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Original Article

Serum Level of Heat Shock Protein 70 in Patients with Type 2 Diabetes Mellitus in Basrah, Iraq

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Abstract

Diabetes mellitus is a chronic metabolic disease with an increasing prevalence, caused by a defect in insulin production, insulin action, or both, and can increase the risk for the development of microvascular as well as macrovascular complications. Heat shock protein70 is considered a family of a larger group of proteins known as heat shock proteins, which their expression is induced when the cells are subjected to environmental stress. They are believed to keep the native folding of proteins in cells under stressful conditions and their therapeutic role. Therefore, this study aimed to investigate the serum level of HSP70 in patients with type 2 diabetes mellitus (T2DM) to assess if there is an association of HSP70 with T2DM and to evaluate the effect of age and duration of disease on the serum level of HSP70. Ninety-one patients with T2DM were recruited, and 85 individuals with the same age range and sex as healthy controls. Serum HSP70, fasting blood sugar, and HbA1c were measured. The results revealed that the level of HSP70 was significantly higher in the diabetic group compared to the control group (*P* value<0.05). The level of HSP70 showed a significant positive correlation with age and duration of disease as well as with fasting blood sugar and HbA1c. The study suggested that HSP70 may have the potential to be used as an indicator of metabolic derangement and a prognostic biomarker in diabetes.

Keywords: Type 2 diabetes mellitus, HSP70, Fasting blood sugar, Glycated hemoglobin, Duration of DM

1. Introduction

Diabetes Mellitus (DM) refers to a group of various metabolic disorders characterized by elevated blood glucose levels caused by insufficient insulin release, resistance to insulin action, or both (1). Type 2 diabetes mellitus (T2DM) is the most common type of diabetes worldwide. It is caused by ineffective use of insulin by the body combined with a gradual progressive loss of β-cells in the pancreas (2). In 2019 the global prevalence of DM in the adult population was estimated to be 9.3 percent, rising to 10.2 percent by 2030 and 10.9 percent by 2045 (3). In Iraq, around 1.4 million have diabetes, and the prevalence ranges from 8.5-13.9%. Basrah's age-adjusted prevalence of diabetes in individuals aged 19-94 is 19.7 percent (4). Heat shock proteins (HSPs) are ubiquitous and well-described proteins, with molecular masses ranging from 15 to 110 kDa (5). They are classified into six major families based on their molecular weight (kDa): HSP110 (or HSPH), HSP90 (or HSPC), HSP70 (or HSPA), HSP60 (or HSPD1), HSP40 (or DNAJ), and other small HSP families (HSPB) (6). They are thought to be protective molecules that play various roles and can be expressed in response to various cellular stresses (7), such as ischemic, thermal, and oxidative stress (8). Heat shock proteins act as chaperone proteins and can assist denatured proteins in refolding (9). Moreover, they have anti-apoptotic and anti-inflammatory

properties (10). Recently their functions have been expanded to include cell signaling control (11), immune response modulation (12), and involvement in chronic disease conditions such as DM, obesity, and insulin resistance (13). One of the HSP family members extensively studied is HSP70 (14). Heat shock protein 70 can induce antagonistic actions, depending on its location. When cells are stressed, intracellular HSP70 (iHSP70) can migrate to circulation via an active mechanism, lipid rafts, and exosome and change its name to extracellular HSP70 (eHSP70). As a result of this migration, HSP70 transforms from an anti-inflammatory molecule, iHSP70, to a pro-inflammatory molecule, eHSP70 (15). Hyperglycaemia is known to play a role in the inflammatory conditions and vascular complications associated with diabetes, resulting from the generation and action of reactive oxygen species (16). Indeed, patients with T1DM (17) and T2DM have higher levels of eHSP72, and this response has been linked to disease duration (18). The goal of the current study is to compare the level of HSP70 in patients with T2DM and control and correlate with age, glycated hemoglobin (HbA1c), fasting blood sugar (FBS), and disease duration.

2. Materials and Methods

2.1. Study Population

This was a case-control study done at Basrah College of Medicine; Department of Biochemistry in Basrah governorate, southern Iraq, from December 2020 to December 2021, on 176 participants categorized into two main categories; 91 patients with T2DM as cases and 85 healthy individuals matched for both age and sex with cases. All the participants attended the FDEMC either for medical consultation or regular check-ups. The diagnosis of T2DM was done according to the Criteria of the ADA (19). Exclusion criteria included pregnancy, smoking, proteinuria, renal involvement (creatinine more than 1.5 mg/dl or glomerular filtration rate (GFR) <70 cc/min), glomerulonephritis, congestive heart failure, and hospital admission in the previous six months. A detailed questionnaire containing demographic data (Age, Gender, Residency, Duration, Type of treatment, whether dietary, oral, insulin, or mixed therapy, and Family history of diabetes) were obtained from each participant.

2.2. Anthropometric Parameters and Blood Pressure

Each participant in the study underwent body weight and height measurement in the morning while wearing light clothes and no shoes. Body mass index (BMI) was calculated as (kg/m^2) (20). Blood pressure (BP) was measured three times following at least 10 min of rest, and the average value was selected for data analysis. Either documented diagnosis of HT defined hypertension (HT), the patient taking antihypertension medication, or the systolic BP being≥140 mmHg and⁄or the diastolic BP ≥90 mmHg following repeated examination (21). Regarding the glycaemic control, the patients were categorized based on HbA1c (22) into good glycaemic control (HbA1c<7%), fair control (HbA1c 7-8%), and poor control (HbA1c>8%), while they were classified based on FBS (23) into good glycaemic control (FBS 80-130 mg⁄dl) and poor glycaemic control (FBS >130 mg⁄dl).

2.3. Sample Collection

After an overnight fast for at least 8 h, five ml venous blood samples were obtained from each participant by venipuncture and divided into two parts: 2 ml was dispensed in a tube containing 1.5 mg/ml ethylene-di amine tetra acetic acid (K_3EDTA) for determination of HbA1c%. The rest of the blood was placed in a serum separator tube (SST) that contained gel and clot activator without anticoagulants and was left at room temperature (20-25) ℃ for 30 minutes and centrifuged at 3000 rpm for 5 minutes to collect serum. Then, a part of the serum was used to promptly estimate the routine biochemical tests. The other part of the serum was frozen in tightly closed Eppendorf tubes and stored at -20˚C for subsequent analysis of HSP70. Fresh urine samples were collected from each subject in the morning while remaining fasting, then they were centrifuged (4-5minutes) at 3000 pm and

used to measure proteinuria. The results were expressed as a ratio to creatinine in urine.

2.4. Laboratory Investigation

FBS, serum and urine creatinine, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were measured by automated colorimetric methods, while albumin in urine was measured by automated Immunoturbidimetric assay using kits provided by Roche diagnostics Gmbh, Germany. Glycated hemoglobin was measured by ion exchange high-performance liquid chromatography (HPLC) using VARIANT II TURBO HbA1c Kit-2.0 provided by Bio-Rad, USA. The estimation of HSP70 was done by sandwich enzyme-linked immunosorbent assay (ELISA) kit, according to the instruction of the manufacturer (My Biosource, USA, REF MBS760396). Absorbance was measured at 450 nm, and a standard curve was constructed from the known dilution of HSP70. Results were compared with the standard curve, and the detection range was 31.25-2000 Pg⁄ml. The inter-assay precision was <10%, while intra-assay precisions were <8%.

2.5. Statistical Analysis

A computer program called Statistical Package analyzed the data of this study for Social Science (SPSS) version 23, and the results were expressed as Mean±Standard Deviation (SD) and percentage. For analysis of continuous data Independent t-test was used, while categorical data; were analyzed using the Chi-square test (χ^2 test). Bivariate Pearson correlation was used to determine the parameters' correlation coefficient (r-value). *P*-values <0.05 were considered the lowest limit for significance.

3. Results

The participants' demographic, clinical, and biochemical data are shown in (Table 1). There were no significant differences between the patients and the controls regarding age, gender, TC, LDL, and creatinine (*P*>0.05). More than half of the individuals were females (54.9% and 55.3%) for patients and controls, respectively. The mean BMI was significantly higher in diabetes than in the controls (*P*<0.05). Hypertension was significantly more frequent in diabetic patients than in controls (*P*<0.05). In addition, the majority of diabetes (76.9%) with a family history of DM. There was a significant difference between the diabetic patients and the controls in FBS, HbA1c, TG, VLDL-C, and HDL-C (*P*<0.001, *P*<0.05). The mean value of serum HSP70 was significantly higher in diabetes than in controls (3.48±0.27 vs. 3.19±0.38pg⁄ml; *P*<0.05).

Table 1. Demographic, clinical, and biochemical data of the participants

Variables		Cases n=91	Controls n=85	*P* value*
Age (years)		50.29±10.32	49.59±10.18	NS ‡
Gender	Male	41(45.1%)	38(44.7%)	NS♣
	Female	50(54.9%)	47(55.3%)	
BMI(kg/m$_2$)		31.81±5.57	30.19±4.89	<0.05 ‡
Hypertension (n, %)		49(53.8%)	25(29.4%)	<0.05♣
Family medical history of DM		70 (76.9%)	47 (55.3%)	<0.05♣
Duration of disease(years)		----	6.80±4.93	-----
Fasting blood sugar (mg/dL)		210.51±76.13	89.45±11.20	<0.001 ‡
HbA1c (%)		9.07±2.21	5.13±0.46	<0.001 ‡
Serum total cholesterol(mg/dL)		175.46±40.67	173.31±38.17	NS ‡
Triglyceride (mg/dL)		168.80±90.93	119.71±44.50	<0.001 ‡
HDL-C (mg/dL)		44.20±9.80	49.02±9.43	<0.05 ‡
LDL-C (mg/dL)		120.70±36.37	118.02±35.70	NS ‡
VLDL-C (mg/dL)		33.75±18.09	24.06±8.90	<0.001 ‡
Creatinine (mg/dL)		0.73±0.19	0.77±0.19	NS ‡
HSP70 (pg/ml)		3.48±0.27	3.19±0.38	<0.05 ‡

Data were represented as mean ± SD or percent. **P* Level of significance between cases and controls
‡ Student t-test ♣Chi-square test

To investigate the effect of age on the serum level of HSP70, participants were categorized into five age groups (Table 2). The mean of HSP70 increased with the age of participants, but it did not reach a statistically significant value (P>0.05). Furthermore, HSP70 levels in the diabetes patients were higher compared with healthy controls concerning their similar age groups with significant differences between the ages of 36-65 years (P<0.05).

A comparison of HSP70 levels by disease duration as a function of disease revealed that the mean of HSP70 levels was significantly higher in patients with a disease duration of >5 years than those with a duration ≤5 years (3.55±0.24 vs. 3.41±0.28 pg⁄ml; P<0.05). A comparison of HSP70 among the studied groups according to FBS found that diabetes with FBS≥130 mg⁄dl had higher HSP70 than those with FBS<130 g⁄dl; means of HSP70 were (3.50±0.24 and 3.28±0.42 pg⁄ml) respectively (P<0.05). Furthermore, the results revealed that there was a statistically highly significant difference in the levels of HSP70 in patients concerning HbA1c% (P<0.001); patients with poor glycaemic control (HbA1c>8%) had higher HSP70 than those with good glycaemic control (HbA1c<7%) and fair control (HbA1c 7-8%); means of HSP70 were (3.58±0.23, 3.30±0.19 and 3.32±0.26 pg⁄ml) respectively (Table 3). There was a significant positive correlation between serum HSP70 with FBS, HbA1c, and disease duration in patients with the known disease and with age in all studied populations (Table 4).

Table 2. Distribution of HSP70 of the study population according to age groups

Age (Years)	HSP70 (Mean±SD) (pg⁄ml)		*P* value*
	Cases n=91	Controls n=85	
26-35 years	3.28±0.45#	3.00±0.44#	NS
36-45 years	3.42±0.20#	3.11±0.37#	<0.05
46-55 years	3.48±0.24#	3.21±0.40#	<0.05
56-65 years	3.54±0.31#	3.27±0.34#	<0.05
≥66 years	3.61±0.29#	3.44±0.21#	NS

Data were represented as mean ± SD
*Level of significance between cases and controls
Level of significance between age categories
P> 0.05
Student t-test

Table 3. Distribution of HSP70 according to the duration of disease and glycaemic control

Parameter		HSP70 (Mean±SD) (pg⁄ml)	*P* value*
Duration of disease	≤5 years (n=46)	3.41±0.28	<0.05
	> 5 years (n=45)	3.55±0.24	
FBS (mg⁄dl)	<130(n=9)	3.28±0.42	<0.05
	≥130(n=82)	3.50±0.24	
HbA1c%	Good control <7% (n=14)	3.30±0.19	<0.001
	Fair control 7-8% (n=24)	3.32±0.26	
	Poor control >8% (n=53)	3.58±0.23	

Data were represented as mean ± SD
*Level of significance
P<0.05 statistically significant P<0.001 highly significant
Student t-test

Table 4. Correlation of HSP70 with other variables of the study

HSP70	Variable	r-value	*P*-value
	FBS(mg⁄dl)	0.436**	0.001
	HbA1c (%)	0.474**	0.001
	Age(years)	0.215**	0.004
	Duration of disease	0.259*	0.013

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

4. Discussion

Diabetes mellitus type 2 is the most common type of diabetes, a multifactorial chronic metabolic disorder with a global prevalence that has been steadily increasing (24). The data of the present study revealed that more than 66% of diabetic patients were between 36-55 years, and this was not unexpected as well as can support and agree with the idea that middle age individuals have the highest numbers of T2DM (8). The present study found that more than half of the patients (54.9%) were females. The high proportion of females in this study could be attributed to the population admitted to this hospital, in which more women seek medical attention than men to have more free time because most of them were housewives. The study revealed that the mean BMI was higher in the diabetic group than in the control group (as shown in table 1) (*P*. value<0.05), which could be explained by the fact that T2DM is frequently associated with obesity and insulin resistance. In the present study, serum HSP70 in patients with diabetes was significantly higher than in the control group. Similar results were reported by several other studies (10, 18, 25, 26), while Kavanagh, Flynn (27) reported lower HSP70 levels in diabetes compared to controls.

On the other hand, another study reported that the levels of HSP70 in diabetic patients approximate that in the control group (28). The higher plasma levels of HSP70 in T2DM patients compared to healthy control subjects are most likely due to higher systemic levels of toxic metabolites capable of increasing the expression of several major heat shock proteins, including HSP70 (29). A characteristic feature of DM is uncontrolled oxidative stress (30). Indeed, increased HSP70 expression by many cell types appears to be an adaptive cellular response to hyperglycemia-induced oxidative stress (31). It has been found that eHSP70 may play a role in the development of T2DM by increasing insulin resistance (25). Krause, Bock (32) reported that HSP70 plays dual roles; iHSP70 is anti-inflammatory, whereas eHSP70 is pro-inflammatory and linked to insulin resistance and beta cell dysfunction in diabetic patients. The current study found that the mean value of the HSP70 level increased with the participant's age, but not to a statistically significant level.

In addition, the serum HSP70 levels in diabetic patients were significantly higher compared to healthy controls in similar age groups ($P<0.05$). Moreover, serum HSP70 correlated positively with the participant's age in the study population. These findings agreed with the results of other studies (15, 18). Krause, Keane (33) have found a positive association between insulin resistance and eHSP70 in elderly individuals, which could imply that eHSP72 plays a role in impairing insulin signaling in skeletal muscle that occurs with advanced age and in T2DM. On the contrary, Atkin, Moin (34), and Zubair and Ahmad (35) found a negative correlation between HSP70 and age in diabetic patients. The results found that the serum HSP70 in patients with diabetes was positively correlated with disease duration (r=0.259, $P=0.013$). These results were supported by other studies (18, 36), which found that patients with long-term diabetes (>5 years) had higher levels of HSP70 than those who were newly diagnosed ($P<0.001$). Moreover, there was a significant difference in the circulating level of HSP70 concerning glycaemic control. Serum HSP70 correlated positively with FBS (r=0.436, $P=0.001$). These results agreed with the results reported by Angelini, Salinari (37). In a hyperglycaemic environment, HSP70 can be glycated, which reduces its chaperone activity, but it is unknown whether this also affects plasma and tissue levels (38).

On the contrary, other studies showed a significant negative correlation between FBS and HSP70 in diabetic patients (17, 18, 34). Regarding HbA1c, the study found a significant positive correlation between HbA1c and HSP70 in the population (r=0.474, *P*=0.001), consistent with the results of another study (35). On the contrary, Nakhjavani reported no association between serum levels of HSP70 and HbA1c in diabetic patients, demonstrating the acute response of serum levels of HSP70 to hyperglycemia independent of HbA1c as an indicator of the long-term average of serum glucose levels (18).

A higher level of HSP70 was found in patients with T2DM compared to control, with a significantly higher level in poorly controlled diabetic patients and in those who have had diabetes for a long time, implying that HSP70 may be involved in the development of disease and suggest that HSP70 may have the potential to be used as an indicator of metabolic derangement in DM. The higher level of HSP70 in elderly individuals suggests that HSP70 may contribute to the pathogenesis of T2DM via increasing insulin resistance.

Authors' Contribution

Study concept and design: A. A. A.
Acquisition of data: A. A. A.
Analysis and interpretation of data: A. A. A.
Drafting of the manuscript: A. A. A.
Critical revision of the manuscript for important intellectual content: A. A. A.
Statistical analysis: A. A. M.
Administrative, technical, and material support: A. A. A. and A. A. A.

Ethics

Informed written consent was obtained from all the participants, and the ethical committee approved the Basrah Faculty of Medicine study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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