VCAM-1 Is Associated With High Cardiovascular Risk Predicted by the Framingham Score: A Cross-Sectional Study With Diabetic Women

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Abstract

Background

The best strategy to establish cardiovascular risk (CVR) in women has yet to be defined, although risk scores are widely used. The inclusion of endothelial biomarkers, such as vascular cell adhesion molecule-1 (VCAM-1), to the risk scores could increase their discriminatory power and improve risk assessment.

Objective

To evaluate the association between endothelial biomarkers and CVR in women with type 2 DM (T2DM) and without previous cardiovascular disease (CVD).

Methods

Cross-sectional study, including women with T2DM from Fortaleza, Brazil, from January to October 2017. Women aged 30 to 74 years were included and those with CVD were excluded. Clinical and laboratory data were evaluated (age; time of T2DM diagnosis; hypertension presence and treatment; smoking; body mass index - BMI; systolic blood pressure - SBP and diastolic blood pressure - DBP; glycemic and lipid profiles and serum biomarkers VCAM-1, fibroblast growth factor-23, Syndecan-1 and Angiopoietin-2). The CVR was stratified using the Framingham Risk Score - FRS (version with laboratory tests - laboratory FRS and the one with the BMI - non-laboratory FRS) and the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.

Results

In total, eighty-eight diabetic women were evaluated, with mean age 56 ± 10 years and T2DM time of 5 (3–9) years, most of them hypertensive (71.6%), overweight or obese (74%), with altered blood glucose levels (blood glucose 8.53 ± 3.65 mmol/L and hbA1c of 65 ± 1 mmol/mol) and lipids (LDL 27.06 ± 8.99 mmol/L), in addition to high risk according to laboratory (72.7%) and non-laboratory (81.8%) FRS and low risk for any UKPDS score outcome. Regarding the biomarkers, only VCAM-1 showed an association with CVR, mainly with a high-risk classification using laboratory FRS ($p = 0.024$), even in this population without CVD.

Conclusion

The assessed diabetic women without previous CVD had high CVR in traditional scores (FRS scores) and low CVR in specific score for diabetics (UKPDS score). VCAM-1 may be useful in detect subclinical

endothelial dysfunction in women with T2DM and its incorporation into scores may improve the accuracy in risk stratification of women with T2DM.

Introduction

The world's population of individuals with diabetes mellitus (DM) is approximately 463 million people.^[1] Among women, the overall prevalence of DM is lower than in men (9.0% and 9.6%, respectively); however, in Central and South America, the number of diabetic women is higher than that of men.^[1, 2]

About 1.6 million deaths are attributed to DM worldwide, with cardiovascular diseases (CVD) being the main cause of death in diabetic individuals, which is equivalent to about 2/3 of the total deaths. Considering gender differences, globally, in 2019, there were more deaths associated with DM in women (2.3 million) than in men (1.9 million).^[1, 2] Moreover, it is suggested that diabetic women have a higher risk of coronary artery disease (CAD), cerebrovascular disease, cardiac death and all-cause mortality compared to men.^[3]

The cardiovascular risk assessment in women population is essential to prevention of cardiovascular events. Risk scores can be carried out, such as the Framingham Risk Score (FRS) and the score derived from the United Kingdom Prospective Diabetes Study (UKPDS).^[4, 5] However, the literature shows conflicting results about risk estimate when using these scores in women, with a previous risk overestimation by the FRS and the UKPDS having been demonstrated. The latter, although being a specific score for the diabetic population, has already demonstrated a risk overestimation of 51.3% in women.^[6, 7]

There is yet no definition regarding the best CVR stratification strategy in diabetic individuals^[4, 7] A meta-analysis evaluated different tools for CVR stratification in diabetics and found that their performance varied significantly, even among those developed specifically for this population.^[8] Moreover, there are important differences between genders regarding the epidemiological and statistical determinants of the performance of risk prediction models.^[9] Therefore, improvements in the predictive capacity of these models, such as the possible addition of specific biomarkers, such as vascular cell adhesion molecule-1 (VCAM-1), fibroblast growth factor-23 (FGF-23), Syndecan-1 (Sdc-1) and Angiopoietin-2 (Ang-2), would be required to understand the short and long-term risks before implementing them into clinical practice.^[8]

However, there is no definition of which biomarkers could be used for the best estimate of the occurrence of cardiovascular events and the studies still bring conflicting results regarding the role of biomarkers in predicting CVR in the overall population and the diabetic populations.^[4, 10, 11] Research conducted by Ren et al.^[12] and Llauradó et al.^[13] found no association between VCAM-1 and the occurrence of CVD, respectively, in the overall population and in diabetics. As for the study by Gardner et al.^[14], it showed higher levels of VCAM-1 in Caucasian women, when compared with Caucasian men with peripheral

obstructive arterial disease (POAD). Women in this study still had more severe POAD than men, with worse exercise performance and daily outpatient activity.

Considering the existing gaps in CVR stratification in diabetic women, this study aimed to stratify cardiovascular risk by different scores and evaluate the association between the predicted risk and serum vascular and cardiorenal biomarkers in women with T2DM.

Methods

Study type and location

This is an observational, cross-sectional study that evaluated women with T2DM, aiming at assessing their CVR and its association with new serum endothelial biomarkers. The study was carried out from January to October 2017, in a Primary Health Care (PHC) unit in the city of Fortaleza, state of Ceará, a large urban center located in northeastern Brazil.

Study population

The study population comprised women with T2DM, followed at the aforementioned PHC unit. The criteria defined by the American Diabetes Association^[15] were adopted for the diagnosis of T2DM.

Women with T2DM, aged 30 to 74 years, who lived in the area assigned to PHC unit and who signed the free and informed consent form, were included in the study. This age limit was defined, since the FRS to be used in primary care was validated with individuals who were included in this age group.^[16]

The exclusion criteria comprised women with a previously diagnosed CVD (CAD, stroke, POAD or heart failure - HF) or who had symptoms possibly related to CVD; those with malignant neoplasms of any kind and / or active inflammatory disease; those who had an infection with systemic effects at the time of the assessment; those whose electrocardiogram (EKG) examination performed during the study was suggestive of the presence of CVD, according to previously established criteria^[17] and pregnant or postpartum women.

Patient recruitment

The participants' recruitment was carried out through a public announcement with posters and pamphlets and through a direct invitation to possible participants. Interested individuals were shown the research objectives, their risks and benefits, and explanations of possible doubts were provided.

Women were evaluated according to the following sequence for the sample selection: electrocardiogram (EKG) to assess possible alterations compatible with CVD, performed on a portable 3-channel Philips EP-3 Dixtal™ device, of which reports were given as recommended^[17] and analysis of the research inclusion/exclusion criteria.

Data collection and variables

The participants were instructed to come on a scheduled day and time, so that data collection could be carried out, according to the following steps: 1) Anamnesis - the following parameters were evaluated: sociodemographic data (age, ethnicity, level of schooling) and clinical history (time since the T2DM diagnosis; presence of systemic arterial hypertension - SAH; use of antihypertensive medication; personal pathological history and smoking status); 2) Physical examination - the following data were assessed: height and weight measurement, to calculate the body mass index (BMI), as recommended^[18] and blood pressure (BP) measurement, with the recording of systolic BP (SBP) and diastolic BP (DBP), as previously recommended.^[19]

Women who stated being hypertensive and who regularly used antihypertensive medications for BP control purposes in the past month were considered as having previous SAH. Women who smoked at least one cigarette in the last 30 days were considered to be current smokers.

Blood sample collection and processing

Blood was collected from the participants using the venipuncture technique, after a 12-hour fast. The samples were placed in vacuum collection tubes, with separating gel, for further processing of these samples in the biochemical tests and serum biomarkers, with the exception of glycemia, for which the sample was placed in a tube with sodium fluoride and ethylenediaminetetraacetic acid (EDTA) and glycated hemoglobin A1c (hbA1c), for which the samples were stored in tubes with EDTA. The collected samples were taken for processing within an average time of 2 hours.

The other blood samples were centrifuged at 3500–4000 rpm for 15 minutes to obtain the serum, which was aliquoted for storage in 3 1mL-Eppendorf tubes. Subsequently, these samples were stored in a freezer at -80° C.

Laboratory analysis

HbA1c was measured in the total blood sample of each participant, using the high performance liquid chromatography technique. For the overall biochemical evaluation, the following laboratory tests were performed in the stored serum samples, using the colorimetric enzymatic method: fasting glycemia (FG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG). Levels of low-density lipoprotein cholesterol (LDL-c) were obtained using Friedewald's formula.^[20]

Analysis of biomarkers

All of serum endothelial biomarkers were analyzed using the “sandwich” type Enzyme-Linked Immunosorbent Assay (ELISA) technique, according to the manufacturer's instructions for the assays. Specific kits were acquired for each biomarker: for FGF-23 (Duoset DY2604; R&D Systems®), VCAM-1 (ab47355; Abcam®), Sdc-1 (ab47352; Abcam®) and Ang-2 (Duoset DY623; R&D Systems®). All analysis were performed in isolated serum samples.

Cardiovascular risk stratification through the scores

For the analysis of CVR in this study, the following tools were used: the FRS for primary care using lipid fractions (laboratory FRS), the FRS using the BMI (non-laboratory FRS) and the UKPDS risk engine.

The CVR by laboratory FRS was assessed using the online calculator.^[21] For its calculation, the following data were used: age, presence of diabetes, smoking status, SBP value in mmHg, presence of treatment for SAH, and HDL-c and TC values.^[16, 22] The non-laboratory FRS was also calculated using a tool available online.^[21] For this purpose, the same data used for the laboratory FRS were utilized, with the exception of the TC and HDL-c, which were replaced by the BMI. The risk of CAD, stroke, POAD or HF) in 10 years was assessed using these scores. Regarding the CVR analyzed by these scores, the participants were classified as having low (< 5%), intermediate (calculated risk $\geq$ 5% and $\leq$ 10%) or high risk (> 10%). Additionally, the vascular age of each participant was estimated according to both scores.^[16, 22]

Data on age, T2DM duration, gender, presence of atrial fibrillation, ethnicity, smoking status, hbA1c, SBP, TC and HDL-c values were used to calculate the UKPDS score. With this tool, the risk of fatal or non-fatal CAD and stroke in 10 years was analyzed for each participant.^[23, 24] According to UKPDS risk engine, the participants were classified as having high (> 20%), intermediate (10 to 20%) or low (< 10%) risk for any of the outcomes.^[23, 24]

Statistical analysis

Categorical data were expressed as absolute count and relative frequency in percentages and were compared using the chi-square test. All quantitative variables were assessed regarding a normal distribution, using the Kolmogorov-Smirnov normality test and evaluation of variance and histograms to verify data asymmetry. Variables with normal distribution were presented as mean $\pm$ standard deviation and non-normal data were shown as median and interquartile range. The Analysis of variance (ANOVA) test with Tukey's post-test were applied to compare means of continuous variables. Additionally, Spearman's correlation analyses were performed to assess the association between quantitative variables. Significance was set at $p < 0.05$ (two-way). All analyses were performed using the Statistical Package for Social Sciences (SPSS) version 23.0 for Macintosh (IBM, Armonk, NY, USA).

Ethical aspects

This research was carried out in accordance with Resolution n. 466/2012 of the National Health Council, after approval by the Research Ethics Committee of University of Fortaleza (Opinion n. 1.843.144 / 2016).

Results

A total of 88 women with T2DM were included in this study. Table 1 shows the main characteristics of the assessed population. The mean age of the women was 56 ± 10 years, with the majority being classified as brown (81.8%). Only 4.6% of the sample had a higher education level; 17.2% were illiterate and 70.1% had not entered high school.

Table 1

Sociodemographic and clinical-laboratory characteristics of diabetic women, Fortaleza-Ceará-Brazil, 2021. n = 88

Sociodemographic and clinical-laboratory characteristics	Total group
Age (years)	56 ± 10
Ethnicity/skin color	
White	10 (11.4)
Black	6 (6.8)
Brown	72 (81.8)
Yellow	0 (0)
Indigenous	0 (0)
Level of schooling	
Higher Education	4 (4.6)
High School	22 (25.3)
Elementary School	46 (52.9)
Illiterate	15 (17.2)
Smoking status	
Non-smoker	46 (52.3)
Ex-smoker	34 (38.6)
Current smoker	8 (9.1)
Systemic Arterial Hypertension	
No	25 (28.4)
Yes	63 (71.6)
Time of Diagnosis of Type 2 Diabetes Mellitus (years)	5 (3–9)
Body Mass Index Classification	
Normal weight	8 (9.1)
Overweight	32 (36.4)

SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL-c = High-density lipoprotein cholesterol; LDL-c = Low-density lipoprotein cholesterol; HbA1c = Glycosylated hemoglobin A1c.

Quantitative data expressed as mean ± standard deviation or as median and interquartile range in parentheses. Categorical data expressed as absolute count and percentages in parentheses.

Sociodemographic and clinical-laboratory characteristics	Total group
Obesity Grade I	29 (33)
Obesity Grade II	17 (19.3)
Obesity Grade III	2 (2.3)
Body Mass Index (Kg/m²)	31.13 ± 4.76
SBP (mmHg)	134 ± 19
DBP (mmHg)	83 ± 15
Total Cholesterol (mmol/L)	48.02 ± 10.25
HDL-c (mmol/L)	11.6 ± 2.51
LDL-c (mmol/L)	27.06 ± 8.99
Fasting plasma glucose (mmol/L)	8.53 ± 3.65
HbA1c (mmol/mol)	65 ± 2
SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL-c = High-density lipoprotein cholesterol; LDL-c = Low-density lipoprotein cholesterol; HbA1c = Glycosylated hemoglobin A1c.	
Quantitative data expressed as mean ± standard deviation or as median and interquartile range in parentheses. Categorical data expressed as absolute count and percentages in parentheses.	

When evaluating the clinical characteristics, 47.7% were smokers at some point in their lives (current or past smoker) and 71.6% had SAH as a comorbidity. The median time of T2DM diagnosis was 5 years (IQR 3–9 years). The analysis of body composition showed that most of the study sample was overweight (32%) or obese (42%).

The laboratory data showed high blood glucose levels, with a mean fasting plasma glucose of 8.53 ± 3.65 mmol/L and hbA1c of 65 ± 1 mmol/mol. The analysis of lipid levels showed high mean levels of LDL-c (27.06 ± 8.99 mmol/L).

Table 2 shows the stratification of the CVR of the assessed population according to different scores, as well as the vascular age calculated by the laboratory FRS and non-laboratory FRS. The median vascular age assessed by both FRS variants was 86 (IQR 76–86) years. When evaluating the classification of the CVR by the laboratory FRS and by the non-laboratory FRS, it is observed that, in both, most of the population was classified as high risk (72.7% and 81.8%, respectively). When assessing the UKPDS Risk Engine score, regarding the risk of CAD (fatal or non-fatal), as well as the risk of stroke (fatal or non-fatal), the highest percentage of the population was classified as low risk.

Table 2

Distribution of diabetic women according to risk stratification by cardiovascular risk scores, Fortaleza-Ceará-Brazil, 2021. n = 88.

Risk classification and vascular age	Diabetic women (n = 88)
Laboratory FRS (%)	16.3 (9.35–25)
Vascular age Laboratory FRS (years)	86 (71–86)
Risk categories according to Laboratory FRS	
Low risk	9 (10.2)
Intermediate risk	15 (17)
High risk	64 (72.7)
Non-laboratory FRS (%)	23.75 (12.7–31)
Vascular age non-laboratory FRS (years)	86 (76–86)
Risk categories according to non-laboratory FRS	
Low risk	5 (5.7)
Intermediate risk	11 (12.5)
High risk	72 (81.8)
UKPDS Risk Engine – CAD outcome (%)	4.3 (2.6–7.2)
Risk categories according to UKPDS Risk Engine – CAD outcome	
Low risk	78 (91.8)
Intermediate risk	5 (5.9)
High risk	2 (2.4)
UKPDS Risk Engine – Fatal CAD outcome (%)	2.7 (1.2–5.3)
Risk categories according to UKPDS Risk Engine – Fatal CAD outcome	
Low risk	80 (94.1)
Intermediate risk	5 (5.9)
High risk	0 (0)
UKPDS Risk Engine – Stroke outcome (%)	3.7 (1.9–7.9)

FRS = Framingham Risk Score; UKPDS = United Kingdom Prospective Diabetes Study; CAD = coronary artery disease.

Quantitative data expressed as median and interquartile range in parentheses. Categorical data expressed as absolute count and percentages in parentheses.

Risk classification and vascular age	Diabetic women (n = 88)
Risk categories according to UKPDS Risk Engine – Stroke outcome	
Low risk	71 (83.5)
Intermediate risk	11 (12.9)
High risk	3 (3.5)
UKPDS Risk Engine – Fatal stroke outcome (%)	0.4 (0.2–1.2)
Risk categories according to UKPDS Risk Engine – Fatal stroke outcome	
Low risk	85 (100)
Intermediate risk	0 (0)
High risk	0 (0)
FRS = Framingham Risk Score; UKPDS = United Kingdom Prospective Diabetes Study; CAD = coronary artery disease.	
Quantitative data expressed as median and interquartile range in parentheses. Categorical data expressed as absolute count and percentages in parentheses.	

Table 3 shows the correlations between CVR scores and vascular biomarkers. Among the biomarkers, VCAM-1 was the one that showed the best correlation with the assessed risk scores, although the correlation was weak.

Table 3
Correlation between cardiovascular risk scores and different biomarkers in diabetic women, Fortaleza-Ceará-Brazil, 2021. n = 88.

	FGF-23 (pg/mL)		Sdc-1 (ng/mL)		VCAM-1 (ng/mL)		Ang-2 (ng/mL)	
	Rho	p	Rho	p	Rho	p	Rho	p
Laboratory FRS (%)	0.018	0.895	0.064	0.553	0.213	0.049	0.104	0.598
Vascular age according to laboratory FRS	-0.011	0.938	0.039	0.722	0.283	0.008	0.225	0.250
Non-laboratory FRS (%)	-0.028	0.842	-0.017	0.875	0.209	0.054	0.051	0.797
Vascular age according to non-laboratory FRS	-0.119	0.389	0.013	0.908	0.258	0.016	0.203	0.300
UKPDS Risk Engine - CAD outcome	0.053	0.702	0.104	0.345	0.260	0.018	0.148	0.462
UKPDS Risk Engine - Fatal CAD outcome (%)	0.039	0.778	0.105	0.343	0.272	0.013	0.161	0.422
UKPDS Risk Engine - Stroke outcome (%)	-0.108	0.439	0.136	0.216	0.234	0.033	0.088	0.664
UKPDS Risk Engine – Fatal Stroke outcome (%)	-0.043	0.757	0.109	0.325	0.223	0.043	0.135	0.501
FGF-23 = Fibroblast growth factor-23; Sdc-1 = Syndecan-1; VCAM-1 = Vascular cell adhesion molecule-1; Ang-2 = Angiopoietin-2; FRS = Framingham Risk Score; UKPDS = United Kingdom Prospective Diabetes Study; CAD = coronary artery disease.								

When performing a more detailed analysis (Table 4) and when correlating the values of VCAM-1 with the risk stratification categories, we observed that, for the laboratory FRS, there was a statistically significant association between the CVR categories and VCAM-1 ($p = 0.024$), so that individuals with a higher CVR also had higher values of serum VCAM-1. For the other assessed scores, there was no statistical significance in the observed associations.

Table 4

VCAM-1 levels according to cardiovascular risk degrees according to different scores in diabetic women, Fortaleza-Ceará-Brazil, 2021. n = 88.

Diabetic women			
VCAM-1 (ng/mL)			
	n	Mean ± SD	p*
Laboratory FRS			0.024
Low risk	9	914 ± 315.5	
Intermediate risk	15	1128.3 ± 272	
High risk	64	1163.4 ± 234.9	
Non-laboratory FRS			0.261
Low risk	5	1001.8 ± 347.2	
Intermediate risk	11	1055 ± 312.3	
High risk	72	1152.9 ± 241.8	
UKPDS Risk Engine – CAD outcome			0.105
Low risk	78	1116.3 ± 259	
Intermediate risk	5	1368.4 ± 118.2	
High risk	2	1162.8 ± 296.5	
UKPDS Risk Engine - Fatal CAD outcome			0.318
Low risk	80	1125.4 ± 261.8	
Intermediate risk	5	1245.1 ± 180.5	
High risk	0	-	
UKPDS Risk Engine – Stroke outcome			0.698
Low risk	71	1122 ± 263	
Intermediate risk	11	1186.4 ± 248.2	
High risk	3	1200.8 ± 219.7	
VCAM-1 = Vascular cell adhesion molecule-1; FRS = Framingham risk score; UKPDS = United Kingdom Prospective Diabetes Study; CAD = coronary artery disease.			
* ANOVA-test with Tukey's posttest was used for all comparisons. The significance occurred among "low risk" versus "high risk" group.			

Figure 1 shows that, when the low-risk group is compared with the high-risk group according to the Laboratory FRS, those with the highest CVR have, in fact, higher values of VCAM-1 ($p < 0.05$).

Discussion

The present study is the first to attempt to establish a relationship between biomarkers and the CVR predicted by different risk tools in diabetic women using the scores and biomarkers evaluated by us. We demonstrated that the majority of these women had high CVR according to the scores derived from the Framingham cohort and low CVR according to the UKPDS score. Moreover, serum levels of VCAM-1 were directly associated with a high CVR estimated by the laboratory FRS, even in patients without previous cardiovascular disease.

Cardiovascular risk scores are instruments that are widely used in clinical practice to predict the incidence of future CVD. A meta-analysis reviewed studies that investigated the predictive performance of widely used scores such as Framingham Wilson 1998, Framingham Adult Treatment Panel III 2002 and the Pooled Cohort Equations 2013, in the general population. It was observed that the performance of all three models was consistently better in women than in men, which makes us think that the reproducibility degree in the implementation of these scores in the population of our study may have been reliable, as our sample was consisted by women only. Moreover, great differences were observed regarding performance between the validations of the same model in different populations,^[25] which shows the need to create or adapt a score validated for specific populations, such as the Brazilian one. However, all of these models overestimated the risk of CVD development, especially in high-risk populations,^[25] which corroborates the idea that it is necessary to correlate other factors, by adding other criteria to the scores, as in the case with biomarkers, to increase their degree of reliability.

In a study designed to assess the risk of CVD in adults with T2DM and metabolic syndrome and to compare the Framingham risk scores and the UKPDS, Korean adults were evaluated and these scores were compared in this population. No significant differences were observed between the two scores and it was shown that approximately a quarter of the adults had a high risk of CVD ($> 20\%$).^[26] In another study that compared several scores (UKPDS, FRS, Atherosclerotic Cardiovascular Disease - ASCVD, and Joint British Societies for the prevention of Cardiovascular Disease - JBS3) in South Asian and Caucasian populations with T2DM, a high prevalence of subclinical CAD was verified in high-risk patients through all scores, with JBS3 showing the highest correlation, despite the higher rate of low-risk classification in the studied population according to all scores.^[27] Another study that evaluated the performance of the Framingham and UKPDS scores showed that the UKPDS was the score with the best 10-year CVD risk prediction in patients with T2DM, when compared to the Framingham equation.^[28]

As for the comparison of scores in the female population, the study by Cook et al.^[29] showed that Reynolds score was more effective than the Framingham score for risk stratification, but this result was not observed in diabetic women. In the present study, when the CVR was classified by the laboratory FRS and the non-laboratory FRS, it was observed that the majority of the population was classified as high

risk in both of them (72.7% and 81.8%, respectively). When using the UKPDS, both for CAD risk and for the risk of stroke (fatal or non-fatal), the highest percentage of the women was classified as low risk. With the conflicting results in the analysis of these scores, including the discrepancies found in the results of the present study, it will be necessary to improve the scores in an attempt to enhance their predictive power, so they can be used with greater reliability in the female population with T2DM.

Regarding the Framingham risk scores and the laboratory profile, our sample showed that most of the participants were classified as high risk, with the laboratory data showing high glycemic levels, with a mean FG of 8.53 ± 3.65 mmol/L and hbA1c of 65 ± 1 mmol/mol. It has been defined in the literature that goals not reached by diabetic patients imply a higher risk of unfavorable outcomes. This was also demonstrated in the study by Kim et al,^[26] in which the inadequate glycemic control was associated with a high risk of CVD. The mean level of hbA1c in the high-risk group (hbA1c = 69 mmol/mol) was higher than in the low-risk group (hbA1c = 56 mmol/mol), corroborating this association.

Because it is a disease that results in endothelial dysfunction, T2DM can predispose to cardiovascular complications and thus, some biomarker measurements can be considered for CVD assessment and their alterations may constitute an increased risk of future complications.^[30] A study evaluated the relationship of T2DM and glycemic control with circulating cell adhesion molecules (CAMs) and showed that VCAM-1 was significantly higher in patients with T2DM than in healthy individuals.^[31] Another study evaluated 23 biomarkers of different pathophysiological pathways to improve the risk prediction of cardiovascular events in patients with T2DM, in addition to the traditional risk factors. It was observed that markers such as the N-terminal fragment of B-type natriuretic peptide (NT-proBNP), osteopontin, metalloproteinase-3 of the extracellular matrix and their combination improved the prediction of the cardiovascular event risk in this population.^[32] These studies point to the fact that the use of serum biomarkers in predicting CVD can be an objective tool in the evaluation of these patients, considering that the traditional risk scores do not show uniform results.

In a prospective study with diabetic patients without manifest macrovascular disease followed for 5 years, some biomarkers (intercellular adhesion molecule-1 - ICAM-1, VCAM-1, P-selectin and E-selectin) were measured in beginning and during follow-up. Baseline ICAM-1 was found to be significantly higher in patients who developed macrovascular disease than in the ones who did not.^[33] Kocijancic et al.^[34] demonstrated, in patients with chronic dialysis kidney disease, that the concentrations of ICAM-1 and VCAM-1 had a strong independent correlation with carotid intima-media thickness (IMT) and that ICAM-1 and omentin-1 were strong predictors of cardiovascular death and progression of IMT. The present study does not correlate biomarkers with the incidence of CVD but assesses the relationship between such biomarkers and the event risk prediction assessed by scores. This association can generate hypotheses that can be confirmed with subsequent studies of more robust incidence analysis.

Another study assessed the baseline activity of a disintegrin and metalloproteinase domain 17 (ADAM17), by measuring the levels of the four main circulating substrates (VCAM-1, ICAM-1, Interleukin-6 and the soluble tumor necrosis factor-receptor 1) and correlated them with a second major

cardiovascular event (cardiovascular death, peripheral arterial surgery and non-fatal acute myocardial infarction and stroke). A score was created based on the substrates of ADAM17, correlating it to the Framingham Recurring-Coronary-Heart-Disease-Score and a significant increase was observed in the score prediction accuracy, with an important improvement of the correct classification in 10% of events and 41% of non-events.^[35]

Few studies in the literature have attempted to correlate the use of biomarkers, especially those analyzed in the present study, and the CVR predicted by scores in the diabetic population.^[36–41] One of these studies evaluated the association between a set of biomarkers that analyze different metabolic pathways, such as Asymmetric dimethylarginine (ADMA), Soluble endothelin-1 (ET-1), Placental growth factor-1 (PIGF-1) and NT-pro-BNP and the CVR predicted by the UKPDS and Action in Diabetes and Vascular disease (ADVANCE) scores in diabetic patients. This study showed that ADMA and PIGF-1 were not associated with CVR stratification with any of the scores, while ET-1 was associated with the risk of stroke by the UKPDS and NT-proBNP was associated with CVR predicted by both tools.^[39] Different from our research, these studies did not evaluate the female diabetic population, or the biomarkers analyzed by us.

In our study, it was observed that, of the assessed biomarkers, VCAM-1 is the one that showed the best correlation with the risk scores, although this correlation was weak, which may have been due to the small study sample. We also observed that higher levels of VCAM-1 were found in patients classified as having high CVR by the laboratory FRS. VCAM-1 is a protein that mediates the adhesion of lymphocytes, monocytes, eosinophils and basophils to the vascular endothelium and can play a role in atherosclerosis development. It is thought that one of the reasons why this can occur in patients with T2DM is the fact that their HDL-c has less anti-inflammatory capacity. This fact was demonstrated in the study carried out by Ebtehaj et al.,^[42] which showed that, in individuals with T2DM, the anti-inflammatory capacity of HDL-c was strongly impaired, with a greater increase in the expression of VCAM-1 messenger ribonucleic acid, which indicates less anti-inflammatory capacity.

Studies are contradictory when they try to correlate the CAMs, such as VCAM-1 and ICAM-1, with cardiovascular events in a population of individuals without previous CVD and it seems that the same occurs with studies in the subpopulation of diabetic patients, as previously reported.^[30] According to Derosa et al.,^[43] pro-atherogenic CAMs (ICAM-1, VCAM-1 and E-selectin) are elevated in T2DM and their increased expression and release would contribute to accelerated atherogenesis in diabetic patients. Also correlating VCAM-1 and diabetes, a study group evaluated patients with a recent diagnosis of DM and measured several markers in serum, including VCAM-1, and correlated them with cardiovascular function (represented by the measurement of flow-mediated vasodilation, intima-media thickness and arterial stiffness). In this study, a positive relationship was found between IMT and between arterial stiffness with VCAM-1.^[44] This demonstrates the presence of an increase in the expression of VCAM-1 in diabetic patients with atherosclerotic disease evidenced by these methods.

The studies show that CAMs such as VCAM-1 are established markers of endothelial dysfunction, corroborating the data from our study, which showed higher levels of this biomarker in patients at higher cardiovascular risk. It will be necessary to monitor our studied sample to document future cardiovascular events and verify the discriminatory power of both risk scores and the measurement of biomarkers, especially VCAM-1.

Study limitations

The main limitation of this study is related to its cross-sectional design, not being possible to establish an association between the assessed biomarkers and cardiovascular outcomes, which would be able to assess the discriminating power of both the biomarkers and the applied risk scores in this population. Moreover, other important points are the fact that no risk scores have been validated for the Brazilian population and the fact that the present study was carried out in a single center. These factors may also have caused bias in the present study. However, the data described by us in this study are important because they correlate biomarkers, especially VCAM-1, with CVR predicted by scores widely used worldwide in clinical practice for the risk stratification in the female population and constitute an important topic to be further studied in future research.

Conclusion

We evaluated women with T2DM without previous CVD and found that most of them had high CVR according to the Framingham scores and low risk according to the score that is more specific for diabetics (UKPDS). Moreover, we found that the VCAM-1 biomarker was directly associated with the CVR estimated by the laboratory FRS, which may indicate the presence of endothelial injury and subclinical atherosclerosis. The findings described here may point to the need to find biomarkers, such as VCAM-1, that can further refine the risk analysis by cardiovascular risk scores, tools that are widely used in CVR stratification in the female population with and without diabetes in the most diverse levels of health care.

Abbreviations

DM = Diabetes Mellitus

CVD = Cardiovascular disease

T2DM= Type 2 Diabetes Mellitus

CAD = Coronary artery disease

CVR = Cardiovascular risk

FRS = Framingham risk score

UKPDS = United Kingdom Prospective Diabetes Study

VCAM-1 = Vascular cell adhesion molecule-1

FGF-23 = Fibroblast growth factor-23

Sdc-1 = Syndecan-1

Ang-2 = Angiopoietin-2

HF = Heart failure

POAD = Peripheral obstructive arterial disease

PHC = Primary health care

EKG = Electrocardiogram

SAH = Systemic arterial hypertension

BMI = Body mass index

BP = Blood pressure

SBP = Systolic blood pressure

DBP = Diastolic blood pressure

EDTA = Ethylenediaminetetraacetic acid

HbA1c = Glycated hemoglobin A1c

FG = Fasting glycemia

TC = Total cholesterol

HDL-c = High-density lipoprotein cholesterol

TG = Triglycerides

LDL-c = Low-density lipoprotein cholesterol

ELISA = Enzyme-Linked Immunosorbent Assay

ANOVA = Analysis of variance

SPSS = Statistical Package for Social Sciences

ASCVD = Atherosclerotic Cardiovascular Disease

JBS3 = Joint British Societies for the prevention of Cardiovascular Disease

CAMs = Cell adhesion molecules

NT-pro-BNP = N-Terminal brain natriuretic pro-peptide

IMT = Carotid intima-media thickness

ICAM-1 = Intercellular adhesion molecule-1

ADMA= Asymmetric di-methylarginine

ET-1= Soluble endothelin-1

PIGF-1= Placental growth factor – 1

ADVANCE= Action in Diabetes and Vascular Disease

ADAM-17 = Metalloproteinase domain 17

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The present study is in full agreement with Resolution n. 466/2012 of the National Health Council and obtained approval from the Research Ethics Committee of Universidade de Fortaleza (Opinion n. 1.843.144 / 2016). Each study participant provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

Not applicable.

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Authors' contributions

AKMN and GBSJ contributed with study concept and design, data collection, analysis and interpretation, as well as the writing of the manuscript. GCM contributed with data collection, statistical analysis and review of the manuscript. DOCL, AMCM, APPL, RVBMC, JHSJ and JMOL contributed with data collection and writing of the manuscript. RPS contributed with data interpretation, review of the manuscript and final approval of the version to be published. All authors read and approved the final manuscript.

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Figures

Figure 1

Levels of VCAM-1 in relation to cardiovascular risk degrees according to the Laboratory Framingham risk score in diabetic women. VCAM-1 = Vascular cell adhesion molecule-1. * $p < 0.05$ for "high risk" vs. "low risk", using the ANOVA test with Tukey's post-test.