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Research Article

Association of Serum Protein Levels in the Diabetic Patients with Risk of Cardiovascular Disease and Nephropathy in Pakistani Population -

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ABSTRACT

Albuminuria has been recognized as a marker for prognosis of renal and cardiovascular risk in diabetic patients. Role of microalbuminuria in cardiac disease and nephropathy has not been surveyed in Pakistani population and its foretelling importance in diabetic individuals is undetermined. In this study we examined the relation between microalbuminuria, HbA1c and serum albumin levels in association with diabetes in population of Pakistan based on equal number of male and female subjects with and without prevalent baseline diabetes. We found that increased levels of micro albuminuria are associated with cardiovascular disease, HbA1c with nephropathy and serum albumin with cardiovascular disease, nephropathy and hypertension in the diabetic patient.

Keywords: Diabetes; Microalbuminuria; Serum Albumi; Pakistani Population; Lahore; Punjab University.

INTRODUCTION

Hyperglycaemia is a significant characteristic of diabetes, a condition which results from alteration in insulin secretion or its action or defect in both. Hyperglycaemia in this metabolic disorder is related with lasting malfunction of different organs, for example, eyes, blood vessels, heart, nerves, and kidney [1,2].

In 2017, 451 million people were estimated to be affected with diabetes worldwide and the figures are expected to raise to 693 million by 2045. 49.7% people are estimated to be living undiagnosed and approximately, 5 million deaths were caused by diabetes in 2017 [3].

Diabetes is increasing worldwide at an incredibly alarming pace. With the prevalence of 8.3%, 387 million people are currently and by 2035, 592 million people will have diabetes. It is estimated that 316 million people exhibiting glucose intolerance have chances to develop diabetes mellitus type 2 [4].

The diagnostic criteria for diabetes depends on one out of these plasma glucose standard: increased (i) Plasma glucose during fasting (FPG) (≥126 mg / dL), (ii) Haemoglobin A1c (HbA1c) ≥ 6.5% (iii) Oral glucose tolerance test (OGTT) ≥ 200 mg / dl) (iv) typical symptoms of hyperglycemic crisis with random plasma glucose levels ≥ 200 mg / dl. Complications, like macrovascular diseases (e.g., cardiovascular and cerebrovascular diseases etc.) and microvascular diseases (e.g., nephropathy, neuropathy and retinopathy etc.) are linked with elevated HbA1c levels [5].

A major protein part of blood serum is Human Serum Albumin (HSA) that carries many essential metabolites drugs and its glycation is involved in biological abnormalities. Several studies suggest its glycated form to be a marker for diabetes [6].

Microalbuminuria is the condition resulting from hyperglycation of HSA. More than 30 mg/g is the most common used clinical indication of diabetic nephropathy's elevated risk. In diabetes it is a marker for monitoring treatment and prediction of risk [7]. DN is the major cause of deaths due to renal failure and cardiovascular disease and is characterized by increasing albuminuria (>300 mg / day) and declining renal function. Also, microalbuminuria has been the most acknowledged way for the evaluation of early renal injury in diabetes [8]. This study demonstrates the significant relationship between increased levels of micro albuminuria and serum albumin with type 2 diabetes.

MATERIALS AND METHOD

Patient Selection

A 100 confirmed type 2 diabetic patients were selected and 100 control individuals. Detailed disease histories, blood pressure, fasting sugar level of all patients were recorded with their consent.

Inclusion criteria for patient's includes female and male both subjects, have diabetic symptoms, have micro albuminuria (30-300 mg / 24h) and blood pressure ≤ 130 / 80. Exclusion criteria include subjects with alcoholic abuse, with infectious diseases, with psychiatric disorder and pregnant women.

Sample collection

Urine and blood samples were collected in morning. A 2-3c.c blood was drawn from median cubital vein of the patient and stored in sterile EDTA vials for separation of plasma and in vials without having anticoagulant for separation of serum separately at -20°C. Also, 24hrs urine from the patients and controls were measured and concentrated with freeze drying method (lyophilisation) stored at -20°C and then analysed. Sampling was done during the period of October 2014 to December 2014.

Estimation of physical parameters

Age, weight and height of 200 subjects (100 controls and 100 diabetic) were recorded and BMI was determined using formula

$$BMI = \text{Weight in kg} / (\text{Height in meters})^2$$

Estimation of biochemical parameters

Different biochemical parameters were assessed for all selected diabetic and control samples as below.

Estimation of total proteins: Total serum protein was estimated by Bradford assay kit (ab102535) and Biuret protein assay using standard protocol. Total protein content was estimated for all 200 samples

Estimation of blood glucose: Glucose Assay Kit (ab65333) was used to quantitatively determine glucose in serum of both control and diabetic samples.

Determination of urine creatinine: Urine creatinine was estimated by the Creatinine Assay Kit (ab65340) from urine samples.

SDS-PAGE: Proteins were separated using SDS PAGE analysis. It is a procedure used for separation of proteins by electrophoresis, a method to separate proteins with discontinuous polyacrylamide gel and sodium dodecyl sulphate (SDS). The samples of all 200 individuals, 100 of diabetic patients and 100 of control were analysed by SDS-PAGE [9].

Statistical analysis

Two tail T-test, mean and ANOVA was applied using excel sheet and IBM-SPSS.

RESULTS AND DISCUSSION

In this study, the samples were initially analysed for the physical parameters: age, weight, height and BMI of all individuals. Two study

groups were formed, normal control individuals were enlisted in Group 1, diabetic females and diabetic males in Group 2. Biochemical parameters like total protein estimation, creatinine, serum albumin, MAU, HbA1c levels were estimated for selected individuals.

The average BMI of diabetic subjects (31.92 $lbft^{-1}$ ± 6.15) was greater than control (22.81 $lbft^{-1}$ ± 5.10) and shows positive significant ($p < 0.05$) (table 1) when statistical rules were applied, which means that higher the BMI higher the chances of having diabetes and complications. These results are in accordance with Bays *et al.*, 2007 that worldwide presence of hypertension and diabetes mellitus exist in all age groups but it is more likely to occur in individuals with higher BMI [10]. Previously, increased BMI increases the risk of developing diabetes type 2 [11]. The average Blood Glucose levels of control is 94.52 ± 19.45 while for diabetic subjects is 196.49 ± 80.65 which shows statistically significant correlation showing an elevated blood glucose levels of diabetic patients as compared with control individuals($p < 0.001$) (Table 1).

The average HbA1c levels of control subjects is 5.24 % ± 0.89 while of diabetic subjects is 8.39 % ± 1.31 which shows statistically significant correlation ($p < 0.05$) indicating HbA1c is directly related to diabetes (table 1). As concluded in the previous paper that it is a key marker of the glycaemic control. Levels HbA1c are also associated with complication in diabetes [12]. As shown in another paper HbA1c is the standard test to determine the linkage between cardiovascular risk, nephropathy and glycaemic control [13]. In our study, HbA1c levels also show association with BMI ($p < 0.008$), *age* ($p < 0.002$) and nephropathy ($p < 0.001$) of diabetic individuals > (Table3).

Microalbuminuria levels had been associated with age, duration of diabetes and cardiovascular diseases. Increase age and duration of disease are positive factor for diabetes. In type 2 diabetes its prevalence is 37% [14]. In our study, microalbuminuria average levels for diabetic individuals is 33.52 mg / dL ± 9.05 while for control subjects is 26.84 mg / dL ± 4.86 which shows statistically significant correlation ($p < 0.05$) (table 1). Also, there is significant association of age, duration of disease, blood glucose level and cardiovascular disease ($p =< 0.001$) of diabetic individuals and their microalbuminuria level. This association has been reported in the previous studies discussed above (Table 2).

Average levels of serum albumin for diabetic individuals was lower 2.16 ± 0.95 than control subjects is 4.036 ± 1.89 which shows statistically significant correlation ($p < 0.05$) (table 1). It is shown in previous studies that risk of cardiovascular disease and diabetes increases with the lower levels of serum albumin [15]. In another paper, levels of serum albumin has been associated with nephropathy. The microalbuminuria (30 - 300 g / 24 hours) test is clinically used to diagnose kidney disease in diabetic individuals [16]. In our study, serum albumin level is associated with all the anthropometric factors ($p = < 0.001$) (Table 4).

Average levels of creatinine for diabetic individuals (0.86 ± 0.12 mg / dL) is less than control subjects (1.06 ± 0.36 mg / dL) which shows statistically significant correlation ($p < 0.05$) (table 1). Renal damage increases with increasing age of diabetic individuals [17]. Lower serum creatinine increased the risk of type 2 diabetes [18,19]. Low serum creatinine predicts the occurrence of diabetes in overly obese patients, anthropometric measures, age independent, and hypertension, family history of diabetes, gender, current smoking and history of disease running in family [20].

Table 1: Showing association of anthropometric traits with patients and control using statistical test (two tail T-test). The *values show that they are significant.

Parameters	PATIENTS (n=100)	CONTROLS (n=100)	P-value
Age	52.89±10.40	49.25±11.89	0.0222
Weight(lb)	139.79±29.62	57.09±10.56	< 0.0001*
Height(ft.)	66.1±7.31	158.92± 9.41	< 0.0001*
BMI($lbft^{-1}$)	31.92±6.16	22.82±5.10	< 0.0001*
Duration of disease(years)	7.16±5.51	-	-
Blood glucose (mg/dl)	196.49±80.66	94.52±19.45	< 0.0001*
Microalbumin(mg/dl)	33.52±9.05	26.85±4.87	< 0.0001*
HbA1C%	8.39±1.31	5.24±0.89	< 0.0001*
Total serum proteins(g/dl)	9.53±1.28	6.78±1.82	< 0.0001*
Albumin Serum (mg/dl)	2.17±0.95	4.04±1.89	< 0.0001*
Creatinine(mg/dL)	0.86±0.12	1.06±0.36	< 0.0001*
Systolic BP	145±15	110±15	< 0.0001*
Diastolic BP	90±10	76±8	< 0.0001*

Table 2: Showing association of anthropometric traits with microalbuminuria, HbA1C and Serum protein levels using statistical test (ANOVA). The *values show that they are significant.

Parameters	P-value of microalbuminuria	P-value of HbA1C	P-value of Serum protein
Age	0.001*	0.002*	<0.001*
Weight	0.006*	0.012	<0.001*
High	<0.001*	0.250	<0.001*
BMI	0.203	0.008*	<0.001*
Duration	<0.001*	0.023	<0.001*
Blood Glucose	<0.001*	0.088	0.066*
Creatinine	<0.001*	<0.001*	<0.001*
Nephropathy	0.019	<0.001*	<0.001*
Cardiovascular	<0.001*	0.161	<0.001*
Obesity	0.019	0.017	<0.001*
Hypertension	0.017	0.292	<0.001*

The average levels of systolic blood pressure in diabetic patients is 145 ± 15 while in control individuals is 110 ± 15 and average levels of diastolic pressure in diabetic patients is 90 ± 10while for control subjects is 76 ± 8 (table 1). As shown in the previous research Diabetes and hypertension are closely related to each other i.e. if one occurs the risk of occurrence of other increases. This is possibly due to a common pathway or genetic process [21]. Detection and management of hypertension can effectively prevent deleterious events in diabetic patients and one of the best way in primary care for maintaining blood pressure levels is pharmacotherapy [22].

Microgram quantities of protein were quantified using Bradford method. Bovine Serum Albumin was used to make standard curve and concentration of protein in each sample was calculated. Biuret assay was also used for calculation of protein concentrations in each sample. The total proteins for controls were 52.6 ± 1.97 g / dl, while for diabetic female patients were 94.6 ± 1.98 and for diabetic male patients were 97.63 ± 1.99 g / dl. Increased protein concentrations were observed in samples of patients as compared with control.

SDS-PAGE Analysis

SDS-PAGE analysis of serum and urine samples of patients and control showed significant albumin presence in diabetic subjects as compared to control. It is the most abundant protein in serum and urine of patients.

SDS Page analysis of serum albumin levels is shown in figure 1a. (Vertical lines labelled 1-4 indicate samples of diabetic patients, while 5 indicates sample of control)

In figure 1a, Line 1 shows the high concentration of albumin protein in sample while Lines 2, 3 and 4 shows relatively less protein concentrations. Protein ladder is used as a standard for comparison of molecular weights of samples. Molecular weight of albumin is 66 KD.

Figure 1b shows SDS page analysis of urine albumin levels. (Lines 1, 2 shows the urine sample of control while urine sample of patients is shown in lines 3-9)

Line 3 in figure 1b, indicate very high concentration of albumin protein. Lines 4-9 indicate relatively less amounts of protein. Protein marker as a standard is used.

CONCLUSION

It is proved by the experiments that serum albumin levels, microalbuminuria, cardiovascular disease and diabetic nephropathy are closely related in diabetic subjects of Punjab University premises. Diabetic patients have elevated levels of urine albumin.

This is novel original research work done in Pakistan and never been published anywhere.

DECLARATION

Ethics approval and consent to participate

Ethical approval for the study was given by the Ethics Committee of School of Biological Sciences, in March 2014.Sampling was performed from September to December 2014.The study was assigned the reference number as SBS/191/14, dated: 31.03.2014.

Competing interests

Conflict of Interest: The author(s)declare(s) that there is no conflict of interest regarding the publication of this article.

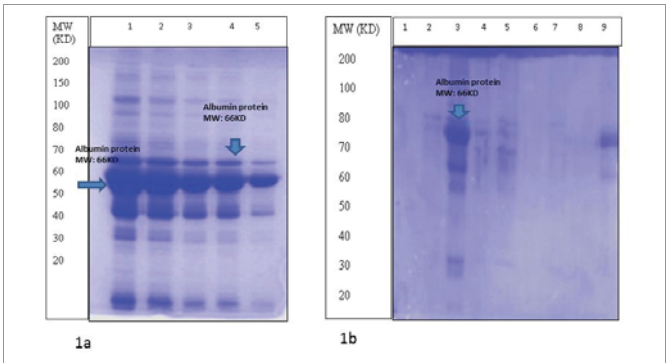

Figure 1: (a). 12% SDS Page analysis of serum albumin levels in diabetic and control samples. (Lines 1,5=High molecular weight protein ladder, Line 5 shows control sample while Lines 1-4 shows samples of diabetic patients).
(b). 12 % SDS Page analysis of urine albumin levels in diabetic and control samples (Lines 1-9 shows high molecular weight protein ladder, Lines 1and 2 shows samples of control, while lines 3-9 shows samples of diabetic patients).

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AUTHORS' CONTRIBUTIONS

Manal Tariq is a research student of M.Sc. who performed this research and wrote this paper.

Sara Aslam is co-author of this work who helped in data analysis.

Dr. Samreen Riaz is the supervisor and co-author of this work who designed the project, provided funds and facilities to the student for lab work.

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