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RANTES as a novel biomarker for atherogenic dyslipidemia and metabolic disturbances in patients with Type 2 Diabetes

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Abstract

RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted) is a chemokine engaged in the pathophysiology of diabetes type 2 (T2DM), related cardiovascular complications, dyslipidemia and hypertension that are major modifiable risk factors of T2DM. VEGF (Vascular endothelial growth factor) plays a role in diabetic vascular complications. Atherogenic dyslipidemia (AD) is a lipid aberration defined as the incidence of raised triglycerides (TG) and dimished high-density lipoprotein cholesterol (HDL-C) and associated with residual cardiovascular risk. However, the association between chemokine and type 2 diabetes (T2DM) and metabolic disturbances is still unclear, and few data are available. The aim of this study was to estimate serum RANTES, serum lipid composition, VEGF, and metabolic syndrome (MS) in patients with T2DM and elucidate their relationship in a sample of Egyptian premenopausal women. Serum level of RANTES, VEGF, lipids and body composition were assessed in 100 premenopausal women with T2DM (mean age 35 years old) and 100 healthy controls. Significant increase in serum RANTES level and VEGF was observed in T2DM compared to the control group. Positive correlations between elevated RANETS and increased values of VEGF, body mass index (BMI), waist circumference (WC), fat mass, LDL-C, TG, total cholesterol and presence of MS were observed in T2DM patients. Elevated RANTES concentration is associated with AD risk, abnormal metabolic components and VEGF levels. This study pinpoints the importance of RANTES as a novel biomarker for dyslipidemia and metabolic disturbance in T2DM patients.

Keywords: RANTES, VEGF, type 2 diabetes, Atherogenic dyslipidemia, BMI

1. Introduction

Patients with diabetes mellitus are at risk of developing hyperlipidemia, hypertension, and obesity [1][2][3][4]. Diabetes mellitus leads to the development of premature atherosclerosis [5]. The premature atherosclerosis in T2DM and related cardiovascular complications are major public health challenges worldwide[6]. The leading cause of death among patients with T2DM is coronary artery disease (CAD) with two- to four-fold increased risk[7]. Hypertension and dyslipidemia are chief amendable risk factors for T2DM and correlated CAD, that account for more than 87% of incapacity in middle - and low -income countries[8]. Moreover, prediabetes (an intermediate metabolic state between T2DM and normoglycemia) have also been found to be accompanied with an augmented risk for cardiovascular disease [9][10][11]. Many researches revealed the association of type 2 diabetes and acute-phase proteins or proinflammatory cytokines, much less is known about the probable role of chemokines in the development of type II diabetes[12]. Chemokines are a superfamily of secreted low–molecular weight proteins with decisive roles in pathophysiological and physiological processes such as angiogenesis, hematopoiesis, atherosclerosis, inflammation and infectious, autoimmune or allergic diseases[13] . Previous studies lack the data on the role of chemokines in type II diabetes among

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Egyptian patients that led to the current investigation. Metabolic syndrome (MS) is defined by a cluster of risk factors, including hypertension, dyslipidemia, obesity and insulin resistance. They increase the risk of developing cardiovascular disease (CVD) and diabetes when occurring together [14]. Previous researches have shown that the defined risk factors of MS cannot explain all CVD events observed in these subjects. The study aimed to assess RANTES in T2DM patients and detect to what extent it correlates with serum lipid abnormalities and metabolic disturbances.

2. Subjects and Methods

Diagnosis of Patients with T2DM

The study group composed of 100 premenopausal T2DM women and 100 control normal healthy women matched age (aged 29–38). This study was conducted during February 2019 and March 2020 in the National Research Centre, Egypt; Medical Research Centre of Excellence. The study was approved by the Ethical Committee form of National Research Centre, Egypt (number = 16361), in accordance with the World Medical Association's Declaration of Helsinki.

3. Methods

All the T2DM patients were diagnosed at the National Research Centre (NRC), in accordance with the World Health Organization International Society of Diabetes Guidelines [15]. Blood samples were collected after an overnight fasting (minimum 12 h).

4. Statistical analysis

All statistical analyses were performed using SPSS16.0 for Windows (SPSS Inc). The Kolmogorov–Smirnov test of normality was used to verify whether the distribution of variables followed a Gaussian pattern. Normally distributed data in groups were expressed as means ± SDs. All parametric were analyzed by Student's t-test. All non-parametric data were analyzed by Chi-square test. Pearson correlation was used to evaluate the correlation between dependent variables. P-value was set at <0.05 for statistical significance.

5. Biochemical Analyses

5.1. Serum lipids

Fasting serum lipids (total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and plasma glucose were evaluated by enzymatic colorimetric methods [16]. Low density lipoprotein cholesterol (LDL-C) was calculated via the equation (LDL-C= Total cholesterol – Triglycerides/5+HDL-C). Participants were considered with dyslipidemia if they had elevations of LDL-C, triglycerides, non-HDL-C, and decreases in plasma HDL-C [17].

5.2. Quantification of RANTES

Serum RANTES was evaluated by sandwich enzyme linked immunosorbent assay (R&D system, Wiesbaden, Germany).

5.3. Quantification of VEGF

Serum VEGF was measured by the ELISA (enzyme-linked immunosorbent assay) method using an ELX-800 system (RayBiotech, Norcross, GA, USA)

Anthropometric parameters, blood pressure measurements and blood collection

Body weight and height were measured unclothed and without shoes in the morning. Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m2). Waist and hip circumferences were measured with paper tape horizontally at the umbilicus in the standing position after normal expiration. Body fat % was assessed by Tanita Body Composition Analyzer (SC-330). Blood pressure was read from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo).

MS was defined according to NCEP ATP III guidelines [18]. MS was diagnosed if at least three of the following five features were present: (i) waist circumference >88 cm, (ii) serum triglyceride ≥1.70 mmol/l, (iii) serum high-density lipoprotein (HDL)-cholesterol <1.30 mmol/l or the use of lipid lowering medication, (iv) blood pressure ≥130/85 mm Hg or the use of anti-hypertensive medication, (v) fasting plasma glucose ≥6.11 mmol/l.

6. Results and Discussion

In total, 200 subjects were enrolled in the study (100 patients with T2DM and 100 age-matched normal female controls. RANTES values were measured. The clinical features of control subjects and T2DM patients are shown in Table 1. T2DM patients had significant higher levels of RANTES, VEGF, BMI, WC, WHR, body fat %, the systolic and diastolic blood pressure value than controls.

Table 2 shows the correlation between the levels of RANTES, clinical characteristics and serum lipids. Significant positive correlations were observed between elevated levels of RANTES and VEGF, obesity measures, systolic and diastolic blood pressure levels, abnormal serum lipids and presence of MS. Table 3 shows risk factors associated with different types of dyslipidemia in T2DM patients. Results showed the highest incidence of central obesity, hypertension, MS%, elevated levels of RANTES in diabetic patients with AD followed by hypertriglyceridemia then low-HDL-C.

Coronary artery disease is associated with high serum concentrations of RANTES [19][3][20]. Chemokines are stimulated by inflammatory cytokines, pathogenic stimuli and growth factors [21][22]. They mainly assist in the regulation of immune cells motility to lymphoid organs during inflammation and in normal hematopoiesis. Chemokines facilitate the recruitment and migration of the activated leukocytes into the synovium at the areas of inflammation in rheumatoid arthritis, this triggers tissue impairment and plays a central role in inflammation. Several reports have confirmed the inflammatory role of TNF-alpha, IL-6, and IL-10[23]. Few studies have observed the associations between nephropathy in type 2 DM and chemokine gene polymorphisms[24][25]. Previous investigators, stated that the major RANTES receptor (CCR5 promoter 59029A genotype) and the RANTES promoter (28G genotype) linked to diabetic nephropathy [24]. Type 2 DM constitutes about 90% of diabetic individuals, and within this group, 10% represents the monogenic forms such as maturity onset diabetes of the young (MODY) or late-onset autoimmune diabetes of the adult, which is actually late-onset type 1 diabetes and mitochondrial diabetes. Furthermore, 20-30% of type II DM patients may develop a significant degree of renal damage while remaining normoalbuminuric [26]. Moreover, a significant increase in serum RANTES level was detected in the active Egyptian rheumatoid arthritis patients, and controls and positively correlated with the disease activity parameters [27]. Other authors concluded that the prevalence of AD varies approximately from 5.7% - 9.9% in patients with at least one cardiovascular risk factor [28]. The major traditional risk factors for cardiovascular disease (CVD) include both non-modifiable factors (e.g., familial predisposition, age, or gender) and modifiable factors such as physical inactivity, obesity, smoking, hypertension and dyslipidemia) [29,30][31].

Our current research identified considerable differences between T2DM and controls in RANTES, VEGF, obesity indices, blood pressure levels and serum lipids[32][4]. The present study is in consistent with the supposition that CCL2 and RANTES are rather biomarkers of the presence of atherosclerotic lesions, than being markers of severity[33].

Previous studies, have established a pivotal role of VEGF in the progression and incidence of diabetic retinopathy (DR) [34]. Moreover, in the filtration of macromolecules in the kidney, neo-angiogenesis in the end organs in case of hypoxia and regulating blood-retinal barrier [35]. The current study reveals that VEGF can be an important factor prompting complications[36]. Furthermore, the characteristics of the study contributors based on categories of different types of dyslipidemia showed strong association with AD risk[22][37]. The role of serum triglycerides levels as an independent risk factor for cardiovascular disease remains provocative, some researches revealed no significant association with CHD[38]. Some Researches, including 262,525 participants shown highly and moderate significant correlations between TG levels and CHD, however the impact of TG decreased after adjusting other factors [39]. On the other hand, meta-analysis of 68 long-term prospective studies comprising 302,430 people revealed no significant association between CVD and TG [40]. Besides, the current results displayed significant correlation between the presence of MS and different types of dyslipidemia in T2DM patients. The combination of elevated triglycerides and reduced HDL cholesterol more strongly associated with an increased risk of T2DM [41][42].

In conclusion our study identifies the role of RANTES in the pathophysiological conditions of T2DM and could be potentially used as a diagnostic biomarker for dyslipidemia and metabolic disturbances. Moreover, VEGF might be a biomarker for the disease progression.

Table 1 Clinical and lipid profile characteristics of T2DM and controls

	T2DM	Controls	P-value
	Mean ± SD	Mean ± SD	
Age	35.5 ± 4.5	36.7 ±3.7	0.06
BMI (kg/m^2)	34.42±5.62	23.46±2.56	<0.001
WC	100.71±11.830	78.44±9.787	<0.001
WHR	.8420±.05853	.7892±.07971	<0.001
Fat %	36.10±10.88	16.20±5.64	<0.001
Systole (mmHg)	109.50±16.041	97.88±12.185	<0.001
Diastole (mmHg)	72.09±10.23	65.91±6.18	<0.001
RANTS (ng/mL)	27.59±8.69	14.26±1.98	<0.001
VEGF(pg/mL)	150.55 (125–259) *	52.54 (20–117)	<0.001
Total cholesterol (mg/dL)	201.92±45.016	174.81±43.865	<0.001
Triglycerides(mg/dl)	111.40±48.16	87.77±52.43	<0.001
HDL-C(mg/dl)	51.87±13.966	51.05±13.053	<0.001
LDL-C(mg/dl)	127.56±41.078	110.10±43.834	<0.001

*Values are medians with interquartile ranges in parentheses. BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol*p <0.05, ** p<.001

Table 2 Correlation coefficients of RANTES levels with lipid parameters, VEGF and clinical features.

	RANTES	
Variables	r	p-Value
BMI (kg/m^2)	0.653**	0.001
WC	0.698**	0.001
WHR	0.638**	0.006
Fat %	0.694**	0.001
Systole (mmHg)	0.667**	0.001
Diastole (mmHg)	0.640**	0.001
Total cholesterol (mg/dL)	0.651**	0.001
Triglycerides(mg/dl)	0.448*	.032
HDL-C(mg/dl)	-0.099	.652
LDL-C(mg/dl)	0.435*	0.001
VEGF	0.633**	0.001
MS %	0.664**	0.001

BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol*p <0.05, ** p<.001

Table 3 Risk factors for different types of dyslipidemia in T2DM patients

Criteria	Atherogenic dyslipidemia	Hypertriglyceridemia	Low-HDL-C
	OR (95% CI), p value	OR (95% CI), p value	OR (95% CI) p value
MS (%)	2.11 (1.14–2.30), 0.001	1.12 (1.04–1.34), 0.01	1.13 (1.94–2.34), 0.01
Central Obesity (%)	2.61 (1.90–2.14), 0.001	2.45 (1.84–2.36), 0.001	2.10 (1.90–2.14), 0.01
Elevated RANTES	2.63 (1.94–2.94), 0.001	2.23 (1.78–2.44), 0.001	1.34 (1.93–2.74), 0.01
Hypertension,	2.67 (1.65–2.99), 0.001	2.33 (1.94–2.34), 0.001	1.53 (1.94–2.34), 0.01

7. Conclusions

Elevated RANTES concentration is associated with AD risk, abnormal metabolic components and VEGF levels. This study pinpoints the importance of RANTES as a novel biomarker for dyslipidemia and metabolic disturbance in T2DM patients.

8. Conflicts of interest

There are no conflicts to declare

9. Formatting of funding sources

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