Sree Lekshmi R. S. et al /J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2805-2807

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Association of Serum Total Bilirubin Level with Diabetic Retinopathy in Type 2 Diabetes Mellitus

Sree Lekshmi R. S.[1], *P. Shanmugasundaram[2]

[1]Pharm. D. (student),School of Pharmaceutical Sciences,Vels Institute of Science, Technology and Advanced Studies,Chennai.
[2]Director, School of Pharmaceutical Sciences,Vels Institute of Science, Technology and Advanced Studies,Chennai.

Abstract

Aim and Objective: Oxidative stress holds clinically a major role in the pathogenesis of Diabetic Retinopathy (DR) and serum bilirubin has been shown to have antioxidant properties. It is considered as a protective substance against atherosclerotic and microvascular complications of Diabetes Mellitus. This study was formulated to find the association between total serum bilirubin concentration and diabetic retinopathy (DR).

Methods: This case control study was conducted in the Department of Ophthalmology and Department of General Medicine of a tertiary care hospital. 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.

Results: A total of 136 patients, 68 cases and 68 controls were included. Serum bilirubin concentration was found inversely and independently (p 0.000) associated and inversely co related (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), HbA1C (p=0.016) and longer duration of DM (p=0.012) 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.

INTRODUCTION

As per International Diabetes Federation (IDF) report 2014, 375 million people were living with DM and another 316 million with pre-diabetes. Diabetes Mellitus (DM) claimed the lives of 4.9 million people around the globe. India leads the world in terms of its diabetic population and estimates have revealed that there are 62.4 million people with diabetes, and 77.2 million with prediabetes in India [1]. It is predicted that by 2030, DM may affect up to 79.4 million in India [2]. Due to these sheer numbers, the socioeconomic burden as a result of diabetes in India is among the highest in the world [3].

In the economically productive sub class of population in the developed world, Diabetic Retinopathy (DR) is the prime root of blindness and visual impairment [4] Prevalence of DR varies a lot among different parts of the world from as low as: 4.01% in Finland, 5.05% in Denmark, 4.81% in Netherlands and rural France[5] to as high as: 17.61% in Chennai

India [6] and 33.25% in certain areas of the United States [7]. After 20 years, population based studies suggest that 70% DM patients will have some form of DR.[8]

In the pathogenesis of DR, Oxidative stress is appraised to be one of the key factors [9]. Because of its high energy demands and exposure to light, retina is especially more susceptible to oxidative damage [10]. Various mechanisms presenting to the pathogenesis of DR such as inflammation, polyol pathway, accumulation of Advanced Glycation End Products (AGEs), flux of hexosamine Pathway and Protein Kinase C (PKC) activation are associated with overproduction of Reactive Oxygen Species (ROS) by the mitochondria [11].

Although, bilirubin has long been reviewed as a toxic waste product of heme catabolism, its role as a prospective endogenous anti-oxidant under physiological conditions is being increasingly acknowledged [10]. Bilirubin is also said to have anti-inflammatory effects, apart from its ability to move stealthily over produced ROS [12].

Several population-based studies have reported a negative association between serum bilirubin concentrations and the risk of diabetes [14], coronary artery disease [15], chronic kidney disease [16], peripheral arterial disease [17] and diabetic peripheral neuropathy [18] based on these physiological effects of bilirubin.

Bilirubin level and its effects on DR are published currently. In patients with DR, Serum bilirubin levels may have a protective role and independent of known risk factors for DR.[19] In China, a study demonstrated that compared to those with lower levels, diabetic patients having higher serum bilirubin level have low prevalence of DR.[20] We hypothesized that the same association exists in our study population as well. This study was sketched 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 in the Department of Ophthalmology and Department of General Medicine of a tertiary care hospital. 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 outpatient 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. 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 subcategorized 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 (antituberculous, anti epileptic) and women on oral contraceptive pills were excluded.

Under the direct supervision of researchers, all the patients were subjected to ophthalmic examination by well trained and experienced optometrist and finding were revalidated by consultant ophthalmologist. screening for DR was done by direct ophthalmoscopy after pupil dilation with 1% Tropicamide (Mydriacyl) eye drop.

For complete blood profile, serum total bilirubin level, complete blood count, random blood glucose level, serum creatinine level and HbA1c, blood was drawn. Serum bilirubin level was measured and HbA1c was performed.

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≤0.40; Q20.41–0.51; Q3 0.51–0.60; Q4 ≥0.61). The *p* value <0.05 was considered statistically significant during all kind of statistical analysis.

RESULTS

A total of 136 participants, cases (n=68) and controls (n=68) 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* 0.002)

Serum total bilirubin level was significantly lower in cases compared to controls (*p* 0.019) 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 34 patients had NPDR, 11 (%) mild, 16 (%) moderate, 7 (%) severe NPDR. Most of the controls (35.30%) were concentrated in 3rd quartile and highest quartile contained more control than cases. On the contrary cases were more concentrated in 1st (36.76%) and 2nd (29.41 %) quartiles and in the lowest quartile only had patients with DR (Table-3).

Table-1: Baseline characteristic of cases and controls (n=136)

CHARACTERISTICS	GROUP A	GROUP B	P VALUE
Age	52.6±10.6	50.6±12.2	0.901
Males	39	36	0.293
Females	29	32	
Duration of Diabetes	12.10±3.2	8.06±2.6	0.002
HbA1c	9.62±2.02	10.61±2.10	0.281
Creatinine	1.16±0.32	1.20±0.62	0.954
SBP	134.7±20.62	131.05±16.20	0.314
DBP	89.6±10.32	84.6±11.17	0.310
FBS	194.2±26.12	186.24±1.26	0.210
LDL	110.42±24.16	104.6±10.12	0.098
HDL	30.6±10.49	37.6±12.19	0.021
TG	186.2±21.42	168±3.21	0.072
TC	146.42±6.20	164±5.20	0.121
Hb	11.13±1.97	10.6±1.62	0.201
cataract	6	0	-
bilirubin	0.609±0.06	0.759±0.02	0.019

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

Risk factor	OR	95% CI	p-value
Age	1.03	0.96-1.111	0.386
Duration of Diabetes	2.05	1.172-3.592	0.012 *
HbA1c	2.42	1.177-4.984	0.016 *
Serum T.Bilirubin	0.001	0.000- 0.054	0.001*
HDL-C	0.96	0.920-1.010	0.116

* p-value ≤0.05

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

	Quartiles of Serum Total Bilirubin					
	< 0.40	**0.41-0.51**	**0.51-0.60**	**>0.61**	**TOTAL**	**p-value**
Controls	12	14	24	18	68	0.010
No DR	2	5	4	9	20	
Mild DR	3	4	2	2	11	
Moderate DR	6	6	2	2	16	
Severe DR	5	1	0	1	7	
PDR	9	4	1	0	14	

DISCUSSION

In this study, the concentration of serum bilirubin was equated negatively with the severity of retinopathy in patients with Type 2 DM. Multiple regression analysis also revealed serum total bilirubin as an independent risk factor associated with DR. When the enzyme Heme Oxygenase (HO) catalyzes the degradation of heme, bilirubin is generated. This leads to the emergence of biliverdin which is transformed to bilirubin by biliverdin reductase.Unconjugated (lipid-soluble) bilirubin is conjugated by uridine diphosphate glucuronyl transferase (UDP-GT) to a water soluble form for excretion in the hepatocytes. In healthy individuals, total bilirubin is the sum of unconjugated (indirect) and conjugated (direct) bilirubin and generally ranges from 0.2 to 1.2 mg/dl. Until now bilirubin was onsidered as a physiological antioxidant. Several clinical studies that is now being evaluated is showing possible associations between serum bilirubin and the risk of diabetes (and its complications) which has been published [21,22].

In this study, we noted that Type 2 diabetics with retinopathy had significantly lower serum bilirubin concentrations than those without retinopathy. Our observations are similar to that of epidemiologic and hospital based studies elsewhere which have suggested an inverse relation between serum total bilirubin and DR [20,23,24].

Longer duration of Diabetes Mellitus, low Hb levels, low HDL-C levels were also found significantly varying among cases and controls, and thus predictors of DR in diabetic patients. Duration of DM, low haemoglobin level and serum total bilirubin concentration found out to be independent risk factor of progression of DR in multivariate logistic regression analysis after adjusting for confounding variables. DM for longer duration and poorly managed blood glucose level are popularly liberated risk factors for the evolution of DR.[4,23]

Association of increasing age with DR prevalence revealed controversial results. We didn't find direct association of increasing age with advancement of DR unlike others.[25]

Low haemoglobin concentration has been studied and considered as independent risk factor for promotion of DR.[26]

We found that the prevalence of DR rises with increasing serum total bilirubin level and when
correlated with progressive NPDR and PDR. 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*[20] and Dave A *et al*[27] also suggested that higher total serum bilirubin level is inversely co related with DR in T2DM patients.
We found that in patient who advanced 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. Our study has some limitations. First, our sample size was limited; second, we performed only a single measurement of serum bilirubin which might have within subject variation. However, all subjects including controls were examined after overnight fast.

CONCLUSION

In conclusion, our cross-sectional study suggested that low total serum bilirubin level was independently associated with higher risk of DR in patients with type 2 diabetes, regardless of other conventional risk factors. Further, prospective studies on large samples are required to better assess the effects of bilirubin on DR.

ACKNOWLEDGEMENTS

The authors would like to thank School of Pharmaceutical Sciences, Vels University for granting us necessary permission for the study. The authors also wishes to express their appreciation to the management of Tertiary care hospital for providing the enabled environment that made this research successful.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

RFERENCES

1. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R, et al. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical Research India Diabetes (ICMR INDIAB) study. Diabetologia. 2011; 54:3022-27.
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
3. Joshi SR, Das AK, Vijay VJ, Mohan V. Challenges in diabetes care in India: sheer numbers, lack of awareness and inadequate control. J Assoc Physicians India. 2008;56:443–50.
4. Marozas LM, Fort PE. Diabetic retinopathy-update on prevention techniques, present therapies, and new leads. US Ophthalmic Rev 2014;7(1):54–8.
5. Sivaprasad S, Gupta B, Crosby-Nwaobi R, Evans J. Prevalence of diabetic retinopathy in various ethnic groups: A Worldwide Perspective. Surv Ophthalmol 2012;57(4):347– 70.
6. Rema M, Premkumar S, Anitha B, Deepa R, Pradeepa R, Mohan V. Prevalence of diabetic retinopathy in urban India: the Chennai Urban Rural Epidemiology Study (CURES) Eye Study, Invest Ophthalmol Vis Sci 2005;46(7):2328–33.
7. Wong TY, Klein R, Islam FM, Cotch MF, Folsom AR, Klein BE, *et al*. Diabetic retinopathy in a multi-ethnic cohort in the United States. Am J Ophthalmol 2006;141(3):446–55.
8. Diabetic Retinopathy - Asia Pacific - American Academy of Ophthalmology [Internet]. [cited 2014 Sep 21]. Available from: http://www.aao.org/topic-detail/diabetic-retinopathy--Madsen-Bouterse SA, Kowluru RA.
9. Oxidative stress and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord. 2008;9:315-27.
10. Kumari S, Panda S, Mangaraj M, Mandal MK, Mahapatra PC. Plasma MDA and antioxidant vitamins in diabetic retinopathy. Indian J Clin Biochem. 2008;23:158-62.
11. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615-25.
12. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. Science. 1987;235:1043–46.
13. Vítek L, Schwertner HA. The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem. 2007;43:1-57.
14. Cheriyath P, Gorrepati VS, Peters I, Nookala V, Murphy ME, Srouji N, et al. High total bilirubin as a protective factor for Diabetes Mellitus: An analysis of NHANES Data From 1999 - 2006. J Clin Med Res. 2010;2:201-06.
15. Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem. 1994;40:18- 23.
16. Tanaka M, Fukui M, Okada H, Senmaru T, Asano M, Akabame S, et al. Low serum bilirubin concentration is a predictor of chronic kidney disease. Atherosclerosis. 2014;234:421-25.
17. Perlstein TS, Pande RL, Beckman JA, Creager MA. Serum total bilirubin level and prevalent lower extremity peripheral arterial disease: National health and nutrition examination survey (NHANES) 1999 to 2004. Arterioscler Thromb Vasc Biol. 2008;28:166-72.
18. Kim ES, Lee SW, Mo EY, Moon SD, Han JH. Inverse association between serum total bilirubin levels and diabetic peripheral neuropathy in patients with type 2 diabetes. Endocrine. 2015;50:405-12.
19. Mazzone GL, Rigato I, Ostrow JD, Bossi F, Bortoluzzi A, Sukowati CH, *et al*. Bilirubin inhibit the TNFalpha-related induction of three endothelial adhesion molecules. Biochem Biophys Res Commun 2009;386(2):338–44
20. Yasuda M, Kiyohara Y, Wang JJ, Arakawa S, Yonemoto K, Doi Y, *et al*. High serum bilirubin levels and diabetic retinopathy: the Hisayama Study. Ophthalmology 2011;118(7):1423–8.
21. Han SS, Na KY, Chae DW, Kim YS, Kim S, Chin HJ, et al. High serum bilirubin is associated with the reduced risk of diabetes mellitus and diabetic nephropathy. Tohoku J Exp Med. 2010;221:133-40.
22. Jung CH, Lee MJ, Kang YM, Hwang JY, Jang JE, Leem J, et al. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: A 4 year retrospective longitudinal study. Metabolism. 2014;63:87- 93.
23. Cho HC. The relationship among Homocysteine, Bilirubin, and diabetic retinopathy. Diabetes Metab J. 2011;35:595-601.
24. Sekioka R, Tanaka M, Nishimura T, Itoh H. Serum total bilirubin concentration is negatively associated with increasing severity of retinopathy in patients with type 2 diabetes mellitus. J Diabetes Complications. 2015;29:218-21.
25. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin Epidemiologic Study of Diabetic Retinopathy II. Prevalence and Risk of Diabetic Retinopathy When Age at Diagnosis is Less Than 30 Years. Arch Ophthalmol 1984;102(4):520–26.
26. Qiao Q, Keinänen-Kiukaanniemi S, Läärä E. Relationship between hemoglobin levels and diabetic retinopathy. J Clin Epidemiol 1997;50(2):153–8.
27. Dave A, Kalra P, Gowda BHR, Krishnaswamy M, Association of Bilirubin and Malondiaaldehyde levels with retinopathy in type 2 Diabetes Mellitus. Indian J Endocrinol Metab 20 15;19(3):373–7.