Original Research Article

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

Santosh Eknath Bidwe1, Prashant J Hisalkar2,*, Neerja Mallick3

1 Dept. of Biochemistry, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India
2 Dept. of Biochemistry, Government Medical College and Hospital, Dungarpur, Rajasthan, India
3 People’s University, Bhopal, Madhya Pradesh, India

ARTICLE INFO

Article history:
Received 19-07-2019
Accepted 23-08-2019
Available online 21-09-2019

Keywords:
Cardiovascular disease
Homocysteine
Nitric Oxide
Prediabetes
Type 2 diabetes

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 and considered as 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.

© 2019 Published by Innovative Publication.

1. 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. 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. Thus, vascular complications may already start in prediabetic stage and accelerated atherosclerosis has been documented in patients with DM. 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.

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated levels of homocysteine are toxic to vascular endothelium inducing endothelial dysfunction and contributing to development of atherosclerosis independent of standard CVD risk factors in diabetic subjects. Hyperhomocysteinemia (HHcy) has been widely considered as the major determinant of the various cardiovascular diseases. Although endothelial dysfunction, oxidative damage, and inflammation have...
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 Hcy risk factor for CVD were clinically clear in diabetes mellitus, it is not clear whether increased concentrations of Hcys 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](image)

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. (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 < 0.001. 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\textsuperscript{18} |
| 2      | Cholesterol           | CHOD-POD Method\textsuperscript{19} |
| 3      | TG                    | Glycerol phosphate oxidase- Peroxidase (GPO-POD Method)\textsuperscript{20} directly enzymatic colorimetric Quantitative determination.\textsuperscript{21} |
| 4      | HDL                   | Friedewald equation assuming that total cholesterol is composed primarily. |
| 5      | LDL                   | By calculation |
| 6      | VLDL                  | Enzymatic method\textsuperscript{22} |
| 7      | HbA1c                 | E LISA method\textsuperscript{23} |

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

| Variables        | Control       | Prediabetes   | T2D         | p value |
|------------------|---------------|---------------|-------------|---------|
| Age (years)      | 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 statistically significant

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 |
| PPBG             | -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. 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. Hyperhomocysteinemia induce proliferation of smooth muscle cell and synthesis of collagen which in turn promote intima-media thickening. 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. Lentz in the study concluded that serum Hcy levels are an independent risk factor for cardiovascular disorders in T2DM patients. Similarly with our study Shaikh MK et al found significant increased homocysteine levels in the prediabetic subjects and type 2 diabetes compared to control, 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 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). 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.

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.

References
1. European Diabetes Epidemiology Group. Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? Study Group. 2003;26:688–96.
2. Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, et al. Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract. 2007;78(3):305–12.
3. Surdacki A, Stochmal E, Szurkowski M, Bode-Bger SM, Martens-Lobenhoffer J. Nontraditional atherosclerotic risk factors and extent of coronary atherosclerosis in patients with combined impaired fasting glucose and impaired glucose tolerance. Metabolism. 2007;56(1):77–86.
4. Levitan EB, Song Y, Ford ES, Liu S. Is nondiabetic hyperglycemia a risk factor for cardiovascular disease? A meta-analysis of prospective studies. Arch Intern Med. 2004;164:2147–55.
5. Defronzo RA, M AG. Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. Am J Cardiol. 2011;108(3):23–24. Suppl.
6. Gerstein HC. Glucose: a continuous risk factor for cardiovascular disease. Diabet Med. 1997;14(3):25–31. Suppl.
7. Munshi MN, Stone A, Fink L, Fonseca V. Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. Metabolism. 1996;45(1):133–5.
8. Catena C, Colussi G, Nait F. Elevated homocysteine levels are associated with the metabolic syndrome and cardiovascular events in hypertensive patients. Am J Hypertens. 2015;28(7):943–50.
9. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998;338:1042–1050.
10. Hoogeveen EK, Costense PJ, Beks PJ, Mackaay AJ, Jakobs C, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non-insulin-dependent diabetes mellitus: a population-based study. Arterioscler Thromb Vasc Biol. 1998;18:133–8.
11. Hoogeveen EK, Costense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hordum Study. Circulation. 2000;101:1506–11.
12. Okada E, Oida K, Tada H, Asazuma K, Eguchi K, et al. Hyperhomocysteinemia is a risk factor for coronary arteriosclerosis in Japanese patients with type 2 diabetes. Diabetes Care. 1999;22:484–490.
13. Stehouwer CD, Gall MA, Hougaard P, Jakobs C, Parving HH. Plasma homocysteine concentration predicts mortality in non-insulin-
dependent diabetic patients with and without albuminuria. *Kidney Int*. 1999;55:308–314.

14. Mahalle N, Kulkarni MV, Garg MK, Naik SS. Vitamin B12 deficiency and hyperhomocysteinemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease. *J Cardiol*. 2013;61(4):289–94.

15. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med*. 1998;338(15):1042–50.

16. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest*. 1986;77(4):1370–6.

17. Zeng X, Dai J, Remick DG, Wang X. Homocysteine-mediated expression and secretion of monocyte chemotactant protein-1 and interleukin-8 in human monocytes. *Circ Res*. 2003;93(4):311–20.

18. Kaplan L. Carbohydrates and metabolite, In Clinical Chemistry: theory, Analysis and co-relation. KLA, PAJ, obsy ECV, editors. Toronto; 1984.

19. Allain CC, Poon L. Enzymatic Determination of Total Serum Cholesterol. *Clin Chem*. 1974;20:470.

20. Bachorik PS, Albers JJ, Rifai N. Editors Tietz Textbook of Clinical Chemistry. 3rd ed. CA B, ER A, editors. Philadelphia: W.B Saunders Company; 1999.

21. Williams P, Robinson D, Bailey A. High density of lipoprotein and coronary risk factor in normal man. *Lancet*. 1979;1:72.

22. Edited by the Japan Diabetes Society: Treatment Guide for Diabetes 2010. Bunkodo; 2010.

23. Thambhyrajah J, Townsend JN. Homocysteine and atherothrombosis—mechanisms for injury. *European Heart J*. 2000;21(12):967–74.

24. Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, et al. Fasting Plasma Homocysteine Levels in the Insulin Resistance Syndrome. *Diabetes Care*. 2001;24:1403–10.

25. Fach D, Chiolerio A, Paccaud F.

26. Kanani PM, Sinkey CA, Browning RL, Allaman M, Knap HR, et al. Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocysteinemia in humans. *Circ*. 1999;100:1161–8.

27. Tawakol A, Omland T, Wijt GM, Creager MA. Hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilation in humans. *Circ*. 1997;95:1119–21.

28. Malinow MR, Neito FJ, Szklo M, Chambless LE, Bond G. Carotid artery intimal-medial wall thickening and plasma homocysteine is asymptomatic adults. The Atherosclerosis Risk in Communities Study. *Circulation*. 1993;87:1107–1113.

29. Majors A, Ehrhart LA, Pezacka EH. Homocysteine as a risk factor for vascular disease. Enhanced collagen production and accumulation by smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 1997;17:2074–2081.

30. Voutilainen S, Morrow JD, G Roberts Lj 2nd A, Alho H, Nyyssonen K. Enhanced in vivo lipid peroxidation at elevated plasma total homocysteine levels. *Arterioscler Thromb Vasc Biol*. 1999;19:1263–6.

31. Lentz SR. Mechanisms of homocysteine-induced atherothrombosis. *J Thromb Haemost*. 2005;8:1646–54.

32. Shaikh MK, Devrajani BR, Shaikh A, Shah S, Shaikh S. Plasma Homocysteine Level in Patients with Diabetes mellitus. *World Appl Sciences Journal*. 2012;16(9):1269–1273.

33. Feng X, Xu Y. Hyperhomocysteinemia as a metabolic risk factor for glucose intolerance among high-risk groups of chinese adults. *Med Sci Monit*. 2017;23:2775–2781.

34. Bansal S, Kapoor S, Singh GP, Yadav S. Serum Homocysteine Levels in Type 2 Diabetes Mellitus Patients. *International Jr of Contemporary Medical Research*. 2016;3(11):3393–3396.

Author biography

Santosh Eknath Bidwe Assistant Professor

Prashant J Hisalkar Professor and HOD

Neerja Mallick Professor and Registrar

Cite this article: Bidwe SE, Hisalkar PJ, Mallick N. Homocysteine (Hyc) in prediabetes: An early indicator of Cardiovascular disease. *Int J Clin Biochem Res*. 2019;6(3):348–352.