ORIGINAL ARTICLE

Detection of Microalbuminuria by Rocket Electrophoresis in Patients Suffering from Diabetes Mellitus with Soft Tissue Infections

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Abstract

Background: *Diabetes Mellitus is an important public health problem because of their health, social and economic consequences. Diabetes progressively leads to many systemic abnormalities including diabetic nephropathy which initially causes microalbuminuria. High risk patients such as cases with recurrent infections can be screened by detecting microalbuminuria. Various methods are available for detection of microalbuminuria and we have tried rocket electrophoresis for detection of microalbuminuria in diabetic patients with soft tissue infections.* ***Material and Methods:*** *A total of 305 Diabetes Mellitus patients without associated complications but with or without soft tissue infections were recruited for the study. Microalbuminuria was detected in these patients by Rocket Electrophoresis using the locally raised antialbumin antibodies. Rocket electrophoresis was done according to the technique described by Laurell CB, (1966). Height of rocket (in mm) was plotted on Y axis and corresponding albumin concentration on X – axis.* ***Results:*** *Microalbuminuria was observed in 70 cases out of whom 45(64.28%) were on oral therapy and 25(35.72%) were on insulin therapy. 38(54.28%) were hyperglycemic and 44(62.85%) were hypertensive. 55(78.57%) microalbuminuric patients had duration of illness above 5 years.* ***Conclusion:*** *Microalbuminuria is not uncommon feature of diabetes mellitus. It is commonly seen in raised blood sugar, raised blood pressure and mores with increased duration of diabetes. The method of rocket electrophoresis used in the study is simple, rapid, economical and feasible. Routine screening of patients of diabetes for microalbuminuria especially in presence of associated factor is recommended.*

Key words: *Diabetes Mellitus, Microalbunuria, Rocket Electrophoresis.*

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Introduction

Diabetes mellitus is one of the most common serious metabolic disorders. The diabetic patients are susceptible to a series of complications that cause morbidity and premature mortality. These include circulatory abnormalities, retinopathy, nephropathy, neuropathy, diabetic foot ulcers and infections. Diabetic nephropathy is not uncommon cause of death in diabetic patients. Diabetic nephropathy may be functionally silent for years. It initially leads to microproteinuria (microalbuminuria) before landing up in the stage of macroproteinuria. Identification of those patients with risk of developing diabetic nephropathy is possible by screening microalbuminuria[1]. Microalbuminuria is an excretion of albumin in the range of 30 to 300 mg per 24 hours[2]. At the stage of microalbuminuria, if suitable treatment in the form of Angiotensin Converting Enzyme (ACE) inhibitors, control of sugar and hypertension is given, the albumin excretion rate can be lowered which postpones or prevents the clinical nephropathy. A number of quantitative assays

are available to detect urinary albumin at low level. These are based on immunochemical methods using antibodies to human albumin. These methods include Nephalometry, Turbidimetry, Radioimmunoassay, ELISA, Rocket electrophoresis and Single Radial immunodiffusion[3]**.** High cost, frequent non availability of kits and lack of infrastructure limit the utility of some of these tests in the peripheral laboratories. Therefore for routine screening of these patients, a simple, rapid and economical test is desired.

Microalbuminuria has been well studied as a useful marker for impending complications like diabetic nephropathy[4]**.** It has been also determine to be useful marker for retinopathy. The association of microalbuminuria with other associated factors in diabetes mellitus like insulin therapy, hyperglycemia and increased duration has been documented. However, its role in yet other complication i.e. soft tissue infections remains to be ascertained. In diabetes mellitus patients, ischemia and peripheral neuropathy are the major factors leading to ulcer, a lesion that usually serves as portal of entry for soft tissue, bony or even systemic infection[5]. Detection of microalbuminuria may also serve as useful marker for an individual's susceptibility to soft tissue infection in diabetes mellitus. High risk patients such as cases with recurrent infections can be screened by detecting microalbuminuria. Various methods are available for detection of microalbuminuria and we have tried rocket electrophoresis for detection of microalbuminuria in diabetic patients with soft tissue infections.

Materials and Methods

This study comprises of 305 patients of diabetes mellitus with or without soft tissue infections attending or admitted to Government Medical College and Hospital. Presence of any metabolic complications of diabetes mellitus like Diabetic ketoacidosis/ketonuria, Diabetic hyperosmolar coma were excluded from this study. These patients were thoroughly evaluated and investigated to determine presence of complications like retinopathy, neuropathy, nephropathy, coronary artery disease, peripheral vascular disease, cardiovascular events etc. The findings in each patient were recorded. A total of 100 age and sex matched controls were also included in the study. The statistical analyses were done using the standard statistical methods.

Assessment of Albuminuria

After detailed history, clinical examination, patients were advised to collect 24 hour urine output. The collected urine output was measured in liters and samples of urine was examined by uristix were considered macroalbuminuria and excluded for microalbuminuria detection while the sample which was negative for albuminuria by uristix was subjected to rocket electrophoresis for microalbuminuria estimation[6].

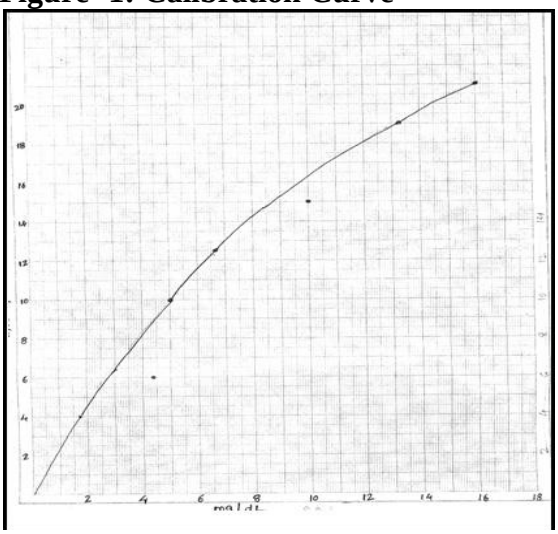
Rocket Electrophoresis [7]

Rocket electrophoresis was done according to the technique described by Laurell CB, (1966). In 10 ml of Barbitone buffer (pH 8.6, 0.05, 1.5%). Agarose was taken, boiled and cooled at to 56°c in water bath. The albumin antibody (120:1) was thoroughly mixed with the molten gel at 56°C and poured on the microscope slides (3 ml each). The antibody incorporated gel was allowed to set at 4°C for 1 hour. Using suitable templates the wells of 2 mm diameter each, six wells were punched in the gel, and filled with albumin standards. The electrophoresis was carried out using barbitone buffer (Diluted 1:1) in the tank at the constant current of 7mA per gel for 3 hours. Following the electrophoresis the heights of rocket shaped immune precipitates were measured and a calibration curve was plotted. Height of rocket (in mm) was plotted on Y axis and corresponding albumin concentration on X – axis (Figure -1).

Urinary albumin was estimated by loading urine samples in the wells and measuring the heights subsequent to the electrophoresis. The albumin concentrations were derived by noting the intercepts on the calibration graph for the heights of rocket obtained. Finally, drying and staining of gels was done.

The 24 hour urinary albumin excretion was calculated from albumin concentration in urine (mg/dl) and the 24 hours urine output. The samples with 24 hour albumin excretion between 30 mg and 300 mg were considered to be having microalbuminuria, < 30 mg as normoalbuminuria and more than 300 mg as macroalbuminuria.

Figure- 1: Calibration Curve



Results

Out of 305 patients of diabetes mellitus 203(66.55%) patients were without soft tissue infections and 102(33.45%) were with soft tissue infections. 220(72.15%) of these were males and 85(27.85%) were females. The male to female ratio was 2.6 to 1 with mean age of 49.66. Out of 305 cases, 166(54.45%) patients were on oral therapy and 139(45.55%) on insulin therapy. From 102 patients with infections, 76(74.50%) were on insulin therapy while in without infection group 75(36.95%) patients were on insulin (Table- 1).

From all the cases, 160(52.45%) patients were hyperglycemic in which 84(41.38%) were without infections while 76(74.50%) with infections. 171(56.06%) patients were normotensive and 134(43.94%) were hypertensive. 61(59.80%) patients infected patients were normotensive as compared to 110(54.20%) patient without infection. 160(52.46%) patients had duration of illness upto 5 years while 145(47.54) patients were having above 5 years duration of illness. In infected group 60(58.80%) patients had duration of illness above 5 years while number of patients were less in without infection group 100(49.27%) table- 1.

Microalbuminuria was detected in a total of 91(23.83%) patients from whom 70(34.48%) patients were without infection and 21(20.58) were with infections. It was observed that 39(38.23%) patients with infection had overt albuminuria as detected by dipstick. Out of 70 microalbuminuric patients of without infection group, 45(64.28%) were on oral therapy and 25(35.72%) were on insulin therapy. 38(54.28%) were hyperglycemic and 32(45.72%) were normoglycemic. 44(62.85%) were hypertensive and duration of illness in 55(78.57%) was above 5 years (Table- 2).

Table- 1: Association of different factors in diabetic patients with & without infection

S.N.	Associated Factors	Patient without infection (n=203)	Patient with infection (n=102)	Total (n = 305)
1	**Treatment**			
	Oral	128 (63.05%)	38 (37.25%)	166 (54.42%)
	Insulin	75 (36.95%)	64 (62.75%)	139 (45.57%)
	Total	230(100%)	102(100%)	305(100%)
2	**Blood sugar**			
	Normal	119 (58.62%)	26 (25.50%)	145 (47.55%)
	Raised	84 (41.38%)	76 (74.50%)	160 (52.45%)
	Total	203(100%)	102(100%)	305(100%)
3	**Blood pressure**			
	Normal	110 (54.18%)	61 (59.80%)	171 (56.06%)
	Raised	93 (45.82%)	41 (40.20%)	134 (43.94%)
	Total	203(100%)	102(100%)	305(100%)
4	**Duration of illness**			
	Upto 5 Years	103 (50.73%)	42 (41.20%)	145 (47.54%)
	Above 5 Years	100 (49.27%)	60 (58.80%)	160 (52.46%)
	Total	203(100%)	102(100%)	305(100%)

Table- 2: Association of factors with microalbuminuria

Factors	Microalbuminuria
Treatment	**Patients (%)**
Oral (n =128)	45 (64.28)
Insulin (n = 75)	25 (35.72)
Total (n = 203)	70 (100)
Blood sugar	
Normal (n = 119)	32(45.72)
Raised (n = 84)	38 (54.28)
Total (n = 203)	70 (100)
Blood pressure	
Normal (n = 110)	26 (37.15)
Raised (n = 93)	44 (62.85)
Total (n = 203)	70 (100)
Duration of illness	
Upto 5 Years (n = 103)	15 (21.42)
Above 5 Years (n = 100)	55 (78.58)
Total (n = 203)	70(100)

Patients of without infections with microablimunuria group further subdivided into sub groups to access the co-relation of associated factors with prevalence of microalbuminuria (Table- 3). Each associate factor was named as factor A, factor B, factor C and factor D as given below.

Insulin therapy : Factor A
Raised Blood sugar : Factor B
Raised Blood Pressure : Factor C
Increased duration : Factor D

In group I out of 21 patients which were having only single factor D, there were 5(23.80%) microalbuminuric patients followed by 3(21.42%) microalbuminuric patients which were having only factor B out of 14 patients. In 15 patients who were having only factor C there were 3(20%) microalbuminuric patients. In group II, out 16 patients who were having factor C and factor D there were 10(62.5%) microalbuminuric patients. In group III, maximum number of microalbuminuric patients i.e. 15(75%) belonged to 20 patients which were having factor B, factor C and factor D followed by 05(71.42%) microalbuminuric patients in which 7 patients were having factor A, factor B & factor D. In group IV, out of 11 patients who were having all factors (factor A, factor B, factor C and factor D) there were 8(72.72%) microalbuminuric patients. In group V, 26 patients were without all factors and in this group 3(11.53%) were microalbuminuric patients.

Discussion

In the present study, out of 305 patients of diabetes mellitus, 203(66.55%) were without soft tissue infections and 102(33.45%) were with soft tissue infections. 220(72.15%) of these were males and 85(27.85%) were females. The male to female ratio was 2.6 to 1. The mean age of patients was 49.66(12.57%). Charles S Sharp el al. (1979)[8] studied on superficial and deep tissue infected diabetic gangrene on 52 patients which was conducted during a period of eight months. The mean age of patients was 60.1 years with range 40-80 years. Smriti Agnihotri et al (2001)[9] observed that in type II patients majority of cases (about 69%) belonged to age group of 41-60 years and in type I most of the cases (about 70%) were found in age group of 11-30 years. The age and sex distribution of present study is more or less similar to all these studies.

Microalbuminuria was detected 70 out of 203 (31.92) patients without infections and 21 out of 102 (28.43) with infections. However, in 39 (38.23) patients with infections macroalbuminuria was already present. They were included in this study to assess infection profile. Nevertheless, in diabetes patients with infections albuminuria was evident in as many as 60 out of 102 (58.82) patients which is substantially high.

The microalbuminuria varies with associated factors in diabetes mellitus like insulin therapy, raised blood pressure, raised blood sugar and increased duration of illness. In the present study, only 11.53% patients without any of these associate factors had microalbuminuria. The prevalence of microalbuminuria increased to 17.91% if any one of these factor were present, became 41.07% if any two of these factors, further rose to 55.81% with any 3 associated factor, to reach 72.72% in presence of all associated factors. But if all factors absent in 26 patients, only 3(11.53%) patient were microabluminuric.

Table- 3: Factors associated with microalbuminuria in without infection group

Groups	Associated Factors	Number of Patients	Microalbuminuric Patients (%)
	Only single factor		
I	Factor A	17	01 (5.88)
	Factor B	14	03 (21.42)
	Factor C	15	03 (20)
	Factor D	21	05 (23.80)
	Total	67	12 (17.91)
	Any two factors		
II	Factor A + Factor B	8	01 (12.5)
	Factor A + Factor C	4	01 (25)
	Factor A + Factor D	12	05 (41.66)
	Factor B + Factor C	11	03 (27.27)
	Factor B+ Factor D	5	03 (60)
	Factor C + Factor D	16	10 (62.5)
	Total	56	23 (41.07)
	Any three factors		
III	Factor A+ Factor B+ Factor C	8	00 (0)
	Factor A + Factor B+ Factor D	7	05 (71.42)
	Factor A+ Factor C+ Factor D	8	04 (50)
	Factor B + Factor C+ Factor D	20	15 (75)
	Total	43	24 (55.81)
	All four factors		
IV	A + B + C + D	11	08 (72.72)
	Total (I + II + III + IV)	177	67 (37.85)
V	**All factors absent**	26	3 (11.53)
	Total (I + II + III + IV + V)	**203**	**70 (34.48)**

Insulin therapy - Factor A, Raised Blood Sugar - Factor B, Raised Blood Pressure - Factor C, Increased duration - Factor D.

Gall MA et al (1991)[10] observed that prevalence of hypertension increased from 48% (range 45 - 54%) in NIDDM with normoalbuminuria to 68% (range 60-74%) in patients with microalbuminuria to 85% (range 75-92%) in macroalbuminuria. Bharipanyo P et al (1992)[11] had noted hypertension in 22.2% of 207 NIDDM patients while Multicentre Thai Group on NIDDM (1994) noted hypertension in 38.4% patients of NIDDM. Krolewski AS et al (1995)[6] examined correlation between the degree of hyperglycaemia and urinary albumin excretion rate. The prevalence of microalbuminuria was found to be 18% in IDDM. It increased non linearly with HbA1C value, for HbA1C below 10.1%. The slope of relation was almost flat whereas for values above 10.1% the prevalence of microalbuminuria rise steeply ($p<0.001$). In diabetes control and complications trial (1993)[12] intensive treatment of IDDM patients with insulin to maintain blood glucose range to normal and its effect on vascular complications were examined. Vijay V et al (1994)[13] observed direct correlation between duration and prevalence of albuminuria. The prevalence of albuminuria (200-400 mg %) was 7.8% at 5 years of duration, 16.4% at 5-10 years duration and 17.4% in patients of NIDDM with duration of diabetes more than 10 years. Ghai R et al (1994)[14] observed similar correlation between albuminuria and duration of diabetes. He noted that mean duration of diabetes in normoalbuminuria, microalbuminuria and

macroalbuminuria in patients were 10.74 ± 6.89 years and 15.08 ± 536 years respectively.

All thses findings suggest that routines screening of all diabetic patients for microalbuminuria is necessary, especially if the patients have either of these associated factors present. This can reduce morbidity and mortality in these patients by prompt and proper treatment at this stage itself.

WHO study group on prevention of diabetes (1998) also have recommended annual screening for microalbuminuria who have diabetes of over 5 years. For routine screening of patients for microalbuminuria a simple, rapid and economical test with good sensitivity and specificity is needed. Rocket electrophoresis, used in the present study fulfills these requirements. Rocket electrophoresis is simple, rapid, and cost effective and has high sensitivity and specificity. The reagents and equipments are easily available in moderately equipped laboratories with limited resources. The raising of antisera in the local laboratory makes the set up viable by regular and uninterrupted supply of albumin antiserum. In contrast to these, immunoassays used for the microalbuminuria estimation are costly, not available everywhere and kits and reagents have very short shelf life. Therefore, Rocket electrophoresis appears to be an excellent option for screening of diabetes mellitus patients for microalbuminuria.

The question, whether microalbuminuria acts as a marker for predisposition to the infections suggest that, in the present study, in 21 (28.43) out of 102 patients with infection had microalbuminuria. But 39 (38.23) others already had macroalbuminuria; thus albuminuria in 60 (58.82) patients. This indicate that microalbuminuria starts fairly early than occurance of infections. As vasculitis and polyneuritis are considered to be predisposing factor for infection in diabetes mellitus and microalbuminuria is an earliest marker of both microangiopathy and macroangiopathy, it automatically serves as a marker for risk of infections. More over routine screeing for microalbuminuria will as such denote possible predisposition to complications like nephropathy, retinopathy, neuropathy as well as soft tissue infections.

Conclusion

It is concluded that microalbuminuria is not uncommon feature of diabetes mellitus as it is 34.48% in our study population of diabetes without infection and also more common in associated infections. It is commonly seen in raised blood sugar, raised blood pressure and mores with increased duration of diabetes. Prevalence of microalbuminuria substantially increases with increase in number of associated factors.

The method of rocket electrophoresis used in the study is simple, rapid, economical and feasible in moderately equipped laboratories with limited resources. It is very sensitive and raising of antiserum in the local laboratory not only makes it more cost effective but also assist greatly in maintaining the viability of the setup due to its uninterrupted supply.

Routine screening of patients of diabetes for microalbuminuria especially in presence of associated factor like insulin therapy, hyperglycemia, hypertension or increased duration of diabetes is recommended. Rocket electrophoresis will be useful for such routine screening.

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