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Inflammations mediators and circulating levels of matrix metalloproteinases: Biomarkers of diabetes in Tunisians metabolic syndrome patients

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ABSTRACT

Aims: This study investigates the relationships between matrix metalloproteinases, inflammations mediators and type 2 diabetes mellitus in Tunisians metabolic syndrome (Mets) patients.
Methods: The study has included 239 MetS patients and 247 controls. Mets was defined according to the NCEP–ATPIII report. Mets patients were also divided into two categories: 29 MetS non-diabetics and 210 MetS diabetics. Dysglycemia markers, matrix metalloproteinase-9 (MMP-9), Tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2), tumor necrosis factor α (TNF-α), C-reactive protein (CRP) levels and White Blood Cells (WBC) counts were determined in patients and controls.
Results: In our study, the level of inflammatory markers WBC, TNF-α and matrix metalloproteinases (MMP-8 and MMP-9) were significantly higher in diabetic patients with MetS, as compared with non-diabetic MetS patients. Inflammation mediators and MMP-9 were significantly associated with many clinical characteristics of MetS. The use of ROC "Receiver Operating Characteristic" analysis revealed the impact of TNF alpha on diabetes patients with MetS. In fact TNF alpha was found as a sensitive parameter in these patients with a sensitivity of 85%.
Conclusion: Inflammation, matrix metalloproteinases and dysglycemia markers are not expressed in isolation but rather concurrently and are continuously interacting with each other, in MetS and diabetics patients. These markers fit with an early stage of cardiovascular disease (CVD); and measuring them could improve the risk evaluation, an early diagnosis, and the prognosis of CVD.

1. Introduction

Metabolic syndrome (MetS) is a set of several factors including hyperglycemia, dyslipidemia, characterised by high triglycerides and low high density lipoprotein (HDL)-cholesterol, elevated blood pressure and visceral obesity. The worldwide prevalence of MetS is between 8% and 43% for men and 7% and 56% for women [1] and it keeps rising day by day. Patients with MetS have a 5-fold increased risk for type 2 diabetes mellitus (T2DM) and an approximately two-fold increased risk of developing cardiovascular disease (CVD) [2].

MetS was reported to be associated with a low-grade systemic inflammation as evidenced by the elevated levels of inflammatory markers, such as C-reactive protein (CRP), fibrinogen, and pro-inflammatory cytokines [3].

Furthermore, the identified association of MetS and its components with endothelial dysfunction could influence the synthesis and the activity of metalloproteinases (MMPs) who are a variety of endopeptidases, synthesized in several tissues, act principally in remodelling tissues by degrading the extracellular matrix (ECM) and are mainly regulated by tissue inhibitors of metalloproteinases (TIMPs) [4–6]. Gonçalves and collaborators showed that patients with MetS presented an increase in pro-MMP-9, MMP-8, and TIMP-1 circulating levels and have been associated with increased concentrations of the pro-inflammatory mediators. Thereby, MMPs may be involved in the cardiovascular

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modifications, and the pro-inflammatory state associated with MetS [7].

On the other side, several studies have shown that glucose can regulate the production, expression and activity of specific MMPs [8,9].

Thus, the aim of this study investigates the relationships between matrix metalloproteinases, inflammations mediators and type 2 diabetes mellitus in Tunisians metabolic syndrome (Mets) patients.

2. Materials and methods

2.1. Subjects and study protocol

For this case-control study, 239 MetS patients were recruited by the Endocrinology Department of La Rabta Hospital (Tunis, Tunisia) and 247 Control subjects were selected from a large population recruited from the Greater Tunis by the Biochemistry department The study participants matched in for age and gender. All participants were of ages between 35 and 70 years. Patients and controls have benefited of a clinical and biological examination. Diagnosis of MetS was confirmed by a senior experienced endocrinologist based on the NCEP–ATPIII report [10]. Those with three or more of the following criteria were considered as patients with MetS:

- Waist circumference (WC) > 102 cm in men and WC > 88 cm in women;
- HDL-cholesterol (HDL-c) < 40 mg/dL (<1.04 mmol/L) in men and serum HDL-c < 50 mg/dL (<1.29 mmol/L) in women;
- Triglycerides (TG) $\geqslant$ 150 mg/dL ($\geqslant$1.69 mmol/L) and/or taking hypolipidemic medication;
- Blood sugar $\geqslant$100 mg/dL ($\geqslant$5.6 mmol/L) and/or taking diabetes medications;
- Blood pressure $\geqslant$130/85 mmHg and/or taking hypertension medications;

Mets patients were also grouped into two categories: 29 MetS non-diabetics and 210 MetS diabetics and diabetes was confirmed, based on biochemical and clinical features according the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997 [11].

Patients with acute disease (renal, cardiovascular, hepatic, cancer...), as well as those with infectious or autoimmune diseases and pregnant women were excluded from the study. The control group consisted of healthy persons with no history of disease, randomly selected from the great Tunis population. This diagnosis was confirmed by a physical examination and laboratory testing. Weight and height were taken with the participants wearing light clothing and no shoes and body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Waist circumference was measured between the lower edge of the rib cage and the iliac crest at the end of normal expiration. Systolic blood pressure and diastolic were measured in the morning, in individuals at fasting and at rest for 15 min. Written informed consent was obtained from each participants and the design of the study was approved by Rabta Hospital ethics committee.

2.2. Biochemical measurements

Blood specimens were collected after overnight fasting and were centrifuged at 3000*g* for 15 min at 4 °C. Immediately after centrifugation, plasma samples for analysis of MMP-8, MMP-9, tissue inhibitor of matrix metalloproteases (TIMP-1, TIMP-2) and TNFα were frozen and stored at −80 °C, while all the other analyses were carried out within 4 h. Cholesterol and triglycerides were assayed by enzymatic colorimetric method (GPO-PAP method) using commercial kits (Roche, Mannheim, Germany). Serum HDL-cholesterol was measured after precipitation with magnesium chloride phosphotungstate and the determination of LDL-c level was calculated according to the Friedewald formula [12]. Glucose concentration was measured using the glucose-oxidase method. CRP was measured by immunoturbidimetric method. Glycated hemoglobin (%HbA1c) was measured by TINIA (turbidimetric inhibition immunoassay) (Roche, Mannheim, Germany). All biochemical analyses were performed by automatic chemistry analyzer (Hitachi 912, Roche) and the coefficient of variation of the different parameters ranged from 1% to 5%.

Plasma insulin levels were studied by immunoradiometric assay kit for the *in vitro* quantitative measurement of human insulin in plasma was assured by INS-IRMA kit from IBL (Immuno-Biological Laboratories – America). Homeostasis Model Assessment insulin resistance (HOMA-IR) (μU/mg) was calculated using the formula: (fasting insulin × fasting glucose)/22.5. MMP-8, MMP-9, Tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) and TNF-α levels were measured in citrate plasma by ELISA sandwich kits (R&D Systems, Lille, France). All samples from the same patients were measured in duplicated on the same ELISA kit. The inter-assay and intra-assay coefficients of variation were <6%. *White blood cell* (WBC) and lymphocytes was obtained by the determination of the complete blood count (CBC). The determination of hematological parameters was performed by hematology automated "ABX Micros 60".

2.3. Statistical analysis

Statistical analysis was performed using the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Normality of data was tested using numerical Shapiro-Wilk test and by Kolmogorov-Smirnov Test. Normally distributed variables were compared using parametric tests (Student *t*-test). In case of non-normal variable distribution, nonparametric tests were used (Mann-Whitney test). Findings are expressed as mean ± SD or median (minimum-maximum).

Categorical variables were shown as percentages. Study of bivariate correlations was performed using the Spearman rank correlation coefficient. Stepwise logistic regression analysis was applied to assess the prediction of diabetes (as dependent variable) by pro-inflammatory parameters ratios (as independent variables) in two models: model I, crude and model II, adjusted for components of MetS. In each model, results of regression are presented as odds ratios (OR) 95% confidence intervals (95% CI). Receiver-operating characteristic (ROC) plot based on the findings from binary logistic regression analyses. ROC plot of sensitivity (true positive rate, y-axis) versus 1 – specificity (false positive rate, x-axis). Statistical significance was accepted when $p \leqslant 0.05$.

3. Results

Clinical and anthropometric parameters of metabolic syndrome patients and controls are summarized in Table 1. MetS patients and controls have comparable gender distribution and age. BMI, Waist circumference, systolic blood pressure and diastolic blood pressure were significantly higher in Mets compared than normal subjects ($p < 0.001$).

Among biological parameters, fasting glucose, total cholesterol, triglycerides, insulin, HbA1c, TNF-α, MMP-8, MMP-9, TIMP-2, CRP and lymphocytes were significantly higher in MetS patients than controls. In contrast to HDL-cholesterol, which was significantly lower (Table 2).