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INCIDENCE OF MICROALBUMINURIA IN HYPERTENSIVE PATIENTS

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ABSTRACT

The prevalence of microalbuminuria was assessed in 174 albustix negative hypertensive patients by estimating albumin in the morning random urine samples by immunoturbidimetric method within four hours of voiding of urine. The urine samples were not stored and collected without any preservatives. The urinary albumin was calculated in terms of ratio with respect to urinary creatinine and expressed as albumin creatinine ratio (mg/g). Out of 174 albustix negative hypertensives, 58 (33.3%) patients were found to have microalbuminuria. The prevalence of microalbuminuria in males and females was found to be 34% and 30.7% respectively. No correlation was found between the Body Mass Index (BMI) and albumin excretion (r^2 = 0.0271) and between duration of hypertension and urinary albumin excretion (r^2 = 0.0042). Prevalence of microalbuminuria in nonsmokers and non-alcoholic hypertensives was 20%. The prevalence in alcoholics, smokers and both smokers and alcoholics was found to be 35%, 42% and 41% respectively. The high prevalence of microalbuminuria than the various reported studies on the subject demands establishment of a screening programme for microalbuminuria, implementation of specific intervention methods and education of hypertensive patients about the consequences of smoking and alcohol on possible involvement of renal system.

KEY WORDS

Hypertension, Microalbuminuria, Albumin : Creatinine ratio.

INTRODUCTION

Hypertension is one of the most common cardiovascular disorders. Benign arteriolar nephrosclerosis seen in hypertensive patients (blood pressure more than 140/90 mm Hg) for an extended period of time may manifest as a mild to moderate elevation of serum creatinine, microscopic haematuria and/or microalbuminuria. The association between microalbuminuria and hypertension was described by Parving et al in 1974 (1). Microalbuminuria has a major impact on cardiovascular risk (2) During the past few years microalbuminuria has become a prognostic marker for cardiovascular disorders. In essential hypertensives, an increased transglomerular passage of albumin may result from several mechanisms—hyperfiltration, glomerular basal membrane abnormalities, endothelial dysfunction and nephrosclerosis (3) Microalbuminuria which represents albumin excretion rate (AER) of 30 to 300 mg/24 hours or 20-200 micrograms/minute (4) or 30-299 mg/g creatinine (5) is defined as elevated urinary albumin excretion below the level of clinical albuminuria (4), undetected by Albustix and can only be detected by special methods such as immunochemical (6) and is reversible with euglycaemic control.

Microalbuminuria has been proved to be a prognostic marker for the development of nephropathy in long standing diabetic patients (7). Treatment of such patients with drugs like angiotensin converting enzyme inhibitors has been shown to be useful in retarding progress of nephropathy, although the exact mechanism of protection remains to be elucidated (8). A similar prognostic role of microalbuminuria is possible in early detection and intervention in patients of hypertensive nephropathy. Therefore it is pertinent to detect nephropathy as early as possible to take proper precaution and to initiate appropriate management.

The objective of this study was to detect microalbuminuria in albustix negative hypertensive patients quantitatively by immunoturbidimetric method and study its prevalence, without storing and adding any preservatives to urine as it has been

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reported that storing of urine sample affects the albumin values depending upon the time and storage conditions (9)

MATERIALS AND METHODS

In this cross sectional study, morning random urine samples of clinically documented hypertensive patients attending Civil OPD of Command Hospital (SC), Pune and Military Hospital (Cardio-Thoracic Centre), Pune were screened for albuminuria by using Albustix. After excluding Albustix positive patients, Albustix negative urine samples centrifuged at 2000 rpm for 10 minutes, were used for quantitation of creatinine and albumin whereas the corresponding urine sample collected in sterile plastic bottles were sent to Dept of Microbiology for culture to rule out any urinary tract infection. The blood samples of the selected Albustix negative patients were analyzed for glucose, urea and creatinine levels while urine samples were analyzed for creatinine and albumin levels. Patients with diabetes (fasting plasma glucose values > 110 mg/dl and post prandial > 140 mg/dl), pre-existing renal insufficiency and urinary tract infection were also excluded.

Out of 204 patients screened, 188 Albustix negative patients were evaluated of which 174 patients were finally included in the study as “Cases” or “Patients”. Forty eight, apparently healthy, normotensive (Blood pressure <130/85 mm Hg, no previous history of hypertension / anti-hypertensive drugs), non-diabetic (Blood glucose values < 110 mg/dl (fasting) and < 140 mg/dl (post prandial) individuals were selected as “Controls” or “Normals” (Table 1).

Glucose was estimated by GOD-POD method, urea by Marbach, Scott, Chawney and Fawcett method based on Berthlot’s reaction and creatinine by fixed time kinetic method based on Jaffe’s reaction. Urinary albumin was estimated by immunoturbidimetric method using antibodies developed and purified in-house using affinity chromatography kit obtained from Bangalore Genei private limited Bangalore. The in-house developed and purified antibodies were standardized to estimate urinary albumin as per the procedure of Teppo (10) and compared with the immunoturbidimetric kit – Tina – quant product No 1875400, Boerhinger, Germany. The method is based on the principle that Anti-human albumin antibodies react with the antigen human albumin in the sample to form antigen-antibody complexes which following agglutination is measured turbidimetrically at 340 nm. The calibrators (Human Albumin in phosphate buffer, 50mmol/L, pH 8.0) of concentration 0.0, 22.5, 30.9, 167, 394 mg/L provided with the Boerhinger kits were used to plot the standard curve using our in-house developed antibodies. Urinary albumin in the samples was estimated from the standard curve. All the analytes were analyzed by using Shimadzu CL-750 (micro-flow spectrophotometer) within 4 hours of sample collection.

Body Mass Index (BMI) of all the subjects was calculated by the formula BMI = Weight (Kg) / Height (m^2). Weight and height were recorded by standard instruments.

The “Gold Standard” for the presence of microalbuminuria in random urine samples was defined as urinary albumin excretion in the range of 30 - 300mg/g creatinine. Statistical analysis was done by using software EPI 2000.

Urinary albumin was expressed as the ratio of milligrams of urinary albumin to grams of urinary creatinine (A:C ratio)

RESULTS

Microalbuminuria was studied in 174 hypertensive subjects (patients or cases). The age and sex distribution in 174 cases and 48 controls is as per Table 1. The mean duration of hypertension in the patients was 5.15 years with SD of 3.93 and ranged from 1-17 years.

The albumin:creatinine ratio (A:C ratio) in controls and cases is shown in Table 2. The mean ± SD of A:C ratio in controls was found to be 20.11 ± 6.65 mg/g and ranged from 4.3 - 29.5 mg/g. The mean ± SD of A:C ratio in total 174 patients was 40.07 ± 56.21 mg/g and ranged from 3.8 -296.3 mg/g.

The value of urinary albumin between 30 and 300 mg/g of creatinine was considered to be positive for microalbuminuria.

Table 1: Age (in years) & Sex Distribution in Controls and Patients

	CONTROLS					PATIENTS				
	No.	%	Range	Mean	SD	No.	%	Range	Mean	SD
Female	19	39.6	23-75	47.8	14	38	21.7	45-65	56.84	5.75
Male	29	60.4	20-68	42.92	16.02	136	78.3	41-72	58.14	8.2
Total	48	100	20-75	45.25	15.12	174	100	41-72	57.8	7.7

Table 2: Albumin : Creatinine Ratio (mg/g) in Control and Patients

	CONTROLS			PATIENTS		
Group	Range	Mean	SD	Range	Mean	SD
Female	4.6-29.3	18.3	6.92	3.8-210.2	41.0	58.61
Male	4.3-29.5	22.0	5.89	4.7-296.3	39.7	56.18
Total	4.3-29.5	20.11	6.65	3.8-296.3	40.07	56.21

Fifty eight patients were found to have albumin excretion of more than 30 mg/g of creatinine in random morning samples and therefore positive for microalbuminuria. The overall prevalence thus amounts to be 33.3 percent. The A:C ratio in normoalbuminuric and microalbuminuric hypertensive patients and its distribution in males and females is as per Table 3. The prevalence of microalbuminuria in females was found to be 30.7 percent and in males, it was found to be 34%.

Body mass index (BMI) of the patients ranged from 19.5 to 31.2 kg/m^2. No correlation was found between A:C ratio and BMI of the patients studied.

On the basis of duration of hypertension, patients were divided into three groups; Group-I with duration between 0-5 years, Group-II with duration between 6-10 years and Group-III with duration >10 years. The mean ± SD of A:C ratio in the Group I, II and III was found 42.48±59.90, 28.33±33.73 and 35.28±32.01mg/g respectively. p value between Group I & II, Group II & III, Group I & III and between Group I, II & III was found to be 0.4726, 0.570, 0.885 and 0.740, respectively. The correlation between duration of hypertension and urinary A:C ratio in overall 174 hypertensive yielded the coefficient of correlation (r^2) was 0.0042.

All the females in the study were nonalcoholic and nonsmoker. Therefore, only males were further divided into subgroups to study the effect of smoking and alcohol on microalbuminuria. It was found that in patients who were both nonsmoker and nonalcoholic, the prevalence was 20% whereas in patients who were both smokers and alcoholics, the prevalence was 41%.

DISCUSSION

Hypertension is one of the most common cardiovascular disorders. The higher blood pressure is more likely to accelerate atherosclerosis causing various cardiovascular complications prematurely. Benign arteriolar nephrosclerosis seen in hypertensive for an extended period of time may manifest as a mild to moderate elevation of serum creatinine and/or microalbuminuria. The association between microalbuminuria and hypertension was described as early as in 1974. Microalbuminuria which represents albumin excretion rate of 30 to 300 mg/24 hours or 20-200 micrograms/minute (4) is reversible with euglycaemic control. In this study we detected microalbuminuria in albustix negative hypertensive patients by immunoturbidimetric method and studied its prevalence without storing and adding any preservatives to urine as storing was reported to decreases albumin levels (9).

Albumin to creatinine ratio (A:C ratio) in the morning urine sample is a reliable estimate of 24 hours AER and better than albumin concentration alone (11-13). The AER of two hours timed urine samples has been reported to correlate well with the A:C ratio (r^2=0.91) (14). It is reported by Caduff that the A:C ratio in spot urine is a reliable test for microalbuminuria (15). Moreover, as per the latest recommendations of ADA 2001 (16), A:C ratio can be used for urinary albumin estimation to detect microalbuminuria. Therefore, in this study urinary albumin is estimated by using random urine samples and expressed as ratio of mg of albumin per gram of creatinine.

Based on this, the prevalence of microalbuminuria in

Table 3: Distrubution of Normoalbuminuric and Microalbuminuric Hypertensive Patients

	NORMOALBUMINURIC (n=116)			MICROALBUMINURIC (n=58)		
Group	Range	Mean	SD	Range	Mean	SD
Female	3.8-29.5	21.6	5.13	31.8-296.3	52.3	70.8
Male	4.7-29.5	27.2	4.39	33.0-288.3	59.7	66.04
Total	3.8-29.5	25.3	5.35	31.8-296.3	53.7	69.89

hypertensive patients was found to be 33.3 percent and it is in agreement with the study of Rosa (17), Cerasola (18) and Agrawal (19). Also the higher prevalence in males as compared to females is in agreement with the study of Halal (20). However our prevalence is comparatively higher than the studies by Hornych (21), Mimran (22), Halal (20), Col M (23), and MAGIC Study (Microalbuminuria: A Genoa Investigation on Complications) (24). This may be due to the fact that in this study urinary albumin was estimated within 4 hours of voiding of urine without adding any preservatives and storage of samples, as storage has been reported to reduce urinary albumin. Moreover large variation has been reported in various published studies. The large study on the prevalence of microalbuminuria by Bigazzi et al in 1992 reported the figure of 40% (25), while subsequent studies reported the variation from 4.7% to 40% (17).The difference in prevalences are expected as the differences can also be due to differences in definition of microalbuminuria (i.e. cut off values), method of urine collection (timed versus random), quality of albumin assay and mode of expression of urinary albumin (excretion rate, ratio to creatinine, or concentration), size of study (small cohort versus large population based) and ethnic background of patients.

No correlation was found in this study between the BMI and albumin excretion ($r^2 = 0.0271$) even though it is reported that microalbuminuria is more prevalent in obese hypertensive (26). This is in contradiction to the study by Redon (27) where correlation was reported between BMI and albumin excretion. However, Nishijo et al in a study of 245 nondiabetic Japanese men concluded that urinary albumin was significantly related to systolic and diastolic blood pressure in a manner independent of other factors such as BMI and plasma insulin (28). Correlation between duration of hypertension and urinary albumin excretion also yielded no correlation with $r^2 = 0.0042$.

As compared to overall prevalence of 33.3%, prevalence in nonsmokers and non-alcoholic hypertensive patients was 20%. The prevalence in alcoholics (which included both smoker-alcoholics, and non-smoker-alcoholics) was found to be 35%, whereas in the smoker group (which included both alcoholic-smokers and non-alcoholic-smokers) it was found to be 42%. It is reported that hypertensive smokers have a worse cardiovascular risk profile than non-smokers as smoking has been hypothesized to impair the pharmacological effects of antihypertensive drugs (29). The prevalence in these groups could not be statistically validated due to the small sample size of the subgroups. However this suggests that both smoking and alcohol add insult to renal function in hypertensive patients.

This study strongly recommends introduction of "microalbuminuria screening, intervention and education" programme. This programme should be designed to obtain data of the prevalence of microalbuminuria in hypertensive patients treated in various hospitals, establishing an easy screening programme for microalbuminuria, implementation of specific intervention methods and educate the hypertensive patients about the consequences of smoking and alcohol. Such programmes can substantially modify the natural history of renal involvement and possibly reduce the incidence of end-stage renal failure in the hypertensive patients.

REFERENCES

1. Parving HH, Jensen HE, Mogensen CE, Evrin PE. Increased urinary albumin excretion rate in benign essential hypertension. Lancet 1974; i: 1190-92.
2. Zuccala A, Fiorenza S, Rapana R, Santoro A. Hypertension, atherosclerosis and kidney. G Ital Nefrol 2005; 22 Suppl 31:S9-14.
3. Redon J, Pascual JM. Development of microalbuminuria in essential hypertension. Curr Hypertens Rep 2006; 8(2): 171-7
4. Parving HH. Microalbuminuria in essential hypertension and diabetes mellitus. J Hypertens 1996 Suppl; Sep: 14(2): S89-93: discussion S93-4.
5. Toto RD J. Microalbuminuria: definition, detection, and clinical significance. Clin Hypertens (Greenwich). 2004; 6 (11 Suppl 3): 2-7.
6. Lehmann R, Spinas GA, Schweiz Rundsch. Diabetic nephropathy: significance of microalbuminuria and proteinuria in Type I and Type II diabetes mellitus. Med Prax 1995; 84(44): 1265-71.
7. Sheth J, Trivedi B, Shah L, Sheth VS. Prevalence of microalbuminuria in diabetic subjects. J Assoc Physicians India 1993; 41(9):562-4.
8. Kshirsagar AV, Joy MS, Hogan SI, Falk RJ, Colinders RE. Effect of ACE inhibitors in diabetic and nondiabetic chronic renal disease: a systematic overview of randomized placebo controlled trials. Am J of Kidney Dis 2000; 35(4): 695-707.
9. Shield JP, Hunt LP, Morgan JE, Pennock CA. Are frozen urine samples acceptable for estimating albumin excretion in research? Diabet Med 1995; 12(8): 713-6
10. Teppo A M Immunoturbidimetry of albumin and immunoglobulin G in urine. Clin Chem 1982; 28 : 1359-61
11. Rodby RA, Rohde RD, Sharon Z, Pohl MA, Bain RP, Lewis EJ. The urine protein to creatinine ratio as a predictor of 24-hour urine protein excretion in type 1 diabetic patients with nephropathy. The Collaborative Study Group. Am J Kidney Dis 1995; 26(6): 904-9.

12. Eshoj O, Feldt-Rasmussen B, Larsen ML, Mogensen EF. Comparison of overnight, morning and 24-hour urine collections in the assessment of diabetic microalbuminuria. Diabet Med 1987; 4(6): 531-3.

13. Goldschmid MG, Domin WS, Ziemer DC, Gallina DL, Phillips LS. Diabetes in urban African-Americans. II. High prevalence of microalbuminuria and nephropathy in African-Americans with diabetes. Diabetes Care 1995; 18(7): 955-61

14. Somani BL, Saha TK, Arora MM, Sharma YV. Modified bromophenol blue dye binding method for quantitation of microalbuminuria in diabetes mellitus. MJAFI 1995; 51: 110-16.

15. Caduff F, Spinas GA, Engler H, Achermann HR, Berger W. The practical assessment of microalbuminuria as a marker for beginning diabetic nephropathy in: which urine sampling method ? Schweiz Med Wochenschr 1991; 121(10): 324-31.

16. ADA Standards of medical care for patients with diabetes mellitus: Diabetic nephropathy. Diabet Care 2001; 24 Suppl I: S33-S43

17. Rosa TT, Palatini P. Clinical value of microalbuminuria in hypertension. J Hypertens 2000; 18(6): 645-54.

18. Cerasola G, Cottone S, Mule G. Microalbuminuria, renal dysfunction & cardiovascular complications in essential hypertension. J Hypertens 1996; 14:915-20.

19. Agrawal B, Berger A, Wolf K, Luft FC. Microalbuminuria screening by reagent strip predicts cardiovascular risk in hypertension. J Hypertens 1996; 14:223-28.

20. Hallan H, Romundstad S, Kvenild K, Holmen J Microalbuminuria in diabetic and hypertensive patients and the general population—consequences of various diagnostic criteria—the Nord-Trondelag Health Study (HUNT). Scand J Urol Nephrol 2003; 37(2): 151-8.

21. Hornych A, Marre M, Mimran A, Chaignon M, Asmar R, Fauvel JP. Microalbuminuria in arterial hypertension. Measurement, variables, interpretation, recommendations. Arch Mal Coeur Vaiss 2000; 93(11): 1304-8.

22. Mimran A, Ribstein J, Du Cailar G. Microalbuminuria in essential hypertension. Current opinion in nephrology and hypertension 1999; 8(3): 359-63.

23. Col M, Ocaktan E, Ozdemir O, Yalcin A, Tuncbilek A Microalbuminuria: prevalence in hypertensives and diabetics.Acta Med Austriaca 2004 ; 31(1):23-9.

24. Pontremoli R, Sofia A, Ravera M. Prevalence and clinical correlates of microalbuminuria in essential hypertension: the MAGIC Study. Microalbuminuria: A Genoa Investigation on Complications. Hypertension 1997; 30(5): 1135-43.

25. Bigazzi R, Binachi S, Campese VM,Baldari G. Prevalence of microalbuminuria in a large population of patients with mild to moderate essential hypertension. Nephron 1992; 61(1): 94-7.

26. Pedrinelli R, Dell'Omo G, Penno G, Di Bello V, Giorgi D, Pellegrini G, Del Prato S, Mariani M. Microalbuminuria, a parameter independent of metabolic influences in hypertensive men. Microalbuminuria is more frequent in obese hypertensives J Hypertens 2003 ; 21(6): 1163-9.

27. Redon J, Liao Y, Lozano JV, Miralles A, Baldo E, Cooper RS. Factors related to the presence of microalbuminuria in essential hypertension. Am J Hypertens 1994; 7 (9 Pt 1): 801-7.

28. Nishijo M, Nakagawa H, Morikawa Y. Microalbuminuria and hypertension in nondiabetic Japanese men. Am J Hypertens1999; 12(1Pt 1): 16-20.

29. Journath G, Nilsson PM, Petersson U, Paradis BA, Theobald H, Erhardt L. Hypertensive smokers have a worse cardiovascular risk profile than non-smokers in spite of treatment—a national study in Sweden. Blood Press 2005; 14(3): 144- 50.