SAR Journal of Medicine

Abbreviated Key Title: *SAR J Med*
Home page: https://sarpublication.com/journal/sarjm/home
DOI: https://doi.org/10.36346/sarjm.2025.v06i01.001

OPEN ACCESS

ISSN 2707-773X (P)
ISSN 2709-6920 (O)

Original Research Article

Evaluation of Cystatin C, Glomerular Filteration Rate and Vanillylmandelic Acid in Comorbid Diabetic and Hypertensive Patients Attending University Teaching Hospital, Owerri

Uneze Odimma Chiamaka[1*], Nwanjo Harrison[1], Nwosu Dennis[1], Ambition Anyanwu[2], Edward Ukamaka[1], Nnodim Johnkennedy[1]

[1]Department of Medical Laboratory Science, Imo State University, Owerri
[2]University College Hospital London

***Corresponding Author:** Uneze Odimma Chiamaka
Department of Medical Laboratory Science, Imo State University, Owerri

Article History: | Received: 21.12.2024 | Accepted: 27.01.2025 | Published: 30.01.2025 |

Abstract: The present study evaluates the cystatin C, glomerular filteration rate (GFR) and vanillylmandelic acid (VMA) in comorbid diabetic and hypertensive patients attending Federal University Teaching Hospital, Owerri, Nigeria. A total number of 200 persons were recruited for the research; 50 controls, 50 hypertensive, 50diabetic, and 50 hypertensive-diabetic persons. The respondents were Federal Teaching Hospital, Owerri. Verbal consent was sort and questionnaires were used to extract information regarding biodata and patients' history of diabetes and hypertension. Blood pressure was determined. A standard venepuncture method was used to obtain seven milliliters (7ml) of blood from all the subjects under asceptic conditions. 3milliliters was dispensed into a plain container capped, labeled appropriately and allowed to clot at room temperature. The serum were separated from the red cell by spinning at 4,000 r.p.m for 5minutes. The supernatant serum obtained were stored frozen at -20 ^{0}C until the day of analysis. The remaining 2ml of blood was dispensed into a fluoride oxalate container for the estimation of fasting blood glucose. 5ml of urine was collected into sterile universal bottle, then aliquot into cryovials and stored at -20^{o}C till the day of assay. All reagents were commercially purchased and the manufacturer's standard operating procedures was strictly adhered to. The fasting blood sugar levels was higher in diabetic-hypertensive group (199.50 ± 25.36 mg/dl) and diabetic groups (209.85 ± 19.95 mg/dl) compared to hypertensive (84.10 ± 9.03 mg/dl) and control group (84.20 ± 9.40 mg/dl). The mean value of the diastolic pressure of the diabetic-hypertensive group (102.90 ± 6.87 mmHg) decreased which was statistically significant (P<0.05) when compared with the diabetic group (75.10 ± 4.58 mmHg). The mean value of the systolic pressure of the diabetic-hypertensive group (160.00 ± 7.36 mmHg) was decreased which was statistically significant (P<0.05) when compared with the diabetic group (115.30 ± 6.14 mmHg). Serum creatinine increased in diabetic-hypertensive group when compared with other groups. The cystatin C levels was higher in hypertensive group (0.90 ± 0.14 mg/l) and lower in diabetic-hypertensive group (0.84 ± 0.17 mg/l) but the mean difference between the groups was not significant (P >0.05). The mean value of VMA was higher in diabetic-hypertensive group (12.48 ± 2.25 mg/24hr) compared to diabetic (11.26 ± 1.74 mg/24hr) and control (9.96± 1.43 mg/24hr) group. There was decrease in the mean value of GFR of diabetic-hypertensive group (71.80 ± 8.30 ml/min/1.73m^2) which was not statistically significant (P>0.05) when compared with other groups. While there was an increase in GFR value of hypertensive group when compared with other groups. There was a positive correlation between creatinine and cystatin C (r = 0.337) and their association was significant (p<0.05). There was a negative correlation between creatinine and VMA (r = -0.164) and their association was significant (p<0.05). There was a negative correlation between creatinine and GFR (r = -0.558) and their association was significant (p<0.05). There was a negative correlation between cystatin C and VMA (r = -0.078) and their association was not significant (p>0.05). There was a positive correlation between cystatin C and GFR (r = 0.019) and their association was not significant (p>0.05). In conclusion, good control of blood glucose and blood pressure level reduces the likelihood of the development of renal impairment which is usually associated with both diabetes and hypertension. Co-morbidity of diabetes and hypertension poses a higher risk of developing renal disease. Both serum creatinine, cystatic C and GFR are important biomarkers for renal impairment hence should be monitored on a regular basis for diabetic and hypertensive patients and much more frequently for hypertensive-diabetic patients.

Keywords: Cystatin C, Glomerular Filteration Rate, Vanillylmandelic Acid, Comorbid Patients Owerri.

Citation: Uneze Odimma Chiamaka, Nwanjo Harrison, Nwosu Dennis, Ambition Anyanwu, Edward Ukamaka, Nnodim Johnkennedy (2025). Evaluation of Cystatin C, Glomerular Filteration Rate and Vanillylmandelic Acid in Comorbid Diabetic and Hypertensive Patients Attending University Teaching Hospital, Owerri, *SAR J Med*, *6*(1), 1-10.

INTRODUCTION

Diabetes Mellitus is a chronic disease associated with a high incidence morbidity and mortality rate in the world. Type 2 diabetes mellitus makes up 90% of the cases of diabetes [1]. This disease is relentlessly affecting on economically affluent nations and afflicting developing country like Nigeria. The regional prevalence of type 2 diabetes mellitus in Nigeria has been reportedly variable across different parts of the country which could be a reflection of cultural, tribal, diet and life style [2]. The International Diabetic Federation estimated predicted rise of affected individuals of 19 million type 2 diabetes mellitus individual to 41.5 million by 2035 [3]. In Imo State, the prevalence of 8.7% was reported among geriatric individuals [4].

Type 2 diabetes mellitus formerly known as non-insulin dependent diabetes mellitus or adult-onset diabetes is a metabolic disorder that is characterized by hyperglycaemia in context of insulin resistance, decline insulin production and eventual pancreatic cell failure [5]. The tissues that must prominently demonstrate reduced insulin sensitivity include skeleton muscle, liver and adipose tissue due to requirement for glucose uptake and metabolism at *these sites. Indeed, insulin secretory defects appear to be* critical to overt type 2 diabetes mellitus although residual insulin β cells can persist for prolonged period despite considerable disease progression. This is characterized by hyperglycaemia, polyuria, polydypsia, polyphagia, blurred vision, weakness and weight loss [6].

Type 2 Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to serious damage to the heart, blood vessels, eyes, kidneys and nerves. It is a dysfunction characterized by hyperglycemia resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion. Microvascular complications of diabetes include retinal, renal, and possibly neuropathic disease. Macrovascular complications include coronary artery and peripheral vascular disease [7].

Diabetes mellitus is a chronic disease that requires long-term medical attention to limit the development of its devastating complications and to manage them when they do occur. Unlike patients with type 1 diabetes mellitus, patients with type 2 are not absolutely dependent on insulin for life. This distinction was the basis for the older terms for types 1 and 2, insulin dependent and non–insulin dependent diabetes. Type 2 diabetes is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids and proinflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased breakdown of fat. A role for excess glucagon cannot be underestimated; indeed, type 2 diabetes is an islet paracrinopathy in which the reciprocal relationship between the glucagon-secreting alpha cell and the insulin-secreting beta cell is lost, leading to hyperglucagonemia and hence the consequent hyperglycemia [8].

The prevalence rates of diabetes are increasing worldwide. The International Diabetes Federation predicts that the number of people living with diabetes will to rise from 366 million in 2011 to 552 million by 2030. Type 2 diabetes mellitus is less common in non-Western countries where the diet contains fewer calories and daily caloric expenditure is higher. However, as people in these countries adopt Western lifestyles, weight gain and type 2 diabetes mellitus are becoming virtually epidemic [9].

The top 10 countries in number of people with diabetes are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The greatest percentage increase in rates of diabetes will occur in Africa over the next 20 years. Unfortunately, at least 80% of people in Africa with diabetes are undiagnosed, and many in their 30s to 60s die from diabetes related complications [10].

The global diabetes prevalence in 2019 is estimated to be 9.3%. The prevalence is higher in urban (10.8%) than rural (7.2%) areas, and in high-income (10.4%) than low-income countries (4.0%) [11].

In Nigeria, the current prevalence of DM among adults aged 20–69 years is reported to be 1.7% (IDF, 2017). The pooled prevalence of DM in the six geopolitical zones of Nigeria were 3.0% (95% CI 1.7–4.3) in the north-west, 5.9% (95% CI 2.4–9.4) in the north-east, 3.8% (95% CI 2.9–4.7) in the north-central zone, 5.5% (95% CI 4.0–7.1) in the south-west, 4.6% (95% CI 3.4–5.9) in the south-east, and 9.8% (95% CI 7.2–12.4) in the south-south zone (Andrew *et al.*,2018). A cross-sectional observational survey in Imo state put the prevalence of type 2 diabetes at 11.0% [12].

The etiology of type 2 diabetes mellitus appears to involve complex interactions between environmental and genetic factors. Presumably, the disease develops when a diabetogenic lifestyle (ie, excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed on a susceptible genotype. The body mass index (BMI) at which excess weight increases risk for diabetes varies with different racial groups [13].

For example, compared with persons of European ancestry, persons of Asian ancestry are at increased risk for diabetes at lower levels of overweight. Hypertension and prehypertension are associated with a greater risk of developing diabetes in whites than in African Americans. In addition, an in utero environment resulting in low birth weight may predispose some individuals to develop type 2 diabetes mellitus. Infant

weight velocity has a small, indirect effect on adult insulin resistance, and this is primarily mediated through its effect on BMI and waist circumference [14].

About 90% of patients who develop type 2 diabetes mellitus are obese. However, a large, population-based, prospective study has shown that an energy-dense diet may be a risk factor for the development of diabetes that is independent of baseline obesity. Obesity arises when there is excessive intake of food, especially high calorie diet and decreased physical activity (sendentary lifestyle), during these events, the mass of adipose tissue increases [15].

Insulin resistance is the most significant predictor of type 11 DM and is one of the main component of metabolic syndrome. Adipose tissue responds to the actions of insulin, where insulin stimulates the storage of energy in the form of triglyceride via a rise in the absorption of fatty acids from circulating lipoproteins, insulin also inhibits lipolysis in the adipose tissue [16].

Cardiovascular diseases (CVD) are a group of diseases that affects the cardiovascular system, especially the heart and blood vessels. Cardiovascular diseases are the leading causes of death worldwide and mostly results from atherosclerosis and hypertension. Obesity is an established risk factor of CVD and dyslipidemia [17].

Elevated blood pressure (BP) values are a common finding in patients with type 2 diabetes mellitus (T2D) and are thought to reflect, at least in part, the impact of the underlying insulin resistance on the vasculature and kidney. Moreover, accumulating evidence suggests that disturbances in carbohydrate metabolism are more common in hypertensive individuals, thereby indicating that the pathogenic relationship between diabetes mellitus and hypertension is actually bidirectional [18].

The development of hypertension in diabetic individuals not only complicates treatment strategy and increases healthcare costs but also heightens the risk for macrovascular and microvascular complications considerably. Although BP lowering is followed by a significant reduction in cardiovascular and microvascular morbidity and mortality, a large proportion of diabetic subjects exhibit poorly controlled hypertension. This observation may reflect not only delayed recognition of the presence of hypertension, clinical inertia, and poor adherence to the prescribed regimen but also uncertainty regarding the treatment targets and pathogenic correlation.

Cystatin C is an endogenous protein produced by all nucleated cells in the body. Cystatin C is a nonglycosylated basic protein that is produced and secreted at a constant rate by all nucleated cells [19]. Cystatin C is more reliable marker than the serum creatinine because it is less affected by external factors such as gender, race and muscle mass. It is the early marker for nephropathy and it can also predict peripheral neuropathy, retinopathy and arterial sclerosis . Apart from renal function, cystatin C is also associated with cardiovascular events and limb ischemia. Associated complications deteriorate the quality of life of patients and increase the morbidity rate. Cystatin C is involved in arterial wall remodeling, blood vessel integrity, neovascularization, inflammation and neuronal degenerative pathology. Moreover, serum cystatin C level is the superior marker of GFR than serum creatinine or serum creatinine-based estimates of GFR, and a better predictor of cerebrovascular disease deaths, coronary heart disease, lower extremity arterial disease [20].

Traditional risk factors such as age, gender, body mass index (BMI), smoking status, dyslipidemia, and chronic kidney disease have been shown to be associated with the incidence of hypertension in persons with and without diabetes [21]. The kidney plays a significant role in the regulation of blood pressure (BP) by controlling blood volume and extracellular electrolytes, glomerular hemodynamics, and the renin–angiotensin system. Serum cystatin C has also been shown to be associated with the incidence of hypertension in the general population independent of chronic kidney disease as estimated by serum creatinine using the Modification of Diet in Renal Disease (MDRD) equation [22].

Glomerular filteration rate provides an excellent measure of the filtering capacity of the kidneys. A low or decreased GFR is equal to the sum of the filteration rates in each of the functioning nephrons, the total GFR can be used as an index of functioning renal mass.

Current clinical practice guidelines recommend the use of estimated glomerular filtration rate for the evaluation and classification of renal diseases. The commonly used formulae including Cockcroft and Gault, the Modification of Diet in Renal Disease, and the Chronic Kidney Disease Epidemiology Collaboration formulae all use serum creatinine for estimating GFR. Creatinine-based measurements of GFR have several limitations, including the inability to detect early renal dysfunction due to low sensitivity [23]. As a result of these limitations, plasma cystatin C has been proposed as an alternative endogenous marker for GFR. cystatin C is freely filtered without tubular secretion and is completely catabolized at the proximal tubule [23].

Vanillylmandelic acid (VMA) is the end product of catecholamines breakdown and is secreted in the urine. As the majority of metanephrines are produced within chromaffin cells of the adrenal medulla by a process independent of exocytotic catecholeamine released [24]. Vanillylmandelic acid is found in

the urine, along with other catecholamine metabolites, including homovanillic acid (HVA), metanephrine, and normetanephrine. In timed urine tests the quantity excreted (usually per 24 hours) is assessed along with creatinine clearance, and the quantity of cortisols, catecholamines,
and metanephrines excreted is also measured [25]. Catecholamines include dopamine (found mostly in the central nervous system), norepinephrine (mainly in the sympathetic nervous system) and epinephrine (mainly in the adrenal medulla). They are stored as inactive complex. Released catecholamines, having a short half-life, are taken up by sympathetic nerve endings, or metabolized by the liver and kidney and excreted. Vanillylmandelic acid (VMA) and 4-hydroxy-3-methoxy-mandelic acid (HMMA) are the end product of both epinephrine and norepinephrine catabolism. Catecholamines are produced in the central portion of the adrenal glands, the adrenal medulla. Adrenal glands are small triangular organs located on top of each kidney. The primary catecholamines released are dopamine, epinephrine, and norepinephrine. These hormones are released into the bloodstream in response to physical or emotional stress. They help transmit nerve impulses in the brain, increase glucose and fatty acid release for energy, dilate bronchioles, and dilate the pupils. Norepinephrine also constricts blood vessels, increasing blood pressure, and epinephrine increases heart rate and metabolism. After completing their actions, the catecholamines are metabolized to inactive compounds. Dopamine becomes homovanillic acid (HVA), norepinephrine breaks down into normetanephrine and VMA, and epinephrine becomes metanephrine and VMA. Both the hormones and their metabolites are eliminated from the body in the urine. VMA is usually present in the urine in small fluctuating amounts that only increase appreciably during and shortly after the body is exposed to a stressor. Moreover, increased cathecoleamines which causes hypertension in turn also causes diabetes mellitus by stimulating gluconeogenesis and glycogenolysis [26].

In many societies diabetes and its associated macrovascular/microvascular complications such as hypertension, renal disorders have reached epidemic proportions and consequently attract commensurate medical attention as well as early intervention programmes. However, most developing countries are yet to come to terms with its gradual increase as reported in various epidemiological studies. Serum creatinine level, the most commonly used surrogate measure of glomerular filtration rate (GFR), does not increase until renal function decreases to approximately 50% of its normal value; its excretion rate varies with age, sex, physical exercise, and lean body mass. The population variance of serum creatinine level is large, making it a poor measure for comparison with a reference range. creatinine clearance, measured from a 24-hour urine collection and a concurrent plasma creatinine concentration, is unwieldy and often inaccurate but is widely used in clinical practice, formulas such as the Cockcroft and Gault formula and the MDRD formula try to adjust for these variables ‘gold standard’ tests such as clearance methods using radioisotope (such as 51Cr-labeled EDTA, 99mTc-labeled DTPA, and 125i-labelled iothalamate) or iohexol (krutzen *et al.*, 1984) are too cumbersome to use in the clinic settings [27].

Many researches and interventions over the past years have demonstrated outstanding results in the research of type 2diabetes and hypertension impact on the kidney. Some of these developments are believed to lead to better understanding of the disease, treatments and management of the disease. One of the newly identified early marker of kidney injury is cystatin C and decreased level of VMA. Cystatin C has been proposed as a good marker of GFR. particularly in patients with moderate to severe renal impairment. Plasma cystatin C fulfills a number of the criteria that would make it suitable as a marker of GFR, cystatin C has a low molecular weight (approximately 13.3 kilo Daltons), and it is removed from the bloodstream by glomerular filtration in the kidneys. If kidney function and glomerular filtration rate decline the blood levels of cystatin C rise. Serum levels of cystatin C are a more precise test of kidney function (as presented by the glomerular filtration rate GFR) than serum creatinine levels. This finding is based mainly on cross-sectional studies (on a single point in time). Longitudinal studies (that follow cystatin Cover time) are scarcer; some studies show promising results. cystatin C levels are less dependent on age, sex, race and muscle mass compared to creatinine. The production of cystatin C is not altered by inflammatory conditions, is not related to lean muscle mass and does not have a circadian rhythm. The function of cystatin seems to be to protect connective tissue from destruction by intracellular enzymes. it has been suggested that cystatin C might predict the risk of developing chronic kidney disease thereby signaling a state of ‘preclinical’ kidney dysfunction. Thus, a more precise and accurate study of marker of GFR as an assessment of renal function would be clinically useful in early detection and management of diabetic and hypertensive induced kidney disease.

METHODS AND MATERIALS

Study Area

The study was conducted at Federal Teaching Hospital, Owerri. This hospital is located in Owerri and is a tertiary referral center which provide adequate medical care to individuals with type 2 diabetes mellitus. They are also center for training of health personnel.

Study Population

Two hundred (200) subjects of both sexes between the ages of twenty and sixty-five years was recruited for the study. One hundred (100) type 2 diabetics and hypertensive subjects attending clinic of Federal Teaching Hospital, Owerri, for at least six months were eligible for the study. Also, fifty apparently

healthy individuals who came for check up for medical fitness served as the control subjects. They were further grouped into three:
Diabetic patients with hypertension (n = 50),
Diabetic patients without hypertension (n = 50),
Non diabetic patients with hypertension (n = 50) and
Apparently healthy subjects (n = 50).

SELECTION CRITERIA

1. Inclusion Criteria

The subjects were selected based on the criteria that

A. They are type 2 diabetes mellitus individuals who had been attending diabetic clinic for at least six months.
B. They met World Health Organization (WHO) and International Diabetics Federation (IDF) diagnostic criteria:
   - Fasting plasma glucose >126mgl/di (7.0mmol/L)
   - Two hours plasma glucose >200mg/dl (11.1 mmol/L)
C. They are within the age of twenty to sixty- five years
D. They are apparently healthy individuals who served as control subjects
E. They agreed to be given informed written consent.
F. They are hypertensive with blood pressure of greather than 140/80 mm/Hg

Exclusion Criteria

This study will exclude

A. Type 1 diabetes mellitus individuals
B. Subjects below twenty and about sixty-five years of age.
C. Pregnant women
D. Subjects who had other diseases or severely ill

Sample Collection

A standard venepuncture method was used to obtain seven milliliters (7ml) of blood from all the subjects under asceptic conditions. 3milliliters was dispensed into a plain container capped, labeled appropriately and allowed to clot at room temperature. The serum were separated from the red cell by spinning at 4,000 r.p.m for 5minutes. The supernatant serum obtained were stored frozen at -20 ^{0}C until the day of analysis. The remaining 2ml of blood was dispensed into a fluoride oxalate container for the estimation of fasting blood glucose. 5ml of urine was collected into sterile universal bottle, then aliquot into cryovials and stored at -20^{o}C till the day of assay.

Laboratory Assays

All reagents were commercially purchased and the manufacturer's standard operating procedures was strictly adhered to.

Estimation of Fasting Plasma Glucose

This method was carried out as modified by Randox Laboratories, United Kingdom. Catalog number GL 2059.

Estimation of Serum Creatinine

Serum creatinine was determined using modified Jaffe Slot method. The test kit was obtained from Sigma Company, USA. Catalog number SP226-001.

Determination of Serum cystatin C

Serum cystatin C was determined using the enzyme linked immunosorbent assay. The test kit was obtained from Calbiotech Company, USA. Catalog number CBT-0144.

Determination of urine VMA

Urine VMA level was determined using Bioassay ELISA kit from Melson company. Catalog number MLL B3005.

Estimation of GFR

The principle of GFR is based on the concept of clearance. The renal clearance of a substance is defined as the volume of plasma from which the substance is completely cleared by the kidneys per unit time. Creatinine clearance is the volume of plasma from which creatinine is completely cleared by the kidneys per unit time. The amount of creatinine c filtered at the glomerulus = GFR multiplied by plasma concentration of creatinine (GFR X Pc). The amount of c excreted equals the urine concentration (Uc) multiplied by the urinary flow rate (V; volume excreted per unit of time).
Since filtered creatinine = Excreated creatinine
GFR X Pc = Us x V
GFR= (Uc X V)/ Pc
Procedure

A patient is allowed to urinate into a given large container for a 24 hours interval. The blood and the urine creatinine concentration is determined (principle and procedure for creatinine estimation are seen in page 74 above). The volume of the urine is also ascertained.
GFR= (Uc X V)/ Pc
Where GFR is Glomerular filteration rate,
Uc is urine concentration of creatinine,
Pc is plasma concentration of creatinine,
V is urinary flow rate (volume excreted per unit of time).

Estimation of GFR using Cockcroft-Gault Equation

Cockcroft-Gault equation is often used as a method of estimating GFR (although it was developed as a method of predicting creatinine clearance) from knowledge of serum creatinine, age and weight.

Therefore creatinine clearance = (140 - age in years) x (wt in kg) / (serum creatinine in mg/dl) X 72.
For women multiply the result of calculation by 0.85.

Statistical Analysis

Statistical analysis was performed using SPSS software version 21 (California Inc.). Values were expressed as mean ± standard deviation. Values from the groups were compared using using independent sample

t-test. Pearson's correlation analysis was used to determine the inter-variable association between the various parameters. Value of P<0.05 was considered statistically significant Results was presented in tables.

RESULTS

Table 1: The mean and Standard deviation values of measured parameters in diabetic and control subjects

Parameters	Diabetic subjects (n=50) Mean ± SD	Control Subjects (n = 50) mean ±SD	t-test	P-value
Age (Years)	43.70 ± 7.86	45.95 ± 7.37	1.028	0.311
FBS (mg/dL)	209.85 ±19.95	84.20 ± 9.40	25.49	0.0001*
Diastolic Pressure (mmHg)	75.10 ± 4.57	75.40 ± 5.96	0.184	0.855
Systolic pressure (mmHg)	115.30 ± 6.14	118.30 ± 7.55	1.379	0.176
Creatinine (mg/dl)	1.05 ± 0.26	0.92 ± 0.27	1.553	0.129
Cystatin C (mg/L)	0.88 ±0.18	0.85 ± 0.16	1.835	0.074
VMA (mg/24hr)	11.26 ± 1.74	9.96 ± 1.43	2.591	0.013*
GFR (ml/min/1.73m^2	74.90 ± 15.64	80.10 ± 17.65	2.470	0.018*

Key: n: Number of subjects in each group
***:** statistically significant (P<0.05).
FBS: Fasting Blood Sugar
VMA: Vanillylmandelic acid,
GFR: Glomerular filtration Rate

Table 2: The mean and Standard deviation values of measured parameters in hypertensive and control subjects

Parameters	Hypertensive subjects (n = 50) mean ± SD	Control Subjects (n = 50) mean ±SD	t-test	P-value
Age (Years)	46.05 ± 6.57	45.95 ± 7.37	0.850	0.715
FBS (mg/dL)	84.10 ± 9.03	84.20 ± 9.40	0.034	0.973
Diastolic Pressure (mmHg)	101.80 ± 1.88	75.40 ± 5.96	11.648	0.0001*
Systolic pressure (mmHg)	167.50 ± 11.59	118.30 ± 7.55	15.907	0.0001*
Creatinine (mg/dl)	1.03 ± 0.30	0.92 ± 0.27	1.227	0.227
Cystatin C (mg/L)	0.90 ± 0.14	0.85 ± 0.16	2.506	0.017*
VMA (mg/24hr)	13.73 ± 1.65	9.96 ± 1.43	7.739	0.0001*
GFR (ml/min/1.73m^2)	85.90 ± 21.94	80.10 ± 17.65	0.304	0.763

KEY
n: Number of subjects in each group
***:** statistically significant (P<0.05).
FBS: Fasting Blood Sugar
VMA: Vanillylmandelic acid
GFR: Glomerular filtration Rate

Table 3: The mean and Standard deviation values of measured parameters in Comorbid diabetic-hypertensive and control subjects

Parameters	Diabetic and Hypertensive subjects (n=50) mean ± SD	Control Subjects (n = 50) mean ±SD	t-test	P-value
Age (Years)	47.20 ± 7.91	45.95 ± 7.37	0.500	0.620
FBS (mg/dL)	199.50 ± 25.36	84.20 ± 9.40	13.315	0.0001*
Diastolic Pressure (mmHg)	102.90 ± 6.87	75.40 ± 5.96	13.784	0.0001*
Systolic pressure (mmHg)	160.00 ± 7.36	118.30 ± 7.55	17.696	0.0001*
Creatinine (mg/dl)	1.13 ± 0.24	0.92 ± 0.27	2.592	0.013*
Cystatin C (mg/L)	0.84 ± 0.17	0.85 ± 0.16	1.061	0.095
VMA (mg/24hr)	12.48 ± 2.25	9.96 ± 1.43	4.225	0.001*
GFR (ml/min/1.73m^2)	71.80 ± 8.30	80.10 ± 17.65	3.720	0.001*

KEY
n: Number of subjects in each group
***:** statistically significant (P<0.05).
FBS: Fasting Blood Sugar
VMA: Vanillylmandelic acid
GFR: Glomerular filtration Rate

Table 4: The mean and Standard deviation values of measured parameters in Comorbid diabetic-hypertensive and diabetic subjects

Parameters	Diabetic and Hypertensive subjects (n=50) mean ± SD	Diabetic subjects (n=50) Mean ± SD	t-test	P-value
Age (Years)	47.20 ± 7.91	43.70 ± 7.86	1.404	0.168
FBS (mg/dL)	199.50 ± 25.36	209.85 ±19.95	1.435	0.160
Diastolic Pressure (mmHg)	102.90 ± 6.87	75.10 ± 4.57	15.044	0.0001*
Systolic pressure (mmHg)	160.00 ± 7.36	115.30 ± 6.14	20.864	0.0001*
Creatinine (mg/dl)	1.13 ± 0.24	1.05 ± 0.26	1.013	0.318
Cystatin C (mg/L)	0.84 ± 0.17	0.88 ±0.18	0.702	0.487
VMA (mg/24hr)	12.48 ± 2.25	11.26 ± 1.74	1.907	0.064
GFR (ml/min/1.73m^2)	71.80 ± 8.30	74.90 ± 15.64	0.783	0.438

KEY

n: Number of subjects in each group

***:** statistically significant (P<0.05).

FBS: Fasting Blood Sugar

VMA: Vanillylmandelic acid

GFR: Glomerular filtration Rate

Table 5: The mean and Standard deviation values of measured parameters in Comorbid diabetic-hypertensive and hypertensive subjects

Parameters	Diabetic and Hypertensive subjects (n=50) mean ± SD	Hypertensive subjects (n = 50) mean ± SD	t-test	P-value
Age (Years)	47.20 ± 7.91	46.05 ± 6.57	0.147	0.884
FBS (mg/dL)	199.50 ± 25.36	84.10 ± 9.03	19.173	0.0001*
Diastolic Pressure (mmHg)	102.90 ± 6.87	101.80 ± 1.88	0.454	0.653
Systolic pressure (mmHg)	160.00 ± 7.36	167.50 ± 11.59	2.443	0.019*
Creatinine (mg/dl)	1.13 ± 0.24	1.03 ± 0.30	1.173	0.248
Cystatin C (mg/L)	0.84 ± 0.17	0.90 ± 0.14	1.139	0.262
VMA (mg/24hr)	12.48 ± 2.25	13.73 ± 1.65	2.003	0.052
GFR (ml/min/1.73m^2)	71.80 ± 8.30	85.90 ± 21.94	2.688	0.011*

KEY

n: Number of subjects in each group

***:** statistically significant (P<0.05).

FBS: Fasting Blood Sugar

VMA: Vanillylmandelic acid

GFR: Glomerular filtration Rate

Table 6: Pearson Correlation of studied parameters in diabetic-hypertensive groups

Parameters	Pearson Coefficient	P-value
Creatinine vs Cystatin C	0.337	0.009*
Creatinine vs VMA	-0.164	0.0001*
Creatinine vs GFR	-0.558	0.0001*
Cystatin C vs VMA	-0.078	0.552
Cystatin C vs GFR	0.019	0.886

KEY

n: Number of subjects in each group

***:** statistically significant (P<0.05).

VMA: Vanillylmandelic acid

GFR: Glomerular filtration Rate

DISCUSSION

The recent rise in incidence and prevalence of End Stage Renal Disease has risen progressively and is very much attributable to the rapidly increasing worldwide incidence of diabetes and hypertension. This study examined the effects of hypertension and diabetes on renal function using serum creatinine, cystatin C and as markers for renal function. This was done by comparing the levels of serum creatinine, cystatin C, and GFR in patients who are hypertensive, diabetic, and hypertensive-diabetic with a control group. Glycemic control reflects the risk of nephropathy and other diabetic complications. Increase in creatinine level indicates the impairment or damage to the kidney and creatinine is also a marker of GFR. Increase in both creatinine & urea with increased blood sugar clearly indicates the damage of kidney [29].

Our study shows significant increase of serum creatinine, blood glucose and a decrease in GFR rate in diabetic-hypertensive and diabetic patients when compared with hypertensive and control subjects. This may be an indicative of pre-renal damage. This study is similar to the study of [30], as they explained the relationship of long-standing plasma glucose level with blood creatinine level. This is also similar to the study of other scholars as they found increased sugar and creatinine value in diabetic-hypertensive and diabetic patents which leads to progressive renal damage. As our study shows increased level of serum creatinine, it clearly indicates prolonged hyperglycemia which causes irretrievable damage to the nephrons of the kidney. The tiny filtering units of kidneys i.e., nephrons are damaged due to high blood sugar level [31].

As the main function of kidney is to maintain the fluid electrolyte balance, this function got impaired. Increase in serum creatinine & blood cystatin C is due to diminishing of GFR as the creatinine is an indirect measure of glomerular filtration and indicating reduced filtration capacity of the kidney [32].

Cystatin C was slightly lower in diabetic-hypertensive subjects when compared with diabetic subjects. There was also a positive correlation between creatinine and cystatin C. This is as a result of the association between insulin resistance and inflammation. It was shown that cystatin C level, independent of renal function, was associated with insulin resistance and inflammation. This may explain the association between cystatin C and cardiovascular disease in type 2 diabetes. Furthermore, there is mounting evidence that cystatin C may be a predictor of adverse outcomes independent of renal function. Higher levels of cystatin C among the hypertensive group as shown in our study have been associated with a twofold increased risk of cardiovascular events even after adjusting for well-known risk factors in addition to higher mortality in patients with acute coronary syndromes [32]. In unadjusted models, higher concentrations have been associated with the degree of endotheliosis in conditions believed to be attributable to endothelial damage. Studies have tried to explore the link between cystatin C and anthropometric measures and its influence on cardiovascular mortality, and they found that cystatin C corelated better with measures of visceral adiposity that included waist circumference and waist to hip ratio compared to BMI. It appeared to predict cardiovascular outcomes better in those with measures that do not suggest obesity than those who have abnormal anthropometric measures.

The mean (± SD) serum VMA was higher in the hypertensive-diabetic group and hypertensive group compared with diabetic group and control group. There was also a negative correlation between urine VMA and creatinine and between urine VMA and cystatin C. Vanillylmandelic acid (VMA) and 4-hydroxy-3-methoxy-mandelic acid (HMMA) are the end product of both epinephrine and norepinephrine catabolism. They are stored as inactive complex. Released catecholamines, having a short half-life, are taken up by sympathetic nerve endings, or metabolized by the liver and kidney and excreted. Catecholamines are produced in the central portion of the adrenal glands, the adrenal medulla. The primary catecholamines released are dopamine, epinephrine, and norepinephrine. These hormones are released into the bloodstream in response to physical or emotional stress as seen in diabetics and hypertensive patients. Norepinephrine also constricts blood vessels, increasing blood pressure, and epinephrine increases heart rate and metabolism. After completing their actions, the catecholamines are metabolized to inactive compounds. Dopamine becomes homovanillic acid (HVA), norepinephrine breaks down into normetanephrine and VMA, and epinephrine becomes metanephrine and VMA. Both the hormones and their metabolites are eliminated from the body in the urine. Moreover, increased cathecoleamines which causes hypertension inturn also causes diabetes mellitus by stimulating gluconeogenesis and glycogenolysis [33].

CONCLUSION

Good control of blood glucose and blood pressure level reduces the likelihood of the development of renal impairment which is usually associated with both diabetes and hypertension. Co-morbidity of diabetes and hypertension poses a highe rrisk of developing renal disease. Both serum creatinine, cystatic C and GFR are important biomarkers for renal impairment hence should be monitored on a regular basis for diabetic and hypertensive patients and much more frequently for hypertensive-diabetic patients. Both serum creatinine levels increase significantly in hypertensive and diabetic patients with a marked increase in the co-morbid state hence should be monitored more frequently to ensure early detection of renal impairment.

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