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Correlation of Serum Bilirubin with Inflammatory Marker hsCRP in Metabolic Syndrome Disorder

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Abstract: *In India the incidence and prevalence of metabolic syndrome (MS) are on the rise as suggested by various surveys [1]. Different cross sectional and longitudinal studies revealed that MS is strongly associated with inflammation [2-4]. Increased proinflammatory cytokines are seen in patients of MS. The increases in proinflammatory cytokines, including Interleukin-1 (IL-1), Interleukin-6(IL-6), Interleukin-18(IL-18), tumour necrosis factor (TNF), and C-reactive protein (CRP), gamma interferon (IFN gamma) reflect overproduction by the expanded adipose tissue mass. Among them, CRP is strongly associated with Insulin resistance or metabolic syndrome [5-9]. For years, bilirubin has been recognized as a powerful antioxidant in the human body. However no studies could be found in India describing protective role of bilirubin on MS. Accordingly, we carried out the present study to evaluate a correlation between fasting serum bilirubin levels with inflammatory panel markers like hs-CRP in proved cases of MS of Indian origin. In this present study, it was found that in patients of MS, serum bilirubin is inversely correlated with inflammatory marker hs-CRP and thus serum bilirubin has a protective role against MS.*

Keywords: *Metabolic syndrome, Bilirubin, hs-CRP, Antioxidant*

I. Introduction

Metabolic syndrome (MS) or insulin resistance syndrome is a group of risk factor those occur together and increase the risk of stroke, and type 2 diabetes mellitus (DM) [10]. Cross-sectional surveys indicate that, in the United States (U.S), one-third of adults [11] and an alarming proportion of children have the MS [12]. Furthermore, a relatively high prevalence of the MS is a worldwide phenomenon. In the America, in Europe, and in India, at least one-fourth of the adults carry the syndrome [1]. It is increasing in developing country like India and is becoming more prevalent among adolescent and younger people. Although, the exact mechanism of underlying MS has not yet been properly elucidated, a number of risk factors are associated with occurrence of MS. Insulin resistance, type II diabetes, hypertension, dyslipidemia and visceral obesity, altogether increase oxidative stress [13-15] and reduce antioxidant defences [13-15] thereby inducing MS. Clinically, the serum biomarkers like IL-1, IL-6, IL-18, TNF, CRP, IFN gamma are readily measured and alteration in these biomarkers may predict development of MS. Obesity, IR and type 2 DM have been characterized as chronic inflammatory states that are associated with abnormal concentrations of cytokines, acute-phase reactants and other inflammatory signalling markers [16-20].

Serum bilirubin is a newly developed biomarker for MS. As atherosclerosis is characterized by a chronic state of low-grade inflammation and oxidative stress of the vascular wall its development may be delayed by bilirubin [21-24]. A protective action of increased levels of bilirubin has been found against the damages incurred upon by MS. The protective action of bilirubin is mainly due to its antioxidant properties [25]. Accordingly, we carried out the present study to evaluate a correlation between fasting serum bilirubin levels with inflammatory panel markers like hs-CRP in proved cases of MS of Indian origin.

II. Materials And Methods

The present study was undertaken as a cross sectional observational study in a tertiary care hospital in the department of Biochemistry in association with department of Medicine**.** During study period, 49 subjects having BMI more than 25 but not suffering from MS were selected as control subjects following screening for exclusion and inclusion criteria. On the other hand, 71 patients having BMI greater than 25 and meeting the criteria for diagnosis of MS following NCEP guidelines were selected as case group after meeting the requisite inclusion and exclusion criteria.

Inclusion criteria- Any three of the following five conditions-

(1) Blood pressure more than or equals to 130/85 mm of Hg
(2) Fasting blood glucose more than or equals to 110 mg/dl
(3) Waist circumference: In men more than or equals to 40 inches in women more than or equals to 35 inches
(4) HDL: Men- less than 40 mg/dl Women- less than 50 mg/dl
(5) Serum triglycerides - 150 mg/dl or more

The above criteria were according to National Cholesterol Education Programme (NCEP) [26]

Exclusion criteria
(1) Subjects with any hormonal disorder other than DM
(2) Neonate and immunocompromised
(3) History of alcoholism or hepatitis
(4) Liver function test abnormality

12 hours overnight fasting venous blood samples were collected from cases and controls and estimated for fasting blood glucose, serum total bilirubin, serum triglycerides, serum total cholesterol, HDL, hs-CRP. All the biochemical investigations were carried out on a semi automated chemistry analyzer using standard kits. Estimation of hs-CRP was done by immunoturbidimetry [27, 28] method in semiautomatic analyser.

III. Results

Table 1A shows distribution of gender in both case and control groups. It is evident from the data that there is no significant difference between the case and control groups as far as distribution of males and females are concerned. Pearson Chi-Square value is 2.296 and p value is 0.155 which is not significant. In **Table 1B**, significance of difference between age in case and control group is analysed. Age distribution shows no significant difference between the two groups. So it can be said this study is age matched. In the **Table 1C**, distribution of different test parameters are shown. Group 1 and group 0 indicated the case and control subjects respectively. Differences between the mean rank values of the parameters suggest a significant difference in the distribution of study parameters except their ages, between the case and control population. The results of the Table 1 were validated by the Mann-Whitney test in Table 2. In **Table 2**, significance of difference between the study parameters in case and control group is analysed. Significant difference is found between case and control group in these study parameters: Serum bilirubin, serum hs-CRP, Serum TG, Serum HDL, plasma FBG, waist circumference. Results of the correlation study in **Table 3** show that the hs-CRP level shows significant negative relationship with the bilirubin level among the case group. A correlation coefficient of r = -.513 between bilirubin and hs-CRP in the case group with p value of <.001 (2 tailed) is highly significant.

IV. Discussion

It is well known from various studies that, MS has components like IR, type II diabetes, hypertension, dyslipidemia, and visceral obesity, which increase oxidative stress [13] and reduce antioxidant defences [29]. It is well known that serum bilirubin is potent antioxidant, so it must have some beneficial effect on prevention of MS and atherosclerosis. Accordingly, many studies on different population have shown its inverse relation with MS [30, 31]. Serum bilirubin has been proved to have an inverse correlation with MS in Korean population [30]. In a study among Japanese people inverse relationship was found between serum bilirubin and high sensitivity CRP (hs-CRP) [32].

The present study was proposed to validate the result in Indian scenario with an object to explore a correlation between serum bilirubin levels with inflammatory panel markers like hs-CRP in proved cases of MS of Indian origin. The values from the Table 1 C and 2 show higher values of FBG, waist circumference and TG level is significantly higher and HDL level is significantly lower in case group in comparison to control groups, which strongly validate the diagnosis of MS in our case group . Table 1A and 1B show there is no significant difference in sex and age parameters between case and control groups, so it can be said that this study is age and sex matched. In the present study, hs-CRP level has been found to be significantly elevated in MS patients (Table 1C and 2). Role of CRP in association with atherosclerosis is well documented in variety of race and age group [33, 34]. Among all proinflammatory markers, its role in MS patients is well established [6-8].

So far, many studies have demonstrated role of proinflammatory markers like hs-CRP and gamma interferon in MS. These markers are elevated in MS and are associated with CAD, CVA and other co morbidities. On the other hand, various studies have demonstrated that serum bilirubin is significantly low in MS. But, the present investigator could not find any report regarding correlation of proinflammatory markers with serum bilirubin in the Indian patients. Keeping this in mind, this present study was done with 71 MS cases and 49 control subjects. During study period, 49 subjects having BMI more than 25 but not suffering from MS

were selected as control subjects following screening for exclusion and inclusion criteria. On the other hand, 71 patients having BMI greater than 25 and meeting the criteria for diagnosis of MS following NCEP guidelines were selected as case group after meeting the requisite inclusion and exclusion criteria.

In this present study, it was found that in patients of MS, serum bilirubin is inversely correlated with inflammatory marker hs-CRP (Table no. 3,Correlation coefficient r = - 0.513 between bilirubin and hs-CRP among case group with a p value of < 0.001 in 2 tailed study which is highly significant). Level of significance was considered $p \leq 0.05$. Differences in mean rank and median values of the parameters were found between the case and control population, which suggest a significant difference in the distribution of study parameters between the case and control population (Table 1C and 2). The result was validated by the Mann-Whitney test for determination of the significance of difference. Mann-Whitney test results show that FBS, hs-CRP, waist circumference are significantly higher in the case group. There is significant difference in TG and HDL level between case and control .The bilirubin level also has significant difference between the two groups (Table 2). These findings strongly suggested that the selected proinflammatory marker in our study population were significantly higher in the case group who did not show a bilirubin level as much high as observed in the normal control subjects.

Results of the study showed that the hs-CRP level had significant negative relationship with the bilirubin level among the case group. Correlation coefficient r = -0.513 between bilirubin and hs-CRP among case group with a p value of <0.001 in 2 tailed stud. These results have important implications on the complications of MS. From the above results it can be concluded that, in patients of MS, hs-CRP level are increased and they have important role for causation of CAD, CVA. It can also be concluded that serum bilirubin has protective role against MS due to its antioxidant properties. It reduces oxidative stress caused by various inflammatory markers and increased bilirubin level within physiological limit is beneficial. In our study subjects of MS, hs-CRP is inversely correlated with serum bilirubin. This result tells that, MS patients have decreased bilirubin level which leads to reduced antioxidant defences against proinflammatory markers. . The findings of the present study further establish the need for further studies exploring the exact mechanism of the protective role of serum bilirubin in patients of MS

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Tables

Table 1A: Distribution of male and females in both cases and control groups

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.296[a]	1	.130		
Continuity Correction[b]	1.719	1	.190		
Likelihood Ratio	2.274	1	.132		
Fisher's Exact Test				.155	.095
N of Valid Cases	120				
a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.29.					
b. Computed only for a 2x2 table					

Table 1B: Mann-Whitney test to analyze the significance of difference between age in case and control group:

	Case group mean rank	Control roup mean rank	Case group (median)	Control group (median)	Mann-Whitney U	Z value	p value asymp. Sig. (two tailed)
Age in year	64.65	54.48	49	45	1444.500	-1.577	0.115

Table1C: Non parametric assay for analysing of parameters between case and control group: (Case group = 1, Control group = 0).

Ranks				
	Grouping	N	Mean Rank	Sum of Ranks
Age in yrs	.00	49	54.5	2669
	1.00	71	64.6	4590
	Total	**120**		
Serum TG in mg/dl	.00	49	27.00	1323.00
	1.00	71	83.62	5937.00
	Total	**120**		
Serum HDL in mg/dl	.00	49	82.16	4026.00
	1.00	71	45.55	3234.00
	Total	**120**		
Serum bilirubin (mg/dl)	.00	49	68.00	3196.00
	1.00	71	53.87	3825.00
	Total	**120**		
	1.00	71	82.66	5869.00
	Total	**120**		
FBG (mg/dl)	.00	49	24.91	1171.00
	1.00	71	82.39	5850.00
	Total	**120**		
hs-CRP (mg/l)	.00	49	40.54	1905.50
	1.00	71	72.05	5115.50
	Total	**120**		
	1.00	71	66.85	4746.00

	Total	**120**		
WC (cm)	.00	49	31.62	1486.00
	1.00	71	77.96	5535.00
	Total	**120**		

Table 2: Mann-Whitney test to analyze the significance of difference between the study parameters in case and control group:

	Case group mean rank	Control group mean rank	Case group (median)	Control group (median)	Mann-Whitney U	Z value	p value asymp. Sig. (two tailed)
Serum bilirubin in mg/dl	53.87	68.00	0.69	0.83	1269.000	-2.197	.028
Serum hs-CRP in mg/L	72.05	40.54	2.31	1.00	777.500	-4.900	<0.001
Plasma FBG in mg/dl	82.39	24.91	162	88	43.000	-8.936	<0.001
Waist circumference in cm	77.96	31.62	107	92	358.000	-7.215	<0.001
Serum HDL in mg/dl	45.55	82.16	42	48	678.000	-5.680	<0.001
Serum TG in mg/dl	83.62	27.00	215	144	98.000	-8.766	<0.001

Table 3: Non parametric correlation analysis to the significance of strength between different parameters of the case group:

			FBS (mg/dl)	Serum bilirubin (mg/dl)	hs-CRP (mg/l)	Waist circumference in cm
Spearman's rho	FBS (mg/dl)	Correlation Coefficient	1.000	-.027	.085	.048
		Sig. (2-tailed)	.	.820	.479	.694
		N	71	71	71	71
	Serum bilirubin (mg/dl)	Correlation Coefficient	-.027	1.000	-.513**	-.121
		Sig. (2-tailed)	.820	.	<0.001	.313
		N	71	71	71	71
	hsCRP (mg/l)	Correlation Coefficient	.085	-.513**	1.000	.127
		Sig. (2-tailed)	.479	<0.001	.	.291
		N	71	71	71	71
		Sig. (2-tailed)	.662	.05	.030	.211
		N	71	71	71	71
	WC (cm)	Correlation Coefficient	.048	-.121	.127	1.000
		Sig. (2-tailed)	.694	.313	.291	.
		N	71	71	71	71