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Evaluation of alterations in the urine biochemical profiles of type 2 diabetes mellitus patients in Southwest, Nigeria

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Diabetic nephropathy is a major sign of kidney dysfunction in diabetes mellitus. The identification of biochemical markers for the clinical course of kidney dysfunction in diabetes mellitus remains a major challenge in the disease management. We investigated the changes in 24 h urine excretion of biochemical indices of type 2 diabetes mellitus. We estimated 24 h urinary excretion of sodium, potassium, bicarbonate, chloride, calcium, inorganic-phosphate, total protein, uric-acid, urea, creatinine, specific gravity, and Ph in 150 type 2 diabetic subjects and their age matched controls by standard methods. Plasma urea and creatinine were also estimated and creatinine and urea clearances calculated for both subjects. The demographic and anthropometric indices of the subjects were obtained from the case files. Results were analyzed statistically by standard statistical spss package version 11.0 to draw conclusion from the data generated. Urinary excretion of total protein, inorganic-phosphate, bicarbonate, urine volume and pH values were significantly higher ($p<0.05$) in the diabetics than in none diabetic controls, while urinary excretion of calcium, urea, creatinine, uric-acid, urea and creatinine clearances were significantly reduced ($p<0.001$, $p<0.05$ respectively) when compared with the controls. Urinary excretion of total protein and fasting glucose have significant direct correlation with the disease duration, while a significant inverse correlation exist between urea, inorganic-phosphate and the disease duration ($P<0.05$). Significant proteinuria becomes detectable in diabetes mellitus 1-5 years after diagnosis. Urine biochemical indices are altered in type 2 diabetes mellitus and the alterations correlate with the disease duration.

Key words: Diabetes mellitus, diabetes nephropathy, urinary excretion, biochemical indices, disease duration.

INTRODUCTION

Diabetes mellitus is a chronic debilitating disease, which is costly concerning both human suffering, and health care expenditure (ADA, 2009). Severe complications such as renal disease, cardiovascular diseases, myocardial infarction, stroke and blindness are common in diabetes mellitus of long term duration (ADA, 2013). Major kidney dysfunction in diabetes mellitus is regarded as diabetic nephropathy and is known to be a serious

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Table 1. Grouping of the diabetic subjects according to disease duration.

Group	Duration (years)
1	1-5
2	6-10
3	11-15
4	16-20
5	21-25

N=30 in each group.

complication of both type 1 and 2 diabetes mellitus. Diabetic nephropathy progresses to end stage renal disease when left untreated (Randa` et al., 2010; Vivek bhalla et al., 2013). Identification of biochemical markers for the clinical course of kidney dysfunction in diabetes mellitus remain a major challenge in the management of this disease (Diahzi et al., 2007). Detection of urine microalbumine has for long been considered as the gold standard for the evaluation of kidney dysfunction in diabetes mellitus (Giuseppe, 2002; Gunter and Eberhard, 2003). There is paucity of information on the value of other urine biochemical indices in the assessment of kidney dysfunction in diabetes mellitus. Recent research reports (Rao et al., 2007; Randa et al., 2010) indicate that measurement of microalbumine for the assessment of kidney dysfunction has some limitations such as inadequate specificity and sensitivity. These researchers reported that kidney dysfunction in form of diabetic nephropathy is multifactorial which requires the application of combined markers as a screening or diagnostic tool (Yang et al., 2007). There is limited information on the assay of urine biochemical profiles in type 2 diabetes mellitus of varying duration of onset. This study was conducted to fill this research gap to establish a data base of the alteration of urine biochemical profiles in these subjects.

MATERIALS AND METHODS

One hundred and fifty (150) (70 males 80 females) subjects with type 2 diabetes mellitus of 1- 25 years duration and aged 45-70years who have been regular at the diabetic clinic (for treatment and management) were recruited from the diabetic Clinics of a University Teaching Hospital in south west, Nigeria after consenting to participate in the study. The subjects were under oral hypoglycemic agents and were classified in to five groups based on disease duration. Each group comprises thirty (30) diabetic subjects. 100 age matched apparently healthy non-diabetic recruited as control subjects. The grouping of the diabetic subjects were as follows: Group 1 comprise of diabetic subjects of 1-5 years disease duration; Group 2 comprises diabetics of 6-10 years duration; Group 3 comprises diabetic subjects with diabetes mellitus of 11-15 years duration; Group 4 comprises diabetic subjects of16-20 year's disease duration; Group 5 comprises diabetics with diabetes mellitus 21-25 years duration. Fasting glucose analyses were determined in the diabetic subjects for three consecutive times and the average value taken to represent the glycaemic level of the subjects.

Blood collection

Seven milliliter (7 ml) of venous blood were collected from both subjects between 8.00 and 9.00 am. 5ml of the blood was dispensed into lithium heparin tubes to obtain plasma for the estimation of creatinine and urea by standard method (Kazmierczack, 1996b). 2 ml of the blood was dispensed in fluoride oxalate bottles for the estimation of fasting blood glucose by standard method (Kazmierczack, 1996a).

Urine collection

A 24 h urine sample was also collected from both diabetic and control subjects after being properly instructed on procedures for obtaining the 24 h urine specimen. The volume of the urine voided was measured and aliquot was dispensed and stored in 5 ml plastic tubes at -20°C or analyzed immediately for the urine biochemical parameters. Reference methods were used for the estimation of urinary sodium and potassium (Korzum, 1996), bicarbonate (Sega, 1955), chloride (Mathew, 1982), calcium, creatinine, inorganic phosphate, urea, uric acid total protein (Kazmierczack, 1996b), specific gravity (First, 1996) and pH (Schumann, 1996). The blood pressures of both subjects were measured with sphygmo-manometer; the weight and height of both subjects were also measured by trained health personnel. The body mass index of both subjects was computed by dividing the body weight by square of the height.

Data analysis

The data generated were analyzed with the Statistical Package for the Social Sciences (SPSS) statistics programme (version 116). Means were computed, test of significance was done using student t-test and correlation test was also done to draw conclusion from the study.

RESULT

The results obtained from this study are shown in Tables 1-4. The groupings of the diabetic subjects according to the disease duration are shown in Table 1. The comparison of the mean:body weight, body mass index, blood pressures fasting blood glucose and urine biochemical indices of all the diabetic subjects and control subjects are shown in Table 2. The systolic and diastolic blood pressure, fasting blood glucose, urine volume, urine:total protein, inorganic phosphate creatinine, bicarbonate were significantly elevated ($p0<05$) in the diabetic subjects than in the controls, while urinary excretion of calcium, urea, creatinine, uric- acid, creatinine and urea clearances and urine pH values were significantly lower ($p<0.001P<0.05$) in the diabetic subjects than in the controls (Table 2). Urinary excretion of sodium, potassium were significantly higher in the diabetics than the non-diabetic controls but the mean differences were not statistically significant ($p>00.5$). On the contrary, urinary excretion of chloride, was lower in

Table 2: Comparison of age, weight, body mass index, blood pressures fasting blood glucose, 24 h urine volume and urine biochemical parameters between diabetics and controls.

Parameter	Diabetics N = 150 (Mean ± SD)	Controls(n=100) (Mean ± SD)	P Value
Age (year)	61 ± 8.2	59 ± 6. 9	P > 0.05
Weight (Kg)	72 ± 15. 8	69 ± 10. 8	P > 0.05
Body mass index (kg / M^2)	29. 6 ± 6.8	29. 2 ± 4.2	P > 0.05
Systolic blood pressure (SBP) (mmHg)	136 ± 24. 8	113 ± 12. 8	P< 0.001
Diastolic blood pressure (DBP) (mm Hg)	83 ± 13. 0	73 ± 8.0	P< 0.05
Fasting blood glucose (mmol/L)	9. 3 ± 3. 4	3. 9 ± 0.6	P< 0.001
24 h urine volume (L / 24 h)	2.3 ± 0.8	1. 7 ± 0.6	P<0.05
Total Protein (g / 24 h)	1.2 ± 0.9	0.29± 0.3	P< 0. 001
Sodium (Na^+)+mmol / 24h	149 ± 59. 5	146 ± 55.4	P< 0. 05
Potassium (K^+) mmol / 24 h	49. 0 ± 22. 6	38. 9 ±25. 3	P> 0.05
Chloride (Cl^-) mmol / 24 h	188 ± 85	200 ± 95	P>0.05
Calcium (Ca^{2+} mmo1 / 24 h	1. 8 ± 3.3	4.1 ± 3.3	P< 0.001
Inorganic phosphate (PO_4^{3-}) mmol / 24 h	5 .1 ± 3.2	3. 40 ± 1.61	P<0.05
Bicarbonate (HCO_3) mmol/ 24 h	43 ± 21.2	32 ± 11.6	P<0.05
Uric acid mmol / 24 h	1.35 ± 0.85	1.90 ±.86	P<0.05
Urea mmol / 24h	75 .2 ± 47. 1	100.3 ± 47. 6	P<0.05
Urea clearance ml / min	48 ± 18. 3	60 ± 17.5	P< 0.05
Creatinine µmol / 24 h	4198 ± 2263	4823 ± 2076	P<0.05
Creatinine clearance ml / Min.	39 ± 15.6	70 ± 17	P< 0.05
Specific gravity -	1. 015 ± 0. 011	1. 020 ± 009	P>0.05
pH	5.2 ± 1. 0	7.0 ± 0.2	P<0.05

SD, Standard deviation; Kg, kilogramme; Mmol, milimol; Ml, milliliter; µmol, micromole; hrs, hours.

the diabetics than in the non-diabetic controls, but the mean difference was not statistically significant (p>0.05) (Table 2).

The mean urine volume, urinary excretion of the biochemical indices, body weight, body mass index, blood pressure and fasting blood glucose of the diabetic subjects in each of the five groups are shown in Table 3. The mean urine volume, urinary excretion of total protein, potassium and urine pH in the diabetics changed (increased) with increase in the duration of the diabetes, while urinary excretion of calcium, urea, creatinine, uric-acid, inorganic-phosphate, urea and creatinine clearances were also altered (declined) with increase in the duration of diabetes (Table 3). However urinary excretion of sodium and, chloride and specific gravity did not exhibit a definite pattern of alteration, while the body weight, bodymass index and the blood pressures of the diabetics remain apparently constant with the duration of the disease. The glycaemic control of the diabetics becomes diminished with the duration of the diabetes (Table 3).

The results of the multiple correlation between bodyweight, body mass index, blood pressures, urine biochemical indices and the duration of the disease are shown in (Table 4). Fasting blood glucose and urine total protein have direct significant correlation with the duration of the diabetes, while urinary excretion of inorganic-phosphate and urea have significant inverse correlation with the duration of the diabetes (Table 4). The correlations of other urine biochemical indices with the duration of the diabetes mellitus were not statistically significant (p>0.05).

DISCUSSION

Alterations of urine biochemical indices in type2 diabetes mellitus of various duration were demonstrated by the findings of this study. The reduced pH, urinary excretion of calcium, chloride, creatinine, urea, creatinine and urea clearances as well as the elevated urinary excretion of total protein, inorganic phosphate, bicarbonate, elevated urine volume recorded among the diabetics (Tables 2 and 3) was an evidence that these urine biochemical indices are altered. Our experiment on relationship between urinary excretion of the biochemical indices and duration of the diabetes further confirmed that the biochemical indices are not only altered but the alteration varies with the duration of the disease (Tables 3 and 4). The mechanism for the alteration of these urine biochemical indices in the diabetic subjects might be attributed to the development of kidney dysfunction: a known complication of diabetes mellitus in these subjects (Erkins et al., 2007; Vivek bhalla et al., 2013). The onset of gradual glomerular basement membrane damage in the diabetics after diagnosis (Ness Perrin et al., 2006;

Table 3. Anthropometric indices, (age, weight, body mass index), blood pressures (sbp &dbp) fasting blood glucose (FBG), 24hours urine volume and urine biochemical parameters of type II diabetics in groups 1-5.

Parameter	Group I (1 - 5 years)	Group II (6 - 10 years)	Group III (11 - 15years)	Group IV (16 - 20 years)	Group V (21-25 years)
Age (Year)	57 ± 10	62 ± 8	61 ± 10	57 ± 10	70 ± 8
Weight (Kg)	72 ± 20	72 ± 19	71 ± 10	72 ± 10	72 ± 10
BMI (kg/m^2)	30. 7 ± 8	28.8 ± 9	28 ± 5	29.9 ± 3	29.9 ± 2
SBP mmHg)	137 ± 18	137 ± 20	140 ± 21	137 ± 17	137 ± 15
DBP mmHg)	82 ± 13	82 ± 10	83 ± 11	81 ± 7	76 ± 10
FBG (mmol. /L)	8. 4 ± 3.1	8.2 ± 2.4	8.8 ± 3.0	11.1 ± 4.5	13. 0 ± 2. 8
Urine volume (L/24 h)	1.9 ± 1.2	1.4 ± 0.5	1.7 ± 0.6	2.1 ± 0.6	2.1 ± 0.5
Total protein (g/24 h)	0.85 ± 0.64	1.0 ± 0.67	1.5 ± 0.92	2.0.8 ± 0.6	2.5 ± 0.02
Sodium (mmol/24 h)	158 ± 71	129 ± 52	160 ± 56	159 ± 51	158 ± 52
Potassium (mmol / 24 h)	42 ± 25.7	46.0 ± 20.6	50. 9 ± 25.3	56.8 ± 16. 2	62.7 ± 18.2
Bicarbonate (mmol / 24 h)	37 ± 16	40 ± 14	43 ± 20	46 ± 14	58 ± 9
Chloride (mmol / 24 h)	203 ± 54	161 ± 65	210 ± 62	184 ± 64	180 ± 58
Calcium (mmol / 24 h)	2.3 ± 1. 7	1.83 ± 0.71	1. 60 ± 0.92	1.00 ± 1.1	0.85 ± 0. 84
Inorganic phosphate (mmol / 4 h)	6.25 ± 1. 09	4.65±1. 84	5. 03 ± 1. 79	4. 34 ± 1.65	3.77 ± 1. 70
Uric acid (mmol / 24 h)	2. 9 ± 0. 85	1.81 ± 0. 49	1.58 ± 0.27	1. 3 ± 0. 39	0.95 ± 0. 27
Urea (mmol / 24 h)	109.4 ± 15.5	106. 2 ± 14.2	104 ± 16.3	79. 2 ± 18.2	76.5 ± 20. 2
Creatinine (mmol / 24 h)	4618 ± 2433	4390 ± 2544	4161 ± 2495	4089 ± 1976	3759 ± 691
Urea Clearance ml /m	36 ± 8.5	35 ± 7.5	34 ± 12.5	30 ± 8.5	28 ± 9.8
Creatinine clearance (ml /m)	50 ± 16	44 ± 19	42 ± 20	32 ± 22	29 ± 19
Specific gravity	1.016 ± 0. 011	1.013 ± 0.008	1.022 ± 0. 017	1.007 ± 0.006	1 020 ± 0.004
pH	5.4 ± 0.9	5.7 ± 1. 2	5.9 ± 1.2	6.1 ± 1.3	6.6 ± 0.9 5

SD, Standard deviation; Ml, milliliter; µmol, micromole; FBG, fasting blood glucose; Kg, kilogramme; BMI, body mass index; Dbp, diastolic blood pressure; Mmol, milimol; Sbp, systolic blood pressure.

Simerjot et al., 2010) might be responsible for the progressive elevation of urine voided, urinary excretion of total protein, bicarbonate, as well as the progressive reduction of pH and urinary excretion of calcium, inorganic-phosphate, uric-acid, urea, creatinine, urea and creatinine clearances with respect to the disease duration (Table 3). Significant proteinuria reported in this study confirms previous findings in Nigeria (ADA, 2013) and among the Caucasian (WHO 1999; Vivek Bhalla, 2013). Our study further revealed that significant proteinuria in diabetes can be detected as early as between 1-5 years post diagnosis (Table 3). This is contrary to previous reports (Meier et al., 2005; Vivek Bhalla et al., 2013) of significant proteinuria detectable only after 10 years post diagnosis of diabetes mellitus. The presence of significant proteinuria at early stages (1-5 years) of diabetes might be suggestive of existing gradual glomerular basement membrane damage in the kidney before the manifestation of the diabetes and before the patient actually presents for diagnosis and management. The direct and significant correlation between the urinary excretion of protein reported and the duration of the diabetes confirms this speculation or theory. However the relationship between proteinuria and duration of diabetes reported in this study is at variance with previous findings of Erasmus and Okesina (1982), Mogensen (1999) and Bhaskar Thakkar et al. (2011). These researchers reported that proteinuria in diabetes is independent of disease duration but greatly influenced by diastolic blood pressure and age of the subject.

We reported hyperphosphaturia and significant inverse relationship between the hyperphosphaturia and the disease duration (Tables 2, 3 and 4). Urinary phosphate excretion was at peak at onset of the diabetes but progressively declines with the duration of the disease (Table 4). Diabetes mellitus influences renal tubular damage and might have caused the alteration in urinary phosphate excretion in the diabetic subjects (Adrian, 2012; Simerjot et al., 2010). The elevated urinary excretion of bicarbonate reported in this study confirms previous findings which attributed elevated urinary excretion of bicarbonate to hyperglycaemic status of the subjects (Leszek Szablewski, 2011). Reduced renal function in diabetes which might impair renal tubular reabsorption of bicarbonate is another explanation for the elevated urinary excretion of bicarbonate.

Diabetic subjects produce acidic urine irrespective of the disease duration; this confirms earlier reports of Leszek Szablewski and 2011, Erkins Hovind et al., 2007. The mechanism behind this might be that the depletion of bicarbonate through increased excretion will adversely affect the bicarbonate-carbonic acid system leading to

Table 4. Correlation of duration of diabetes mellitus and urinary excretion of biochemical indices

Parameter	r-value	P-value
Total protein	0.244	P<0.05
Inorganic-phosphate	-0.250	P<0.05
Urea	-0.225	P<0.05
Urine volume	0.094	P>0.05
Sodium	0.013	P>0.05
Potassium	0.189	P>0.05
Bicarbonate	0.153	P>0.05
Chloride	0.041	P>0.05
Calcium	-0.201	P>0.05
Uric-acid	-0.137	P>0.05
Creatinine	-0.020	P>0.05
Creatinine clearance	-0.014	P>0.05
Urea clearance	-0.121	P>0.05
Specific-gravity	-0.015	P>0.05
pH	0.110	P>0.05
Fasting blood glucose	0.384	P<0.05

reduction of the hydrogen ion reserve and eventually reducing the pH (acidosis). We also reported reduced urinary excretion of calcium among the diabetics and declining calcium excretion with the disease duration (Tables 2, 3 and 4) although the inverse correlation between calcium excretion and the disease duration was not statistically significant (Table 4). This alteration might be due to the loss of the regulatory role of kidney in urinary calcium excretion as a result of the compromise of kidney function in diabetes mellitus.

Urinary excretion of creatinine, urea, uric-acid, urea and creatinine clearances which are known indices of renal function and glomerular filtration rate (GFR) were significantly lower in the diabetics (Table 2). However a significant inverse relationship exists between urinary excretion of urea and the disease duration, but the inverse relationship between urinary excretion of creatinine, uric-acid, creatinine and urea clearances were not statistically significant (Table 4). In spite of this, it still implies that urinary excretion of creatinine, urea, uric-acid as well as their clearances from the urine will be compromised with increase in duration of the diabetes. The mechanism behind this observation may be attributed to a reduced GFR as well as reduced blood flow to the glomerulus coupled with acute and chronic tubular damage precipitated by the diabetes (Vivek Bhalla et al., 2013).

In conclusion, we have established a data base of urine biochemical profiles of kidney excretory products in type 2 diabetes mellitus. Our study has also shown that apart from microalbumine, other excretory products of the kidney are significantly altered in diabetes mellitus and the alterations vary with the disease duration. The identification of kidney dysfunction in diabetes mellitus is still a major challenge in the disease management; we therefore recommend the application of a combined screening indices or markers as diagnostic tool for the assessment of clinical sign of renal dysfunction in diabetes mellitus. The assay of these urine biochemical profiles may provide a novel clinical assessment or guide of renal complications in type 2 diabetes mellitus.

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