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ORIGINAL ARTICLE

Plasma Alpha-defensin Levels in Smoker and Non-smoker Male Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: *Alpha defensins were known to have broad spectrum potent antimicrobial acdtivities.* ***Objectives:*** *To evaluate plasma alpha-defensin 1- 3 levels in smoker and non-smoker male patients with type 2 diabetes mellitus (T2DM).* ***Methodology:*** *The subjects included 50 consecutive current smoker men with T2DM attending the outpatient clinic, Zagazig University Hospitals (Egypt), matched for age and diabetes duration with 50 consecutive non-smoker men with T2DM and 100 healthy men (50 smokers; 50 non-smokers). Associations between α-defensin plasma levels and characteristics of participants were evaluated.* ***Results:*** *Plasma levels of α-defensins were significantly higher in smokers compared with non-smokers in diabetic and non-diabetic subjects (P<0.001 for all comparisons). Ten characteristics of participants that significantly correlated with α-defensins were further analyzed using stepwise multiple linear regression. The final model included four variables explaining 47.0% of the variation in α-defensin level. Presence of diabetes alone accounted for 32.6% of the variation. However, 40.1% of the variation in α-defensins could be explained by including current smoking.* ***Conclusion:*** *Plasma α-defensin 1-3 is elevated in patients with T2DM, and current smoking is combined with a further elevation. Given the known association between α-defensin and serious complications of diabetes, results of the current study should have important clinical implications in the management of patients with T2DM who smoke or think about smoking.*

INTRODUCTION

Alpha-defensins, or human neutrophil peptides (HNP), constitute a group of small (3 - 5 kDa) cationic cysteine-rich peptides. Most of these peptides are in the form of α-defensin-1, -2, and -3. These are very similar in their amino-acid sequence, but they only differ in the first N-terminal amino-acid [1, 2]. They are all synthesized constitutively and stored mainly in the azurophilic granules of neutrophils. Alpha-defensins were initially identified on account of their broad-spectrum potent antimicrobial activities [3]. In addition, they are essential elements of innate immunity, and may augment the production of pro-inflammatory cytokines [4]. Studies have demonstrated accumulation of α-defensins in the intima of atherosclerotic vessels [5,6]. Also, a significant correlation has been reported between α-defensin deposition in the skin biopsies and coronary artery disease[7]. Thus, α-defensins may be considered a link between inflammation and atherosclerosis.

While it has long been acknowledged that people with diabetes are at particular risk of developing atherosclerotic cardiovascular disease (CVD) [8], only few studies have assessed α-defensins in such people. In one study, serum α-defensin concentrations were found to be elevated in patients with type 1 diabetes mellitus (T1DM) with nephropathy in comparison to patients with either normo- or microalbuminuria [9]. Also, increased plasma levels of α-defensins have been reported in patients with T1DM with CVD [10]. It is rather surprising that although type 2 diabetes mellitus (T2DM) is commonly regarded as an inflammatory disease [11, 12], studies addressing α-defensins in patients with T2DM are scarce.

Tobacco smoking is another powerful risk factor for atherosclerotic vascular disease [13, 14]. Tobacco smoking may also be an independent risk factor for T2DM [15]. Although roles of α-defensin in smoking-related inflammation have been addressed in conditions such as chronic obstructive pulmonary disease (COPD) [16, 17], the combined effect of tobacco smoking and T2DM, whether synergistic or antagonistic, on blood levels of α-defensin has not yet been elucidated despite possible important theoretical and practical risk management implications. One study of apparently healthy Caucasian men suggested that smoking reduced rather than increased plasma levels of α-defensin [18].

Of note, the majority of available studies have come from high-income countries although tobacco use is decreasing in these countries whereas it is increasing in many low- and middle-income countries [19].

In this study, we aimed to evaluate the effect of current tobacco smoking on plasma α-defensins 1-3 in Egyptian middle-aged men with T2DM in comparison to healthy control.

METHODOLOGY

Patients:

The study sample consisted of 50 consecutive current smoker men with T2DM attending the outpatient Diabetes Clinic of Zagazig University Hospitals, Zagazig, Egypt, matched for age and diabetes duration with 50 consecutive non-smoker men with T2DM attending the same Outpatient Clinic during the same study period from November 2016 to October 2017. All included patients were diagnosed according to the American Diabetes Association [20] with a duration of >6 months and in the age range of 45 to 69 years. A control group of 100 healthy men (50 current smokers; 50 non-smokers), matched for age and body weight, was recruited from unrelated hospital visitors and staff. Current smoking was defined as consumption of one or more cigarettes per day within at least the last six months. Non-smokers included both lifelong never-smokers and former smokers with a five pack year history of smoking.

Before patient's inclusion into the study, full medical history, thorough physical examination, and routine laboratory and further investigations as indicated were carried out to exclude persons with evidence of any cardiovascular, hepatic, renal, neurological, or endocrine disease (apart from T2DM in the patient group), acute and/or chronic inflammatory disease, and malignancy. Patients treated with insulin were excluded. Subjects with history of substance abuse were also excluded. Patients who had a WBC count above 10,000/mm3 or a CRP concentration above 10 mg/l were considered to represent an acute or chronic infection or inflammation. These patients were excluded.

All participants signed informed consents before study enrollment. The Research Ethics Committee of the Faculty of Medicine, Zagazig University approved the study.

Methods:

Clinical examinations, anthropometric measurements, blood sample collections and laboratory procedures, including complete blood cell count (CBC), analysis of plasma lipids, glycemia and high-sensitivity C reactive protein (hs-CRP) measurements were performed along lines that we detailed elsewhere [21]. Alpha defensins 1–3 were measured in EDTA plasma samples using a commercially available Human HNP 1-3 enzyme-linked immunosorbent assay (ELISA) kit (Hycult Biotechnology, Uden, the Netherlands) following the manufacturer's instructions. Each sample was diluted 2000-fold and was assayed in duplicate. Sensitivity of the assay is .150µg/L. Values below the assay detection limit were assigned a value of .150µg/L. The intra- and inter-assay coefficients of variation (CVs) for analytic variation were respectively, 7.0% and 9.5%.

Statistical analysis:

Continuous data were presented as the arithmetic mean and standard deviation, unless specified otherwise. Data that were not normally distributed, as tested by the Kolmogorov-Smirnov test, were logarithmically transformed before being used in analysis but back-transformed for presentation as geometric means. Comparisons between groups were analyzed by one-way analysis of variance (ANOVA) and, if *P*-value was <0.05, post-hoc comparisons (Tukey HSD). Also, in the case of comparisons between two groups Student's t-test was performed. Correlations of log α-defensins with other continuous variables were assessed using Pearson's *r* test, and correlations with categorical variables were assessed using Kendall's tau-b (τ_b). Variables that showed significant correlations were further analyzed using multiple linear regression method to determine their influence (as independent variables) on the change of the logarithmically transformed α-defensin levels (as the dependent variable). Multicollinearity was evaluated by the estimation of the tolerance test and its reciprocal variance inflation factors (VIFs). The assumption of non-multicollinearity was not violated in this study. Dummy variables were used for presence of diabetes (yes=1; no=2) and current smoker (yes=1; no=2). All statistical analyses and sample size estimations were performed with IBM SPSS version 19.0.1 software (SPSS Inc, Chicago, Ill). Two-tailed P values <0.05 were considered statistically significant.

RESULTS

Table 1 shows demographic and clinical characteristics of the study participants. All groups were not significantly different regarding age, BMI and blood pressure. Also, smoker and non-smoker diabetic groups were not significantly different as regard diabetes duration and treatment used. In addition, as shown in table 2, the two diabetic groups were not different regarding glycated hemoglobin (HbA_{1c}), total cholesterol (TC) and triglycerides (TG). However, all counts of white blood cells (WBCs) and platelets, and plasma levels of both hs-CRP and α-defensin were increased in smokers compared with non-smokers in subjects with and without diabetes. Also, groups of patients with diabetes (smokers and non-smokers) had higher WBC count, platelet count, and hs-CRP and α-defensin plasma levels compared with groups of subjects without diabetes.

We reported significant correlations between α-defensins (logarithmically transformed DEFA1–3) and

10 variables including (1) presence of diabetes (τ_b = 288; P<0.001), (2) current smoker (τ_b =276; P<0.001), (3) BMI (r=0.180; P=.011), (4) SBP (r=0.149; P=0.035), (5) TC (r=0.170; P=0.016), (6) HbA_{1c} (r=0.233; P=0.001), (7) counts of total WBCs (r=0.179; P=0.011), (8) neutrophils (r=0.166 ; P=0.019) and (9) platelets (r=0.161; P=0.023) and (10) hs-CRP levels (r=0.186 ; P=0.008).

Table 1: Demographic and clinical characteristics of participants.

Subjects	With diabetes			Without diabetes			Significance
Current smoking status ($N^{o.}$)	CS (50)	N-S (50)	All (100)	CS (50)	N-S (50)	All (100)	
Age (years)	57.9 ±6.50	57.1 ±7.52	57.5 ±7.00	57.8 ±7.43	56.4 ±8.16	57.1 ±7.80	P^a=0.712
Diabetes duration (years)	8.4 ±3.14	8.1 ±2.93	8.2 ±3.03	--	--	--	P^b= 0.682
Medication use: N (%)							
Biguanides	11 (22)	13 (26)	24 (24)	--	--	--	P^b= 0.640
Sulphonylurea	17 (34)	21 (42)	38 (38)	--	--	--	P^b=0 .410
TZDs	7 (14)	9 (18)	16 (16)	--	--	--	P^b= 0.585
α-GI	16 (32)	19 (38)	35 (35)	--	--	--	P^b= 0.529
Statins	18 (36)	16 (32)	34 (34)	--	--	--	P^b= 0.673
Diuretics	12 (24)	17 (34)	29 (29)	--	--	--	P^b= 0.271
β-blockers	11 (30)	6 (22)	17 (17)	--	--	--	P^b= 0.183
ACEI	13(26)	8 (16)	21 (21)	--	--	--	P^b= 0.220
ARB	12 (24)	10 (20)	22 (22)	--	--	--	P^b=0 .629
CCB	12 (14)	11 (22)	23 (23)	--	--	--	P^b= 0.812
BI	577 ±500	-- --	-- --	524 ±486	-- --	-- --	P^c=0.594
BMI (kg/m^2)	30.1 ±2.74	30.5 ±3.25	30.3 ±3.00	29.5 ±3.22	30.8 ±6.78	30.2 ±5.32	P^a=0.469
SBP (mmHg)	138 ±12.9	135 ±13.9	137 ±13.4	135 ±12.5	132 ±15.2	134 ±13.9	P^a=0.191
DBP (mmHg)	82.7 ±9.21	80.3 ±9.60	81.5 ±9.44	81.4 ±7.40	80.7 ±6.99	81.0 ±7.17	P^a=0.491

CS, current smoker; N-S, non-smoker; TZDs, Thiazolidinediones; α-GI, Alpha-glucosidase inhibitors; ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin II receptor blockers; CCB, Calcium channel blockers; BI, Brinkman index (cigarettes/day × years); BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Values represent the arithmetic mean ± SD.

[a] ANOVA followed by post-hoc Tukey HSD test.

[b] Current smoker patients *versus* non-smoker patients.

[c] Current smokers with diabetes *versus* current smokers without diabetes.

Table 2: Laboratory characteristics of participants.

Subjects	With diabetes			Without diabetes			Significance
Current smoking status (N°.)	CS (50)	N-S (50)	All (100)	CS (50)	N-S (50)	All (100)	
HbA_{1c} (%)	7.40 ±.397	7.27 ±.587	7.3 ±.503	5.21 ±.539	5.04 ±.560	5.2 ±.536	P^a<0.001 P^b=0.207
TC (mmol/L)	5.20 ±.576	5.13 ±.610	5.2 ±.591	5.14 ±.650	5.03 ±.582	5.1 ±.548	P^a=0.516 P^b=0.545
TG (mmol/L)[d]	1.78 ±.649	1.63 ±.465	1.7 ±.566	1.67 ±.658	1.54 ±.430	1.6 ±.557	P^a=0.216 P^b=0.208
WBC: Total (/µl)	8413 ±1215	6879 ±1272	7646 ±1409	7188 ±1101	5524 ±1081	6356 ±1349	P^a<0.001
Neutro (/µl)	4546 ±818	3602 ±866	4074 ±963	4295 ±1103	3021 ±999.6	3658 ±1184	P^a<0.001
Lympho (/µl)	2818 ±932	2454 ±846	2636 ±904	2246 ±439	1982 ±485	2114 ±479	P^a<0.001
Mono (/µl)	585 ±83.1	538 ±68.9	561 ±79.5	348 ±90.4	271 ±92.3	442 ±92.2	P^a<0.001
Eos (/µl)	330 ±92.9	214 ±70.8	272 ±99.9	229 ±62.3	200 ±53.2	215 ±59.5	P^a<0.001
Platelets ($\times 10^4$/µl)	23.7 ±3.57	22.0 ±3.22	22.9 ±3.49	21.0 ±3.26	19.2 ±2.40	20.1 ±.2.99	P^a<.001
Hs-CRP (mg/L)[d]	2.29 ±1.30	1.31 ±1.14	1.8 ±1.32	1.13 ±1.06	.751 ±.723	.94 ±.924	P^a<.001
DEFA1–3 (µg/L)[d]	395 ±59.4	320 ±55.6	358 ±57.5	215 ±63.7	158 ±61.5	187 ±62.6	P^a<.001

CS, current smoker; N-S, non-smoker; TC, total cholesterol; TG, triglycerides; HbA_{1c}, glycated hemoglobin; Total WBC: total white blood cell count; Neutro: neutrophils; Lympho: lymphocytes; Mono: monocytes; Hs-CRP, high sensitivity C-reactive protein; DEFA1–3, α-defensins 1- 3.
Values represent the arithmetic mean (or geometric mean, as indicated) ± SD.
[a] ANOVA followed by post-hoc Tukey HSD test.
[b] Current smoker patients *versus* non-smoker patients.
[c] Current smokers with diabetes *versus* current smokers without diabetes.
[d] Geometric mean and geometric standard deviation.

To further analyze participants' data that significantly correlated with α-defensin levels, we performed stepwise multiple regression analysis. This yielded four models. The R^2 for these models were 0.326, 0.401, 0.447, and 0.470 respectively. As shown in table 3, the variables included in the final model were the presence of diabetes, current smoker, BMI and TC. Taken together, variables in the final model accounted for 47.0% of the variation in the levels of α-defensins. Presence of diabetes alone explained 32.6% of the variation but with the inclusion of current smoking 40.1% of the variation in α-defensins could be explained.

Table 3: Stepwise multiple regression analysis of variables significantly related to plasma α-defensin level in all participants: final model.

	B	SE	β (Beta)	t	P
(Constant)	16.532	0.047		9.843	<0.001
Presence of diabetes	3.849	0.095	0.435	7.144	<0.001
Current smoker	2.973	0.146	0.201	3.405	0.001
BMI	2.753	0.171	0.196	3.392	0.001
TC	0.967	0.569	0.104	2.619	0.025

BMI, Body mass index; TC, total cholesterol.

DISCUSSION

Results of the present study suggested that middle-aged Egyptian men with T2DM, as compared with normal control subjects, have increased levels of plasma α-defensins, and in contrast to some earlier studies, which claimed that smoking reduces α-defensin plasma levels [18], our findings indicate that α-defensin is further increased with smoking. In our regression analysis, current tobacco smoking emerged as one of the important determinants of plasma α-defensin levels in subjects with or without diabetes. The higher levels of α-defensins that we found in smokers with diabetes compared with non-smokers with diabetes and smokers or non-smokers without diabetes are presumably due, in part, to the combined effects of diabetes mellitus and smoking. We do not have comparable studies to interpret and support our findings, but there are reports of increased α-defensin levels in T1DM associated with nephropathy [9] or with CVD-related morbidity and mortality [10]. Together with such literature indicating that α-defensins are involved in the pathogenesis of serious diabetic angiopathies, our results may further support the clinical importance of discouraging smoking, particularly among people with diabetes.

However, because the study was cross-sectional, we could not verify the causality between each variable and the elevation of α-defensins. Moreover, the intra-individual variation over time in plasma α-defensins is unknown and remains to be explored. Another limitation is because our sample consisted of men only as we had difficulty in recruiting female smokers. Thus, we should be careful in generalizing our findings to women. Finally, smoking history was not detailed here. Type of cigarettes was not reported (e.g., whether filter-tipped, hard or soft pack, etc.). Impact of exposure to passive smoking was not considered. Smoking status was based on self-reports and was not verified with biochemical markers, such as breath carbon monoxide or serum cotinine assays. Further studies are needed to address these limitations.

CONCLUSION

Plasma α-defensin 1-3 is increased in middle-aged men with T2DM, and in contrast to some earlier studies, which claimed that smoking reduces α-defensin plasma levels [18], we found that current smoking is associated with further increase of plasma α-defensin 1-3. Given the known association between α-defensin and serious complications of diabetes[9,10], data provided by the present study should have important clinical implications in the management of patients with T2DM who smoke or think about smoking.

Conflict of interest statement

The authors declare that they have no competing interests.

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