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ORIGINAL ARTICLE

Association between homocysteine and Glycated Hemoglobin in pre-diabetic and diabetic patients of Karachi Pakistan

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ABSTRACT

Background: Diabetes Mellitus (DM) is a metabolic disorder related to high morbidity and mortality. This study aims to investigate the relationship between plasma homocysteine (Hcy) levels and Glycated Hemoglobin A1c (HbA1c) levels in individuals with pre-diabetes and diabetes mellitus.

Methods: This study was conducted at the Department of Chemical Pathology, Dow University of Health Sciences (DUHS), Karachi, involving 148 participants (74 pre-diabetic, 74 diabetic). Plasma Hcy levels and HbA1c levels were measured using the Siemens Atellica Analyzer. Statistical analysis was performed using SPSS version 26. Mann-Whitney U test, Spearman's correlation, and linear regression were used to analyze data, considering level of significance ($p<0.005$).

Results: Diabetic participants had significantly higher Hcy levels (31.94 ± 8.88 µmol/L) compared to pre-diabetic individuals (26.55 ± 12.57 µmol/L) ($p < 0.001$). HbA1c levels were also significantly higher in diabetics (7.97 ± 0.98%) compared to pre-diabetics (6.05 ± 0.18%) ($p < 0.001$). Weak but significant positive correlation was observed between Hcy and HbA1c ($r = 0.225$, $p = 0.006$). Linear regression analysis showed that Hcy levels explained 4.9% of the variance in HbA1c levels ($R = 0.222$, $p = 0.007$).

Conclusion: We observed a weak but statistically significant association between hcy and HbA1c levels. It highlights the need for further research to explore potential mechanisms and confounding factors.

Keywords: Diabetes Mellitus, Glycated Hemoglobin A1c, Glycemic Control, Homocysteine, Prediabetic State

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Introduction

Diabetes mellitus (DM) is a widespread and escalating multifactorial disorder of global health concern, that is characterized by persistent hyperglycemia resulting from shortcomings in insulin secretion, insulin action, or both (1). The disorder manifests through various complications, grouped into systemic microvascular and macrovascular damage (2). Among the latter, cardiovascular diseases (CVD) are prevalent, with diabetic individuals exhibiting a 2-4 times higher risk of developing coronary artery disease (CAD)

compared to non-diabetic individuals (3). The pathogenesis of cardiovascular complications in diabetes is multifactorial, with hyperglycemia, dyslipidemia, and endothelial dysfunction being key contributors (4).

Homocysteine (Hcy), a sulfur-containing amino acid that is produced during the methionine metabolism pathway, has emerged as an important biomarker in CVD (5). Elevated levels of plasma Hcy have been linked to endothelial cell damage, promoting the development of atherosclerosis, thrombosis, and subsequent vascular complications (6). Hcy-induced endothelial dysfunction is mediated through mechanisms such as oxidative stress, collagen sulfation, and the generation of Hcy thiolactone (7). In patients with diabetes and pre-diabetes, elevated plasmaHcy levels are commonly observed, with insulin resistance and hyperinsulinemia further exacerbating these levels. However, the relationship between Hcy levels and glycemic control, particularly as indicated by HbA1c, remains underexplored in clinical research, particularly in local populations.

HbA1c serves as a primary biomarker for long-term glycemic control, reflecting the average blood glucose concentration over a 2-3 month period (8). HbA1c is utilized as a diagnostic tool for diabetes and a predictor of diabetes-related complications, including CVD (9). High HbA1c levels are associated with an increased risk of both microvascular and macrovascular complications, underlining the importance of maintaining optimal glycemic control in diabetic patients (10, 11). The potential relationship between HbA1c and Hcy levels, particularly in pre-diabetic and diabetic populations, could provide the interplay between glycemic control and cardiovascular risk.

Recent studies suggest that plasma Hcy levels are influenced by insulin concentrations and anti-diabetic therapies such as metformin, glitazones, and insulin itself, which may either elevate or reduce Hcy levels (12-14). However, the association between Hcy and HbA1c levels has shown mixed results in the existing literature (15-17). While some studies report a positive correlation between Hcy and HbA1c in diabetic patients, others suggest a negative or no significant relationship (16). Despite this, the association between these two markers remains poorly defined, particularly in local settings where diabetes and CVD are on the rise. Therefore, the study was aimed to investigate the relationship between Hcy and HbA1c in individuals with pre-diabetes and diabetes mellitus patients.

Methods

This study was carried out in the Department of Chemical Pathology, DUHS, Ojha Campus, Karachi. The study was carried out over a period of six months, following the ethical approval from the institutional review board of DUHS via letter no. IRB-3723/DUHS/Approval/2024/05.

A non-probability consecutive sampling method was used to recruit participants into the study. Sample size calculation was based on a study by Ebesunun et al. (18), where the mean value of total homocysteine (tHcy) in the male group was 12.57 ± 4.7 µmol/L, and in the female group, it was 9.6 ± 2.8 µmol/L. Using these values, a sample size of 56 was estimated with a confidence interval of 95%, a power of 80%, and a ratio of sample size between the tested and control groups set to 1. After accounting for a 30% attrition rate, the adjusted sample size was determined to be 74 participants per group. With two

groups (pre-diabetic and diabetic), the total sample size was 148 participants.

Diabetic patients included those with HbA1c > 6.5% aged 18–60 years, while pre-diabetic participants had FBG levels between 100–125 mg/dL and HbA1c 5.7–6.4%, also aged 18–60 years. Exclusion criteria for both groups included pregnant women, diabetics (for pre-diabetics), and those on medications like methotrexate, niacin, fibric acid derivatives, folate, or vitamins B6/B12. Furthermore, patient with renal and liver diseases were also excluded.

Data collection took place in the main laboratory and OPD block of DUHS, Ojha Campus, Karachi. Written informed consent was obtained from all participants. Demographic data, including age, and gender, were recorded on an approved proforma.

A 5 ml blood sample was drawn under aseptic conditions using a disposable syringe. The blood was readily transferred to EDTA tube and was processed using a centrifuge at 3000 revolutions per minute for 10 minutes to separate the plasma, which was then transferred into Eppendorf tubes. Homocysteine levels were measured using the Siemens Atellica Analyzer and the Atellica IM Hcy Assay, a competitive immunoassay utilizing direct chemiluminescent technology.

SPSS version 26.0 was used to analyze the data. Descriptive statistics, were calculated for continuous variables such as age, homocysteine, and HbA1c. The frequency and percentage distributions were calculated for categorical variables. Shapiro-Wilk test was applied to determine normality. The Mann-Whitney U test was employed to compare homocysteine and HbA1c levels between the pre-diabetic and diabetic groups. Spearman's rank correlation coefficient was applied to find the relationship between homocysteine and HbA1c. To determine the strength and direction of the relationship between homocysteine and HbA1c, a simple linear regression analysis was conducted. The significance of the regression coefficients was assessed using t-tests. A p-value of <0.05 was considered statistically significant for all tests.

Results

The study sample consisted of 148 participants, aged 27 to 68 years, with a mean age of 51.35 ± 9.2 years. Of these participants, 74 were classified as pre-diabetic and 74 as diabetic. In the pre-diabetic group, 54.1% (n = 40) were male and 45.9% (n = 34) were female, while in the diabetic group, 56.8% (n = 42) were male and 43.2% (n = 32) were female.

The Mann-Whitney U test showed that diabetic patients had significant higher homocysteine levels (mean 31.94, S.D 8.88 µmol/L) compared to pre-diabetic group (mean 26.55, S.D 12.57 µmol/L), ($p < 0.001$). Similarly, HbA1c levels were also differed between the groups. The pre-diabetic group had a mean HbA1c of 6.05 ± 0.18%, in contrast, the diabetic group showed a mean HbA1c of 7.97 ± 0.98% with statistically significant difference ($p < 0.001$) (Fig 1).

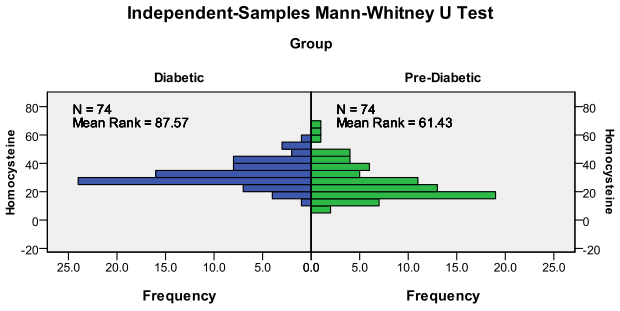

Figure 1: Homocysteine and HbA1c levels Difference between Pre-Diabetic and Diabetic Patients

A Spearman's rank correlation analysis revealed a strong, positive correlation between homocysteine and HbA1c (r = 0.225, p = 0.006), indicating that with the increment of Hcy levels, the HbA1c levels also increases and vice versa. All the results are shown in table 1 below.

Table 1: Correlation between Homocysteine and HbA1c

Correlations				
			Homocysteine	Hba1c
Spearman's rho	Homocysteine	Correlation Coefficient	1.000	.225**
		Sig. (2-tailed)	.	.006
		N	148	148
	Hba1c	Correlation Coefficient	.225**	1.000
		Sig. (2-tailed)	.006	.
		N	148	148

** demonstrates p-value at 0.01.

Linear regression analysis revealed significant relationship between homocysteine and HbA1c (R = 0.222, p = 0.007), with homocysteine explaining 4.9% of the variance in HbA1c levels. For each unit increase in homocysteine, HbA1c increased by 0.024 units (p = 0.007). (Table 2)

Table 2: Linear Regression

Model	R	R Square	Adjusted R Square	Change Statistics				
				R Square Change	F Change	df1	df2	p-value
1	.222[a]	.049	.043	.049	7.570	1	146	.007

Model		Unstandardized Coefficients		t	p-value
		B	Std. Error		
1	Constant	6.316	.269	23.435	.000
	Hcy	.024	.009	2.751	.007

Discussion

One of the major complications related to DM is damaging cardiovascular and renal systems. (1). One such complication is hyperhomocysteinemia (HHcy), an elevated level of homocysteine (Hcy) in the plasma, which has been recognized as an independent risk factor for cardiovascular disease (CVD) (16, 19). The relationship between Hcy and glycemic control, as measured by glycated hemoglobin (HbA1c), is of significant interest, as both high Hcy levels and poor glycemic control are associated with an increased risk of vascular complications. Previous studies have suggested that hyperglycemia-induced oxidative stress may impair Hcy metabolism, thereby contributing to its accumulation in diabetic individuals. (11, 17, 18).

Our study examined the relationship between plasma Hcy levels and glycemic control, measured by glycated hemoglobin (HbA1c), in pre-diabetic and diabetic individuals. The findings demonstrated significantly higher Hcy and HbA1c levels in diabetic patients compared to pre-diabetic participants. A weak yet statistically

significant positive correlation was observed between Hcy and HbA1c levels, suggesting a potential relationship between poor glycemic control and elevated Hcy levels.

Our study found significantly higher Hcy levels in diabetic patients (mean 31.94 ± 8.88 µmol/L) compared to pre-diabetic individuals (mean 26.55 ± 12.57 µmol/L), with a statistically significant difference ($p < 0.001$). These findings are consistent with several previous studies that have reported elevated Hcy levels in diabetic populations. For example, Bansal et al. (20) reported a significant positive correlation between Hcy and HbA1c in diabetic patients, suggesting that hyperglycemia-induced oxidative stress impairs Hcy metabolism and leads to its accumulation. Similarly, Abdulhameed et al. (21) observed markedly elevated Hcy levels in diabetic patients compared to non-diabetic controls, highlighting the role of oxidative stress and inflammation in disrupting Hcy clearance. Chen et al. (22) and Shen et al. (23) also demonstrated that poorly controlled diabetes (HbA1c > 7%) is associated with higher Hcy levels, likely due to hyperglycemia-induced renal dysfunction. These studies, along with our own, reinforce the idea that diabetes and poor glycemic control exacerbate Hcy, contributing to an increased risk of vascular complications. However, not all studies have consistently supported this relationship. Aghamohammadi et al. (24) found no significant correlation between Hcy and HbA1c in diabetic patients, suggesting that factors such as dietary patterns, and vitamin supplementation may obscure the association. Khan et al. (25) reported a negative correlation between Hcy and HbA1c, attributing their findings to improved renal function in individuals with better glycemic control, which facilitates Hcy clearance. Similarly, another study also found elevated Hcy levels in diabetic patients but negative correlation with HbA1c, emphasizing the potential influence of confounding factors such as renal function, nutritional status, vitamin B12, and folate levels (26). These discrepancies highlight the complexity of the relationship between Hcy and glycemic control, which is likely modulated by population characteristics, methodological variations, and unaccounted confounders. In our study, a Spearman's rank correlation revealed a weak but statistically significant positive correlation ($r = 0.225$, $p = 0.006$) between Hcy and HbA1c, indicating that as Hcy levels increase, HbA1c levels also tend to increase. Furthermore, linear regression analysis in our study showed that Hcy explained 4.9% of the variance in HbA1c levels ($R = 0.222$, $p = 0.007$), indicating a weak but significant relationship. For each unit increase in Hcy, HbA1c increased by 0.024 units ($p = 0.007$). This may be due to hyperglycemia-induced renal dysfunction and vitamin B12 deficiency. However, the effect size observed in our study is modest, suggesting that other factors may also contribute to poor glycemic control in diabetic individuals. The role of anti-diabetic medications in influencing Hcy levels further complicates the interpretation. Metformin, a commonly prescribed medication for type 2 diabetes, has been associated with reduced vitamin B12 absorption, which may contribute to elevated Hcy levels with prolonged use (27). Infante et al. (28) and Kim et al. (29) have observed this effect, suggesting that medication use may partially explain the elevated Hcy levels observed in diabetic populations. The current study did not account for medication use, which may have influenced the observed Hcy levels. Future studies should include detailed

medication histories to better elucidate these effects. The clinical implications of our findings highlight the importance of comprehensive metabolic monitoring in diabetic and pre-diabetic individuals. Elevated Hcy levels, in conjunction with poor glycemic control, may serve as early indicators of heightened cardiovascular risk. Interventions targeting both glycemic control and Hcy reduction—such as folate and vitamin B12 supplementation—could potentially mitigate the risk of vascular complications in these populations.

Conclusion

Our study supports the association between elevated Hcy levels and poor glycemic control in diabetes, it also highlights the complexity of this relationship. The association between hcy and HbA1c is weak suggesting that further research is needed to explore the underlying mechanisms. and the role of confounding factors such as medication use, renal function, and vitamin levels.

Study limitations

The role of confounding variables like deficiency of vitamin B12, renal functions and drugs that affect hcy levels should be evaluated to further strengthen the association between hcy and HbA1c levels.

Future Recommendation

Longitudinal studies, along with a more comprehensive control for confounders, will be essential to clarify the temporal relationship between Hcy levels and glycemic control, and to better understand the potential of Hcy as a biomarker for vascular risk in diabetic populations.

Conflict of interest

All the authors declares no conflict of interest

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All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.	