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Serum Levels of Heat Shock Protein 27 as a Potential Marker of Diabetic Nephropathy in Egyptians with Type 2 Diabetes

Shahenda Mahgoub [1], Mahmoud Youns[1], Atef Bassyouni[2], Zeinab Hassan[1]
[1]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Ain Helwan, Cairo, Egypt
[2] National Institute of Diabetes and Endocrinology, Kaser Elainy Cairo, Egypt.

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ABSTRACT

Heat shock protein 27 (Hsp27) is over-expressed after cells exposure to stressful conditions that include oxidative stress like diabetes as well as chronic kidney disease. Here, the serum Hsp27 levels in Egyptian type 2 diabetic subjects with and without diabetic nephropathy (DN) were investigated. Serum Hsp27 levels were determined using The AssayMax Human Hsp27 ELISA kit in 72 individuals: 14 diabetic control subjects, 28 diabetic subjects with hypertension and/or dyslipidemia as risk factors for DN and 30 individuals with different DN stages (DN1= 6, DN2= 9 and DN3= 15 patients) according to the estimated glomeular filtration rate. Serum Hsp27 concentrations were significantly higher in patients at risk to and with DN compared to diabetic control subjects ($p<0.01$). Moreover, before microalbuminuria becomes evident, serum Hsp27 levels showed higher sensitivity and area under the curve compared to common traditional markers for diagnosis of DN as creatinine and microalbuminuria. In conclusion, our results showed, for the first time, that serum Hsp27 concentrations appear to be related to the incidence of DN as a microvascular complication in patients with type 2 diabetes mellitus and we concluded that serum HSP 27 may be used as an early marker for diagnosis of diabetic nephropathy.

INTRODUCTION

The prevalence of insulin resistance is increasing globally. At the same time, it is a consistent feature of metabolic syndrome (Zimmet *et al.*, 2005) and obesity (Kahn and Flier, 2000).The molecular mechanisms of hyperglycemia induced effects on inflammation and vascular complications are thought to involve the action of reactive oxygen species within the cell nucleus (Wright *et al.*, 2006). It has been proposed that oxidative stress is one pathogenic factor underlying the onset and progression of insulin resistance and diabetes and consequently on vascular complications (Brownlee, 2005; Ceriello and Motz, 2004; Evans *et al.*, 2003).

Exposure of cells to environmental stressors as heat and oxidative stress, subsequently leads to over-expression of a group of highly conserved proteins known as the heat shock proteins (Hsps). In addition to the function of many of these proteins as molecular chaperones, facilitating the correct folding of nascent peptides and the refolding of denatured or mis-folded proteins (Georgopoulos and Welch, 1993), they may also have other vital roles. Furthermore, insulin resistance and type 2 diabetes mellitus (T2DM) are associated with impaired expression of heat shock proteins by insulin sensitive tissues and thus those tissues become susceptible to damage by oxidative stress (Hooper and Hooper, 2009). Hsp27 is a member of the small HSP family that is over-expressed when cells are exposed to oxidative stress (Mehlen *et al.*, 1995). Several mechanisms were proposed to show how Hsp27 enables cells to adapt to exposure to oxidative stress including the up-regulation of glucose-6- phosphate dehydrogenase and glutathione peroxidase and by decrease intracellular iron levels (Preville *et al.*, 1999; Arrigo *et al.*, 2005).

In vitro studies have shown that Hsps are released from cells exposed to stress (Child *et al.*, 1995; Liao *et al.*, 2000), which would explain their presence in serum in vivo and why they may stimulate an autoimmune response (Xu, 2002). Studies have reported that antibody titres to some Hsps, such as Hsp60, are related to circulating antigen concentrations (Xu *et al.*, 2000) and that there are elevated concentrations of auto-antibodies to HSPs

** Corresponding Author*
Shahenda Mahgoub, Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Helwan,University campus, Ain Helwan, Cairo, 11511, Egypt.
Tel: 002 25540804, 002 01005056094, Fax: 002 25541601

in patients with atherosclerosis (Xu *et al.*, 1993), and furthermore, elevated concentrations of serum Hsp60 are associated with higher risk of coronary heart disease (Zhang *et al.*, 2008). It has been proposed that oxidative stress associated with hyperglycaemia may be involved in the vascular complications of type 1 diabetes (Evans *et al.*, 2003), and in a recent study, serum Hsp27 levels were found to be independently associated with the presence of distal symmetrical polyneuropathy in type 1 diabetic patients (Gruden *et al.*, 2008), though Hsp27 antibody levels did not correlate with the presence of the antigens in the same group of patients (Burt *et al.*, 2009). Recently, Hsp27 and its antibody concentrations appeared to relate to the presence of cardiovascular complications in patients with Glucose intolerance (Burut *et al.*, 2010).

Diabetes mellitus (DM) remains the most common cause of end-stage renal disease (ESRD) in the developed world, and diabetic kidney disease (DKD) is the leading specific primary renal diagnosis for patients commencing renal replacement therapy(Shaw *et al.*, 2010). Poorly controlled glucose levels, blood pressure (BP), and cholesterol activate inflammatory mediators, and patients with a genetic predisposition progress to advanced stage nephropathy (Balakumar *et al.*, 2009). Moreover, these patients are at the greatest risk for cardiovascular disease (CVD), morbidity and premature mortality caused by the combination of DM and chronic kidney disease (CKD) , thus imposing a large burden on both patients and health care costs (Gaede *et al.*, 2008; Keith *et al.*, 2004). During progression of CKD increased cell death or cell damage can occur, as indicated by the elevated serum levels of cell death markers (Ankersmit *et al.*, 2001; Roth *et al.*, 2011), which could also lead to a release of HSP into extracellular space (Musial and Zwolinska, 2011).

The aim of this present study was to identify additional diagnostic tool that would allow the early identification of diabetic patients at risk for developing diabetic nephropathy.

MATERIAL AND METHODS

A cross-sectional survey has been performed on 72 patients with T2DM attending the out clinic of National Institute of Diabetes and Endocrinology, Kasr EL Einy, Cairo, Egypt.

The criteria for inclusion were age 30–60 years old. Exclusion criteria included smokers and patients suffering from urinary tract infection, patients suffering from acute inter-current infection, a known history of other chronic diseases such as cancer, hyperthyroidism, Alzheimer disease, chronic analgesic abuse, and chronic gluco-corticoids treatment. The patients were divided into three groups: diabetic control group (DC) of 14 diabetic patients; diabetic nephropathy group (DN) of 30 diabetic patients and Risk group (DR) of 28 diabetic patients who had one or more of the known risk factors for Diabetic nephropathy.

Diabetic nephropathy group was selected by detection of micro-albuminuria < 300 mg/dl with no evidence of dialysis or kidney transplantation. Diabetic nephropathy group was subdivided into: diabetic nephropathy stage 1 –DN1- (6 patients), diabetic nephropathy stage 2 -DN2- (9 patients) and diabetic nephropathy stage 3 –DN3- (15 patients) based on their estimated glomerular filtration rate (eGFR); normal GFR with microalbuminuria, GFR of 60-89 ml/min with microalbuminuria and GFR of 30-59 ml/min with microalbuminuria, respectively.

Diabetic risk group patients were selected by having one or more risk factor for diabetic nephropathy but without overt microalbuminuria and this group was subdivided into: diabetic hypertensive (H) group, Blood Pressure (BP)>140/90, (9 patients), diabetic dyslipidemia (L)group (11 patients) and combined hypertensive and dyslipidemia (H+L) group (8 patients).

Blood samples

After 12 hours of fasting, blood samples were collected, centrifuged, and kept at −50°C until analysis. Serum creatinine, urea, fasting blood sugar (FBS), total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and HbA1c was measured for all participants.

Glucose measurements were carried out using the hexokinase method using a Bayer Advia 1650 analyzer, while glycated haemoglobin, HbA1c, was analyzed by ion-exchange HPLC technique using Bio-Rad D-10 Hemoglobin testing system. Cholesterol, HDL-C, LDL-C, and triglycerides were determined using direct enzymatic methods (Greiner Diagnostic GmbH, Germany).

Urine samples

Random urine spot sample was collected from each patient for measurement of microalbuminuria (turbidimeteric assays) and urine creatinine using ADVIA® 1650 clinical chemistry system, Siemens, Germany to calculate the albumin to creatinine ratio (ACR).

Diabetic Kidney Disease (DKD) progression and staging

The staging of DKD was evaluated by rate of decline of estimated glomerular filteration rate (eGFR) as calculated by using The Cockcroft-Gault (C-G) equation for ceatinine clearance, described in 1976 (Cockcroft and Gault, 1976).

$$\mathrm{CrCl(mL/min)} = \frac{(140 - \mathrm{age}(years) \times \mathrm{weight}(in\ kg))}{72 \times \mathrm{Scr\ level}(mg/dL)}$$

if female, this result is multiplied by 0.85

In this formula, CrCl approximates GFR, if the serum creatinine is stable, taking into account age-related decline in body weight and sex. The C-G equation is easiest to use and approximates eGFR from CrCl, whereas the other equations use computer-assisted calculations to derive eGFR (Dharmarajan *et al.*, 2012).

Determination of serum Hsp27

HSP27 serum concentrations were measured by The AssayMax Human Hsp27 ELISA kit (Assaypro, USA, Catalog No. EH5001-1 and purchased from Indomedix, Egypt). This assay employs the quantitative sandwich enzyme immunoassay technique. Briefly, a monoclonal antibody specific for HSP27 was pre-coated onto 96-well microtitre plate. Samples and standards were incubated along with a polyclonal HSP27 antibody in the microtiter plate. After incubation and a wash step, a horseradish peroxidase enzyme/IgG antibody conjugate was added. After another incubation and wash to remove unbound substances, an enzyme substrate was added and color was generated that was proportional to the amount of HSP27 present in the sample.

Statistical analysis

Statistical analyses were performed using Graph pad prism 5. All measured values were presented as the mean ± standard error. Correlation between serum concentration of HSP27 and other clinical parameters was analyzed using the Spearman's correlation coefficient. For comparison of clinical parameters between different groups, the non-parametric Mann–Whitney-test was used. P values less than 0.05 were taken as significant. SPSS software (Statistical Package for the Social Sciences, version 10, SPSS Inc, Chicago, Ill, USA) was used to make ROC curve.

RESULTS

The characteristics of the participants are presented in table 1. There were no significant differences between groups with respect to age, body mass index (BMI), FBG, A1C and serum urea levels. Serum TC, TG levels and TG/HDL were significantly higher, while HDL level was significantly lower in Risk group than diabetic control (DC) ($p<0.01$). LDL and LDL/HDL were significantly higher in Risk subjects (DR) than diabetic control ($p<0.05$). Microalbuminuria was significantly higher in diabetic nephropathy (DN) patients than Diabetic control ($p<0.01$) and Risk cases ($p<0.001$). ACR was significantly higher in DN group than Diabetic control and Risk groups ($p<0.001$). Serum creatinine was significantly higher in DN patients than Diabetic control ($p<0.05$) and Risk ones ($p<0.01$). eGFR was significantly lower in patients with DN than Diabetic control ($p<0.01$) and Risk individuals ($p<0.001$). Serum HSP27 levels in diabetic patients with risk for diabetic nephropathy were higher than in diabetic controls [1.30 ± 0.20] vs. [0.45 ± 0.06] ng/ml; ($p<0.01$). Likewise, HSP27 levels were higher in patients with DN than diabetic control ones [1.11± 0.14] vs. [0.45 ± 0.06] ng/ml; ($p<0.01$); Fig. 1), however, there was no significant difference in serum HSP27 levels between risk and DN groups.

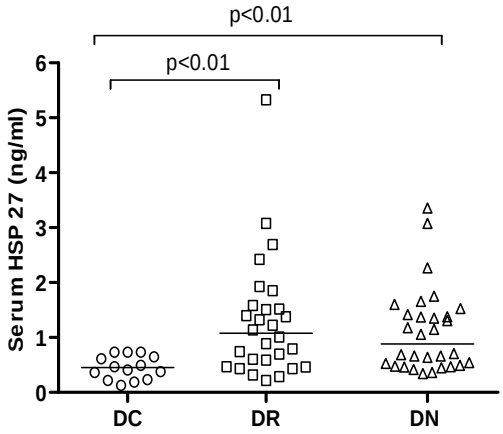

Fig. 1: Scatter plot of the distribution of serum Hsp27 concentrations in diabetic control (DC) individuals, diabetic individuals at risk for diabetic nephropathy (DR) and individuals with diabetic nephropathy (DN). Horizontal lines represent the median of the distribution.

The characteristics of the participants' subgroups of risk and DN groups are presented in table 2. There were no significant differences considering age, FBG, A1C and serum urea levels among the different subgroups of Risk and DN patients. BMI was significantly higher in hypertensive diabetic patients (H) than

Table. 1: Demographic, clinical and biochemical characteristics of study subjects groups.

Parameters	Diabetic control group (DC)	Diabetic risk group (DR)	Diabetic nephropathy group (DN)
N	14	28	30
Gender (female %)	43%	54%	50%
Age (years)	47.00 ± 2.28	48.36 ± 1.66	49.93 ± 1.77
BMI (Kg/m^2)	28.42 ± 0.99	31.90 ± 0.98	32.19 ± 1.17
FBG (mg %)	189.92 ± 14.29	210.43 ± 12.37	224.20 ± 15.71
A1C %	9.02 ± 0.67	9.22 ± 0.45	9.07 ± 0.43
Lipid profile (mg %) TC	174.29 ± 4.82	206.04 ± 6.56**	190.60 ± 6.91
TG	161.14 ± 37.48	207.75 ± 41.33**	192.47 ± 57.20
HDL	51.07 ± 2.92	32.75 ± 3.42**	41.63 ± 3.48
LDL	91.00 ± 4.59	128.54 ± 8.33*	109.27 ± 8.51
LDL/HDL	1.88 ± 0.17	5.58 ± 0.63*	3.92 ± 0.60
TG/HDL	3.42 ± 0.38	8.46 ± 0.85**	6.16 ± 0.73
Microalbuminuria (mg/dl)	22.79 ± 2.43$^{\#\#}$	21.29 ± 2.76$^{\#\#\#}$	87.57 ± 14.88
ACR (mg/g cr.)	18.93 ± 1.85$^{\#\#\#}$	14.49 ± 1.39$^{\#\#\#}$	78.90 ± 10.62
Serum creatinine (mg %)	1.03 ± 0.04$^{\#}$	1.03 ± 0.04$^{\#\#}$	1.35 ± 0.08
Urea (mg %)	23.35 ± 1.71	27.76 ± 1.32	29.20 ±1.88
eGFR (ml/min)	98.03 ± 1.97$^{\#\#}$	99.09 ±1.96$^{\#\#\#}$	83.19 ± 8.21

Data are presented as mean ± SEM, n: number

#, # #, # # #: significant from DN group at $p< 0.05$, 0.01and 0.001

*, **: significant from Diabetic control at $p < 0.05$, 0.01

Table. 2: Demographic, clinical and biochemical characteristics of study subjects sub groups.

Parameters	DC group	DR group			DN group		
		H	L	H+L	DN1	DN2	DN3
n	14	9	11	8	6	9	15
Age(years)	47.00 ± 2.28	50.22 ± 1.91	44.64 ±2.87	51.38 ±3.43	43.00 ± 2.53	49.11 ± 3.11	52.20 ± 2.55
BMI(Kg/m^2)	28.42 ± 0.99	35.23± 4.46^{*+}	28.91±1.33	31.99 ± 1.71	37.07 ± 4.67	32.36 ±1.26	30.15 ± 1.00
FBG (mg %)	189.92 ± 14.29	210.43 ± 12.37	224.20± 15.71	225.89 ±23.94	196.17 ±32.72	270.22 ±37.10	207.80 ± 16.28
A1C %	9.02 ± 0.67	9.51± 0.85	8.92± 0.80	9.31± 0.71	9.51± 0.85	8.92 ± 0.80	9.31± 0.71
Lipid profile (mg %)TC	174.29± 4.82	166.11± 8.43^{+++}	231.25± 24.23***	226.73± 6.02**	160.67 ±14.66	190.00± 8.34	202.93± 10.32
TG	161.14 ±37.48	167.89 ±8.62^{+}	224.00 ±10.99**	230.25± 10.56**	192.33± 43.28	190.11± 10.58	193.93 ±12.06
HDL	51.07 ±2.92	53.22 ± 3.83^{++oo}	20.55 ±1.06***	26.50± 5.91**	54.83 ± 4.64	44.44± 7.71	34.67± 4.24
LDL	91.00 ±4.59	79.44 ± 10.84^{+++}	154.27 ±6.68***	148.38 ±11.12**	67.50 ± 7.33††	107.67 ±14.39	126.93± 12.21
LDL/HDL	1.88 ± 0.17^{+++oo}	1.62 ± 0.26^{++o}	7.65 ±0.38	7.19 ±1.12	1.29 ± 0.19†	3.88 ± 1.14	5.00 ±0.88
TG/HDL	3.42 ±0.38^{+++oo}	3.27 ± 0.27^{+++oo}	11.25 ± 0.90	10.46 ± 1.29	2.96 ± 0.19	6.06 ±1.39	7.62 ±1.10
Microalbumi-nuria (mg/dl)	22.79 ± 2.43	21.56 ± 6.14	22.73 ±4.46	19.00 ±3.76	45.00± 14.60	92.00 ±19.49*	101.93± 26.26**
ACR(mg/g cr.)	18.93± 1.85	14.63± 2.27	13.15± 2.28	16.18 ±2.65	41.50 ± 4.22	93.56 ± 26.58***	85.07 ±13.06***
Serum creatinine(mg%)	1.03 ± 0.04†††	1.02± 0.06	1.07 ± 0.06	0.99 ± 0.08	0.74 ± 0.03†††	1.31 ±0.09 ^{Λ}	1.61 ± 0.07
Urea(mg %)	23.35± 1.71	29.95± 2.70	26.12 ± 2.44	27.53± 1.09	27.16 ± 4.43	30.79 ± 2.58	29.07 ± 3.06
eGFR (ml/min)	98.03 ± 1.97†††	102.11± 2.96	97.16 ± 2.99	98.33 ± 4.53	163.02 ± 13.22†††	81.13± 3.08^{ΛΛ}	52.50 ± 1.30

Data are presented as mean ± SEM, n: number
*, **, ***: significant from control at p<0.05, 0.01and 0.001 respectively
+, ++, +++: significant from Dyslipidemia (L) group at p<0.05, 0.01and 0.001, respectively
o, oo: significant from H+L group at p< 0.05and 0.01, respectively
Λ, Λ Λ: significant from DN1 at p< 0.05 and 0.01, respectively
†, ††, †††: significant from DN3 at p< 0.05, 0.01and 0.001, respectively

control diabetic and dyslipidemia (L) ones (p<0.05), while there was no significant difference in BMI among DN subgroups. Considering lipid profile, TC and TG levels were significantly higher in dyslipidemia diabetic patients than diabetic control (p<0.001and 0.01, respectively) and hypertensive ones (p<0.001and 0.05, respectively). At the same time TC and TG levels were significantly higher in combined hypertensive and dyslipidemia (H+L) than diabetic control patients (p<0.01). There was no significant difference among DN subgroups with respect to TC and TG.

LDL/HDL was significantly higher in dyslipidemia and H+L patients than diabetic control (p<0.001 and p<0.01, respectively) and Hypertensive individuals (p<0.01and p< 0.05), respectively. LDL/HDL was significantly higher in DN3 than DN1 (p<0.01). Also, TG/HDL was significantly higher in dyslipidemia and H+L patients than diabetic control (p<0.001, 0.01) and Hypertensive ones (p<0.01and p< 0.01, respectively) but there was no significant difference in DN subgroups considering TG/HDL.

Subjects of DN2 and DN3 subgroups, differed significantly from Diabetic control group for microalbuminuria (p<0.05 and p< 0.01) and ACR (p<0.001 for both subgroups), while there were no significant differences considering microalbuminuria and ACR among individuals of risk group. Serum creatinine remained insignificant among risk subgroups, while it was significantly higher in DN3 individuals compared to diabetic control (p<0.001) and DN1 ones (p<0.001), furthermore DN2 patients differed significantly from DN1 for serum creatinine (p<0.05).

There was no significant difference for mean eGFR between the subgroups of risk group. In contrast, eGFR was significantly different between DN3 and subjects of diabetic control (p<0.001) and DN1 (p<0.001), respectively as well as, between DN2 and DN1 subjects (p<0.01). Serum HSP27 levels were significantly higher in diabetic patients with hypertension and H+L than in diabetic controls [1.64 ± 0.51] and [1.34 ± 0.28] vs. [0.45 ± 0.06] ng/ml; (p<0.05; Fig. 2). Likewise, HSP27 serum levels were higher in patients with DN1 [0.90 ± 0.20] ng/ml than diabetic control subjects. However, the significant increase in HSP27 levels were in DN2 and DN3 compared to Diabetic control ones [1.31 ± 0.33] and [1.08 ±0.19] vs. [0.45 ± 0.06] ng/ml; (p<0.05); Fig. 3.

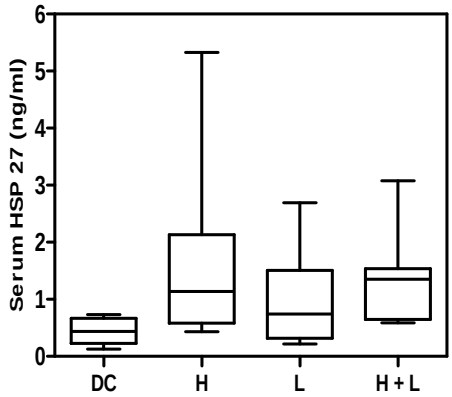

Fig. 2 Box plot demonstrating the higher serum level of HSP27 (ng/ml) in Hypertensive (H) (p<0.05) and combined hypertensive & dyslipidemia (H+L) groups (p<0.05) vs. diabetic control (DC) Horizontal lines represent the median of the distribution

Correlation analysis was undertaken between serum Hsp27 and other biochemical variables and the results reported in Table 3. We did not find any association between serum HSP27 levels and other biochemical variables in all studied population.

Table. 3: Correlation between HSP27 plasma concentration and baseline characteristics for all participants.

Variable	Spearman correlation coefficient	P-value
Age	-0.102	0.395
BMI	0.022	0.852
FBG	0.122	0.309
A1C	0.102	0.396
TC	0.059	0.622
TG	0.155	0.194
HDL-C	-0.041	0.735
LDL-C	0.002	0.989
LDL/HDL	0.026	0.830
TG/HDL	0.134	0.261
Microalbuminuria	-0.019	0.877
ACR	0.182	0.127
Serum creatinine	0.048	0.691
Urea	0.059	0.621
GFR	-0.143	0.231
Diabetes duration	-0.189	0.112

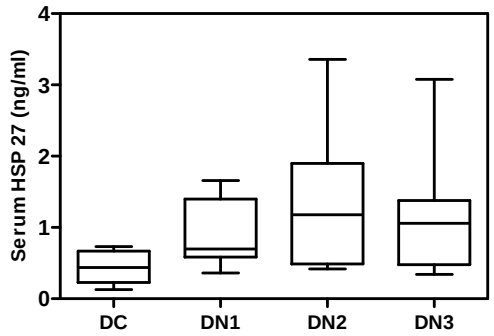

Fig. 3: Box plot demonstrating the higher serum level of HSP27 (ng/ml) in diabetic nephropathy stage 2 (DN2) ($p<0.05$) and stage 3 (DN3) groups ($p<0.05$) vs. diabetic control (DC) Horizontal lines represent the median of the distribution

ROC curve analysis of HSP27 serum levels

As depicted in fig. 4 ROC curve analysis revealed an area under the curve of 0.801 ($p<0.001$). Moreover, serum HSP27 showed higher AUC than that of ACR (0.67) and serum creatinine (0.62) as well as higher sensitivity to the stressful conditions implicated in pathogenesis of DN than ACR and serum creatinine, indicating a possibility of HSP27 serum levels to serve as an early diagnostic marker for DN before it becomes manifest.

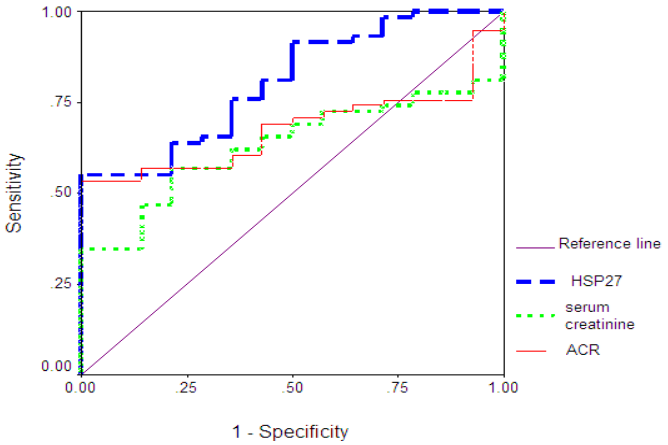

Fig.4: ROC curve for serum HSP27 compared to traditional markers of nephropathy. HSP27 shows higher AUC than all other markers.

DISCUSSION

In sight of the alarming increase in the number of people with DM , a growing number of patients with DKD, ESRD as well as CVD is forecasted (Altemtam *et al.*, 2011). Knowledge of the HSP engagement in CKD is still increasing, although not all aspects of their role in this process are wholly understood. Data showed that the intracellular forms of HSP, mainly Hsp70, delay the progression of CKD through the anti-apoptotic activity and cytoprotection. While, the extracellular HSP in CKD show more variability in their action, because they may actively protect against stress conditions as Hsp27 and Hsp70 or act detrimentally like Hsp60 (Musial and Zwolinska, 2011).

In this study we proposed that Hsp27 concentrations may be associated with the incidence of DN as a microvascular complication with various stresses in type 2 diabetic patients. Therefore, the relationship between serum Hsp27 levels and the risk for DN as well as its relation with different stages of DN were investigated. Serum Hsp27 concentrations were found to be significantly higher in individuals with risk for DN, as well as in DN patients, compared to diabetic control subjects. Investigation of serum HSP27 levels after sub grouping of the risk group showed that individuals with hypertension and with combined hypertension and dyslipidemia showed significant increase in serum HSP27 compared to diabetic control ones. This effect was not observed in individuals with dyslipidemia only which may suggest that hypertension may be related to an increased cellular expression of Hsp27 leading to the higher serum concentration.

Hyperglycemia and glomerular capillary hypertension were reported to be crucial determinants in the pathogenesis of DN. They produce cellular stresses on renal target cells which make them potential inducers of a stress response that may counterbalance the deleterious effects of these insults (Smoyer *et al.*, 1996). Barutta *et al.*, reported that diabetes and diabetes related insults differentially modulate HSP27, HSP60, and HSP70 expression/phosphorylation in the glomeruli and in the medulla which may affect the ability of renal cells to build up an effective cytoprotective response (Barutta *et al.*, 2008).

With regard to hypertension, Rossing *et al.* reported that baseline Systolic BP was one of the main predictors of GFR decline in patients with DKD (Rossing *et al.*, 2004). Furthermore, a recent study by Altemtam *et al.*, indicated that hypertension is a strong predictor for the progression of DKD (Altemtam *et al.*, 2011).The above findings are consistent with ours and support what was observed in our study regarding the elevated levels of HSP27 in serum of patients suffering from hypertension as a stressful risk factor for DN.

Additionally, findings of interventional and observational studies suggest that early improvement in BP control in patients with both T2DM and hypertension is important in preventing cardiovascular and renal complications in diabetic patients (Holman *et al.*, 2008). Oxidative stress is increased throughout the decline of kidney function (Shah *et al.*, 2007; Fujii *et al.*, 2011) which is manifested by accumulation of dysfunctional oxidized

proteins (Himmelfarb *et al.*, 2000), subsequently promoting inflammation (Iadecola and Alexander, 2001) and apoptosis (Mattson, 2006), thus leads to cardiovascular complications in uremic patients (Himmelfarb *et al.*, 2000; Fujii *et al.*, 2011). Moreover, this pathological condition may be intensified through the loss of extracellular reducing substances by the kidney, which leads to induction the heat shock response (Meyer and Hostetter, 2007). That response is indicated by an increased expression of HSPs at high stress levels in order to maintain cell integrity and minimize cell injury. HSP27 inhibits apoptosis by blocking caspase activation in addition to different antioxidant capabilities (Arrigo *et al.*, 2005). However, cell damage or necrosis can lead to the release of intracellular HSP and in consequence to elevated HSP serum concentrations (Srivastava, 2002). Due to increased systemic oxidative stress it is not surprising to find elevated serum HSP concentrations in patients with DN, released from disintegrated cells that alter HSP expression before death of the cells (Lebherz-Eichinger *et al.*, 2011). Increased serum HSP27 levels in patients with DN compared to diabetic control subjects was detected in the present research which agrees with the findings of the above studies. In our study the rise in serum HSP27 in diabetic hypertensive patients together with the elevated levels observed in established DN patients may suggest that the kidney cells suffering from hypertension, as a stress condition, increase expression of HSP27 as an adaptive response which helps podocytes to survive this stressor in addition to the other stressors implicated in the pathogenesis and progression of DN itself.

A clinical study concerning HSP27 and diabetic microvascular and /or macrovascular complications was performed. It was a cross-sectional sample of type 1 diabetic patients from the EURODIAB Prospective Complications Study. It was the first study measuring serum HSP27 in a large group of subjects and showed significantly greater age-adjusted HSP27 levels in cases with distal symmetrical polyneuropathy as well as with micro-macroalbuminuria, providing a proof that serum HSP27 levels are independently associated with distal symmetrical polyneuropathy in type 1 diabetic patients (Gruden *et al.*, 2008).

The present study is consistent with the above report concerning increase in serum HSP27 level in microalbuminuria patients; however our study showed that serum HSP27 levels were not age dependant. Nonetheless, the present research does not show a correlation between serum HSP27 concentration and other clinical and biochemical parameters, it should be taken into consideration that the natural history of CKD in T2DM is heterogeneous and is mainly associated with atherosclerosis (Plutzky *et al.*, 2002). The progression of CKD in T2DM extensively varies between individuals. Moreover, the risk factors that determine the prognosis of the disease have not been fully understood (Altemtam *et al.*, 2011). Confirmation of these findings by other prospective studies is warranted.

In conclusion, the present study showed increased serum HSP27 levels in DN patients suggesting that HSP27 may be related to DN. Furthermore, the rise of serum HSP27 was seen in diabetic patients with hypertension, a main risk factor implicated in the pathogenesis of DN, independently from any other common diagnostic marker for renal function. HSP27 may be an early marker for diagnosis of DN. Further studies using larger numbers of subjects are required to confirm these findings.

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