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Association between Hyperhomocysteinemia and Microangiopathic Complications (Neuropathy and Nephropathy) in Subjects with Type 1 and Type 2 Diabetes

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Abstract

This prospective case-control study aimed to assess the prevalence of hyperhomocysteinemia and explore its potential correlation with microangiopathic complications, specifically nephropathy and neuropathy, in a cohort of both type 1 and type 2 diabetic patients. Conducted at the Marc Sankalé Center of Abass Ndao Hospital in Dakar from June to September 2018, the study enrolled a total of 106 diabetic patients, comprising 93 type 2 diabetics and 13 type 1 diabetics, who were matched with control subjects free from clinically detectable pathologies, based on sex and age ± 2 years. The mean age of type 1 and type 2 diabetic patients was 24.46 ± 8.41 years and 57.28 ± 11.28 years, respectively. Our findings revealed a statistically significant elevation in mean homocysteine levels among patients when compared to controls (12.63 vs. 9.88; $p < 0.0001$). Hyperhomocysteinemia was observed in 24.5% of the patients, exclusively among those with type 2 diabetes. Within the hyperhomocysteinemia subgroup, 58% were male, and 42% were female. The analysis of neuropathy and nephropathy frequencies among type 2 diabetic patients, stratified by homocysteine concentrations, demonstrated a notably higher prevalence of diabetic nephropathy in patients with hyperhomocysteinemia compared to those with normohomocysteinemia (23.07% vs. 8.75%; $p = 0.052$). Similarly, diabetic neuropathy exhibited a significantly greater fre-

quency in patients with hyperhomocysteinemia as opposed to normohomocysteinemia (80.76% vs. 50%; p = 0.005). Furthermore, our results established a significant positive correlation between homocysteine concentrations and both age (r = 0.402; p < 0.0001) and creatinine levels (r = 0.461; p < 0.0001). Bivariate logistic regression analysis indicated that patients with hyperhomocysteinemia faced 3 times and 6 times higher risks of developing neuropathy (OR = 3.5; p = 0.061) and diabetic nephropathy (OR = 6.092; p = 0.014), respectively.

Keywords

Homocysteine, Hyperhomocysteinemia, Diabetes Mellitus, Type 1 Diabetes, Type 2 Diabetes, Nephropathy, Neuropathy

1. Introduction

The escalating prevalence of diabetes over the past decade, driven by a global surge in obesity rates, poses a significant challenge to public health. As of 2021, estimates from the International Diabetes Federation (IDF) indicate that approximately 537 million individuals worldwide are living with diabetes. If substantial action is not taken to prevent and manage this epidemic, projections suggest that this number could surpass 700 million by 2045 [1]. The consequential burden of premature morbidity, mortality, diminished life expectancy, and soaring treatment costs underscores the urgent need for effective intervention strategies.

Microangiopathic complications, such as neuropathy and nephropathy, stand as serious and distressing outcomes of diabetes, contributing substantially to heightened morbidity and mortality within the diabetic population. The etiology of these microangiopathic complications is intricate, arising from a multifaceted interplay of genetic, environmental, and metabolic factors.

Among the potential contributory factors, hyperhomocysteinemia, characterized by elevated blood homocysteine levels, has emerged as a noteworthy candidate in the initiation and progression of these complications among diabetic patients. Elevated homocysteine levels have been linked to a cascade of detrimental processes, including endothelial dysfunction, heightened oxidative stress, and increased inflammation—all of which collectively contribute to the characteristic vascular damage seen in microangiopathic complications.

Recent years have borne witness to an increasing number of studies investigating the role of homocysteine, suggesting its implication in the pathogenesis of microangiopathic complications in individuals with diabetes. Research conducted among diabetic patients has unveiled an association between hyperhomocysteinemia and the presence of microangiopathic complications. Indeed, several studies have underscored the prevalence of hyperhomocysteinemia in diabetic patients afflicted by these complications [2] [3] [4] [5] [6].

However, the literature presents a certain degree of discordance in its findings. Serum homocysteine levels exhibit notable elevation in type 2 diabetes [7] [8] [9] [10] [11]. On the other hand, contrasting outcomes have been reported concerning type 1 diabetes, with serum homocysteine levels being described as normal, elevated, and even reduced among patients [12] [13] [14].

Given the evolving landscape of this research, it is noteworthy that, despite the controversy in the literature, a dearth of data exists in Senegal on this subject. In this context, studies of this nature take on heightened significance, as they offer an opportunity to enhance patient care and treatment strategies within our specific context. The prevalence of microangiopathic complications related to diabetes is particularly high in our setting, underscoring the potential benefits of identifying biomarkers to improve patient management. Therefore, our study aims to elucidate the intricate relationship between hyperhomocysteinemia and microangiopathic complications, with a specific focus on neuropathy and nephropathy, in individuals diagnosed with both type 1 and type 2 diabetes.

2. Patients et Methods

2.1. Study Designs and Subjects

This prospective case-control study was conducted from June to September 2018. The study population consisted of ninety-three (93) patients with type 2 diabetes and thirteen (13) patients with type 1 diabetes, all of whom were under the care of the Marc Sankalé Center at Abass Ndao Hospital in Dakar. These participants were meticulously matched with 106 control subjects who were devoid of clinically detectable pathologies, based on sex and age criteria within a range of ±2 years. Ethical approval for this study was granted by the Scientific Ethics Committee of the Faculty of Medicine, Pharmacy, and Dentistry at Cheikh Anta Diop University in Dakar. Informed written consent was diligently obtained from the patients themselves and/or their legal guardians.

Patients presenting with conditions or receiving medications that could potentially influence serum homocysteine concentrations were intentionally excluded from the study. For instance, individuals with chronic kidney disease, hypothyroidism, hematological disorders such as megaloblastic anemia, or gastrointestinal malabsorption disorders were excluded due to their potential to impact homocysteine levels. Similarly, patients who were taking medications known to affect serum homocysteine concentrations, such as methotrexate for autoimmune disorders, anticonvulsants like phenytoin or carbamazepine were excluded. Furthermore, individuals who were regularly taking vitamin supplements containing folic acid, vitamin B12, or vitamin B6 were also excluded. In addition, heavy smokers and those engaging in excessive alcohol consumption were excluded, given that these lifestyle factors are known to impact homocysteine metabolism. Pregnant women were also excluded from the study due to the temporary changes in homocysteine levels that can occur during pregnancy.

2.2. Data Collection

Epidemiological and demographic data were collected using a questionnaire. For each patient, blood samples were taken after a 12-hour fast through venous puncture at the elbow fold. A 24-hour urine collection was also performed to determine microalbuminuria. The blood samples were centrifuged at 3000 rpm for 5 minutes and immediately processed or stored at −20˚C until use. Homocysteine levels were measured using the Architect ci 4100® (Abbott) analyzer, HbA1c levels were measured using the D10® system (Bio Rad, USA), and other parameters were measured using the Cobas 6000/c501® analyzer (Roche, Hitachi, Germany). Diabetic nephropathy was defined as microalbuminuria levels greater than 30 mg/24h, and the diagnosis of diabetic neuropathy was clinically established.

2.3. Estimation of Serum Homocysteine

The estimation of serum homocysteine levels employed a chemiluminescence technique, which is routinely implemented in our laboratory due to its robust and satisfactory analytical performance. In this approach, the bound or dimerized form of homocysteine (oxidized form) underwent reduction to free homocysteine, achieved through the action of dithiothreitol (DTT). Subsequently, recombinant S-adenosylhomocysteine hydrolase (rSAHHase) enzyme catalyzed the conversion of free homocysteine to S-adenosylhomocysteine (SAH), utilizing an excess of adenosine as a substrate. This newly formed SAH then entered into competition with acridinium-labeled S-adenosyl-cysteine for binding to a monoclonal antibody immobilized on microparticles. Post-immobilization, subsequent washing, and magnetic separation, the resulting chemiluminescence generated a quantifiable signal measured in relative light units (RLUs). Notably, the concentration of homocysteine within the sample exhibited an inverse relationship with the magnitude of RLUs detected, a relationship meticulously quantified by the ARCHITECT iSystem optical system.

Hyperhomocysteinemia was defined as homocysteine concentrations > 15 μmol/L.

2.4. Statistical Analysis

The data were collected using Excel 2019, and the statistical analysis was performed using XLSTAT 2020 software. The Shapiro-Wilk test was used to assess the normality of the distribution of quantitative variables. The comparison of means was conducted using the student's t-test for variables with a normal distribution and the Mann-Whitney test for variables without a normal distribution. The chi-square test was used for comparing frequencies, and the association between variables was assessed using the Spearman correlation test. Additionally, logistic regression analysis was conducted to evaluate hyperhomocysteinemia as a risk factor for diabetic neuropathy and nephropathy. A p-value < 0.05 was considered statistically significant for all analyses.

3. Results

Epidemiologically, the mean age of patients was 53.14 ± 15.47 years constituted of 13 type 1 diabetics with an average age of 24.46 ± 8.41 years and 93 type 2 diabetics with an average age of 57.28 ± 11.28 years. The mean duration of diabetes was 5.15 ± 2.96 years and 7.55 ± 6.88 years for type 1 and type 2 diabetes respectively.

Diabetic nephropathy was found in 30.76% of patients with type 2 diabetes and 9.67% of patients with type 1 diabetes. Neuropathy was found only in type 2 diabetics patients with a rate of 65.59% (Table 1).

As for the control group, they had an average age of 53.67 ± 15.12 years, ranging from 16 to 88 years.

Our results revealed a significantly higher mean homocysteine level in patients compared to controls (12.63 vs 9.88; $p < 0.0001$) (Figure 1).

Table 1. Characteristics of the population according to the type of diabetes.

	Diabetes mellitus		Controls
\	Type 1 (13)	Type 2 (93)	
Average age (years)	24.46 ± 8.41	57.8 ± 11.28	53.67 ± 15.12
Minimum age (years)	16	32	18
Maximum age (years)	40	87	88
Sex Ratio	0.44	0.60	0.58
Duration of Diabetes (years)	5.15 ± 2.96	7.55 ± 6.88	-
Neuropathy (%)	0	61 (65.59%)	-
Nephropathy (%)	4 (30.76%)	9 (9.67%)	-

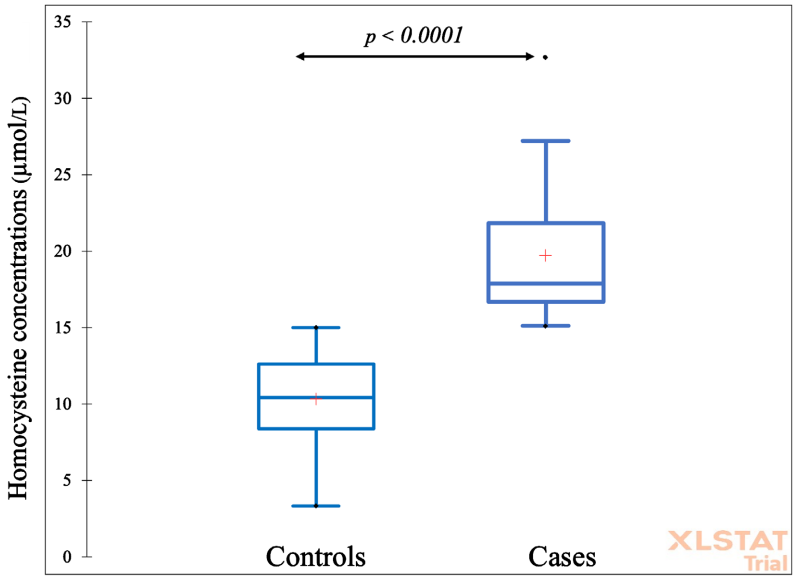

Figure 1. Comparison of mean homocysteine levels between cases and controls.

We then divided our study population into two groups based on the type of diabetes: the group of type 1 diabetes patients and the group of type 2 diabetes patients. Comparing the results obtained, we found that all type 1 diabetes patients had normal homocysteine levels, while in the group of type 2 diabetes patients, 27.96% had hyperhomocysteinemia, and 72.04% had normal homocysteine levels (Figure 2). This suggests that hyperhomocysteinemia is more prevalent in the type 2 diabetes group compared to the type 1 diabetes group.

In our study, a male predominance was observed among patients with hyperhomocysteinemia (Table 2).

The comparison of biological parameters based on homocysteine levels among diabetic patients revealed significantly higher levels of homocysteine and creatinine in the group of patients with hyperhomocysteinemia, with p-values of <0.0001 and 0.001, respectively. However, no significant differences were found for the other parameters (Table 3).

The comparison of neuropathy and nephropathy frequencies among type 2 diabetic patients based on homocysteine concentrations revealed a significantly higher frequency of diabetic nephropathy in patients with hyperhomocysteinemia compared to those with normohomocysteinemia (23.07% vs. 8.75%; p = 0.052). Similarly, diabetic neuropathy had a significantly higher frequency in patients with hyperhomocysteinemia compared to those with normohomocysteinemia (80.76% vs. 50%; p = 0.005) (Figure 3).

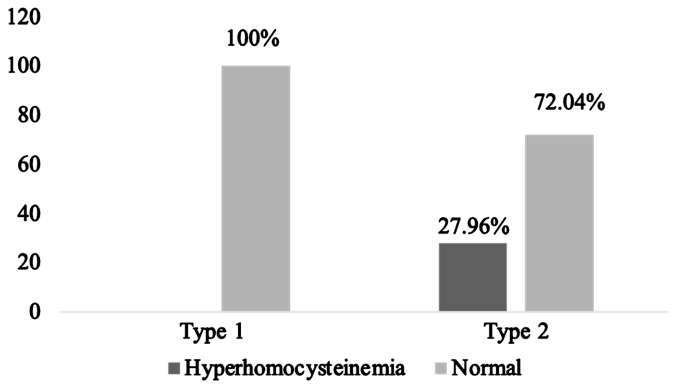

Figure 2. Frequency of hyperhomocysteinemia in patients with diabetes mellitus.

Table 2. Gender distribution in relation to homocysteine level in patients with diabetes mellitus.

	Homocysteine			
	Hyperhomocysteinemia	Normal	Total	p value
Gender				
Male	15 (58%)	24 (30%)	39 (36.8%)	0.00002*
Female	11 (42%)	56 (70%)	67 (63.2%)	
Total	26 (100%)	80 (100%)	106 (100%)	

*p-value is statistically significant; $X^2 = 3.84$; df = 1.

Table 3. Comparative table of epidemiological and biological parameters according to homocysteine levels in diabetic patients.

	Homocysteine		
	Normohomocysteinemia	Hyperhomocysteinemia	p
Homocysteinemia (µmol/L)	10.33	19.69 ± 4.37	<0.0001 *
Age (years)	51.2 ± 16.17	52.64 ± 11.42	1.000
Duration of Diabetes	6.6 ± 5.87	7.12 ± 8.22	0.415
Fasting glucose (g/l)	1.64 ± 0.81	1.66 ± 1.11	0.775
HbA1c	8.22 ± 2.31	8.14 ± 2.28	0.633
Total cholesterol (g/l)	2.14 ± 0.49	2.14 ± 0.56	0.749
HDL-cholesterol (g/l)	0.69 ± 0.22	0.70 ± 0.23	0.904
LDL cholesterol (g/l)	1.27 ± 0.44	1.27 ± 0.48	0.736
Triglycerides	0.84 ± 0.47	0.84 ± 0.35	0.817
Urea (g/l)	0.24 ± 0.10	0.23 ± 0.11	0.227
Creatinine (mg/l)	9.49 ± 2.31	10.03 ± 2.80	0.001 *
Microalbuminuria	13.32 ± 34.10	21.18 ± 32.37	0.120

*p-value is statistically significant.

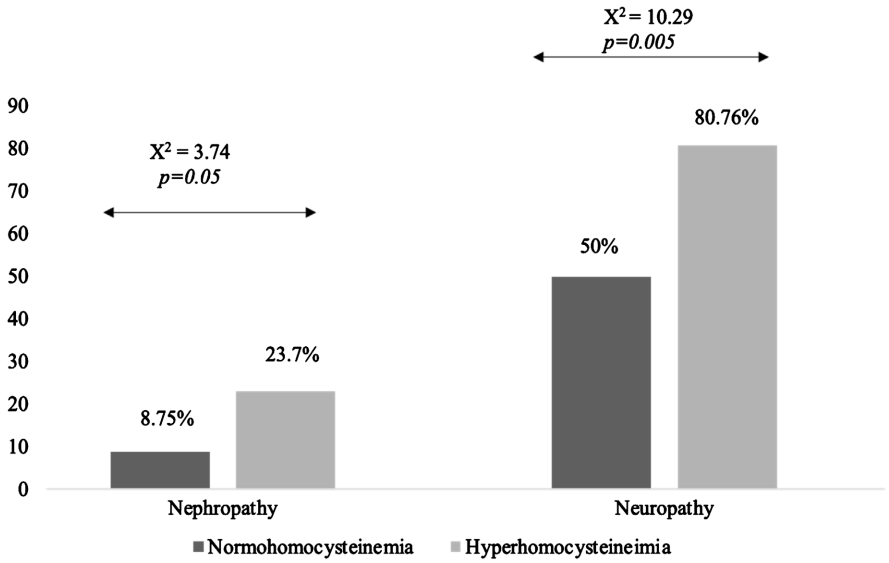

Figure 3. Frequency of nephropathy and neuropathy according to homocysteine levels in the diabetic population.

Our results revealed a significant positive correlation between homocysteine concentrations and age (r = 0.402; p < 0.0001) as well as creatinine levels (r = 0.461; p < 0.0001). However, no significant correlations were observed with the other parameters (p > 0.05) (Table 4). This suggests that higher homocysteine levels are associated with older age and higher creatinine levels in the study

Table 4. Correlation between epidemiological/biological parameters and homocysteine concentrations.

Parameters	r	*p*
Age	0.402	<0.0001*
Duration of diabetes	0.120	0.120
Fasting glucose (g/l)	-0.168	0.085
HbA1c	-0.89	0.053
Total cholesterol (g/l)	-0.016	0.874
HDL cholesterol (g/l)	-0.052	0.598
LDL cholesterol (g/l)	-0.004	0.967
Triglycerides	0.015	0.875
Urea	0.127	0.196
Creatinine	0.461	<0.0001*
Microalbuminuria	0.055	0.576

**p*-value is statistically significant.

Table 5. Association between hyperhomocysteinemia, diabetic nephropathy, and diabetic neuropathy in the study population.

Parameters	Odds Ratio	*IC* 95%	*p*
Neuropathy	3.486	−2.338; −0.059	0.01
Nephropathy	6.920	−0.5940; −0.087	0.009

population, while no significant associations were found with the other parameters examined.

The bivariate logistic regression analysis revealed that patients with hyperhomocysteinemia have 3 times and 6 times higher risks of developing neuropathy (*OR* = 3.5; p = 0.061) and diabetic nephropathy (*OR* = 6.092; p = 0.014), respectively (Table 5). These results suggest that hyperhomocysteinemia is associated with an increased risk of neuropathy and diabetic nephropathy in the study population. However, please note that the p-value for the association with neuropathy is not statistically significant (p = 0.061), indicating weaker evidence of association compared to the statistically significant association with diabetic nephropathy (p = 0.014).

4. Discussion

Diabetes is a chronic, insidious disease characterized by a metabolic disorder of various etiologies. It is accompanied by long-term complications that affect multiple organs, particularly the eyes, kidneys, nervous system, and cardiovascular system. Various risk factors are known to contribute to these degenerative complications. The aim of this study was to determine the frequency of hyperhomo-

cysteinemia in both type 1 and type 2 diabetes, as well as its association with diabetic nephropathy and neuropathy.

To achieve this, we conducted a prospective case-control study involving 106 patients with type 1 and type 2 diabetes, who were matched with 106 controls based on gender and age with a maximum difference of 2 years. In terms of epidemiology, the patients had a mean age of 53.14 years, ranging from 16 to 87 years. Similar findings were reported in the studies by *Shaikh et al.*, *Karabag et al.*, and *Emoto et al.*, with mean ages of 51.82 years ± 4.92 years, 53.4 years ± 8.6 years, and 56 years ± 1.1 years, respectively, for type 2 diabetic subjects [2] [11] [15].

In our study, the average duration of diabetes was 7.14 years. Similar results were found in other studies, such as those by Shaikh *et al.* and *Lanfredini et al.*, with respective durations of 5 years and 7.75 years [2] [16]. This prolonged duration reflects the insidious nature of the disease, which increases the risk of complications for patients.

The frequency of neuropathy and nephropathy in our diabetic patients was 57.5% and 12.2% respectively. We find similar results in the study by *Davies et al.*, which reported a neuropathy frequency of 54%, and the study by *Wirta et al.* in Finland, which estimated a nephropathy frequency of 15% - 20% in type 2 diabetes [17] [18]. However, the prevalence of diabetic neuropathy can vary widely, ranging from 0% to 93% according to *Vinik et al.* [19].

This disparity can be explained by the fact that clinical symptoms are not specific to diabetic neuropathy, and the prevalence depends on the diagnostic criteria used and whether electrophysiological tests are utilized, which have variable sensitivity. Our study revealed a significant difference in mean homocysteine levels ($p < 0.0001$) between the controls and the patients, with hyperhomocysteinemia present in 25% of the population. This finding is consistent with results from most studies, including those by Davies *et al.* in Australia, *Heydari-Zarnagh et al.* in Iran, *Ozmen et al.* in Izmir, Turkey, and *Ndrepepa et al.* in Germany [7] [10] [17] [20].

In our study, as well as in many others, patients with type 1 diabetes had normal homocysteine levels [13] [21] [22] [23] [24] [25]. This could be attributed to the low number of type 1 diabetic patients in our study population.

Moreover, several factors can explain this. Firstly, the different pathophysiological mechanisms between type 1 and type 2 diabetes play a role. Type 1 diabetes is characterized by autoimmunity, while type 2 diabetes involves metabolic alterations such as insulin resistance and obesity, which can contribute to the development of hyperhomocysteinemia. Secondly, the duration of the disease can influence homocysteine levels, with type 1 diabetes typically being diagnosed at a younger age. Lastly, associated risk factors, such as poor dietary habits, inadequate vitamin B levels, and impaired nutrient metabolism, may be more prevalent in type 2 diabetes, particularly among individuals who are overweight or obese.

Twenty-eight percent (28%) of the patients with type 2 diabetes had hyperhomocysteinemia, while 72% had normal homocysteine levels. This finding is consistent with many studies and is relatively close to the results reported in the Hoorn Study by *Hoogeven et al.* and the study by *Buysshart et al.*, where hyperhomocysteinemia was found in 25.8% and 31% of the type 2 diabetic population, respectively [26] [27].

Our results reflect the ongoing debate regarding whether homocysteine is a risk factor in diabetic patients.

In this study, the frequency of hyperhomocysteinemia was 58% in males and 42% in females. This is consistent with findings from many studies that have shown higher homocysteine levels in males compared to females. Indeed, studies on this topic have yielded conflicting results, and several potential factors could contribute to this observation 1) hormonal factors: some researchers have suggested that estrogen levels in females may reduce homocysteine levels compared to males (28) 2) behavior and associated risk factors: factors such as higher alcohol consumption, smoking, and less balanced diet in males may contribute to elevated homocysteine levels 3) genetic differences may also influence homocysteine metabolism.

We found a significantly higher frequency of diabetic neuropathy in patients with hyperhomocysteinemia compared to those with normal homocysteine levels, with frequencies of 80.76% and 50%, respectively ($p = 0.005$). Recent clinical research has demonstrated that hyperhomocysteinemia increases the prevalence of neuropathy in diabetic patients and exacerbates pre-existing diabetic neuropathy [28] [29]. Regarding diabetic nephropathy, the frequency was also significantly higher in patients with hyperhomocysteinemia compared to those with normal homocysteine levels, with frequencies of 23.07% and 8.75%, respectively ($p = 0.05$). We found significant positive correlations between homocysteine and certain parameters, including age ($r = 0.402$; $p < 0.0001$) and creatinine levels ($r = 0.461$; $p < 0.0001$). Like other studies, this association between homocysteine and age has been demonstrated in the study by *Chico et al.* [22]. Indeed, the association between homocysteine and age involves various mechanisms, depending on sex. One of these mechanisms is the age-related deterioration of renal function, which can lead to increased homocysteine levels [30]. The association between homocysteine and creatinine levels has also been observed in the study by Chico *et al.* and other studies [22] [30] [31].

No significant correlation was found between homocysteine and microalbuminuria. However, the relationship between albuminuria and homocysteine is more complex, with positive correlations reported by some authors [16] [22] [32] but not confirmed by others [33] [34] [35].

Finally, in this study, a bivariate logistic regression analysis revealed that hyperhomocysteinemia was a risk factor for developing neuropathy ($OR = 3.5$; $p = 0.061$) and diabetic nephropathy ($OR = 6.092$; $p = 0.014$). This finding is consistent with the study by *Cohen JA et al.*, which also found a significant associa-

tion between hyperhomocysteinemia and the risk of developing neuropathy (*OR* = 1.071; p = 0.041) [4].

Indeed, according to the study by *Welch et al.* [36], homocysteine is known to decrease the production of nitric oxide (NO), which is an important vasodilator and neurotransmitter. In diabetic patients, alterations in the polyol pathway can lead to depletion of NADPH, resulting in reduced synthesis of NO. This decrease in NO affects muscle tone and decreases sodium and potassium levels, leading to a decrease in ATPase activity, vasoconstriction, and reduced neural blood flow. The decrease in blood flow results in ischemia and nerve fiber loss.

Furthermore, hyperhomocysteinemia is strongly associated with inflammation. Inflammatory pathways play a central role in the development and progression of diabetic nephropathy. Hyperhomocysteinemia promotes inflammation by inducing the production of pro-inflammatory cytokines and facilitating macrophage infiltration [37] [38]. Hyperhomocysteinemia also increases the risk of oxidative stress, which can lead to renal damage [39].

Moreover, plasma homocysteine levels are proportional to insulin concentration. Patients with insulin resistance have elevated homocysteine levels [40]. Possible mechanisms may involve insulin resistance with endoplasmic reticulum dysfunction, elevated blood glucose levels, and increased regulation of phosphoenolpyruvate carboxykinase [37] [41].

5. Conclusions

In this study, a significant frequency of hyperhomocysteinemia was observed, particularly among patients with type 2 diabetes, highlighting a distinct metabolic profile in this group. Furthermore, discernible disparities in homocysteine levels were evident when comparing diabetic patients to controls, signifying the potential relevance of homocysteine as a biomarker in diabetes.

Strikingly, none of the type 1 diabetic patients exhibited hyperhomocysteinemia, indicating a unique metabolic contrast between type 1 and type 2 diabetes with respect to homocysteine regulation. Moreover, our findings underscored a substantially elevated prevalence of diabetic nephropathy and neuropathy within the hyperhomocysteinemic group, mirroring results from diverse studies and underscoring a plausible association between hyperhomocysteinemia and microangiopathic complications.

The bivariate logistic regression analysis reaffirmed the established trend, highlighting hyperhomocysteinemia as a discernible risk factor for the development of both diabetic nephropathy and neuropathy, consolidating the relevance of homocysteine as a potential clinical marker.

While our study offers valuable insights, it is essential to acknowledge its limitations. The cross-sectional nature of the study precludes causal inferences, and the relatively small sample size warrants caution in generalizing the results. Furthermore, the intricacies of other confounding factors and interactions merit exploration in future investigations.

A notable limitation of our study is the lack of assessment of the vitamin B status of the patients, except for the absence of supplementation. Given the potential interplay between homocysteine metabolism and vitamin B levels, future research could delve into the influence of vitamin deficiencies on the observed outcomes.

In conclusion, this findings illuminate the intricate interplay between hyperhomocysteinemia and diabetic microangiopathic complications. While further research is required to elucidate the underlying mechanisms and potential therapeutic implications, our study suggests that managing homocysteine levels, alongside comprehensive diabetes management, could hold promise in attenuating the onset and progression of these intricate complications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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