ORIGINAL ARTICLE

SERUM BILIRUBIN AND ANTIOXIDANT LEVELS IN FIRST DEGREE RELATIVES OF PATIENTS WITH ISCHEMIC HEART DISEASE AND NORMAL SUBJECTS

Nadia Mahmood, Tahira Naseem, Farhana Mukhtar, Rubina Basheer*

Department of Pathology CMH and Sheikh Zayed Hospital Lahore,
***Department of Biochemistry, Lahore Medical & Dental College, Lahore, Pakistan**

Background: Coronary diseases appear to result from an overbalance between radical-generating, compared with radical-scavenging systems, a condition called as oxidative stress. Total antioxidant status (TAS) in human plasma reflects the balance between oxidants and antioxidants in each system. Bilirubin has been considered an antioxidant, with capacity to remove reactive species of oxygen. Present study tried to measure the total antioxidant status of first degree relatives of patients with IHD. Study also tried to evaluate the prognostic role of serum bilirubin in disease prevention or progression. **Methods:** Seventy five apparently healthy subjects in age group 20–50 years, comprising equal number of males and females, who were first degree relatives of ischemic heart disease patients, were included in the study. Family members were divided on the bases of their numbers, i.e., one family member (Group-A), 2 family members (Group-B) and more than 3 family members (Group-C). Study was cross sectional and carried out in a period of 6 months (Jun 2008–Jan 2009). Subjects with letter of consent were taken from general population. Seventy five healthy age matched people with no history of ischemic heart disease in family were taken as control. An overnight fasting blood sample was taken. Total antioxidant status was determined using a commercially available kit. Serum bilirubin was estimated by auto analyzer. **Results:** Family history of ischemic heart disease with serum bilirubin showed a significant negative correlation ($p<0.05$). But the values of TAS failed to show any significant correlation with the family history. It was observed that the value of serum bilirubin was decreased significantly ($p<0.05$) with an increased number of family members. Total antioxidant status failed to show any significant difference among all the three groups. **Conclusion:** Our data demonstrated that reduced serum levels of bilirubin were seen in people with a higher prevalence of coronary artery disease in the family. The levels of serum bilirubin showed a downward trend with an increase in number of family members affected with ischemic heart disease. Present study failed to show a definite association of total antioxidant status with family history of ischemic heart disease. Additional studies are still necessary on large number of first degree relatives to confirm and demonstrate the association of these findings with clinical outcomes.

Keywords: Ischemic heart disease, first degree relatives, total antioxidant status and bilirubin

INTRODUCTION

Ischemic heart disease (IHD), is a disease characterized by ischemia of the heart muscle, usually due to coronary artery disease. It is the leading cause of death in most population of the world. From 1990–2020 deaths due to cardiovascular diseases (CVD) is expected to rise from 28.4–33.4%.[1]

Its risk increases due to some non-modifiable risk factors which are age, sex, family history of IHD and serum bilirubin levels. IHD in male relatives with onset at age ≤55 years or female relative with onset at age of ≤65 is considered as positive family history, the larger the number of relatives with early onset of IHD or the younger the age of IHD onset in the relative, the stronger the predictive value.[2]

Coronary diseases appear to result from an overbalance between radical-generating, compared with radical-scavenging systems, a condition called oxidative stress.[3] Reactive oxygen species (ROS) can damage endothelial cells in many ways, either directly orindirectly.[4] They can also increase endothelial permeability and thereby accelerate the accumulation of athrogenic factors, such as LDL, in the sub endothelial cell space.[5]

Total antioxidant status (TAS) in human plasma reflects the balance between oxidants and antioxidants in each system. Oxidative stress has recently been implicated in the pathogenesis of various diseases such as diabetes and coronary artery disease.[6,7] The TAS is a complex trait reflecting homeostasis of redox metabolism, which is highly relevant to pathological processes, including aging, atherosclerosis etc. It is affected by the relative contributions of each antioxidant species and the stress of oxidative free radicals. The measure of TAS does represent a continuous spectrum in the function of antioxidants and free radicals in plasma. For a given level of antioxidants in plasma, increased production of ROS will result in a reduced TAS level.[8]

Total serum bilirubin has a strong relationship with coronary artery lesion types.[9] It has a protective effect on CVD and CVD related diseases.[10] Bilirubin has

been considered an antioxidant, with capacity to remove reactive species of oxygen. Studies have suggested that an increased bilirubin level promotes protection against atherosclerosis. It inhibits the inflammatory response and the proliferation of vascular smooth muscle cells.[11,12] Bilirubin, once considered simply the metabolic end product of haem degradation, has emerged as a potential endogenous inhibitor of atherosclerosis.[13] Reduced serum levels of bilirubin were shown to be associated with a higher prevalence of coronary artery disease emerging as a new potential risk factor marker.[14]

Study hypothesizes that oxidative stress and its subsequent pathological processes are under significant genetic regulation. In this context, present study measured the total antioxidant status of first degree relatives of patients with IHD. Study was also tried to evaluate the prognostic role of serum bilirubin in disease prevention or progression.

MATERIAL AND METHODS

Seventy five apparently healthy subjects in age group 20–50 years, comprising equal number of males and females, who were first degree relatives of ischemic heart disease patients, were included in the study. Family members were divided on the bases of their numbers, i.e., one family member (Group-A), 2 family members (Group-B) and >3 family members (Group-C). Study was cross sectional and carried out in a period of 6 months. Subjects were taken from general population. Seventy five healthy people with no history of ischemic heart disease in family, matching age and gender were also studied as a control group. An inclusion criterion was the first degree relatives patients with IHD. Patients with diabetes, known hypertension, smoking, obesity and abnormal lipid profile were excluded from the study. An over night fasted blood samples were taken. The plasma was collected and an aliquot was refrigerated for total antioxidant status using a commercially available TAS manual kit of Randox Laboratories UK as a quantitative measure of circulating antioxidant status. Subjects gave written informed consent.

RESULTS

Pearson's coefficient of correlation was applied to determine the relationship of family history with serum bilirubin and serum total antioxidant status. Family history of ischemic heart disease with serum bilirubin showed a significant negative correlation ($p<0.05$). But the values of TAS failed to show any significant correlation with the family history (Table-1).

First degree relatives were divided into 3 groups. Group A with history of 1 family member affected from IHD. Group B with history of 2 family members affected and group C with history of more than 2 members in family suffering from IHD. It was observed that the values of serum bilirubin was decreased significantly ($p<0.05$) with an increased number of family members (Group-A vs Group-B and C). Total antioxidant status failed to show any significant difference among all the three groups.

Table-1: Pearson's coefficient of correlation for family history of ischemic heart disease with serum bilirubin and serum total antioxidants

Groups	History of IHD vs serum bilirubin	History of IHD vs total antioxidants
Pearson's Correlation	-0.243*	-0.10
Significance 2-tailed	0.034	0.931

*$p<0.05$= Significant

Table-2: Descriptive analysis of serum bilirubin and total antioxidant status with the incidence of family history of ischemic heart disease (Mean±SD)

	Group A (I Family Member)	Group B (2 Family Members)	Group C (>2 Family Members)
No. of Cases	37	32	7
Serum Bilirubin	0.583±0.287*	0.444±0.162	0.467±0.197
TAS	1.409±0.225	1.349±0.220	1.475±0.181

*$p<0.05$= Significant

DISCUSSION

Ischemic heart disease (IHD) is becoming a major health problem world wide. This disease seems to follow an accelerated course with ischemic events occurring a decade earlier in Pakistani population as compared to those reported from developed countries.[15]

Present study tried to find out the correlation between family histories of IHD with serum bilirubin. These parameters were correlated by using Pearsons correlation formula which found a negative correlation of serum bilirubin with family history. Our results are in accord with a study that serum bilirubin has a protective effect on CVD and CVD-related diseases, and UGT1A1 is the major gene controlling serum bilirubin concentrations.[10] Two studies involving the offspring and spouses of the original Framingham cohort evaluated the relationship of bilirubin and the risk of myocardial infarction in >5000 participants.[16,17] Studies reported that higher bilirubin levels, ~0.9 mg/dL, were associated with lowered risk of myocardial infarction and other cardiovascular disease events compared with individuals with lower bilirubin levels of 0.5 mg/dL. Another study have also found lower bilirubin levels associated with cigarette smoking, adiposity, and a family history of myocardial infarction.[18] A study proved that circulating bilirubin may function most predominantly in vascular disease.[19] It is stated that higher serum total bilirubin inhibits the inflammatory response and the proliferation of vascular smooth muscle cells.[11]

Oxidative stress plays a critical role in the development of ischemia/reperfusion injury.[20] It is reported that decrease in TAS could be due to increased free radical production causing oxidative stress.[21]

Present study observed that the values of TAS failed to show a significant correlation with IHD. These findings were consistent with previous studies like the study done in Andhra Pradesh India in 2004, failed to show any correlation in TAS and family history of IHD in South Indian population.[9] However a study stated, that oxidative stress should be included as important markers in the early detection of acute coronary syndrome.[22] The reason of insignificant correlation between oxidative stress and IHD is explained by number of studies. These studies reported that increased values of TAS is usually observed in those first degree relatives who shave shown a strong history of smoking habits, alcohol use, sedentary lifestyle, exercise, and dietary fat intake as well as disease of hypertension and diabetes.[7,23–25] A study stated that an increased oxidative stress and vascular inflammation have been observed in patients with cardiac syndrome X (CSX).[13]

CONCLUSION

Our data demonstrated that reduced serum levels of bilirubin were shown to be associated with a higher prevalence of coronary artery disease emerging as a new potential risk factor marker. The levels of serum bilirubin showed a downward trend with an increase in number of family members affected with ischemic heart disease. Present study failed to show a definite association of total antioxidant status with family history of ischemic heart disease. Additional studies are still necessary to confirm and demonstrate the association of these findings with clinical outcomes.

LIMITATIONS

Study was carried out on small number of subjects.

REFERENCES

1. Paffenbarger RS Jr, Hyde RT, Wing AL, Lee IM, Jung DL, Kampert JB. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. N Engl J Med 1993;328:538–45.
2. Stewart MW, Humphriss DB, Mitcheson J, Webster J, Walker M, Laker MF. Lipoprotein composition and serum apolipoproteins in normoglycaemic first degree relations of non insulin dependent diabetic patients. Atherosclerosis 1998;139:115–21.
3. Fukagawa NK. Aging: is oxidative stress a marker or is it causal? Proc Soc Exp Biol Med 1999;222:293–8.
4. Harrison DG. Endothelial function and oxidant stress. Clin Cardiol 1997;20(11 Supp 2):II-11–7.
5. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, *et al*. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. J Clin Invest 1993;92:1866–74.
6. Alho H, Leinonen J. Total antioxidant activity measured by chemiluminescence methods. Methods Enzymol 1999;299:3–15.
7. Trapani L, Segatto M, Incerpi S, Pallottini V. 3-Hydroxy 3-Methylglutaryl Coenzyme A Reductase Regulation by Antioxidant Compounds: New Therapeutic Tools for Hypercholesterolemia? Curr Mol Med 2011;11:790–7.
8. Wang XL, Rainwater DL, Vande Berg JF, Mitchell BD, Mahaney MC. Genetic contribution to plasma antioxidant activity. Arterioscler Thromb Vasc Biol 2001;21:1190–5.
9. Wei S, Gao C, Wei G, Chen Y, Zhong L, Li X. The level of serum bilirubin associated with coronary lesion types in patients with coronary artery disease. J Cardiovasc Med (Hagerstown) 2011;27: [Epub ahead of print]
10. Lin JP, Vitek L, Schwertner HA. Serum bilirubin and genes controlling bilirubin concentrations as biomarkers for cardiovascular disease. Clin Chem 2010;56:1535–43.
11. Kuwano T, Miura S, Shirai K, Ike A, Mori K, Shimizu T, *et al*. Serum levels of bilirubin as an independent predictor of coronary in-stentrestenosis: a new look at an old molecule. J Atheroscler Thromb 2011;18:574–83.
12. Yoshino S, Hamasaki S, Ishida S, Kataoka T, Yoshikawa A, Oketani N, *et al*. Relationship between bilirubin concentration, coronary endothelial function, and inflammatory stress in overweight patients. J Atheroscler Thromb 2011;18:403–12.
13. Huang SS, Huang PH, Leu HB, Wu TC, Lin SJ, Chen JW. Serum bilirubin predicts long-term clinical outcomes in patients with cardiac syndrome X. Heart 2010;96:1227–32.
14. Ghem C, Sarmento-Leite RE, de Quadros AS, Rossetto S, Gottschall CA. Serum bilirubin concentration in patients with an established coronary artery disease. Int Heart J 2010;51(2):86–91.
15. Gul R, Anjum T, Yasmin R, Ijaz RM, Saleem M. Serum total antioxidant status in cardiovascular patients and in healthy individuals. Pak J Biochem Mol Biol 2010;43(3):140–3.
16. Djousse L, Rothman KJ, Cupples LA, Levy D, Ellison RC. Effect of serum albumin and bilirubin on the risk of myocardial infarction (the Framingham Offspring Study). Am J Cardiol 2003;91:485–8.
17. Djousse L, Levy D, Cupples LA, Evans JC, D' Agostino RB, Ellison RC. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. Am J Cardiol 2001;87:1196–200.
18. Madhavan M, Wattigney WA, Srinivasan SR, Berenson GS. Serum bilirubin distribution and its relation to cardiovascular risk in children and young adults. Atherosclerosis 1997;131:107–13.
19. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 3rd ed. Oxford UK: Oxford University Press; 1999.
20. Wang X, Yu Y, Ji L, Liang X, Zhang T, Hai CX. Alpha-lipoic acid protects against myocardial ischemia/reperfusion injury via multiple target effects. Food Chem Toxicol 2011;49:2750–7.
21. Olisekodiaka MJ, Igbeneghu CA, Onuegbu AJ, Oduru R, Lawal AO. Lipid, Lipoproteins, Total Antioxidant Status and Organ Changes in Rats Administered High Doses of Cadmium Chloride. Med Princ Pract 2011. [Epub ahead of print]
22. Gururajan P, Gurumurthy P, Nayar P, Chockalingam M, Bhuvaneshwari S, Babu S, *et al*. Lipid profile and non-enzymic antioxidant status in patients with acute coronary syndrome in South India. Heart Lung Circ 2010;19(2):75–80.
23. Higgins M. Epidemiology and prevention of coronary heart disease in families. Am J Med 2000;108:387–95.
24. Centers for Disease Control (CDC). The Surgeon General's 1989 Report on Reducing the Health Consequences of Smoking: 25 Years of Progress. MMWR Morb Mortal Wkly Rep 1989;38(Suppl 2):1–32.
25. Potter AS, Foroudi S, Stamatikos A, Patil BS, Deyhim F. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. Nutr J 2011;10:96.

Address for Correspondence:

Dr. Nadia Mahmood, Department of Pathology Combined Military Hospital, Lahore, Pakistan. **Cell:** +92-300-4325114
Email: nadia_ather@hotmail.com