Serum Heat Shock Protein 27 (sHSP27) In a Group of Type 1 Diabetic Children and Adolescents: Relationship to Microvascular Complications

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Abstract

Background: There is increased prevalence of T1DM in children and adolescents with its adverse complications especially microvascular ones (retinopathy, nephropathy and neuropathy) that might cause multiple organ damage. Heat shock protein 27 (HSP27 or HSPB1) appears to be generally cytoprotective; its decrease may be a primary factor leading to the development of diabetes and its diverse chronic microvascular complications with widespread organ damage.

Aim: to measure serum Heat shock protein 27 (sHSP27) level in a group of type 1 diabetic Children and adolescents and to investigate its relationship with diabetic microvascular complications.

Subjects and Methods: This case control study was executed in the Diabetes Clinic and the outpatient clinic, Children's Hospital, Ain Shams University. June 2013- June 2015 This study was carried out on 60 children and adolescents with type 1 diabetes mellitus, there ages ranged between (8- 18) years compared with 60 apparently healthy children matched as regards their age, sex, and socioeconomic Status.
Serum heat shock protein 27 (sHSP27) estimation was done by ELIZA technique.

Results: Serum HSP27 was statistically significantly lower in diabetic patients with complications (6.19± 1.17) than diabetic patients without complications (8.72± 1.95) and controls (12.29± 2.08). The diabetic neuropathy was accompanied by most decrease in the level of HSP27 among diabetic patients with microvascular complications.

Conclusion: decreased Serum heat shock protein 27 (sHSP27), indicative of decreased defense mechanism in type 1 diabetes, this decrease was more profound in patients with microvascular complications especially these who developed diabetic neuropathy.

Key Words: Diabetes Mellitus Type 1– Microvascular Complications- Heat Shock Protein 27.

بروتين صدمة الحرارة٢٧ لدى مجموعة من الأطفال والمراهقين المصابين بداء السكرى النوع الأول وعلاقته بمضاعفات الأوعية الدموية الدقيقة

مقدمة: إن معدل حدوث داء السكرى النوع الأول فى إزدياد ملحوظ وما ينجم عنه من مضاعفات خاصةً مضاعفات الأوعية الدموية الدقيقة والتى تشمل (إعتلال الكلى وشبكية العين وإعتلال الأعصاب الطرفية) وماينجم عنها من تلف لكثير من اجهزة وأعضاء الجسم. يعتبر بروتين صدمة الحرارة٢٧ ذو خصائص تعمل على حماية الخلايا ويعد نقص مستواه كأحد العوامل الفعاله التى تؤدى الى حدوث مرض السكرى ومضاعافاته وماينجم عنها من تلف لأعضاء الجسم المختلفه.

الهدف: قياس مستوى بروتين صدمة الحرارة ٢٧ فى مصل الدم وتوضيح علاقته بمضاعفات الأوعية الدموية الدقيقة لمجموعة من الأطفال والمراهقين المصابين بداء السكرى النوع الأول.

طرق إجراء البحث: قد أجريت دراسة مقارنة بعيادة السكر والعيادة الخارجية بمستشفى الأطفال جامعة عين شمس خلال الفترة من يونيو٢٠١٣ حتى يونيو ٢٠١٥ واشتملت على ستين طفلا ومراهقا يعانوا من داء السكرى النوع الأول تتراوح أعمارهم من ٨ إلى ١٨عام وستين طفلا ومراهقا من الأطفال الأصحاء من نفس العمر والجنس والمستوى الاجتماعى.ت م قياس مستوى (بروتين صدمة الحرارة ٢٧) فى مصل الدم بواسطة .ELISA

النتائج: أوضحت نتائج تلك الدراسة انخفاض مستوى بروتين صدمة الحراره فى مصل الأطفال المصابين بداء السكرى النوع الأول (٨,٧٢± ١,٩٥نانوجرام/ ملليتر) عن مستواه فى مصل الأطفال والمراهقين الأصحاء (١٢,٢٩± ٢,٠٨ نانوجرام/ ملليليتر) مع مزيد من الانخفاض فى مستواه فى أولئك الذين يعانون من وجود أحد مضاعفات السكرى الخاصه بالأوعيه الدمويه الدقيقة وكان هذا الانخفاض ملحوظا أكثر مايكون فى أولئك المصابين باعتلال الأعصاب الطرفية الناجمه عن مرض السكرى.

الخلاصة: انخفاض مستوى بروتين صدمة الحراره ٢٧ فى مصل الاطفال المصابين بداء السكرى النوع الاول مع مزيد من ذلك الانخفاض بهؤلاء المصابين بمضاعافات الاوعية الدموية الدقيقة خاصه اعتلال الاعصاب الطرفى.

الكلمات الإفتتاحية: داء السكرى النوع الأول– مضاعفات الأوعية الدموية الدقيقة– بروتين صدمة الحرارة ٢٧.

Introduction:

Diabetes Mellitus is a complex metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Inadequate insulin secretion and/or diminished tissue responses to insulin in the complex pathways of hormone action result in deficient insulin action on target tissues, which leads to abnormalities of carbohydrate, fat, and protein metabolism. Impaired insulin secretion and/or action may coexist in the same patient (Criag et.al., 2014)

T1DM incidence and prevalence showed a progressive increase over a period of 18 years among children aged from0 to 18years living in the Nile Delta region. Higher T1DM occurrence was observed in rural areas and female predominance was evident. Seasonality in T1DM diagnosis was documented with a peak occurring in winter prevalence increased from 1996- 2006- 2011 from 1.9/ 100000 to 15.5/ 100000 to 26.8/ 100000 (Abd- El Monem et.al., 2014).

The heat shock protein family is associated with a range of functions that promote cell survival during times of cellular stress (Korngut et.al., 2012). Heat shock protein 27 (HSP27 or HSPB1) appears to be generally cytoprotective by promoting cell survival through the stabilization of the actin filament cytoskeleton, the inhibition of apoptotic processes, the reduction of oxidative stress, and by functioning as a chaperone (Brerro-Saby et.al., 2010).

The level of HSPs and their response to stress stimuli are decreased in insulin responsive tissues in diabetes suggest that the loss of cellular stress response is a central event in the pathogenesis of the disease (Hooper, et.al. 2014). In turn, compromised HSP expression may contribute to diabetic complications, resulting in a vicious cycle (Hooper, 2003).

Aims:

Aim of the study was to measure serum Heat shock protein 27 (sHSP27) level in a group of type 1 diabetic Children and adolescents and to investigate its relationship with diabetic microvascular complications.

Subjects And Methods

This case control study included 60 children and adolescents with type 1 diabetes mellitus recruited from the regular attendants of the Diabetes Clinic, Children's Hospital, Ain Shams University.

They were subdivided into two groups according to the presence or absence of microvascular complications.

- Group (I): Included 30 children and adolescents diagnosed with type1diabetes without evidence of developing any of the chronic microvascular complications.
- Group (II): Included 30 children and adolescents diagnosed with type1diabetes, they had developed one or more diabetic microvascular complications (diabetic nephropathy, neuropathy or retinopathy).
- Control Group: Included 60 healthy children and adolescents with no obvious medical disorders and not receiving any medication they were matched for age, sex and socio economic levels with the study diabetic patients.

1. Inclusion Criteria:
   a. Age: 8- 18 years.
   b. Gender: Both Sexes
   c. Cases diagnosed with Type 1 diabetes mellitus.
   d. Patients on regular visits to clinic.
   e. Patients receiving human insulin therapy.
2. Exclusion Criteria:
   a. Cases diagnosed with Type 1 diabetes mellitus and associated with another chronic disease (e.g. chronic renal failure, cardiac diseases chronic chest disease, … etc.).
   b. Persons with Biological or clinical signs of acute infection or inflammation on the day of taking the blood sample.
3. Ethical aspect of the study: Written informed consent was obtained from the parents after explanation of the aim of the study, its benefits and expected risks for their children if they participate in the study. Informed verbal assent was taken also from all the patients as their age exceeds eight years after a simplified explanation of the aim and benefits of the study for them. Approval was taken to conduct this research from the Ethical Committee of the Institute of Postgraduate Childhood Studies Ain Shams University, the Ethical Committee of the Faculty of Medicine Ain Shams University and the Ethical Committee of the National Research Centre (NRC).

Methods:

1. Full medical history: Laying stress on Sociodemografic Data; name, age, sex and socio- economic class.
2. Medical history of diabetes: For cases (Including age of onset, duration of the disease, regimen of insulin treatment, and frequency of hypoglycemia or ketoacidosis.
3. Thorough Clinical Examination: With particular emphasis on full neurological examination, puberty assessment according to Tanner stages (Tanner, 1988). and fundus examination.
4. Auxological Assessment: Growth was assessed through auxological measurements of weight and height. Weight for age, height for age and body mass index for age was recorded according to World Health Organization (WHO) standards using AnthroPlus software for personal computers (WHO, 2007).
5. Lab Investigations: Routine investigations for all cases were done:
   a. Routine random blood sugar using glucocard II blood glucose.
   b. Monitoring system Glycosylated Hb (HbA1c) by HPLC (high performance liquid chromatography), Calculation of mean random blood glucose and mean HbAlc in the last one year prior to the study was done by retrospective study of the patient's filing system.
   c. Quantitative determination of urinary microalbumin for diabetic nephropathy. Microalbuminuria was defined as excretion rate (30-300 mg/ urinary creatinine).
   d. Biochemical Analysis for Serum heat shock protein 27 (sHSP27) estimation by ELIZA technique using kits supplied by SunRed technology comp.

Results:

The study included 60 children and adolescents with type 1 diabetes mellitus; they were 22 (36.7%) males and 38 (63.3%) females, there ages ranged between (8- 18) years with a mean of 13.03± 2.78 years. They were subdivided into two groups according to the presence or absence of microvascular complications.

Group (I): Included 30 children and adolescents diagnosed with type 1 diabetes without evidence of developing any of the chronic microvascular complications. Their ages ranged between (8- 15) years with a mean of 11.25± 1.92 years. They were 12 (40.0%) males and 18 (60.0%) females.

Group (II): Included 30 children and adolescents diagnosed with type 1 diabetes, they had developed one or more diabetic microvascular complications (diabetic nephropathy, neuropathy or retinopathy). Their ages ranged between (11- 18) years with a mean of 14.80± 2.35 years. They were 10 (33.3%) males and 20 (66.7%) females.

Control group: Included 60 healthy children and adolescents with no obvious medical disorders and not receiving any medication they were matched for age, sex and socio economic levels with the study diabetic patients. Their ages ranged between (8- 18) years with a mean of 12.43± 2.66 Years. They were 23 (38.3%) males and 37 (61.7%) females.

Table (1) Demographic and clinical data of the studied diabetic and control groups.

Variables	Diabetic Group N= 60	Control N= 60
	Mean± SD Range	Mean± SD Range
Age (Years)	13.03± 2.78 (8- 18)	12.43± 2.66 (8- 18)
Age Of Onset Of Disease (Years)	5.68± 2.77 (1- 12)	-
Disease Duration (Years)	7.34± 4.24 (1- 17)	-
Height (Cm)	151.23± 10.17 (125- 167)	150.00± 12.51 (120- 172)
Weight (Kg)	45.29± 12.54 (21- 70)	42.16± 10.46 (22- 72)
BMI (kg/ m²)	19.33± 3.31 (13.4- 27.3)	18.35± 2.51 (12.1- 28.1)
Mean Insulin Dose (IU/kg/d)	1.04± . 30 (0.48- 1.70)	-
Systolic Blood Pressure (Mmhg)	110.33± 10.73 (90- 130)	104.58± 10.05 (90- 120)
Diastolic Blood Pressure (Mmhg)	71.75± 9.69 (50- 90)	65.00± 6.57 (50- 80)

A.O.D: age of onset of disease; D. D: Disease duration; BMI: Body mass index; IU/kg/d: international unit per kilogram per day.

Table (2) Laboratory data of the studied diabetic and control groups.

Variables	Diabetic Group N= 60	Control N= 60
	Mean± SD Range	Mean± SD Range
MRBG (mg/dl)	214.15± 49.94 (105- 360)	84.53± 9.25 (70- 105)
Hbalc (%)	8.88± 1.56 (5.6- 12.6)	-
HSP27 (ng/ml)	7.46± 2.04 (4.5- 12.3)	12.29± 2.08 (8.3- 16)

MRBG= Mean random blood glucose; mg/dl: milligram per deciliter; HBA1C= Glycated hemoglobin A1C; HSP27= heat shock protein 27; ng/ml: nanogram per millilit

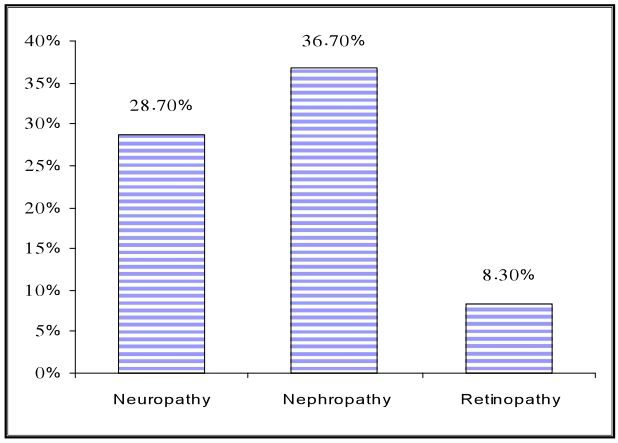

Fig (1) Frequency of microvascular complications among studied diabetics.

The most common microvascular complication encountered in the studied patients was diabetic microalbuminuria.

Table (3) Comparison between the three studied groups as regards their mean serum HSP27 levels

HSP27 (ng/ml)	Non Complicated	Complicated	Control Group	One Way Anova	
	No.= 30	No.= 30	No.= 60	F	P- Value
Mean± SD	8.72± 1.95	6.19± 1.17	12.29± 2.08	82.021	<0.001
Range	(6- 12.3)	(4.5- 8.7)	(8.3- 16)		
Post Hoc Analysis Using Lsd					
Non Complicated Vs Complicated		Non Complicated Vs Control		Complicated Vs Control	
<0.001		<0.001		<0.001	

Serum HSP27 was statistically significantly lower in diabetic patients with complications (6.19± 1.17) than diabetic patients without complications (8.72± 1.95) and controls (12.29± 2.08) fig (2).

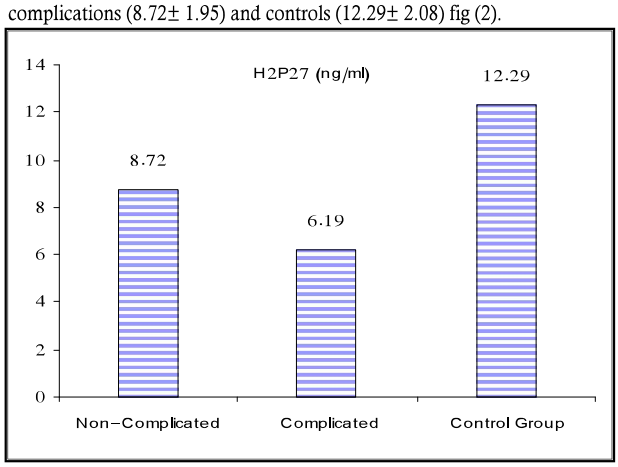

Fig (2) Comparison between the three studied groups as regards their mean serum HSP27 levels.

Table (4) Levels of serum HSP27 in different microvascular complications.

		HSP27 (ng/ml)		Independent T- Test	
		Mean± SD	Range	t	P- Value
Neuropathy	No	7.2± 0.86	6.2- 8.7	7.609	0.000
	Yes	5.31± 0.47	4.5- 6.2		
Nephropathy	No	5.6± 0.39	5.1- 6.2	1.720	0.096
	Yes	6.4± 1.29	4.5- 8.7		
Retinopathy	No	6.41± 1.13	4.9- 8.7	2.530	0.017
	Yes	5.08± 0.65	4.5- 6.2		

Serum HSP27 was statistically highly significantly lower in diabetic patients with neuropathy and statistically significantly lower in diabetic retinopathy although it was higher in patients with diabetic nephropathy

in respect to other microvascular complications but not reaching significant difference.

The diabetic neuropathy was accompanied by most decrease in the level of HSP27 among diabetic patients with microvascular complications.

Discussion:

The most common chronic complication encountered in the studied patients was diabetic microalbuminuria (nephropathy) being in 22 out of 60 patients representing 36.7% of the studied diabetic patients, followed by peripheral neuropathy being in 16 patients representing 26.7% and retinopathy being in 5 patients representing 8.3% of the studied diabetic patients. The frequency of micro- vascular complications in type 1 diabetes varies between studies and depends on several factors including disease duration and glycemic control (Zgibor et.al., 2002). The incidence of nephropathy in type 1 diabetes was between 20% and 40% (Gross et.al., 2005; Molitch et.al., 2004). Approximately 30% of type 1 diabetic patients may develop diabetic nephropathy within 25 years of diabetes (Rossing et.al., 2005).

In the present study Serum HSP27 was statistically significantly lower in diabetic patients with complications (6.19± 1.17) than diabetic patients without complications (8.72± 1.95) and controls (12.29± 2.08)

Hooper P. L and Hooper J. J (2005) Proposed that diabetes produces a vulnerable condition with impaired defenses against stress, resulting in widespread unprotected organ systems. More specifically, the ultimate natural history of diabetes and its complications is determined by the net effect of diabetes- induced inflammation, oxidation, and glycation, as well as an induced deficiency of heat shock factor- 1 (HSF- 1) that subsequently reduces the stress proteins that HSF- 1 stimulates heat Shock proteins.

This was in accordance with Pourhamidi et.al. (2014) who reported that patients with type 1 diabetes had lower HSP27 concentrations than nondiabetic healthy controls.

"There were rather controversial data on the level of sHsp and autoantibodies to these proteins in serum of diabetics" reported by Sudnitsyna M.V. and Gusev N.B. (2015) who tried to analyze the probable participation of small heat shock proteins in different cellular processes of diabetes, they concluded that there was increased expression of small heat shock proteins. Therefore, diabetes increases the level of small heat shock proteins in the heart and retina and certain brain regions and in the kidney cells. They explained their conclusion by that diabetes disturbs homeostasis and evokes metabolic stress. This stress leads to accumulation of unusual metabolites, covalent protein modification, modulation of activity of certain regulatory enzymes (such as different protein kinases), increase of reactive oxygen species, and changes of many other important parameters. These conditions dramatically increase the role of small heat shock proteins that prevent aggregation of denatured proteins, stabilize cytoskeleton, participate in regulation of key enzymes, and control redox state, proliferation and apoptosis.

In the present study Serum HSP27 was statistically highly significantly lower in diabetic patients with neuropathy and statistically significantly lower in diabetic retinopathy although it was higher in patients with diabetic nephropathy in respect to other microvascular complications but not reaching significant difference. The diabetic neuropathy was accompanied by most decrease in the level of HSP27 among diabetic patients with microvascular complications.

There were many studies that came in accordance with this finding and concluded the neuroprotective role of small heat shock proteins. From these studies was that conducted by (Pourhamidi et.al., 2011) who founded that Patients with diabetes had significantly lower sHSP27 levels than those with IGT and healthy controls, they also found that Participants with few signs of neuropathy had significantly higher sHSP27 levels than participants with many signs, concluding that Higher sHSP27 levels were associated with better nerve function and fewer neuropathic signs in diabetics with diabetic neuropathy, that was indicative of a potential neuroprotective function of HSP27, which was in accordance with the neuroprotective effects of HSP27 upregulation that have been proposed based on animal studies done by Dodge et.al., 2006.

Another study by (Pourhamidi et.al., 2014) had been done and reported that patients with type 1 diabetes had lower HSP27 concentrations than nondiabetic healthy control subjects. They concluded that correlation between progression of large nerve fiber dysfunction and a relative decrease in serum HSP27 concentrations during the follow- up period could be indicative of an association between neuropathy and HSP27.

The neuroprotective role of Heat shock proteins (Hsps) had been explained by Hooper, 2003. Who reported that NO synthesis was observed to increase Hsp expression, whereas blocking NO synthesis was found to lower Hsp expression. Relevantly, medications that have been associated with improved outcome in diabetes as adrenergic blockers, HMG CoA reductase inhibitors, ACE inhibitors and thiazolidinediones have all demonstrated restoration of endothelial NO synthase, which might result in Hsp expression and cytoprotection from the metabolic stresses of diabetes. He deducted that Exercise increases NO production and increases Hsp expression, and that perhaps contributing to the improved outcomes associated with exercise and diabetes. Importantly, he reported that a drug designed to increase Hsp expression, bimoclomol, improved diabetic retinopathy, neuropathy, nephropathy, wound healing, cardiac ischemia, and insulin resistance in laboratory diabetic animal models. Also, he observed that heat therapy, via hot tub immersion, had improved diabetic glycemic control and symptomatic diabetic neuropathy in diabetic patient.

Finally he concluded that Decreased Hsps might be a primary factor leading to the development of diabetes and its diverse, widespread organ damage.

Conclusion:

The present study revealed decreased Serum heat shock protein 27 (sHSP27), indicative of decreased defense mechanism in type 1 diabetes, this decrease was more profound in patients with microvascular

complications especially these who developed diabetic neuropathy.

Recommendations:

Further studies to postpone the effects of serum HSP27 in development of diabetes and its adverse complications.

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