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THE ROLE OF HOMOCYSTEINE AND VITAMIN D IN ASSESSING CARDIOVASCULAR RISK IN TYPE 2 DIABETIC PATIENTS

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Abstract

New biomarkers are currently promising to improve cardiovascular risk (CVR) prediction to prevent cardiovascular complications and their associated morbidity and mortality. In practice, homocysteine (Hcy) and vitamin D (Vit D) have shown statistical associations with the incidence of cardiovascular events. Our study aimed to investigate the association between each of these biomarkers and atherosclerosis and to evaluate their contribution to the stratification of CVR in type 2 diabetic patients (T2D).

Methods:
This was a cross-sectional and comparative study that included 53 T2D patients with at least one cardiovascular disease (CVD) and 67 T2D patients without any CVD. Measurements of Hcy and 25-OH Vit D were obtained for 116 and 71 patients, respectively. Hyperhomocysteinemia (HHC) was defined as a level greater than 15 µmol/L, and Vit D deficiency was defined as a level below 10 ng/mL.

Results:
The average level of Hcy was 27.65 ± 31.95 µmol/L. It was significantly correlated with age ($p = 0.008$), body mass index ($p = 0.008$), fasting blood glucose ($p < 0.01$), HbA1c ($p = 0.05$), total cholesterol ($p < 0.01$), LDL cholesterol ($p = 0.01$), and HDL cholesterol ($p = 0.01$). Hcy was significantly higher in smokers ($p = 0.02$) and in patients with macroangiopathy ($p < 0.01$), coronary artery disease ($p = 0.03$), diabetic retinopathy (DR) ($p < 0.01$), and diabetic nephropathy ($p = 0.03$). HHC was significantly associated with macroangiopathy (OR = 3.81), coronary artery disease (OR = 2.13), peripheral artery disease (OR = 2.52), DR (OR = 4.11), and diabetic nephropathy (OR = 3.75). Hcy had a sensitivity of 56% and a specificity of 63% in diagnosing macroangiopathy. The performance of Hcy compared to the Framingham score showed significantly higher areas under the ROC curves for Hcy in the diagnoses of macroangiopathy, coronary artery disease, and DR.

The average level of 25-OH Vit D was 14.81 ± 6.15 ng/mL. It was significantly correlated with the duration of diabetes, total cholesterol, LDL cholesterol, and HDL cholesterol. Levels were significantly higher in men ($p < 0.001$) and in smokers. Vit D deficiency was significantly associated with diabetic neuropathy (OR = 2.84) and coronary artery disease (OR = 3.73). Vit D deficiency had a sensitivity of 57% and a specificity of 76% in diagnosing macroangiopathy, but it was not effective in diagnosing coronary artery disease, exhibiting low sensitivity (42%) and an area under the ROC curve of 0.365.

Conclusion:
Our study demonstrated an association between Hcy, macroangiopathy, and coronary artery disease in T2D patients; however, Vit D was not effective in diagnosing these conditions.

Keywords: Homocysteinemia, Low serum levels of 25-hydroxyvitaminD , Cardiovascular risk, Atherosclerosis, Type 2 diabetes

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INTRODUCTION

Cardiovascular (CV) disease is currently the leading cause of death worldwide, with coronary heart disease and stroke being the most common causes of death [1]. Although the CV event rate in diabetics has been halved in recent years, the incidence remains very high [2]. One in every two diabetics dies from a cardiovascular event, and diabetics are two to four times more likely to develop coronary heart disease than non-diabetics [2]. It is therefore important to identify high-risk patients to develop prevention strategies and reduce morbidity and mortality.

The risk of developing atherosclerosis over a lifetime depends on the level of certain cardiovascular risk factors and the duration of exposure to these factors. These risk factors are numerous and can be classified into modifiable risk factors (such as hypertension, diabetes, smoking, dyslipidemia, obesity, and physical inactivity) and non-modifiable risk factors (including age, gender, heredity, and menopause) [3]. These factors have a cumulative effect and combine to increase overall risk [3].

To date, it has been estimated that conventional cardiovascular risk factors (diabetes, hypertension, smoking, and cholesterol) account for about half of the variance in the occurrence of coronary death. However, in 50% of cases, no known risk factor is identified, which may be related to other unknown or underestimated factors [4].

New biomarkers are now promising to better identify patients at risk. Their use aims primarily to refine risk prediction and to better identify individuals at high cardiovascular risk. Non-traditional cardiovascular risk factors that appear to have the greatest clinical interest include apolipoprotein A (ApoA), apolipoprotein B (ApoB), C-reactive protein (CRP), microalbuminuria, type B natriuretic peptide (BNP), homocysteine (Hcy), and 25-hydroxy vitamin D (25-OH Vit D) [5].

For several years, hyperhomocysteinemia (HHC) and vitamin D deficiency have been suggested as biological factors related to atherosclerosis and an increased risk of CV events [6]. Vitamin D (Vit D) has been known and studied for many years for its major role in calcium-phosphate homeostasis [7]. Studies have also reported that it has numerous extra-skeletal effects and is directly correlated with certain cardiovascular risk factors, including insulin resistance, hypertension, and dyslipidemia [8].

This article aims to investigate the correlation of each of these biomarkers with atheromatous disease and assess their role in the stratification of cardiovascular risk (CVR): specifically, to study the association between homocysteine and cardiovascular risk in patients with type 2 diabetes and to assess the relationship between vitamin D and cardiovascular risk among patients with type 2 diabetes (T2DM).

METHODS

Study population

This is a prospective, cross-sectional, descriptive, and comparative study. We recruited patients with type 2 diabetes mellitus (T2DM) who were consulting in the endocrinology department. The participants were divided into two groups: one group consisting of patients diagnosed with atherosclerosis (which included coronary artery disease, cerebrovascular accidents (CVA), or established peripheral arterial disease (PAD)), and a second group of patients who did not have any signs of atherosclerosis.

Exclusion Criteria: Patients with other types of diabetes, those who are pregnant or breastfeeding, individuals with thyroid dysfunction, and patients with severe renal or hepatic impairment were excluded.

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Data Collection

For each patient, we collected the following data: age, sex, physical activity level, smoking status (active or ceased for less than 3 years), history of alcoholism, presence of other diabetes-related conditions, duration of diabetes (in years), type of antidiabetic treatment, and known degenerative complications, as indicated by recent medical evaluations.

Clinical parameters recorded included weight, height, BMI, waist circumference, blood pressure, peripheral pulse palpation, evaluation of superficial and deep sensitivity, tendon reflex assessment (patellar and Achilles), and electrocardiogram results. Biological parameters included fasting glucose, HbA1c, total cholesterol, triglycerides, HDL cholesterol, creatinine, microalbuminuria, homocysteine, and 25-hydroxy vitamin D (25-OH Vit D).

The plasma homocysteine (Hcy) level was measured in 116 patients using enzyme immunoassay with fluorescence polarization detection. A level of Hcy > 15 µmol/L was considered pathological, as this threshold is widely used in the literature [18]. Hyperhomocysteinemia (HHC) was classified as follows: High: 15 < Hcy < 30 µmol/L; Intermediate: 30 < Hcy < 100 µmol/L; Severe: Hcy > 100 µmol/L [18].

The measurement of 25-hydroxy vitamin D (25-OH Vit D) was conducted for 71 patients, with normal values established as > 30 ng/mL. Vitamin D insufficiency was defined as values between 10-30 ng/mL, and vitamin D deficiency was defined as < 10 ng/mL.

Regarding macrovascular complications, we examined the history of myocardial infarction (MI), stroke, transient ischemic attack (TIA), and/or obliterating arteriopathy of the lower limbs (OALL). Coronary artery disease was identified based on any of the following criteria: history of acute coronary syndrome, previous percutaneous angioplasty or aorto-coronary bypass, or positive results in stress tests, transthoracic echocardiography, myocardial scintigraphy, or coronary angiography indicating ischemic signs. PAD was diagnosed based on known PAD, significant arterial stenosis in Doppler ultrasound of the lower limbs, or pathological ankle-brachial index (ABI) values (ABI < 0.9 or > 1.3).

The cardiovascular risk (CVR) was calculated using the Framingham score (FS), which estimates the 10-year risk of a cardiovascular event based on sex, age, smoking status, diabetes presence, and cholesterol and blood pressure values. CVR was categorized as low (FS < 10), moderate (FS 10-20), or high (FS ≥ 20).

Statistical Analysis

Data entry and statistical analysis were conducted using SPSS 19.0 software. Comparisons between qualitative variables were made using the Chi-square (X^2) test, and when this test was not valid, the Fisher exact test was applied. Comparisons between quantitative variables were performed using Student's t-test, and if this was not valid, the nonparametric Mann-Whitney test was used.

Correlations between homocysteine and 25-OH Vit D values and various clinical and biological parameters were assessed using bivariate correlation tests. A p-value of less than 0.05 was considered statistically significant. To compare the performance of Hcy and 25-OH Vit D, we calculated the sensitivity (Se) and specificity (Sp) for each respective test.

RESULTS

We included 120 patients with type 2 diabetes mellitus (T2DM), divided into two groups: 53 patients with atherosclerotic disease and 67 without atherosclerosis. The average age of the study population was 58.29 ± 8.06 years, with a sex ratio (M/F) of 1.22. The average duration of diabetes was 8.28 ± 6.4 years. Family histories of diabetes, hypertension, dyslipidemia, and cardiovascular disease were reported in 73%, 69%, 38%, and 32% of the patients, respectively. Diabetes treatment was as follows: lifestyle changes in 1.7% of cases, a

combination of insulin and oral antidiabetic agents (OAD) in 26.6%, oral medications alone in 54.2%, and insulin alone in 17.5%. Poor diabetes control was observed in 72.2% of the cases, and 60% of the study population had at least one microangiopathy.

In Group 1, the prevalence of coronary insufficiency, obliterating arteriopathy of the lower limbs (OALL), and stroke or transient ischemic attack (TIA) was noted in 25, 33, and 3 patients, respectively. No significant differences were found between the two groups regarding age, sex, duration of diabetes, family history of cardiovascular disease, anthropometric parameters, blood pressure, blood glucose control, lipid parameters, prevalence of metabolic syndrome, or Framingham score (FS).

The prevalence of dyslipidemia was significantly higher in Group 1 compared to Group 2 ($p = 0.015$). The rate of raised reactive species (RRS) was elevated in 75.8% of the study population, with 100% in Group 1 and 58% in Group 2 ($p < 0.001$).

The average homocysteine (Hcy) level was 27.65 ± 31.95 µmol/L, significantly correlated with age ($p = 0.008$; $r = 0.24$), BMI ($p = 0.008$; $r = 0.92$), fasting plasma glucose (FPG) ($p < 0.01$; $r = -0.285$), HbA1c ($p = 0.05$; $r = -0.58$), total cholesterol (TC) ($p < 0.01$; $r = -0.28$), LDL cholesterol (LDLc) ($p = 0.01$; $r = -0.22$), and HDL cholesterol (HDLc) ($p = 0.01$; $r = -0.23$). No significant correlation was found between Hcy and gender, diabetes type, weight, blood pressure, triglycerides (TG), or FS.

Additionally, higher Hcy levels were observed in smokers ($p = 0.02$) and patients with macrovascular complications ($p < 0.01$), coronary heart disease ($p = 0.03$), diabetic retinopathy (DR) ($p < 0.01$), and diabetic neuropathy (DN) ($p = 0.03$). The prevalence of hyperhomocysteinemia (HHC) was 41.4% and was significantly associated with macroangiopathy (OR = 3.81; $p = 0.001$), coronary artery disease (OR = 2.13; $p = 0.04$), OALL (OR = 2.52; $p = 0.02$), DR (OR = 4.11; $p = 0.02$), and DN (OR = 3.75; $p = 0.03$).

For a threshold of 15 µmol/L, Hcy showed a sensitivity of 58% and a specificity of 73% for diagnosing macrovascular disease, and a sensitivity of 56% and specificity of 63% for diagnosing coronary artery disease. The performance of Hcy was superior to FS in diagnosing macrovascular disease, coronary artery disease, and DR; however, Hcy was less efficient than FS in diagnosing DN.

The prevalence of vitamin D deficiency was very high in T2DM (99% of patients), with an average 25-OH vitamin D level of 14.81 ± 6.15 ng/mL. This level was significantly correlated with the duration of diabetes ($p = 0.01$; $r = -0.3$), TC ($p = 0.006$; $r = -0.32$), LDLc ($p = 0.01$; $r = -0.29$), and HDLc ($p < 0.01$; $r = -0.415$). However, no significant correlation was found between 25-OH vitamin D and age, weight, BMI, waist circumference (WC), blood pressure, glucose control parameters, TG, or FS. Additionally, 25-OH vitamin D levels were significantly higher in men than in women ($p < 0.001$) and in smokers ($p = 0.002$).

The prevalence of vitamin D deficiency was 32% and was significantly associated with diabetic nephropathy (DNP) (OR = 2.84; $p = 0.04$) and coronary heart disease (OR = 3.73; $p = 0.032$). Vitamin D deficiency had a sensitivity of 57% and a specificity of 76% for diagnosing macrovascular disease, but was not effective in diagnosing coronary artery disease, showing low sensitivity (42%) and an area under the ROC curve of 0.365 (not discriminative).

DISCUSSION

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The Framingham Score (FS) is commonly used to assess cardiovascular risk (CVR), incorporating factors such as age, sex, total cholesterol (TC), high-density lipoprotein (HDL), systolic blood pressure, smoking status, and the presence of diabetes [9]. This score estimates the probability of coronary events occurring within a 10-year period. However, FS and similar risk scores do not account for certain additional risk factors known to play a role in atherosclerosis [10]. Emerging biomarkers have been proposed to enhance predictive value beyond traditional risk factors and multifactorial risk scores.

The prevalence of hyperhomocysteinemia (HHC) in the general population is estimated to be around 5-7%, varying by age and ethnicity [11]. The lowest concentrations of homocysteine (Hcy) are found among Asian Americans, while the highest levels are reported in Caucasians [11]. In our study, HHC was present in 41.4% of patients, with 33.6% classified as having moderate HHC and 7.8% with intermediate HHC.

We found no significant correlation between Hcy levels and the FS ($p = NS$). Furthermore, no significant association was observed between the FS and HHC ($p = 0.585$). The average Hcy level increased with the level of CVR (13 ± 4.67 μmol/L for low risk, 16.06 ± 8.74 μmol/L for moderate risk, and 17.37 ± 10.17 μmol/L for high risk), although these differences were not statistically significant ($p = 0.065$). In contrast, a study by Meltem et al. involving 1,698 patients over the age of 65 found a significant increase in average Hcy levels corresponding to CVR levels (15.96 ± 7.81, 17.75 ± 8.31, and 18.62 ± 9.08 μmol/L for low, moderate, and high CVR, respectively; $p < 0.001$). For Hcy, the odds ratio (OR) for moderate versus high CVR was 1.016 (95% CI [1.001 to 1.030]; $p = 0.032$). Additionally, a 1 mg/dL increase in Hcy was linked to a transition from low to moderate CVR [12]. Gariglio et al. also found a significant risk increase in the highest quartile of Hcy compared to the lowest [13]. The discrepancies between our results and those reported in the literature may stem from differences in the populations studied, as all our patients had T2DM, unlike those in previous studies.

In our cohort, Hcy levels were higher in the group with macrovascular complications compared to those without (18.96 ± 8.78 μmol/L vs. 14.66 ± 9.55 μmol/L; $p < 0.01$). The prevalence of HHC was significantly higher in patients with macrovascular disease (59% vs. 27%; $p = 0.001$). We also reported a significant association between Hcy and macrovascular complications, with an OR of 3.81 (95% CI [1.74 to 8.31]). Hcy demonstrated a sensitivity of 58% and a specificity of 73% for diagnosing macrovascular disease, consistent with other studies showing a significant association between HHC and vascular disease [14]. A study involving 122 diabetic patients found that the prevalence of macroangiopathy was higher in those with HHC compared to those with normal Hcy levels (70% vs. 42%; $p = 0.01$) [14]. In the Hoorn study, an increase in Hcy by 5 μmol/L was associated with a relative risk of developing cardiovascular disease (CVD) of 1.38 in non-diabetics, 1.55 in glucose-intolerant individuals, and 2.33 in diabetics [15]. Proposed mechanisms for the atherogenic effects of HHC include stimulation of smooth muscle cell proliferation, endothelial injury via oxidative stress, and reduced synthesis of HDL [16]. Hcy may also activate factor V and inactivate protein C [16], contributing to the formation of atheromatous plaques and subsequent thrombotic complications.

Regarding the association of Hcy with coronary artery disease, our results indicated significantly higher Hcy levels in patients with coronary artery disease (19.32 ± 9.96 μmol/L vs. 15.88 ± 9.18 μmol/L; $p = 0.03$). Additionally, the frequency of coronary insufficiency was significantly higher in patients with elevated Hcy levels compared to those with normal levels (56% vs. 37%; $p = 0.05$). We identified a significant association between Hcy and coronary insufficiency with an OR of 2.13 (95% CI [0.86 to 5.57]). Becker et al. suggested that Hcy is significantly associated with coronary artery disease in individuals with T2DM, independent of traditional CV risk factors [17]. A meta-analysis from two large studies (MESA and NHANES III) demonstrated that HHC significantly predicted coronary heart disease (OR 2.22, 95% CI [1.20 to 4.09]; $p = 0.01$) and mortality from coronary artery disease (OR 2.61,

95% CI [1.83 to 3.73]; $p < 0.001$) after adjusting for traditional risk factors and C-reactive protein [18]. For screening purposes, our results indicated an area under the ROC curve of 0.637 (95% CI [0.525 to 0.749]; $p = 0.036$) for Hcy, with a sensitivity of 56% and a specificity of 63%. In contrast, the study by Sun Yu et al. reported a sensitivity of 81.1% and a specificity of 54.3% [19]. The difference in findings may be attributed to variations in the threshold value for HHC (cutoff of 9.49 µmol/L vs. 15 µmol/L, respectively).

The high prevalence of vitamin D deficiency in the general population and the identification of vitamin D receptors in the heart and blood vessels have sparked interest in its potential cardiovascular effects [20]. While the role of vitamin D in bone metabolism is well established, recent decades have focused on its potential extraneous effects. Low circulating levels of 25-OH vitamin D, which indicate vitamin D status, have been linked to an increased risk of developing T2DM [21]. Consequently, vitamin D deficiency may contribute to poor glycemic control and act as a risk factor for the development or progression of diabetes-related complications [21].

In our study, no significant difference was observed in the average 25-OH vitamin D levels between patients with macrovascular disease and those without ($p = NS$). Our findings contrast with other studies; for instance, the Framingham Offspring Study, which prospectively analyzed 1,739 individuals with 25-OH vitamin D levels <15 ng/mL, found an adjusted OR of 1.6 (95% CI [1.11 to 2.36]; $p = 0.01$) for cardiovascular events [22]. A meta-analysis of 19 prospective studies involving 65,994 participants revealed a significant association between vitamin D deficiency and cardiovascular events, with a cumulative relative risk of 1.52 (95% CI [1.30 to 1.77]) for total CVD and 1.42 (95% CI [1.19 to 1.71]) for CVD mortality [22]. The divergence between our results and those of other studies may be attributed to the relatively small number of patients in our cohort who underwent 25-OH vitamin D testing.

CONCLUSION

Cardiovascular complications are the leading cause of death among individuals with diabetes, with a significant proportion of these fatalities occurring in asymptomatic patients. This highlights the urgent need for strategies to detect atherosclerosis at its subclinical stage, thereby reducing the prevalence of cardiovascular events. Recent years have seen the development of various methods for evaluating cardiovascular risk (CVR), including blood biomarkers such as homocysteine (Hcy) and vitamin D. Our study demonstrates the superiority of Hcy over the Framingham Score (FS) in predicting CVR. Hcy serves not only as a marker for CVR but also as a potential biological tool for screening macrovascular disease. At a threshold of 15 µmol/L, Hcy exhibited a sensitivity of 58% and 56% for diagnosing macrovascular disease and coronary artery disease, respectively, with specificities of 73% and 63%. In contrast, vitamin D showed low sensitivity in diagnosing coronary artery disease. However, these findings warrant further validation through prospective studies involving larger populations.

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