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Serum Bilirubin level and its correlation with white blood cells as risk factor for cardiovascular disease in adult healthy population.

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ABSTRACT… Objectives: Evaluate serum bilirubin in adult healthy subjects and its correlation with white blood cells as risk factor for cardiovascular disease. **Study Design:** Cross- sectional study. **Setting:** Department of Pharmacology and Medicine, Suleman Roshan Medical College. **Period:** January - December 2017. **Material & Methods:** A sample of 100 male and 100 female adult healthy subjects were recruited for study protocol. Blood glucose, Serum creatinine, Blood lipids, liver enzyme levels, White blood cell counts and Serum bilirubin levels were analyzed. Pearson`s correlation was used for the correlation coefficient and its statistical significance for the association of serum bilirubin and white blood cells. Data variables were analyzed by statistical software SPSS (ver 21.0) at 95% CI ($P \leq 0.05$). **Results:** Mean± SD age of male and female was found 47.02±8.42 and 48.59±7.80 years respectively (P=0.071). Serum bilirubin shows statistically significant negative correlation with blood glucose (r= - 0.257, P=0.0001) and LDLc (r= - 0.155, P=0.027) and WBC (r= - 0.871, P=0.0001). **Conclusion:** The present study shows the elevated serum total bilirubin levels within reference range correlated negatively with total white blood cells in adult healthy population.

Key words: Bilirubin, Cardiovascular Disease, White Blood Cells.

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INTRODUCTION

Bilirubin is a lipid soluble metabolite of heme-porphyrins. Heme is released when senile red blood cells are hemolyzed. The monocyte macrophage system scavenges the fragile red blood cells. Heme is catabolized into biliverdin and bilirubin. In large concentrations, the bilirubin is neurotoxic. Bilirubin is transported by serum albumin in vascular system. In liver cells, the bilirubin is conjugated with glucuronic acid. Bilirubin mono- and di- glucuronides are produced in liver. Bilirubin glucuronides are water soluble, hence excreted in bile through small intestine.[1] Bilirubin is a potent anti-oxidant against oxidants in the blood.[1,2] Anti- inflammatory property of bilirubin has also been reported.[3] It is suggested that the serum bilirubin is negatively related to oxidative stress in chronic inflammatory disease such as atherosclerosis that is forerunner of cardiovascular diseases (CVD).[4] Negative relation of serum total bilirubin has been suggested against the coronary artery disease (CAD), brain ischemia and peripheral arterial disease (PAD).[5] Elevated serum bilirubin can retard and prevent atherosclerosis through suppression of oxidizing radicals and lipid peroxides[6], particularly oxidation of low-density lipoprotein cholesterol (oxidized-LDL).[7] In- vitro human studies have demonstrated the serum bilirubin prevents vascular stickiness through vascular endothelial factors that are involved in the inflammation and vascular endothelial damage. Serum bilirubin exerts anti-inflammatory action through inhibition of tumor necrosis factor-α (TNF-α). TNF-α up regulates the activity of vascular cell adhesion molecule-1(VCA-1), E- selectin and other intercellular adhesion molecule[8] that are expressed on vascular endothelia and play role in vascular inflammation. As there is accumulating evidence that the atherosclerosis is initiated by inflammation that is caused by white blood cells. WBCs release many oxidants, and one of safety guard against this is the serum bilirubin that if found low may aggravate the inflammatory

diseases.[9] It is suggested that serum bilirubin may protect against inflammatory oxidants, atherosclerosis and cardiovascular disease (CVD). Several epidemiological studies have reported an elevated WBC count may indicate the inflammation and are associated multiplied risk of CVD.[9,10] The research is lacking on the correlation of serum bilirubin and WBC counts. Hence there is a need to evaluate serum bilirubin as simple markers of oxidant load and its correlation with white blood cells as risk factor for cardiovascular disease in adult healthy population.

MATERIAL & METHODS

The study protocol of present cross sectional study was planned to be conducted after ethical permission by the ethical review committee of the institute. The study took place at the Department of Pharmacology and Medicine, Suleman Roshan Medical College from January - December 2017. A proforma was designed for data collection. A sample of 100 male and 100 female adult healthy subjects were recruited for study protocol. The participants were recruited through convenient sampling. Sample for study protocol was calculated by "sampling-for- proportions". Participants were recruited through inclusion criteria of age 40- 60 years, healthy adults of both genders, with overnight fast presenting in early morning. Exclusion criteria were; acute bacterial disease, chronic kidney disease, viral hepatitis, bilirary tract disease, hemolytic anemia, chronic liver disease, hypersplenism, alcoholism, hepatotoxic drug intake, malnutrition, malabsorption syndrome and NSAIDs drug intake. Proper history of participants with physical examination was carried out to fulfill the criteria of study protocol. Volunteers were asked of dietary habits, drug and alcohol intake. Participants were informed that the Biodata and blood tests will benefit the society through medical publications and personal data shall be confidential. Medical officer were directed for data collection once they were briefed of study protocol and variables to be tested. Medical officers helped to rule out the exclusion criteria and fulfill the inclusion criteria. Volunteers were requested for written consent and blood sampling. 10 ml Disposable syringe (BD, USA) was used for venesection. 5 ml blood was taken from a peripheral vein preferably in the ante- cubital fossa. Sterilization measures were taken and vein was made prominent by a tourniquet above the ante- cubital fossa. After venesection, the area was covered by saniplast. 2 ml blood was poured into EDTA bottles for complete blood counts. Blood formed elements counting was performed on fully automated Sysmex KX-21 hematology analyzer. 3 ml blood was centrifuged to get sera for the analysis of serum total bilirubin, blood glucose, serum creatinine, blood lipids and liver aminotransferase enzymes on Hitachi Chemistry Analyzer. White blood cell counts and serum bilirubin levels checked and re-checked. Data was analyzed by statistical software SPSS (ver 21.0). Pearson`s correlation was used for the correlation coefficient and its statistical significance for the association of serum bilirubin and white blood cells. The α-level of 95% Confidence interval was taken as statistically significant ($P \leq 0.05$).

RESULTS

Mean± SD age of male and female was found 47.9±8.02 and 48.59±7.80 years respectively (P=0.071). Blood glucose, serum creatinine, cholesterol, triglycerides, LDLc, and HDLc are summarized in Table-I. Alanine transaminase, Alkaline phosphatase, Lactate dehydrogenase reveals significant difference between male and female (P<0.05). White blood cell counts in male were 6478±2.78/μL and in female 6781±3.1/μL (P>0.05). Serum bilirubin was noted as 1.19±0.51 mg/dl in male and 1.12±0.39 mg/dl in female. Correlation of serum bilirubin is shown in Table-II. Serum bilirubin shows positive correlation with creatinine (r=0.155 P=0.028), triglycerides (r=0.179, P=0.011), ALT (r= 0.465, P=0.0001), AST(r= 0.206, P=0.003), ALP (r=0.248, P=0.0001), LDH (r=0.44, P=0.0001) and GGT (r=0.44, P=0.0001). Statistically significant negative correlation of bilirubin was found for blood glucose (r= - 0.257, P=0.0001) and LDLc (r= - 0.155, P=0.027). WBC shows strongly negative correlation with bilirubin (r= - 0.871, P=0.0001). Scatter plot-1 shows strongly negative correlation of bilirubin and WBC.

Variable	Male	Female	P-Value
Blood Glucose (R) (mg/dl)	136.4±15.76	142.7±16.78	0.87
Creatinine (mg/dl)	1.2±0.04	0.98±0.12	0.91
Cholesterol (mg/dl)	153.3±20.87	149.7±19.8	0.83
Triglycerides (mg/dl)	124.6±9.48	126.5±13.5	0.93
LDLc (mg/dl)	87.6±9.4	76.8±11.7	0.43
HDLc (mg/dl)	41.5±5.71	40.3±6.31	0.76
ALT (U/L)	39.0±7.13	35.6±3.13	0.03
AST (U/L)	43.4±7.13	41.3±9.11	0.23
ALP (U/L)	145.4±19.76	125.5±15.5	0.001
LDH (U/L)	150.9±13.7	121.5±12.5	0.003
GGT (U/L)	54.2±7.9	41.1±11.8	0.001
WBC (μL)	6478±2.78	6781±3.1	0.93
Bilirubin (mg/dl)	1.19±0.51	1.12±0.39	0.76

Table-I. Biochemical findings and White blood cell counts of study subjects (n=200)

	r-value	P-Value
Age	0.045	0.52
Blood Glucose	- 0.257	0.0001
Creatinine	0.155	0.028
Cholesterol	0.091	0.198
Triglycerides	0.179	0.011
LDLc	- 0.155	0.027
HDLc	0.166	0.019
Alanine transaminase	0.465	0.0001
Aspartate transaminase	0.206	0.003
Alkaline phosphatase	0.248	0.0001
Lactate dehydrogenase	0.440	0.0001
Gamma GLutamyl transferase	0.440	0.0001
White blood cells	- 0.871	0.0001

Table-II. Correlation of serum bilirubin (n=200)

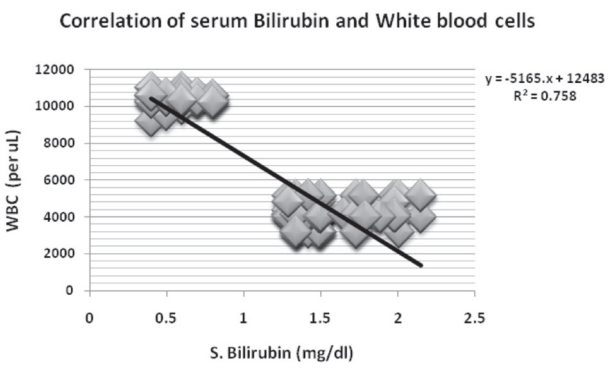

Figure-1. Scatter plot showing strongly negative correlation of serum bilirubin and white blood cells

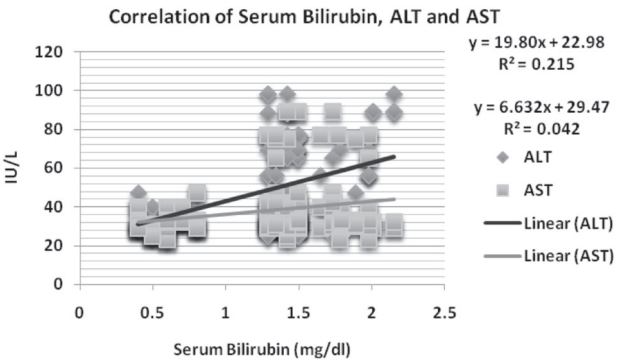

Figure-2. Scatter plot showing strong positive correlation of serum bilirubin with Alanine transaminase (ALT) but weak correlation with Aspartate transaminase (AST)

DISCUSSION

The present study is the first cross sectional report on evaluating serum bilirubin in adult healthy subjects and its correlation with white blood cells as risk factor for cardiovascular disease. The present study reports statistically significant negative correlation of bilirubin and WBC (r= -0.871, P=0.0001) that is considered as risk factor for the cardiovascular disease.[4,5,9] The present study observed age (mean± SD) of male and female as 47.02±8.42 and 48.59±7.80 years respectively (P=0.071). Alanine transaminase, Alkaline phosphatase, Lactate dehydrogenase reveals significant difference between male and female (P<0.05). Serum bilirubin was noted as 1.19±0.51 mg/dl in male and 1.12±0.39 mg/dl

in female (P>0.05). The white blood cells counts were found equal in both male and female; noted as 6478±2.78/μL and 6781±3.1/ μL (P>0.05) respectively, these findings are supported by previous studies.[5.6,7,10] In present study, we observed strong negative correlation of serum bilirubin and WBC counts; correlation co-efficient (r= - 0.871) that was statistically highly significant (P=0.0001). The findings are in line with previous studies.[11-14] Various epidemiological studies have demonstrated inverse correlation of serum bilirubin with cardiovascular risk factors such as the systemic blood pressure, blood lipids and glucose levels, and obesity.[11-14] Hence, the findings of serum bilirubin, white blood cells, and lipid profile are in agreement with above studies. The finding is of clinical importance as regards the risk of CVD and role of WBC that is already proven.[11-13] Epidemiological studies on Gilbert's syndrome patients demonstrated high normal levels of serum bilirubin carry low risk of carotid atherogenesis and coronary artery disease.[15,16] It has been suggested that the correlation of serum bilirubin and WBC counts is protean and different mechanisms have been postulated. For example in-vitro studies, both human and animal studies, had reported inverse association of serum total bilirubin and inflammatory markers such as the CRP (C-reactive protein), interleukin-1α (IL-1α), vascular endothelial ligands such as the P- selectin and CD40 ligand which have been implicated in the inflammation and leucopoiesis.[17,18] Above studies support our present observations of negative correlation of serum bilirubin and WBC counts as risk factor for the cardiovascular disease. Another mechanism of low serum bilirubin and high WBC counts is an enhanced free radical production by later that depletes the former natural anti oxidant.[19] Still another postulated mechanism says an indirect linking factor might be influencing the inverse association of serum bilirubin and WBC counts.[11-14] A previous study[11] proposed the inverse correlation of serum bilirubin and metabolic syndrome is because of the regulatory effects of serum bilirubin upon the insulin resistance that is the core defect of pathophysiology of the metabolic syndrome. Other in-vivo animal studies.[21,22] demonstrated the serum bilirubin improves insulin sensitivity through expression of adiponectin. On contrary, persons with raised WBC counts have increased risk of metabolic syndrome as chronic inflammation induces insulin resistance.[23,24] Hence it is proposed the negative correlation of serum bilirubin and WBCs may underlie the chronic inflammatory phenomena leading to metabolic syndrome through insulin resistance. The present study has limitations of; 1st – cause effect relationship of serum bilirubin and WBC counts cannot be ascertained due to cross sectional study design, 2nd – present study could not measure the inflammatory mediators such as the CRP, interleukin-1α (IL-1α) and Vascular endothelial ligands because of economic problems. Strength of study lies in its inclusion and exclusion criteria and prospective study design. Hence, the observations of present study are worth to report for further studies to be conducted with large sample size.

CONCLUSION

The present study shows elevated serum total bilirubin levels within reference range correlated negatively with total white blood cells in adult healthy population. This suggests low serum bilirubin with elevated white blood cells indicates the risk factor for cardiovascular disease; however, further studies are recommended.

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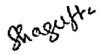
2	Shumaila Shaikh	Materials handling, Interpretation lab investigations, Manuscript write up, Proof reading.	
3	Shagufta Memon	Concept, Materials handling Collection of materials, compilation of resutls, statistical analysis, manuscript write up, Correspondence.	
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