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Research Article

Association of Hyperhomocysteinemia with Poor Glycemic Control and Duration of Diabetes Mellitus: A Cross-Sectional Study

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ABSTRACT

Background: Hyperhomocysteinaemia promotes oxidative stress, endothelial dysfunction, and vascular injury and may contribute to the microvascular and macrovascular complications of diabetes mellitus. Poor glycaemic control and prolonged duration of diabetes may further influence homocysteine metabolism. This study evaluated the association of hyperhomocysteinaemia with glycaemic control and duration of diabetes mellitus. **Aim:** To determine the association of hyperhomocysteinaemia with poor glycaemic control and duration of diabetes mellitus among adult patients with diabetes. **Materials and Methods:** This hospital-based observational cross-sectional study included 131 adult patients with type 1 or type 2 diabetes mellitus attending the Department of General Medicine, Victoria Hospital, Bangalore Medical College and Research Institute, Bengaluru, from August 2022 to January 2024. Demographic and clinical information, diabetes duration, and treatment history were recorded. Serum homocysteine, HbA1c, fasting and postprandial blood glucose, folate, and vitamin B12 were assessed. HbA1c >7.5% was classified as poor glycaemic control, and serum homocysteine >15 µmol/L was considered hyperhomocysteinaemia. Categorical variables were compared using the chi-square or Fisher's exact test. A p value <0.05 was considered statistically significant. **Results:** Of the 131 patients, 75 (57.3%) were male, 122 (93.1%) had type 2 diabetes, and 61 (46.6%) had diabetes for more than five years. Serum homocysteine was normal in 96 (73.3%), borderline elevated in 22 (16.8%), and elevated in 13 (9.9%) patients. Poor glycaemic control was present in 102 (77.9%; 95% CI: 70.0%-84.1%) patients. In the binary analysis of 109 patients, hyperhomocysteinaemia was present in 13 of 80 (16.3%) patients with HbA1c >7.5%,

compared with none of the 29 patients with HbA1c ≤7.5% (p=0.019). Its prevalence increased from 2.1% among patients with diabetes for 0-3 years to 24.1% and 27.3% among those with disease durations of >5-10 and >10-15 years, respectively (χ^2=19.39, p=0.002). Hyperhomocysteinaemia was present in 3.1% of patients with diabetes for ≤5 years and 25.0% of those with diabetes for >5 years (p<0.001). A diabetes duration exceeding five years was associated with approximately 10.5-fold higher odds of hyperhomocysteinaemia. **Conclusion:** Hyperhomocysteinaemia was significantly associated with poor glycaemic control and a diabetes duration exceeding five years. Serum homocysteine assessment may help identify patients with prolonged and inadequately controlled diabetes who could be at increased vascular risk. Prospective multicentre studies with adequate control of renal, nutritional, and treatment-related confounders are warranted.

KEYWORDS: Hyperhomocysteinaemia; glycated haemoglobin; diabetes duration.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Prolonged hyperglycaemia promotes oxidative stress, endothelial dysfunction, inflammation, and vascular injury, thereby increasing the risk of microvascular and macrovascular complications. Glycated haemoglobin (HbA1c) reflects average blood glucose levels over the preceding two to three months and is widely used to assess long-term glycaemic control. Poor glycaemic control and a longer duration of diabetes are associated with cumulative metabolic and vascular damage. Homocysteine is a sulphur-containing amino acid formed during methionine metabolism. Its metabolism depends on folate, vitamin B12, vitamin B6, and the normal functioning of remethylation and transsulfuration pathways. Hyperhomocysteinaemia may occur because of nutritional deficiencies, genetic abnormalities, impaired renal function, medications, or metabolic disorders. Elevated homocysteine promotes endothelial injury, oxidative stress, reduced nitric oxide bioavailability, vascular smooth-muscle proliferation, platelet activation, and thrombosis [1]. These mechanisms may accelerate atherosclerosis and contribute to cardiovascular and cerebrovascular complications in patients with diabetes. Abnormal homocysteine metabolism has also been linked to insulin resistance and diabetic microvascular complications. Meigs et al. observed an association between fasting plasma homocysteine and features of insulin resistance [2]. Majumder et al. reported that serum homocysteine was associated with glycaemic control among patients with type 2 diabetes [3]. A systematic review and meta-analysis by Wang et al. demonstrated that homocysteine levels were significantly higher among patients with type 2 diabetes than among healthy individuals, particularly in patients with diabetic nephropathy or retinopathy [4]. Hyperhomocysteinaemia may therefore represent an additional biochemical marker of metabolic disturbance and vascular risk in diabetes. Furthermore, longer exposure to hyperglycaemia may progressively alter homocysteine metabolism through renal dysfunction, oxidative stress, and nutritional or metabolic changes. Muzurović et al. highlighted the potential involvement of homocysteine in both macrovascular and microvascular diabetic complications [5]. However, the relationship between serum homocysteine, glycaemic control, and duration of diabetes has not been consistent across studies and may vary according to patient characteristics and associated conditions. Evaluating these relationships may help identify patients with

poor metabolic control who are at increased risk of future vascular complications.

AIM

To determine the association of hyperhomocysteinaemia with poor glycaemic control and duration of diabetes mellitus among adult patients with diabetes.

OBJECTIVES

1. To estimate serum homocysteine and HbA1c levels among patients with diabetes mellitus.
2. To determine the association between hyperhomocysteinaemia and poor glycaemic control.
3. To assess the association between hyperhomocysteinaemia and duration of diabetes mellitus.

MATERIALS AND METHODOLOGY

Source of Data

The study participants were recruited from known cases of diabetes mellitus attending the outpatient and inpatient services of the hospitals attached to Bangalore Medical College and Research Institute, Bengaluru. Patients fulfilling the eligibility criteria were consecutively enrolled until the required sample size was achieved.

Study Design

This was a hospital-based observational cross-sectional study.

Study Location

The study was conducted in the Department of General Medicine, Victoria Hospital, Bangalore Medical College and Research Institute, Bengaluru, Karnataka.

Study Duration

The study was conducted over 18 months, from August 2022 to January 2024.

Sample Size

A total of 131 patients with diabetes mellitus were included in the study. The sample size was based on the availability of eligible participants during the study period and was adequate for assessing the relationship between serum homocysteine, HbA1c, and duration of diabetes. All 131 enrolled participants were included in the final analysis.

Inclusion Criteria

1. Patients aged more than 18 years were included.
2. Patients of either sex with a known diagnosis of type 1 or type 2 diabetes mellitus were included.
3. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria.
4. Patients attending either outpatient or inpatient services during the study period were included.
5. Patients who provided written informed consent were included.

Exclusion Criteria

1. Patients aged 18 years or younger were excluded.
2. Patients with hypertension were excluded.
3. Patients receiving anticonvulsants, theophylline, methotrexate, levodopa, corticosteroids, or other medications known to influence homocysteine metabolism were excluded.
4. Patients with anaemia or hypothyroidism were excluded.
5. Patients with autoimmune diseases, including psoriasis and systemic lupus erythematosus, were excluded.
6. Patients already receiving folic acid, pyridoxine, or vitamin B12 supplementation were excluded.
7. Patients with documented folic acid or vitamin B12 deficiency were excluded.
8. Patients who did not provide informed consent or had incomplete clinical or laboratory data were excluded.

Data Collection

Approval was obtained from the Institutional Ethics Committee before commencement of the study. Eligible patients were informed about the purpose, procedures, potential benefits, and confidentiality provisions of the study. Written informed consent was obtained from each participant.

Data were collected using a predesigned and pretested case-record form. Information regarding age, sex, type of diabetes, age at diagnosis, duration of diabetes, treatment history, medication use, comorbidities, dietary or vitamin supplementation, and relevant clinical symptoms was recorded. The duration of diabetes was calculated from the date of initial diagnosis to the date of enrolment and was categorized as 0-3, 3-5, 6-10, 11-15, 15-20, and more than 20 years.

A detailed clinical examination was performed. Pulse rate, blood pressure, respiratory rate, temperature, height, and weight were recorded. Cardiovascular, respiratory, abdominal, and central nervous system examinations were performed to identify associated clinical abnormalities.

Procedure and Methodology

Patients were screened according to the predefined inclusion and exclusion criteria. Diabetes mellitus was confirmed from the clinical record and biochemical evidence according to the American Diabetes Association criteria: fasting plasma glucose ≥126 mg/dL, two-hour plasma glucose ≥200 mg/dL during an oral glucose-tolerance test, HbA1c ≥6.5%, or random plasma glucose ≥200 mg/dL in a patient with classical symptoms of hyperglycaemia.

Following enrolment, demographic and clinical data were documented, and venous blood samples were collected for complete blood count, fasting blood glucose, postprandial blood glucose, HbA1c, serum folate, vitamin B12, and serum homocysteine. Folate and vitamin B12 were evaluated to exclude nutritional deficiencies that could independently increase homocysteine levels.

Glycaemic control was assessed using HbA1c. In accordance with the study protocol, an HbA1c value ≤7.5% was classified as relatively good glycaemic control, whereas HbA1c >7.5% was classified as poor glycaemic control. Serum homocysteine was classified as normal or elevated according to the reference range followed by the institutional laboratory; a value >15 µmol/L was considered hyperhomocysteinaemia. Serum homocysteine status was compared across HbA1c categories and categories of diabetes duration.

Sample Processing

After an overnight fast, venous blood was collected under aseptic precautions. Samples required for complete blood count and HbA1c estimation were collected in ethylenediaminetetraacetic acid-containing tubes. Samples for fasting glucose, serum homocysteine, folate, and vitamin B12 estimation were collected in appropriate laboratory tubes. A postprandial sample was obtained two hours after food for estimation of postprandial blood glucose.

Serum samples were allowed to clot and were centrifuged to separate the serum from cellular components. The separated serum was analysed promptly in the institutional clinical biochemistry laboratory. If immediate analysis was not possible, the samples were stored under laboratory-recommended conditions until testing. Haemolysed, improperly labelled, insufficient, or inadequately stored samples were rejected and recollected. All investigations were performed using standardized laboratory procedures, calibrated equipment, and appropriate internal quality-control measures.

Statistical Methods

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics, version

23.0. Continuous variables were summarized using mean and standard deviation or median and interquartile range, depending on the distribution of data. Categorical variables were presented as frequencies and percentages. The prevalence of hyperhomocysteinaemia was reported with a 95% confidence interval.

The independent-samples *t*-test or Mann-Whitney *U* test was used to compare continuous variables between patients with normal and elevated homocysteine levels, as appropriate. The chi-square test was used to assess the association of homocysteine category with glycaemic-control category and duration of diabetes. Fisher's exact test was applied when expected cell frequencies were less than five. Pearson's or Spearman's correlation coefficient was used to evaluate the relationship of serum homocysteine with HbA1c and duration of diabetes, depending on data distribution.

Where applicable, binary logistic regression was performed with hyperhomocysteinaemia as the dependent variable to estimate odds ratios and 95% confidence intervals for poor glycaemic control and longer diabetes duration after adjustment for relevant covariates. All tests were two-tailed, and a *p* value <0.05 was considered statistically significant.

OBSERVATION AND RESULTS

Table 1. Overall clinical profile relevant to the association of hyperhomocysteinaemia with glycaemic control and duration of diabetes mellitus (N=131)

Parameter	n (%)	95% CI	Test of significance†	P value
Male sex	75 (57.3%)	48.7%-65.4%	z=1.66	0.097
Female sex	56 (42.7%)	34.6%-51.3%	z=−1.66	0.097
Type 1 diabetes mellitus	9 (6.9%)	3.7%-12.5%	z=−9.87	<0.001*
Type 2 diabetes mellitus	122 (93.1%)	87.5%-96.3%	z=9.87	<0.001*
Hyperhomocysteinaemia	13 (9.9%)	5.9%-16.2%	z=−9.17	<0.001*
Borderline homocysteine	22 (16.8%)	11.4%-24.1%	-	-
Normal homocysteine	96 (73.3%)	65.1%-80.1%	-	-
Poor glycaemic control, HbA1c >7.5%	102 (77.9%)	70.0%-84.1%	z=6.38	<0.001*
Relatively good glycaemic control, HbA1c ≤7.5%	29 (22.1%)	15.9%-30.0%	z=−6.38	<0.001*
Duration of diabetes ≤5 years	70 (53.4%)	44.9%-61.8%	z=0.79	0.432
Duration of diabetes >5 years	61 (46.6%)	38.2%-55.1%	z=−0.79	0.432

†One-sample proportion z-test against a reference proportion of 50%.
*Statistically significant at p<0.05.

Among the 131 patients with diabetes mellitus, 75 (57.3%; 95% CI: 48.7%-65.4%) were male and 56 (42.7%; 95% CI: 34.6%-51.3%) were female, with no statistically significant difference from an equal sex distribution (p=0.097). Most participants had type 2 diabetes mellitus (93.1%), whereas only 6.9% had type 1 diabetes mellitus; this distribution was statistically significant (p<0.001). Hyperhomocysteinaemia was observed in 13 (9.9%; 95% CI: 5.9%-16.2%) patients, while 22 (16.8%) had borderline and 96 (73.3%) had normal homocysteine levels. Poor glycaemic control, defined as HbA1c >7.5%, was present in 102 (77.9%; 95% CI: 70.0%-84.1%) patients and was significantly more prevalent than the reference proportion of 50% (z=6.38, p<0.001). Only 29 (22.1%) patients had relatively good glycaemic control. Regarding disease duration, 70 (53.4%) patients had diabetes for five years or less and 61 (46.6%) had diabetes for more than five years. The difference between these duration categories was not statistically significant (p=0.432).

Table 2. Distribution of serum homocysteine status and HbA1c-defined glycaemic control among patients with diabetes mellitus (N=131)

Biochemical parameter	Category	n (%)	95% CI	Test of significance	P value
Serum homocysteine	Normal	96 (73.3%)	65.1%-80.1%	χ^2=95.01†	<0.001*
	Borderline elevation	22 (16.8%)	11.4%-24.1%		
	Hyperhomocysteinaemia	13 (9.9%)	5.9%-16.2%		
HbA1c	≤7.5%: relatively good control	29 (22.1%)	15.9%-30.0%	z=6.38‡	<0.001*
	>7.5%: poor control	102 (77.9%)	70.0%-84.1%		

†Chi-square goodness-of-fit test comparing the three homocysteine categories.
‡One-sample proportion z-test comparing the prevalence of poor glycaemic control with 50%.
*Statistically significant at p<0.05.

Serum homocysteine levels were normal in 96 (73.3%; 95% CI: 65.1%-80.1%) patients, borderline elevated in 22 (16.8%; 95% CI: 11.4%-24.1%), and elevated to the hyperhomocysteinaemic range in 13 (9.9%; 95% CI: 5.9%-16.2%). The distribution across the three homocysteine categories was statistically significant (χ^2=95.01, p<0.001), with normal levels constituting the largest category. Based on HbA1c, 102 (77.9%; 95% CI: 70.0%-84.1%) patients had poor glycaemic control, whereas only 29 (22.1%; 95% CI: 15.9%-30.0%) had relatively good control. The prevalence of poor glycaemic control was significantly higher than the reference proportion of 50% (z=6.38, p<0.001). Thus, although definite hyperhomocysteinaemia affected approximately one-tenth of the participants, more than three-fourths had inadequate glycaemic control.

Table 3. Association between hyperhomocysteinaemia and poor glycaemic control

Glycaemic control	Hyperhomocysteinaemia, n (%)	Normal homocysteine, n (%)	Total analysed	95% CI for hyperhomocysteinaemia	Test of significance	P value
HbA1c ≤7.5%	0 (0.0%)	29 (100.0%)	29	0.0%-11.7%	Fisher's exact test	0.019*

HbA1c >7.5%	13 (16.3%)	67 (83.8%)	80	9.7%-25.8%		
Total	**13 (11.9%)**	**96 (88.1%)**	**109**	**7.1%-19.3%**		

*Statistically significant at $p<0.05$.

Of the 109 patients included in the binary homocysteine analysis, 13 (11.9%; 95% CI: 7.1%-19.3%) had hyperhomocysteinaemia and 96 (88.1%) had normal homocysteine levels. None of the 29 patients with HbA1c ≤7.5% had hyperhomocysteinaemia, whereas all 13 cases of hyperhomocysteinaemia occurred among patients with HbA1c >7.5%. The prevalence of hyperhomocysteinaemia among patients with poor glycaemic control was 16.3% (95% CI: 9.7%-25.8%), compared with 0.0% (95% CI: 0.0%-11.7%) among patients with relatively good control. Fisher's exact test demonstrated a statistically significant association between hyperhomocysteinaemia and poor glycaemic control ($p=0.019$).

Table 4. Association between hyperhomocysteinaemia and duration of diabetes mellitus

Duration of diabetes	**Hyperhomocysteinaemia, n (%)**	**Normal homocysteine, n (%)**	**Total analysed**	**Prevalence of hyperhomocysteinaemia (95% CI)**	**Test of significance**	**P value**
0-3 years	1 (2.1%)	47 (97.9%)	48	2.1% (0.4%-10.9%)	$\chi^2=19.39$, df=5†	0.002*
>3-5 years	1 (5.9%)	16 (94.1%)	17	5.9% (1.0%-27.0%)		
>5-10 years	7 (24.1%)	22 (75.9%)	29	24.1% (12.2%-42.1%)		
>10-15 years	3 (27.3%)	8 (72.7%)	11	27.3% (9.7%-56.6%)		
>15-20 years	1 (100.0%)	0 (0.0%)	1	100.0% (20.7%-100.0%)		
>20 years	0 (0.0%)	3 (100.0%)	3	0.0% (0.0%-56.1%)		
Total	**13 (11.9%)**	**96 (88.1%)**	**109**	**11.9% (7.1%-19.3%)**		

†Pearson chi-square test.

*Statistically significant at $p<0.05$.

A clinically more stable analysis was obtained after combining the duration categories:

Duration category	**Hyperhomocysteinaemia, n (%)**	**Normal homocysteine, n (%)**	**Total**	**95% CI for hyperhomocysteinaemia**	**Test of significance**	**P value**
≤5 years	2 (3.1%)	63 (96.9%)	65	0.8%-10.5%	Fisher's exact test	<0.001*
>5 years	11 (25.0%)	33 (75.0%)	44	14.6%-39.4%		

Odds ratio: OR=10.50; patients with diabetes for more than five years had approximately 10.5 times higher odds of hyperhomocysteinaemia than those with diabetes for five years or less.

The prevalence of hyperhomocysteinaemia generally increased with the duration of diabetes. It was 2.1% among patients with diabetes for 0-3 years, 5.9% among those with diabetes for more than 3-5 years, 24.1% among those with a duration of more than 5-10 years, and 27.3% among those with a duration of more than 10-15 years. One patient in the more than 15-20-year category had hyperhomocysteinaemia, while none of the three patients with diabetes for more than 20 years had elevated homocysteine. The overall association between duration of diabetes and hyperhomocysteinaemia was statistically significant (χ^2=19.39, df=5, p=0.002). Nevertheless, the estimates for the longest-duration categories had wide confidence intervals because of their very small sample sizes and should therefore be interpreted cautiously.

After combining the duration categories, hyperhomocysteinaemia was present in only 2 of 65 (3.1%; 95% CI: 0.8%-10.5%) patients with diabetes for five years or less, compared with 11 of 44 (25.0%; 95% CI: 14.6%-39.4%) patients with diabetes for more than five years. This difference was statistically significant on Fisher's exact test (p<0.001). Patients with a diabetes duration exceeding five years had approximately 10.5 times higher odds of hyperhomocysteinaemia than those with a duration of five years or less (OR=10.50), demonstrating a strong association between prolonged diabetes and elevated homocysteine levels.

DISCUSSION

Table 1: Overall clinical profile

The present study included 131 patients with diabetes mellitus, with a modest male predominance of 57.3%; however, the sex distribution did not differ significantly from 50% (p=0.097). Type 2 diabetes constituted 93.1% of cases, while type 1 diabetes accounted for only 6.9%, reflecting the substantially greater population burden and hospital attendance associated with type 2 diabetes. Bansal et al. (2016)[1] similarly studied predominantly adult patients with type 2 diabetes and reported higher serum homocysteine levels in diabetic patients than in healthy controls. Majumder et al. (2017)[2] also observed that type 2 diabetes formed the principal clinical setting in which the relationship between homocysteine and glycaemic control was evaluated.

Definite hyperhomocysteinaemia was identified in 9.9% of participants, while another 16.8% had borderline elevation. Thus, approximately one-fourth of the participants had either borderline or elevated homocysteine levels. Choudhary et al. (2018)[3] reported higher serum homocysteine among patients with type 2 diabetes and demonstrated its relationship with an adverse lipid profile. Differences in the prevalence reported across studies may be attributable to variations in age, renal function, folate and vitamin B12 status, metformin exposure, dietary patterns, assay methods, and the cut-off used to define hyperhomocysteinaemia. Aroda et al. (2016)[4] demonstrated that long-term metformin use increased the risk of biochemical vitamin B12 deficiency, which may indirectly elevate homocysteine. The comparatively low prevalence of definite hyperhomocysteinaemia in the present study may partly be explained by the exclusion of patients with folate or vitamin B12 deficiency and those already receiving vitamin supplementation.

Poor glycaemic control, defined as HbA1c >7.5%, was present in 77.9% of participants, indicating a high burden of inadequate long-term glucose control. Noor et al. (2021)[5] similarly reported HbA1c >7% in 83.5% of their patients with type 2 diabetes. The duration of diabetes was five years or less in 53.4% and more than five years in 46.6%, with no significant difference between these two overall proportions (p=0.432). Although

this comparison merely describes the sample distribution, the large subgroup with disease exceeding five years was clinically important because prolonged exposure to hyperglycaemia may be accompanied by renal impairment, oxidative stress, endothelial dysfunction, and disturbances in homocysteine metabolism.

Table 2: Homocysteine status and glycaemic control

Normal homocysteine levels were observed in 73.3% of patients, borderline elevation in 16.8%, and definite hyperhomocysteinaemia in 9.9%. The distribution differed significantly across the three categories (χ^2=95.01, p<0.001), mainly because normal homocysteine was the predominant category. Nevertheless, the combined prevalence of borderline and definite elevation was clinically relevant. Bansal et al. (2016)[1] reported that patients with type 2 diabetes had higher serum homocysteine than non-diabetic controls. Choudhary et al. (2018)[3] similarly found elevated homocysteine among patients with type 2 diabetes and suggested that its coexistence with dyslipidaemia could contribute to increased cardiovascular risk.

Majumder et al. (2017)[2] reported a significant association between serum homocysteine and glycaemic control in patients with type 2 diabetes, supporting the present finding that homocysteine abnormalities occurred in a population with a high prevalence of poor glycaemic control. A systematic review and meta-analysis by Wang et al. (2021)[6] found that circulating homocysteine was significantly higher in patients with type 2 diabetes than in healthy individuals and was particularly elevated in patients with diabetic nephropathy or retinopathy. Rijal et al. (2023)[7] also reported higher homocysteine among young patients with newly diagnosed diabetes than among healthy controls, suggesting that disturbed homocysteine metabolism may occur even before long-standing diabetic complications become established.

In the present study, 77.9% of participants had HbA1c >7.5%, whereas only 22.1% had relatively good control. Noor et al. (2021)[5] reported a comparable burden of poor control and found a direct relationship between HbA1c and serum homocysteine. The biological relationship between these markers is plausible because persistent hyperglycaemia promotes oxidative stress and endothelial dysfunction, while elevated homocysteine further reduces nitric oxide bioavailability and promotes vascular injury. However, homocysteine is influenced by renal function, vitamin status, genetic variation, age, sex, smoking, and medication exposure; therefore, HbA1c cannot be considered its sole determinant.

Table 3: Hyperhomocysteinaemia and poor glycaemic control

Among the 109 patients included in the binary analysis, hyperhomocysteinaemia was found in 16.3% of those with HbA1c >7.5%, whereas none of the patients with HbA1c ≤7.5% had hyperhomocysteinaemia. Fisher's exact test demonstrated a significant association between poor glycaemic control and hyperhomocysteinaemia (p=0.019). This finding was consistent with Majumder et al. (2017)[2], who reported that serum homocysteine was associated with glycaemic control among patients with type 2 diabetes. Noor et al. (2021)[5] likewise found a direct correlation between HbA1c and homocysteine and reported that severe hyperhomocysteinaemia was concentrated among patients with HbA1c >7%.

The present findings are also supported indirectly by interventional research. Satapathy et al. (2020)[8] reported that folic acid and methylcobalamin supplementation reduced serum homocysteine in patients with type 2 diabetes, while methylcobalamin-containing regimens also improved HbA1c and insulin resistance. Elbarbary et al.

(2020)[9] found that vitamin B-complex supplementation reduced homocysteine, HbA1c, urinary albumin excretion, and cystatin C among young patients with type 1 diabetes and early nephropathy. El-khodary et al. (2022)[10] similarly observed that folic acid supplementation lowered homocysteine and improved glycaemic indices and insulin resistance among patients with type 2 diabetes. These studies suggest that one-carbon metabolism, vitamin status, glycaemic regulation, and renal function may be interrelated.

Nevertheless, not all intervention studies have demonstrated parallel changes in glycaemic indices and homocysteine. Gheflati et al. (2019)[11] found improvement in fasting blood glucose and oxidative-stress indices after apple-vinegar supplementation but no significant change in homocysteine. Therefore, the significant cross-sectional relationship in the present study should not be interpreted as proof that poor glycaemic control directly caused hyperhomocysteinaemia. Reverse causation and residual confounding remain possible.

An important analytical consideration is that the 22 patients with borderline homocysteine levels were excluded from the binary analysis because their HbA1c distribution was unavailable. Consequently, Table 3 analysed 109 rather than all 131 participants. Although Fisher's exact test was appropriate because one cell contained zero cases, the result should be interpreted with the available-case denominator and confirmed using a complete three-category analysis in future studies.

Table 4: Hyperhomocysteinaemia and duration of diabetes

The prevalence of hyperhomocysteinaemia increased from 2.1% among patients with diabetes for 0-3 years and 5.9% among those with diabetes for more than 3-5 years to 24.1% among those with diabetes for more than 5-10 years and 27.3% among those with diabetes for more than 10-15 years. The overall association between duration category and hyperhomocysteinaemia was statistically significant (χ^2=19.39, p=0.002). Noor et al. (2021)[5] similarly demonstrated a significant association between longer duration of type 2 diabetes and increasing homocysteine levels and recommended closer evaluation of patients with disease exceeding five years.

When duration was dichotomised, hyperhomocysteinaemia was present in 3.1% of patients with diabetes for five years or less and 25.0% of those with diabetes for more than five years. Patients in the longer-duration group had approximately 10.5 times higher odds of hyperhomocysteinaemia ($p<0.001$). This result supports the hypothesis that prolonged diabetes may contribute to disturbances in homocysteine metabolism through cumulative glycaemic exposure, progressive renal dysfunction, chronic inflammation, oxidative stress, metformin-associated vitamin B12 depletion, and increasing vascular injury. Aroda et al. (2016)[4] showed that vitamin B12 deficiency became more frequent with prolonged metformin exposure, providing one possible mechanism linking diabetes duration with elevated homocysteine.

The clinical importance of this association extends beyond biochemical abnormality. Lei et al. (2018)[12], in a systematic review and meta-analysis, found that elevated homocysteine was associated with a higher risk of diabetic retinopathy. Muzurović et al. (2021)[13] concluded that homocysteine may participate in both macrovascular and microvascular complications of diabetes through endothelial dysfunction, oxidative stress, inflammation, and prothrombotic mechanisms. Wang et al. (2021)[6] also reported greater homocysteine concentrations among patients with diabetic nephropathy and retinopathy than among patients with uncomplicated type 2 diabetes.

The apparent 100% prevalence in the >15-20-year subgroup and absence of hyperhomocysteinaemia in the >20-year subgroup should not be interpreted as a true biological reversal because these categories contained only one and three patients, respectively. Their confidence intervals were correspondingly wide. Furthermore, multiple cells had expected frequencies below five, weakening the assumptions of the Pearson chi-square test. The dichotomised Fisher's exact analysis was therefore more statistically stable, although it also involved only 109 patients because the borderline group was unavailable for cross-classification.

CONCLUSION

Hyperhomocysteinaemia was significantly associated with poor glycaemic control and longer duration of diabetes mellitus. It was observed exclusively among patients with HbA1c >7.5%, and its prevalence was significantly higher among patients who had diabetes for more than five years. Patients with diabetes for more than five years had approximately 10.5 times higher odds of hyperhomocysteinaemia than those with a shorter disease duration. These findings suggest that serum homocysteine may serve as an additional biochemical marker for identifying patients with prolonged diabetes and inadequate glycaemic control who may be at increased vascular risk. Nevertheless, longitudinal studies are required to establish causality and determine the clinical value of routine homocysteine screening.

LIMITATIONS OF STUDY

This study had several limitations. Its cross-sectional design established associations but could not determine the temporal or causal relationship between hyperhomocysteinaemia, poor glycaemic control, and duration of diabetes. As a single-centre, hospital-based study with a relatively small sample, the findings may not be generalizable to the wider diabetic population. The number of patients with type 1 diabetes and those in the longest-duration categories was small, resulting in imprecise estimates and wide confidence intervals. Several duration categories had low expected cell frequencies, limiting the reliability of the Pearson chi-square analysis. Twenty-two participants with borderline homocysteine levels could not be included in the binary association analyses because their cross-classification by HbA1c and diabetes duration was unavailable; consequently, Tables 3 and 4 included only 109 of the 131 participants. Potential confounders such as age, renal function, metformin exposure, dietary intake, smoking, alcohol consumption, socioeconomic status, genetic polymorphisms, and subtle variations in folate and vitamin B12 status were not fully adjusted for. Homocysteine and HbA1c were measured at a single time point, and intra-individual biological or laboratory variation could not be assessed. Furthermore, the absence of a non-diabetic control group prevented comparison of hyperhomocysteinaemia prevalence between diabetic and healthy populations.

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