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Early Biochemical Changes in Renal Status of Diabetic Rats

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Abstract: ***Aim:*** ***The aim of this study was to compare the diagnostic value of serum creatinine and cystatin c in detection of minor changes at early stages of development of diabetic kidney disease.*** ***Background:*** ***Loss of kidney function is grossly under diagnosed due to the inherent weakness in the traditional kidney function markers, and cumbersome and uneconomical nature of the gold standard. The imperativeness to evaluate the ability of newer renal function markers becomes inevitable.*** ***Methods:*** ***Male rats (albino rats) were aged-matched and weighed at the onset of the study. Diabetes was induced by a single tail vein injection of Alloxan monohydrate (60mg/kg body weight) dissolved in normal saline. Diabetes was confirmed by glucose estimation after 48hours. Only rats with blood glucose levels greater than 11.0mmol/L were used for the study. The rats were grouped to into four (4) categories to represent duration of diabetes. The rats were sacrificed weekly and blood samples collected. Determination of creatinine, urea, potassium, sodium, chloride, blood glucose and cystatin-c were done weekly for the test groups and control.*** ***Results:*** ***There was significant high mean blood glucose in the test group compared to the control across the weekly duration of diabetes. There were was no significant difference in mean creatinine levels between the test cases and controls. There was significant increase in the cystatin-c levels of the test groups compared with the control across the four weeks duration.*** ***Conclusion:*** ***The weakness and inability of creatinine to detect minor changes in kidney function is apparent in the face of rising incidence of kidney disease. This provides sufficient rationale for development of modern relevant tool. Cystatin-c proved to have better ability to detect early changes in kidney function than creatinine and should be considered a veritable tool detection of diabetic kidney disease.***

Keywords: Diabetes, kidney, renal markers, early detection

1. Introduction

The global epidemic of diabetes type 2 has left an increasing prevalence of kidney and end-stage renal diseases worldwide [1]. Diabetes type 2 is the single largest cause of end-stage renal diseases (ESRD) worldwide, accounting for over one-third of all patients enrolling onto renal support therapy programmes [2][3]. Diabetic kidney diseases are significantly under-diagnosed due to the limitations and weaknesses of the traditional diagnostic tools. . Even though serum creatinine determination remains the traditional renal marker for estimation of GFR, it is known to have a number of inherent difficulties which limit its clinical reliability [4]. Attempts to improve the accuracy of serum creatinine measurement yielded some equations. These include the modification of Diet in Renal Diseases (MDRD) and Cockroft-Gault (CG). Each of these equations has strengths and weaknesses for use in specific patient sub-populations. Although creatinine based GFR equations such as the MDRD improved the accuracy of serum creatinine measurements the concentrations of creatinine can be within the normal ranges even with a GFR of around 40ml/min per 1.73m² resulting in the so called "creatinine blind" rang [4]. A substantial body of evidence has developed over the past years which support the use of Cystatin C as an alternative and more sensitive endogenous marker for the estimation of GFR [4-6]. We investigated an animal model whether cystatin-c is cause or a marker in early detection of diabetic kidney disease. Using Alloxan, we induced diabetes in male rats and monitored the cystatin-c levels and traditional kidney function markers, for a period of four (4) weeks.

2. Methodology

2.1 Experimental protocol

Male rats *(albino* rats) sourced from University of Port Harcourt and Rivers State University of Science and Technology all in Port Harcourt, were used for this study. The rats were allowed to acclimatize to the laboratory conditions for seven (7) days following their arrival at the laboratory. All the rats were age-matched at the onset of the study and weighed. The rats were randomly divided into five groups; controls and those that had been diabetic for durations of one week, two weeks, three weeks and four weeks, with eight rats in each group (n=8 per group). The animals were housed individually in plastic-bottomed cages with free access to a standard commercial feed and water.

2.2 Induction of diabetes

Diabetes was induced by a single tail vein injection of Alloxan monohydrate (60mg/kg – body weight) in normal saline solution, after an overnight fast. Diabetes was confirmed by blood glucose estimation after 48 hours of Alloxan injection. Only rats with blood glucose levels

greater than 11.0mmol/L were used for the study. The rats were given free access to food and water, in accordance with the National Institute of Health Guideline for the care and use of laboratory animals (NRC, 1985).
The non-diabetic controls were given 1ml/kg normal saline. The one-week, two-week, three-week and four-week post-Alloxan induced diabetic rats were observed daily and changes in weight and general behaviour were recorded.

2.3 Collection of blood samples

The rats were sacrificed weekly and 6ml of blood collected and dispensed into plastic bottles for assays of Creatinine, urea, potassium, sodium, chloride, blood glucose and Cystatin C. The samples were centrifuged at 6000r/min for 5minutes and serum separated, and stored frozen until the determination of the biochemical parameter. Blood glucose monitoring was done weekly for all the groups by measuring the blood glucose.

3. Results

3.1 Biochemical parameter of diabetic rats after one week duration of diabetes compared with control

Results of the biochemical parameters of diabetic rats after one week duration of diabetes compared with controls are presented in Table 4.1. There was no difference in body weights (WT1) between test group and controls before diabetic induction. The mean blood glucose (FBG1) for the test group was significantly higher than the control group after induction ($p<0.05$). The body weights (WT2) of diabetic rats after one week duration of diabetes were significantly lower than the control cases ($p< 0.05$). The blood glucose levels after one week duration diabetic rats were significantly higher than those of the controls ($p<0.05$). The mean difference between creatinine values for test and control cases were not statistically significant ($p>0.05$). The Urea, Potassium and Sodium for test animals were significantly higher than the controls ($p<0.05$). The Cystatin C levels of the one week diabetic rats were significantly higher than the control ($p< 0.05$).

3.2 Biochemical parameter of diabetic rats after two weeks duration of diabetes

The details of biochemical parameters of diabetic rats after two weeks duration of diabetes compared with controls are shown Table 4.2. There was significant increase in blood glucose (FBG1) of diabetic rats compared with controls after induction ($p<0.05$). It was observed that there were significant decrease in body weight of the two weeks duration diabetic rats as compared with the control ($p<0.05$).

After two weeks of diabetes, the mean blood glucose of the diabetic rats increased significantly compared with values for the controls ($p< 0.05$). The creatinine values of the diabetic rats at two weeks were significantly lower than those of the control animals ($p=0.005$). The urea levels of the diabetic group were significantly higher than the control ($p< 0.05$). Similarly, significantly higher mean values of potassium and sodium were observed in the diabetic rats compared with controls ($p< 0.05$). There was no significant difference observed in the means of the chloride levels for both groups. The Cystatin C levels of the diabetic rats were also significantly higher than values for the control group ($p<0.05$).

3.3 Biochemical parameter of diabetic rats after three weeks duration of diabetes compared with the controls.

Results of the biochemical parameters of diabetic rats after three weeks duration of diabetes and their control are presented in Table 4.3. There was no significant difference in body weight and blood glucose of the rats at onsets of the experiment. The blood glucose (FBG1) after induction of diabetes was significantly higher in the diabetic group compared with the controls ($p<0.05$). At the expiration of three weeks after induction, the body weight and blood glucose were significantly higher in the diabetic rats than the control group ($p<0.05$). Similar trends of significant increases were observed in the mean levels of Creatinine, Urea and Potassium at ($p<0.05$). However, there was no significant difference in means of Sodium levels in both groups. Interestingly, the chloride levels of the diabetic group were significantly lower than the control ($p<0.05$). The Cystatin C level of the diabetic group were significantly higher than the control at ($p<0.05$).

3.4 Biochemical parameter of diabetic rats after four weeks duration of diabetes compared with the controls

The details of the biochemical parameters of four weeks duration of diabetic rats and their control are shown in Table 4.4. There was significant increase in the mean of the blood glucose (FBG1) of diabetic rats after induction of diabetes compared with the none- diabetic control rats, ($p<0.05$). At the expiration of four weeks, it was observed that the blood glucose (FBG2) was significantly higher in the diabetic group compared with the controls. The mean values of the diabetic group increased significantly from the control group for Creatinine, Urea, Potassium, Sodium, Chloride and Cystatin C compared with the control at ($p<0.05$).

4. Discussions

There was significant loss in the body-weight of the diabetic rats across the duration of diabetes (week 1- 4), compared to the control where there was gain in body-weight. This could be attributed to excessive break down of tissue protein caused by hyperglycemic conditions induced by Alloxan. This is in agreement with earlier reports of [7-8] they reported that diabetic rats showed polyphagia and muscle wasting over the controls caused by hyperglycemic conditions. Again, the blood glucose levels of the diabetic rats were significantly higher than the control ($p <0.05$) thereby implicating hyperglycemic condition.

In this study, urea levels of diabetic rats were significantly higher than the controls($P <0.05$) in. Hyperglycemia generates reactive oxygen species that in turn cause lipid peroxidation and membrane damage in kidney, liver and

brain of diabetic rats [9-10]. This may account for the significant increase in urea levels of diabetic rats across the 4 weeks duration of diabetes.

There was significant increase in Potassium and sodium levels of the diabetic rats compared with the controls ($p<0.05$). [11], reported that dehydration leads to increases in sodium and potassium levels of diabetic rats. Again urinary loss of fluid is a common observation in diabetic conditions, leading to hyperkalaemia and natraemia. It was observed that in week one, there was no significant difference in the creatinine level of the diabetic rat compared to the control ($P<0.05$). Creatinine is a product of metabolism of creatine and phosphocreatine in skeletal muscles, and in an early on-set of diabetic kidney condition, creatinine can be normal due to tubular secretion of creatinine [12].Thus indicating that creatinine levels do not increase until there is moderate decrease in glomerular filtration rate (GFR). This is in agreement with the reports of [13], where it was indicated that creatinine is insensitive in detecting small decreases in GFR, suggesting the existence of the so called creatinine blind range GFR (40-70 ml/min/ $1.73m^2$).

In week-two, it was observed that creatinine levels were not significantly different from that in week-one. The reason for this trend is not clear, however, it has been reported by [14] creatinine levels tend to decrease due to hyperfilteration. There were significant increases in creatinine levels in week 3 and week 4. At one week duration of diabetes there was significant increase in Cystatin C levels the diabetic rats compared with the controls ($P<005$). This observation is in consonance with earlier studies that Cystatin C is an earlier indicator of mild renal failure [15-17]Cystatin C is freely filtered by the glomerulus and largely reabsorbed and catabolized in the proximal tubules [18]. This implies that its clearance cannot be measured because of this catabolism; as such its plasma concentration is a good measure of GFR [19]. In an observational study, [20] reported that the potential utility of serum Cystatin C is the ability to detect early renal failure at stage 2.
Similarly it was observed that there were significant differences in the mean levels of Cystatin C of diabetic rats in week 2 – week4. Again these observations were in agreement with the earlier findings of [21], that Cystatin C was more sensitive than serum creatinine for mild decreases in GFR by TC - DTPA clearance as evidenced by ROC analysis (AUC: 0.996 for Cystatin C and 0.870 for serum creatinine) respectively.

5. Future Scope

Future prospects of this study are hinged on the fact that diabetic renal disease connotes specific changes in function and structure of the kidney. Hence the need to prospectively look at the structural changes in diabetic rats and subsequent application of findings in detection of diabetic kidney disease in humans.

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Table 4.1: Biochemical parameters of diabetic rats after on e week duration of diabetes compared with controls.

Parameters	*WK 1 DMR*	*Control*	*P-Value*
Body WT1	150 ± 6.70	150 ± 6.2	0.5000
FBGI(48hrs)	21.56 ± 2.0	5.0 ± 0.42	0.0001
Body WT2	126.25 ± 5.2	167.5 ± 3.5	0.0001
FBG2	22.73 ± 1.9	5.0 ± 0.60	0.0001
Creatinine	89.83 ± 5.6	81.2 ± 5.5	0.1666
Urea	6.58 ± 8.4	4.5 ± 0.40	0.0080
Potassium	5.45 ± 0.99	4.75 ± 0.1	0.0433
Sodium	137.84 ± 3.0	132.5 ± 7.0	0.0009
Chloride	101.13 ± 2.5	103.9 ± 2.0	0.1170
Cystatin C	0.86 ± 0.08	0.6 ± 0.01	0.0001

Parameters	*WK 3 DMR*	*Control*	*P-Value*
Body WT1	165.62 ± 6.6	175.5 ± 0.71	0.0985
FBGI	27.76 ± 1.9	4.75 ± 0.21	0.6672
Body WT2	31.04 ± 2.2	4.8 ± 0.42	0.0001
FBG2	21.04 ± 2.2	4.8 ± 0.42	0.0001
Creatinine	80.41 ± 3.9	71.8 ± 1.7	0.0366
Urea	10.47 ± 1.2	5.0 ± 0.14	0.0001
Potassium	9.2 ± 0.58	4.7 ± 0.14	0.0001
Sodium	134.85 ± 2.2	133.5 ± 1.5	0.2557
Chloride	85.82 ± 3.1	100.05 ±	0.0018
Cystatin C	1.75 ± 0.09	0.62 ± 0.02	0.0001

Table 4.2: Biochemical parameters of diabetic rats after two weeks duration of diabetes compared with controls.

Parameter	*WK 2 DMR*	*Control*	*P-Value*
Body WT1	150.36 ± 0.4	150 ± 7.60	0.5341
FBGI(48hrs)	28.36 ± 3.1	4.75 ± 0.11	0.0001
Body WT2	131.87 ± 4.5	172.5 ± 3.5	0.0001
FBG2	31.61 ± 3.1	4.75 ± 0.10	0.0001
Creatinine	58.73 ± 4.6	75.95 ± 3.3	0.1666
Urea	8.73 ± 0.88	5.25 ± 0.51	0.0080
Potassium	8.56 ± 0.88	4.65 ± 0.10	0.0343
Sodium	142.75 ± 0.44	134.0 ± 1.4	0.0009
Chloride	102.71 ± 6.1	102.5 ± 7.1	0.0948
Cystatin C	1.31 ± 0.11	0.63 ± 0.02	0.0001

Table 4.4: Biochemical parameters of diabetic rats after four weeks duration of diabetes compared with controls

Parameters	*WK 4 DMR*	*Control*	*P-Value*
Body WT1	150.71 ± 5.1	150 ± 7.1	0.5021
FBGI	23.42 ± 2.3	4.65 ± 0.35	0.0001
Body WT2	135 ± 7.70	165 ± 8.30	0.0089
FBG2	25.92 ± 1.9	5.0 ± 0.14	0.0019
Creatinine	111.88 ± 5.0	80.95 ± 0.19	0.0009
Urea	9.92 ± 1.10	5.0 ± 0.14	0.0001
Potassium	9.08 ± 0.84	4.9 ± 0.14	0.0002
Sodium	159.36 ± 9.6	137.5 ± 0.7	0.0036
Chloride	112.54 ± 6.0	104 ± 1.4	0.0155
Cystatin C	1.55 ± 0.08	0.62 ± 0.2	0.0001

Author Profile

Dr. Brown Holy obtained Bachelor of Medical Laboratory Science BMLS, Master of Science in Chemical Pathology and Doctor of Philosophy in Chemical Pathology, from Rivers State University of Science and Technology Port Harcourt, in 1998, 2007 and 2013 respectively. He is currently a lecturer in Department of Medical Laboratory Science, of same university. He has worked in many clinical laboratories. He is happily married with three children.