Original Article

Protocol of an observational study to evaluate diabetic nephropathy through detection of microalbuminuria in Indian patients

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ABSTRACT

Aim: To assess the prevalence of persistent microalbuminuria (MAU), its clinical correlates by dip stick method, its predictive value for potential kidney disease and the utility of this test as objective cue for health care seeking behavior in adult Indian patients with type 2 diabetes mellitus. **Materials and Methods:** Approximately 400,000 patients shall be enrolled in this multicentric, cross sectional study. Patients meeting eligibility criteria shall be screened for MAU through urine dipstick test using random daytime single spot urine specimen. Result shall be expressed either positive or negative based on the presence or absence of albumin in the urine and will be correlated with the corresponding random blood glucose. Height, weight, waist circumference and blood pressure shall be assessed. There will be three visits with a minimum interval of 28 days between two visits, to be completed within 180 days, and at least two of three urine tests measured in this period must show elevated albumin levels to diagnose MAU. **Conclusion:** Detection of MAU through the dipstick method is postulated to be a rapid, reliable test for early detection of diabetic nephropathy, which, in turn will help the physician to plan treatment strategy. Further, it will help to identify the disease burden on the individual and society, and may serve as an objective cue for improved health care seeking behavior, as well as a catalyst for health policy change.

Key words: Diabetic nephropathy, dipstick test, health policy, India, microalbuminuria

Introduction

The world is experiencing a definite surge in the prevalence of diabetes mellitus (DM) from 366 million people in 2011, which is expected to increase to 522 million by 2030. It is one of the most challenging clinical entities without appropriate preventive measures. Around 70% of diabetic patients are from developing and underdeveloped countries indicating, the burden of the disease on their crippling economy. Further, the disease affects people in the productive age group of 40-59 years, adding to the disease burden. India is expected to become the global capital for DM, with the number of patients increasing from 61.3 million in 2011 to about 101.2 million by 2030.[1]

DM is associated with multiorgan damage, with renal and cardiovascular involvement being highly critical.

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.111655

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Diabetic nephropathy (DN) is one of the most evident common causes of chronic kidney disease (CKD),[2] which, without timely intervention, can lead to end stage renal disease. In India, 30% of chronic renal failures can be attributed to DN.[3] The earliest clinical evidence of DN is microalbuminuria (MAU), which is also a strong risk factor for cardiovascular disease (CVD).[4-6] Routine screening for MAU is an easy, inexpensive predictive procedure for DN and can guide appropriate intervention, thereby minimizing disease progression. This procedure can also prove cost-effective.[7] Routine screening of diabetic patients for MAU will alert the authorities to prioritize and aid in bringing modification in the preventive strategies in health policies.

Figure 1 is the schematic representation of routine screening in patients with diabetes for MAU.[8]

The dip stick test is considered as a rapid, easy, valid, economical and reliable method for MAU screening in diabetic patients as a urinary albumin excretion rate (AER) above the threshold value of 20 μg/min can easily be demonstrated. In this article, we discuss the protocol of a clinical study, which is intended to assess the suitability and reliability of an easy, rapid and cost-effective method to test MAU in a larger population. We planned this study in Indian patients considering the high disease burden and associated economic costs. Moreover, the prevalence of MAU is not uniform throughout the world, being slightly higher in South Asian patients (31%) than in European patients (20%). Also, South-Asian type 2 diabetic patients are more prone to develop nephropathy and progressive renal failure than the European diabetic patients.[9]

Study design

This study is a multicentric, cross-sectional, observational, epidemiological study without any intervention to assess the prevalence of persistent MAU among type 2 diabetic patients. We intend to enroll approximately 400,000 adult patients aged >30 years and <74 years.

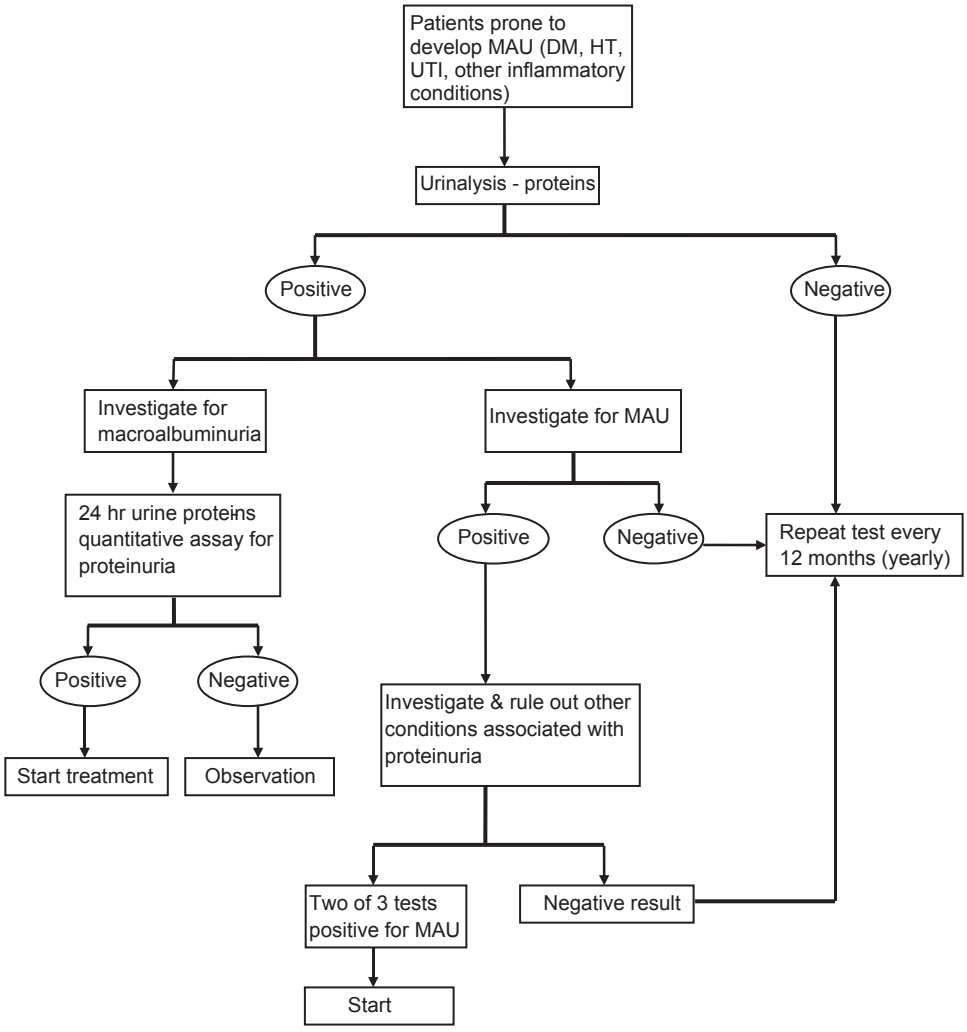

Figure 1: Schematic representation of dipstick test in patients with diabetes

Study objectives

The primary objective of the study is to assess the prevalence of persistent MAU in type 2 diabetics by the dip stick method (spot test), with the secondary objective being assessment of the clinical correlates of MAU and the exploratory objective being aimed to assess the utility of the dip stick test as an objective cue for health care-seeking behavior in type 2 diabetes.

Sample eligibility and enrollment process

This study shall be initiated after all regulatory prerequisites are met and conducted as per good clinical practice principles.

Inclusion criteria shall include those who are willing to participate voluntarily and provide written informed consent. Adult patients of both genders aged >30 years and <74 years, with a confirmed type 2 DM not having received insulin for the first 6 months, are eligible to be considered for this study.

Patients with blood pressure (BP) 160/100 mm Hg, having clinically evident inflammatory state including pyrexia, chronic use of non-steroidal anti-inflammatory drugs, individuals with hematuria/pyuria/urinary tract infections/ketonuria at the time of screening, patients with history of established renal disease and those who had performed strenuous physical exercise on the day of screening shall not be enrolled. Women, if in their menstrual cycle, pregnant or lactating, will also to be excluded.

Sample size calculation

A reasonably large sample size has been selected to give a better indication of the disease prevalence in the population. Approximately 400,000 patients are expected to be enrolled in this study. Major diabetic complications such as CKD, CVD, hypertension (HT) and dyslipidemia, which have a probability of 0.999 occurring as an event to an individual and probability of 0.001 in rare events, were considered among the population. Based on these, the sample size for this study has been calculated using nQuery statistical solutions. At alfa correction, the multiple outcome is 0.0125, 98.75 confidence interval (CI). To get at least one event, 4603 subjects are required. Assuming 75 such rare events to occur, the sample size required is 345,225, including 15% drops out. The final sample size of this study is 406,147 patients, which is rounded off to 400,000.

Materials and Methods

The study shall be conducted by 20 principal investigators. Around 1060 co-investigators shall facilitate the data collection and assist in the study.

Patient's eligibility shall be determined based on history and screening investigations, which shall be performed after obtaining a written informed consent from the participants. Study duration will be approximately, but not more than, 180 days (6 months), and shall include three visits having a minimum interval of 28 days between consecutive visits (including final visit at the end of the study).

At screening, patient's basic information, i.e., patient demography consisting of age, gender, height, weight, waist circumference (WC) and body mass index (BMI), will be recorded in the case report form (CRF). Baseline disease characteristics, i.e., h/o DM, duration of DM, treatment received, complications such as symptoms and evidence of DN and diabetic retinopathy will be recorded. History of smoking, alcohol consumption, any cerebrovascular attacks and peripheral vascular disease were also be recorded.

Information regarding preexisting conditions such as HT, dyslipidemia, concomitant drugs such as antihypertensive agents, lipid-lowering agents and other relevant medical information will also be collected.

Evaluation method

The urine dip stick test will be used for the evaluation of MAU at baseline. These dip sticks (QDX URINE TEST 11 MAU) are in use as part of clinical practice to detect the urinary levels of various compounds, including microalbumin.

Random daytime single spot urine specimen shall be collected from participants and presence of MAU will be assessed by the dip stick test. At least two of these three consecutive urine tests measured within a 180 day period of this ongoing study (last sample taken at 180 days) is required to show elevated albumin levels to confirm the diagnosis of MAU in these patients. The baseline visit will be considered as visit 1, followed by visit 2 and visit 3, which shall be completed within 180 days.[9]

Estimating random blood sugar (RBS) during these visits will help to correlate the diabetic status of the patient, which can be estimated using the glucose oxidase and peroxidase method.

Specimen collection and preparation

Fresh urine (first morning sample) shall be collected in a clean, dry container, which should be tested within 1 h of collection. The un-centrifuged sample will be mixed well before testing. Figure 2 is the schematic representation of the procedure for visual testing of dip sticks.

Interpretation of the urine test with dip stick

Color developed on the dip stick will be matched with

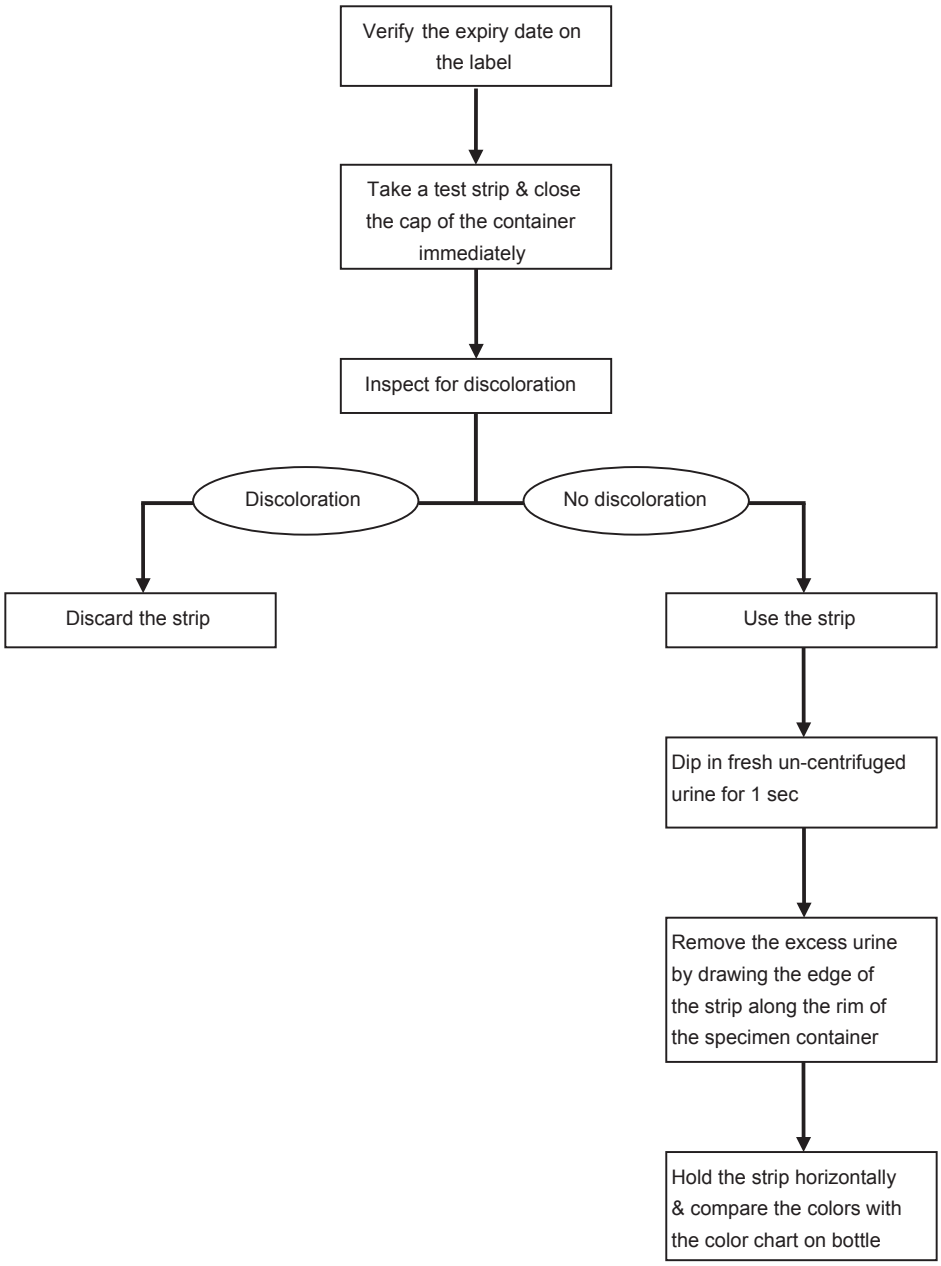

Figure 2: Procedure for visual inspection of dipstick

that on the label of the dip stick container [Figure 3]. The results of MAU are interpreted either as positive or negative based on the presence or absence of albumin specimen, respectively. Based on the result, further visits are planned [Figure 4].

The sensitivity and the test range of urinalysis strips are as shown in Table 1.[10]

If the result was negative, the dip stick test shall be be repeated only after 12 months. If the result is positive on two consecutive visits out of three, the investigating physician may advise screening of serum creatinine.

Secondary objective measures

The secondary objective measures shall include measurement of height, weight, WC, BMI, and BP to assess any other risk factors involved. Height and weight will be measured using the standard method of measurement.

Measurement of height

The patients will be made to stand with their feet together and their back pressed on to a wall having a measuring scale. The patient's buttocks, back and occiput shall touch the wall. The head shall be placed in the "Frankfurt plane." A book shall be placed perpendicular to the wall touching the highest point of the head of the patient while the patient is

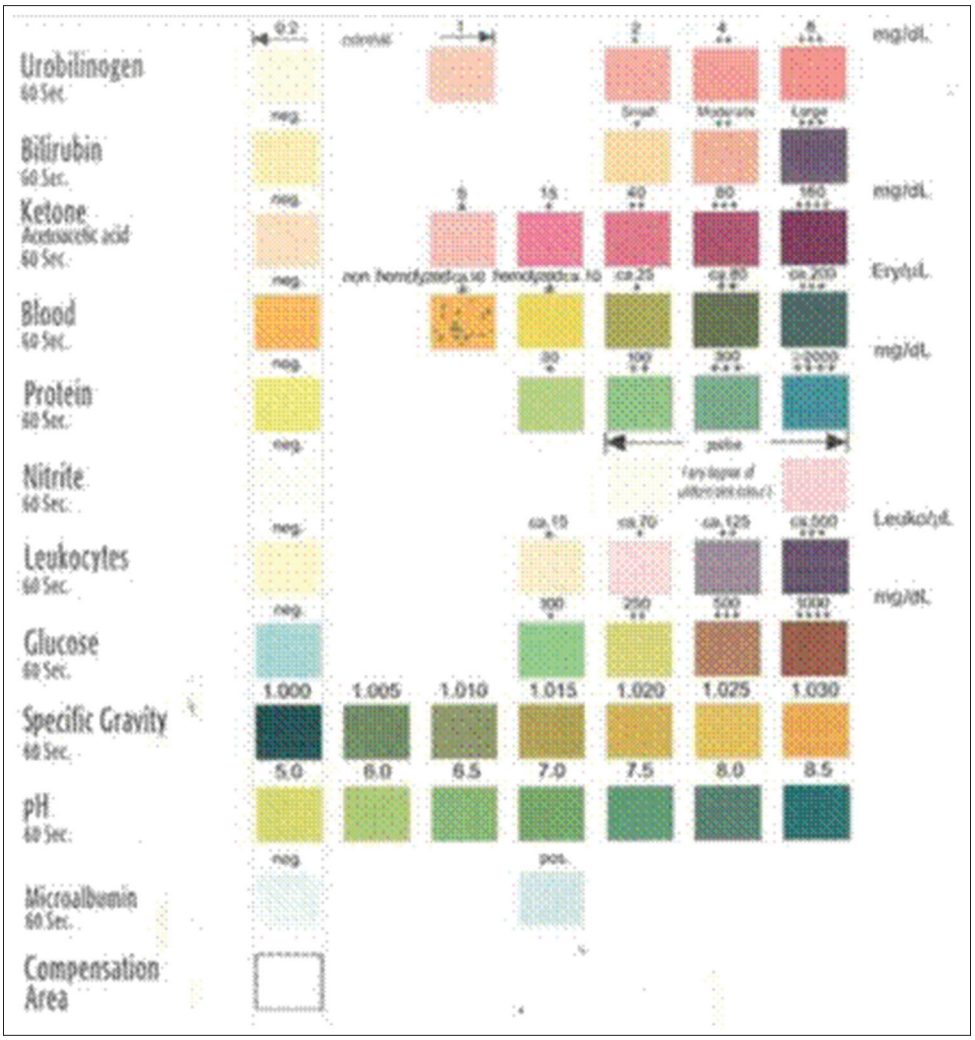

Figure 3: Tests and reading time for dipstick printed on the label. (Source: Label QDX URINE TEST 11 MAU Piramal Health Care India [dated 2012 Apr 01])

Table 1: Sensitivity and test range of urine test urinalysis strips

Test parameters	Sensitivity	Visual test range	Instrument test range
Uribilinogen (mg/dL)	0.05-0.3	0.05-2.50	
Bilirubin (mg/dL)	0.15-0.30	Negative-1.8	
Ketone (acetoacetic acid) (mg/dL)	10-20	Negative-300	Negative-150
Blood (Ery/µL)	5-15	Negative-200	
Protein (mg/dL)	15-30	Negative-2000	Negative-300
Nitrite (mg/dL)	0.25-0.4	Negative-Positive	
Leukocytes (leuko/µL)	5-15	Negative-500	
Glucose (mg/dL)	50-100	Negative-1000	
Specific gravity		1.000-1.030	1.005-1.030
pH		5.0-8.5	5.0-9.0
Microalbumin (mg/dL)	10-15	Negative-15	Negative->15

(Source: Package insert QDX URINE TEST 11 MAU-Reagent strips for urinalysis, Piramal Health Care Ltd., India [dated 2012 Apr 01])

breathing quietly and measurement (in centimeters) from the measuring scale after avoiding visual parallax shall be read.

Measurement of weight

The patient shall stand on the weighing scale after removing their footwear, belt and other heavy objects. The patient shall stand straight without looking at the weighing scale, with the feet kept together. The examiner shall read the measurement (in kilograms) from the weighing scale after avoiding visual parallax.

Different studies have used different anatomic landmarks to measure WC. In our study, the midpoint between the lowest rib and the iliac crest will be used for obtaining WC.[11]

Body mass index is calculated as weight divided by height squared (kg/m^2).[11] BMI above 25 kg/m^2 is considered as overweight as per the World Health Organization standards.[12] Indian guidelines has set a much lower value for BMI in Indians compared with the global standards at 23 kg/m^2 and those with BMI ≥23 kg/m^2 will be considered as clinically obese.[13]

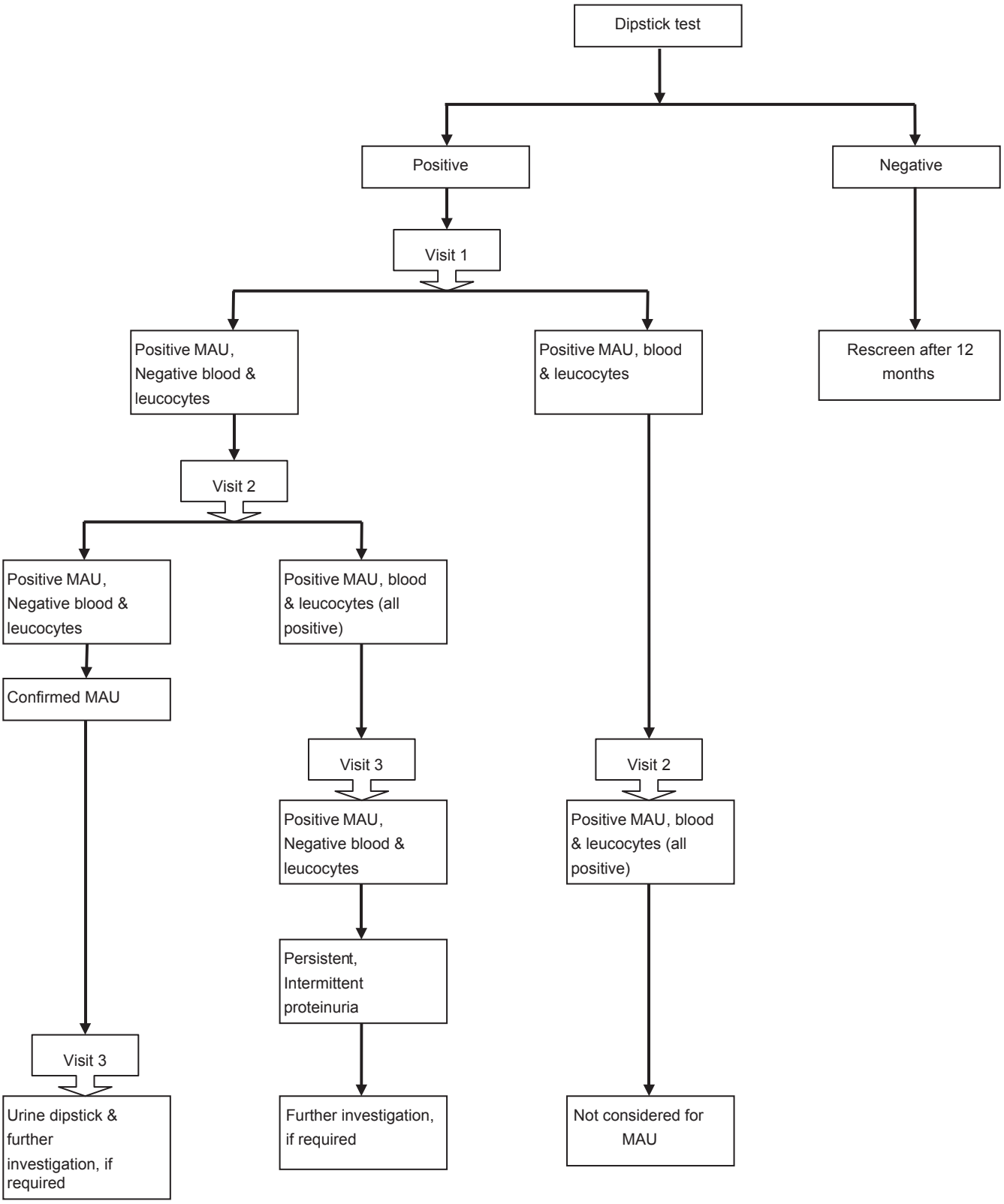

Figure 4: Flow diagram of test

Sitting BP shall be measured using a standard sphygmomanometer.

Because a study of such magnitude is being conducted for the first time anywhere in the world, certain unexpected findings may be encountered. Appropriate corrective action regarding the conduct of this study will be taken if deemed necessary by the panel.

Data collection

Demographic details, BP, RBS, result of dip stick test performed and any other relevant details that are recorded in the CRF shall be collected by the sponsor's representative(s) at regular intervals. The patients shall be provided with a Patient Profile Card – for patient identification and to track the visit-wise screening details.

Statistical analysis

Collected data will be entered in an appropriate format for analysis. Data processing, tabulation of descriptive statistics, calculation of inferential statistics and graphical representations will be performed using statistical software statistical analysis software (SAS) version 9.1.3 for Windows. Statistical testing will be performed at the 5% level of significance. Probability (P) < 0.05 will be regarded as being statistically significant. Appropriate statistical tests (parametric or non-parametric) will be used to determine the statistical significance between variables. Descriptive statistical analysis will be performed for all variables. Continuous variables will be summarized as mean, standard deviation, median and range (minimum, maximum) and 95% confidence interval of mean, while categorical variables shall be summarized as proportions (counts and percentages). Reason for drop out will be mentioned in the report as tables with its percentage value. Data analysis findings will be displayed in tables.

Discussion

Identifying and managing DN at an early stage of MAU, which is potentially reversible, is of utmost importance. The American Diabetes Association position statement (2012) recommendations for nephropathy screening advises estimation of urinary albumin annually in patients with type 1 diabetes of duration of ≥5 years and type 2 diabetic patients at diagnosis; it also recommends estimation of serum creatinine annually in all adult diabetic patients to evaluate renal functions.[14]

Early detection of MAU at an early and reversible stage is most critical as intervention at this stage is thought to have a potential positive impact on the disease outcome. Although the guidelines recommend routine screening in high-risk groups, unfortunately, we have not been able to implement it as these procedures are either not easily available or affordable.[15]

Hence, the first step in screening for MAU should be the measurement of albumin in a urine sample by a reliable method such as spot (first-morning or random sample), 24-h collection or timed collection.[16] Timed urine collection, although cumbersome, is considered the reference method, which established the predictive role of MAU for the development of overt nephropathy.

Estimation of the albumin/creatinine ratio of an early morning urine sample to detect the overnight AER >30 μg/min has been found to be convenient, and this method is also less error prone to detect MAU. It has shown 100% sensitivity, 95% specificity, and 64% predictive value.[17] This ratio was higher among Indian diabetic patients than among Indian controls and European diabetic patients. Early onset of type 2 DM observed in Indians than in other populations indicated that DN can be a significant health burden in this population.[18]

Overall prevalence of MAU in Indian patients has been reported to be around 37% in type 2 DM.[19,20] A study by Varghese *et al*.[20] has shown that the prevalence of MAU in Indian patients with type 2 DM is similar to that in the European population. Islam *et al*.[21] have seen no difference in the prevalence of MAU among Indian, Malay, and Chinese patients, although urinary albumin excretion was higher in Indians. The UK Prospective Diabetes Study Group too did note any ethnic difference in the prevalence of MAU.[22]

Measurement of urinary albumin in random urine samples is considered as the best procedure to screen MAU in diabetic patients, as the results are accurate and the procedure is cost-effective.[16] Many studies have suggested early morning urine sample to test MAU,[17] but the easiest and most useful tool to screen is using a spot urine specimen, especially in larger populations with type 2 DM.[23] This test has been found to valid, reliable[24] and equivalent to the measurement of AER.[25] It has also been suggested that spot test can be used as a screening test for MAU in a large population as satisfactory results have been observed with this test.[25] In clinical settings, where a standard quantitative technique to measure urinary albumin is not available, a semiquantitative test such as the dip and read test strips could be used to screen for MAU.[16]

The urine test reagent strips for urinalysis are dip strips used as an *in vitro* diagnostic aid to determine eight different compounds, specific gravity, pH and microalbumin in urine. These strips can provide both qualitative and semiquantitative determination. The results obtained on these strips are read visually and used.[10]

The sulfonaphthalein dye has high sensitivity to detect microalbumin, which is interpreted as positive or negative. To rule out pyuria and hematuria, leukocytes and blood, respectively, can also be detected by these strips.

Granulocyte leukocyte esterases in urine that catalyze the hydrolysis of the pyrrole amino add ester to liberate 3-hydroxy 5-phenyl pyrrole. This pyrrole produces a purple color when it reacts with diazonium. Hemoglobin reacts with peroxidase causing the release of neoecotypes oxide (O), which oxidizes the indicator and produces the color change on the dip stick.[10] The advantage of this dip stick method is that it includes random urine sampling for analysis, unlike the first morning sample as required by other assays. This test can be performed on un-centrifuged urine sample immediately after sample collection, and gives a rapid and reliable result.

Several methods such as enzyme-linked immunosorbent assay, conventional radioimmunoassay, immunoturbidimetry, immunonephelometry, chemiluminescence immunoassay, fluorescence immunoassay, time-resolved fluorescence, resonance scattering spectral assay, high-performance liquid chromatography, nephelometry and immunoturbidimetry in clinical practice to detect MAU can be used.[7] Urinary measurement of transforming growth factor-β and type IV collagen[26] elevated plasma concentrations of soluble vascular adhesion molecule-1, soluble intercellular adhesion molecule-1 and soluble E-selectin[27] and various other endogenous markers including cystatin-c as predictors of early renal damage have been implicated.[28] However, they are not readily available, particularly for general practitioners, and not affordable by many patients. Furthermore, the advantages offered by the dip stick method make this more applicable in routine clinical practice, even in distant, rural areas.

Obesity-related factors have been considered as predictors of risk factors of DM, especially in type 2, HT and CVD and, among this, WC is considered as a strong and reliable predictor.[29] Body fat at the lumbar region (L2-3, L4-5), WC and BMI among others have been considered as strong predictors of DM; their association is well established all over, including the Asian population.[30,31] The literature indicates an association between WC, BMI with HT, DM and cardiovascular risk factors, and the same has been noted in Indian women by Gijo *et al*.[32] WC is a measure of abdominal/central obesity and is considered as a decisive factor determining CKD.[33] Hence, we intend to measure these factors to correlate the association between WC, BMI, DM and CKD. This will help in risk stratification, planning the treatment strategy, optimizing patient care by focusing on obesity management and preventive measures in high-risk patients.

Available data on MAU in Indian diabetic patients in the recent past is limited. Considering a large number of existing DM patients, and with an increase in the number annually, disease complications are expected to be higher in the future. Detection at the early stage of MAU helps in not only early diagnosis but also in better glycemic control, prevention of disease complications and other associated disorders such as HT, CVD and CKD. This study will be the first to study the accuracy and cost-effectiveness of the dip stick method to detect MAU in Indian diabetic patients, and is expected to provide a better insight to the existing health problem that is surging rapidly and reaching epidemic proportions.

Conclusion

Diagnosing and managing MAU is very important to health care providers as well as to policy makers to assess the extent of the disease problem. Rapid detection through an easy, reliable method, apart from cost-effectiveness and affordability, makes this procedure highly relevant to a developing country like India, where the number of patients with DN is increasing exponentially. Detection of MAU through this dip stick method can be a reliable test for early detection, which can help the physician to assess the progress and complications of the disease and can bring positive changes in health care policies.

Acknowledgments

The authors acknowledge Dr. Yajnik CS, Diabetologist, KEM Hospital; Dr. Subhankar Chowdary, Endocrinologist, SSKM Hospital, Kolkata; Dr. Subhash Wangnoo, Endocrinologist, Apollo Hospital, Delhi; Dr. Sunil M Jain, Endocrinologist, Total Diabetes Hormone Institute, Indore; Dr. Narashima Shetty, Endocrinologist, Karnataka Institute of Diabetology, Bengaluru; and Dr. Nishit Mohanty, Nephrologist, Bhubaneshwar, India. Authors also thank the following staff from Dr Reddy's Laboratories Ltd., Hyderabad India- Dr V P Pandya, former Associate Director; Kumar S, Director and Division Head Zenura 3; Mr Ameet Kishor Suratkar,Associate Director; Mr Prahlad Katte, Group product Manager; Mr Ramesh Katipally, Product Manager, Dr. Akhilesh Sharma, Vice-President, Global Medical Affairs; and Dr. Rajan Mittal, Associate Director, Medical Affiars, Dr. Reddy's Laboratories Ltd., Hyderabad, India for supporting the team with their scientific inputs. The support of Mr. *P* Krishnanand, CRA, Global Medical Affairs, Dr. Reddy's Laboratories Ltd., India, in the preparation of this manuscript is highly acknowledged. Authors also acknowledge Dr Latha, Manager, Medical writing, Dr Reddy's Laboratories Ltd., Hyderabad, in the preparation, editing of this manuscript.

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Cite this article as: Gokulnath, Sahay M, Kalra S, Vishwanathan V, Zargar AH, Talwalkar PG, *et al*. Protocol of an observational study to evaluate diabetic nephropathy through detection of microalbuminuria in Indian patients. Indian J Endocr Metab 2013;17:496-504.

Source of Support: This study is sponsored by Dr Reddy's Laboratories Ltd., Hyderabad, India. Dr Jasmeet Singh Sahni is an employee of Sponsor Company and is a stakeholder in Dr Reddy's Laboratories Ltd., by means of salary and stocks. Other authors are the investigators who will be conducting this study and do not have any conflict of interest. **Conflict of Interest:** Author 14 –Dr Jasmeet Singh Sahni is having conflict of interest; other authors do not have any conflict of interest.