

Overexpression of HSP90 in Skin of Diabetic Rats Impacts Wound Healing Process

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Abstract: Diabetes has become an epidemic problem at global level and it has molecular impacts on various body tissues among which is the skin. Diabetes has been associated with adverse effects on wound healing to explore the expression of HSP90 in the skin of diabetic rats compared with control group. Twenty albino rats were randomly assigned into two groups, control group (N = 10) and diabetic group (N = 10). Diabetes was induced by peritoneal injection of streptozotocin (60 mg kg⁻¹) of body weight. After the end experiment, all animals were anesthetized with ether for 5 min, then blood were withdrawn from heart to measure glucose. Skin samples were taken and put in containers of formaldehyde (10%). Indirect immunoperoxidase staining was carried out to evaluate the expression of HSP90 in skin samples. The expression of HSP90 was assessed by using Adopyphotoshop Software Version 7.2. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colors (blue and brown), then the brown color (the color of the marker under study) was computed and divided by the total number of pixels. Data was represented as means and standard deviations while the relationships between variables were examined using independent t test. The significance was considered at alpha level <0.05. Glucose level in diabetic group was 330.5±63.60 and in control group was 101.3±4.57. The difference in means was statistically significant (p = 0.000). Diabetic skin samples exhibited expression level of HSP90 (0.578) which was significantly higher than that of skin samples in control group (0.15%) (p = 0.000). Diabetes has molecular impacts in wound healing through upregulating the expression of HSP90 in skin tissue.

Key words: Diabetes, wound healing, HSP90, upregulation Jordan

INTRODUCTION

Diabetes Mellitus (DM) has been considered as a part of metabolic disorders which are characterized by having hyperglycemia (Alemu, 2015). DM is viewed as a chronic metabolic disorder and is considered as a global health problem. Its main feature is the defect in insulin secretion or its function. It has been estimated that DM affects >366 million people and the number may exceed 552 million by 2030 (Danaei *et al.*, 2011).

According to study of Schreml *et al.* (2010), one of the major issues in current medicine is the delayed wound healing from a therapeutic and economic points of view. Diabetes has been associated with high frequencies of

chronic wounds that are not easily treated (Schreml *et al.*, 2010). Diabetic skin is susceptible to destruction and if injured, it has the tendency to be recurrent (Ge *et al.*, 2015).

The recent trends regarding chronic wound healing have brought the molecular link between Advanced Glycation End Products (AGEs) and microvascular as well as macrovascular complications in diabetes (Huijberts *et al.*, 2008; Sibbald and Woo, 2008).

According to He *et al.* (2013), HSP90 plays roles in several cellular processes including cell proliferation, differentiation and apoptosis. HSP90 is one of most available proteins and estimated to make about 1-2% of cytosolic proteins (Helmbrecht *et al.*, 2000).

Lee *et al.* (2013) found that the use of AUY922, a selective HSP90 inhibitor resulted in various beneficial effects including cytoprotection and improved insulin signaling in cells. Furthermore, the researchers showed that continuous exposure to HSP90 inhibitors exhibited anti-hyperglycemia activities in the diabetic db/db mouse model and also exhibited an improved insulin sensitivity in the diet-induced obese mouse model of insulin resistance. In type 2 diabetes, intracellular stress pathways have been associated with metabolic functions including insulin sensitivity and glycemic state (Kaneto *et al.*, 2004; Hotamisligil, 2005).

The present study was conducted to explore the expression of HSP90 in the skin of diabetic rats compared with control group.

MATERIALS AND METHODS

Animal preparation: Twenty albino rats weighing (180 grams on average) were randomly assigned into 2 groups (N = 10) the first group, control group and the second group, diabetic group. Rats in both groups were put in cages in a private room booked for this experiment in the animal house at Jordan University of Science and Technology. There was one week running before starting the experiment to let animals adapting to environmental conditions in which the experiment is going to be carried out.

Induction of diabetic model: The animals in diabetic group were intraperitoneally injected by streptozotocin (60 mg kg^{-1}) of body weight. Streptozotocin was freshly prepared in sodium citrate buffer (pH = 6). We followed other protocols for using streptozotocin to induce diabetes (Akbarzadeh *et al.*, 2007). About 2 days later, blood glucose was measured for all animals using glucose measuring device (Glucocheck). During the experiment, glucose was measured every 3 days, to monitor diabetic animals having glucose level 250 mg or more. Two animals received another dose of streptozotocin. After the end of experiment (30 days), animals were anesthetized with ether for 5 min, then blood were withdrawn from heart to measure glucose. Skin samples were taken and put in containers of formaldehyde (10%). Immunohistochemistry protocols.

Skin tissues were processed using tissue processor and sections were obtained on charged slides, then sections were deparaffinized using oven at 65°C for 1 h, then slides passed into solutions from xylene to distilled water. Sections were treated with 1% hydrogen peroxide in absolute methanol for 20 min to minimize or inhibit

endogenous peroxidase activity, then slides were washed by phosphate buffer saline (pH = 7.2-7.4) for 5 min, then slides were incubated with 1% bovine serum albumin for 30 min to minimize or prevent non-specific bindings. During that time, the primary antibody and other immunohistochemistry reagents were prepared and brought to room temperature. The monoclonal antibody (HSP90, Santa Cruz Biotechnology) was prepared (1:100) and incubated with slides for 1 h in humid chamber. After that, slides were washed with phosphate buffer saline (pH = 7.2-7.4) for 5 min, then incubated with secondary biotinylated antibodies for 20 min, then washed with phosphate buffer saline (pH = 7.2-7.4) for 5 min, then incubated with streptavidin conjugated with horseradish peroxidase enzyme for 20 min, then washed with phosphate buffer saline (pH = 7.2-7.4) for 5 min. Finally, immunohistochemical reaction was visualized through incubation with DAB (diaminobenzidine) till the development of brown reaction, then sections were washed with tap water to stop the reaction. Section then were stained with hematoxylin for 30 sec as a counterstain, then sections were washed with water, dehydrated and mounted with mounting medium.

Interpretation of the results and statistical analysis: The expression of HSP90 was assessed by using Adobe Photoshop Software Version 7.2. Photos for sections were taken and divided into pixels. The total number of pixels was computed and represented both colors (blue and brown), then the brown color (the color of the marker under study) was computed and divided by the total number of pixels (Al-Jaraha *et al.*, 2010, 2012; Al-khatib, 2013). Data was represented as means and standard deviations while the relationships between variables were examined using independent t-test. The significance was considered at alpha level <0.05 .

RESULTS AND DISCUSSION

Glucose level in study and control groups: The glucose level in control group was $101.3 \pm 4.57 \text{ mgdL}^{-1}$, while glucose level in diabetic group was $330.5 \pm 63.60 \text{ mgdL}^{-1}$. There was significant difference in glucose level between study and control groups ($p = 0.000$) (Table 1).

The expression of HSP90 in study and control groups The data of our results showed that the mean expression of HSP90 in control group was 0.1499 ± 0.044 and this was increased further in diabetic group 0.5773 ± 0.090 . The difference in means was statistically significant ($p = 0.000$) (Table 2, Fig. 1 and 2).

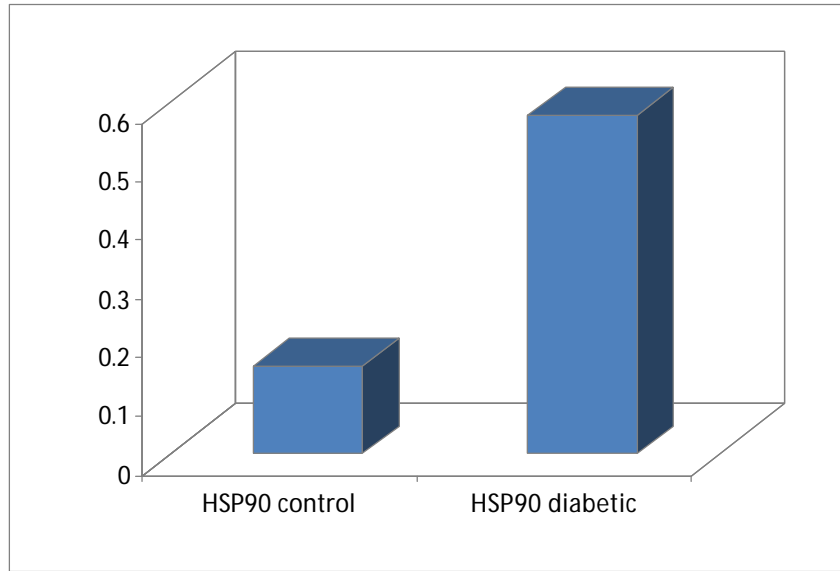


Fig. 1: The average expression of HSP90 in the skin of diabetic rates

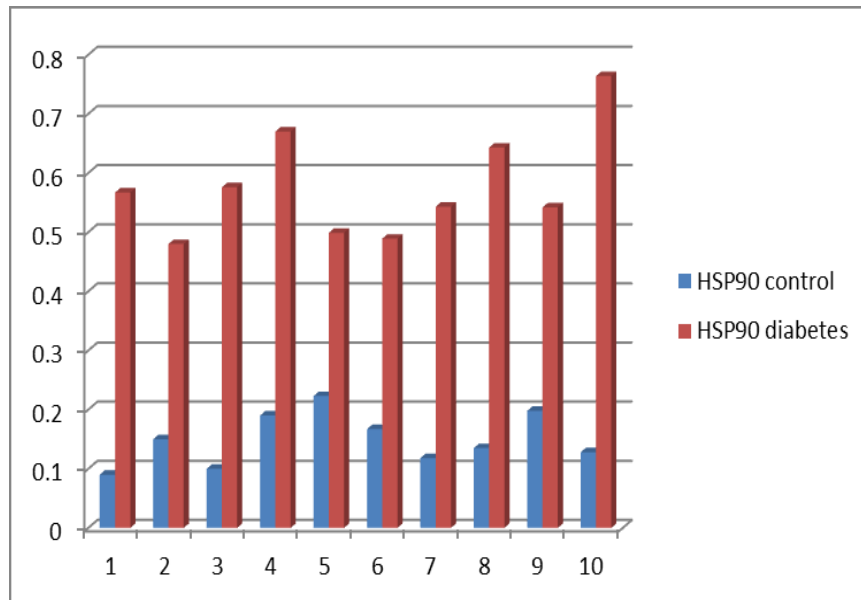


Fig. 2: The average expression of HSP90 in the skin of diabetic and control rates

Table 1: Glucose level among study groups (N = 10)

Animal No.	Control group (mg dL ⁻¹)	Diabetic group (mg dL ⁻¹)
1	99	280
2	102	260
3	97	320
4	106	350
5	107	420
6	98	270
7	95	290
8	103	335
9	108	360
10	98	450
Total	M±SD (101.3±4.57)	M±SD(330.5±63.60)*

*p = 0.000 at the mean level

Table 2: The expression of HSP90 in study and control groups

Group	M	S	f-value	t-value	df	p-values
Control	0.150	0.044	3.258	-13.438	18	0.000
Diabetic	0.578	0.090	-	-	-	-

The present study was conducted to explore the impacts of over expression of HSP90 in wound healing in skin of diabetic rats. We depended in the induction of diabetes in rats using streptozotocin. The first set of data showed that streptozotocin is an effective model to induce diabetes over 250 mgdL⁻¹. In the current study,

glucose level was 330.5 ± 63.60 compared with control group (101.3 ± 4.57) and this difference in means was statistically significant ($p = 0.000$). This finding is consistent with numerous studies in which streptozotocin can induce diabetes in animal models (Holemans *et al.*, 1997; Akbarzadeh *et al.*, 2007).

The other set of our data showed that the skin of diabetic rats exhibited significant expression of HSP90 compared with control group ($p = 0.000$). This finding is consistent with other studies in which diabetes participates into chronic wounds that are not easily treated (Schreml *et al.*, 2010). This finding may explain the findings of other studies in diabetic skin is susceptible to destruction and the tendency to recurrent injuries (Ge *et al.*, 2015). Our findings are also consistent with other studies in which the use of AU922, a selective HSP90 inhibitor resulted in various beneficial effects including cytoprotection and improved insulin signaling in cells (Lee *et al.*, 2013).

Up to the best knowledge of researchers, no previous studies reported the expression of HSP90 in diabetic skin which makes this study as a unique.

CONCLUSION

The present study showed that diabetes has molecular impacts in wound healing through upregulating the expression of HSP90 in skin samples.

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