Original Research Article

Homocysteine (Hyc) in prediabetes: An early indicator of Cardiovascular disease

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ABSTRACT

Introduction: Diabetes mellitus (DM) is associated with an increased risk of cardiovascular disease (CVD) and mortality. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are well characterized prediabetes conditions and about half of those proceed with the development of type 2 diabetes. During the pre-diabetic state, the risk of a CVD is modestly increased. The aim of present study is to evaluate serum Homocysteine (Hyc) level in prediabetes and type 2 diabetes patients and find the association between Hyc and other variables.

Materials and Methods: This cross sectional descriptive study was conducted on 450 participants. Out of this 150 were type 2 diabetic patients, 150 prediabetes and 150 controls. Homocystine and biochemical parameters were measured.

Results: Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine consider as a an early detection marker in cardiovascular disease elevated in prediabetes patients, type 2 diabetes compared to controls.

Conclusion: We observed that homocysteine significantly increased in prediabetes compared to healthy control. Homocysteine levels can indicate the risk of CVD in prediabetic stage. Prediabetes people are under risk of CAD and type 2 diabetes. The evaluation of the Homocysteine levels may improve the early diagnosis of CVD in prediabetes patients.

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1. Introduction

Diabetes mellitus (DM) is associated with an increased risk of cardiovascular disease (CVD) and mortality.[1] Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are well characterized prediabetes conditions and about half of those proceed with the development of type 2 diabetes.[2] During the pre-diabetic state, the risk of a CVD is modestly increased.[3–5] even in healthy people, increased blood glucose levels may lead to an increased risk of CVD. It has been proposed that high glucose level is a cardiovascular risk factor, similar to hypercholesterolemia and hypertension.[6] Thus, vascular complications may already start in prediabetic stage and accelerated atherosclerosis has been documented in patients with DM.[2,6] Homocysteine is a secreted into the plasma from endothelial. It is cytotoxic amino acid, which is plasma and red blood cells as a product of incomplete conversion of methionine to cysteine, reflecting elevated oxidative stress. It may promote insulin resistance and β - cell dysfunction through its adverse metabolic effects, ultimately contributing to the pathogenesis of type 2 diabetes and associated complications.[7,8]

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated levels of homocysteine are toxic to vascular endothelium[9] inducing endothelial dysfunction and contributing to development of atherosclerosis independent of standard CVD risk factors in diabetic subjects[10–13] Hyperhomocysteinemia (HHcy) has been widely considered as the major determinant of the various cardiovascular diseases[14] Although endothelial dys-function,[15] oxidative damage,[16] and inflammation[17] have

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been demonstrated to be involved in the detrimental effects of Hcy on the cardiovascular system, the mechanisms by which Hcy accelerates cardiovascular diseases still need further investigation. Most of studies done on Hyc. It was risk factor for CVD were clinically clear in diabetes mellitus, it is not clear whether increased concentrations of Hycs are present in patients with impaired glucose tolerance (IGT). Homocysteine is a product of incomplete conversion of methionine to cysteine, reflecting elevated oxidative stress hence oxidative stress is a triggering factor to induce serum Homocysteine levels In our view, there are hardly any studies available in Indian population regarding these biomarkers in Prediabetes. The aim of present study is to evaluate serum Hyc level in prediabetes and type 2 diabetes patients and find the association between Hyc and other variables.

2. Material and Methods

This study was conducted in the Department of Biochemistry at People's College of Medical Sciences and Research Center (PCMS & RC) and Centre for Scientific Research and Development (CSRD), People's University Bhopal. This cross sectional descriptive study was included 150 type 2 diabetes patients, 150 prediabetes and 150 healthy controls after applying inclusion and exclusion criteria. All socio-demographic data of the participants was entered in a self-designed questionnaire during the period June 2017 to December 2018.

Inclusion Criteria for T2D according to the American Diabetic Association : Age 18-60 yrs, Known case of T2D (1-5 year) and HbA1c 6.5%; Exclusion Criteria for T2D: Prolonged diabetes (more than 5 year), Patients on Statin therapy, Pregnant woman

Inclusion Criteria for prediabetes according to the American Diabetic Association: Age between 18-60 yrs, FBG level 100-125 mg and HbA1c 5.7–6.4%, PPBG level (after 2 hours of 75 g oral glucose) 140-199 mg/dl

Exclusion Criteria for prediabetes: Age more than 60 yrs and Age less than 18, Diagnosed diabetic patients, AIDS Patients, Pregnant women.

The study protocol was approved by Institutional ethics committee. All the participants were screened for age, gender, FBG level, PPBG level, HbA1c, Family history, any medication history. Prediabetic cases were included and excluded with the help of physician, Dept. Medicine, People's College of Medical Sciences and Research Centre (PCMS & RC), Bhopal. Routine biochemical investigations as per Table 1

## 3. Materials and Methods

3.1. Statistical analysis

SPSS (Chicago, IL, USA) version 21 was used for statistical analysis of data. Descriptive statistics for quantitative variables were presented as mean ± SD. Analysis of variance (ANOVA) was used to compare between the three groups, $P<0.05$. Association between homocysteine and variables were tested using pearson's correlation. $P < 0.05$ was considered statistically significant.

4. Observations and Results

The observations and inference obtained from this study were summarized in following tables:
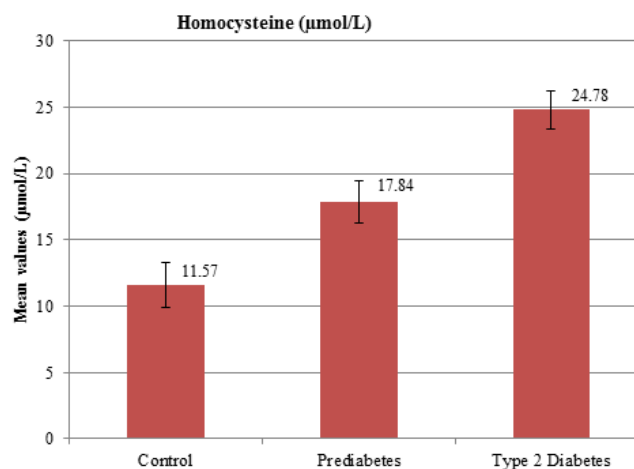
Graph 1: Mean score of homocysteine

The levels of homocysteine in prediabetes was 17.84 ± 1.59 and type 2 diabetes 24.78 ± 1.47 was significantly increased compared to controls 11.57 ± 1.67. p value was < 0.05. w(Table. No.2 & Graph.No.1). Mean score of cholesterol in prediabetes was 243.39 ± 19.98 and type 2 diabetes was 311.06 ± 61.25 significantly increased compared to controls 170.52 ± 22.28. p value was <0.001. Mean score of Triglyceride in prediabetes was 169.85 ± 14.42 and type 2 diabetes was 191.08 ± 41.35 significantly increased compared to controls 90.96 ± 16.46. p value was <0.001 which was significance. Mean score of HDL in prediabetes was 31.15 ± 4.36 and type 2 diabetes was 27.98 ± 4.99 significantly decreased compared to controls 42.19 ± 5.82. p value was < 0.001. Mean score of LDL in prediabetes was 171.89 ± 20.03 and type 2 diabetes was 236.63 ± 56.61 significantly increased compared to controls. 108.80 ±18.60. p value was < 0.. Mean score of VLDL in prediabetes was 36.07± .04 and type 2 diabetes was 47.53 ± 10.51 significantly increased compared to controls 19.03 ± 4.85. p value was < 0.001. Mean score of TG/HDL in prediabetes was 5.57 ± 1.00 and type 2 diabetes was 7.22 ± 2.23 significantly increased compared to controls 2.18 ± 0.47. p value was < 0.05. Mean score of LDL/HDL in prediabetes was 5.73 ± 1.06 and type 2 diabetes was 8.93 ± 3.44 significantly increased compared to controls 2.60 ± 1.67. p value was < 0.001.(Table 2)

Table 1: Methods of biochemical parameters

S. No.	Biochemical parameters	Method
1	Blood Glucose	GOD-POD Method [18]
2	Cholesterol	CHOD-POD Method [19] [19]
3	TG	Glycerol phosphate oxidase- Peroxidase (GPO-POD Method) [20]
4	HDL	directly enzymatic colorimetric Quantitative determination. [21]
5	LDL	Friedewald equation assuming that total cholesterol is composed primarily.
6	VLDL	By calculation
7	HbA1c	Enzymatic method [22]
8	Homocysteine	E LISA method [23]

Table 2: Comparison of endothelial dysfunction biomarkers in prediabetes and type 2 diabetes compared to controls

Variables	Control	Prediabetes	T2D	p value
Age	37.78 ± 10.49	45.58 ± 9.07	43.04 ± 10.96	< 0.001
Sex (M/F)	82/68	93/57	87/63	
FBG (mg/dL)	83.01 ± 08.36	115.12 ± 5.93	161.70 ± 21.77	< 0.001
PPBG mg/Dl	115.53 ± 10.55	163.50 ± 13.43	244.09 ± 29.15	< 0.001
HbA1c %	5.07 ± 0.34	6.01 ± 8.17	8.17 ± 1.15	< 0.001
Cholesterol (mg/dL)	170.52 ± 22.28	243.39 ± 19.98	311.06 ± 61.25	< 0.001
TG (mg/dL)	90.96 ± 16.46	169.85 ± 14.42	191.08 ± 41.35	< 0.001
HDL (mg/dL)	42.19 ± 5.82	31.15 ± 4.36	27.98 ± 4.99	< 0.001
LDL (mg/dL)	108.80 ± 18.60	171.79 ± 20.03	236.63 ± 56.61	< 0.001
VLDL(mg/dL)	19.03 ± 4.85	36.07 ± 7.07	47.53 ± 10.51	< 0.001
TG/HDL	2.18 ± 0.47	5.57 ± 1.00	7.22 ± 2.23	< 0.05
LDL/HDL	2.60 ± 0.55	5.73 ± 1.06	8.93 ± 3.44	< 0.001
Homocysteine (μ mol/L)	11.57 ± 1.67	17.84 ± 1.59	24.78 ± 1.47	< 0.001

$p < 0.05$ consider as statisticallysignificant

Table 3: Association of Homocysteine with Biochemical Parameters

Parameters	Prediabetes		Type 2 Diabetes	
	R	P	R	P
FBGL	-0.145	0.017	0.233	0.000
PPBGL	-0.037	NS	0.074	NS
HbA1c	-0.082	NS	0.377	0.000
Cholesterol	0.049	NS	0.097	NS
TG	0.090	NS	0.253	0.000
HDL	-0.042	NS	-0.081	NS
LDL	0.010	NS	0.104	0.097
VLDL	0.074	NS	0.136	0.030
TG/HDL	0.081	NS	0.182	0.004
LDL/HDL	0.031	NS	0.102	NS

Correlation is significant at the $p < 0.05$, NS- Not significant

In group type 2 diabetes, significant and positive correlation was observed between plasma Homocysteine level and FBSL (r = 0.233; p= 0.000), HbA1c (r=0.377; p=0.000), TG(r=0.253; p=0.000), VLDL (r=0.136; p=0.03. In group Prediabetes, negative correlation was observed between plasma Homocysteine level and FBSL (r=-0.145; p= 0.017). (Table 3)

5. Discussion

Homocysteine is a cytotoxic amino acid, which is secreted into the plasma from endothelial and red blood cells as a product of incomplete conversion of methionine to cysteine, reflecting increased oxidative stress due to hyperglycemia. In the present study, it was observed that mean value of homocysteine in prediabetes was 17.84 ± 1.59 and type 2 diabetes 24.78 ± 1.47 was compared to controls 11.57 ± 1.67. p value was < 0.05 which shows significance. (Table 2Graph 1).

Studies have confirmed that, lipid and lipoprotein abnormalities play a major role in the pathogenesis and progression of CVD. Studies on the connection between plasma homocysteine level and CVD risk have been controversial, with some authors claiming an association and some denying the efficacy of lowering the homocysteine levels as a means of preventing the CVD incidence. There is however consensus about the prothrombotic and prooxidant properties of homocysteine, which can cause the formation of ROS and contribute to endothelial dysfunction. .[24,25] Increased levels of serum Hcy levels leads to endothelial impairment by enhancing oxidative stress and declines the release of nitric oxide (NO), which impairs vascular dilatation.[26,27] Hyperhomocysteinemia induce proliferation of smooth muscle cell and synthesis of collagen which in turn prom otes intima-media thickening.[28,29] High serum Hcy levels considered to have thrombogenic activity by altering coagulation system and stimulate on of platelet aggregation. Excess of serum Hcy level is also seen to be related with enhanced lipid peroxidation which also predisposes to atherosclerosis.[30] Lentz in the study concluded that serum Hcy levels are an independent risk factor for cardiovascular disorders in T2DM patients.[31] Similarly with our study Shaikh MK et al found significant increased homocysteine levels in the prediabetic subjects and type 2 diabetes compared to control[32]. hyperhomocysteinemia in patients with diabetes mellitus which may contribute to the development of chronic complications. The pressure of diabetes treatment on Hcy levels requires additional advance observations. Now our study extended in prediabetes which confirmed similar results with Feng X et al[33] He found increased level of homocysteine in prediabetes compared to control.

In group type 2 diabetes, significant and positive correlation was observed between plasma Homocysteine level and FBSL (r = 0.233; p= 0.000), HbA1c (r=0.377; p=0.000), TG(r=0.253; p=0.000), VLDL (r=0.136; p=0.03). In group Prediabetes, negative correlation was observed between plasma Homocysteine level and FBSL (r=-0.145; p= 0.017). (Table 3) Bansal et al found similar result like our study, positive association between homocysteine and FBSL and HbA1c.[34]

In group Prediabetes, Negative correlation was observed between plasma Homocysteine level and FBSL (r=-0.145; p= 0.017) and. Similarly Feng et al observed positive correlation between plasma Homocysteine with TG, BMI and HDL-C in prediabetes patients.[33]

6. Conclusion

Based on findings of the present study, we concluded that, increased level of Homocysteine indicate the risk of CVD in prediabetic stage and T2D patients. Early detection of this biochemical parameter along with routine investigations is mandatory for the diagnosis for the clinicians to plan line of treatment. Well balanced nutrition, patient education, diet counseling and supplementation therapies for high risk group of diabetic patients is strongly recommended.

7. Source of Funding

None.

8. Conflict of Interest

None.

9. Abbreviations

Hyc - Homocysteine, BMI- Body mass index, TC-total cholesterol, TG- triglycerides, HDL- high density lipoprotein cholesterol. LDL- low density lipoprotein cholesterol. VLDL- very low density lipoprotein cholesterol, DM- Diabetes Mellitus.

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