

CORRELATION BETWEEN SERUM HOMOCYSTEINE AND TOTAL BILIRUBIN IN METABOLIC SYNDROME

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ABSTRACT

BACKGROUND

Metabolic syndrome, also known as syndrome X, is a well-known cluster of clinical features which precipitate diabetes mellitus, coronary artery disease (CAD) and many other life threatening conditions. Homocysteine (Hcy), a non-standard amino acid, has been found by a number of workers as a marker for CAD. Association of Metabolic syndrome with general inflammatory condition has been reported in our previous work among the population in Kolkata, and its neighbourhood. There is not enough data regarding association of serum Hcy and metabolic syndrome in the above-mentioned population. In the present work, we have concentrated to find out any association of serum Hcy and total bilirubin levels in proved cases of metabolic syndrome.

MATERIALS AND METHODS

A total of 100 subjects were identified with metabolic syndrome according to the guidelines provided by NCEP (ATP III) criteria, USA. A total of 125 normal individuals with no history of previous coronary artery diseases were chosen as control population. BMI, fasting blood glucose (FBG), fasting serum Insulin, Hcy, serum total bilirubin, lipid profile, TSH were estimated both in control and test groups. HOMA-IR, Waist-hip ratio were calculated by standard procedure. Hcy was estimated with ELISA kit, Axis-Shield, Ranbaxy.

RESULTS

Mean Serum Total Bilirubin and Hcy for Control population were 0.74 ± 0.11 and 6.62 ± 1.07 respectively. Mean Serum Total Bilirubin and Hcy for Metabolic Syndrome diagnosed population were 0.36 ± 0.06 and 13.91 ± 0.84 respectively.

CONCLUSION

Dyslipidaemia, obesity, sedentary lifestyle, hypertension are markers of metabolic syndrome. Metabolic syndrome is associated with atherosclerosis and cardiac disorders. Type II diabetes is also associated with metabolic syndrome and CAD. Serum Hcy level has direct relationship with risk of CAD. In this work, we observed an inverse relationship between serum Hcy with serum total bilirubin which is a potent physiological antioxidant.

KEYWORDS

Metabolic Syndrome, Coronary Artery Disease (CAD), Homocysteine, Serum Bilirubin, HOMA-IR.

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BACKGROUND

Metabolic syndrome has been a serious threat to our life due to modern day lifestyle. Beside genetic causes metabolic syndrome is an effect of sedentary habits. Subjects of Metabolic Syndrome are prone to develop risk of battery of non-communicable diseases, such as type 2 diabetes mellitus and cardiovascular diseases. Metabolic syndrome is associated with inflammatory process and atherosclerosis. In this study, serum homocysteine, a potent marker for atherosclerosis (CAD) and serum total bilirubin, a

physiological antioxidant have been measured in 100 subjects of Metabolic Syndrome and compared with 125 control subjects. We observed an inverse relationship between serum homocysteine and total bilirubin. The metabolic syndrome was diagnosed as per NCEP (ATP III) criteria. BMI, waist-hip ratio, lipid profile and HOMA-IR score of the test and control groups have been considered for diagnostic criteria.

Serum homocysteine estimation has been a tool to assess risk of coronary artery diseases in present time. Many researchers have found direct relationship of serum homocysteine and risk of CAD.^{1,2} The Homocysteine (Hcy) is an amino acid, is a homologue of the amino acid cysteine, differing by an additional methylene (-CH₂-) group. It is biosynthesised from methionine by the removal of its terminal methyl group and can be recycled into methionine or converted into cysteine with the aid of B-vitamins.³ Metabolic syndrome or Syndrome-X is recognised as one of the most serious risk factors for developing type II diabetes mellitus, as well as cardiovascular disease.⁴ Criteria for diagnosis of metabolic syndrome are the criteria of the National Cholesterol Education Programme (NCEP) Adult Treatment

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Panel III (ATP-III), with combination of body mass index (BMI) and waist circumference.^{5,6,7} The key points of this criteria are: 1) Triglyceride levels ≥ 150 mg/dL; 2) HDL-C (High density lipoprotein) levels < 40 mg/dL in men or < 50 mg/dL in women; 3) Fasting plasma glucose levels ≥ 110 mg/dL or taking an antidiabetic medication; 4) Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg (or receiving drug therapy for hypertension); and 5) BMI > 25 Kg/Metre².⁸

The exact mechanism underlying metabolic syndrome has not yet been elucidated completely. Many cross sectional or longitudinal studies have shown that metabolic syndrome is strongly associated with inflammation,^{9,10,11} insulin sensitivity,¹² endothelial dysfunction.¹³ As a result, several biological markers have been proposed as for metabolic syndrome. These are associated with an increase in the risk of metabolic syndrome: homeostasis model assessment insulin resistance index¹⁴ (HOMA-IR; as a marker of insulin resistance), homocysteine¹³ (As a marker of endothelial dysfunction). As there is no available data for the study in Indian population, the object of present study was to establish any correlation between serum homocysteine a marker of endothelial dysfunction and fasting serum bilirubin an oxidative stress marker in established cases of metabolic syndrome around Kolkata and its neighbourhoods in India.

Bilirubin acts as a potent physiological antioxidant,¹⁵ the object of present study is to establish any correlation between serum homocysteine and fasting serum bilirubin in proved cases of metabolic syndrome among the patients visiting outpatient departments of Calcutta National Medical College, Kolkata, India. Informed consent was duly taken from each subject under study and the entire procedure was done as per the institutional ethical committee's permission.

Inclusion Criteria

In this hospital based cross sectional study, subjects of metabolic syndrome were diagnosed with high BMI, high waist-hip ratio, high HOMA-IR score, hypertension and known type 2 diabetes mellitus. Healthy controls were chosen from the spouse of metabolic syndrome patients, medical students, teaching and non-teaching staff of the Calcutta National Medical College, Kolkata.

Exclusion Criteria

Subjects with chronic diseases such as known tuberculosis, hepatitis due to any cause, Cushing's syndrome, pregnancy, chronic alcoholism, renal dysfunction, long treated diabetes mellitus, taking treatment for thyroid disorders, recent history of fever and infection, history of known heart disease were excluded from the study.

MATERIALS AND METHOD

Blood samples were collected from the patients visiting the outpatient department of Calcutta National Medical College, Kolkata, India. Necessary clearance from Institutional ethical committee was obtained. Blood was collected from antecubital vein. Cells were separated by centrifugation at 5000 rpm for 5 minutes. Blood glucose, serum bilirubin were estimated immediately. Serum was separated and stored in -20°C for future use. Patient's Body Mass Index was measured using standard measuring tape and weighing machine. Height was

measured against wooden vertical scale. Body mass index was measured using the following formula mass in Kg/height in metre². Fasting plasma glucose and total bilirubin was estimated using autoanalyser (A125-Transasia). HbA1c was estimated using Bio-Rad D-10. Fasting insulin was estimated using ELISA kit of Monobind Inc. Waist-hip ratio was measured using standard measuring tape in the outpatient department. Insulin resistance was estimated by calculating HOMA-IR originally described by Mathew et al¹⁶ (Homeostatic model assessment-Insulin resistance). HOMA-IR was calculated using the following formula.

$$\text{HOMA-IR} = \frac{\text{Fasting glucose (mg\%)} \times \text{fasting insulin } (\mu\text{U/mL})}{405}$$

Serum homocysteine was estimated using ELISA kit of Axis Shield, Ranbaxy in Tecan ELISA reader, Imperial Biotech. Subjects were divided in two groups - control and test. Control group had 125 healthy individuals and test group had 100 proved cases of metabolic syndrome. Test group subjects were selected using standard criteria for metabolic syndrome. Individuals with chronic disease manifestation were excluded from study. Fasting blood samples were collected in EDTA tubes and plasma was separated immediately within one hour and stored at -20°C till analysis. All reagents and samples were brought to room temperature ($18-25^{\circ}\text{C}$) before use. A standard graph was prepared with standards provided with the kit. Standards were diluted by serial dilution method. 100 μL sample each from standard and test were taken into appropriate wells. Wells were covered and incubated for 2 hours at room temperature with gentle shaking. The solution was then discarded but not washed. 100 μL of Detection Reagent A working solution was added to each well. Plate was covered and incubated for 1 hour at 37°C and mixed gently. Each well was aspirated and washed with wash solution for three times. 100 μL of Detection Reagent B working solution was added to each well and incubated for 1 hour at 37°C . Contents were aspirated and washed for 5 times. Then 90 μL of Substrate Solution was added to each well and Incubated for 15-30 minutes at 37°C . 50 μL of Stop Solution was added to each well and mixed properly. Reading was taken immediately at 450 nm using a plate reader.

Data was analysed using Microsoft Excel and graph prism software. Value of individual parameters were expressed as mean \pm standard deviation. Significance of difference of the means within the groups was tested by unpaired Student's t-test. Everywhere $P < 0.05$ is considered as significant.

RESULTS

Analysed data are furnished in the tables below. Table-1 shows the physical parameters including HOMA-IR score of control and test group subjects. Obesity markers were also estimated which are not included in this article. Table 1 shows the average age of the subjects. Male and female ratio of the subjects is also close among the groups.

	Control (n=125)		Test (n=100)	
	Number	Mean Age in Years	Number	Mean Age in Years
Male	64	32.73 \pm 15.09	59	49.6 \pm 12
Female	61	31.43 \pm 9.61	41	43.85 \pm 13.15

Table 1: Age and Gender Distribution in sample Population with Physical Parameters

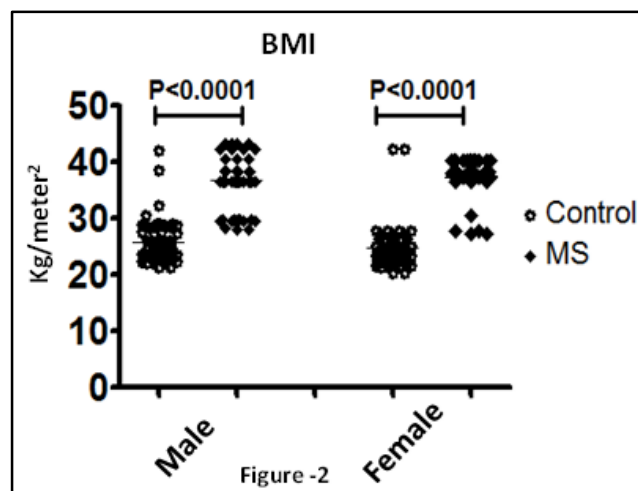
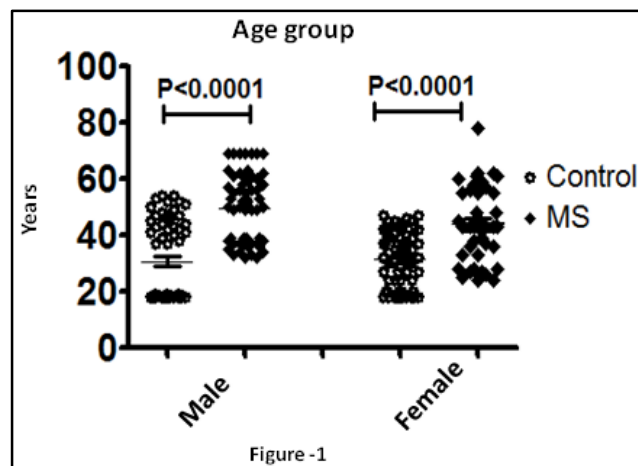
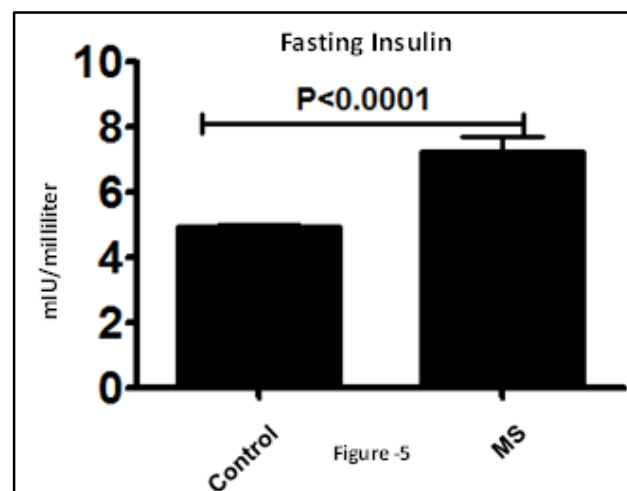
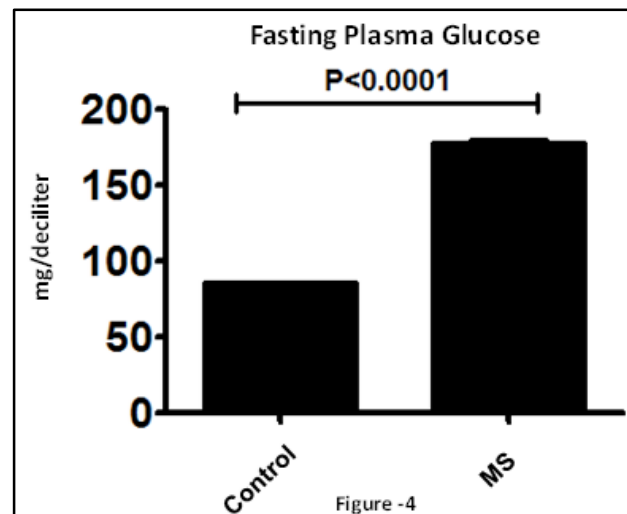
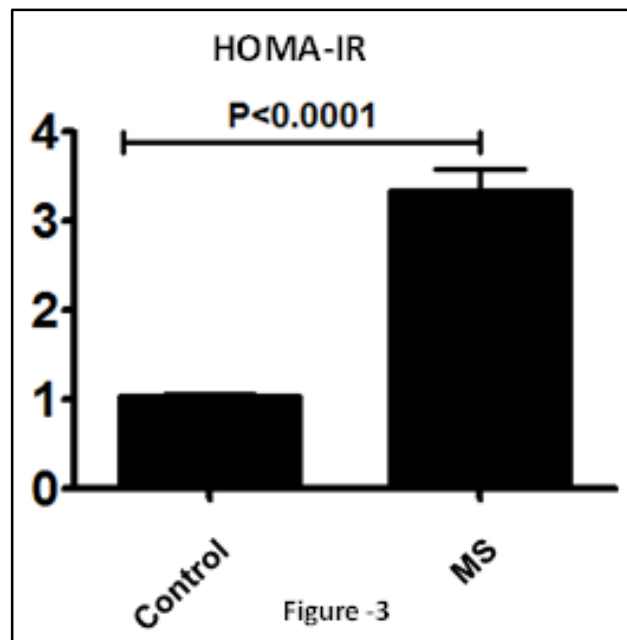
Parameter	Control Group N=125	Test Group n=100
Age in Years	32.01±12.78	47.26±12.87
Male:Female	1.05	1.44
Waist/Hip Ratio	0.81±0.03	0.97±0.05
Body Mass Index (Kg/metre ²)	Male	25.7±3.73
	Female	24.88±3.86
HOMA-IR Score*	1.04±0.15	3.34±2.44

Table 2: Physical Parameters and HOMA-IR score of Sample Population

*p value <0.0001

Parameter	Unit	Control		Test		P value
		Mean	±SD	Mean	±SD	
Fasting Plasma Glucose	mg/deciliter	85.98	2.73	178.39	15.03	<0.0001
Fasting Insulin	mIU/milliliter	4.94	0.80	7.25	4.78	<0.0001
HbA1c	NGSP (%)	5.71	0.16	10.52	0.90	<0.0001
Serum Total Bilirubin	mg/deciliter	0.75	0.12	0.37	0.06	<0.0001
Homocysteine	µmol/litre	6.62	1.07	13.91	0.84	<0.0001

Table 3: Special Parameters of Metabolic Syndrome and Control Subjects



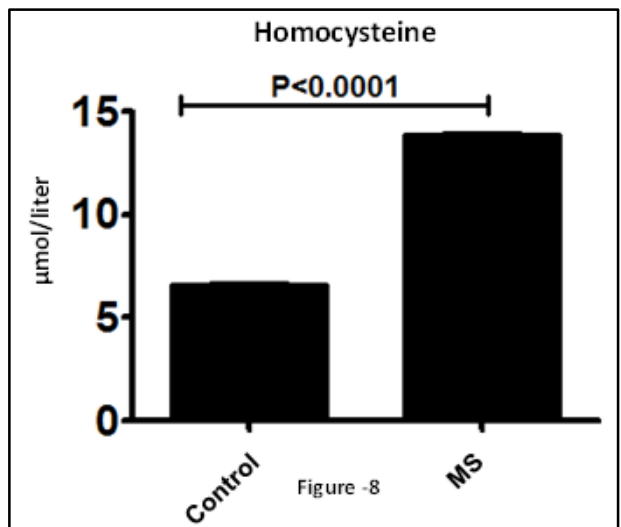
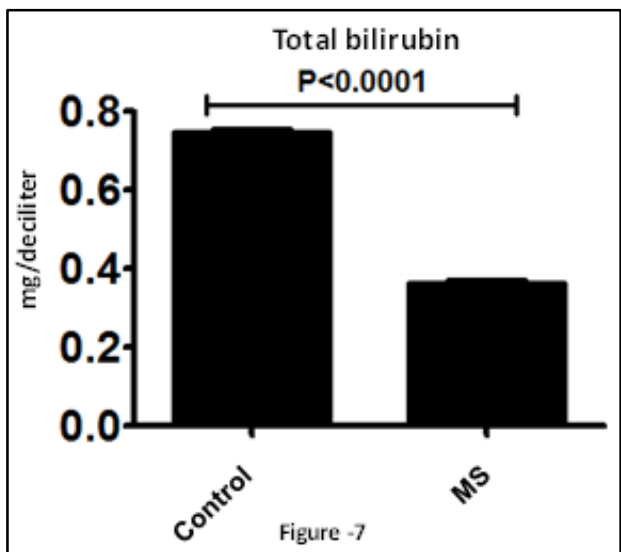
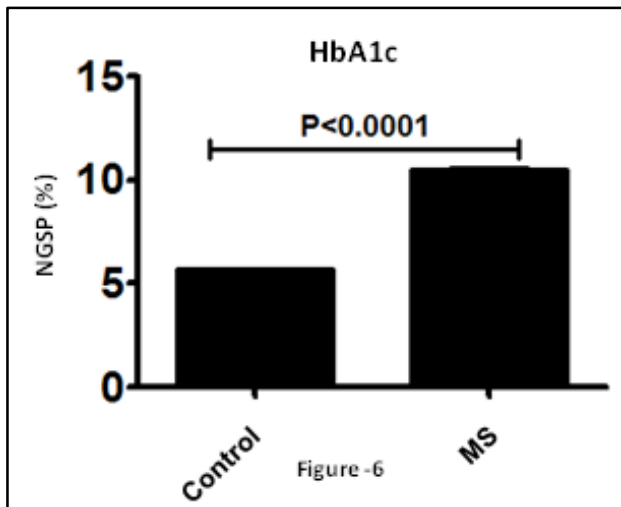


Table 1 and Figure 1 shows the gender wise distribution of age of subjects participated in the study.

Waist-Hip Ratio

Waist-hip ratio is an important parameter for assessment of obesity.¹⁷ WHR was found to be 0.81±0.03 and 0.97±0.05 in control and metabolic syndrome groups respectively. [Table 1].

Body Mass Index

Mean and SD of body mass index of male and female control group were 25.7±3.73 kg/metre² and 24.88±3.86 kg/metre² respectively. Mean and SD of body mass index of male and female metabolic syndrome group were 36.59±4.15 kg/metre² and 37.41±3.69 kg/metre² respectively. The p values for both sexes are <math>< 0.0001</math>. [Table 2, Fig. 2].

HOMA IR Score

Control group and metabolic syndrome group were found to have significantly different HOMA IR score i.e., 1.04±0.15 and 3.34±2.44 respectively. The p value is <math>< 0.0001</math>. [Table 2, Fig. 3].

Fasting Plasma Glucose

Mean and SD of fasting plasma glucose of control group was 85.98±2.73 mg/deciliter. Mean and SD of fasting plasma glucose of metabolic syndrome group was 178.39±15.03 mg/deciliter. The p value is <math>< 0.0001</math>. [Table 3, Fig. 4].

Fasting Insulin

Mean and SD of fasting insulin of control group was 4.94±0.80 mIU/millilitre. Mean and SD of fasting insulin of metabolic syndrome group was 7.25±4.78 mIU/millilitre. The p value is <math>< 0.0001</math>. [Table 3, Fig. 5]. HOMA-IR is better parameter than fasting insulin for detection of metabolic syndrome cases.¹⁸

HbA1c: Mean and SD of HbA1c of control group was 5.71±0.16%. Mean and SD of HbA1c of metabolic syndrome group was 10.52±0.90%. The p value is <math>< 0.0001</math>. HbA1c or glycated haemoglobin A1 is a diagnostic as well as prognostic parameter for hyperglycaemic state of an individual.¹⁹ [Table 3, Fig. 6].

Serum Total Bilirubin

Mean and SD of Serum Total Bilirubin of control group was 0.75±0.12 mg/deciliter. Mean and SD of Serum Total Bilirubin of metabolic syndrome group was 0.37±0.06 mg/deciliter. The p value is <math>< 0.0001</math>. [Table 3, Fig. 7].

Serum Homocysteine

Mean and SD of Serum homocysteine of control group was 6.62±1.07 micromoles/litre. Mean and SD of Serum homocysteine of metabolic syndrome group was 13.91±0.84 micromoles/litre. The p value is <math>< 0.0001</math>. [Table 3, Fig. 8].

DISCUSSION

Smoking, high blood pressure, elevated serum total cholesterol and elevated serum glucose are major risk factors for cardiovascular disease (CVD).²⁰ Metabolic syndrome or syndrome-X also shares common diagnostic criteria with CVD risk factors.^{21,22} The definition of hyperhomocysteinaemia differs between studies.²³ Hyperhomocysteinaemia is defined as a medical condition characterised by an abnormally high level (Above 15 μmol/L) of homocysteine in the blood according to some studies.²⁴ Concentration of homocysteine in plasma of healthy humans (Fasting) is low and its level is between 5.0 and 15.0 μmol/L when assessed with the use of HPLC, and 5.0-12.0 μmol/L when immunoassay methods are used.²⁵ The level between 16-30 μmol/L is considered as moderate and 31-100 μmol/L is considered intermediate and a value above 100 μmol/L is considered as severe hyperhomocysteinaemia.²⁶ Homocysteine level in blood

among Indians have been reported by some workers.²⁷ But these are all in case of proved CVD patients. In our work, we have chosen individuals without any reported history of cardiac disease. A number of articles have been published worldwide regarding association of serum bilirubin and metabolic syndrome. The study group was diagnosed according to NCEP (ATP 111) protocol. There is clear evidence that two groups of subjects are significantly distinct. Fasting plasma glucose, HOMA-IR, waist-hip ratio, BMI, HbA1c, serum homocysteine and fasting serum total bilirubin levels are significantly distinct between control and study group, each parameter is having p value <0.001. There is a report of inverse relationship of fasting serum bilirubin and metabolic syndrome in Korean population.^{28,29} There is another report of increase in serum homocysteine in individuals with metabolic syndrome.¹³ Our study includes a new finding that serum homocysteine has an inverse relationship with fasting serum total bilirubin. Metabolic syndrome is a condition in which there is increase in oxidative stress in the affected subjects. Researchers from other part of the globe also reported increased oxidative stress in metabolic syndrome.^{8,10,13,14} Bilirubin functions as an antioxidant in association with glutathione.³⁰

CONCLUSION

We propose that decrease in fasting serum total bilirubin may be caused by increased oxidative stress in metabolic syndrome and this is inversely related to serum homocysteine which is a marker of CAD. Yet we end this paper with a hope that same work may be continued with a much bigger population sample for better correlation.

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