ANNALS OF DIABETES, METABOLIC DISORDERS & CONTROL Open Access

SCIENTIFIC LITERATURE

RESEARCH ARTICLE

Linkage of Micro Albuminuria and Serum Albumin Levels in the Diabetic Patients of Punjab University Premises

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ARTICLE INFO

Article history:
Received: 03 May 2018
Accepted: 06 July 2018
Published: 10 July 2018

Keywords:
Diabetes;
Microalbuminuria;
Serum albumin;
Pakistani population;
Lahore;
Punjab university

Citation this article: Riaz S, Tariq M. Linkage of Micro Albuminuria and Serum Albumin Levels in the Diabetic Patients of Punjab University Premises. Ann Diabetes Metab Disord Contr. 2018; 2(1):117.

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ABSTRACT

Microalbuminuria and serum albumin levels both are associated with an increased risk of cardiovascular and renal disease in patients with diabetes and hypertension. The role of microalbuminuria as a predictor protein biomarker of diabetes nephropathy and heart disease has not been examined in large general-population of Pakistan, and its prognostic significance in persons with established diabetes is uncertain. In our study we examined the relation between microalbuminuria and serum albumin levels in a Pakistani population-based same number of men and women aged 40–79 years with and without prevalent baseline diabetes and evaluated the levels of microalbuminuria and serum albumin levels significance. The samples were collected from the Health Centre, Diabetic Clinic, University of the Punjab, Lahore during January 2014 to June 2014. Samples were then estimated from referred standard protocols and results were shown in the tables and graphs form. Statistical analysis was then done on the collected data. Microalbuminuria and elevated serum albumin levels may be useful in identifying persons at increased risk of diabetes and nephropathy and subsequent death in the general population.

Introduction

Hyperglycemia is a significant characteristic of diabetes, a condition which results from alteration in insulin action, or its secretion (type 1- β-Cell) or defect in both of it. The persistent hyperglycemia in diabetes is related with lasting damage, dysfunction, along with malfunction of different organs, for example, eyes, blood vessels, heart, nerves, and kidneys leading to hyperglycemia.

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 with impaired glucose tolerance have chances to develop type 2 diabetes [1].

American Diabetes Association (ADA) diagnostic criteria for diabetes mellitusinclude fasting plasma glucose level≥ 126 mg/dL or 7 mmol/L under no caloric intake fo≥8 h, Hemoglobin A1c (HbA1c) ≥ 6.5%, 2 -h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an 75-g Oral Glucose Tolerance

Test (OGTT), or classic symptoms of hyperglycemia or hyperglycemic crisis with a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) [2].

Self-management of diabetes focuses on glycemic control by the determination of HbA1c concentrations. Development of complications, such as micro vascular diseases (e.g., neuropathy, retinopathy, and nephropathy), and macro vascular diseases (e.g., cerebrovascular, peripheral vascular and cardiovascular disease etc) is associated with the decrease in HbA1c concentrations [3].

Human Serum Albumin (HSA) is a major protein part of blood serum that carries many drugs and essential metabolites and its glycation is involved in biological abnormalities. Several studies suggest its glycated form to be a marker for diabetes [4].

Microalbuminuria is the condition resulting from hyperglycation of HSA. More than 30 mg/g is the earliest and most commonly used clinical hallmark of increased risk for DN and has evolved into a key marker for risk prediction and treatment monitoring in diabetes [5]. Diabetic Nephropathy (DN), characterized by declining renal function and increasing albuminuria (>300 mg/day), is the leading cause of End-Stage Renal Disease (ESRD) in the Western world, and is associated with significant cardiovascular morbidity and mortality [6-10]. Microalbuminuria has been the most acknowledged way for the evaluation of early renal injury in diabetes [11].

Experimental

1. Selection of patients

Samples were collected from the Health Centre, University of Punjab. 100 confirm diabetic patients were selected; out of which 50 were females and 50 males. A detailed individual patient´s present and past history, socio-economical history, protein estimation, blood pressure, sugar level during fasting were recorded in this study. Informed consent of each patient was taken.

2. Sample collection

Samples as 10c.c blood and 24 hrs urine from the patients and controls were collected according to rule for the collection of blood and urine for clinical analysis midstream morning samples from the diabetic patients and control groups were collected. From 10 c.c half of blood is transferred into sterile vacutainer containing K-EDTA for separation of plasma and other half is transferred into sterile tubes without having anticoagulant for separation of serum. Then 24 hrs urine samples were collected, measured and concentrated by freeze drying (lyophilisation) and all samples were stored at -80°C until analysis.

3. Ethical approval

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.

4. Estimation of physical parameters

4.1. Age: Age of all 100 subjects that consists of 50 diabetic patients and 50 controls subjects was calculated from the history of the subjects and it is calculated in years.

4.2. Weight: Weight of all 100 subjects that consists of 50 diabetic patients and 50 control subjects was measured by the weighing machine and it is calculated in kilograms.

4.3. Height: Height of all 100 subjects that consists of 50 diabetic patients and 50 control subjects was measured and is listed in inches.

4.4. The BMI (Body Mass Index): BMI of all 100 subjects consisting of 50 diabetic patients and 50 control subjects was calculated by the formula

BMI = Weight in kg

(Height in meters) 2

5. Estimation of biochemical parameters

Blood and 24 hrs urine samples were collected from the selected diabetic patients and controls. Different parameters were assessed for all selected diabetic and control samples.

5.1. Estimation of total proteins: Total proteins were estimated by Bradford and Biuret protein assay using standard protocol. Total proteins were estimated for all 100 samples, 50 of diabetic patients and 50 of control.

5.2. Estimation of blood glucose: Blood glucose was estimated by the GLU method used as in vitro diagnostic

test for the quantitative determination of glucose in plasma, urine, cerebrospinal fluid and serum. Blood glucose was estimated for the selected 50 diabetic and 50 control samples.

5.3. Determination of urine creatinine: Urine creatinine was estimated by the CREA method used as an in vitro diagnostic test projected for the computable determination of creatinine in plasma, urine and serum. Urine creatinine was determined of all 100 samples.

5.4. SDS Page: Proteins were separated using SDS PAGE analysis. It is a very common procedure used for the separation of proteins by electrophoresis, a very common method for separating proteins by using a discontinuous polyacrylamide gel as a support medium and Sodium Dodecyl Sulphate (SDS) to denature the proteins. Hence, the name Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The samples of all 100 individuals, 50 of diabetic patients and 50 of control were analyzed by SDS-PAGE.

Results and Discussion

In this study, the samples were initially analyzed for the physical parameters: Age, weight, height and BMI of all individuals. Three study groups were formed. Normal control individuals were grouped in Group 1, diabetic females in Group 2 and diabetic males in Group 3.Biochemical parameters like total protein estimation, creatinine, MAU, HBA1C levels were estimated of the selected individuals.

1. Estimation of physical parameters

1.1. Age: The study groups comprised of 100 subjects, of whom 50 were diabetic patients, i.e. 25 male and 25 female and 50 were controls. The mean age of the diabetic cases were 53±2.3 years and that of control group was 56±3.4years. The difference in the mean age was not statistically significant (P >0.05).

1.2. Weight: Weight for all study groups was measured that comprised of 100 subjects i.e. 50 diabetic and 50 controls. The mean weight of the diabetic cases was 65.93±3.5 kg and that of control group was 59.3±3.7 kg. The difference in the mean weight was not statistically significant (P > 0.05).

1.3. Body mass index: Body Mass Index was calculated in kg/m2 for both diabetic patients and control. The average BMI of control is 24.6 ± 0.61 while for diabetic subject is 31.62 ± 0.59 which is 22% more than the control. The results show increase BMI in diabetic patients as compared with control.

2. Estimation of biochemical parameters

2.1. Estimation of fasting blood sugar: The average value of fasting blood sugar for diabetic female patients is 180±18.65 and for diabetic male patients is 183±17.85. While for control is 94.3±2.87. The diabetic patients show a highly significant increase in blood sugar than the control ($p<0.001$).

2.2. Estimation of creatinine: The average value of creatinine for diabetic female patients is 0.875±0.07 and for diabetic male patients are 0.893±0.06while for control is 0.76±0.04. The diabetic female and male patients both show significant increase in serum creatinine as compared with control ($p>0.01$).

2.3. Total protein estimation in serum and urine: Bradford assay was executed for the quantification of microgram quantities of the protein whereas optical densities were taken at 595 nm. Then the protein concentration of each sample was calculated from the standard curve of Bovine Serum Albumin (BSA). Protein concentration of every sample was also determined using the biuret method. The total proteins for control were 52.6±1.97, while for diabetic female patients were 94.6±1.98 and for diabetic male patients were 97.63±1.99. In both Bradford and kit method increased serum protein concentration was observed for the diabetic patients as compared with control.

Pie charts of different biochemical parameters are shown in (Figure 1).

Average values of different parameters of controls as compared with diabetic patients are shown in (Table 1).

Graph between average percentages of different parameters are shown in (Figure 2).

Comparison of physical and biochemical parameters of control and diabetic female and male patients is shown in (Table 2).

2.4. SDS-PAGE analysis: The protein analysis of human serum and urine samples was done using SDS-PAGE

analysis which indicated significant presence of albumin in diabetic patients as compared to normal controls. The albumin is the most abundant protein observed in the patient's serum and urine samples as compared to the normal control.

SDS Page analysis of serum albumin levels in control and diabetic patients is shown in (Figure 3a). (Lane 1-4 shows the serum samples of diabetic patients, Lane 5 shows the serum sample of control)

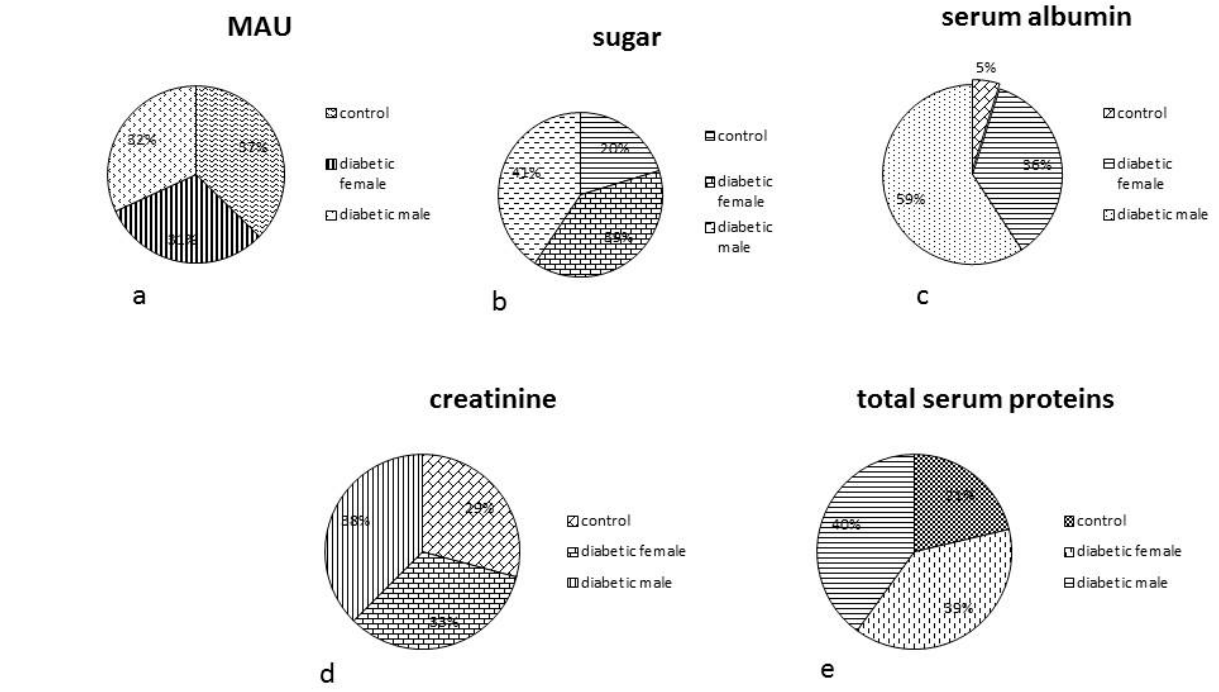

Figure 1: The basic physical parameters comparison in control and diabetic patients with the help of pie charts.

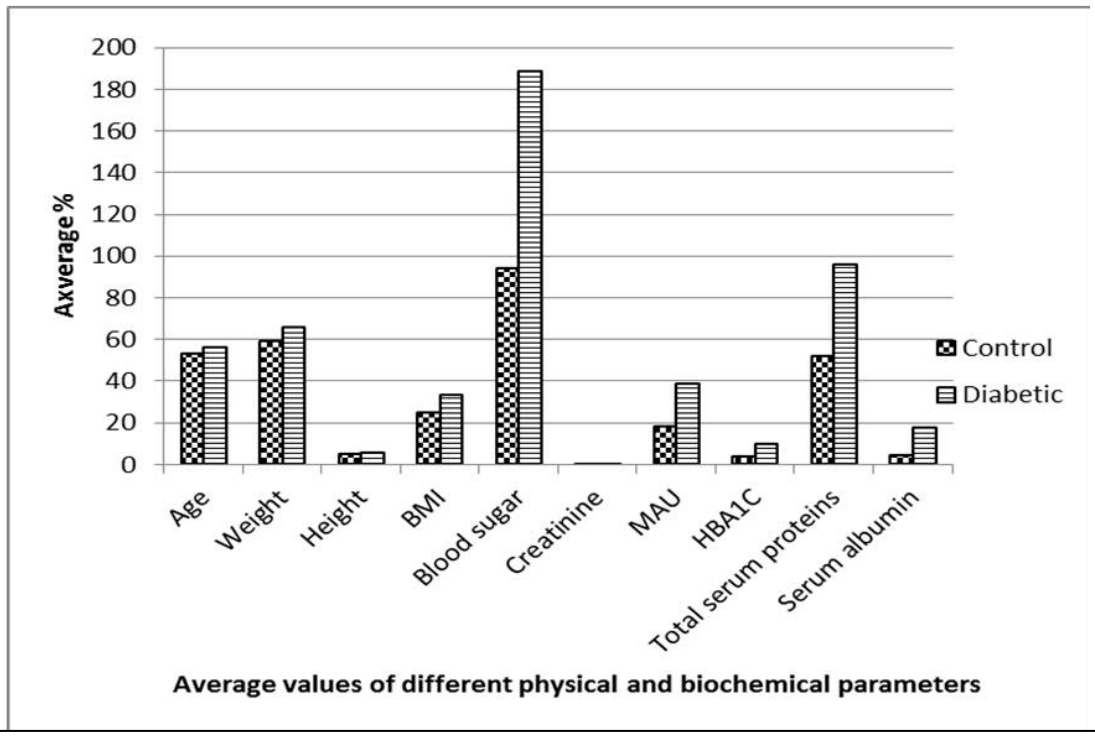

Figure 2: Average Values of different physical and biochemical parameters with help of Bar graph.

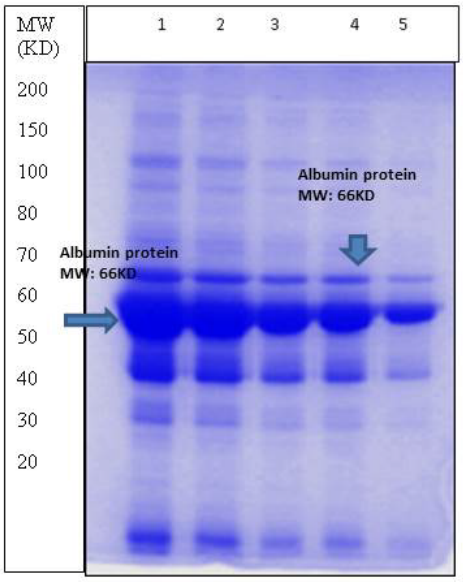

Figure : 12% SDS Page analysis of serum albumin levels in control and diabetic patients.(Lane 1,5=High molecular weight protein marker or ladder, Lane 5 shows the serum sample of control while Lane 1-4 shows the serum samples of diabetic patients).

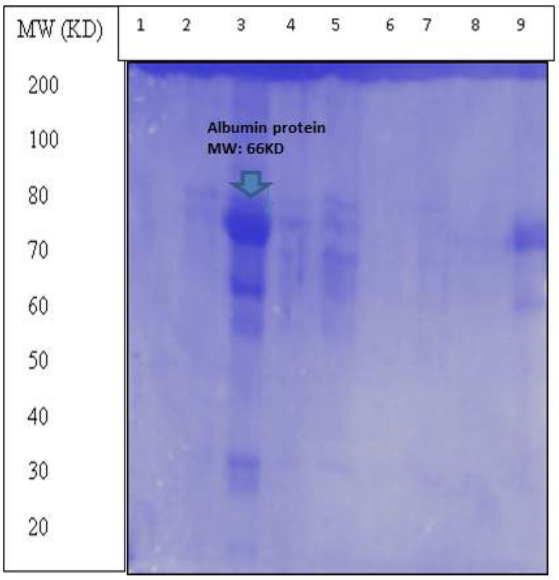

Figure: 12 % SDS Page analysis of urine albumin levels in control and diabetic patients. (Lane 1, 9= High molecular weight protein marker or ladder, Lane 1and 2 shows the urine sample of control, Lane 3-9 shows urine sample of diabetic patients).

Figure 3: SDS 12% PAGE to show the a) serum albumin and b) Urine albumin in the control and diabetic patients.

Table 1: Average values of different parameters of control as compared with diabetic patients.

Serial No.	Parameters	Normal values
1	Fasting bood sugar(mg/d1)	80 mg/d1 to perhaps 110 mg/dl
2	Creatinine(mg/d1)	Norma Adult Range: .7-1.4 mg/d1
3	Total serum proteins(g/d1)	Normal Adult Range: 6.0-8.5 g/d1
4	Microalbuminuria (mg/d1)	< 30 mg/d1
5	Serum albumin(g/d1)	3.5-5.5 g/d1
6	HBA1C (%)	4-6 %
7	BMI(kg/m^2)	19-25 kg/m^2

Table 2: Comparison of physical and biochemical parameters of control with that of diabetic female and male patients.

Parameters	Control(n=50)	Diabetic(n=50)
Age(years)	53±2.3	56*±3.4
Weight(kg)	59.3±3.7	65.93*±3.5
Height(inches)	5.3±1.5	5.4*±1.8
BMI(kg/m^2)	24.6±0.6	33±1.3
Blood sugar(mg/d1)	94.3±2.87	188.3***±17
HBA1C (%)	3.6±0.83	9.8**±0.56
Total proteins(g/dl)	52±0.1	96**±0.3
MAU(mg/d1)	18.5±0.1	38.9**±0.3
Serum albumin(g/d1)	4.1±1.24	17.57***±1.42
Creatinine(mg/d1)	0.76±0.04	0.87 * *±0.07

Data are means ± SD. *=p>0.05 (statistically not significant), **=p<0.01(statistically significant), ***=p<0.001 (statistically highly significant) comparison of control with diabetic baseline. Group 1 is control; group 2 contains diabetic female patients while group 3 consists of diabetic male patients.

In figure 3a, Lane 1 shows the serum sample of diabetic patient in which albumin protein is present in very high concentration. Lane 2, 3 and 4 shows serum samples of other diabetic patients, which contains relatively less amounts of albumin as compared to Lane 1. Protein marker or ladder is used to compare the molecular weights of our samples with the standard. Albumin protein has a molecular weight of 66 KD.

SDS Page analysis of urine albumin levels in control and diabetic patients is shown in (Figure 3b). (Lane 1and 2 shows the urine sample of control, Lane 3-9 shows a urine sample of diabetic patients)

In figure 3b, Lane 3 shows the urine sample of diabetic patient in which albumin protein is present in very high concentration. Lanes 4-9 shows urine samples of other diabetic patients, which contains relatively less amounts of albumin as compared to Lane 3. Protein marker or ladder is used to compare the molecular weights of our samples with the standard. Albumin protein has a molecular weight of 66 KD.

Conclusion

It is proved by the experiments that there is a linkage between microalbuminuria and serum albumin levels in diabetic patients of Punjab University premises. Diabetic patients have elevated levels of serum albumin and urine albumin. So, if we take the test for microalbuminuria we can easily detect the diabetes in persons before symptoms are shown.

Acknowledgment

We are very grateful to Dr. Nausair A Malik Incharge Diabetic Clinic Health Centre, University of the Punjab Lahore and Prof. Dr. SaadiaShahzadAlam Head of Pharmacology Department FPGMI Shaikh Zayed Hospital, Lahore for their valuable suggestion's and support of this research work. This project is funded by University of the Punjab, Lahore.

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