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Serum Levels of Insulin-Like Growth Factor 1, Interlukin-6, Heat-Shock Proteins-27 and Heat-Shock Proteins-60 as Inflammatory Markers of Type 2 Diabetes: A Case-Control Study

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Abstract

Background: Inflammatory biomarkers such as insulin-like growth factor 1 (IGF-1), interleukin 6 (IL-6), and heat-shock proteins 27 (HSP-27) and 60 (HSP-60) may contribute to the development of type II diabetes mellitus. This study aimed to compare these inflammatory biomarkers among individuals with and without type II diabetes mellitus.
Methods: This case-control study was conducted on fifty patients with type II diabetes mellitus and fifty individuals without it. None of the participants suffered from inflammatory diseases like rheumatoid arthritis and systemic lupus erythematosus. Patients in the diabetic group were matched with individuals in the non-diabetic group respecting their age and gender. Serum levels of IGF-1, IL-6, HSP-27, and HSP-60 were measured through enzyme-linked immunosorbent assay kits. Data were analyzed using the SPSS software (v. 15.0).
Results: The mean serum levels of IGF-1, IL-6, HSP-27, and HSP-60 in the diabetic group were significantly greater than the non-diabetic group ($P < 0.05$).
Conclusions: This study shows that IGF-1, IL-6, HSP-27, and HSP-60 inflammatory markers play roles in the development of type II diabetes mellitus.

Keywords: Diabetes Mellitus Type 2, Insulin-Like Growth Factor 1, Interleukin-6, Heat-Shock Proteins, Inflammation

1. Background

Recently, the incidence of diabetes mellitus (DM), a chronic metabolic disorder, increased so that the number of diabetic people will rise to 592 million in 2035 (1). Type 2 diabetes mellitus (T2DM) was described by inadequate secretion of insulin or insulin dysfunction. It is caused by a blend of genetic and environmental risk factors (2). On the other hand, complications of DM include nephropathy, retinopathy, neuropathy, cardiovascular disease, and inflammation (3, 4).

Several studies suggested that Inflammation may play as an intermediary role in development of T2DM; however, clinical data addressing for this issue are limited. Of course, there are differences of opinion in this regard in the researches done, for example; some of clinical studies showed that markers of inflammation have not been consistently associated with development of diabetes. Also, some of studies expressed that there was no relationship between the development of diabetes and markers of inflammation (HSP and IGF-1). On the other circulating biomarkers of inflammation may be associated with development of type 2 diabetes. Similarly, studies have shown that these biomarkers may be associated with development of diabetic nephropathy and retinopathy (4-7).

Insulin-like growth factor (IGF-1), as a polypeptide hormone, is modulating the cellular proliferation, differentiation, apoptosis, and inflammation. Both its secretion and function are also regulated by the pro-inflammatory cytokines (6). Interleukin-6 (IL-6), a pro-inflammatory cytokine, has an extensive range of biological activities in inflammation, immunity and oncogenes is pathways (8). When inflammatory mechanisms are activated, IL-6 has a key role in the acute phase response (9).

Additionally, other cellular inflammation biomarkers

of T2DM are heat shock proteins (HSP), for instance HSP-27 and HSP-60 (10). Heat shock proteins (HSP) are known as molecular chaperones with various functions. Moreover, HSPs play a key role in modulating of inflammation (11). HSP-27 is a member of the small HSP family (12). In addition, there is expanding evidence which mitochondrial dysfunction that may be terminated to the development of T2DM. HSP-60 is a mitochondrial stress protein and can be induced under mitochondrial impairment conditions (7).

2. Objectives

Due to the fact that inflammation may play a role in the development of T2DM, our aim for the present study is to measure some serum biomarkers of the inflammation that increase in T2DM.

3. Methods

3.1. Study Population

This case-control study was conducted from March 2016 to July 2016 in Birjand, Southern Khorasan, Islamic Republic of Iran. The diabetic group included people with T2DM, who had no inflammatory disease, such as rheumatoid arthritis, lupus, erythematosus and etc., and referred to a clinic at the Imam Reza Hospital of Birjand to follow their sickness. In addition, they had a glycated hemoglobin level of $\geq$ 7.0% and $\leq$ 10.5% as well as the presence of glucose and protein in their urine. The non-diabetic group contained of 50 individuals with a glycated hemoglobin level of $\geq$ 5.7% and an absence of glucose and protein in their urine; in addition, they referred to the Imam Reza clinic for annual checkup and they had no inflammatory diseases, such as rheumatoid arthritis, lupus erythematosus, and etc.. The non-diabetic group and diabetic group were matched in age and sex. Notably, we used the sample size from the approved project (grant No. 3908) at Birjand University of Medical Science.

3.2. Biochemical Analysis

Informed consent was obtained from all participants who were included in the study. A total of 5 mL of venous blood was collected from all participants after a 12-hour fasting period in plain tubes and centrifuged at 3000 rpm for 10 minutes as soon as they had been collected. Fast blood sugar (FBS), hemoglobin A1c (HbA1c), and lipid profile including total cholesterol (TC), triglyceride (TG), High-density lipoprotein (HDL) and low-density lipoprotein (LDL) determined using the automated clinical chemistry analyzer (prestige, Tokyo). In addition, atherogenic index plasma (AIP) was assayed in two groups by logarithm of the ratio of plasma concentration of triglycerides to HDL-cholesterol (Log[TG/HDL-C]).

3.3. Pathogenesis Assay

All serum IL-6, IGF-1, HSPs-27, and 60 biomarkers were detected by human ELISA kits (Boster Biological Technology Co. USA).

3.4. Statistical Analysis

The results were expressed as the mean $\pm$ standard deviation (SD) for continuous or discrete variables. Data were analyzed with the SPSS software, version 15. The *t*-test was used to compare both groups.

4. Results

The diabetic group (24 (48%) men and 26 (52%) women, 45.5 $\pm$ 11 years ranging from 27 to 70) and non-diabetic group (28 (56%) men, 22 (44%) women, and 48.5 $\pm$ 12 years ranging from 22 to 71) were matched according to age (P = 0.20) and sex (P = 0.54). FBS, TG, TC, and LDL cholesterol levels of diabetic group were higher when compared with non-diabetic group, while HDL cholesterol level significantly was lower in diabetic than non-diabetic group (P < 0.05) (Table 1). The results of this study showed that the mean of IGF-1 and IL-6 in the diabetic group was significantly higher in comparison to the non-diabetic group (P < 0.001) (Table 2). In addition, the mean of HSP-27 and HSP-60 in the diabetic group considerably incremented in comparison to the non-diabetic group (P < 0.001) (Table 2).

Table 1. Comparison of Biochemical Results of Non-Diabetic and Diabetic Groups

Variables	Non-Diabetic (N = 50)	Diabetic (N = 50)	P Value
FBS, mg/dL	94.40 $\pm$ 7.23	171.30 $\pm$ 76	< 0.001
TG, mg/dL	113 $\pm$ 33.43	165.66 $\pm$ 77.82	< 0.001
TC, mg/dL	172.66 $\pm$ 23.56	191.63 $\pm$ 44.45	0.02
LDL, mg/dL	105.36 $\pm$ 24.44	121.5 $\pm$ 33.94	0.01
HDL, mg/dL	46.83 $\pm$ 7.79	41.23 $\pm$ 5.42	0.001
AIP	0.37 $\pm$ 0.11	0.57 $\pm$ 0.19	< 0.001

Table 2. Comparison of Inflammatory Factors IGF-1, IL-6, HSP-27 and HSP-60 in the Studied Groups

Factors	Non-Diabetic	Diabetic	P Value
IGF-1, ng/mL	185 $\pm$ 11	310 $\pm$ 21	< 0.001
IL-6, pg/mL	4 $\pm$ 0.82	15 $\pm$ 1.02	< 0.001
HSP-27, ng/mL	10.61 $\pm$ 3.11	33.61 $\pm$ 7.21	< 0.001
HSP-60, ng/mL	8.38 $\pm$ 2.54	21.33 $\pm$ 4.54	< 0.001

5. Discussion

Results of this study show that inflammatory factors like IGF-1, IL-6, HSP-27, and HSP-60 in the diabetic group are higher than the non-diabetic group. Some evidence supports that inflammation is nearly involved in the development of T2DM (13). Furthermore, some investigations have suggested that markers of inflammation are predictors of the development of T2DM (1, 14). IGF-1 is involved in many signaling pathways in cells, which its deregulated may be connected to some metabolic disorders, such as T2DM. Glucose homeostasis and insulin requirements in human were improved by administration of IGF-1 (15). The previous study was indicated that in T2DM, a chronic elevation of insulin might lead to higher levels of bioavailable IGF-1 (6). In this study, it was shown that the diabetic group has higher IGF-1 than the non-diabetic group. Teppala and Shankar found a positive association with lower serum IGF-1 levels and diabetes (16), while Rajpathak et al. did not detect any association between IGF-1 and glucose intolerance in their study (17). Serum concentration of IGF-1 association with T2DM incidence were studied by Drogan in 2016 (15). Their results do not admit an association between IGF-1 concentrations and development of T2DM. We were not able to present supporting evidence in regards to a previous finding done by Deleskog et al. who demonstrated that men with high IGF-1 have a reduced risk of development of T2DM. However, in this study it was shown that IGF-1 in male diabetic group were more than non-diabetic males (320 $\pm$ 15 ng/mL and 197 $\pm$ 13 ng/mL, respectively) (18). Therefore, we can conclude that increase in the level of IGF-1 enhances the risk of development of T2DM incidence.

IL-6 is a cytokine that involves in inflammation adjustment of metabolic, regenerative, and neural processes (5). Elevated concentration of IL-6 can predict the development of T2DM (7).

Some studies illustrated that IL-6 in the diabetic group was more than the non-diabetic group, our results confirm the data (4, 19). From these results, it can derive that an increase in IL-6 enhances the risk of development of T2DM.

HSPs are a family of stress-responsive proteins that modulate cell function and contribute to protein homeostasis (15). Actually, HSPs are protective protein chaperones where some of them may also modify other proteins known to be involved in inflammation (20).

HSP-27 is a multitask protein that acts as a protein chaperone and plays a role in the inhibition of apoptosis (21). Mahgoub et al. offered that concentration of HSP-27 may be associated with inflammation and micro vascular complication in the diabetic group (10). Results of another research showed that concentration of HSP-27 in subjects with at least one complication of type I diabetes was significantly more than the non-diabetic group, which has no complication (22). Our results show that concentration of HSP-27 in the diabetic group was more than the non-diabetic group.

HSP-60 is an intracellular protein that has been displayed to be present at higher levels of systemic circulation in T2DM. Hall and Martinus indicated that hyperglycemic conditions can propel to the induction of HSP-60 expression. Moreover, they suggested that the higher serum levels of this molecular stress protein observed in the diabetic group could also be due to uncontrolled hyperglycemia (23). In addition, it reported that HSP-60 has a remarkable role in the T2DM pathology (24). The present study showed that serum HSP-60 of subject group was more than the non-diabetic group.

5.1. Conclusions

Our results showed that inflammatory markers such as IGF-1, IL-6, HSP-27, and HSP-60 higher in T2DM can suggest that these biomarkers play roles in the development of T2DM.

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Footnotes

Conflict of Interests: All contributing authors declare no conflicts of interest.

Ethical Considerations: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. In addition, it was also in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, as reflected in apriority approval by the Birjand University of Medical Sciences ethic code IR.bums.1395.61).

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Patient Consent: Informed consent was obtained from all individual participants who were included in the study.

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