**KIDNEY INJURY MOLECULE-1, CYSTATIN C AND MICROALBUMINURIA LEVELS AMONG DIABETICS ATTENDING RIVERS STATE UNIVERSITY TEACHING HOSPITAL, PORT HARCOURT, NIGERIA**

**ABSTRACT**

|  |
| --- |
| **Background:** Diabetes mellitus affects over 422 million people globally, with projections estimating 643 million cases by 2030. Diabetic nephropathy remains one of its most common complications, underscoring the need for early detection.**Aim:** To assess the levels of Kidney injury Molecule-1 (KIM-1), cystatin C, and microalbuminuria among diabetic patients at Rivers State University Teaching Hospital (RSUTH).**Methodology:** An analytical cross-sectional study was carried out in Rivers State University Teaching Hospital (RSUTH) from September 2024 to December 2024. A total of 140 participants were recruited—70 diabetic patients and 70 age- and sex-matched healthy controls residing in Port Harcourt. Demographic and clinical data were collected using questionnaires. Laboratory analyses included HbA1c, KIM-1, cystatin C, fasting blood glucose, urea, creatinine, sodium, chloride, potassium, bicarbonate, and microalbuminuria were conducted. Data were analyzed using GraphPad Prism v9.0.0, with significance set at *P*<0.05.**Results:** KIM-1 levels were significantly higher in the test 23.97 ± 18.94 pg/mL compared with control group 6.678 ± 5.347 pg/mL, (*P*<0.0001). Cystatin-C levels were significantly higher in test 18.54 ± 7.441 ng/mL in comparison with the control group 10.42 ± 4.051 ng/mL (*P*<0.0001). Microalbuminuria levels were significantly higher in the test group, 62.00 ± 61.07 mg/L when compared with control group 21.43 ± 28.09 mg/L (*P*<0.0001). HbA1c levels were significantly higher in the test group 8.581 ± 1.803 compared with the control group 5.483± 0.4249 (*P*<0.0001). No significant correlation was found between Glycated hemoglobin (HbA1c) and KIM-1, cystatin C nor microalbuminuria. KIM-1, Cystatin C, Microalbuminuria and Bicarbonate were statistically significantly higher in the test group for both males and females. Creatinine and Urea were statistically significantly higher in the male test group. KIM-1 and Cystatin-C were significantly higher in test groups in all age ranges except 26-35 years. Cystatin-C levels were significantly higher for individuals with over 20 years diabetes diagnosis. **Conclusion: T**he study revealed significantly elevated renal biomarkers in diabetic individuals, independent of glycemic control. the rise in cystatin C with longer disease duration suggests progressive kidney decline. These findings highlight the value of combining multiple biomarkers for early and accurate detection of diabetic kidney disease. |

*Keywords: Diabetes Mellitus; Kidney injury Molecule, Cystatin C, Microalbuminuria, Diabetic Kidney Disease, Rivers State.*

1. INTRODUCTION

Diabetes mellitus (DM), is a complex metabolic disorder characterized by hyperglycemia which results from anomalies in either insulin secretion, insulin action or both and manifests in a heterogeneous manner like carbohydrate, fat, and protein metabolic dysfunctions [1]. Globally, diabetes mellitus affects over 422 million people and this value is predicted to rise to 643 million by 2030 and 783 million by 2045 [2].

Diabetic kidney disease (DKD) is one of the most frequent complications of diabetes mellitus (DM). It is a condition characterized by progressive decline in kidney function and is an under recognized global health threat [3]. People with diabetic kidney disease are at high risk of kidney failure, atherosclerotic cardiovascular disease, heart failure, and premature mortality [4]. Additionally, recent studies show that DKD is not only a major driver of end-stage kidney disease (ESKD) worldwide [5] but that it also accounts for approximately 50% of Chronic Kidney Disease. Undoubtedly, early detection and intervention are crucial for preventing end-stage Kidney disease.

Traditional kidney function markers (urea, creatinine, sodium, potassium, chloride, bicarbonate) typically show abnormal values when about 80% of the kidney function has been lost [6]. Diabetic nephropathy is diagnosed by routine testing of estimated glomerular filtration rate (eGFR) and detection of microalbuminuria. Although microalbuminuria is recognized as the gold standard for diagnosing early DKD, it may appear late in the disease course and kidney damage in diabetics may occur without increased albuminuria. Additionally, decrease in eGFR, which is typically measured by serum creatinine levels, is not an early indicator of diabetic renal damage as abnormalities may only be noticed after significant amount of the nephrons have been damaged [7]. Recent research suggests that damage to the kidney tubules, alongside glomerular dysfunction, plays a significant role in the pathogenesis of DKD and implicates kidney injury molecule-1 (KIM-1) and cystatin C as early biomarkers of renal dysfunction [8].

The International Diabetes Federation reported a prevalence rate of about 3.7% in Nigeria [9]. This increasing prevalence is a growing public health concern that has propelled an in-depth study of the condition within the country. The current study aims to assess kidney injury molecule-1 (KIM-1), cystatin c and microalbuminuria levels among diabetics attending Rivers State University Teaching Hospital, Port Harcourt, Nigeria

2. materialS and methods

**2.1 Study Design**

The study was an analytical cross-sectional study carried at Rivers State University Teaching Hospital (RSUTH) between September 2024 and December 2024. Blood test on glycated hemoglobin, kidney injury molecule, cystatin C, plasma sodium, potassium, chloride and bicarbonate were carried out alongside microalbuminuria.

**2.2** **Study Population and** **Sample Size Determination**

The study population comprised diabetic patients receiving care at the Rivers State University Teaching Hospital (test group) and apparently healthy individuals randomly selected from within Port Harcourt (control group). The minimum required sample size was determined using Cochran’s formula, based on a prevalence rate of 3.7% [9]. All participants were selected through random sampling.

* 1. **Eligibility Criteria**

All participants were adults (18 years and above). Those with a laboratory confirmed diagnosis of diabetes mellitus were recruited as cases while apparently healthy adults (age > 18 years) were recruited as control participants. All participants were residents of Port Harcourt for at least the past 6 months and only those willing to give consent were recruited. Patients suffering from HIV, pregnant or breastfeeding women, people with known ongoing or past kidney disease and/or urinary tract infection were exempted from participating.

* 1. **Specimen Collection**

Two (2) mililiters of venous blood was collected into K₃EDTA (Tripotassium ethylenediaminetetraacetic acid) anticoagulant vacutainer tubes for the analysis of glycated haemoglobin (HbA1c), while 2.5 milliliters was collected into a plain tube. All samples were collected using standard venepuncture techniques as described by Cheesebrough [10]. KIM-1 and cystatin C were analyzed with the serum obtained from the plain tube, HbA1c analyzed with whole blood from EDTA tube, and microalbuminuria (albumin to creatinine ratio) analyzed using spot urine sample collected into a universal bottle.

* 1. **Laboratory Analysis**
		1. **Estimation of Glycated Hemoglobin Levels**

Glycated haemoglobin levels were estimated by the fluorescence immunoassay method described by Hicks [11], as specified by Finecare™ (Wondfo® Finecare machine, SN: FS1132102101372), China.

* + 1. **Estimation of Kidney injury Molecule-1 Levels**

The concentration of Kidney injury Molecule-1 was estimated by Sandwich-Enzyme Linked Immunosorbent Assay using Elabscience Biotechnology ELISA Kit (Catalog No: E-EL-H6029), according to manufacturer’s instruction. Analysis was performed using Emp 201 microplate reader.

* + 1. **Estimation of Cystatin C (Cys-C) Levels**

the concentration of Cystatin C was estimated by Sandwich-Enzyme Linked Immunosorbent Assay using Elabscience Biotechnology ELISA Kit (Catalog No: E-EL-H3643), according to manufacturer’s instruction. Analysis was performed using Emp 201 microplate reader.

* + 1. **Estimation of Microalbuminuria Levels**

Microalbuminuria levelswere estimated using Combina 13 test strips (REF 22132) and combilyzer13 (REF 17600, SN 200075, Human GmbH Max Planck-Ring 21 65205 Wiesbaden Germany) and according to manufacturer’s instruction.

* + 1. **Determination of Plasma Creatinine Concentration**

Plasma Creatinine Concentration was measured using the enzymatic oxidation method described by Jaffe’s Slot [13], following the protocol provided by Randox Laboratories Limited, United Kingdom, and analyzed with the Mindray Semi-Auto Chemistry Analyzer (Model: BA-88A).

* + 1. **Determination of Plasma Urea Concentration**

Plasma urea concentration was measured using the Urease Berthelot enzymatic reaction method described by Armstrong, 1927 [14], following the protocol provided by Randox Laboratories Limited, United Kingdom, and analyzed with the Mindray Semi-Auto Chemistry Analyzer (Model: BA-88A).

* + 1. **Determination of Plasma Electrolytes (sodium, potassium, chloride and bicarbonate)**

Plasma sodium, potassium, chloride and bicarbonate were analyzed by Ion Selective Electrode method following the protocol provided by Labomiz Scientific Limited, United States, and analyzed with the Labomiz Electrolyte Analyzer (Model: MELA-1A).

* 1. **Statistical Analysis**

Obtained data were analyzed using GraphPad Prism software version 9.0.0 (121), San Diego, CA. Numerical data was expressed as Mean ± Standard deviation. Statistical comparisons of means and standard deviations for all parameters were done using independent sample t-tests and ANOVA to evaluate potential differences between the test and control groups. Tukey’s multiple comparison (post hoc tests) was used to obtain specific significant differences among the various groups and Pearson’s correlation used to obtain association between groups. Results were considered statistically significant at 95% confidence interval (P<0.05).

3. results and discussion

Table 1: Demographic Characteristics of the Studied Subjects

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Category |  | Control | Test | Total |
| Gender | Male | 30 (21.43%) | 30 (21.43%) | 60 (42.86%) |
| Female | 40 (28.57%) | 40 (28.57%) | 80 (57.14%) |
| Age (Years) (Years) | 26-35 | 3 (2.14%) | 3(2.14%) | 6 (4.29%) |
| 36-45 | 12 (8.57%) | 13 (9.29%) | 25 (17.86%) |
| 46-55 | 23 (16.43%) | 24 (17.14%) | 47 (33.57%) |
| 56-65 | 19 (13.57%) | 24 (17.14%) | 43 (30.71%) |
| 66-75 | 13 (9.29%)  | 6 (4.29%) | 19 (13.57%) |
| Duration of Dm Diagnosis (Years) (Years) | 1-5 | Nil | 31(44.29%) | 31(44.29%) |
| 6-10 | Nil | 17 (24.29%) | 17 (24.29%) |
| 11-15 | Nil | 11 (15.71%) | 11 (15.71%) |
| 16-20 | Nil | 5 (7.14%) | 5 (7.14%) |
| >20 | Nil | 6 (8.57%) | 6 (8.57%) |

Table 2: Mean ± standard deviation, *p-value* of glycated hemoglobin (HbA1c) of diabetic subjects attending RSUTH and control subjects in Port Harcourt

|  |  |
| --- | --- |
| Parameter/Groups | HbA1c (%) |
| Control (N= 70) | 5.483± 0.4249 |
| Test (N= 70) | 8.581 ± 1.803 |
| *P-Value* | <0.0001 |
| Remark | S |

Key: S=Significant

Table 3: Mean ± Standard Deviation, *P-Value* of KIM-1, Cystatin C and Microalbuminuria of Diabetic Subjects Attending RSUTH and Control Subjects in Port Harcourt

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters/Groups | KIM-1 (pg/ml) | Cystatin C (ng/ml) | Microalbuminuria (mg/l) |  |
| Control (N= 70) | 6.678± 5.347 | 10.42± 4.051 | 21.43± 28.09 |  |
| Test (N= 70) | 23.97± 18.94 | 18.54± 7.441 | 62.00± 61.07 |  |
| *P-Value* | <0.0001 | <0.0001 | <0.0001 |  |
| Remark | S | S | S |  |

Key: S=Significant

Table 4: Mean ± Standard Deviation and *P-Value* of Traditional Markers of Kidney Function (Plasma Sodium, Chloride, Potassium, Bicarbonate, Urea and Creatinine) of Diabetic Subjects Attending RSUTH and Control Subjects in Port Harcourt

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters/Groups | Urea (mmol/l) | Creatinine (mmol/L) | Sodium (mmol/l) | Potassium (mmol/l) | Chloride (mmol/l) | Bicarbonate (mmol/l) |
| Control (N= 70) | 3.419± 0.4906 | 78.19±17.86 | 140.1± 1.671 | 4.117± 0.2604 | 100.3± 2.083 | 24.61± 1.609 |
| Test (N= 70) | 4.893± 4.576 | 80.91± 9.671 | 140.3± 2.049 | 5.710± 7.316 | 100.3± 2.997 | 25.89± 2.602 |
| *P-Value* | 0.0082 | 0.0204 | 0.5279 | 0.0709 | 0.8445 | 0.0007 |
| Remark | S | S | NS | NS | NS | S |

Key: NS= Non-Significant S=Significant

Table 5: Correlation Analysis of HbA1c and Some Early Markers of Kidney Function (KIM-1, Cystatin C and Microalbuminuria) of Diabetic Subjects Attending RSUTH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | HbA1c (%) | KIM-1 (pg/ml) | Cystatin C (ng/ml) | Microalbuminuria (mg/l) |
| r | *P* | r | *P* | r | *P* | r | *P* |
| HbA1c (%) | 0.9999 | 0 | 0.0778 | 0.5216 | 0.1183 | 0.3290 | 0.0219 | 0.8571 |
| KIM-1 (pg/ml) | 0.0778 | 0.5216 | 1 | 0 | 0.4803 | 2.5774 | 0.1458 | 0.2284 |
| Cystatin C (ng/ml) | 0.1183 | 0.3290 | 0.4803 | 2.5774 | 0.9999 | 0 | 0.0552 | 0.0552 |
| Microalbuminuria (mg/l) | 0.0219 | 0.8571 | 0.1458 | 0.2284 | 0.0552 | 0.2284 | 1 | 0 |

**Table 6: Correlation Analysis of Glycated Hemoglobin (HbA1c) and Traditional Markers of Kidney Function (Urea. Creatinine, Sodium, Chloride, Potassium and Bicarbonate) of Diabetic Subjects Attending RSUTH**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **HbA1c (%)** | **Urea (mmol/l)** | **Creatinine(Μmol/L)** | **Sodium (mmol/l)** | **Potassium (mmol/l)** | **Chloride (mmol/l)** | **Bicarbonate (mmol/l)** |
| **r** | ***P*** | **r** | ***P*** | **r** | ***P*** | **r** | ***P*** | **r** | ***P*** | **r** | ***P*** | **r** | ***P*** |
| HbA1c (%) | 0.999 | 0 | 0.165 | 0.171 | 0.024 | 0.837 | 0.028 | 0.817 | 0.063 | 0.599 | 0.053 | 0.658 | 0.040 | 0.736 |
| Urea (mmol/l) | 0.165 | 0.171 | 1 | 0 | 0.277 | 0.020\* | 0.199 | 0.097 | -0.027 | 0.820 | 0.304 | 0.010\* | -0.057 | 0.634 |
| Creatinine (Μmol/L) | 0.024 | 0.837 | 0.277 | 0.020\* | 1 | 0 | 0.032 | 0.792 | -0.012 | 0.917 | 0.096 | 0.427 | 0.555 | 0.000\* |
| Sodium (mmol/l) | 0.028 | 0.817 | 0.199 | 0.097 | 0.032 | 0.792 | 1 | 0 | 0.017 | 0.883 | 0.306 | 0.009\* | -0.207 | 0.085 |
| Potassium (mmol/l) | 0.063 | 0.599 | -0.027 | 0.820 | -0.012 | 0.917 | 0.017 | 0.883 | 1 | 0 | -0.194 | 0.106 | -0.030 | 0.799 |
| Chloride (mmol/l) | 0.053 | 0.658 | 0.304 | 0.010\* | 0.096 | 0.427 | 0.306 | 0.009\* | -0.194 | 0.106 | 1 | 0 | 0.157 | 0.192 |
| Bicarbonate (mmol/l) | 0.040 | 0.736 | -0.057 | 0.634 | 0.555 | 0.000\* | -0.207 | 0.085 | -0.030 | 0.799 | 0.157 | 0.192 | 1 | 0 |

Key: NS= Non-Significant S=Significant

**Table 7: Correlation Analysis of Traditional Markers of Kidney Function (Plasma Sodium, Chloride, Potassium, Bicarbonate, Urea and Creatinine) and Early Markers of Kidney Damage (KIM-1, Cystatin C and Microalbuminuria) in Diabetic Subjects Attending Rivers State University Teaching Hospital**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameters |  | KIM-1 (pg/ml) | Cys-C (ng/ml) | Malb (mg/l) | Urea (mmol/l) | Cr (Mmol/L) | Na (mmol/l) | K (mmol/l) | Cl (mmol/l) | HCo3 (mmol/l) |
| KIM-1 (pg/ml) | r | 1 | 0.48 | 0.145 | -0.094 | 0.247 | -0.083 | -0.017 | -0.065 | 0.199 |
| *P* | 0 | 2.577 | 0.228 | 0.435 | 0.038\* | 0.492 | 0.886 | 0.591 | 0.097 |
| Cystatin-C (ng/ml) | r | 0.48 | 0.999 | 0.055 | -0.124 | 0.045 | -0.0832 | -0.074 | -0.125 | 0.113 |
| *P* | 2.577 | 0 | 0.649 | 0.304 | 0.709 | 0.493 | 0.537 | 0.301 | 0.349 |
| Microalbuminuria (mg/l) | r | 0.145 | 0.055 | 1 | 0.049 | 0.238 | -0.012 | -0.186 | -0.148 | 0.128 |
| *P* | 0.228 | 0.649 | 0 | 0.682 | 0.047\* | 0.918 | 0.122 | 0.219 | 0.29 |
| Urea (mmol/l) | r | -0.094 | -0.124 | 0.049 | 1 | 0.277 | 0.199 | -0.027 | 0.304 | -0.057 |
| *P* | 0.435 | 0.304 | 0.682 | 0 | 0.020\* | 0.097 | 0.82 | 0.010\* | 0.634 |
| Creatinine (mmol/L) | r | 0.247 | 0.045 | 0.238 | 0.277 | 1 | 0.032 | -0.012 | 0.096 | 0.555 |
| *P* | 0.038\* | 0.709 | 0.047\* | 0.020\* | 0 | 0.792 | 0.917 | 0.427 | 0.000\* |
| Sodium (mmol/l) | r | -0.083 | -0.0832 | -0.012 | 0.199 | 0.032 | 1 | 0.017 | 0.306 | -0.207 |
| *P* | 0.492 | 0.493 | 0.918 | 0.097 | 0.792 | 0 | 0.883 | 0.009\* | 0.085 |
| Potassium (mmol/l) | r | -0.017 | -0.074 | -0.186 | -0.027 | -0.012 | 0.017 | 1 | -0.194 | -0.03 |
| *P* | 0.886 | 0.537 | 0.122 | 0.82 | 0.917 | 0.883 | 0 | 0.106 | 0.799 |
| Chloride (mmol/l) | r | -0.065 | -0.125 | -0.148 | 0.304 | 0.096 | 0.306 | -0.194 | 1 | 0.157 |
| *P* | 0.591 | 0.301 | 0.219 | 0.010\* | 0.427 | 0.009\* | 0.106 | 0 | 0.192 |
| Bicarbonate (mmol/l) | r | 0.199 | 0.113 | 0.128 | -0.057 | 0.555 | -0.207 | -0.03 | 0.157 | 1 |
| *P* | 0.097 | 0.349 | 0.29 | 0.634 | 0.000\* | 0.085 | 0.799 | 0.192 | 0 |

N=70, \* Represents Statistical Significance at P<0.05, Cys-C = Cystatin-C, Malb = Microalbuminuria, Cr= Creatinine, Na = Sodium, K = Potassium, Cl = Chloride, HCo3 = Chloride

**Table 8: Mean ± Standard Deviation, *P-Value* of Early Kidney Markers (KIM-1, Cystatin C and Microalbuminuria) and Traditional Markers of Kidney Function (Plasma Sodium, Chloride, Potassium, Bicarbonate, Urea and Creatinine) in Male and Female Diabetic Subjects Attending RSUTH and Control Subjects in Port Harcourt**

|  |  |  |
| --- | --- | --- |
| Parameters/Groups | Female | Male |
| Control | Test | *P-Value* | Control | Test | *P-Value* |
| KIM-1 (pg/ml) | 5.508± 0.4364 | 8.478± 1.776 | <0.0001\* | 7.890± 5.624 | 23.90± 18.37 | <0.0001\* |
| Cystatin C (ng/ml) | 9.554± 4.091 | 17.35± 6.040 | <0.0001\* | 11.58± 3.755 | 20.12± 8.839 | <0.0001\* |
| Microalbuminuuria (mg/l) | 21.00± 21.46 | 61.25± 61.61 | 0.0002\* | 22.00± 35.47 | 63.00± 61.37 | 0.002\* |
| Urea (mmol/l) | 3.380± 0.4784 | 4.610± 4.562 | 0.0939 | 3.470± 0.5100 | 5.270± 4.643 | 0.0391\* |
| Creatinine (Μmol/L) | 75.30± 11.00 | 105.3± 105.8 | 0.0783 | 82.03± 23.85 | 96.27± 21.95 | 0.0194\* |
| Sodium (mmol/l) | 140.2± 1.810 | 140.6± 1.973 | 0.3476 | 140.1± 1.494 | 140.0± 2.141 | 0.8893 |
| Potassium (mmol/l) | 4.133± 0.2759 | 5.998± 7.907 | 0.1400 | 4.097± 0.2414 | 5.327± 6.558 | 0.3089 |
| Chloride (mmol/l) | 100.2± 2.099 | 100.6± 2.716 | 0.4633 | 100.4± 2.092 | 100.0± 3.358 | 0.6462 |
| Bicarbonate (mmol/l) | 24.73± 1.648 | 25.78± 2.833 | 0.0462\* | 24.47± 1.570 | 26.03± 2.297 | 0.0031\* |

N=70 \* Represents Statistical Significance at *P*<0.05

**Table 9: Mean ± Standard Deviation, *P-Value*, of Some Early Markers of Kidney Function (KIM-1, Cystatin C & Microalbuminuria) of the Different Age Groups of Diabetic Subjects Attending RSUTH and Control Subjects in Port Harcourt**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters/Age (Years) | 26-35 Years | 36-45 Years | 46-55 Years | 56-65 Years | 66-75 Years |
| KIM-1 (pg/ml) | Control  | 5.113± 4.415 | 5.475± 3.937 | 7.740± 5.992 | 6.682± 5.428 | 5.803± 6.110 |
| Test  | 31.01± 17.54 | 12.46± 5.614 | 25.54± 18.91 | 21.15± 12.47 | 34.31± 28.55 |
| *P-Value* | 0.068 | 0.001\* | <0.0001\* | <0.0001\* | 0.0290\* |
| Cystatin-C (ng/ml) | Control | 12.17± 4.162 | 9.485± 4.509 | 11.66± 3.957 | 9.490± 4.033 | 10.37 ± 2.860  |
| Test | 16.28± 4.346 | 14.49± 3.366 | 17.85± 5.226 | 19.74± 7.957 | 22.26± 11.14 |
| *P-Value* | 0.302 | 0.004\* | <0.0001\* | 0.0007\* | 0.0212\* |
| Microalbuminuria (mg/l) | Control | 10.00± 0.000 | 18.46± 19.94 | 20.00± 28.89 | 25.83± 33.22 | 75.30± 11.00 |
| Test | 80.00± 70.00 | 46.67± 62.57 | 70.00± 66.06 | 54.21± 54.70 | 21.67± 28.58 |
| *P-Value* | 0.1583 | 0.1360 | 0.0015\* | 0.0417\* | 0.0982 |
|  |  |  |  |  |  |  |

\* Represents Statistical Significance At P<0.05

**Table 10: Results of one-Way Analysis of Variance (Anova) of Glycated Hemoglobin (HbA1c) in Diabetic Subjects Attending RSUTH Based on the Duration of Diabetes Diagnosis**

**Table** **11: Mean ± Standard Deviation, *P-Value* and F-Value of one Way Analysis of Variance (Anova) of Early Kidney Markers (KIM-1, Cystatin C and Microalbuminuria) in Diabetic Subjects Attending RSUTH Based on the Duration of Diabetes Diagnosis**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters/Duration of Diabetes Diagnosis (Years) | KIM-1 (pg/ml) | Cystatin-C (ng/ml) | Microalbuminuria (mg/l) |
| Mean ± Sd | Mean ± Sd | Mean ± Sd |
| 1-5 (N=31) | 21.76 ± 20.02 | 16.48± 4.498e | 51.61± 60.56 |
| 6-10 (N= 17) | 21.84± 18.32 | 17.91± 4.386 e | 61.18± 56.22 |
| 11-15 (N= 11) | 18.65± 10.01 | 17.15± 7.515 e | 98.18± 61.78 |
| 16-20 (N= 5) | 32.61± 15.25 | 16.30± 1.273 e | 70.00± 73.48 |
| >20 (N= 6) | 37.66± 25.08 | 32.13± 12.19 a,b,c,d | 45.00± 58.57 |
| F-Value | 1.584 | 10.25 | 1.355 |
| *P-Value* | 0.1889 | <0.0001 | 0.2592 |
| Remark | NS | S | NS |

Key: NS= Non-Significant, S=Significant, a,b,c,d,e Significant for Tukey’s Comparison of the different diabetic durations.

**Table 12: Mean ± Standard Deviation, *P-Value* and F-Value of one Way Analysis of Variance (Anova) of** **Traditional Markers of Kidney Function (Plasma Sodium, Chloride, Potassium, Bicarbonate, Urea and Creatinine) in Diabetic Subjects Attending RSUTH Based on the Duration of Diabetes Diagnosis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters/Duration of Diabetes Diagnosis (Years) | Urea (mmol/l) | Creatinine (mmol/L) | Sodium (mmol/l) | Potassium (mmol/l) | Chloride (mmol/l) | Bicarbonate (mmol/l) |
| Mean ± Sd | Mean ± Sd | Mean ± Sd | Mean ± Sd | Mean ± Sd | Mean ± Sd |
| 1-5 (N=31) | 4.435±4.635 | 85.23± 24.57 | 140.3± 1.990 | 5.397± 6.429 | 100.6± 2.592 | 25.81± 2.024 |
| 6-10 (N= 17) | 6.406±6.489 | 125.5± 140.6 | 140.1± 2.193 | 6.147± 8.731 | 99.47± 3.826 | 26.00± 3.937 |
| 11-15 (N= 11) | 4.182±1.207 | 86.91± 20.33 | 139.8± 2.316 | 7.327± 10.84 | 100.5± 3.357 | 25.45± 1.572 |
| 16-20 (N= 5) | 5.400±3.425 | 148.0± 142.4 | 142.0± 1.225 | 4.400± 0.400 | 100.6± 2.702 | 25.80± 2.588 |
| >20 (N= 6) | 3.850±1.104 | 104.8± 14.73 | 140.8± 1.722 | 4.217± 0.248 | 101.0± 2.191 | 26.83±2.714 |
| F-Value | 0.6892 | 1.205 | 1.178 | 0.2548 | 0.4877 | 0.2791 |
| *P-Value* | 0.6020 | 0.3170 | 0.3288 | 0.9057 | 0.7447 | 0.8905 |
| Remark | NS | NS | NS | NS | NS | NS |

Key: NS= Non-Significant

**3.2 DISCUSSION**

The study was an analytical cross-sectional design involving two groups; diabetic patients (test group) and apparently healthy individuals (control group), all residing in Port Harcourt. Both groups were matched for age and sex. As recorded in Table 1, female participants were numerically greater than males in both the diabetic and control groups. This distribution was similar to that of Jenewari *et al*., [15] but was in contrast with the study of Umahi-Ottah *et al*., [16] who recorded a greater percentage of male participants in the test and control groups. The participants of the current study were within the ages of 26-75years. This distribution is similar to the 20–79 years global prevalence estimates for diabetes reported by the international Diabetes Federation [9].

Majority of the participants in the current study fall within and above the middle age group; 46-55 and 56-65 accounting for 33.57% and 30.71% of the study population respectively. This distribution aligns with that of the systematic review by Olamoyegun *et al*. [19 17], which highlighted age over 45 as a significant risk factor for type 2 diabetes in Nigeria. As shown in Table, the highest proportion of diabetic subjects (31 individuals, 44.29%) had been diagnosed within the past 1–5 years, while those with a diagnosis duration of over 20 years represented the least frequent group, comprising only six participants (8.57%). The fewer number observed in the last groups could be attributed to higher mortality rates [20 18], whether related or unrelated to diabetes, poor follow-up leading to patient attrition in long-term studies, or improvements in diabetes management resulting in better quality of life and disease control.

In individuals with diabetes, persistent hyperglycemia accelerates the glycation process of hemoglobin, leading to elevated HbA1c levels [21 19]. The results from our study revealed a significant elevation (*P*< 0.0001) of HbA1c in the diabetic group, confirming the diabetic state of the test participants.

In diabetes, chronic hyperglycemia can lead to oxidative stress, inflammation, and hemodynamic changes in the kidney which leads to proximal tubular damage. KIM-1 expression may increase in response to this injury, and its levels in urine and serum can serve as early indicators of kidney damage [23 20]. The current study observed significantly higher levels of serum KIM-1 in the diabetic subjects (*P*<0.0001). This was in accordance with the findings of Abid *et al*. [22 21]. Similar to the findings of Zadabbas *et al*. [24 22], significantly higher levels of cystatin C were observed in the diabetic subjects (*P*<0.0001) of the current study compared to controls. Early in diabetes, hyperglycemia leads to glomerular hyperfiltration. Similarly to the findings of Shinkafi *et al*., [25 23], The current study observed significantly higher levels of microalbuminuria in the diabetic subjects compared to controls (*P*<0.0001). A microvascular injury to the kidneys may alter filtering capacity of cystatin c and damage the glomerular capillary walls, resulting in albumin leakage into the urine.

Urea and creatinine levels were significantly higher in diabetic patients compared to controls (P=0.008 and P=0.0204, respectively), likely due to reduced glomerular filtration or dehydration from osmotic diuresis. These findings align with previous studies, such as Richard et al. [del 17, 18, 26 27 34 24]. Bicarbonate levels were also significantly elevated in the diabetic group (P=0.0007), possibly due to diuretic-induced metabolic alkalosis. No significant differences were found for sodium, potassium, and chloride. Overall, diabetic patients are more prone to acid-base and electrolyte disturbances due to the disease, medications, and related organ damage.

No significant correlations were found between HbA1c and KIM-1 (r = 0.0778, p = 0.52), cystatin C (r = 0.1183, p = 0.3290), or microalbuminuria (r = 0.0219, p = 0.8571). These findings align with studies by Balu et al. [28 25] and Sim et al. [31 26], but differ from others like Ahn et al. [29 26] and Luke & John [30 27], who recorded a statistically significant positive connection between HbA1c and cystatin C in their study although the research area was at Kerala, india. These suggesting population and glycemic differences may influence outcomes.

Similarly, no significant correlations were observed between HbA1c and electrolytes or kidney markers (Na⁺, Cl⁻, K⁺, HCO₃⁻, urea, creatinine), supporting the findings of Nabila et al. [35 28], though differing from Chutani et al. [36 29].

KIM-1 and microalbuminuria were not significantly correlated (r = 0.1458, p = 0.2284), consistent with Balu et al. [28 25]. Their different timing in kidney damage progression may explain this. However, creatinine correlated significantly with KIM-1 (r = 0.247, p = 0.038) and microalbuminuria (r = 0.238, p = 0.047), in line with Khan et al. [38 30] and Dutta et al. [39 31], respectively. Urea also showed weak but significant correlations with creatinine (r = 0.277, p = 0.020), chloride (r = 0.304, p = 0.010), and sodium-chloride (r = 0.306, p = 0.009).

KIM-1 and cystatin C were significantly elevated in diabetics across all age groups except 26–35 years, possibly due to shorter diabetes duration or fewer cases in that age group. This age-related rise aligns with Peng et al. [41 32] and Odden et al. [42 33]. Microalbuminuria was significantly higher in diabetics aged 46–65, suggesting greater renal risk in this group, while differences in younger groups may reflect early disease or undetectable kidney damage.

HbA1c levels rose with diabetes duration up to 15 years before declining slightly, though not significantly—similar to Yang et al. [43 34], who suggested improved management over time may affect this trend. Conversely, Olorunfemi & Adedunmade [44 35] found HbA1c increased with longer disease duration.

**4. CONCLUSION**

The study found significantly elevated levels of serum kim-1, cystatin c, and microalbuminuria in diabetic subjects compared to controls, indicating early kidney damage. However, these markers showed no correlation with hba1c, suggesting that renal injury may occur independently of glycemic control. Cystatin c levels were notably higher in patients with over 20 years of diabetes, reflecting a time-dependent decline in kidney function. The lack of age-related trends in hba1c may be due to variations in disease duration, treatment adherence, and comorbidities. Elevated kim-1 and cystatin c across most age groups even without microalbuminuria highlight early tubular damage, emphasizing their value in the early detection of diabetic nephropathy.

Consent

Signed informed consent was obtained from the respective participants prior to enrolment into the study.

Ethical approval

Ethical clearance was obtained from the Rivers State Health Research Ethics committee with REC NUMBER: RSUTH/REC/2024493.

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