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Indication of Hyperhomocysteinemia in Type 2 Diabetes Mellitus Patients with Cardiovascular Complications

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Abstract: Introduction Cardiovascular disease (CVD) is one of the prominent causes of mortality in cases of chronic Type 2 diabetes mellitus (T2DM) patients and necessitates improving risk categorization. There are few available biomarkers that can assess preceding or current glycemic and cardiac status, but not prognosis. Serum homocysteine (Hcy) has been indicated and reported to be a likely biomarker that can detect cardiovascular complication in patients with T2DM. **Methodology** Present study details the comparative analysis of several biochemical and metabolic biomarkers including Hcy in T2DM patients with and without CVD complications. A total of eighty patients, n = 40 each in T2DM with CVD and T2DM without CVD, were included in the study. Patient's preparation, blood sample collection and analyses of all biochemical, metabolic markers including Hcy were performed as per standard protocols. One way ANOVA was used for independent measures including Tukey HSD with level of significance at $P< 0.05$. **Results** Indication of hyperhomocysteinemia, was significantly apparent in patients with T2DM who have CVD, as compared to those with T2DM without CVD. All other biochemical and metabolic parameters manifested marked significant ($P< 0.00001$) elevations, which was more perceptible in T2DM CVD as compared to T2DM non CVD. Clinical relevance of high Hcy in blood in patients with T2DM CVD thus suggested being prominent risk factor for proceeding renal and cardiac complications.

Keywords: Hyperhomocysteinemia, Cardiovascular complications, Type 2 Diabetes mellitus, prognosis. Biomarkers

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Introduction

Diabetes affects millions worldwide, its major cause of morbidity and in many cases mortality due to impending complications [1-4]. Out of various documented complications, cardiovascular disease (CVD) is one of the prominent causes of mortality and necessitates improving risk categorization. Furthermore, by doing so, clinicians can suggest and implement better individualized treatment regiments, bed-side care, and enhanced management of daily routine to prevent morbidity and mortality [5]. Few biomarkers, such as glycated hemoglobin (HbA1c), Trop I provide preceding or current glycemic and cardiac status, respectively, nevertheless, cannot provide long-lasting association or disease progression, especially in CVDs [6,7]. With

limited prognostic significance, several biomarkers reflect only existing disease anomalies which might not be clinical appropriate, if disease progression and complications are impending and there is a marked risk of overactive morbidity [6,7]. Numerous earlier and recent studies reported evaluation of novel biomarkers to assess risk and prognosis of CVD complication arising due to chronic T2DM [1, 8]. N-terminal neutric peptide (NT-pro BNP), Troponins (I and T), C-reactive proteins, matrix metalloproteinases and Apolipoproteins were reported to be such biomarkers that can assess diagnosis and prognosis of CVD in patients with long term T2DM [1]. More recently, serum homocysteine (Hcy) has been indicated and reported to be a likely biomarker that can detect vascular complication in patients with T2DM [9,10]. Due to its biological mechanism of interacting with blood coagulation, oxidative stress and vascular endothelial and arterial walls injuries, Hcy noted to be a significant prognostic biomarker [11, 12]. Various past and recent studies have reported the association of Hcy with development of atherosclerosis and CVD in T2DM patients [11, 10]. Clinical studies documented determination of hyperhomocysteinemia as a marked risk factor for CVD and having stroke, as 25% increase in blood concentration of Hcy can lead to that risk elevation upto 10% to 20% [8]. Moreover, incremental increase of Hcy can also trigger the occurrence of heart disease with elevated risk upto 52% and mortality upto 32% [9].

In this context, present study details the comparative analysis of several biochemical and metabolic biomarkers including homocysteine in T2DM patients suffering from CVD complication in relation to patients without CVD and control group (without any disease state). Analytical outcome will provide information regarding Hcy significance as diagnostic and prognostic marker in T2DM patients, suspected of developing or suffering from CVD.

Materials and Methods:

Study population:

Around three hundred and fifty patients (males n = 242, females n = 108) with confirmation of T2DM were screened, out of which 121 (males n = 76, females n = 45) were found to be suffering from CVD complications whereas 229 (males n = 167, females n = 62) without cardiovascular complications includes arrhythmias, valvular, coronary artery disease, aortic malfunctioning, pericardial and peripheral diseases, congenital heart disease and deep venous thrombosis.

Study was conducted from January 2022 to December 2022. It was a case control-observational study conducted at Lyari General Hospital and, Liaquat National Hospital, Karachi. Sample size calculation was done via using online calculator https://www.calculator.net/sample-size-calculator.html with screened population three hundred and fifty, population proportion 8.0%, margin of error 5% and confidence level 95%, for which sample size came out to be n = 86. For even distribution, final sample size of 80 was taken, with n = 40 in each group of T2DM without CVD (T1) and T2DM with CVD (T2). Forty individuals, age and gender matched, without any disease were grouped as control (T3).

Analyses of biochemical, metabolic markers and Homocysteine: Patient's preparation and blood sample collection were performed as per protocols reported recently [13]. All biochemical and metabolic markers were analysed by standards methods [14-16]. Hcy was determined by "Homocysteine Enzymatic Assay" based on novel enzyme cycling assay principle that assesses the co-substrate conversion product [17-19]. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalysed by Hcy S-methyltransferase.

Statistical analysis:

All data were analysed by using SPSS version 21, (SPSS Inc, Chicago, Illinois, USA). Data presented as mean ± SD, unless specified otherwise. Comparative data analysed were done with T1: T2, T2: T3 and T1 vs T3 where T1 = T2DM patients without CVD, T2DM patients with

CVD and T3 = Control (non-diseased) group using one way ANOVA for independent measures including Tukey HSD with level of significance at P< 0.05.

Results

Present study details the comparative analysis of alteration in biochemical and metabolic markers in T2DM patients without CVD as compared to those T2DM patients with CVD complications. Moreover, indication of hyperhomocysteinemia, as apparent by higher levels of Hcy in T2DM CVD patients as compared to non-CVDs (and control group), also been revealed (Table 1). Enzymes, ALT, AST comparison amongst T1:T2, T2:T3 and T1:T3 revealed marked significance (P< 0.00001). However, fasting blood glucose, Triglyceride, Cholesterol, HbA1c, insulin, whereas uric acid was moderately significant in one instance T2:T3 (Table 1). Mean Age was noted to between 37.125 to 38.70 years and appeared to be a non-significant parameter. Hcy n control group was estimated to be 6.85 ± 1.955 μmol/L, whereas a higher, more than double concentration was noted in T2DM without CVD group (12.55 ± 1.319 mmol/L) and even highest in T2DM group with CVD (15.7 ± 1.417 mmol/L) suggesting profound effects of T2DM and proceeding complication of CVDs. Insulin, a hormone directly related to carbohydrate metabolism and itself a metabolic biomarker, also seems to have the same pattern of elevation, Data suggested both metabolic, physiological alterations, both in T2DM without CVD, and T2DM in addition to hyperhomocysteinemia with CVD, however more profoundly in later than former group (Table 1).

Table 1: Comparative analysis of biochemical and metabolic biomarkers, including Hcy in T2DM patients with and without CVD

Parameters	**CONTROLS T1**	**T2DM WITHOUT CVDT2**	**T2DM WITH CVD T3**
Age years.	37.25 ± 4.024	37.125 ± 3.487	38.70 ± 3.68
	T1:T2 Q = 0.21 p = 0.98774	T2:T3 Q = 2.67 p = 0.14767	T1:T3 Q = 2.45 p =0 .19655
ALT IU/L	24.3 ± 3.023	27.775 ± 2.5063	36.825 ± 3.2964
	T1:T2 Q = 7.42 p = 0.000001	T2:T3 Q = 19.34 p = 0.000001	T1:T3 Q = 26.76 (p =0 .000001
AST IU/L	23.7 ± 2.2441	29.275 ± 2.2071	40.05 ± 2.5415
	T1:T2 Q = 15.10 p = 0.000001	T2:T3 Q = 29.18 p = 0.000001	T1:T3 Q = 44.27 p =0 .000001
FBG mg/dl	84.225 ± 5.126	111.25 ± 12.04	130.575 ± 10.210
	T1:T2 Q = 17.84 p = 0.000001	T2:T3 Q = 12.75 p = 0.000001	T1:T3 Q = 30.59 p = 0.000001
CHOLESTEROL mg/dl	126.6 ± 11.327	163.1 ± 21.687	189.875 ± 10.503
	T1:T2 Q = 15.01 p = 0.000001	T2:T3 Q = 11.01 p = 0.000001	T1:T3 Q = 26.02 p =0 .000001
TRIGLYCERIDE mg/dl	109.275 ± 8.302	176.125 ± 11.78	201.4615 ± 18.546
	T1:T2 Q = 31.16 p = 0.000001	T2:T3 Q = 11.81 p = 0.000001	T1:T3 Q = 42.97 p =0 .000001

URIC ACID mg/dl	4.27 ± 0.589	6.9173 ± 0.563	7.4633 ± 0.561
	T1:T2 Q = 29.29 p =0 .000001	T2:T3 Q = 6.04 p =0 .00012	T1;T3 Q = 35.33 p = 0.000001
HbA1c %	5.2995 ± 0.292	7.3731 ± 0.545	8.3074 ± 0.474
	T1:T2 Q = 28.55 p =0 .000001	T2;T3 Q = 12.86 p =0 .000001	T1;T3 Q = 41.42 p =0 .000001
Insulin □U/ml	8.85 ± 2.476	15.05 ± 1.986	17.275 ± 1.467
	T1:T2 Q = 19.42 p =0 .000001	T2:T3 Q = 6.97 p = 0.00001	T1:T3 Q = 26.39 p = .000001
HCY □mol/L	6.85 ± 1.955	12.55 ± 1.319	15.7 ± 1.417
	T1:T2 Q = 22.69 p = 0.000001	T2:T3 Q = 12.54 p = 0.000001	T1:T3 Q = 35.23 p = 0.000001

Q = indicates a significant result, Data analysed T1 vs T2 (T1:T2), T2 vs T3, T1 vs T3

Discussion

Higher levels of Hcy have been attributed to diabetic state and more profoundly in T2DM patients with microvascular and CVD [20, 21] as compared to those individuals devoid of T2DM and/or CVDs. Furthermore, several earlier studies reported that hypercholesterolemia, diabetes mellitus, hypertension, smoking can contribute in the development of hyperhomocysteinemia, however it could be, in most cases, shall remain an independent indicator of impending CVDs, in addition to peripheral arterial and coronary arteries disease [20, 21]. A recent study reported correlation of higher levels of Hcy in T2DM patients with impending cause specific mortality, secondary to chronic heart disease [22]. The research also showed a strong connection between Hcy and an increased risk of developing end-stage conditions related to both overall health and the heart. Additionally, it demonstrated a significant ability to predict the risk of mortality over a period of ten years [22]. This report provides further evidence that supports previous research and our own study, confirming that Hcy is indeed linked to the development and progression of cardiovascular CVDs, neurological diseases, and malignancies [23-26]. Clinical studies documented through case control and cross- sectional cohorts suggested and evidently showed a correlation between high circulating total Hcy and occurrence of peripheral, carotid and coronary artery diseases [22]

Hcy is the end product of amino acid methionine metabolism and physiological presence of folic acid, B12 and B6, in addition to kidney functions, it determines the circulating concentrations of Hcy and thereafter its metabolic and physiological effects [22]. As a result of impaired glucose metabolism and imminent T2DM state, hyperhomocysteinemia may occur due to alerted absorption, which tends to increase the risk of CVD development [22, 27]. On the other hand, presenting hyperhomocysteinemia aggravates insulin resistance, vascular endothelial dysfunction in return, thus further complicating clinical wellbeing of T2DM patients. Overall alteration in metabolism of Hcy resulting in hyperhomocysteinemia may be caused by cellular stress, oxidative impairment, unhealthy diet, genetic alterations and low plasma levels of folate, Vitamin B12, vitamin B6, resulting in pathogenesis of CVDs via pathological effects on smooth muscle cells and vascular endothelium, causing variations in subclinical arterial functions, as seen in most cases of T2DM [22, 26, 28]. Reported mechanisms of adverse effects, induced by hyperhomocysteinemia in T2DM patients with CVDs, includes oxidative damages, endothelial dysfunctions, arterial walls deterioration, platelet activation and development of impaired cellular regeneration [22, 26, 28].

Clinical relevance of high Hcy in blood in patients with T2DM and CVD is considered very significant risk factor for proceeding renal and cardiac complications and sometimes may predict prognosis [28]. An earlier study has reported a value of 19.95 mmol/L to predict prognosis for impending CVD complications in T2DM patients [28]. Somewhat similar value has also been observed in our study as well in T2DM patients with CVD.

Conclusion

The current study found a strong connection between high levels of Hcy and the occurrence of CVDs and/or complications in patients with T2DM. The significant findings were noted in the blood levels of metabolic and biochemical markers, including Hcy, when comparing T2DM patients with CVDs to those without CVDs. Cases of T2DM with CVD patients showed higher concentrations of cholesterol, triglycerides, FBG, HbA1c, and metabolic enzymes like AST, ALT, and Hcy compared to T2DM patients without CVDs. This suggests an overall increase in oxidative, hepatic, and cellular stress, as well as an impaired metabolic state in cases of T2DM with CVD.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study is approved by Liaquat National Hospital and Departmental Ethics committee and informed consent of each patient was taken before enrollment.

HUMAN AND ANIMAL RIGHTS

No animals were used in this study. The study on humans was conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

None.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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References

1. Bachmann KN., Wang TJ. Biomarkers of cardiovascular disease: contributions to risk prediction in individuals with diabetes. Diabetologia. 2018; 61(5): 987–995. doi:10.1007/s00125-017-4442-9
2. Emerging Risk Factors C. Sarwar N, Gao P, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet. 2010; 375:2215–2222.
3. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. Diabetes Care. 1979; 2:120–126.

4. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med. 1998; 339:229–234.
5. Scirica BM. Use of Biomarkers in Predicting the Onset, Monitoring the Progression, and Risk Stratification for Patients with Type 2 Diabetes Mellitus. Clin Chem. 2017; 63:186–195. [PubMed: 28062618]
6. St Clair L, Ballantyne CM. Biological surrogates for enhancing cardiovascular risk prediction in type 2 diabetes mellitus. Am J Cardiol. 2007; 99:80B–88B.
7. Berezin AE. Cardiac biomarkers in diabetes mellitus: New dawn for risk stratification? Diabetes Metab Syndr. 2017. 11 Suppl 1:S201-S208. doi: 10.1016/j.dsx.2016.12.032.
8. D'Agostino RB Sr, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008; 117:743–753.
9. Stevens RJ, Kothari V, Adler AI, Stratton IM, United Kingdom Prospective Diabetes Study G. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). Clin Sci (Lond). 2001; 101:671–679
10. Wang TJ. Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. Circulation. 2011; 123:551–565
11. Kengne AP, Patel A, Colagiuri S, et al. The Framingham and UK Prospective Diabetes Study (UKPDS) risk equations do not reliably estimate the probability of cardiovascular events in a large ethnically diverse sample of patients with diabetes: the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) Study. Diabetologia. 2010; 53:821–831.
12. Davis WA, Colagiuri S, Davis TM. Comparison of the Framingham and United Kingdom Prospective Diabetes Study cardiovascular risk equations in Australian patients with type 2 diabetes from the Fremantle Diabetes Study. Med J Aust. 2009; 190:180–184.
13. Bozkaya TA, Ustundag UV, Ates MS, Ulfer G, Yigit P, Kutluay SH, Selek S, Emekli N. The Relations of diabetes, trace element, oxidative stress and homocysteine in patient's undergone off-pump coronary artery bypass surgery. Indian J of App Research, 2022; 12 (2): 72-78.
14. Azeemi HI, Asghar SS, Ansari B, Alam JM, Mahmood SR. Effects of exercise in fasting and postprandial conditions with metabolic profiling of fats and carbohydrates. Int J of Biol-Biotech., 2020; 17 (3): 583-587
15. Azeemi HI, Ansari B, Alam JM.Assessment of serum enzyme levels in various muscular dystrophy conditions. *INT. J. BIOL. BIOTECH., 2018;* 15 (4): 48-51 2018
16. Effects of exercise and metformin on lipid profile and gylcemic index in patients with Type 2 Diabetes Mellitus (T2DM). Humayun I, Azmi, Basit Ansari, Junaid Mahmood Alam, Syed Riaz Mahmood. Int J of Biol-Biotech 17 (1): 527-531 2020 b
17. Diazyme Laboratories. Homocysteine a Strong Risk Factor for Cardiovascular Disease: A guide to Homocysteine, correlation to human disease, and various testing methods, October 2011, 34.
18. Refsum H. Total Homocysteine: Guidelines for Determination in the Clinical Laboratory. Clin Lab News 2002 May;12-14 (www.aacc.org).
19. Refsum H, Smith AD, Ueland PM, et al. Facts and Recommendations about Total Homocysteine Determinations: An Expert Opinion. Clin Chem 2004;50(1):3-32.
20. 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: 133–135. 28.
21. Araki A, Sako Y, Ito H. Plasma homocysteine concentrations in Japanese patients with non–insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. Atherosclerosis. 1993;103:149–157
22. Muzurović E, Kraljević I, Solak M, Dragnić S, Mikhailidis DP. Homocysteine and diabetes:

Role in macrovascular and microvascular complications. Journal of Diabetes and its Complications, 2021; 35 (3) doi.org/10.1016/ j.jdiacomp.2020.107834.

23. Djuric D, Jakovljevic V, Zivkovic V, Srejovic I, Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems. Canadian Journal of Physiology and Pharmacology, 2018; 96(10), 991–1003
24. Ostrakhovitch EA, Tabibzadeh S. Homocysteine and age-associated disorders. Ageing Research Reviews, 2019; 49 144–164.
25. Wu x, Zhang l, Miao Y. Homocysteine causes vascular endothelial dysfunction by disrupting endoplasmic reticulum redox homeostasis," Redox Biology, 2019; 20 46–59 2019.
26. Shiao S, Lie A, Yu CH. Meta-analysis of homocysteine-related factors on the risk of colorectal cancer. Oncotarget, 2018; 9 (39), 25681–25697.
27. Lu J, Chen K, Chen W, Liu C, Jiang X, Ma Z, Li D, Shen Y, Tian H. Association of Serum Homocysteine with Cardiovascular and All-Cause Mortality in Adults with Diabetes: A Prospective Cohort Study. Oxidative Medicine and Cellular Longevity, 2022; doi.org/10.1155/2022/2156483
28. Zhang Z, Gu X, Fang X, Tang Z, Guan S, Liu H, Wu X, Wang C, Zhao Y. Homocysteine and the Risk of Cardiovascular Events and All-Cause Death in Elderly Population: A Community-Based Prospective Cohort Study. Therapeutics and Clinical Risk Management, 2020; 471-481, DOI: 10.2147/ TCRM.S23949.