ORIGINAL ARTICLE

THE DICHOTOMY BETWEEN HEAT SHOCK PROTEIN-27 AND MICROALBUMIN: COVARIATE OF EARLY DIABETIC NEPHROPATHY

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Background: Heat shock protein-27 is the microprotein released from renal parenchyma during diabetic oxidative stress, while microalbumin is the plasma protein that appears in urine in diabetic nephropathy. **Methods:** This case-control study was conducted from Jan to Sep 2021 in the Physiology department, BMC, BMCH, Quetta. The current study included 105 patients with an age range from 30–50 years and was divided into three groups: i) a control group of healthy participants, ii) a diabetic risk group: participants without signs of diabetic nephropathy and diabetic duration from 1–5 years, iii) diabetic nephropathy group: participants having >30 mg/dl of v microalbumin in urine and diabetic duration from 5–10 years. **Results:** There were significant mean differences between all groups concerning anthropometric measurements except in height amongst all groups. Statistically significant mean differences were seen in the risk and nephropathy group concerning serum FBG, RBG, and $HbA1_c$. Elevated microalbumin levels in the diabetic nephropathy group (50.9±8.2) compared with the diabetic risk group (15.4±2.9). Similarly, higher levels of HSP-27 were seen in the diabetic nephropathy group (230.46±23.75) as compared with the diabetic risk group (117.60±14.50). **Conclusion:** HSP-27 is a better biomarker than microalbumin and may show early glomerular injury in the early diabetic stage of diabetic nephropathy.

Keywords: Diabetes mellitus; Microalbumin; Diabetic Nephropathy

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome distinguished and recognized by hyperglycemia in the absence or lack of treatment. More importantly, its pathology comprises deficiency in insulin secretion, its action, or sometimes both. It also contributes to disorders of carbohydrate, protein, and fat metabolism. The longstanding complications of DM were nephropathy, retinopathy, and neuropathy.[1] In diabetic patients, kidney disease has been initially distinguished by increased urinary albumin excretion with slow advance to overt albuminuria and advancement toward end-stage kidney disease (ESRD).[2]

Heat Shock Protein-27 (HSP-27) has its place in the protein family created by renal cells throughout stress, hypoxia, redox oxidation, etc. Its great capacity is to support cell homeostasis, upgrading cell endurance during harmful climates. Intracellularly, HSP-27 regulates significant proteins, for example, transcriptional proteins and protein kinases. HSP goes about as an escort that synchronizes mis-collapsed protein in the atomic milieu. HSP-27 performs an essential job in cell opposition during stress and hotness shock conditions.[3] It also controls unfurled proteins' biosynthesis, gathers, transports cell proteins, and turns away the conglomeration of harmed mis-collapsed proteins. Synergistically, HSP-27, glutathione, and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH) battle oxidation stress.[4]

Albumin is one of the most binding plasma proteins in the body, which is principally produced in an amount of 13.9 grams/day by the liver and has a half-life of around 19 days. The plasma level of albumin is approximately 35–50 mg/dl. Interestingly, albumin is well-regulated by the kidneys and has very important diagnostic value regarding kidney function. Therefore, it is also called the "Marker of Kidney disease".[5,6] Structurally, albumin has 3 homologous domains I, II, and III. Proteinuria is the term that reflects abnormal signs of protein discharge from the kidneys. Nevertheless, the utmost copious protein found in that urine is albumin. In normal individuals, very small amounts of proteins are likely to ultrafiltrate in the

glomerulus. However, only a tiny amount appears in urine.[7] Albuminuria is a pathological and subclinical condition exclusively used to reflect abnormal excretion of albumin in urine.[8,9] More importantly, a study by Thakur *et al*[10] has postulated that the prevalence of microalbuminuria in Pakistan of about 31.56%. Diabetic nephropathy (DN), resulting from microalbuminuria, is a significant cause of morbidity, premature mortality, end-stage renal disease, need for renal replacement therapy, and escalating healthcare costs in diabetic patients.[9] Along with the diabetes epidemic, DN prevalence is increasing rapidly. A third to a half of diabetic patients experience renal manifestations.[10] Due to delayed diagnosis, limited screening and diagnostic resources, poor glycaemic control, and inadequate treatment of microalbuminuria, DN is more prevalent in Africa than in developed nations.[11,12] This current study was hypothesized to find out the role of microalbuminuria among diabetic patients in early diabetics and to identify early diabetic changes in the renal glomerulus. The objective is to estimate microalbumin levels in early diabetics with and without diabetic nephropathy.

MATERIAL AND METHOD

After getting the approval of synopsis and Ethical permission for the current study by the Institutional Review Board (IRB) of Basic Medical Sciences Institute, JPMC, Karachi, data was obtained and was kept extremely confidential. The permission for data collection The data was collected from patients fulfilling the inclusion and exclusion criteria the verbal and written consent was sought from all participants. Only consenting participants were included in the study.

A case-control study was conducted in the Department of Physiology, in collaboration with the diabetic ward in BMC, BMCH, Quetta, from January to September 2021. Patients between 30–50 years of age, of both genders, of diagnosed cases of type-2 DM for control, risk, and nephropathy groups were included in the current study. Inclusion criteria for study members are the duration of diabetes from 1–10 years, with and without diabetic nephropathy, and healthy individuals for controls. The exclusion criteria were hypertension-induced nephropathy, urinary tract infection, renal disease, analgesic abuse, and diabetes duration >10 years. The sampling technique included non-probability purposive sampling, all 105 participants, 35 in each group met the inclusion criteria and were selected for the study. All participants underwent a detailed disease history described by the questionnaire, consisting of age, gender, family history of diabetes mellitus and hypertension and other comorbidities, interval of diabetes, blood sugar, and renal parameters, which were calculated using standard operative procedures. Two average measurements recorded weight and height. The weight was recorded in kilograms while the height was recorded in centimeters using identical techniques, and later the body mass index (BMI) was estimated. A manual mercury sphygmomanometer measured blood pressure, and an average of two readings was selected for the analysis. Other parameters, i.e., serum fasting blood sugar (FBS), random blood sugar (RBS), HbA1c, serum urea, and serum creatinine, were recorded according to standard operative procedures. Assessment of microalbuminuria by mid-stream urine spot test was wholly explained to all participants who were given two separate containers. The participants were guided to provide two separate urine samples to screen urinary albumin excretion subsequently excluding all factors that can increase the urinary albumin levels beyond normal.[11] HSP-27 levels were measured using an ELISA kit (sandwich procedure) (Bioassay Technology Laboratory, Catalog no: E1786Hu). The analyses were performed by using SPSS version 22. Chi-square and independent t-tests were used for data feeding and analysis. For continuous variables, mean, and standard deviation was used. One-way ANOVA and Pearson correlation of coefficient (r) was used to correlate the data, and a *p*-value >0.05 was well-thought-out statistically significant.

RESULTS

Next applying the inclusion and exclusion criteria, 105 individuals were included in the current study. The mean age was 43.51±7.09. There were 60 (57.11%) males and 45 (42.9%) females. The BMI of the diabetic nephropathy group was 26.4±1.9, which was higher than the risk group's 25.0±2.0 and statistically significant with a *p*-value of 0.05. Fasting and random blood sugar levels of the risk group were 152.6±15.9 and 215.3±18.6, respectively, and the nephropathy groups were 169.6±19.8 and 257.0±29.6, respectively. Uncontrolled fasting and random blood sugar levels have shown a significant correlation and positive association with microalbuminuria (r=0.78) with a p-value of 0.001.

Microalbuminuria was significantly higher in the nephropathy group (50.9±8.2) than the risk group (15.4±2.9), but on the other hand, the risk group showed higher levels of microalbuminuria than the control group. The mean level of HSP-27 was significantly increased in the nephropathy group (230.46±23.75) compared to the risk group (117.60±14.50). On the other hand, the risk group had shown higher levels of HSP-27 (117.60±14.50) than the control group (4.97±2.81), which revealed early modification in the renal parenchyma, especially in the glomerulus.

After comparing the correlation and association of microalbumin levels with HSP-27, there are significantly higher levels of HSP-27 in the risk group (r=0.72) than the control, and much-elevated levels in the nephropathy group (r=0.91) than the risk group with a *p*-value 0.001, shown in table 03.

Table-1: Anthropometric measurements & Glucose profile of study participants

Variables	Control Group (n=35)	Diabetic Risk Group (n=35)	Diabetic nephropathy Group (n=35)	*p*-value
Age (Years)	37.2±4.7	43.5±6.5	46.8±6.4	<0.01*
Weight (kg)	70.8±4.6	78.1±5.9	83.1±6.7	<0.01*
Height (cm)	5.8±0.3	5.9±0.2	5.9±0.1	0.79
BMI (kg/m^2)	23.7±1.9	25.0±2.0	26.4±1.9	<0.01*
FBS (mg/dl)	82.5±9.3	152.6±15.9	169.6±19.8	<0.01*
RBS (mg/dl)	118.4±11.2	215.3±18.6	257.0±29.6	<0.01*
HbA1c (%)	4.9±0.1	6.9±0.8	7.5±0.5	<0.01*

Table-2: Renal and Microalbumin & HSP-27 levels of study participants

Variables	Control Group (n=35)	Diabetic Risk Group (n=35)	Diabetic nephropathy Group (n=35)	*p*-value
Serum Urea (mg/dl)	23.8±7.4	32.0±7.2	44.0±9.6	<0.01*
Serum Creatinine (mg/dl)	0.8±0.4	0.97±0.1	1.0±0.1	<0.01*
Blood Urea Nitrogen (gd/dl)	11.15±3.46	14.95±3.38	20.57±4.52	<0.01*
Microalbumin (mg/dl)	00±00	15.46±2.9	50.9±8.2	<0.001*
HSP-27 (ng/dl) Mean±SD	4.97±2.81	117.60±14.50	230.46±23.75	<0.001*

Table-3: Association of HSP-27 with renal parameters

Variables	Diabetic Risk Group (n=35)	Diabetic nephropathy Group (n=35)	*p*-value
Microalbumin (mg/dl)	15.46±2.9	50.9±8.2	<0.001*
HSP-27 (ng/dl)	117.60±14.50	230.46±23.75	<0.001*

DISCUSSION

In a current study, we have understood an excessive incidence of microalbuminuria in early diabetic patients who have 1–5 years of disease duration. A current study revealed that approximately 50% of participants had microalbuminuria. Martin *et al*[11] and Sarafidis *et al*[12] also endorsed the relation of microalbumin with the duration of the disease. In another study, Ahmad *et al*[14] also revealed the current study that microalbuminuria has variations in uncontrolled glycaemic status. Conversely, higher levels of microalbuminuria were seen in the nephropathy and risk groups. When we compared and correlated it, we found a rising trend as the duration of the disease progressed levels of microalbuminuria appeared in urine even though renal parameters (urea and creatinine) were within the normal range. Arus *et al*[15] and Sinkala *et al*[16] also found higher levels of microalbuminuria with normal serum urea and creatinine, have corresponded with other studies.

Another current study showed that microalbuminuria had a significant and positive correlation with disease duration, fasting and random blood sugar, $HbA1_C$, and the association of duration of diabetes mellitus and microalbumin had been well documented and published in previous papers. Kiconco *et al*[17] and Afkhami *et al*[18] have given the logical explanation and correlation of microalbuminuria and increased duration of diabetes to be due to the poor glycaemic status in blood. Huang *et al*[19] had similar findings and reported a positive association of FBS, RBS, and $HbA1_C$ with microalbuminuria.

In a current study, it had also been shown that expression of HSP-27 had a significantly higher and positive association with microalbumin. Still, the expression of HSP-27 appears much earlier than the microalbumin appearance. The association of HSP-27 and microalbumin has been well documented and revealed that HSP-27 is a much better biomarker for detecting diabetic nephropathy in its early stages. More importantly, Mahgoub *et al*[20] and Mather *et al*[21] also has similar results, revealing the early expression of HSP-27 in diabetic patients. Additionally, Jakhotia *et al*[22] endorsed the expression of HSP-27 appearing much earlier in the early phases of diabetic nephropathy. Irfan *et al*[23] also reported that when the renal profile, i.e., serum urea, creatinine, and BUN, were all surrounded by the normal physiological ranges, the levels of HSP-27 were elevated with the advancement and enhancement of kidney disease. This suggests that an abnormal amount of HSP-27 is released throughout the progression of kidney disease. The respective levels can indicate the degree and severity of the DN; there is a pause of kidney act and an escalation of the severity of kidney inflammation (nephropathy). It is further ascertained that HSP-27 can be used as an investigative tool in the early silent stages of DN in early diabetic patients. HSP-27 is a renal biomarker and a predictor for the development of nephropathy in early phases.[23]

Gruden *et al*[24] I suggest that the overexpression of HSP-27 may be planned to prevent the neurological harm caused by DM in diabetic patients. Tikoo *et al* found that constitutional alteration of intracellular protein assembly, turnover, and appreciation results in an accumulation of abnormal proteins in the kidney and other tissues. The HSP-27 changes in fibroblasts might reflect those in the kidney and be pathophysiologically related to the

development of DN.[25] Pourhamidi *et al*[26] also found that comorbid states tend to alter the levels of HSP-27 and that when the levels of HSP-27 are to be measured in humans, comorbid states, such as CVD, should also be considered. The restriction of this study is that it was a single-center study with a small sample size, meaning that these results cannot be applied to a large population. Moreover, we could not enlist any participant with a >10 years of duration of diabetes. In any case, however, it is conspicuous that HSP-27 serves a dual role, both as a renal biomarker and predictor, for DN, and it can be used to detect nephropathy during its early stages.

CONCLUSION

Elevated levels of microalbumin and HSP-27 in the nephropathy group, as compared with the risk groups, have revealed an early prevalence of DN as a microvascular complication in type 2 diabetes mellitus. HSP-27 expression, however, appears much earlier than microalbumin. Thus, it concluded that HSP-27 is a better biomarker than microalbumin and perhaps shows early glomerular injury in an early diabetic stage of diabetic nephropathy.

AUTHORS' CONTRIBUTION

IM did the literature review and manuscript writing. FA designed the methodology. AA assisted in critical review and editing. FMU contributed to the critical review. FA did manuscript writing, and SM performed an Analysis. All authors approved the final manuscript for publication.

Ethical approval

All procedures were by the ethical standards of Helsinki's declaration after being approved by the ethical committee of Bolan Medical College, Quetta, Pakistan NO. IRB-1/ESTB/2021/BMC/2-04), Pakistan.

Patient consent: written and verbal consent was obtained for the research.

Competing interest: The authors declare that they have no conflict of interest.

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