

Association of Microalbuminuria and Estimated Glomerular Filtration Rate With Carotid Intima-Media Thickness in Patients With Type 2 Diabetes Mellitus

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Background: Atherosclerosis is the main cause of cardiovascular diseases, and its risk enhances in type 2 diabetes mellitus.

Objectives: This study aimed to evaluate Carotid Intima-Media Thickness (CIMT) by carotid artery ultrasonography and assess its correlation with microalbuminuria and estimated Glomerular Filtration Rate (eGFR) in the patients with type 2 diabetes mellitus.

Patients and Methods: This cross-sectional study was included 205 patients with Type 2 Diabetes Mellitus (T2DM). We recorded clinical and biochemical data such as FBS, lipid profile, and urinary albumin. Intima-media thickness of carotid arteries was measured in all patients by high frequency ultrasound.

Results: In simple correlation coefficients analysis, CIMT was significantly associated with total cholesterol ($r = 0.197$, $P = 0.008$), serum creatinine ($r = 0.240$, $P = 0.001$), and urinary albumin ($r = 0.420$, $P = 0.000$). Also, CIMT elevated significantly with the stage progression of chronic kidney disease (0.67 ± 0.15 mm in stage 1, 0.73 ± 0.22 mm in stage 2, and 0.82 ± 0.21 mm in stage 3) (P value = 0.024). In multivariate linear regression analysis, the duration of diabetes, weight, HDL, serum creatinine, urinary albumin, and estimated Glomerular Filtration Rate (eGFR) were independently associated with CIMT (P value < 0.05 for all).

Conclusions: Our study shows a relationship between CIMT and renal parameters, including eGFR and albuminuria. This study confirms the importance of intensive examinations for early detection of atherosclerosis and treatment of risk factors.

Keywords: Diabetes Mellitus; Albuminuria; Glomerular Filtration Rate; Carotid Intima-Media Thickness

1. Background

Diabetes is a serious and increasing global health burden. In 2013, 382 million people had diabetes; this number is expected to rise to 592 million by 2035. Most people with diabetes live in low- and middle-income countries, which will experience the greatest increase in cases of diabetes over the next 22 years (1). Compared with people without diabetes, affected individuals are at increased risk for both cardiovascular events and kidney disease (2). Increased urinary albumin excretion (albuminuria) and reduced Glomerular Filtration Rate (GFR) are risk factors for progressive kidney failure and cardiovascular disease (3). Diabetes nephropathy is a major manifestation of microangiopathy that plays a significant role in the prognosis of patients with diabetes mellitus (4).

Atherosclerosis is the main cause of cardiovascular diseases; measurement of IMT enables the detection of atherosclerosis lesions of the arterial wall. CIMT can be mea-

sured by high frequency B mode ultrasonography, which provides a high degree of accuracy in estimating the arterial wall thickness. Atherosclerosis frequently occurs in diabetic patients as diabetic macroangiopathy (4). The carotid IMT is significantly higher in diabetic patients than that in non-diabetic patients (5), and the increased IMT can predict future events of silent brain infarction and coronary heart disease in the patients with T2DM (6, 7). Therefore, IMT is considered to reflect an early stage of macroangiopathy in patients with diabetes. However, association of IMT with both urinary albumin excretion and GFR in type 2 diabetic patients has been investigated in a few reports (4).

2. Objectives

This study aimed to assess the role of CIMT (measured with carotid ultrasonography) and its correlation with microalbuminuria and the chronic kidney disease (CKD) stages (based on eGFR) in patients with T2DM.

3. Patients and Methods

This cross-sectional study included 205 patients with type 2 diabetes mellitus at the diabetes department of Golestan Hospital of Ahvas Jundishapur University. The study was approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences and all participants signed the informed consent prior to enrollment, (ID: AJUMS.REC.1392.321, 2014). Also, we kept patients' privacy. Patients with type 1 diabetes mellitus, smoking, drinking of alcohol, hypertension, history of ischemic heart disease, valvular heart disease, end stage renal disease, glomerulonephritis, history of any malignancy, and chemotherapy were excluded. The clinical conditions that could cause transient elevations in urinary albumin excretion such as exercise, urinary tract infection, febrile illness, pregnancy, and hematuria were also excluded.

3.1. Data Collection

Medical history and physical exam were done in all patients. Information, including age, sex, weight, duration of diabetes, history of smoking, and hypertension was collected. Laboratory tests, including fasting blood glucose, serum creatinine, total cholesterol, High-Density Lipoprotein cholesterol (HDL), Low-Density Lipoprotein cholesterol (LDL), and triglyceride levels were recorded too. Estimated GFR were measured using Cockcroft formula (8). Urinary albumin and creatinine were measured in random urine samples and patients were divided into two groups according to urinary albumin-creatinine ratio (ACR), including normoalbuminuria (ACR < 30 µg/mg creatinine) and microalbuminuria (30 - 300 µg/mg cre-

atinine). Based on eGFR value, CKD stages are defined as: stage 1 (GFR ≥ 89 mL/min/1.73 m²), stage 2 (GFR: 60-89 mL/min/1.73 m²), stage 3 (GFR: 30-59 mL/min/1.73 m²) (9). In this study no patient had eGFR lower than 30 mL/min/1.73 m².

3.2. Carotid Artery Ultrasonography

Carotid artery IMT was measured with ultrasonographic examination by an experienced radiologist using Voluson 730 Expert ultrasound machine (General Electric, New York, USA). B mode imaging of the carotid artery was performed with a 7.5 MHz probe. Measurements involved a primary transverse and longitudinal scanning of the common carotid arteries, bifurcation, and internal carotid arteries. CIMT was measured on the far wall, 1 cm from bifurcation of the common carotid artery as the distance between the lumen-intima interface and the media-adventitia interface. It is done while the patient is in the supine position with a slight neck extension. At least three measurements were performed on both sides, and the average of measurements was taken as the CIMT. All measurements were made at a plaque-free site.

All statistical calculations were done by SPSS 19.0 software package. Data are given as mean ± SD for continuous variables or percentage for categorical variables. An analysis of variance (ANOVA) and the χ^2 test were used for between-group comparisons of the continuous and categorical variables, respectively. Spearman or Pearson univariate regression analysis and multivariate linear regression analysis were performed to determine the association of the carotid IMT with other clinical parameters. Levels of statistical significance were set at a P value < 0.05.

Table 1. Baseline Clinical Characteristics of the Patients, and the Simple Correlation Coefficients With Carotid IMT^a

Variable	Mean ± SD, %	Pearson Correlation Coefficient	P Value
Gender, %			0.506
Female	58.5		
Male	41.5		
Age, y	52.33 ± 10.96	0.035	0.617
Weight, Kg	74.54 ± 12.19	0.012	0.880
Duration of diabetes mellitus, y	8.66 ± 6.07	0.1125	0.081
Fasting blood sugar, mg/dL	159.25 ± 67.32	0.062	0.406
Total cholesterol, mg/dL	173.11 ± 46.82	0.197	0.008
Triglycerides, mg/dL	175.85 ± 100.06	0.008	0.919
LDL cholesterol, mg/dL	103.49 ± 38.23	0.134	0.088
HDL cholesterol, mg/dL	46.00 ± 9.54	0.031	0.690
Serum creatinine, mg/dL	0.87 ± 0.27	0.240	0.001
Urinary ACR, mg/g	67.03 ± 88.68	0.420	0.000
Estimated GFR, mL/min/1.73 m²	104.40 ± 38.96	-0.149	0.076
CKD stage, %			0.016
Stage 1	57.64		
Stage 2	33.33		
Stage 3	9.03		
Carotid IMT, mm	0.67 ± 0.18		

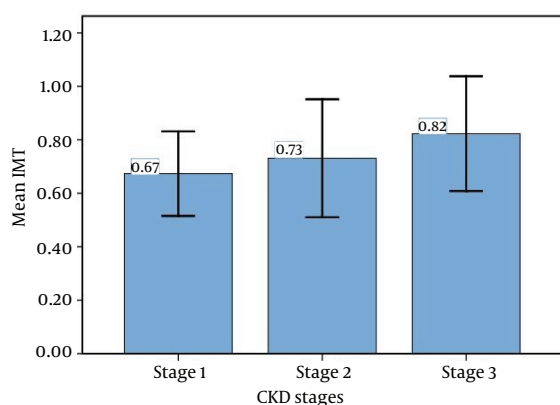
^a Abbreviations: ACR, albumin creatinine ratio; GFR, glomerular filtration rate; CKD, chronic kidney disease; IMT, intima-media thickness.

4. Results

A total of 205 patients were studied, whose overall mean of CIMT was 0.67 ± 0.18 mm. While the mean CIMT in female patients was relatively lower than males (0.66 ± 0.18 mm vs. 0.68 ± 0.17 mm), it was not statistically significant ($P = 0.506$). Table 1 shows the baseline clinical characteristics and the laboratory parameters of the patients, and also shows the simple correlation coefficients of CIMT with these data. Total cholesterol, serum creatinine, and albuminuria were significantly correlated with CIMT. Lower eGFR was associated with higher IMT, but it was not statistically significant. IMT values increased with the stage progression of CKD (0.67 ± 0.15 mm in stage 1, 0.73 ± 0.22 mm in stage 2, and 0.82 ± 0.21 mm in stage 3 of CKD) (Figure 1). The difference of IMT between stage 1 and 3 was statistically significant ($P = 0.003$). Then, subjects were

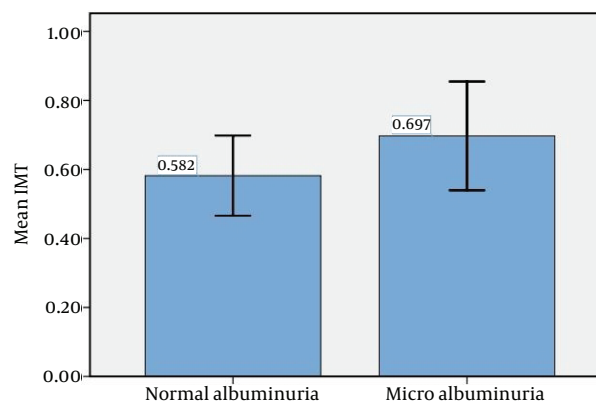
divided into two groups according to their urinary ACR levels: those with normoalbuminuria (ACR < 30 $\mu\text{g}/\text{mg}$ creatinine) and those with microalbuminuria (30 - 300 $\mu\text{g}/\text{mg}$ creatinine). Comparing clinical characteristics and laboratory parameters of these two groups shows that CIMT parameter was the only significant difference (P value = 0.000). Patients with microalbuminuria had higher CIMT (0.69 ± 0.15 mm vs. 0.58 ± 0.11 mm) (Figure 2). In multivariate analysis by multiple linear regression models, among the various variables included, the significant correlation was found between carotid IMT and variables of weight, duration of diabetes, HDL, and creatinine (Table 2). Also, both urinary ACR and eGFR were independently associated with the carotid IMT (P value < 0.05 for all).

Figure 1. The IMT of the Subgroups Divided by the Stages of Chronic Kidney Disease (CKD)



IMT increased with the stage progression of CKD. $P = 0.003$ for the difference between stage 1 and stage 3. IMT, intima-media thickness.

Figure 2. Comparing Mean of IMT Between Normal albuminuria and Microalbuminuria Subjects



$P = 0.000$ for the difference between two groups; IMT, intima-media thickness.

Table 2. Multivariate Analysis of the Variables and Correlation with Carotid IMT^a

Variable	Wilk's Lambda Value	F Test	P Value
Weight, Kg	0.627	5.569	0.012
Duration of diabetes mellitus, y	0.510	4.859	0.029
Fasting blood sugar, mg/dL	0.812	2.201	0.138
Total cholesterol, mg/dL	0.980	0.190	0.829
Triglycerides, mg/dL	0.975	0.242	0.787
LDL cholesterol, mg/dL	0.991	0.089	0.915
HDL cholesterol, mg/dL	0.666	4.761	0.021
Serum creatinine, mg/dL	0.706	3.963	0.036
Urinary ACR, mg/g	0.680	4.475	0.026
Estimated GFR, mL/min/1.73 m ²	0.672	4.633	0.023

^a Abbreviations: ACR, albumin creatinine ratio; GFR, glomerular filtration rate; IMT, intima-media thickness.

5. Discussion

In the present study, the most important finding was the independent association of microalbuminuria and eGFR with CIMT. We noticed that as albuminuria increased, the proportion of patients with raised CIMT also increased. Accordingly, the microalbuminuria group had high CIMT.

Mykkänen et al. investigated the relationship between microalbuminuria and CIMT in 991 non-diabetic and 450 T2DM subjects aged 40 to 69 years. They also reported that subjects with microalbuminuria had greater CIMT than those without microalbuminuria (10). Diercks et al. similarly showed that urine albumin excretion was strongly related to subclinical atherosclerosis in the presence of increased CIMT in patients with T2DM (11). Also Yokoyama et al. reported that a slight elevation of albuminuria was a significant determinant of IMT independent of conventional cardiovascular risk factors in T2DM patients with no clinical nephropathy or any vascular diseases (12). These findings were also reported by Choi et al. (13). Although Chu et al. reported that the IMT did not correlate with urinary albumin excretion (UAE) (14).

To our knowledge, our study is the first report showing that both lower eGFR and higher levels of urinary albumin excretion significantly and independently were associated with increased IMT and atherosclerosis in patients with T2DM. Sjoblom et al. investigated the association between reduced eGFR and microalbuminuria against subclinical organ damage, and showed that levels of urinary albumin excretion, but not reduced eGFR, were associated with increased atherosclerosis in patients with T2DM (15). Ito et al. showed that the carotid IMT increased significantly with the stage progression of CKD (4). However in their study, IMT was not significantly different among the various stages of diabetic nephropathy. Kawamoto et al. demonstrated a negative correlation between the carotid IMT and eGFR without examination for urinary albumin excretion (16). Hermans et al. showed that IMT was significantly associated with both the urinary albumin excretion and the eGFR in 806 subjects from random population (17). However, our findings are in correlation with Ninomiya et al. study, who reported that both the decreased eGFR and proteinuria are independently associated with cardiovascular events in both the random population and in the patients with type 2 diabetes mellitus (18).

Measuring CIMT by safe and non-invasive techniques such as ultrasonography could help in the diagnosis of cardiovascular complications like arteriosclerosis in the early clinical phases, and may help in the detection of non-symptomatic kidney diseases as well. Thus, a rise in the CIMT in association with a change in urinary albumin and eGFR could increase the risk of cardiovascular complications such as arteriosclerosis. On the other hand, the average CIMT in this study was approximately 0.67 mm. Besides, in our study, the mean CIMT was also lower in fe-

male patients than in male patients. It may be due to the supportive role of female hormones, which makes the males more prone to arteriosclerosis (19). In addition, in the present study, there was no significant relationship between age and CIMT. This finding was in agreement with Gayathri et al. (20) and Shin et al. (21) studies that reported no association between age and CIMT. But Yokoyama et al. reported age as an independent predictor that positively correlated with CIMT (12).

In the present study, after multivariate linear regression analysis, we found that the duration of diabetes was significantly correlated with the CIMT. Also Gayathri et al. observed increased CIMT with longer duration of diabetes (20). Our findings indicated that there is a significant correlation between the patient's weight and CIMT. Also, De Michele et al. demonstrated the independent association between general and central obesity increased body mass index with increasing CIMT (22). While Gayathri et al. study showed no significant relationship between body mass index and the CIMT (20).

The results of this study have several limitations that must be considered. First, the evaluations of eGFR, UAE and IMT were performed according to just one assessment of the urinary albumin concentration, the serum creatinine level, and the carotid artery ultrasonography. Second, because of the cross-sectional design, the findings are inherently limited by an inability to eliminate causal relationships between the risk factors and carotid IMT thickening. Third, we have some missing data with lipid profile of patients. However, there is still no investigation showing the effect of measurement on the patient's recovery and more studies are necessary for determining how to use these measurements in promoting patient's outcome.

Atherosclerosis complications in diabetes are the most prevalent and challenging issue in the diabetic management, today. Assessment of CIMT using ultrasound imaging is a noninvasive way of determining atherosclerosis. Our study shows a relationship between the carotid IMT and renal parameters, including eGFR and albuminuria, and these factors are predictors of CIMT and subsequent atherosclerosis. This study confirms the importance of intensive examinations for early detection of atherosclerosis and treatment of risk factors in the patients with type 2 diabetes mellitus, especially when a microalbuminuria or reduced eGFR accompany with IMT thickening is found.

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Authors' Contributions

Aaraf Jenabi developed the original idea and the protocol, abstracted and analyzed data, and wrote the manuscript. Hamid Dehdashti Shahrokh, Mehrnoosh Zakerkish, Mohammad Ghasem Hanafi, Afshin Rezazadeh, and Heshmat Allah Shahbazian contributed to the development of the protocol and abstracted the data. Fakher Rahim contributed to analysis of the data. Aaraf Jenabi, Farzaneh Forouzan, and Mehrnoosh Zakerkish contributed to the collecting of the data. Hamid dehdashti Shahrokh supervised the study and contributed to writing of the manuscript.

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