Evaluation of Heat Shock Protein-27 Levels as A Predictor of Diabetic Nephropathy in Early Diabetics

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Abstract

Objective: To evaluate the levels of heat shock protein-27 in diabetic patients with and without diabetic nephropathy, and to evaluate the functional role of Heat Shock Protein-27 as an early diagnostic biomarker in progressive diabetic nephropathy.

Methods: It was a case control study conducted in Physiology department from March 2019 to September 2020. A total 105 participants between 30-50 years of age were separated into three groups: Group A (n=35) diabetic-patients with history of diabetes < 5 years without any sign of diabetic nephropathy (DN); Group B (n=35) diabetic patients with history of diabetes>5 years' duration of diabetes and microalbuminuria< 30 mg/dl in urine and Group C (n=35) healthy individuals. All participants after given informed written consent, had undergone detailed history and clinical examination, anthropometric measurement, body mass index measurements. Evaluation of serum blood sugar, serum urea, serum creatinine, BUN, urinary albumin, and serum HSP-27 was also estimated

Results: Highly significant elevated levels of HSP-27 were observed in group-A& B participants as compared to Group-C ($p<0.001$). It indicates early renal parenchyma injury. Strong association had been observed for HSP-27 with creatinine ($r=0.69$; $p < 0.001$), and HbA1c ($r =0.72$; $p <0.001$) and urea ($r = 0.72$; $p <0.001$) in all these participants. While statistical highly significant and strong positive association were reported for HSP-27 with microalbumin ($r= 0.86$; $p< 0.001$) in all participants.

Conclusion: Elevated levels of HSP-27 with progression were observed before declining of renal function is seen in current study. These findings suggest that HSP-27 can be used as key diagnostic marker for diabetic nephropathy in early diabetics.

Keywords: Diabetes Mellitus, Diabetic Nephropathy, HSP-27.

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Introduction

Diabetic nephropathy (DN), is one of the major complication of diabetes mellitus and also called as diabetic renal disease (DRD). The conventional explanation of DN is a progressive increase in excretion of urinary albumin followed by rising in blood pressure that leads to hypo glomerular filtration and finally end stage renal disease (ESRD). More importantly, kidney is a vital organ in the body and its function declines during uncontrolled hyperglycemia crisis, which ultimately gives structural and functional variations to renal tissue[1]. Indeed, the pervasiveness of DN is about 50% of diabetic patients can leads to irreversible renal damage lifetime, and approximately 16% of adult populations had been affected by this chronic disease which if not addressed timely can lead to ESRD. Around the world about100 million deaths per annum had been documented due to ESRD[2,3]. Precisely in Asian subcontinent, incidence of diabetic renal injury is mounting rapidly and accounted approximately 18% of finan-

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cial burden universally[4]. DN progression is inconsistent. The key modifiable risk factors are high blood pressure, uncontrolled glycemic status, and hyperlipidemia. Different studies have postulated strong association of smoking with DN, and it is more likely to developed in patients who have family history[3-4]. A clinical symptom that appears in DN is frequent episodes of burning micturition, urinary tract infection, micro-albuminuria that is followed by macro-albuminuria, pedal edema with generalized weakness and easy fatigability and at last escalation of urea / creatinine ratio[3]. Pakistan is one countries of Asian sub-continent that have increased number of cases of DN and were reported around 24.2%[2]. Principally, pathogenesis of DN starts from hyper-filtration with constriction of efferent arterioles, dilation of afferent arterioles, thickening of basement membrane, widening of podocytes gap and hypertrophied mesangial matrix, finally which upon increase in duration of diabetes leads to hypo-filtration. Cellular, endothelial cells, podocytes of glomerulus and mesangial cells, all three cells take part in pathogenesis of DN. At molecular level activation of protein Kinase-C enzyme system and diacylglycerol formation and uncontrolled production of advanced glycosylation end products (AGEs) are anticipated and are the utmost key trigger for development of DN[5]. Other important mechanisms of kidney injury in diabetes comprise hemodynamic modification such as podocyte and mesangial cell hypertrophy with glomerular hyper-filtration, metabolic changes, and finally micro-albuminuria[6].

Prognosis and diagnosis of DN clinically depends on conventional markers, for instance, albuminuria and estimation of glomerular filtration-rate (e-GFR) for diagnosis of DN. The gold standard test is renal biopsy but due to invasive procedure it is not commonly used. Importantly, estimation of serum urea and serum creatinine levels showed renal injury clinically but when 50% of kidney had been damaged. Paradoxically, micro-albuminuria, a renal biomarker was being used for >30 years as a diagnostic tool to assessed renal dysfunction. But now a day, its sensitivity is reduced clinically, because most of the patient's progress to early phase of DN even having no albuminuria. As a result, pathogenesis of DN progress slowly and silently and early phase of DN could be easily missed[7-8]. However, hyperglycemic renal disease emerges before the instigation of micro-albuminuria[9]. The estimated glomerular filtration rate (eGFR) is frequently used to analyze and categorize kidney function[10], but its prognostic accuracy is insensitive and nonspecific. Apart from these; traditional procedures i.e. estimation of serum urea, creatinine and nitrogen are regularly used but all were nonspecific and insensitive, especially in terms of early detection of acute renal-injury (ARI). This paradigm lifts the imperative necessity for novel and new biomarkers which can accurately detect early renal injury[11-12].

HSP-27 is an important intracellular member of protein family. It is formed by renal cells during the time of stress, oxidation and hypoxia etc. Structurally, HSP-27 has 205 amino acids that is arranged in sequence from 1-38, 87 -167 and 179 -182 as N -terminal, α -crystalline domain and C -terminal respectively. Physiologically, HSP-27 is present about 1 -2% in cells but upon phosphorylation its expression increases about 12 fold. Functional part of N -terminal starts from 16 -19 and called as WDPF domain and this domain is called as Trp-Asp-Pro-Phe. Additionally, C-terminal and α-crystalline plays crucial role in phosphorylation, enzymation and is the terminal part of HSP-27 while α-crystalline is in central position which on connection with C-terminal forms small dimer after large oligomerization and phosphorylation of HSP-27. Functionally, major function of HSP-27 is to maintain and balanced cellular environment static and engage cell survival at toxic environment. Intracellularly, HSP-27 controls major proteins function i.e., transcriptional and translational protein and protein kinases. More importantly, HSP-27 protects cell cytoskeleton with F-actin topping action, encourages nitric oxide (NO) formation, and inhibits apoptosis and degradation of misfolded and abnormal proteins. Additionally, Heat shock-protein works as a chaperon that can coordinate misfolded protein in intracellular milieu. Additionally, HSP-27 contributes a key role in cellular

protection during oxidative stress and heat shock circumstances[13]. Moreover, it controls the biosynthesis of misfolded proteins, assembling, and transportation to other cellular proteins and prevents aggregation of abnormal misfolded proteins. Synergistically, HSP-27 along with glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) and glutathione combat oxidative stress[14].

Various studies have evidently reported the over expression of HSP-27 in oxidative stress milieu in renal parenchyma. The renal corticomedullary milieu is conceivably the most persistent area of stress and it is 10 times more exposed to stress than other body organs. More importantly, this abnormal oxidative modification is constantly lethal for entire body organs precisely renal tissue[15]. Hyperglycemia is a primary stimulator and propels the renal tissue towards cellular stress. HSP-27 expression in renal parenchyma more than in tubules of kidney especially when exposed to hyperglycemic status. Molecularly, HSP-27 express in renal podocytes is through p -38 glomerular -8- dependent system which injure podocyte foot-plates and as a result lead to DN. Physiologically, minute quantity of HSP-27 is reported to escape toexternal environment but astonishingly its expression is distinctly increased in renal injury16.Indeed, during oxidative stress, renal cells experience structural modifications, which are relatively different from normal structural design, and causes increased formation of HSP-27[17]. Diabetic induced-nephropathy is an important complication of diabetes mellitus which if untreated can lead to end stage kidney disease (ESKD). In hyperglycemic instigated oxidative pressure can prompt renal framework injury, which clearly raises over-articulation and synthesis of HSP-27. In people, the encoding quality of HSP-27 is situated on chromosome 7 (7q 11,23) which give expression to HSP-27 through transcriptional process. In continuation, an extremely enormous expression of HSP-27 has been accounted in diabetic instigated nephropathy A trial-based review has shown that HSP-27 overexpression could be a better non-intrusive biomarker in DN[13,17].

The objective of the study was to find out the levels of heat-shock protein-27 in healthy and diabetic patients of less than 10 years. of diabetes, and to appraise role of HSP-27 as early diagnostic biomarker in initial phase of diabetic nephropathy.

Patients and Methods

This is case control study and conducted in department of Physiology after the approval of Ethical Review Board. All 105 participants between 30-50 years of age from the Diabetic clinic of Medicine ward 7from March 2019 till September 2020.Whereas patients having chronic disease (cardio-vascular, immunological etc.), uncontrolled hypertension, duration of diabetes >10 years, patients taking nephrotoxic, anti-inflammatory and anti-cancer drugs and on dialysis were excluded from current study. All participants were informed regarding study and had given written and pre-requisite consent. All study participants were separated into three groups: Group A (n=35) diabetic patients with duration of diabetes < 5 years without sign of DN; Group B (n=35) diabetic patients with diabetes >5 years of duration and urinary micro-albumin levels < 30 mg/dl and Group C (n=35) healthy individuals. Blood samples of all participants had been collected in early morning after 8 hours overnight fasting at baseline. Serum was segregated and was used to estimate serum blood sugar, HbA1c percentage, urea/creatinine levels and HSP-27 levels were estimated through ELISA sandwich procedure (Bioassay technology Laboratory, Catalog no: E1786Hu). Random urine was obtained from dipstick urine albumin test at baseline.

For data analysis SPSS version 21.0 software was used. For continuous variable descriptive analysis had been done. For continuous variables data i.e., anthropometric (age, weight, height, BMI) clinical variable (blood-pressure) and biochemical Blood sugar (FBS, RBS), urea/creatinine, and urinary micro-albumin and serum HSP-27 etc.) variables were displayed as mean ± standard deviation (SD). Analysis of variance (one-way ANONA) with post-hoc Turkeys and Scheffe test were applied for com-

parison among groups. Pearson-coefficient of correlation (r) had been applied to correlate the levels of HSP-27. And p-value <0.05 was taken significant.

Results

Mean age, weight and BMI of groups A, group B and group C were significant higher (p<0.01) while mean of height of all groups were not statistically significant (p>0.79). Levels of FBS, RBS, and HbA1c were significantly elevated in diabetic patients as compared to controls (p< 0.001). Levels of serum urea, serum creatinine and BUN were significantly elevated in groups A and B (p<0.001) as compared to controls. The mean values of serum HSP-27of study participants were shown in Table-II. Higher levels of HSP-27 at baseline were reported in patients with >5 years of duration of diabetes as compared to controls and patients with <5 years duration of diabetes(p<0.001). When we compared and correlate the levels of HSP-27 amongst group A, group B and group C: growing trend had been seen for HSP-27 in the absence and presence of microalbuminuria. This shown that renal function was continuously pretentious and there was injury to the kidney itself. Strong association were reported for HSP-27 with creatinine (r=0.69; p < 0.001), and HbA1c (r =0.72; p <0.001) and urea (r = 0.72; p <0.001) in all participants. Whereas highly strong and positive association were seen for HSP-27 with micro-albumin (r = 0.86; p< 0.001) in all participants.

Discussion

In view of the terrifying acceleration in the numbers of cases with diabetes mellitus (DM) in non-industrialized nations, there is also an unprejudiced mounting numbers of patients with diabetes nephropathy (DN). It is one of the main medical care issues that builds the financial burden and as well as obliterates others family members from a normal life18. Despite assessable and praiseworthy endeavors and achievement of medico-clinical science, there is a growing recurrence and rate of DN, tremendous costs of clinical consideration and with helpless anticipation is one of the key difficulties. This current study revealed the clinical significance of serum HSP-27 as an early additional diagnostic bio-marker in diabetic nephropathy. The present study also evaluates the association and correlation of serum HSP-27 to various laboratory investigations amongst diabetic patients. This appearance in rise of detectable range of serum HSP-27 evidently showed the development of diabetes with increase in duration of disease, there is interruption of kidney performance and escalation of severity of kidney inflammation (nephropathy).

Table 1. Baseline status of study participants

Variables	Group A (n=35)	Group B (n=35)	Group C (n=35)	p-value
Age (Years)	43.5 ± 6.5	46.8 ± 6.4	37.2 ± 4.7	<0.01*
Weight (kg)	78.1 ± 5.9	83.1 ± 6.7	70.8 ± 4.6	<0.01*
Height (cm)	5.9 ± 0.2	5.9 ± 0.1	5.8 ± 0.3	0.79
BMI (kg/m2)	25.0 ± 2.0	26.4 ± 1.9	23.7 ± 1.9	<0.01*
FBS (mg/dl)	152.6 ± 15.9	169.6 ± 19.8	82.5 ± 9.3	<0.01*
RBS (mg/dl)	215.3 ± 18.6	257.0 ± 29.6	118.4 ± 11.2	<0.01*
HbA1c (%)	6.9 ± 0.8	7.5 ± 0.5	4.9 ± 0.1	<0.01*
Serum Urea (mg/dl)	32.0 ± 7.2	44.0 ± 9.6	23.8 ± 7.4	<0.01*
Serum Creatinine (mg/dl)	0.97 ± 0.1	1.0 ± 0.1	0.8 ± 0.4	<0.01*
Blood Urea Nitrogen (gd/dl)	14.95 ± 3.38	20.57 ± 4.52	11.15 ± 3.46	<0.01*

Table 2. HSP-27 and urinary microalbumin levels of study participants

Variables	Group A (n=35)	Group B (n=35)	Group C (n=35)	p-value
Microalbumin (mg/dl)	15.46 ± 2.9	50.9 ± 8.2	00 ± 00	<0.01*
HSP-27 (ng/dl)	117.60 ± 14.50	230.46 ± 23.75	4.97 ± 2.81	<0.01*

The levels of HSP-27 in diabetic patients with a shorter duration of diabetes in group-A in current study and higher levels of HSP-27 in Group-B had

shown the development of kidney damage with increase in duration of disease. Diabetes causes kidney dysfunction that remains undiagnosed many years. By determining the levels of HSP-27 with duration and degree of severity of diabetes in study participants; we were capable to illustrate that levels of HSP-27 were elevated with the progression and development of kidney disease even though all renal profile i.e., serum urea, creatinine and BUN were within the normal physiological range. This verdict suggests that as the renal injury progressed, over and abnormal HSP-27 is released; hence, HSP-27 levels could be used as an additional diagnostic tool in initial silent stage of DN in early diabetic patients.

Additionally, different notable research studies have revealed that HSP-27 is one of the glomerular damage biomarker and are prominently high in diabetic patients; even with normal albuminuria in that patients[17-19]. This highlights that serum HSP-27 is an additional diagnostic tool in detection of DN in early phase of diabetes even before the progression of albuminuria[9,19,20]. thus serum HSP-27 possibly recommended as an early biomarker in initial phase of diabetic nephropathy. Importantly, in current study, HSP-27 levels had shown positive and strong association with serum urea, serum creatinine, an important renal function marker, at baseline (r= 0.728; p-value = 0.01). It is hypothesized that HSP-27 is not a only renal biomarker but also a predictor for the development of nephropathy in early phase[21-22-23-24]. More importantly, moderate and positive association had been seen for HSP-27 with age (r=0.288; p-value = 0.001 and duration of diabetes (r=0.290; p-value = 0.01). It was being noticed by different studies as the duration of diabetes increases the frequency of adverse complications of diabetes and its prevalence25SerumHSP-27 may turn into as valuable predictor, specific novel and new biomarker for early detection of kidneys functions in the type 2 diabetes mellitus patients.

The limitation of the study that we didn't performed renal biopsies to validate the severity of renal injury and also was in capable to enlist participants with >10 years of duration of diabetes. The current study also has small sample size and the results that have been obtained could not be applied in all large population. Further research has to be done to explore the role of HSP-27 in DN. The current research and review has reached to the determination that serum HSP-27 is a profoundly explicit indicator, valuable and novel biomarker for early detection of declining kidney function in the diabetic patients. Diabetic kidney damage starts prior to the onset of micro albuminuria, so further biomarkers are needed to diagnose the kidney damage as earliest to find out early therapeutic options. The current study revealed that HSP-27 elevates in early phase of diabetic nephropathy before the commencement of micro albuminuria and derange renal profile levels i.e. serum urea and creatinine. Therefore, it is recommended that HSP-27 can be used as a potential and additional biomarker to detect early silent stage of diabetic nephropathy.

Conclusion:

Elevated levels of HSP-27 with progression were observed before declining of renal function is seen in current study. These findings suggest that HSP-27 can be used as key diagnostic marker for diabetic nephropathy in early diabetics.

Conflict of Interest

Authors have no conflict of interest and no grant/funding from any organization.

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