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Biochemical investigation of gender-specific association between insulin resistance and inflammatory biomarkers in types 2 diabetic patients

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ABSTRACT

Inflammatory mediators play a key role in the pathogenesis of type 2 diabetes mellitus (T2DM) and development of insulin resistance (IR). The purpose of the present study was to investigate the gender-specific association between serum levels of inflammatory biomarkers and development of IR in type 2 diabetic patients. We recruited 90 study participants and collected their blood samples to measure the serum level of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), insulin and glucose. We found that the serum levels of IL-6 (< 0.0001), TNF-α (< 0.0001) and CRP (< 0.0001) in type 2 diabetic patients were significantly high as compared to control participants. Moreover, we also found that in female diabetic patients, a significant association was observed between the elevated levels of IL-6 (r = 0.8819, R^2 = 0.7778), TNF-α (r = 0.9833, R^2 = 0.9669) and CRP (r = 0.9529, R^2 = 0.9080) and increased risk of developing IR when compared with that of the serum levels of IL-6 (r = 0.7977, R^2 = 0.6364), TNF-α (r = 0.9445, R^2 = 0.8920) and CRP (r = 0.9051, R^2 = 0.8192) of male diabetic patients. Additionally, we also found that the Body mass index (BMI) of female diabetic patients was strongly correlated (r = 0.9694, R^2 = 0.9398) with the increased incidence of IR as compared to that of the BMI (r = 0.9188, R^2 = 0.8442) of male diabetic patients. The key findings of present study exhibit that gender differences significantly influence the association of inflammatory biomarkers with the development of IR in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex endocrine-related metabolic disorder in which the body cannot properly regulate the metabolism of carbohydrates, proteins and fats [1]. Several factors notably, hyperglycemia and dyslipidemia are considered key factors responsible for activation of various metabolic and molecular signaling pathways. These pathways may induce the generation of reactive oxygen species (ROS) and oxidative stress [2–4]. Once ROS and oxidative stress is produced, it can lead towards the activation of various cellular inflammatory responses that ultimately induce the activation of various pro-inflammatory mediators and/or cytokines notably, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP) and various other IL-1β-dependent pro-inflammatory cytokines and chemokines [3–7]. Once, inflammatory pathways are activated, they persuade the process of tissue-specific inflammation in several parts of the body. In pancreatic islets, inflammation impairs the normal secretion of insulin whereas, in peripheral tissues and adipocytes, inflammation induces the insulin resistance [5].

Impaired insulin secretion from the β-cells of pancreatic islets and insulin resistance (IR) in peripheral tissues are strongly associated with the induction of inflammatory responses in pancreatic islets and peripheral tissues, respectively [8,9]. Recently, it has been found in various experimental [10,11] and epidemiological studies [2,12–16] that augmented levels of various pro-inflammatory cytokines notably, IL-1β, IL-6 and TNF-α are strongly associated with IR and development of T2DM. These pro-inflammatory mediators are decisively involved to provoke the development of IR and in pathogenesis of T2DM, therefore the increased levels of pro-inflammatory mediators can be used as an independent biomarker for the prediction of development of IR and T2DM, but a very less data exists showing the association of pro-inflammatory mediators with IR and development of T2DM in Pakistani community.

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Table 1
Clinical features and serum levels of biomarkers of study participants.

Parameters	Male participants					Female participants				
	Control males		Diabetic males		*P*-value	Control females		Diabetic females		*P*-value
	N = 15	CV (%)	N = 30	CV (%)	–	N = 15	CV (%)	N = 30	CV (%)	–
Age (years)	29.5 ± 3.2	10.9	39.8 ± 3.1	7.8	–	28.7 ± 2.5	8.8	39.2 ± 3.2	8.1	–
BMI (kg/m^2)	22.4 ± 2.7	12.3	26.8 ± 2.9	10.7	< 0.0001	23.9 ± 3.0	12.6	30.8 ± 3.5	11.3%	< 0.0001
Systolic BP (mmHg)	122.8 ± 1.3	1.1	145.0 ± 3.0	2.1	< 0.0001	106.7 ± 4.2	3.9	135.4 ± 3.1	2.3	< 0.0001
Diastolic BP (mmHg)	86.3 ± 2.4	2.8	82.2 ± 1.4	1.7	< 0.0001	75.7 ± 3.5	4.6	84.9 ± 2.7	3.2	< 0.0001
Glycemic control biomarkers										
Insulin (μIU/ml)	9.5 ± 1.6	17.3	20.9 ± 2.7	13.2	< 0.0001	9.4 ± 2.5	26.5	21.5 ± 4.9	23.0	< 0.0001
Glucose level (mmol/L)	4.9 ± 0.2	5.4	7.2 ± 0.4	6.0	< 0.0001	5.3 ± 0.3	2.5	8.0 ± 0.6	6.9	< 0.0001
Inflammatory biomarkers										
IL-6 (pg/ml)	3.7 ± 0.5	14.8	6.0 ± 1.0	16.1	< 0.0001	2.8 ± 0.5	19.0	9.1 ± 0.6	6.2	< 0.0001
TNF-α (pg/ml)	4.3 ± 0.7	16.1	8.6 ± 0.6	6.4	< 0.0001	4.5 ± 0.36	8.0	10.2 ± 0.6	5.7	< 0.0001
CRP (μg/ml)	2.9 ± 1.2	42.9	5.0 ± 0.3	5.5	< 0.0001	3.1 ± 1.4	44.8	6.2 ± 0.4	5.9	< 0.0001
Insulin resistance										
HOMA-IR	1.9 ± 0.2	12.5	5.9 ± 0.4	7.3	< 0.0001	1.9 ± 0.2	16.4	7.6 ± 0.6	8.1	< 0.0001

In this study, we aimed to investigate the serum levels of inflammatory biomarkers notably IL-6, TNF-α and CRP, in Pakistani population and gender-specific association of these inflammatory biomarkers with the development of IR in type 2 diabetic study participants. Moreover, we also aimed to investigate the correlation of body mass index (BMI) of diabetic patients with that of the development of IR. We found that there a strong association exists between the serum levels of inflammatory biomarkers and development of IR in diabetic patients.

2. Materials and methods

2.1. Study participants

A total of 90 study participants who visited the hospital for their routine checkup from different areas of Faisalabad of Punjab province, Pakistan were randomly selected with pre-informed written consent. A complete physical examination of each study participant was performed by one of the physicians attending the patients. The age of the controls (both males and females) were in the range of 25–35 years whereas, for diabetic patients (both males and females), the age was in the range of 35–45 years. The BMI (kg/m^2) of each study participant was calculated by using the formula ($\mathrm{BMI} = \frac{\mathrm{Weight(kg)}}{(\mathrm{Height})(\mathrm{m})^2}$). The blood pressure (mmHg) of control and diabetic patients was measured by using sphygmomanometer and reading was recorded as systolic and diastolic blood pressure.

2.2. Ethical consideration

The study was conducted in accordance with the ethical standards set by the Ethical Review Committee (ERC) of the Government College University Faisalabad (GCUF). This study was also approved from ERC of GCUF (GCUF/ERC/2016/1698).

2.3. Sample collection

Blood samples were collected in eppendorf tubes from the total of 90 study participants out of which 15 were male controls and 30 were male diabetic patients, whereas, 15 were female controls and 30 were female diabetic patients. The collected blood was centrifuged at 769 × *g* for 15 min and serum was separated and stored at −20 °C till further analysis.

2.4. Biochemical analysis

The serum levels of IL-6, TNF-α, CRP and insulin were estimated using commercially available human IL-6 ELISA kit (catalog Number: E-EL-H0102), human TNF-α ELISA kit (catalog Number: E-EL-H0109), human hs-CRP ELISA kit (catalog Number: E-EL-H0043) and human insulin ELISA kit (catalog Number: E-EL-H5439) of Elabsciences, respectively through micro-plate ELISA reader.

2.5. Statistical analysis

All statistical analyses were performed using Graph Pad Prism 5.01. Multiple linear regression was performed using Microsoft excel 2016 version 16.0.9226.2156. Data were expressed as mean ± SD. Analysis of Variance (ANOVA) was applied to the data to predict the significant difference between the variables. The level of significance was set at two-tailed *P*-value < 0.05. The Pearson's correlation coefficient (r), the coefficient of determination (R^2) and 95% confidence interval (CI) among the incidence of IR (calculated from HOMA-IR) and serum levels of inflammatory biomarkers (IL-16, TNF-α and CRP) and BMI was also calculated. Fasting levels of insulin and glucose were used for the estimation of IR with the help of Homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) and was calculated as follows:- $HOME - IR = \frac{Insulin(\mu U) \times Glu\cos e(mmol/L)}{22.5}$.

3. Results

3.1. Clinical features of study participants

The clinical features of study participants have been mentioned in Table 1, which indicates significant age difference between diabetic and non-diabetic subjects. Observed age for diabetic males was 39.8 ± 3.1, non-diabetic males 29.5 ± 3.2, while for diabetic females 39.2 ± 3.2, non-diabetic females 28.7 ± 2.5. Study subjects with T2DM had significantly (P < 0.0001) higher BMI, 26.8 ± 2.9 (kg/m^2) for male diabetic patients and 30.8 ± 3.5 (kg/m^2) for female diabetic patients and their coefficients of variance (CV) were 10.7% and 11.3% respectively along with increased systolic and diastolic blood pressure when compared with the controls of both male and female diabetic patients. While comparing with control study participants, non-diabetic control males had BMI (kg/m^2) of about 22.4 ± 2.7 while the non-diabetic control females showed BMI (kg/m^2) of about 23.9 ± 3.0.