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Association of Serum Gamma-Glutamyl Transferase Level with Metabolic Syndrome and Insulin Resistance in Adults

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Abstract

Background: MetS is a serious global public health challenge. All the components of MetS are associated with several non-communicable chronic diseases such as T2DM, coronary artery diseases (CAD), cerebrovascular diseases and non-alcoholic fatty liver disease (NAFLD) which are leading cause of death in whole world. Besides these, recent studies have suggested that all components of MetS are independently associated with several cancers. IR is known to play a significant role in the development of MetS.

Materials & Methods: In this study, a total of 110 subjects were selected among them 49 subjects were MetS and 61 were without MetS. Subjects of the study were selected purposively according to the selection criteria from the subjects attending the OPD of Medicine department of SSMC and Mitford Hospital, Dhaka.

Results: Logistic regression analysis showed that after adjustment for age, sex, BMI, ALT, uric acid and LDL-C, the odds ratios (95% CI) for MetS were increased across GGT tertiles (1, 1.22 (0.36-4.12), p=0.738; 5.09 (2.06-12.58), p<0.001). Multiple linear regression analysis showed significant positive association of serum GGT (p<0.001) with HOMA-IR. In model 1, BMI (p<0.05) and TG (p<0.05) showed significant association with HOMA-IR. However, when GGT entered into model 2, the association of TG with HOMA-IR became insignificant and an independent linear association of GGT with HOMA-IR was evident. Insulin, HOMA-IR and all components of MetS except HDL-C are increased in subjects with higher level of GGT.BMI, WC, BP, FPG, insulin, TG and GGT are higher but HDL-C is lower in subjects with MetS and IR as compared to those subjects without MetS and IR. Elevated serum GGT is related with IR and MetS including its components. However, this relationship showed no gender variation. Odds ratios for MetS are increased with increasing tertiles of serum GGT that indicates higher GGT levels are associated with risk for MetS. This association is related with insulin resistance but independent of other confounding factors. Moreover, it is independently associated with insulin resistance.

Conclusion: This study showed that there was strong significant positive correlation of GGT with HOMA-IR and all components of MetS except HDL-C which was negatively correlated with GGT. In either gender, the relationship between serum GGT and the MetS and HOMA-IR components remained substantial.

Keywords: MetS, Gamma Glutamyl Transferase, T2dm, CVD, IR

1. Introduction

Metabolic syndrome (MetS) comprises a combination of several metabolic risk factors including central obesity, high blood glucose, raised blood pressure (BP), elevated serum triglyceride (TG) and low serum high density lipoprotein cholesterol (HDL-C) that directly increase the risk of cardiovascular diseases (CVD), type 2 diabetes mellitus (T2DM) and many other causes of mortality [1]. It usually arises from insulin resistance accompanying abnormal adipose deposition and function [2]. Now a day, MetS is a serious global public health concern. According to International Diabetes Federation [3], approximately

20-25% of the world's adult population have metabolic syndrome, also noticed an increased prevalence of MetS from the age of 20 years to sixth decade of life in US population [4]. The National Cholesterol Education Program-Adult Treatment Panel III (NCEP; ATP III) recommended lipid profile screening starting at age 20, to be repeated every 5 years to decrease the rate of MetS and cardiovascular diseases [5]. An increasing trend of MetS has also been observed in Asian population [6]. Several epidemiological studies showed its increased rate in Bangladeshi adults both in urban and rural areas [7, 8]. Many international organization and expert groups such as the World Health Organization (WHO), American Association of Clinical Endocrinology (AACE), the European Group for the study of Insulin Resistance (EGIR), the NCEP-ATP III, the International Diabetes Federation (IDF) and the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) have attempted to incorporate all the different parameters used to define MetS.

Insulin resistance (IR) is a common condition that plays key role for developing of MetS and strongly associated with an increased risk of diabetes [9]. IR can be defined as the inability of the body to respond fully to insulin[3]. IR is caused by genetic and environmental factors. Impaired insulin sensitivity, also termed insulin resistance, is generally defined as reduced glucose uptake in skeletal muscle, impaired suppression of glucose production by the liver and increased rate of lipolysis in adipose tissue that leads to hyperglycemia and dyslipidemia. IR leads to many of the metabolic abnormalities associated with MetS [10].

Gamma-glutamyl transferase (GGT) is an enzyme located on cell membrane (larger fraction) and cytoplasm (smaller fraction) of different tissues e.g., kidney, liver, pancreas, intestine etc. Renal tissue has the highest concentration of GGT but the enzyme present in serum appears to originate primarily from the hepatobiliary system [11]. Though it is widely used as an indicator of hepatobiliary disease and alcoholism, recent studies have suggested that serum GGT is a sensitive biomarker of oxidative stress and is associated with cardiovascular risk [12]. Serum GGT contributes to the extra cellular catabolism of glutathione. Hydrolysis of glutathione catalyzed by GGT generates reducing substances that causes the reduction of ferric ion to ferrous ion with stepwise production of super oxide and hydrogen peroxide [13]. Elevated levels of GGT thus aggravates oxidative stress that induces inflammation which impairs insulin signaling in the liver, muscle and adipose tissue leading to glucose intolerance and dyslipidemia [14]. Thus GGT has been postulated to be a suitable marker of oxidative stress, inflammation and insulin resistance which are key players in the pathogenesis of MetS [15]. Few prospective studies suggested that serum GGT might be an important predictor for developing of metabolic syndrome and its deleterious consequences like T2DM, CVD, etc [16, 17]. A study reported that increased serum GGT was associated with hypertriglyceridemia, elevated blood glucose and IR [18]. In addition to these, some studies showed that elevated or high normal serum GGT level was strongly associated with the risk of developing hyperglycemia, dyslipidemia, hypertension and obesity [19-20]. Thus, it appears that all of the major components of MetS are linked to the elevation of serum GGT. The aim of this study was to determine the association of serum GGT with MetS and IR.

2. Materials and Methods

It was a cross-sectional analytical study. Department of Biochemistry, Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh. The study was conducted during the period of March' 2019 to Feb' 2020. Study population included subjects attending the outpatient department (OPD) of Medicine of Sir Salimullah Medical College and Mitford Hospital, Dhaka. Sampling technique was Purposive convenient sampling. A total of 110 subjects were recruited to conduct the study. Total 110 subjects were recruited, among them 49 were MetS and 61 were without MetS.

Inclusion criteria of study subjects-

- Apparently healthy subjects of both genders.
- Age range - 20 to 59 years.

Exclusion criteria of study subjects-

- Pregnant and lactating mothers
- Patients with -
  - Acute severe septic condition
  - Cardiovascular disease
  - Liver disease
  - Renal disease
  - Pulmonary disease
  - Chronic debilitating disease: such as malignancy, HIV, etc.
  - Alcoholism, smoking
- Patients receiving drugs that affect liver enzymes.
- Patient using insulin as well as subjects taking oral hypoglycemic agent.

2.1 Study Procedure

This cross-sectional analytical study was conducted from March' 19 to February' 20 among adult individuals. Subjects of the study were selected purposively according to the selection criteria from the subjects attending the OPD of Medicine department of SSMC and Mitford Hospital, Dhaka. Apparently healthy individuals were taken as subjects. Purpose of the study was explained in details and informed written consent was taken from all the study subjects. After proper counseling aim, objectives, and procedure of the study were explained in details to all participants. Only voluntary candidates were recruited as research participant. Socio-demographic as well as other relevant data were taken and recorded in the data collection sheet. A complete physical and relevant clinical examinations were performed and recorded.

2.2 Blood sample collection

Fasting blood samples were collected from all participants. They were allowed to fast overnight (10–12 hours). Blood was collected from the antecubital vein after all aseptic precautions, 5 ml venous blood was taken by sterile disposable syringe. 2 ml of collected blood was taken in a test tube coated with dried sodium fluoride-potassium oxalate mixture and plasma was separated after centrifugation at 3000rpm for five minutes for fasting glucose and insulin. The remaining 3 ml blood was collected in a plain tube. This tube was allowed to stand for 20 to 30 minutes so that blood was cloted properly. Then serum was separated after centrifuging at 3000 rpm for 10 minutes and was collected into eppendorf tubes, labeled properly to measure serum GGT, lipid profile, ALT, uric acid. Samples

were preserved in a deep freezer at-37^{0}C and were analyzed later. Biochemical tests were done in the biochemical laboratory of Sir Salimullah medical college and BSMMU, Dhaka.

2.3 Laboratory analysis

- **Estimation of fasting plasma glucose:** Glucose oxidase method by Humalyzer 3000.
- **Estimation of fasting plasma insulin:** by Chemiluminescent immunoassay by Attelica IM analyzer.
- **Estimation of serum GGT:** By kinetic colorimetric assay.
- **Estimation of serum ALT:** By kinetic colorimetric method.
- **Estimation of serum uric acid:** By uricase method.
- **Estimation of serum total cholesterol:** Enzymatic (CHOD-PAP) method.
- **Estimation of serum TG:** Enzymatic (GPO-PAP) method.
- **Estimation of serum HDL-C:** Enzymatic (CHOD-PAP) method.
- **Determination of serum LDL-C:** LDL–C was calculated from total cholesterol, HDL-C and TG by using Friedwald's formula.

2.4 Data collection and processing

Before collecting specimen, each patient was interviewed and relevant information was recorded systematically in a pre-designed standard data sheet and then data were checked, edited and processed.

2.5 Data analysis

Data were analyzed with the help of software SPSS (Statistical Package for Social Sciences) version 23. The results were expressed as mean ±SD (standard deviation). Unpaired student's t test was performed to compare between MetS and non-MetS subjects as well as between IR and non-resistance subjects. Gender variation of all variables was determined by unpaired student's t test. Serum GGT were divided into tertiles to observe the trend of related variables. ANOVA test was applied to compare three means of quantative variables. Correlation of GGT with components of MetS and HOMA-IR was done by Pearson's correlation test. Odds ratios were calculated for the association of GGT with MetS where multivariate logistic regression analysis was used for adjusted odds ratio. Multiple linear regression analysis was done to observe the association of GGT with HOMA-IR along with confounders. The p-value <0.05 was considered as statistically significant.

3. Results

In this study, a total of 110 subjects were selected among them 49 subjects were MetS and 61 were without MetS.

Table 1: Baseline characteristics of study subjects according to metabolic syndrome (n=110)

Variables	Subjects with MetS (n=49)	Subjects without MetS (n=61)	p-value
Age (years)	40.04±12.77	38.84±12.60	0.621
BMI (kg/m^2)	28.03±04.07	22.00±03.74	<0.001
WC (cm)	102.08±9.69	80.18±07.37	<0.001
SBP (mmHg)	127.76±13.23	110.00±09.13	<0.001
DBP (mmHg)	88.37±10.07	73.52±07.82	<0.001

Data were expressed as mean±SD
Unpaired student t-test was performed to compare between two groups

Table 1 shows mean±SD of age (years), BMI (kg/m^2), WC (cm), SBP (mm of Hg) and DBP (mm of Hg) in study subjects according to metabolic syndrome. Subjects with MetS had significantly (p<0.001) higher BMI, WC and BP than subjects without MetS. There was no significant age difference between two groups.

Table 2: Biochemical parameters of study subjects according to metabolic syndrome (n=110)

Variables	Subjects with MetS (n=49)	Subjects without MetS (n=61)	p-value
FPG (mmol/L)	6.51±1.41	4.78±0.63	<0.001
FPI (µU/ml)	13.47±±5.80	7.39±3.41	<0.001
HOMA-IR	1.85±0.83	0.98±0.47	<0.001
GGT (U/L)	33.18±15.69	17.90±7.15	<0.001
ALT (U/L)	24.78±6.86	23.20±6.00	0.201
Uric acid (mg/dl)	03.96±2.60	03.86±1.75	0.814
TC (mg/dl)	189.47±29.59	168.11±21.11	<0.001
TG (mg/dl)	208.71±55.06	137.41±18.81	<0.001
HDL-C (mg/dl)	32.53±4.91	37.54±5.66	<0.001
LDL-C (mg/dl)	108.65±29.02	104.39±21.19	0.376

Data were expressed as mean±SD
Unpaired student t-test was performed to compare between two groups

Table 2 shows mean±SD of the biochemical parameters in study subjects according to metabolic syndrome. FPG, Insulin and HOMA-IR were significantly (p<0.001) higher in subjects with MetS than in subjects without MetS. GGT was significantly (p<0.001) higher in MetS subjects but there was no significant difference of ALT and uric acid between two groups. Analysis of lipid profile showed that TC and TG were significantly higher whereas HDL-C was significantly lower in subjects with MetS than in subjects without MetS (p<0.001) and there was no significant difference of LDL-C.

Table 3: Characteristics of study subjects according to insulin resistance (n=110)

Variables	Subjects with IR (n=44)	Subjects without IR (n=66)	p-value
Age (years)	39.59±12.37	39.23±12.90	0.883
BMI (kg/m^2)	28.09±4.28	22.42±3.90	<0.001
WC (cm)	99.32±12.51	83.68±10.82	<0.001
SBP (mmHg)	127.73±13.57	111.36±10.36	<0.001
DBP (mmHg)	88.41±9.63	74.62±9.21	<0.001
FPG (mmol/L)	6.51±1.51	4.91±0.72	<0.001
Insulin (µU/ml)	15.30±4.63	6.63±2.50	<0.001
GGT (U/L)	34.07±15.33	18.47±8.50	<0.001
ALT (U/L)	24.57±6.57	23.45±6.32	0.375
Uric acid (mg/dl)	3.95±2.43	3.87±1.98	0.845
TC (mg/dl)	193.73±28.60	166.89±20.38	<0.001
TG (mg/dl)	206.75±57.90	144.12±29.66	<0.001
HDL-C (mg/dl)	31.73±4.28	37.70±5.59	<0.001
LDL-C (mg/dl)	112.77±27.43	101.97±22.34	<0.05

Data were expressed as mean±SD
Unpaired student t-test was done to compare between two groups.

Table 3 shows mean±SD of various parameters in study subjects according to insulin resistance. Subjects with IR had significantly (p<0.001) higher BMI, WC and BP than

subjects without IR. There was no significant age difference between two groups. Serum GGT was significantly ($p<0.001$) higher in IR subjects than in subjects without IR. All components of lipid profile showed significant difference between two groups. Level of serum ALT and uric acid did not differ between insulin resistance and non-resistance subjects.

Table 4 shows characteristics of all study population, male subjects and female subjects. There was no significant gender variation of any parameter except BMI which was higher ($p<0.05$) in female.

Table 4: Characteristics of all study population, male subjects and female subjects (n=110)

Variables	Total (n=110)	Male (n=58)	Female (n=52)	p-value
Age (years)	39.37±12.63	41.07±12.94	37.48±12.13	0.138
BMI (kg/m^2)	24.68±4.91	23.63±4.27	25.86±5.33	<0.05
WC (cm)	89.94±13.81	92.62±11.85	88.69±16.20	0.147
SBP (mmHg)	117.91±14.20	118.10±12.31	117.50±16.19	0.825
DBP (mmHg)	80.14±11.54	80.52±10.33	79.52±12.77	0.652
FPG (mmol/L)	5.55±1.35	5.53±1.30	5.60±1.43	0.795
FPI (µU/ml)	10.10±5.51	10.63±5.90	9.51±5.04	0.287
HOMA-IR	1.37±0.79	1.44±0.77	1.29±0.80	0.341
GGT (U/L)	24.71±13.95	25.86±14.58	23.42±13.24	0.363
ALT (U/L)	23.90±6.42	23.98±6.67	23.81±6.19	0.887
Uric acid (mg/dl)	3.90±2.16	4.12±2.39	3.66±1.86	0.266
TC (mg/dl)	177.63±27.29	177.98±27.86	177.23±26.90	0.886
TG (mg/dl)	169.17±52.89	173.12±55.34	166.96±51.14	0.547
HDL-C (mg/dl)	35.31±5.88	34.46±6.82	36.07±4.81	0.153
LDL-C (mg/dl)	106.29±24.95	105.45±28.08	107.23±21.16	0.710

Data were expressed as mean±SD
Unpaired student t-test was done to compare between males and females

Table 5a: Characteristics of study subjects according to tertiles of serum GGT (n=110)

Variables	Serum GGT tertile (U/L)			p-value
	Tertile 1(<18) (n=37)	Tertile 2 (18-27) (n=37)	Tertile 3 (>27) (n=36)	
Age (years)	38.42±12.85	37.68±13.30	40.58±11.86	0.593
BMI (kg/m^2)	21.65±3.68	25.04±4.83	27.44±4.41	<0.001
WC (cm)	82.03±9.51	87.54±12.26	102.72±11.16	<0.001
SBP (mmHg)	108.11±10.76	115.68±6.03	130.00±14.69	<0.001
DBP (mmHg)	72.57±9.10	78.38±8.34	89.44±10.13	<0.001
FPG (mmol/L)	4.57±0.71	5.31±0.47	6.84±1.49	<0.001
FPI (µU/ml)	6.60±3.11	9.61±4.24	14.19±5.98	<0.001
HOMA-IR	0.84±0.44	1.29±0.54	1.99±0.85	<0.001
ALT (U/L)	21.24±5.50	25.16±6.66	25.33±6.34	<0.05
Uric acid (mg/dl)	3.57±1.67	4.02±2.20	4.12±2.55	0.506
TC (mg/dl)	171.05±22.86	175.22±21.89	186.86±33.90	<0.05
TG (mg/dl)	131.86±20.63	154.92±21.61	225.33±54.63	<0.001
HDL-C (mg/dl)	39.95±4.82	35.46±4.26	30.39±4.16	<0.001
LDL-C (mg/dl)	104.51±23.41	107.22±20.21	107.17±30.82	0.870

Data were expressed as mean±SD
ANOVA test was done to compare among three groups

Table 5a shows characteristics of study subjects according to tertiles of serum GGT. There was no significant age difference among subjects of different tertiles of GGT. Subjects in higher tertile had significantly higher BMI, WC, BP, FPG, Insulin, HOMA-IR, ALT, TC, TG and lower HDL-C. Serum uric acid levels did not differ among different tertiles of GGT.

Table 5b: Post-hoc (Bonferroni test) for multiple comparison between groups based on GGT tertiles

Variables	Post-hoc test		
	Tertile 1 vs Tertile 2 p-value	Tertile 1 vs Tertile 3 p-value	Tertile 2 vs Tertile 3 p-value
WC (cm)	0.102	<0.001	<0.001
SBP (mmHg)	<0.05	<0.001	<0.001
DBP (mmHg)	<0.05	<0.001	<0.001
FPG (mmol/L)	<0.01	<0.001	<0.001
HOMA-IR	<0.01	<0.001	<0.001
ALT (U/L)	<0.05	<0.05	1.000
TG (mg/dl)	<0.05	<0.001	<0.001
HDL-C (mg/dl)	<0.001	<0.001	<0.001

Table 5b shows comparison between two groups using Bonferroni test. It showed that WC was significantly higher in tertile 3 in comparison to tertile 1 and tertile 2 but its values did not differ significantly between tertile 1 and tertile 2. BP, FPG, HOMA-IR, ALT, TG were significantly higher and HDL-C was significantly lower in tertile 3 and tertile 2 in comparison to tertile 1. These parameters except ALT differed significantly between tertile 2 and tertile 3.

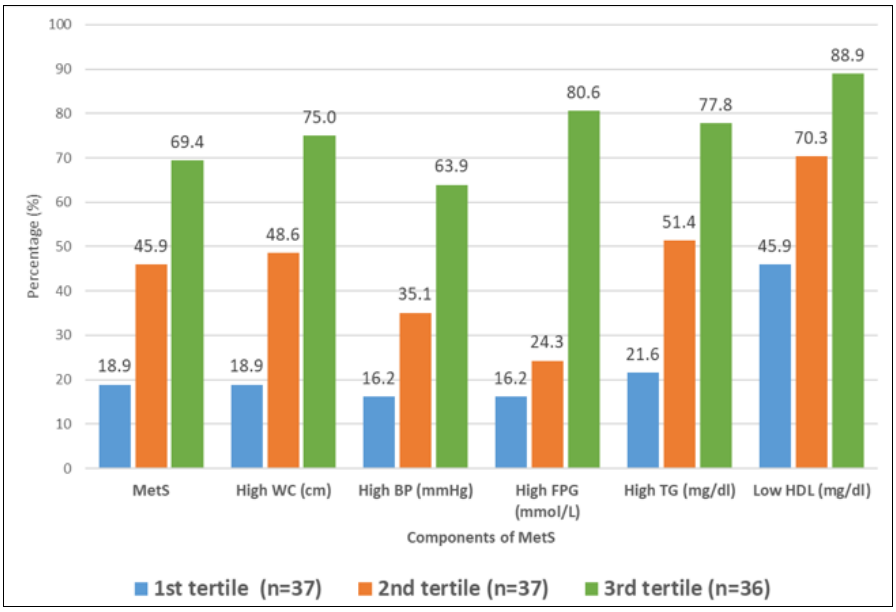

Fig 1: Proportion of MetS and its components according to tertiles of serum GGT

Table 6a: Characteristics of male subjects according to tertiles of serum GGT (n=58)

Variables	Serum GGT (U/L)			p-value
	Tertile 1 (<18) (n=19)	Tertile 2 (18-29) (n=19)	Tertile 3 (>29) (n=20)	
Age (years)	38.89±13.13	40.95±14.12	43.70±11.65	0.452
BMI (kg/m^2)	20.74±3.03	23.97±3.65	26.05±4.33	<0.001
WC (cm)	82.37±7.02	88.05±8.51	100.90±10.94	<0.001
SBP (mmHg)	109.47±9.11	116.32±4.96	128.00±13.12	<0.001
DBP (mmHg)	74.74±9.64	77.89±7.87	88.50±8.13	<0.001
FPG (mmol/L)	4.57±0.59	5.13±0.51	6.81±1.30	<0.001
FPI(µU/ml)	6.81±3.35	9.66±4.49	15.19±6.10	<0.001
HOMA-IR	0.87±0.44	1.33±0.49	2.08±0.79	<0.001
ALT (U/L)	20.95±6.10	25.84±7.13	25.10±6.00	<0.05
Uric acid (mg/dl)	3.87±1.58	4.28±2.41	4.21±3.03	0.856
TC (mg/dl)	170.63±27.14	174.63±22.60	188.15±31.20	0.118
TG (mg/dl)	128.63±22.58	159.68±19.54	228.15±54.74	<0.001
HDL-C (mg/dl)	39.37±3.34	37.79±3.19	31.30±3.39	<0.001
LDL-C (mg/dl)	103.89±26.60	106.74±22.80	105.70±34.62	0.953

Data were expressed as mean±SD

ANOVA test was done to compare among three groups.

Table 6b: Post-hoc analysis (Bonferroni test) for multiple comparisons between groups in male subjects

Variables	Post-hoc test		
	Tertile 1 vs Tertile 2 p-value	Tertile 1 vs Tertile 3 p-value	Tertile 2 vs Tertile 3 p-value
WC (cm)	0.171	<0.001	<0.001
SBP (mmHg)	0.104	<0.001	<0.01
DBP (mmHg)	0.784	<0.001	<0.01
FPG (mmol/L)	0.170	<0.001	<0.001
HOMA-IR	0.064	<0.001	<0.01
ALT (U/L)	0.067	0.145	1.000
TG (mg/dl)	<0.05	<0.001	<0.001
HDL-C (mg/dl)	0.441	<0.001	<0.001

Post-hoc for multiple comparisons between groups

Fig 1 shows proportion of MetS and its components according to tertiles of serum GGT. The proportion of MetS and each component of MetS was increased with increasing tertiles of GGT.

Table 6a shows characteristics of male according to tertiles of serum GGT. There was no significant age difference among subjects of different tertiles of GGT. Subjects in higher tertile had significantly higher BMI, WC, BP, FPG, Insulin, HOMA-IR, ALT, TG and lower HDL-C. Serum uric acid levels did not differ among different tertiles of GGT.

Table 6b shows comparison between two groups using Bonferroni test. It showed that WC, BP, FPG, HOMA-IR and TG were significantly higher and HDL-C was significantly lower in tertile 3 in comparison to tertile 1 and tertile 2 but values of these parameters except TG did not differ significantly between tertile 1 and tertile 2.

Table 7a: Characteristics of female subjects according to tertiles of serum GGT (n=52)

Variables	Serum GGT (U/L)			p-value
	Tertile 1 (<18) (n=17)	Tertile 2 (18-23) (n=17)	Tertile 3 (>23) (n=18)	
Age (years)	37.11±10.43	35.71±13.21	38.65±12.65	0.562
BMI (kg/m^2)	22.44±4.18	24.75±5.21	30.15±3.29	<0.001
WC (cm)	81.41±12.10	83.06±12.95	102.28±13.20	<0.001
SBP (mmHg)	106.47±12.72	114.71±7.17	131.11±16.05	<0.001
DBP (mmHg)	70.29±8.38	77.65±8.31	90.56±12.11	<0.001
FPG (mmol/L)	4.62±0.83	5.22±0.38	6.81±1.64	<0.001
FPI (µU/ml)	6.26±2.94	8.65±3.71	13.38±5.27	<0.001
HOMA-IR	0.80±0.47	1.11±0.52	1.94±0.87	<0.001
ALT (U/L)	21.53±5.11	23.88±6.80	25.89±6.08	0.113
Uric acid (mg/dl)	3.22±1.80	4.16±2.06	3.60±1.70	0.334
TC (mg/dl)	172.18±18.39	170.71±20.50	188.17±35.53	1.000
TG (mg/dl)	135.12±18.91	148.76±18.39	207.89±61.58	<0.001
HDL-C (mg/dl)	40.94±6.00	34.12±4.55	28.67±2.91	<0.001
LDL-C (mg/dl)	105.82±20.69	107.71±17.98	108.11±25.15	0.946

Data were expressed as mean±SD
ANOVA test was done to compare among three groups.

Table 7b: Post-hoc analysis (Bonferroni test) for multiple comparison between groups in female subjects

Variables	Post-hoc test		
	Tertile 1 vs Tertile 2 p-value	Tertile 1 vs Tertile 3 p-value	Tertile 2 vs Tertile 3 p-value
WC (cm)	1.000	<0.001	<0.001
SBP (mmHg)	0.188	<0.001	<0.01
DBP (mmHg)	0.101	<0.001	<0.001
FPG (mmol/L)	0.355	<0.001	<0.001
HOMA-IR	0.532	<0.001	<0.01
TG (mg/dl)	0.948	<0.001	<0.001
HDL-C (mg/dl)	<0.001	<0.001	<0.05

Post-hoc for multiple comparison between groups

Table 8: Correlation of serum GGT with components of MetS and HOMA-IR

Variables	All subjects		Male		Female	
	r-value	p-value	r-value	p-value	r-value	p-value
WC (cm)	+.670	<0.001	+.731	<0.001	+.614	<0.001
SBP (mmHg)	+.735	<0.001	+.764	<0.001	+.729	<0.001
DBP (mmHg)	+.628	<0.001	+.557	<0.05	+.707	<0.001
FPG (mmol/L)	+.804	<0.001	+.820	<0.001	+.806	<0.001
TG (mg/dl)	+.823	<0.001	+.842	<0.001	+.793	<0.001
HDL-C (mg/dl)	-.619	<0.001	-.663	<0.001	-.656	<0.001
HOMA-IR	+.567	<0.001	+.511	<0.05	+.652	<0.001

Correlations were determined by Pearson's correlation coefficient test

Table 7a shows characteristics of female according to tertiles of serum GGT. There was no significant age difference among subjects of different tertiles of GGT. Subjects in higher tertile had significantly higher BMI, WC, BP, FPG, Insulin, HOMA-IR, TG and lower HDL-C. Levels of serum ALT and uric acid did not differ among tertiles of GGT.

Table 7b shows comparison between two groups were using Bonferroni test. It showed that WC, BP, FPG, HOMA-IR and TG were significantly higher and HDL-C was significantly lower in tertile 3 in comparison to tertile 1 and tertile 2 but values of these parameters except HDL-C did not differ significantly between tertile 1 and 2.

Table 8 shows correlation of GGT with components of MetS and HOMA-IR. Significant correlations of GGT with components of MetS and HOMA-IR were observed in all subjects. These correlations were also significant among male and female subjects.

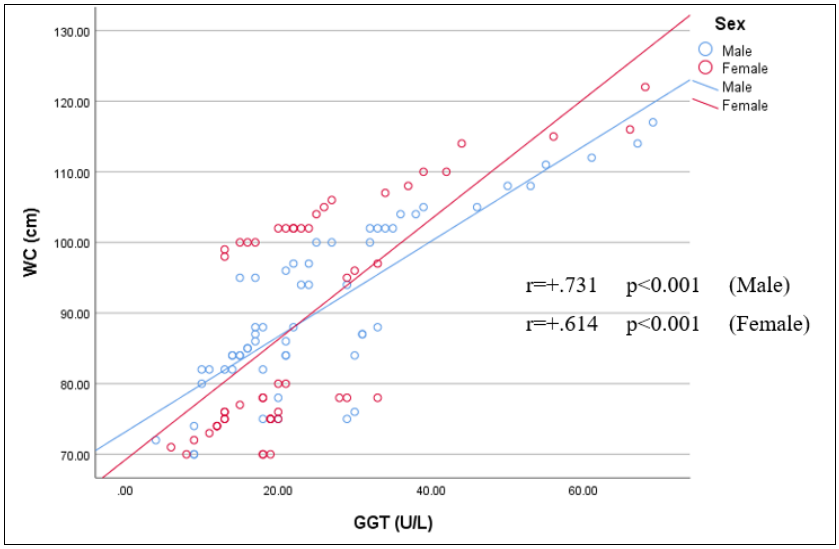

Fig 2: Correlation of serum GGT with WC in male and female

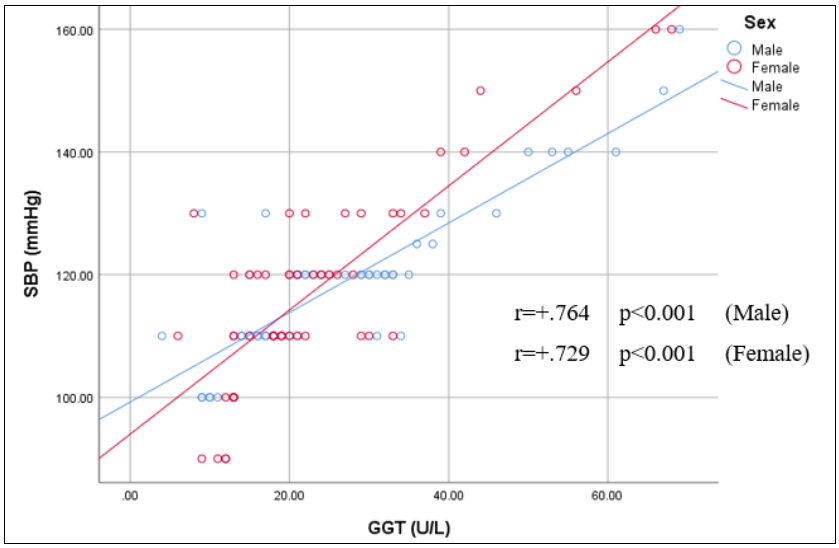

Fig 3: Correlation of serum GGT with SBP in male and female

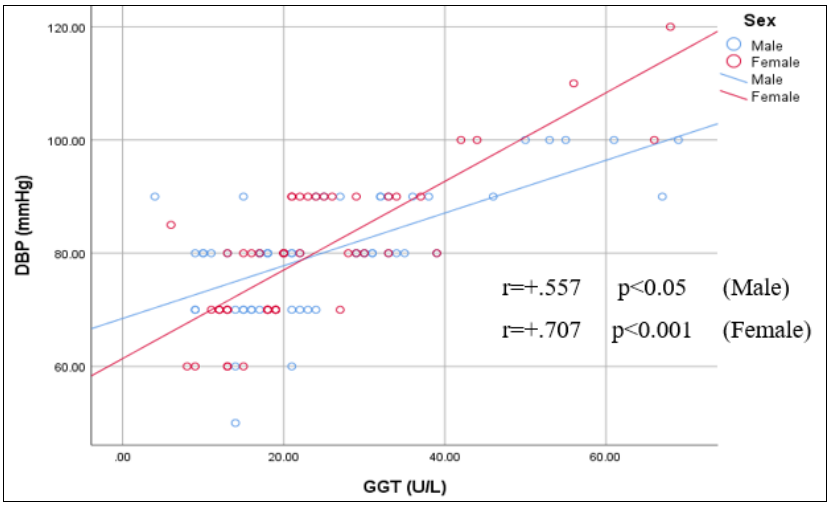

Fig 4: Correlation of serum GGT with DBP in male and female

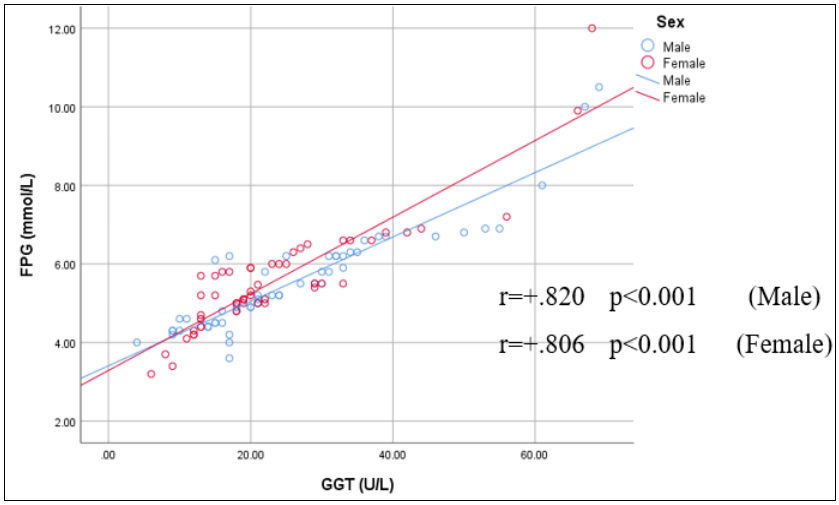

Fig 5: Correlation of serum GGT with FPG in male and female

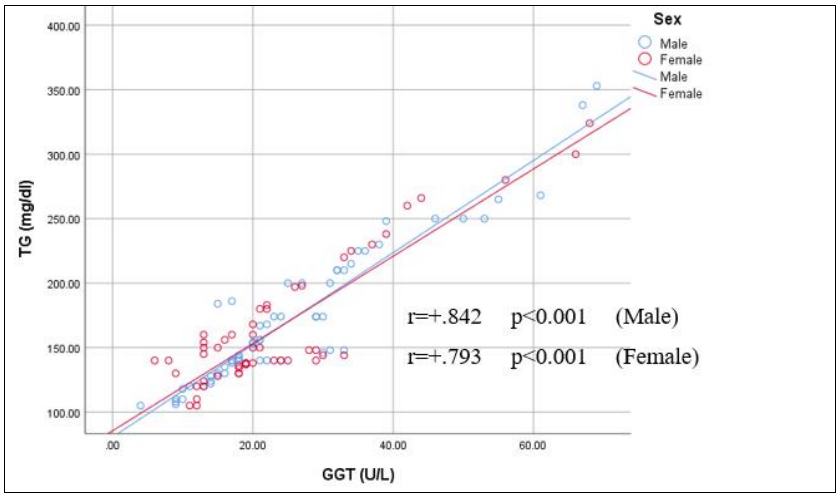

Fig 6: Correlation of serum GGT with TG in male and female

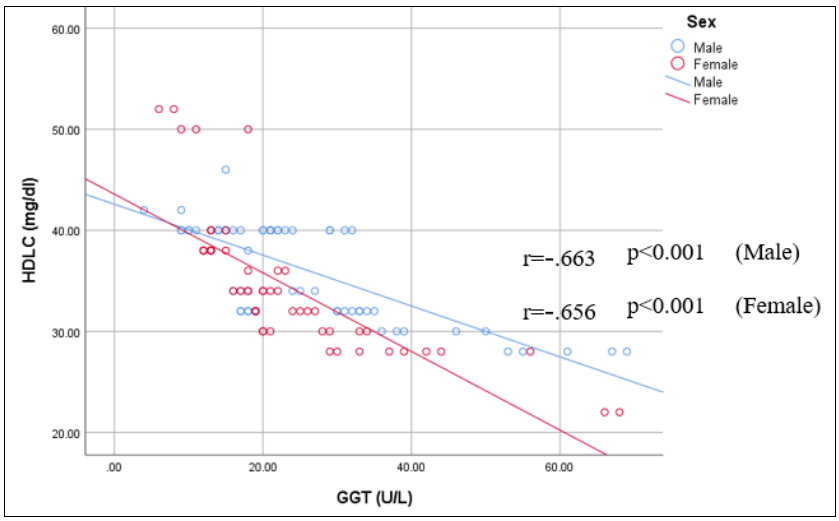

Fig 7: Correlation of serum GGT with HDL-C in male and female

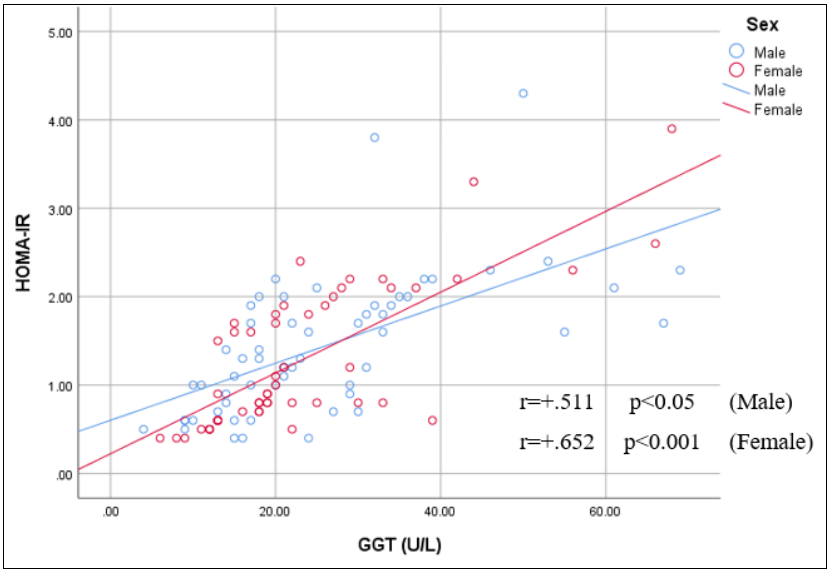

Fig 8: Correlation of serum GGT with HOMA-IR in male and female

Fig 2 showing the correlation between GGT and WC in male and female. It was evident that the two variables were positively and significantly (p<0.001) correlated in both gender.

Fig 3 showing the correlation between GGT and SBP in male and female. It was observed that the two variables were positively and significantly (p<0.001) correlated in both sex.

Fig 4 showing the correlation between GGT and DBP in male and female. It was evident that the two variables were positively and significantly correlated both in male (p<0.05) and female (p<0.001).

Fig 5 showing the correlation between GGT and FPG in male and female. It was evident that the two variables were positively and significantly (p<0.001) correlated in both sex.

Fig 6 showing the correlation between GGT and TG in male and female. It was evident that the two variables were positively and significantly (p<0.001) correlated in either gender.

Fig 7 showing the correlation between GGT and HDL-C in male and female. It was evident that the two variables were negatively and significantly (p<0.001) correlated in both sex.

Fig 8 showing the correlation between GGT and HOMA-IR in male and female. It was evident that the two variables were positively and significantly correlated both in male (p<0.05) and female (p<0.001).

Table 9: Odds ratio (95% CI) for MetS according to tertiles of serum GGT

		Tertile 1 (n=37)	Tertile 2 (n=37)	Tertile 3 (n=36)
	Cases, N (%)	7 (18.9%)	17 (45.9%)	25 (69.4%)
Model 1	OR (95% CI) p-value	1	3.64 (1.29-10.37) <0.05	9.74 (3.28-28.6) <0.001
Model 2	OR (95% CI) p-value	1	2.99 (1.11-8.13) <0.05	8.12(2.74-16.52) <0.001
Model 3	OR (95% CI) p-value	1	1.22 (0.36-4.12) 0.738	5.09 (2.06-12.58) <0.001
Model 4	OR (95% CI) p-value	1	1.08 (0.16-7.29) 0.935	2.20 (0.55-8.87) 0.266

Logistic regression analysis was done for adjusted odds ratio

Model 1: Non-adjusted
Model 2: Adjusted for age and sex.
Model 3: Adjusted for age, sex, BMI, ALT, uric acid and LDL-C.
Model 4: Adjusted for age, sex and HOMA-IR.

Table 9 shows Odds ratio (95% CI) for MetS according to tertiles of serum GGT where lowest tertile was considered as reference category (OR=1). Odds ratios of tertile2 and tertile 3 were significantly increased as compared to tertile 1 in model 1(non-adjusted) and model 2(adjusted for age and gender). In model 3, when BMI, ALT, uric acid and LDL-C were adjusted along with age and gender, the odds ratios were attenuated and the OR of tertile 2 was no longer significant but the OR of tertile 3 was still significant. However, in model 4, when only HOMA-IR was adjusted along with age and sex, the odds ratios were further attenuated and the odds ratios of both tertile became insignificant.

Table 10: Multiple Linear regression analysis of the relation between HOMA-IR and variables of interest in study subjects (n=110)

Variables	Model 1 (R^2=0.608)		Model 2 (R^2=0.606)	
	β	p-value	B	p-value
Age (years)	.004	0.327	.003	0.389
Sex	-.177	0.096	-.152	0.161
BMI (kg/m2)	.040	<0.05	.038	<0.05
WC (cm)	.013	0.059	.011	0.099
SBP (mmHg)	.004	0.518	.003	0.659
DBP (mmHg)	.006	0.385	.002	0.815
TC (mg/dl)	.010	0.221	.008	0.240
TG (mg/dl)	.014	<0.05	.011	0.071
HDL-C (mg/dl)	-.008	0.498	-.001	0.962
LDL-C (mg/dl)	.003	0.394	.001	0.874
ALT (U/L)	.002	0.832	.001	0.934
Uric acid (mg/dl)	.009	0.119	.009	0.117
GGT (U/L)			.022	<0.001

a. Dependent variable: HOMA-IR

Table 10 shows the results of multiple linear regression analysis of HOMA-IR with mentioned confounding independent variables. We observed a positive association of HOMA-IR with BMI ($p<0.05$) and TG ($p<0.05$) in model 1. However, when serum GGT entered into model 2, only BMI remained significant ($p<0.05$) and an independent linear association of serum GGT with HOMA-IR was evident ($p<0.001$).

The analysis was first conducted including all variables except serum GGT (Model 1), then repeated with serum GGT forced into the (Model 2). β for standardized coefficient. R^2 for adjusted R square (multiple coefficients of determination).

4. Discussion

This cross-sectional analytical study was conducted in the department of Biochemistry, Sir Salimullah Medical College Mitford Hospital, and Dhaka, Bangladesh during the period of March ’19 to Feb ’20. The main objective of the study was to find out the association of serum GGT with MetS and IR in adult’s Bangladeshi population. The study population comprised of 110 apparently healthy adult subjects: among them 58 were male and 52 were female. After anthropometric measurement, BP checkup and biochemical tests, 49 subjects were diagnosed as MetS and remaining 61 were non-MetS. According to the cut-off value of HOMA-IR, 44 were IR and 66 were without IR.

In this study, there was no significant gender variation of the parameters except BMI which was higher in female than male (Table IV). GGT, BMI, WC and BP were significantly higher in subjects with MetS than subjects without MetS (Table I and II). Participants belonged to higher tertile of GGT had significantly higher BMI, WC and BP (Table 5, 6 and 7). Significant positive correlations of GGT with WC and BP were observed in this study (Table 8). All of these findings indicated that elevated level of GGT had an association with abdominal obesity and high BP. These findings were consistent with study Serum gamma-glutamyl transferase levels are associated with metabolic syndrome in community-dwelling individuals [19] and the associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase [21]. According to their observations, obesity and hypertension are associated with oxidative stress and IR, influenced by serum GGT.

Serum TG and TC were significantly higher whereas HDL-C was significantly lower in subjects with MetS than non-MetS subjets (Table 2). Subjects in higher GGT tertiles had higher TG and TC but lower HDL-C (Table 5, 6 and 7). Serum GGT concentration showed significant positive correlation with TAG but negative correlation with HDL-C (Table VIII). Similar results were evident in the study named by Association of Gamma-glutamyl transferase with metabolic syndrome [22]. In this study, serum GGT also had a linear relationship with IR (Table 10). IR in adipose tissue may lead to increased lipolysis [23]. The increased flux of FFA from the peripheral tissue to the liver in the IR state stimulates increased hepatic TG synthesis, which in turn inhances the assembly and secretion of VLDL from hepatocyte to systemic circulation [24]. Low HDL-C observed in MetS is considered as secondary to raised TG in blood. In the presence of increased serum TG level, the cholesteryl ester transfers protein (CETP) mediates exchange of TG and cholesteryl ester between LDL and VLDL as well as between VLDL and HDL particles forming TG rich HDL that are more prone to be catabolized [25].

It was evident from the study that subjects with insulin resistance had significantly higher GGT and all components of MetS except HDL-C than subjects without IR (Table III). Participants belonged to higher tertile of GGT had significant higher FPG, insulin and HOMA-IR (Table 5, 6 and 7). Significant positive correlations of GGT with FPG and HOMA-IR were observed in this study (Table 8) suggested an association of GGT with IR. These findings were consistent with the study of Aminotransferase and gamma-glutamyl transpeptidase levels in obesity are associated with insulin resistance and the metabolic syndrome[18]. Also noticed serum GGT had a closer relationship with hepatic insulin resistance regardless of the presence of nonalcoholic fatty liver disease (NAFLD) [26].

In this study, significant correlations of serum GGT with each component of MetS and HOM-IR were observed (Table 8). The odds ratios for MetS were increased with increasing tertiles of serum GGT (Table 9). Serum GGT level was significantly associated with a risk for MetS even after adjusting for age, gender, BMI, ALT, uric acid and LDL-C. After adjustment for only HOMA-IR along with age and gender, the association was attenuated and became insignificant which may indicate that the association was related with insulin resistance but was independent of other confounding factors. This finding explores an important role of HOMA-IR on MetS. This is supported by the observation of multiple linear regression analysis where a significant independent association of serum GGT with HOMA-IR was evident (Table 10). Similar results were evident in a community based cross sectional study on Japanese adults [19]. In a study on 2,579 MetS free Korean adults also demonstrated that serum GGT may be an important predictor for developing MetS. After adjustment for age, sex, alcohol consumption, smoking and family history of DM, higher serum GGT was related to the risk of developing MetS [17].

Serum GGT is a sensitive indicator of biliary obstruction and alcohol consumption. It is also high in patients with other liver diseases e.g., primary biliary cirrhosis (PBC), viral hepatitis, fatty liver disease or drug induced liver injury. These liver diseases are present in community-dwelling individuals and may be asymptomatic [27]; hence

our study participants might include patients with subclinical liver diseases. Nevertheless, the influence of these liver diseases might be small because PBC is rare and history of viral hepatitis were taken and excluded. Further, the relationship between serum GGT and MetS persisted after adjusting for ALT.

The mechanism by which GGT reflects the risk of MetS and IR are not completely understood. Increase in serum GGT activity is responsible for catabolism of glutathione [28]. Hydrolysis of glutathione catalyzed by GGT generates reducing substances that cause the reduction of ferric ion to ferrous ion with stepwise production of super oxide and hydrogen peroxide [14]. Elevated level of GGT thus aggravates oxidative stress that induces inflammation which impairs insulin signaling in the liver, muscle and adipose tissue that leads to MetS [13]. Moreover, subjects with high GGT, even within the normal reference level often exhibit hepatic steatosis which is closely related to the accumulation of visceral fat and increased lipolysis [29]. Hepatic steatosis itself can lead to hepatic insulin resistance and long-term hepatic IR may lead to metabolic abnormalities [30]. Additionally, the inflammatory reactions that are activated by elevated GGT impair insulin signaling in liver and other organs [31].

It can be concluded from the study that serum GGT level is associated with MetS and IR. This association is independent of other confounding factors. So, serum GGT can be considered as an independent predictor of MetS.

5. Conclusion

Subjects with Mets and IR had significantly higher serum GGT than subjects without MetS and without IR respectively. There was no significant gender variation of all variables except BMI which was higher in female. Subjects in higher tertiles of GGT had significantly higher BMI, WC, BP, FPG, insulin, HOMA-IR, TC, TG and lower HDL-C. The variations of parameters based on GGT tertile were almost similar in both gender. Proportion of MetS and its components were increased with increasing tertiles of serum GGT. This study showed that there was strong significant positive correlation of GGT with HOMA-IR and all components of MetS except HDL-C which was negatively correlated with GGT. Relationship of serum GGT with components of MetS and HOMA-IR remained significant in either gender.

6. Limitations

- This study was conducted in only one centre that was not representing population.
- The sampling technique was purposive, so there may be chance of bias which can influence the result.
- It was an analytical study with cross-sectional design; thus, no causal association of GGT with MetS and IR could be explored.
- Ultrasonography or fibro scan was not possible to perform to exclude asymptomatic fatty liver.
- History for viral hepatitis was taken but investigations for hepatitis B surface antigen, hepatitis C antibody were not performed.

7. Recommendations

The following recommendations can be suggested based on the present study:

- A large population based and multi centered study should be undertaken.
- Subjects should be selected by random sampling to avoid biasness.
- Subjects with asymptomatic fatty liver disease and viral hepatitis to be excluded following necessary investigations.
- For community-dwelling healthy individuals, prospective population-based studies are needed to investigate the mechanism underlying the association of GGT with MetS and IR.
- GGT can be used as low-cost biomarker that may help in prediction for development of MetS and thereby reducing the complications and consequences of MetS.

Statement of Ethical approval

This study was approved by the Institutional Ethics Committee of Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh.

Disclosure of conflict of interest

No competing interests exist by the authors. This manuscript has not submitted to, nor is under review at another journal or other publishing venue.

Statement of informed consent

"Informed consent was obtained from all individual participants included in the study."

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