Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20140923053831

Original Research

GESDAV

Assessment of urinary micro albumin levels in Type-1 and Type-2 diabetic subjects

Shreeyas Prasad D. Nilakhe[1], D. V. S. S. Ramavataram[1], Priyanki N. Shah[2], Kirti C. Kanadhia[1], Navneet Kumar R. Singh[2]

[1]Department of Biochemistry, Surat Municipal Institute of Medical Education and Research, Surat, Gujarat, India, [2]Department of Biochemistry, Shri. P. M. Patel College of Paramedical Sciences, Anand, Gujarat, India

Address for correspondence:
Shreeyas Prasad D. Nilakhe, Department of Biochemistry, Surat Municipal Institute of Medical Education and Research, Near Bombay Market, Sahara Darawaja, Surat - 395 010, Gujarat, India. E-mail: sneil35@hotmail.com

Received: May 07, 2014
Accepted: September 23, 2014
Published: October 03, 2014

ABSTRACT

Introduction: Diabetes mellitus is one of the most prevalent metabolic diseases all over the world, including India. Prolonged diabetes is accompanied with many complications including diabetic nephropathy. Diabetic nephropathy is defined clinically as the presence of microalbuminuria or overt nephropathy in patients with diabetes without the manifestations of renal diseases due to other causes. The earliest sign of diabetic nephropathy leading to an end stage renal disease is microalbuminuria and is estimated as urinary albumin excretion in 24 h urine samples and also as urinary albumin creatinine ratio by estimating the albumin/creatinine ratio in spot urine specimens. Indian clinical guidelines strategize the aspects of prevention or prolongation or even reversion of diabetic complications such as diabetic nephropathy at primary, secondary and tertiary stages to yield beneficial effects. With a view of the beneficial effect of assessing the onset of nephropathy in diabetic patients, we analyzed the micro albumin levels for an early detection of the existence of microalbuminuria and its prevalence in both Type-1 and Type-2 diabetic patients. **Materials and Methods:** Morning spot urine samples were collected from diabetic patients. Microalbumin and creatinine were estimated from these urine samples by immuno-turbidometric and alkaline-picrate methods respectively. Microalbumin was expressed in terms of mg/g creatinine. Lipid profile was also estimated in these patients. Student's *"t"*-test was applied for significance. **Results:** A total of 75 samples were analyzed for microalbuminuria, of these 25 were healthy controls and 50 were diabetics (both Type-1 and Type-2) with 4-25 years duration of diabetes. We observed an incidence of 72% microalbuminuria in diabetics (both Type-1 and Type-2) patients and 65% in Type-2 diabetic patients. A good correlation was found between duration of diabetes and incidence of microalbuminuria ($P = 0.02$) in both types of diabetes. **Conclusion:** Percentage of subjects with microalbuminuria was significantly high in both the types of diabetes, and it correlates with higher age and longer duration of diabetes. Early and routine screening for microalbuminuria in diabetic patients is desirable for an early detection and prevention of diabetic nephropathy.

KEY WORDS: Albumin:creatinine ratio, diabetes, duration, incidence, microalbuminuria, screening

INTRODUCTION

Diabetes mellitus is a major health problem in India as well as all over the world. Diabetes mellitus is a disease of abnormal glucose metabolism resulting in hyperglycemia due to deficiency of insulin secretion or insulin resistance, or both. Two major types of diabetes are Type-1 (insulin-dependent) and Type-2 (non-insulin-dependent) diabetes mellitus. According to WHO, the diagnostic criteria for diabetes is as fasting glucose ≥126 mg/dL as well as oral glucose tolerance testoral glucose tolerance testoral glucose tolerance test value at 2 h post-glucose challenge of ≥200 mg/dL [1]. According to WHO, there were 32 million people with diabetes in India in 2000, which is projected to rise to 80 million by the year 2030 [2].

Diabetic nephropathy is one of the serious microvascular complications of diabetes. According to American Diabetes Association (ADA) study, diabetic nephropathy occurs in 20-30% of Type-1 and Type-2 diabetic patients. Without any interventions, 20-40% of Type-2 diabetic patients with microalbuminuria progress to overt nephropathy and approximately 20% will have progressed to end-stage renal disease (ESRD) by 20 years after onset of overt nephropathy [3]. In India, 30% of chronic renal failure cases can be attributed to diabetic nephropathy [4]. In a study carried out in US population, 24.9% of adults of 40 year and older with undiagnosed diabetes had signs of nephropathy, and prevalence for positive test when screening for nephropathy was 26.5% [5]. The prevalence of microalbuminuria was 61% among diabetic patients in urinary albumin excretion (UAE) [6]. ADA

also suggested the guidelines for screening for nephropathy in diabetic subjects as yearly test for microalbuminuria in Type-1 diabetics who have diabetes more than 5 years and all of Type-2 diabetic patients starting at diagnosis (3). The early laboratory evidence of diabetic nephropathy is microalbuminuria leading to proteinuria as the disease progresses toward ESRD. Though, the gold standard method is to detect the albumin excretion rate (AER) also known as UAE by estimating the 24 h urinary albumin [7], practically not convenient and difficult to ensure the compliance by the outpatients regarding strict adherence to the complete collection of urine samples. Albumin creatinine ratio (ACR) is also the proven indicator for microalbuminuria. Using AER by 24 h urine samples, a gold standard method, few studies have revealed equally the better predictive value of albumin: creatinine ratio in spot urine samples for microalbuminuria [8-10]. Similar study was also carried out in children with proteinurea, and it was concluded that urinary ACR (UACR) could be more useful for monitoring of chronic kidney diseases [11]. Sampaio and Delfino [12] also confirmed the usefulness and good correlation with 24 h UAC in diabetic patients. Jafar *et al*. [13] studied the similar correlation in diabetics of Indo-Asian population and found that both UAC and UACR are acceptable tests for screening of albuminuria.

In view of these facts, we screened both Type-1 and Type-2 diabetic patients randomly for the incidence of microalbuminuria to detect the early onset of diabetic nephropathy. As per the guidelines issued by ADA microalbuminuria is confirmed if albumin: creatinine ratio ACR is ≥30 mg/g of creatinine (cut-off value) in a spot random urine samples [14]. Diabetic patients with existing renal infections or complications were excluded from the study.

MATERIALS AND METHODS

The present study was carried out at Surat Municipal Institute of Medical Education and Research Hospital, Surat, India. Samples were collected from hospitalized as well as from outdoor diabetic patients after informed consent. These patients were on medications for the treatment of diabetes and serum cholesterol lowering drugs. In the present study, a total of 50 diabetic patients were screened for microalbuminuria out of which 10 were Type-1 diabetics and remaining 40 were Type-2 diabetic patients. 25 non-diabetic individuals of same age group were also screened for microalbuminuria as healthy controls.

Morning spot urine samples were collected from diabetic patients. Urinary micro albumin was estimated by immuno-turbidometric method and urine creatinine was estimated by alkaline picrate method in urine samples. Microalbumin levels were expressed in terms of mg per gram creatinine. Simultaneously fasting glucose, serum lipid profile was also estimated in these individuals. Serum total cholesterol and triglycerides were estimated by enzymatic methods. High-density lipoprotein cholesterol was measured by direct homogenous assay and low-density lipoprotein cholesterol was calculated by Friedwald's formula. All the investigations were carried out using ERBA XL 300 auto analyzer in the Clinical biochemistry Laboratory. Data analysis was performed using statistical software SPSS version 16.0 (SPSS Inc., Chicago). Student's "*t*"-test was applied for significance.

RESULTS

In the present study, a total of 75 subjects were screened for microalbuminuria out of which 25 were healthy controls, 40 and 10 were Type-2 and Type-1 diabetics respectively [Table 1]. In Type-1 diabetics, out of 10 subjects, 8 have microalbuminuria, and 2 have macroalbuminuria and an incidence of 80% microalbuminuria and 20% of overt nephropathy. In Type-2 diabetes 2 patients have macroalbuminuria and 26 have microalbuminuria and 12 were without the signs of microalbuminuria [Table 1] and overall incidence of microalbuminuria in both the types of diabetes was 72% and 8% had overt nephropathy [Figure 1]. Table 2 shows microalbumin and lipid profile in healthy subjects and type 1 and type 2 diabetic subjects.

Normo and microalbuminuric of Type-2 diabetics a significant correlation was observed among age ($P = 0.03$), duration of diabetes ($P = 0.02$), and micro albumin levels ($P = 0.000$) [Table 3].

Both internal and external quality controls for all the biochemical parameters were performed every day and inter assay mean, standard deviation and coefficients of variation were calculated and found to be within the acceptable limits.

DISCUSSION

Diabetes mellitus is a progressive disease affecting multiple organs over a period with an increasing occurrence across all

Table 1: Details of subjects

Parameter/group	Healthy controls	Diabetic subjects	
		Type-1	Type-2
Subjects	25	10	40
Age (years)	45±14	45±14	58±14
Male	15	6	22
Female	10	4	18
Duration (years)	00	9±10	9±7
Microalbuminurics	00	08 (80%)	26 (65%)
Macroalbuminurics	00	02 (20%)	02 (5%)
Normoalbuminurics	25	00	12 (30%)

Table 2: Microalbumin and other biochemical parameters healthy subjects and diabetics (type 1 & 2)

Parameter/group	Healthy controls	Diabetic subjects	
		Type-1	Type-2
Subjects (n)	25	10	40
Microalbumin (mg/g) (Median)	14.28 (8.33-28.57)	75.42 (39.25-594)	69.17 (10.63-515)
Glucose: Fasting (mg/dL)	85.3±14	257.3±108	185.55±100.44
Cholesterol: Total (mg/dL)	174.8±29.3	163.9±58.2	137.33±38.15
Triglycerides (mg/dL)	96.6±27.5	146.2±89.9	132.10±83.66
HDL-Cholesterol (mg/dL)	39.0±3.52	40.0±7.42	33.74±11.30
LDL-Cholesterol (mg/dL)	116.0±27.0	94.4±43.8	80.97±34.26

HDL: High-density lipoprotein, LDL: Low-density lipoprotein

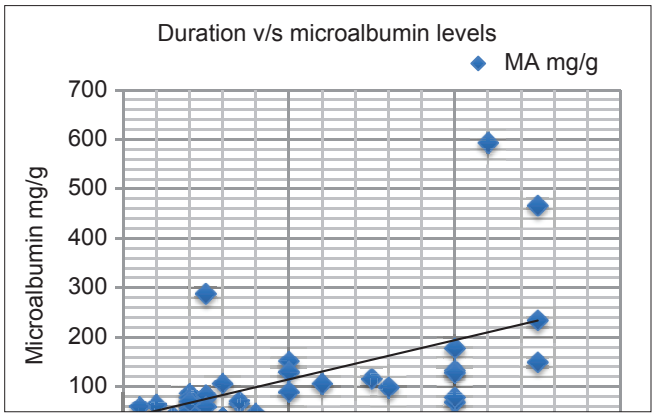

Figure 1: Correlation of microalbuminuria with duration of diabetes in both types of diabetes together

Table 3: Normo and microalbuminurics patients in Type-2 diabetes: A comparison

Parameters/ groups	Normoalbuminurics	Microalbuminurics	Significance
Number (*n*)	12	26	
Age (years)	51±10	61±15	*P*=0.03
Duration (years)	5.5±4.9	11±7.3	*P*=0.02
Glucose: Fasting (mg/dL)	180.7±94.1	192.1±106.9	*P*=0.8
Microalbumin (mg/dL)	18.03±5.8	101.2±59.9	*P*=0.000
Total cholesterol (mg/dL)	132.7±38.2	139.1±41.4	*P*=0.7
Triglycerides (mg/dL)	164.7±113.5	115.5±63	*P*=0.1
HDL-cholesterol (mg/dL)	29.8±10.5	35.5±9.9	*P*=0.1
LDL-cholesterol (mg/dL)	59.5±28.0	83.6±31.6	*P*=0.5

HDL: High-density lipoprotein, LDL: Low-density lipoprotein

the age groups of both the genders. In view of the alarming rise in the incidence of this disease, there is an urgent need to prevent the persistent hyperglycemia at an early stage to ensure a healthy life. Modern lifestyle and alteration in dietary habits etc. are contributing factors to the progression of this disease apart from genetic component. Three-stage strategy has been suggested by the Indian council of medical research for the prevention and better healthcare management of diabetes [2].

Stage I: Primary prevention: Early diagnosis through screening program before the onset of disease.

Stage II: Secondary prevention: Prevention of the onset of complications in diabetics by meticulous control with the help of diet, physical activity and lifestyle changes.

Stage III: Tertiary prevention: The aim was to limit physical disability and taking rehabilitation measures in diabetic subjects with complications to prevent them from going to end stage.

In long-term diabetics there are high chances of development of vascular changes in various organs including kidney. To assess the early signs of diabetic nephropathy one of the good biochemical markers is the measurement of urinary microalbumin. In Indo-Asian population, the prevalence of microalbuminuria was reported to be 11.8% [13].

Microalbuminuria prevalence study reported a prevalence of 39.8% in a total of 6800 hypertensive diabetic adult patients from 10 Asian countries [15] and in a similar type of study in UAE population it was reported to be 61.5% [6]. Similarly, 45.6% prevalence of micro albuminuria in Type-2 diabetic patients was reported in Saudi Arabia [16]. In the present study using one-time morning sample, there was a prevalence of 72% of microalbuminuria in both the types of diabetic patients cumulatively and a prevalence of 65% in Type-2 diabetic patients. Prevalence of microalbuminuria in Type-2 diabetes found in our studies was similar, but slightly higher than that of the study of Al-Maskari *et al.* [6] who reported an incidence of 61.5% in UAE population and much higher than that of reported from different Asian countries and Saudi Arabia. Variations are expected as the studies were conducted in different populations and size of the population and also variations in the methodology of sample collection. Data clearly indicates an increasing trend of prevalence of microalbuminuria over a period compared with that of reported earlier. Follow-up studies are required to assess the microalbuminuria pattern on a periodical basis. There are few reports regarding this aspect. An incidence of 82.3 per 1000 of diabetic patients in Iran was found to have microalbuminuria in 5 years duration [17]. In a follow-up study for 5 years in India 46.9 per 1000 diabetic subjects were found to have microalbuminuria [18]. These studies clearly suggest that the persistent hyperglycemic condition in spite of the patients following the anti-diabetic measures eventually leads to the development of microalbuminuria.

Higher incidence of both micro and macrovascular complications are expected as the duration of diabetes increases due to glycation of various proteins. Longer the duration of diabetes higher the chances of development of nephropathic changes. In 7 years follow-up study of Type-2 diabetic patients, a 42% had microalbuminuria with an increased risk of progression to overt nephropathy when compared to the diabetic subjects without microalbuminuria. It was further observed that 3.7% of the Type-2 diabetics progressed to overt nephropathy every year [19]. In another follow-up study of 10 years duration 25% had developed persistent microalbuminuria and 6% progressed to overt nephropathy [20]. Some other studies also reported the susceptibility of diabetics with microalbuminuria or proteinuria to the development of nephropathy over a period of time [15,17]. The results of the present study were similar to earlier studies as we have observed a good correlation between duration of disease and development of microalbuminuria ($P = 0.02$) in both the types of diabetes [Figure 1]. These studies clearly suggest that the screening for microalbuminuria at regular intervals is mandatory for proper management and monitoring of diabetic patients. According to ADA guidelines, in a position statement 2003, test for microalbuminuria should be performed at the first diagnosis of Type-2 diabetes and screening every year after 5 years in Type-1 diabetes [3]. As Type-1 diabetic patient has high blood glucose levels over a long duration and persistent microalbuminuria over a period of time, an increased

prevalence of kidney disorders is expected compared to Type-2 diabetics. Our study reiterates this aspect as we have found 20% incidence of overt nephropathy in Type-1 compared with 5% in Type-2 diabetics.

With the progression of age, it is expected that more vascular changes take place, and older age diabetics are likely to have more vascular alterations than that of younger age group. In our studies, we observed an independent association between progression of age and increased incidence of microalbuminuria. ($P = 0.03$). A correlation between fasting hyperglycemia and occurrence of microalbuminuria is also expected, but we did not observe any such significant correlation with fasting hyperglycemia. This might be due to the reason that patients visiting out-patient department for their diabetic control were on regular and proper monitoring of their blood glucose level leading to the good control of fasting blood glucose levels or their fasting glucose levels were not true representative of continuous monitoring of control of blood glucose levels as it would be known only through the measurement of hemoglobin A1c (HbA1c).

The association of microalbuminuria to progression of diabetic nephropathy as evidenced by previous studies and higher incidence of microalbuminuria in the present study leads to the conclusion that screening for microalbuminuria should be advocated in diabetic patients as early as possible and carry out the follow-up estimation for microalbuminuria at a defined intervals to monitor the progression toward nephropathy.

Lower incidence of microalbuminuria in Type-2 diabetics compared to Type-1 in our study may be due to consistently high plasma glucose levels found in Type-1 diabetics in spite of usage of anti-diabetic medications. Use of anti-diabetic medications like metformin, pioglitazone and gliclazide, reduce the incidence of microalbuminuria [21,22]. Regular use of anti-diabetic drugs will also be effective to prevent the incidence of diabetic nephropathy. Along with anti-diabetic medications, inclusion of low carbohydrate diet over a period of 1 year lead to the reversal of microalbuminuria to normoalbuminuria in 52% patients of Type-2 diabetics in Japan [23]. In Type-2 diabetic patients, high intake of protein and the low intake of polyunsaturated fatty acids, particularly from plant oils, were associated with the presence of microalbuminuria. Reducing protein intake from animal sources and increasing the intake of lipids from vegetable origin might-reduce the risk of microalbuminuria [24]. In diabetic patients as a long-term goal, it is always better to adopt the natural ways of prevention such as diet control. Dietary control along with anti-diabetic therapy will prevent the diabetic nephropathy and also other complications.

Another most important aspect about microalbuminuria is that its independent association with the presence of other chronic complications such as coronary artery disease and diabetic retinopathy and neuropathy in diabetic patients [6] and this aspect should be considered while screening for microalbuminuria in diabetic patients.

Micro albuminuria predicts cardiovascular diseases and independently associated with hypertension and diabetes [25]. Prevalence of microalbuminuria was reported in 33.3% of hypertensive patients with albustix negative test in spot urine samples [26]. Even in general population microalbuminuria occurs up to 7% people without diabetes and hypertension [27]. Definitely there is a possibility of the greater prevalence of microalbuminuria in diabetic and non-diabetic hypertensive patients. Thus, microalbuminuria not only acts as an indicator of microvascular kidney manifestations of diabetes but also a good indicator of cardiovascular diseases as well. The analysis of urinary microalbumin will serve to assess the complications of diabetes and cardiovascular disease. In the present study, we did not observe significant dyslipidemia in both the types of diabetic patients, rather lipid parameters were well within normal range both in control and diabetic subjects (Table 2). Both Type-1 and Type-2 diabetic subjects were on cholesterol-lowering medications. Lipid levels in all the groups were within the normal levels. The reason for the lower levels of serum cholesterol in the Type-2 subjects may be due to the effect of these medications. However, large samples size is required to analyze the effect of hypocholesterolemic drugs correlation between various lipid parameters and microalbuminuria in both types of diabetic subjects

The most important factor here to mention is the socioeconomic status and lifestyle of these subjects. All subjects were from lower socioeconomic status, and most of them were vegetarians hence the intake of animal fat was not a significant factor. Looking into the variations observed in different lipid parameters it is necessary to establish a reference range in Indian population [28,29]. At present in India, the reference ranges used are mostly of those established for the western population, which will be different from actual reference range in Indian population. Hence, this aspect should also be considered while interpreting the correlation between lipid parameters and micro albumin levels.

A limitation of our study was that we had collected only one sample and did not do follow-up studies and also analysis of other parameters such as HbA1c and other clinical data from the subjects. These aspects can further be studied for better understanding of the effects and control of diabetes in patients in countries like India.

CONCLUSION

In the present study, incidence of microalbuminuria in both types of diabetics was found to be significantly associated with duration of diabetes. The incidence of microalbuminuria was much higher in both the types of diabetes. As microalbuminuria is an early sign of microvascular complications, it is highly recommended to screen all diabetic patients for the incidence of microalbuminuria at the onset and yearly assessment. It is necessary to assess the status of diabetic nephropathy in diabetic patients so as to take measures to prevent it to result into overt nephropathy and to an end-stage kidney disease. Screening for

microalbuminuria in diabetic patients shall be made mandatory at least in the tertiary health care setup for primary prevention and better health care.

REFERENCES

1. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications 1999. Geneva: World Health Organization; 1999.
2. ICMR Guidelines for Management of Type-2 Diabetes. ICMR, New Delhi. Available from: http:\\www.icmr.nic.in/guidelines_diabetes/guide_diabetes.htm ICMR 2005.
3. Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, *et al*. Diabetic nephropathy. Diabetes Care 2003;26 Suppl 1:S94-8.
4. Agarwal SK, Dash SC. Spectrum of renal diseases in Indian adults. J Assoc Physicians India 2000;48:594-600.
5. Koopman RJ, Mainous AG 3rd, Liszka HA, Colwell JA, Slate EH, Carnemolla MA, *et al.* Evidence of nephropathy and peripheral neuropathy in US adults with undiagnosed diabetes. Ann Fam Med 2006;4:427-32.
6. Al-Maskari F, El-Sadig M, Obineche E. Prevalence and determinants of microalbuminuria among diabetic patients in the United Arab Emirates. BMC Nephrol 2008;9:1.
7. American Diabetes Association. Clinical practice recommendations 2001: Diabetic nephropathy. Diabetes Care 2001;24 Suppl 1:S69-72.
8. Incerti J, Zelmanovitz T, Camargo JL, Gross JL, de Azevedo MJ. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial Transplant 2005;20:2402-7.
9. Khawali C, Andriolo A, Ferreira SR. Comparison of methods for urinary albumin determination in patients with type 1 diabetes. Braz J Med Biol Res 2002;35:337-43.
10. Ewald B, Attia J. Which test to detect microalbuminuria in diabetic patients? A systematic review. Aust Fam Physician 2004;33:565-7, 571.
11. Agarwal I, Kirubakaran C, Markandeyulu, Selvakumar. Quantitation of proteinuria by spot urine sampling. Indian J Clin Biochem 2004;19:45-7.
12. Sampaio E, Delfino VD. Assessing albuminuria in spot morning samples from diabetic patients. Arq Bras Endocrinol Metabol 2008;52:1482-8.
13. Jafar TH, Chaturvedi N, Hatcher J, Levey AS. Use of albumin creatinine ratio and urine albumin concentration as a screening test for albuminuria in an Indo-Asian population. Nephrol Dial Transplant 2007;22:2194-200.
14. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: Diagnosis, prevention, and treatment. Diabetes Care 2005;28:164-76.
15. Weir MR. Albuminuria predicting outcome in diabetes: Incidence of microalbuminuria in Asia-Pacific Rim. Kidney Int Suppl 2004:S38-9.
16. Al Shaikh A. Prevalence of microalbuminuria in type 2 diabetes mellitus at diabetic clinic in King Abdulaziz University Hospital. Pak J Med Sci 2007;23:223-6.
17. Amini M, Safaei H, Aminorroaya A. The incidence of microalbuminuria and its associated risk factors in type 2 diabetic patients in Isfahan, Iran. Rev Diabet Stud 2007;4:242-8.
18. John L, Rao PS, Kanagasabapathy AS. Rate of progression of albuminuria in type II diabetes. Five-year prospective study from south India. Diabetes Care 1994;17:888-90.
19. Bruno G, Merletti F, Biggeri A, Bargero G, Ferrero S, Pagano G, *et al*. Progression to overt nephropathy in type 2 diabetes: The Casale Monferrato Study. Diabetes Care 2003;26:2150-5.
20. Rossing P, Hougaard P, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in type 1 diabetic patients: A 10-year prospective observational study. Diabetes Care 2002;25:859-64.
21. Yanagawa T, Araki A, Sasamoto K, Shirabe S, Yamanouchi T. Effect of antidiabetic medications on microalbuminuria in patients with type 2 diabetes. Metabolism 2004;53:353-7.
22. Matthews DR, Charbonnel BH, Hanefeld M, Brunetti P, Schernthaner G. Long-term therapy with addition of pioglitazone to metformin compared with the addition of gliclazide to metformin in patients with type 2 diabetes: A randomized, comparative study. Diabetes Metab Res Rev 2005;21:167-74.
23. Haimoto H, Sasakabe T, Umegaki H, Wakai K. Reduction in urinary albumin excretion with a moderate low-carbohydrate diet in patients with type 2 diabetes: A 12-month intervention. Diabetes Metab Syndr Obes 2012;5:283-91.
24. Almeida JC, Zelmanovitz T, Vaz JS, Steemburgo T, Perassolo MS, Gross JL, *et al.* Sources of protein and polyunsaturated fatty acids of the diet and microalbuminuria in type 2 diabetes mellitus. J Am Coll Nutr 2008;27:528-37.
25. Jafar TH, Qadri Z, Hashmi S. Prevalence of microalbuminuria and associated electrocardiographic abnormalities in an Indo-Asian population. Nephrol Dial Transplant 2009;24:2111-6.
26. Sabharwal RK, Singh P, Arora MM, Somani BL, Ambade V. Incidence of microalbuminuria in hypertensive patients. Indian J Clin Biochem 2008;23:71-5.
27. de Jong PE, Hillege HL, Pinto-Sietsma SJ, de Zeeuw D. Screening for microalbuminuria in the general population: A tool to detect subjects at risk for progressive renal failure in an early phase? Nephrol Dial Transplant 2003;18:10-3.
28. Malati T. Whether western normative laboratory values used for clinical diagnosis are applicable to Indian population? An overview on reference interval. Indian J Clin Biochem 2009;24:111-22.
29. Sairam S, Domalapalli S, Muthu S, Swaminathan J, Ramesh VA, Sekhar L, *et al.* Hematological and biochemical parameters in apparently healthy Indian population: Defining reference intervals. Indian J Clin Biochem 2014;29:290-7.

Source of Support: Nil, Conflict of Interest: None declared.