascular derangements in diabetic retinopathy have been attributed to: increased polyol pathway flux, increased formation of advanced glycation end products (AGE), protein kinase-C activation and increased oxidative stress.[7]

In any aerobic eukaryotic cell, molecular oxygen is sequentially reduced to water via a respiratory chain. During normal intermediary mechanism, reduction of oxygen leads to formation superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals, which initiate a chain reaction leading to lipid hydro-peroxide formation. These reactive oxygen species (ROS) damage other membrane bound enzymes as well as macromolecules.[3] In eye, retina cells owing to their high metabolic activity produce these potent, extremely toxic, free radicals at much larger amount than other cells.[8]

Each cell has both enzymatic and non-enzymatic antioxidant defenses. These include: superoxide dismutase, catalase, glutathione, vitamin E and C, and beta carotene.[3]

Chronic hyperglycemia is an important risk factor for development of diabetic retinopathy. The high extracellular glucose levels, toxic for the endothelium, enter retinal capillary endothelial cells using insulin independent glucose transport systems. Mitochondria metabolize this excessive intracellular glucose to produce free radical reactive oxygen species (ROS). This generates many harmful byproducts that damage endothelial cells, surrounding mural cells, and the pericytes resulting in leakage of capillaries and loss of local control the retinal blood flow. This result in protein and lipid exudation, fluid transudation, increased endothelial sheer stress, capillary closure and retinal hypoxia.[9,10]

Oxidative stress occurs due to disruption of various vital processes in the homeostasis of free radical production, such as the electron transport chain reaction and the scavenging mechanisms designed to neutralize these damaging molecules, because of chronic hyperglycemia.[11] It results into metabolic abnormalities that are linked to the structural and functional changes in the vasculature leading to atherosclerotic and diabetic retinopathy changes. Oxidative stress is also held responsible for alterations in downstream transcription factors which result in change of gene expression and endothelial function.[12]

Bilirubin, owing to its weakly polar nature and poor solubility, is transported in blood tightly bound covalently to albumin. Unbound bilirubin, accounting for less than 0.01% of the total circulating bilirubin, regulates the diffusion of unconjugated bilirubin into tissues, thus, determining beneficial and toxic effects.[8] Bound bilirubin in circulation is taken up by the liver, conjugated with glucuronic acid and then secreted in bile as bile pigments.[13]

Other known significant risk factors found in various studies for development of diabetic retinopathy are longer duration since diagnosis[14], bad glycemic control with HbA1c above 7% [9], systemic hypertension [10], derangement of lipid profile [15], heavy alcohol intake[16], and microalbuminuria. [17]

It was observed that bilirubin levels were significantly lower in patients with diabetic retinopathy compared to patients with no diabetic retinopathy. This result was supported by the results of hospital based study conducted on 102 type 2 diabetic patients by Cho Hc[18].

Ghaffar T et al[19] reported mean total serum bilirubin to be 0.61±0.16 mg/dL in case group and 0.73±0.22 mg/dL in control group. The difference between the two was found to be statistically significant. Similar results were obtained in studies conducted by Zhang D et al[20], Prabhavathi K et al[21], thus, underscoring the protective role of bilirubin in diabetic retinopathy.

Karuppannasamy D et al[22] conducted a study on 86 type 2 South Indian diabetic patients and divided serum bilirubin into quartiles. They found cases clustered more in lower quartiles compared to controls and higher quartiles having significantly more number of controls. These results were similar to those obtained in our study.

Contrary to our study, another study conducted by Shang X et al[23] found no significant association between total serum bilirubin levels and severity of changes in retina of patients with diabetes.

In a study conducted by Shaikh S et al[24] it was found that even if the confounding factor 'duration of diabetes' is kept same in both case and control groups, total serum bilirubin was still significantly lower in patients with diabetic retinopathy than patients with no diabetic retinopathy.

LIMITATIONS

First, due to hospital based study design, the subjects were collected from ophthalmic clinic; hence, there may be selection bias in data collection. Also, due to this our results may not be extrapolated to general population. Secondly, the correlation between total serum bilirubin levels and severity of diabetic retinopathy may not be a causal association because of cross-sectional nature of study. Therefore, long term prospective studies are needed before considering increasing total serum bilirubin as a potential target to delay development or progression of diabetic retinopathy. Thirdly, total serum bilirubin concentration was measured only once and thus, does not reflect fluctuation and mean levels.

CONCLUSIONS

Higher total serum bilirubin level is independently and inversely associated with diabetic retinopathy and also its severity. This has led us to hypothesize that bilirubin is one of the protective factors in cases

of diabetic retinopathy. The higher concentrations of bilirubinbut within physiological limits may act as a reserve anti-oxidant to protect microvasculature against oxidative damage. Once these reserves get used up and bilirubin falls, retinopathy may progress rapidly. Therefore, it could be considered as a potential biomarker of risk of diabetic retinopathy. Also, total serum bilirubin is an inexpensive test that is performed as routine blood test in many hospitals. This may be particularly useful, along with other biomarkers and investigations in those type 2 diabetic patients presenting with mature or hard bilateral cataract where it is impossible to visualize retina using direct or indirect ophthalmoscope.

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