

Comparative Studies of Renal Function Indices in Hypertensive Diabetic and Normotensive Diabetic Subjects

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Annotation: Diabetes mellitus and hypertension are interrelated diseases. The purpose of this study was to compare the renal function indices of hypertensive diabetic and normotensive diabetic subjects. This study was conducted in Osogbo local government in the capital city of Osun state, Nigeria. 40 hypertensive diabetic subjects, 40 normotensive diabetic subjects and 40 healthy subjects were selected for this study. The mean systolic blood pressure of hypertensive diabetic subjects (149.68 ± 7.54 mmHg) was significantly higher when compared to that of normotensive diabetic subjects ($p < 0.01$). Also, the diastolic blood pressure of hypertensive diabetics (88.23 ± 4.13 mmHg) was significantly higher than normotensive diabetics ($p < 0.01$). There was an increase in the mean BMI of hypertensive diabetic subjects (26.11 ± 4.06) when compared to that of the control subjects ($P < 0.05$). The mean concentrations of potassium, bicarbonate, urea and uric acid ($5.29 \pm 1.04, 23.53 \pm 2.89, 6.79 \pm 3.14$ and 0.40 ± 0.17 mmol/l) respectively were significantly high ($P < 0.01$) in hypertensive diabetic when compared to healthy subjects. Also, there were significant differences in the mean concentrations of potassium, bicarbonate, urea and uric acid ($5.26 \pm 1.13, 24.45 \pm 2.90, 6.80 \pm 2.54$ and 0.42 ± 0.17 mmol/l) respectively of normotensive diabetics when compared to control subjects. However, mean concentration of

potassium, sodium, bicarbonate, urea and uric acid were statistically unchanged in hypertensive diabetic subjects when compared to normotensive diabetic subjects. There was inverse correlation between bicarbonate and systolic blood pressure ($r = -0.22$), bicarbonate and creatinine ($r = -0.34$), bicarbonate and chloride ($r = -0.50$). There was positive correlation between systolic and diastolic blood pressures ($r = 0.79$), urea and creatinine ($r = 0.50$), urea and uric acid ($r = 0.75$), creatinine and uric acid ($r = 0.42$). Although, there were no significant differences in the renal function indices of hypertensive diabetic subjects when compared to normotensive diabetic subjects, these findings suggest that there were alterations in the renal function indices of both hypertensive diabetics and normotensive diabetics.

Keywords: renal, diabetes and hypertension.

INTRODUCTION

Hypertension or High Blood Pressure is a medical condition in which constricted arterial blood vessels increase the resistance to blood flow, causing an increase in blood pressure against vessel walls¹. The heart must work harder to pump blood through the narrowed arteries. If the condition persists, damage to the heart and blood vessels is likely, increasing the risk for stroke, heart attack, and kidney or heart failure. Often called the “silent killer,” hypertension usually causes no symptoms until it reaches a life-threatening stage². Diabetes mellitus is caused by an absolute or relative insulin deficiency. It has been defined the World Health Organisation (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration more than or equal to 7.0mmol/L (on more than one occasion or once in the presence of diabetes symptoms) or a random venous plasma glucose concentration more than or equal to 11.1mmol/L³.

Type 1 Diabetes mellitus

This is also called Insulin-dependent diabetes mellitus; it is used to describe the condition in patients for whom insulin therapy is essential because they are prone to develop ketoacidosis. Most of these cases are due to immune-mediated processes and may be associated with other autoimmune disorders such as Addison's' diseases, vitiligo and Hashimoto's thyroiditis⁴.

Type 2 Diabetes mellitus

It was previously called non-insulin-dependent diabetes mellitus; this is the commonest variety worldwide (about 90% of all diabetes mellitus cases). Onset is most usual during adult life. There is a spectrum of disorders ranging from mainly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance⁴.

Gestational Diabetes mellitus

This is associated with increased foetal abnormalities, for example high body weight, cardiac defects and polyhydramnios. In pregnancy, if the plasma glucose concentration is more than or

equal to 9.0mmol/L, then such pregnant woman has gestational diabetes. In addition, birth complication, maternal hypertension and the need for Caesarean section may occur ⁵.

Impaired glucose tolerance (IGT)

The WHO definition of impaired glucose tolerance is a fasting venous plasma glucose concentration between 7.8mmol/L and 11.1mmol/L two hours after an OGTT.

Persons with IGT are borderline cases with slightly raised glucose levels without overt hyperglycaemia. Such cases may later develop diabetes mellitus or revert to normal levels. Generally, they are at high risk of development of cardiovascular diseases ⁶.

RATIONALE OF STUDY

Hypertension and Diabetes mellitus has been well described in Sub-Sahara Africa. For instance, studies revealed that Diabetes mellitus account for about 1.7% of the total mortality in the world while hypertensive heart disease causes about 1.6%.It has been reported that hypertension and diabetes induce significant changes in renal function parameters ⁷. This has been shown severally in various invitro studies and in experimental model invivo. It is hoped that this study will improve and contribute to the understanding of the relationship between the effects of hypertensive diabetes and non-hypertensive diabetes on the renal function parameters.

BIOCHEMISTRY OF RENAL DISORDERS LEADING TO HYPERTENSION

The kidney plays a major role in regulating the water-electrolytes system in the body ⁴.Dysfunction to any part of the kidney may therefore go a long way in affecting many systems in the body; example is the circulatory system ⁸. Different parts of the nephrons are in close anatomical association and are dependent on a common blood supply; renal dysfunction of any kind affects all parts of the nephrons to some extent, although sometimes either glomerular or tubular dysfunction is predominant ⁹.

When kidney retains salt (excess electrolyte solutes) in the bloodstream, it leads to haemoconcentration. This condition causes reduction in renal artery flow, which increases secretion of the hormone-renin from the juxtaglomerular apparatus ⁹.

The renin secretion has a number of important actions:

Renin acts directly on capillary walls, causing vasoconstriction and probably causing arterial constriction and raising blood pressure ⁶.

It also stimulates the cells of zona glomerulosa to synthesize and secrete aldosterone which affects the sodium-potassium and sodium-hydrogen ion exchange across all cell membranes ¹⁰.

It stimulates the thirst centre to secrete antidiuretic hormone (ADH) which enhances water reabsorption in excess of solute from the collecting ducts of the kidney, increasing the volume of the blood fluid. As a higher volume of blood passes through arteries, it increases blood pressure ⁴.

POTENTIAL EFFECTS OF ACID-BASE IMBALANCE IN DIABETIC MELLITUS

The net loss of water and electrolytes through kidney depends on the balance the volume filtered proximally and that absorbed more distally. Any factor affecting either passive filtration or epithelial cellular function may disturb this balance ¹¹.

Sodium is the most abundant extracellular cation ⁴. The metabolism of sodium is so inextricably related to that of water (Jorgensen, 1980). In case of diabetes mellitus, excess glucose remains in the extracellular fluid causing a marked osmotic effect across cell membrane with movement of water from cells into the extracellular compartment causing cellular dehydration. This indicates increased or normal sodium in the blood ¹².

Potassium is the most abundant intracellular cation⁵; it is normally almost completely reabsorbed in the proximal tubules. There is an important inter-relationship between the processes of potassium metabolism and those of water and sodium balance, renal function and acid-base disorders. Potassium is secreted in the distal tubules and collecting ducts in exchange for sodium ions (Na⁺); hydrogen ions (H⁺) compete with potassium ions (K⁺). In diabetes mellitus, there is a shift of potassium ions from the intracellular into the extracellular fluid (ECF) due to acidosis as hydrogen ions (H⁺) shifts intracellularly and potassium ions (K⁺) shifts outward to maintain electrical neutrality. Therefore in most cases of diabetes mellitus, there are usually hyperkalemic conditions¹³.

Also, in diabetic ketoacidosis, there is situation of hydrogen ions (H⁺) shift in inadequate sodium ion-hydrogen ion exchange, leading to reduced reabsorption of bicarbonate ions (HCO₃⁻) in the proximal tubules, causing low concentration of bicarbonate in the blood (metabolic acidosis).

Similarly, imbalances in acid-base system cause alteration in glomerular filtration. This affects the excretion and reabsorption of urea, creatinine and uric acid, thereby leading to accumulation of these metabolites in the blood¹⁴.

MATERIALS AND METHODS

STUDY LOCATION

The study was conducted in Osogbo Local Government Area, in the capital city of Osun State, Nigeria. The areas are in the tropical rain forest belt of South-Western part of Nigeria. The patients were selected from LAUTECH Teaching Hospital (LTH), which is one of the major government hospitals in Osogbo. The other area, where the control patients were selected from, that is, Osogbo, has almost all the important infrastructural facilities.

SUBJECTS

The baseline study population consisted of one hundred and twenty (120) human subjects. The subjects were grouped into three categories. Forty (40) subjects with hypertensive diabetes mellitus, forty (40) subjects with normotensive diabetes mellitus and forty (40) confirmed healthy subjects were randomly selected.

Recruitment of subjects into the test group was done after the completion of a standardized questionnaire. Test populations were recruited by a systemic random sampling of the entire outpatient hypertensive diabetic and normotensive diabetic individuals in the hospital which was used as the study centre.

Ethical consideration: The proposal was submitted for review to the Ladoke Akintola University of Technology (LAUTECH) and LAUTECH teaching hospital (LTH) review board, Osogbo.

CRITERIA FOR THE SELECTION OF SUBJECT

Inclusion criteria

1. Subjects with blood pressure greater than 140/90mmHg.
2. Subjects diagnosed for any type of diabetes mellitus.
3. Subjects with history of heart diseases e.g. myocardial infarction.
4. Subjects who are 18years and above.
5. Subjects suffering from any renal disease.

Exclusion criteria

1. Subjects below 18 years.
2. Pregnant subject

ANALYTICAL METHODS.

DETERMINATION OF UREA CONCENTRATION. ¹⁵

Randox Kit reagent for in vitro quantitative determination of urea in plasma, serum and urine was used.

Principle: The enzyme urease decomposes urea to form ammonium carbonate; the ammonia formed from the enzyme reaction reacts with phenol in the presence of hypochlorite to form indophenol which in an alkaline medium gives reagent that acts a catalyst (sodium nitroprusside), increasing the speed of the reaction, the intensity of colour obtained and reproducibility.

Procedure:

1. 10 μ l of plasma controls and standards was added to each tube.
2. 1000 μ l of Reagent 1 (containing sodium nitroprusside) was added.
3. Each solution was mixed and incubated for 5 minutes at 37° C.
4. 1000 μ l of Reagent 2 was added.
5. Each solution was mixed and incubated for 5 minutes at 37° C.

The absorbance of the sample and standard was read at 578nm against the reagent blank.

DETERMINATION OF CREATININE CONCENTRATION

The method employed for determination of plasma creatinine was Jaffe.

Principle: Creatinine was determined in protein free filtrate of plasma by the Jaffe's reaction. Picric acid reacts with creatinine in free filtrate resulting in the production of an acid tautomer of creatinine picrate in the presence of alkaline solution ¹⁴.

DETERMINATION OF BICARBONATE CONCENTRATION ¹⁶.

The method (back titration) was employed for the determination of plasma bicarbonate concentration was titrimetric method hydrochloric acid, and sodium hydroxide.

Principle: Plasma was added to a solution containing neutral red as indicator and a fixed amount of HCL. The HCL reacts with the HCO₃ in the sample to liberate carbon dioxide. The remaining excess unreacted HCL is determined by titrating to a golden yellow by sodium hydroxide.

Procedure:

1. 100 μ l of plasma and controls was added to tubes
2. 1000 μ l of 0.01N hydrochloric acid was added
3. The tubes were mixed to remove excess carbon dioxide.
4. Drops of neutral red was added
5. The excess HCl was titrated by back titration with 100 μ l of 0.01N.

DETERMINATION OF SODIUM (Na⁺) AND POTASSIUM (K⁺) CONCENTRATION ¹⁷.

Some metal (especially alkali metals) when in solution are aspirated and sprayed in an aerosol in flame, they become excited by heat with sufficiently high temperatures ⁸. These metals will absorb energy from the sources of heat i.e. the flame and then become excited in their atomic form. But as these individual atoms cool, they will fall back to the original ground level and re-emit their absorbed energy by way of photon at specific wavelength.

The emission will be proportional (at a low concentration) to concentration of Na⁺/K⁺ in the sample ¹².

At the end point, excess of Hg^{++} ions react with indicator disphenyl-carbazone, to produce a violet colour.

Procedure:

1. 0.2ml of plasma and control was added to tube
2. 2.Oml distilled water was added to each tube
3. 1 drop of 2/3N H_2SO_4 was then added
4. 6 drops of indicator was added

The tubes were then mixed and titrate with mercuric nitrate to the first appearance of a slight but permanent violet colour.

DETERMINATION OF URIC ACID CONCENTRATION

Plasma uric acid was estimated using direct colorimetric method ¹⁸.

Principle: Uric acid is converted to allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet complex.

STATISTICAL ANALYSIS

The SPSS (Statistical Package for Social Sciences) software package was used for statistical analysis. Values obtained from the study, physical and biochemical parameters in the different study group, were explained and expressed as mean \pm standard deviation, when compared using student t-test. $P < 0.05$ was regarded as significant.

Pearson product moment correlation coefficient was used to determine the level of association between continuous variables.

RESULTS AND ANALYSIS**PHYSICAL PARAMETERS**

Of the one hundred and twenty (120) subjects used, eighty one (81) are female while thirty nine (39) subjects are male.

The mean ages of all the tests subjects-hypertensive diabetics and normotensive diabetics (61.5 ± 9.9 and 60.0 ± 12.0) were not significantly different from that of the control subjects (Table I). The Body Mass Index was significantly higher in hypertensive diabetics when compared to control subjects ($p < 0.05$) as shown in table I. However, there was a marked significant increase ($p < 0.01$) in the systolic blood pressure of the hypertensive diabetics when compared to normotensive diabetics. Also, there was an increase in the diastolic blood pressure of the hypertensive diabetics when compared to that of normotensive diabetics ($p < 0.01$) as indicated in table 1.

BIOCHEMICAL PARAMETERS

The mean concentrations of bicarbonate, potassium, urea and uric acid were significantly higher in hypertensive diabetic subjects when compared with the controls ($p < 0.01$) as it can be seen in Table 2. Also, the mean concentrations of bicarbonate, potassium, urea and uric acid were significantly higher in normotensive diabetic subjects when compared with the controls ($p < 0.01$). However, there were no significant differences between the mean concentration of Sodium, Chloride ions and Creatinine of the hypertensive diabetic subjects and control subjects (Table 2).

Similarly, there were no significant differences in all biochemical parameters between the hypertensive diabetics and normotensive diabetics as shown in table 2.

Table 3 shows the Pearson correlation in the hypertensive diabetics. There was an inverse relationship between Bicarbonate and Chloride ions ($r = -0.44$, $p < 0.01$). However, there was a positive correlation between Systolic and Diastolic blood pressure, Sodium and Chloride ions ($r = 0.80$, $p < 0.01$), Urea and Creatinine ($r = 0.73$, $p < 0.001$), Urea and Uric acid ($r = 0.79$, $p < 0.01$) as well as Creatinine ($r = 0.79$, $p < 0.01$) and Uric acid ($r = 0.60$, $p < 0.01$).

TABLE 1: SHOWING COMPARISON BETWEEN TEST AND CONTROL SUBJECTS FOR PHYSICAL PARAMETERS.

Parameters	Hypertensive Diabetics N=40	Normotensive Diabetics N=40	Controls N=(40)	f-value
	Mean±S.D	Mean±S.D	Mean±S.D	
Age(years)	61.5±9.9	60.0±12.0	60.3±7.2	2.3
BMI	26.1±4.1	24.7±3.9	24.0±3.2	3.3
Systolic BP	149.7±7.5	104.104.9±11.1	110.0±8.2	291.0
Diastolic BP	88.2±4.18	70.8±14.3	80.0±0.0	41.4

TABLE 2: SHOWING COMPARISON BETWEEN TEST AND CONTROL SUBJECTS FOR BIOCHEMICAL PARAMETERS.

Parameters	Hypertensive Diabetics	Normotensive Diabetics	Controls N-(40)	f-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	
HCO ₃ (mmol/l)	23.5±2.9	24.5±2.9	21.3±1.7	16.6
Cr(mmol/l)	102.2±8.7	99.7±6.9	100.9±3.2	14
Na ⁺ (mmol/l)	134.3±7.2	133.8±6.1	133.6±3.40	0.2
K ⁺ (mmol/l)	5.3±1.0	5.3±1.1	3.6±0.4	47.5
Urea(mmol/l)	6.8±3.1	6.8±2.5	4.5±0.9	11.9
Creatinine(μmol/l)	115.8±53.8	110.8±59.0	91.6±12.6	3.0
Uric acid(mmol/l)	0.40±0.2	0.4±0.2	0.3±0.1	8.9

TABLE 3: PEARSON CORRELATION FOR ALL PARAMETERS IN HYPERTENSIVE DIABETICS.

	SP	DP	HCO ₃	CL	Na ⁺	K ⁺	Urea	Creat	Uric
SP r-value	1	0.38*	0.21	-0.29	-0.07	-0.25	-0.01	0.01	0.00
DP r-value	0.38*	1	0.19	-0.04	0.14	-0.19	-0.20	0.30	0.27
HCO ₃ r-value	0.21	0.19	1	-0.44**	-0.14	-0.15	-0.01	-0.31	0.11
CL r-value	-0.29	-0.04	-0.44**	1	0.80**	0.19	0.00	0.23	-0.03
Na ⁺ r-value	-0.07	0.14	-0.14	0.80**	1	-0.13	-0.06	0.20	-0.14
K ⁺ r-value	-0.25	-0.19	-0.15	0.19	-0.13	1	0.08	0.10	0.14
Urea r-value	-0.01	0.20	-0.01	0.00	-0.06	0.08	1	0.73**	0.79**
Creat	0.01	0.30	-0.31	0.23	0.20	0.10	0.73**	1	0.60**

r-value									
Uric r-value	0.00	0.27	0.11	-0.03	-0.14	0.14	0.79**	0.60**	1

DISCUSSION

The estimation of renal function indices plays significant roles in the investigation of hypertensive diabetics and normotensive diabetes mellitus¹⁹. From the result, the mean systolic blood pressure of the hypertensive diabetic subjects is 149.7mmHg which is above the reference of <140mmHg and confirms the WHO definition of hypertension. When the arteries narrow, they increase the resistance to blood flow². Increased blood pressure may damage the small blood vessels within the kidneys, the kidneys then becomes unable to filter blood efficiently, and waste products may build up in the blood.

It was observed from the result that the mean potassium concentration of both hypertensive diabetic and normotensive diabetic subjects were significantly higher when compared with the control subjects; this corroborates the findings of Jorgensen⁸ which explained that hyperkalaemia could occur as a result of extracellular distribution of potassium in insulin deficiency where intracellular potassium is transferred into extracellular when hydrogen ions shift intracellularly. This occurs in acidosis to maintain electrical neutrality. Also, in poorly controlled type 1 diabetes mellitus, there is a shift because normal action of the pump depends on the energy supplied by glucose metabolism²⁰. Also, reduced glomerular filtration or renal function causes decreased excretion of potassium which leads to its retention in the blood²¹.

Similarly, imbalances in acid-base system cause alteration in glomerular filtration. This affects the excretion and reabsorption of urea, creatinine and uric acid, thereby leading to accumulation of these metabolites in the blood.

Also, from the result, the mean concentration of urea was high in hypertensive diabetic and normotensive diabetic subjects when compared to that of the control subjects. This could be due to reduced glomerular filtration. Urea is metabolised in the liver and carried by the plasma to the kidney where it is filtered from the plasma by the glomerulus²². The normal kidney can excrete large amounts of urea, but in any case of renal dysfunction like reduced glomerular filtration, there is accumulation of urea in the systemic circulation leading to uraemia²³.

The elevation of uric acid in the test subjects in the result could occur in situation of diabetic ketoacidosis where there is interference to the tubular secretion of uric acid causing intratubular deposition of uric acid crystals²⁴.

The result showed that there were no significant differences in the mean concentrations of sodium and chloride between the test subjects (hypertensive diabetics and normotensive diabetics) and the control subjects. Sodium is the most abundant extracellular cation and its corresponding anion is chloride ion⁴. The metabolism of sodium is so inextricably related to that of water⁸. In case of diabetes mellitus, excess glucose remains in the extracellular fluid causing a marked osmotic effect across cell membrane with movement of water from cells into the extracellular compartment causing cellular dehydration. This indicates increased or normal sodium in the blood¹². Also, in normal tubules, most filtered sodium ion (Na⁺) is reabsorbed with chloride ion (Cl⁻); the rest is exchanged for secreted hydrogen ion (H⁺) or potassium ion (K⁺). If H⁺ secretion is impaired, and yet the same amount of Na⁺ is reabsorbed, Na⁺ must be accompanied by Cl⁻ or exchanged for K⁺²⁵, this may account for the unchanged levels of sodium ion (Na⁺) and chloride ion (Cl⁻) in the result.

In addition, the result showed significant increases in BMI of both hypertensive diabetics and normotensive diabetics when compared with control subjects. This confirms the findings of⁶, that obesity elevates the adrenocortical hormones especially aldosterone which is regulated by the

Renin-Angiotensin system (RAS), the elevation of the renin constricts arteries resulting in high blood pressure. This corroborates the link of obesity, diabetes mellitus and hypertension.

CONCLUSION

Although, both hypertensive diabetes and normotensive diabetes has been shown to significantly alter some renal function indices, there is no significant difference between the renal function indices of hypertensive diabetic and normotensive diabetic subjects. The renal function markers in diabetes mellitus showed similar results to those of hypertensive diabetes mellitus in this study. Hence, it is suggested that those altered renal function indices associated with hypertensive diabetes and normotensive diabetes could be estimated to detect, control, treat and prevent diabetes mellitus and hypertensive diabetes in order to reduce the mortality arising from the severity of diabetes mellitus.

CONFLICT OF INTEREST STATEMENT

I certify that I have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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