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Research Article

"Clinical & Demographic Profile with Serum Homocysteine Level among Type II Diabetic Patients and IGT Patients with Normal People"

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Abstract: ***Background:*** Detrimental effects of homocysteine on endothelial function are well documented. Plasma Homocysteine (Hcy) levels are elevated in type 2 diabetic patients as well as in pre-diabetic individuals with insulin resistance. In such individuals, plasma Hcy concentration is influenced by the insulin concentration and anti-diabetic therapy such as metformin, glitazones or insulin that can alter the plasma plasma homocysteine. ***Objective:*** To find out the Clinical & Demographic Profile with Serum Homocysteine Level among Type II Diabetic Patients and IGT Patients with Normal People. ***Methods:*** This was a cross-sectional observational study. Total 120 subjects were selected and allocated into three groups, equal number (n=40) in each group. This study was conducted in Mymensingh Medical College Hospital, Mymensingh, Bangladesh from June, 2019 to May, 2020. ***Results:*** The study showed that the mean age of the patients and their BMI were almost similar among the three groups, male female ratio was about 2.5:1. The ccorrelation analysis of different clinical & demographic profile with serum Homocysteine. The correlation between glycemic status and Serum Homocysteine levels of participants, which differ considerably, so that, Pearson correlation & Spearman correlation showed statistically significant difference. Hcy was positively associated with FBG (r=0.296, p<0.001), 2hPG (r=0.078, p=0.004). Similarly positive significant correlation was found with TC (r=7.655, p<0.001), TG (r=13.52, p=<0.05) and HDL-C (r=1.165, p<0.05). Present study showed that levels of Hcy were significantly higher in the type 2 DM group than those in normoglycemic group (19.86 µmol/L *vs.* 8.72 µmol/L, p<0.001). Similarly levels of Hcy were significantly higher in the IGT group than those in normoglycemic group (17.28 µmol/L *vs.* 8.72 µmol/l, p<0.001). The mean values of the serum Homocysteine level of Group -1 and Group – 2 showed statistical significant differences with group-3. In this study, serum Homocysteine levels shows strongly positive correlation with hyperglycemia. ***Conclusions*****:** Present study showed that mean values of the Serum Homocysteine levels were considerably lower in normoglycemic group. Patients with type II DM & IGT, have higher homocysteine levels, which differ considerably to show statistically significant difference. Numerous studies have shown altered serum Hcy concentrations in T2DM & IGT patients.

Keywords: Diabetes mellitus, Serum Homocysteine, Impaired glucose tolerance.

INTRODUCTION

Diabetes Mellitus (DM) is one of major public health concerns in both developing and developed countries. It is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control [1]. At present it is estimated that about 7.1 million cases of diabetes in Bangladesh in 2015 and latest data suggested that prevalence of diabetes in adult (20-59 years) is 7.4% [2]. Type-2 DM is defined as chronic hyperglycemia resulting from either decreased insulin secretion, impaired insulin action or both, in the absence of autoimmune destruction of the pancreatic β-

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cell [3]. Impaired glucose tolerance (IGT), is a pre-diabetic state of dysglycemia that is associated with insulin resistance and increased risk of cardiovascular pathology. IGT is characterized by high postprandial blood glucose level and it may precede type 2 diabetes mellitus by many years. Both diabetic and IGT individuals are highly prone to cardiovascular diseases (MI, Stroke, PVD) and both are frequently related with more damaging effects on the vasculature, including the activation of abnormal vasomotion, up-regulated inflammatory responses, increased oxidative stress, and an extensive procoagulant state [4]. Serum Hcy evaluation may serve to identify diabetic patients predisposed to vascular complications and more importantly the group of patients that may benefit from intensified screening and treatment strategies. The aim of this study was to compare the serum homocysteine level in type 2 Diabetic & IGT patients with normal people. So that preventive measures can be taken at an early stage. However, the mechanism by which IGT interferes with the cardiovascular system has not been fully defined, hence it is necessary to search for advanced markers to assess the risk of cardiovascular complications of Diabetes Mellitus and IGT. During the past 2 decades, hyperhomocysteinemia has emerged as a risk factor for diabetes mellitus, cardiovascular diseases and stroke [5, 6]. Detrimental effects of homocysteine on endothelial function are well documented. Plasma Homocysteine (Hcy) levels are elevated in type 2 diabetic patients as well as in pre-diabetic individuals with insulin resistance [5]. In such individuals, plasma Hcy concentration is influenced by the insulin concentration and anti-diabetic therapy such as metformin, glitazones or insulin that can alter the plasma homocysteine [7]. The results of the positive link between Hcy levels and insulin values and insulin resistance might partially explain the potential mechanism by which HHcy stimulates the earlier initiation of hyperglycemia. Recent studies have documented that insulin resistance is an independent risk factor for the progression of abnormal glucose metabolism [8]. Oxidative stress might also be involved in the precise mechanism linking HHcy to abnormal glycometabolism. HHcy inhibits the synthesis of glutathione (GSH), the major intracellular antioxidant. The depletion of GSH is related to high oxidative stress [10]. Several studies have suggested that oxidative stress occurs in the context of type 2 diabetes pathology [9, 11]. In addition, high levels of plasma homocysteine are known to exert an adverse effect through a mechanism involving oxidative damage [12].

MATERIAL AND METHODS

This was a cross-sectional observational study. Total 120 subjects were selected and allocated into three groups, equal number (n=40) in each group. This study was conducted in Mymensingh Medical College Hospital from June, 2019 to May, 2020. Similar number of nornal subjects were recruited as control. So, 120 cases included among them 40 type 2 diabetic pts, 40 IGT pts. and 40 normal people (control group). Patients with IGT & type 2 Diabetes mellitus were approached and were selected in according to the inclusion and exclusion criteria. Subjects were briefed about the objectives of the study, risk and benefits, freedom for participating in the study and confidentiality. Informed consent was obtained accordingly. Subjects were grouped into three groups, group 1 (patients with DM), group-2 (patients with IGT) & group-3 (normoglycemic people). Compare of homocysteine was investigated among the groups. With all aseptic precaution 5 mL venous blood was drawn from anticubital vein after in a disposable plastic syringe and delivered immediately into a clean dry heparinized tube. Then plasma was separated after centrifuging at 3000 rpm for 5 minutes & collected in ependrop tube, label properly and store in ultra-freezer at -35^0C and all the biochemical tests. Blood samples was collected from study subjects to estimate the fasting blood glucose, serum homocysteine, Fasting lipid profile and then 2 hours after postprandial glucose status was observed. Collected data were recorded in a separate case record form. Hcy concentrations were determined by the clinical chemistry method. Blood glucose levels were detected using the glucose oxidase method. Blood lipids were detected as follows: TC was measured by an enzymatic cholesterol oxidase reaction, HDL-C and LDL- C were measured by the direct assay and TG was measured by a glycerol lipase oxidase reaction. Serum glucose, lipid, and Hcy levels were analyzed using a Dade Behring Dimension RXL Max Chemistry Analyzer (Siemen, German). The data collection sheet filled up by the study physician herself. The data regarding sociodemographic, clinical and biochemical were recorded. Initial evaluation of the subjects were done by history taking and demographic profile and pulse, BP, Height, Weight, BMI etc. measured and recorded in the preformed data collection sheet.

INCLUSION CRITERIA

Patients diagnosed with DM & IGT after admission were included in the sample & peoples with normal blood glucose level as comparison group.

1. Age: 30-60 years
2. Gender: Male & Female
3. Diagnosed type 2 diabetes mellitus by performing fasting blood glucose > 7.0 mmol/l & 2 hours ABF >11.1 mmol/l. Group-1)
4. IGT patients diagnosed by performing fasting blood glucose <5.6mmol/l &after 75g of OGTT blood glucose 7.8-11.0 mmol/l. (Group-2)
5. Normoglycemic people. (Group-3)

EXCLUSION CRITERIA

1. Any gross macrovascular or microvascular disease
2. Pregnancy.

3. Renal disease eg. ARF, CRF.
4. Hepatic disorder eg. CLD, NAFLD, ALD, NASH.
5. Chronic debilitating disease eg. TB, Malignancy.
6. Other hormonal disorder e.g. Hypothyroidism, Hyperthyroidism and Hypercalcemia.
7. Current medication that may influence serum homocysteine level including antifolates (methotrexate, anticonvulsants, trimethoprim), L-Dopa, fibrates, cyclosporine.
8. Cobalamin (vitamin B12), folate or pyridoxine (vitamin B6) supplementation.

DATA ANALYSIS

All statistical analysis was performed using the Statistical Package for Social Science (SPSS) program, version 22.0 and Windows. All data were presented in suitable table, box plot, pie chart and graph according to their affinity. A description of each table and graph was given to understand them clearly. Continuous parameters were expressed as mean ±SD and categorical parameters as frequency and percentage. Comparisons between groups (continuous parameters) were done by Students t- test. Categorical parameters compared by Chi-square test. The significance of the results as determined in 95.0% confidence interval and value of $P<0.05$ was consider to be statistically significant.

RESULTS

This cross sectional descriptive comparative study was conducted in the Mymensingh Medical College Hospital, Mymensingh, Bangladesh. Total 120 patients were divided into three groups; patients with Type 2 diabetes (group-1), IGT (group 2) and normal subject (group-3). This study was conducted to compare serum Homocysteine level among Type 2 DM & IGT patients with normal people. Table 1 shows, the age distribution among the groups. Mean ± SD of age was (48.31 ± 10.62) for Group–1, (47.29 ± 11.0) for Group–2 and (48.28 ± 12.3) for Group–3. P-value = 0.614 (one-way ANOVA); which explains that there was no significant statistical difference among the groups in respect of age.

Table-1: Age distribution of the patients (n=120)

Age (years)	Frequency & Percentage			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
< 40	5 (12.5)	8 (20.0)	7 (17.5)	
40 - 49	11 (27.5)	9 (22.5)	8 (20.0)	
50 - 59	12 (30.0)	13 (32.5)	10 (25.0)	0.566
≥ 60	12 (30.0)	10 (25.0)	15 (37.5)	
Total	40 (100.0)	40 (100.0)	40 (100.0)	
Mean ± SD	48.31 ± 10.6	47.29 ± 11.0	48.28 ± 12.3	0.614
P -value is obtained by Chi- square test.				

One way ANOVA was done to find out the level of significance.

Table-2: Gender distribution of the patients (n=120)

Gender	Frequency & Percentage			Total	p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)		
Male	28 (70.0)	31 (77.5)	27 (67.5)	86	
Female	12 (30.0)	9 (22.5)	13 (32.5)	34	0.925
Total	40 (100.0)	40 (100.0)	40 (100.0)		
Chi square test was done to find out the level of significance.					

Table 2 illustrates that, most of the participants in all Group-1 [28 (70.0%)], Group-2 [31 (77.5%)] and Group-3 [27 (67.5%)] were males. Male: Female ratio was about 2.5:1. There was no statistically significant difference in male-female distribution between the groups.

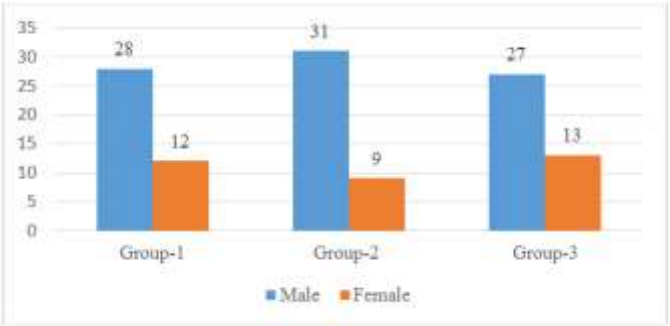

Fig-1: Gender distribution of the patients (n=120)

Table-3: Baseline clinical profile of the participants (n=120)

Variables	Frequency & Percentage			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
Height (m)	62.96 ± 5.26	61.58 ± 4.06	63.29 ± 4.63	0.17
Weight (Kg)	66.58 ± 9.72	62.61 ± 9.91	64.51 ± 8.75	0.61
BMI	21.04 ± 2.92	20.42 ± 3.24	20.66 ± 2.67	0.15
Systolic BP (mm of Hg)	124.68 ± 12.24	119.76 ± 12.74	121.40 ± 16.84	0.15
Diastolic BP (mm of Hg)	78.47 ± 10.11	78.66 ± 8.44	80.12 ± 11.10	0.83
One way ANOVA was done to find out the level of significance.				

Table 3 shows, the clinical and anthropometric measurements of the participants among the groups and there was no significant statistical difference among the groups.

Table-4: Evaluation of serum lipid status among study population (n=120)

Variables	Frequency & Percentage			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
TC (mg/dl)	215.0 ± 40.38	205.1 ± 35.08	196.0 ± 30.59	<0.001
TG (mg/dl)	253.0 ± 71.7	250.2 ± 48.5	226.50 ± 62.5	<0.05
LDL-C (mg/dl)	127.8 ± 33.3	125.2 ± 32.0	124.5± 35.9	>0.05
HDL-C (mg/dl)	36.8 ± 10.4	35.85 ± 9.8	39.8 ± 9.8	<0.05
One way ANOVA was done to find out the level of significance.				

Table 4 shows, the serum lipid status among study population. Among the groups, TC, TG & HDL-C were significant statistical difference among the groups.

Table-5: Distribution of cases according to serum Homocysteine level (n=120)

S. Homocysteine status	Frequency & Percentage			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
Hyperhomocysteinaemia	19 (47.5)	14 (35.0)	0	
Normal Homocysteine	21 (52.5)	26 (65.0)	40 (100.0)	0.001
Total	40 (100.0)	40 (100.0)	40 (100.0)	
Chi-square test was done to find out the level of significance.				

Table 5 shows the distribution of cases according to serum Homocysteine level. All patients in group-3 had normal level of S. Homocysteine. But in group-1 & 2 had raised level of homocysteine, which shows statistical significant difference.

Table-6: Mean Serum Homocysteine level among study people (n=120)

S. Homocysteine status	Mean Serum Hcy level			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
Mean Serum Hcy level Mean ± SD	19.86 ± 5.20	17.28 ± 5.38	8.72 ± 2.96	0.0001
One way ANOVA was done to find out the level of significance.				

Table 6 shows mean serum Homocysteine level. All patients in group-3 had normal level of S. Homocysteine. But in group-1 & 2 had raised level of homocysteine, which shows statistical significant difference.

Table-7: Blood glucose level of the participants among groups (n=120)

Variables	Frequency & Percentage			p-value
	Group-1 (n=40)	Group-2 (n=40)	Group-3 (n=40)	
FBG (mmol/L)	9.4 ± 3.6	6.8 ± 2.7	5.09 ± 1.5	0.048
2hrPPG (mmol/L)	13.5 ± 4.7	9.7 ± 3.1	6.5 ± 1.8	0.025
One way ANOVA was done to find out the level of significance.				

Table-7 represents the blood glucose level of participants among groups and mean values of Fasting blood glucose (FBG), 2-hour plasma postprandial glucose in patients with Type 2 DM & IGT have higher blood glucose levels, which shows statistical significant difference.

Table-8: Correlation analysis of different clinical & demographic profile with serum Homocysteine (n=120)

Parameters	r	p value
Age	-0.092	0.614
Sex	1.772	0.925
BMI	-0.259	0.152
SBP	-6.391	0.153
DBP	0.427	0.831
FBG	0.296	<0.001
2 HABF	0.078	0.004
TC	7.655	<0.001
TG	13.52	<0.05
LDL-C	0.295	>0.05
HDL-C	1.165	<0.05

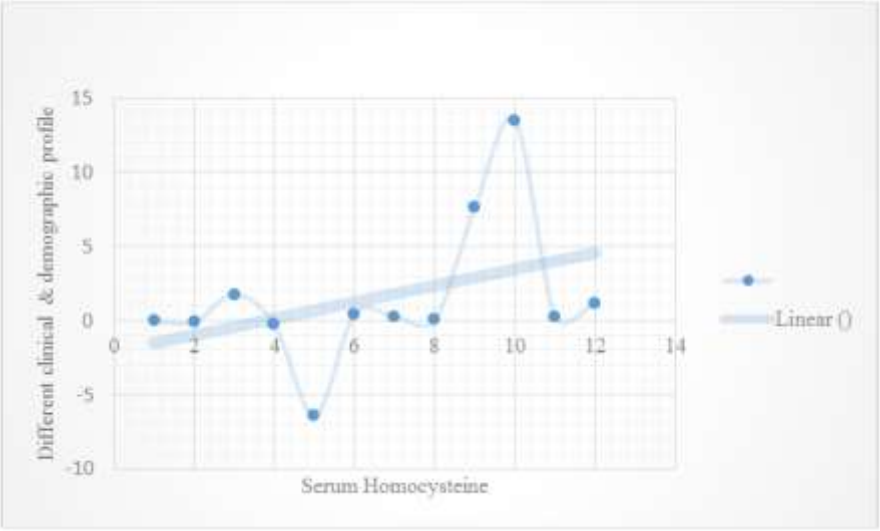

Fig-2: Correlation analysis of different clinical & demographic profile with serum Homocysteine (n=120)

Table 8 shows the ccorrelation analysis of different clinical & demographic profile with serum Homocysteine. The correlation between glycemic status and Serum Homocysteine levels of participants, which differ considerably, so that, Pearson correlation & Spearman correlation showed statistically significant difference. Hcy was positively associated with FBG (r=0.296, p<0.001), 2hPG (r=0.078, p=0.004). Similarly positive significant correlation was found with TC (r=7.655, p<0.001), TG (r=13.52, p=<0.05) and HDL-C (r=1.165, p<0.05).

DISCUSSION

This cross sectional descriptive comparative study was conducted in the department of Medicine & Endocrinology, Mymensingh Medical College Hospital. Total 120 patients were divided into three groups; patients with Type 2 diabetes (group-1), IGT (group 2) and normal subject (group-3). This study was conducted to compare serum Homocysteine level among Type 2 DM & IGT patients with normal people. Table 1 shows, the age distribution among the groups. Mean ± SD of age was (48.31 ± 10.62) for Group–1, (47.29 ± 11.0) for

Group–2 and (48.28 ± 12.3) for Group–3. P-value = 0.614 (one-way ANOVA); which explains that there was no significant statistical difference among the groups in respect of age. Findings are consistent with the results of similar studies at home and abroad, e.g. a cross-sectional study reported that mean age was 62.35 ± 8.88 years [13]. A study in Bangladesh reported that among 1555 study subjects, 731 were male, 824 were female, most of the population was young with a mean age 33 years, and about 78 percent were in age category between 20-40 years [14]. Sex distribution showed that, in group 1, 70% were male, 30% were female. In group 2 , 77.5% male & 22.5% female and in group 3, around 67.5% male & 32.5% female. Male - Female ratio was about 2.5:1 and no significant difference was observed between sex distributions. In the study of Ceriello *et al*., [15] female proportion was higher. In study anthropometric measurements of the participants among the groups showed no significant statistical difference. Among them BMI in group-1(21.09), group -2 (20.42%) and group – 3 (20.66%). Nevin, *et al*., [16] demonstrated that frequency of overweight in men & women was found to be 13.3% & 6.6% respectively. In the current study total cholesterol was 205±35.08 mg/dl in group 1 & 215±40.98 mg/dl in group 2 and 196±30.59mg/dl in controls. Numerous studies have shown an association of serum cholesterol with diabetic complications. Rema, *et al*., [17] reported mean serum cholesterol level in diabetic group was 262±11.39 mg/dl and in control group was 174±7.87mg/dl. That is serum cholesterol level was higher in IGT & DM group than that of normal people. The concentration of TG in the current study was 253±71.76 mg/dl in group 1, 250 ± 65.1mg/dl in group 2and 226 ± 62.59mg/dl in controls. Which is supported by the studies done by Kareem, *et al*.,[18] and Reema, *et al*., [17] found significantly higher TG concentration in cases (174 ± 7.87 mg/dl) than in controls group (151 ± 10.86 mg/dl). In the current study LDL- C level was 127 ± 33.03 mg/dl and 125 ± 32.03mg/dl in group 1 & 2 and 124.58 ± 35.97mg/dl in control. Here, there was no significant difference in LDL- C concentration between cases and controls. The ccorrelation analysis of different clinical & demographic profile with serum Homocysteine. The correlation between glycemic status and Serum Homocysteine levels of participants, which differ considerably, so that, Pearson correlation & Spearman correlation showed statistically significant difference. Hcy was positively associated with FBG (r=0.296, p<0.001), 2hPG (r=0.078, p=0.004). Similarly positive significant correlation was found with TC (r=7.655, p<0.001), TG (r=13.52, p=<0.05) and HDL-C (r=1.165, p<0.05). This findings of no significant difference in LDL-C concentration between the groups is similar with that of [19]. Regarding the concentration of HDL-C this study found significantly lower level in cases (36.86±10.42mg/dl & 35.85±9.86 mg/dl than controls (39.85±9.80mg/dl). This finding differs with that of Nayak, *et al*., [18] and Rema, *et al*., [17]. This conflicting findings regarding different components of lipid profile may be due to different dietary habit, life style and ethnicity of our study subjects than of the studies done abroad. In the present study, patients with DM and IGT presented significantly higher levels of Hcy than subjects with normoglycemic people. Serum Hcy level in group 1 -19.86 ± 5.20 µmol/L & group 2 - 17.28 ± 5.38 µmol/L and in comparison group 3-8.72 ± 2.96 µmol/L. The outcome of earlier studies are variable but many of them have shown increased serum Hcy levels in T2DM & IGT patients [20, 21].

CONCLUSION

Present study showed that mean values of the Serum Homocysteine levels were considerably lower in normoglycemic group. Patients with type II DM & IGT, have higher homocysteine levels, which differ considerably to show statistically significant difference. Numerous studies have shown altered serum Hcy concentrations in T2DM & IGT patients.

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