ABSTRACT

Background/Purpose: Aortic aneurysm early detection is crucial in cases having elevated clinical risk due to its life-threatening sequelae. This study aims to understand the relationship between advanced glycation end products (AGEs), coronary risk factors, abdominal aortic diameter (AAD) and carotid blood flow in elderly diabetes mellitus (DM).

Methods: A case control study conducted on 90 elderly patients, 60 years and above, recruited from the Ain Shams University Hospital. Elderly subjects were divided into 3 groups. Group I (controls): 30 patients without DM; Group II (cases): 30 diabetic cases without co-morbidities; and Group III (cases): 30 diabetic cases with co-morbidities. All groups have undergone assessment of blood sugar, serum lipids, and AGEs, in addition to measurement of carotid intima-medial thickness (CIMT) by carotid duplex scan.

Results: Coronary risk is significantly high in Group III (19.300±9.542) followed by Group II (18.200±11.704) then the control group (7.562±5.241). CIMT is significantly higher in Group III (1.377±0.220) followed by Group II (1.193±0.276) then the control group (0.993±0.246). AGEs are significantly high in Group I followed by Group II then Group III. CIMT is statistically significantly correlated with AGEs in Group I. No statistically significant correlation between AAD and AGEs in study groups. There is highly significant correlation between coronary risk and AGEs in Group II.

Conclusion: Mean carotid intimal thickness is considered as an early marker of atherosclerotic disease.

1. INTRODUCTION

Diabetes mellitus (DM) is a rising medical issue globally, due to long life expectancy arising from advances in medications and management protocols with life style modifications. Over the past two decades, there has
been an considerable rise in the number of diabetic cases in both developed and developing nations.¹

DM could be linked with pathological acceleration of the process of atherosclerosis by either raising the causative risk factors, e.g. dyslipidemia and elevated blood pressure levels, or diabetes-specific risk factors, e.g. advanced glycation end products, increased reactive oxygen species and matrix protein increased production.²

Advanced glycation end-products are highly diverse group of amino-carbonyl compounds whose development is triggered by the reaction of reducing sugars and oxoaldehydes with proteins.³

Raised levels of circulating AGEs in the presence of hyperglycemia are supposed to have a cornerstone role in the pathological and progressive course of development of macro-vascular and micro-vascular disease clinically presented in DM.⁴

Early aortic aneurysm detection is crucial in cases having elevated clinical risk due to its life-threatening sequelae. These forms of aneurysms are usually asymptomatic till occurrence of rupture. Various research studies have mentioned that abdominal aortic aneurysms (AAA) are more widespread in cases that undergo cardiac catheterization for coronary arterial disease or scheduled for coronary artery bypass vascular grafting. Because both issues have similar medical risk factors and pathophysiological pathways, researchers assumed that there is a linkage between AAA and coronary arterial disease.⁵

Diabetes is a chief trigger for pathological developmental course of atherosclerosis within the arterial bed. Cases that are suffering from diabetes are at elevated risk of arterial atherosclerosis causing cardiovascular disorders, particularly coronary heart disease, which is the most frequent complication and the main cause of mortality in type II DM cases. The carotid artery could be considered as a mirror reflecting the status common to all infected arteries. Common carotid artery intimal layer-media thickness parameters are considered a powerful predictability tool of future vascular events and a sonographic marker of atherosclerosis.⁶

Despite the fact that numerous research studies have assessed and investigated the correlation and linkage between diabetes and lower extremity peripheral artery disease, small number of research studies have investigated carotid artery stenosis or AAA in individuals with and without diabetes.⁷,⁸ Thus the aim of the current study is to assess the relation between AGEs, coronary risk factors, AAD and carotid blood flow in elderly diabetics.

2. METHODS

2.1. Participants

A case control study recruited 90 elderly patients, 60 years and above, was conducted at the inpatient wards and outpatient clinics of Ain Shams University Hospitals. Elderly subjects were divided into 3 groups. Group I (controls): 30 patients without DM; Group II: 30 diabetic cases without co-morbidities; and Group III: 30 diabetic cases with co-morbidities. Subjects refused to participate in the research was the only exclusive criteria for this study.

Informed consent was taken from every elderly participating in the current study. The study methodology was reviewed and approved by the Research Review Board of the Geriatrics and Gerontology Department, Faculty of Medicine, Ain Shams University.

Every study subject was subjected to the following:

Comprehensive clinical geriatric evaluation as follows: Detailed history taking, demographic data and past medical history (particularly detailed history of DM, other coronary risk factors, e.g. smoking, hypertension, lack of physical exercise, and unhealthy diet e.g. high caloric diet, increased carbohydrates and fats and low fiber. Coronary risk assessment: using the ASCVD algorithm published in 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk. Detailed clinical examination was done for all participants.

Laboratory work up: Each case recruited have been instructed to fast 12-14 hours for assay of total cholesterol (T.ch), high density lipoproteins (HDL), low density lipoproteins (LDL), and triglycerides (TG) and 8 hours for measurement of fasting blood sugar (FBS). The last sample withdrawn 2 hours after eating for measurement of 2 hour postprandial blood sugar (2hr pp).

Assessment of blood sugar level: The assay was conducted by usage of Synchron CX-9 PRO autoanalyzer (Beckman Coulter, Inc. Fullerton, CA 92835-3100, USA). HbA1c and other hemoglobin fractions could be separated by High Performance Liquid Chromatography (HPLC). The principle of the assay is cation exchange chromatography.

Assay of serum lipids: Total cholesterol, triglycerides were conducted by usage of Synchron CX-9 PRO autoanalyzer (Beckman Coulter, Inc. Fullerton, CA 92835-3100, USA). The assay of high density lipoproteins was performed on Synchron CX-9 PRO autoanalyzer after precipitation with phosphotungstic acid by an enzymatic method by usage of a commercially available kit (Beckman Coulter, Inc. Fullerton, CA 92835-3100, USA).

Assay of advanced glycation end products: Principle of the assay relies on binding of advanced glycation end products in serum samples to microliter plates
coated by purified capture human AGEs antibody, subsequently addition of conjugate AGEs antibody labeled by HRP enzyme (Sandwich ELISA). For the detection step substrate which by the effect of the HRP turned into a blue color and the intensity of the color is proportionate to the concentration levels of the AGEs within the obtained sample. Finally sulphuric acid is added to terminate the reaction after precise period of incubation. The samples concentration levels are obtained by comparison of the optical density of the samples to the calibration curve.

**Radiological studies:** For the measurement of carotid Intima-medial thickness by carotid duplex, all study subjects have been examined in the supine position. Both left and right common carotid arteries were evaluated. Sonographic duplex were conducted by the aid of an Alpinion-Korae- E-CUBE-9 sonography unit equipped with a 7.5-MHz transducer. The sonographers measured carotid intima media thickness of the right and left common carotid arteries posterior wall, 1.5 cm proximal to the bifurcation. The artery was imaged in a longitudinal plane to gain optimal incidence angle, which is described as the plane in which the bifurcation of the carotid bulb into the internal and external carotid arteries could be visualized in a simultaneous manner with the bulb and distal common carotid artery (i.e tuning fork view).9

**2.3. Statistical Analysis**

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS (Statistical Package for Social Sciences) statistics software version 22.0, IBM Corp., Chicago, USA, 2013. Descriptive statistics were done for quantitative research data as minimum and maximum of the range as well as mean±SD (standard deviation), while it was performed for qualitative research data as number and percentage. Inferential statistical analyses were conducted for quantitative research variables by usage of independent t-test in cases of two independent research groups with parametric data. In qualitative data, inferential analyses for independent variables was conducted by usage of Chi square test for differences between proportions and Fisher’s Exact test for variables with small expected numbers.

**3. RESULTS**

A case control study was conducted on 90 elderly subjects that were categorised into 3 groups: Group I (controls) involved 30 patients without DM, Group II involved 30 diabetic cases without co-morbidities and Group III composed of 30 diabetic cases with co-morbidities. As regards demographic data, the study groups are matched for age and sex and this study found that there is no statistical significant difference between study groups as regards marital status, educational level and smoking status (P >0.05) (Table 1).

Regarding laboratory findings, this study found that FBS, 2hr pp, HbA1c, T.ch and LDL were significantly highest among group II, followed by group III and least among control group and HDL was significantly lowest among group III, followed by group I and least among group II and there is no significant difference between study groups regarding TG (P >0.05) (Table 2). Regarding coronary risk, we found that coronary risk is significantly high in group III (19.300±9.542) followed by group II (18.200±11.704) then control group (7.562±5.241) (Figure 1). Regarding CIMT findings, we found that CIMT is significantly higher in group III (1.377±0.220) followed by group II (1.193±0.276) then control group (0.993±0.246) (Figure 2). As regard AAD, there is no statistical significant difference regarding AAD (P >0.05) among Group I (1.793±0.292), Group II (1.853±0.359) and Group III (1.983±0.484). As regard description of AGEs among study groups, we found that that AGEs are significantly high in Group I (211.000±59.181) followed by Group II (204.500±54.935) then Group

![Table 1](image-url)
III (171.83±54.194) (Figure 3). As regard correlation between CIMT and AGEs among study groups, CIMT is statistically significantly correlated with AGEs among Group I ($P=0.038$) and there is no significant correlation among Group II and III ($P=0.120$, $0.441$ consecutively) (Table 3). As regard correlation between AAD and AGEs among study groups, there is no statistical significant correlation between AAD and AGEs among study groups ($P=0.150$, $0.082$, $0.947$ consecutively). As regard correlation between coronary risk and AGEs among study groups, there is highly significant correlation between coronary risk and AGEs among Group II ($P=0.002$) but there is no significant correlation among Group I and III ($P=0.110$, $0.247$ consecutively) (Table 4). Regarding correlation between laboratory findings and AGEs among study groups, this study found highly statistically significantly correlation between FBS, 2hr pp and AGEs among Group I, and also highly statistically significantly correlation between FBS and AGEs among Group II, however there is no statistical significant correlation regarding other laboratory findings (Table 5).

### 4. DISCUSSION

Type II DM is a very complex and multifactorial metabolic illness. In this consideration, the current research was conducted to reveal additional evidence that pathological oxidative stress course have a crucial role in hyperglycemia-triggered tissue injury. On the other hand, the role of AGEs in the pathophysiological development of type II DM and diabetic complications is only partially elucidated, that is why the current research study focused on the correlation between AGEs, coronary risk factors, AAA and carotid blood flow in elderly diabetic cases.

Our study showed that HDL was significantly lower in diabetic patients with co-morbidities in comparison to control group. This result was consistent with the data obtained by Bonakdaran et al. 2011 in which they investigated the prevalence and risk factors of
cardiovascular disease in 752 cases with type II DM, their results denoted that HDL cholesterol was a statistically significant independent predictor for cardiovascular disease in diabetic cases.10

Our study also found that coronary risk is statistically significantly high in Group III followed by Group II then the control group. This was consistent with many studies such as Kim et al. 2018, a cohort study that recruited 1,302 consecutive patients with type II DM and without a prior history of cardiovascular disease, and their results revealed that high hemoglobin glycation index was independently correlated with incident cardiovascular disease in cases with type II DM and cases with high hemoglobin glycation index at baseline had a higher inherent risk for cardiovascular disease.11

Our results showed that AGEs were significantly highest among the control group (211.0±59.2) followed by Group II (204.5±54.9) then Group III (171.8±54.2). On the contrary, Dubourg et al. 2017 that assayed AGEs in 75 cases with poorly controlled type II DM and in 31 non-diabetic controls, and their results showed that AGEs level was statistically significantly raised in diabetic cases in comparison with non-diabetics. This difference in results could be explained by the dietary habits of the most Egyptians and they start to be on healthy diet after being ill, in addition the patients (cases) were on anti-diabetic drugs and lipid-lowering drugs which affect the AGEs level. This fact is verified and proven by several research trials.12 Vlassara and Striker 2013 mentioned that it was originally understood that AGEs arise originally from endogenous sources, and that excessive amounts exist only in cases suffering DM or in aging. It is now apparent that the diet is a chief source of AGEs in normal individuals, in addition to those with DM. Dietary consumption has recently been recognized as a cornerstone environmental source of pro-inflammatory AGEs in humans.13 Foods rich in both protein and fat, and cooked at high and dry heat, e.g. in broiling, grilling, frying, and roasting, tend to be the richest dietary sources of AGEs, whereas low-fat, carbohydrate-rich foods tend to be relatively low in AGEs.14 Healthy subjects young and old, involving cases with diabetes are responsive to low AGE diet with a considerable drop in circulating AGE levels. This decrease in serum AGE concentration levels is correlated by a simultaneous decrease in inflammatory markers, oxidative stress, and endothelial dysfunction.14 There are various drugs that reduce AGEs serum levels (listed in the ascending order of efficacy of binding AGEs): metformin, vitamin B analogues such as pyridoxamine and benfotiamine, and aminoguanidine. The investigators denoted that metformin decreases AGEs serum levels in a dose-dependent manner and that this impact is independent of its influence on glycemic levels.15 The most recent promising anti-AGEs agents are statins and thiazolidinediones. A research study revealed that fluvastatin has an inhibitory influence on AGE-induced vascular smooth muscle cell

Figure 3. Description of AGEs among study groups.

Table 3. Correlation between CIMT and AGEs among study groups.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>CIMT</th>
<th>AGEs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>Group I</td>
<td>-0.381</td>
<td>0.038*</td>
</tr>
<tr>
<td>Group II</td>
<td>0.290</td>
<td>0.120</td>
</tr>
<tr>
<td>Group III</td>
<td>-0.146</td>
<td>0.441</td>
</tr>
</tbody>
</table>

CIMT, carotid intimal medial thickness; AGEs, advanced glycation end products.

Table 4. Correlation between coronary risk and AGEs among study groups.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Coronary</th>
<th>AGEs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>Group I</td>
<td>-0.321</td>
<td>0.110</td>
</tr>
<tr>
<td>Group II</td>
<td>0.548</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group III</td>
<td>-0.346</td>
<td>0.247</td>
</tr>
</tbody>
</table>

AGEs, advanced glycation end products.

Table 5. Correlation between laboratory findings and AGEs among study groups.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Variables</th>
<th>AGEs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>P Value</td>
</tr>
<tr>
<td>FBS</td>
<td>0.456</td>
<td>0.011*</td>
</tr>
<tr>
<td>2hr pp</td>
<td>0.648</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.022</td>
<td>0.908</td>
</tr>
<tr>
<td>LDL</td>
<td>0.150</td>
<td>0.430</td>
</tr>
<tr>
<td>HDL</td>
<td>0.096</td>
<td>0.615</td>
</tr>
<tr>
<td>T.ch</td>
<td>0.167</td>
<td>0.377</td>
</tr>
<tr>
<td>TG</td>
<td>0.126</td>
<td>0.508</td>
</tr>
</tbody>
</table>

FBS, fasting blood sugar; 2hr pp, 2 hour post prandial; LDL, low density lipoprotein; HDL, high density lipoprotein; T.ch, total cholesterol; TG, triglycerides.
proliferation, migration, and production of extra cellular matrix, which were linked and correlated with connective tissue growth factor secretion.15 This difference in results could be explained by Goh and Cooper 2008 which revealed that there is no universally established protocol to measure AGEs, with no international standard values or an internationally accepted standard unit of assay, thus making statistical comparison of research results between different laboratories very complex. Additionally, blood is more accessible for repeated measurements of AGEs than tissue-requiring biopsies, but plasma AGE assays have not yet been revealed to be directly correlated to the tissue AGE content. Additionally, it stays indistinct which circulating AGEs should be assayed, and specifically, which chemical moiety is most relevant biologically.16

On the other hand, many studies showed significant results using ELISA such as the study of Ashraf et al. 2017 which suggested that the AGE level can be used as a biomarker for diagnosis and prognosis of the diabetes and its complications.17 In addition, our study findings revealed statistically significant correlation between coronary risk and AGEs among Group II and there is no statistical significant correlation within Group I and III. This is confirmed by Rhee and Kim 2018 that in chronic hyperglycemia cases, AGEs are actively synthesized and accumulated within the circulation and different tissues. AGEs also speed up the receptors for AGEs expression at molecular levels, and have a cornerstone role in the pathological development of diabetic vascular complications via cellular and molecular pathways.18 Also, a cross-sectional research study of 1,159 men aged 71-92 years with no clinical history of cardiovascular disease conducted by Wannamethee et al. revealed that greater circulating soluble receptor levels for AGEs was correlated with raised level of cardiac markers in men both with and without DM.19 And a Japanese study conducted by Hangai et al. published in 2016 which recruited 122 Japanese study subjects with type II DM in a cross-sectional manner, and its results displayed a statistical significant correlation between AGEs levels and coronary artery calcification.20

The current study findings displayed that the mean carotid intimal thickness was the highest (1.377±0.22 mm) in diabetic patients with co-morbidities, and then (1.193±0.276) in diabetic patients without comorbidities, and the least (0.993±0.246) in the control group and this difference is significant statistically (P <0.001). The same issue was confirmed by Kota et al. 2013 which was conducted on 80 subjects within the age group of 30-75 years and found that the mean CIMT was statistically significantly higher in diabetic study subjects in comparison to healthy subjects.21 Mostaza et al. 2015 cross sectional study recruited 1,475 study subjects revealed that the prevalence of carotid plaques was 34.2% in the control group, 45.1% in prediabetics, 64.2% in newly diagnosed diabetic cases, and 72.9% in established diabetic cases. These numbers were 0.3%, 1.1%, 5.0% and 7.7% for carotid stenosis, consecutively.22

In addition, our study showed that CIMT is significantly correlated with AGEs among Group I (P=0.04) and there is no significant correlation among Group II and III. Wannamethee SG et al. found that there is no association between circulating soluble receptor for advanced glycation end product (sRAGE) and central aortic blood pressure, CIMT or arterial stiffness as determined by pulse wave velocity (PWV) in men with and without diabetes, however sRAGE was significantly and positively associated with NT-proBNP (N terminal pro b-type natriuretic peptide) and other markers of arterial stiffness (central augmentation pressure [AP] and augmentation index [Aix]) in both groups of men after adjustment for CVD risk and metabolic risk markers, renal function and inflammation.19 This study may explain our finding that if we measure NT-proBNP and markers of arterial stiffness as central augmentation pressure (AP) and augmentation index (Aix), and we may find a significant relations among patients with and without diabetes. In the contrast, a study have evaluated skin autofluorescence (SAF), a non-invasive tool of tissue AGE accumulation measurement, in cases with carotid artery stenosis. The SAF measurement was implemented in 56 cases with carotid artery stenosis and in 56 age- and sex-matched healthy controls without diabetes. SAF was greater in cases having carotid artery stenosis in comparison to the control group.23

The controversy of results, could be explained as there are other environmental and genetic factors are implemented in the pathophysiological development of cardiovascular complications in DM cases. AGEs are heterogeneous and complex structures, which could represent crucial causative mediators of diabetic vascular complications at cellular and molecular levels, some research studies have revealed linkage between AGE serum levels and the development/severity of vascular disease.12 Moreover, others failed to establish linkage of some specific plasma AGEs with cardio vascular disease in individuals with normal glucose metabolism or type II DM. Because of their heterogeneous structures, the contribution of each AGE molecule to the pathophysiology remains unknown. Among AGEs, fluorescent ones are cross linking structures such as pentosidine and crossline and known to contribute to the development of vascular complications. Several studies in diabetic patients reported a correlation between serum pentosidine levels and arterial wall stiffness, a recognized component in the determination of vascular risk.24

As regard, comparison between study groups regarding abdominal aortic diameter (AAD), our results showed that there was no statistical significant difference between study groups regarding AAD. These findings have been confirmed by Taimour et al. 2017 who found that among 65 year old men, aortic diameter and AAA
prevalence do not differ between those with newly diagnosed type 2 diabetes and those without diabetes. Putative protective effects of type II DM against aortic dilatation and AAA development therefore probably occur later after diagnosis of diabetes.

Wang et al. conducted a study revealed that although individuals with diabetes have a higher risk of occlusive atherosclerotic disease, data have shown a paradoxically lower prevalence of AAA in diabetic patients. Abdominal aortic aneurysm progresses more slowly in diabetics and diabetic cases are less probable to develop a ruptured AAA at the time point of performing repair, denoting that diabetics or its medications could have protective effect against the pathological course of development and improve the prognosis of AAA. Several reports have shown that diabetic patients develop smaller AAA, as demonstrated by significantly lower aortic diameters compared to non-diabetic subjects. Besides several studies highlighted lower growth rates of AAA in diabetic patients compared to controls. While DM appears as a protective factor of AAA formation and expansion, the prognosis and outcome after AAA treatment also differs between diabetics and non-diabetic patients. Heterogeneous results were found among different studies, some reports revealing increased operative mortality in diabetics, others showing no difference and some reporting lower mortality. Besides, morbidity following AAA repair was analysed, and DM was identified either as a negative or protective factor of specific post-operative outcomes. Higher rates of complications such as myocardial infarction, infection, or pancreatitis were observed in diabetic patients after AAA open repair, and higher incidence of device-related complications following endovascular AAA repair were found. On the opposite, DM had a protective effect on AAA growth and re-interventions after endovascular repair, but no significant difference in the occurrence of neck dilatation or type 1 endoleaks was identified between diabetics and controls.

This study showed that there was no significant correlation between AAD and AGEs among study groups. This is consistent with Koole et al. 2017 study in which researchers collected abdominal aortic artery biopsies from 30 diabetic cases and 30 matched nondiabetic study subjects at the time of open surgical repair, and concentrations of the AGE cross-link pentosidine were assayed. The findings revealed that cross-linking AGEs like pentosidine play a protective role in AAA progression in diabetic patients.

The current research study highlighted the correlation between AGEs, coronary risk factors, abdominal aortic diameter and carotid blood flow in elderly diabetics.

5. CONCLUSION

The mean carotid intimal thickness is considered as an early marker of atherosclerotic disease, it was the greatest in diabetic cases with cardiovascular complications in comparison to the other groups and thus, the measurement of carotid intimal media thickness by sonographic examination provides a quantitative basis for the extent of atherosclerosis. In the contrast, lower prevalence of AAA was reported in diabetic patients suggesting that diabetes or its medications may protect against the development and improve the prognosis of AAA.

Noninvasive screening tests for carotid artery intima-media thickness by sonographic examination is recommended in all elderly population with diabetic mellitus to reveal the patients who are at high risk of development of atherosclerotic disease. Further studies with larger sample size are recommended to explore the relation between AGEs and DM in Egyptian elderly.

CONFLICTS OF INTEREST

There is no conflict of interest of any kind.

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