ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 4, Issue – 1, January - February - 2021, Page No. : 01 – 07

Renal Parameters in Diabetes with Different Age Groups

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How to citation this article: B. Priya, R. Selvam, B. Gopalakrishnan, D. Saravanan, "Phenotypic analysis of female reproductive tract disease of cattles", IJMACR- January - February - 2021, Vol – 4, Issue -1, P. No. 01 – 07.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Diabetes mellitus is a common cause of pyelonephritis. Glucose in the bloodstream stimulates the pancreas to produce insulin. Insulin helps glucose to move from the blood into the cells. Once inside the cells, glucose is converted to energy, which is used immediately, or the glucose is stored as fat or the starch glycogen until it is needed. Diabetic nephropathy affects your kidneys' ability to do their usual work of removing waste products and extra fluid from your body. The best way to prevent or delay diabetic nephropathy is by maintaining a healthy lifestyle and treating your diabetes and high blood pressure. Chronic kidney disease is a collective clinical problem in aging patients and is linked with increased morbidity and mortality. The exact reason this occurs in people with diabetes is unknown, but high blood sugar levels and high blood pressure are thought to contribute to diabetic nephropathy. Persistently high blood sugar or blood pressure levels are two things that can damage your kidneys, making them unable to filter waste and remove water from your body. Understanding the link between kidney and visual disease can lead to the development of new treatment and screening strategies for diabetes.

Keywords: Diabetes mellitus, Glucose, Insulin, Nephropathy, Chronic kidney disease.

Introduction

Diabetes mellitus has become a major health catastrophe of great magnitude and a major public health concern. Studies have demonstrated that diabetes affects upto 10% of the total population in several countries. This rate may be doubled, if those with impaired glucose tolerance (IGT) are also considered (**Gillies, 2007**). The manifestations of diabetes mellitus can cause extensive human sufferings and a huge economic burden. The increase in the number of people with diabetes in association with a longer duration of diabetes alters the disease profile in many

populations in the world, which is due to a higher incidence of diabetes-specific complications such as retinopathy, kidney failure and peripheral arterial disease (**Harding**, **2018**). Despite the high prevalence of diabetes and its secondary complications, the availability of successful prevention strategies, essential health care requirements and facilities for self-care are often inadequate. Attention is required at all levels of health care to improve health care delivery systems to people with diabetes mellitus (**Lawrence, 2012**).

Diabetes mellitus is a multifactorial, multisystemic endocrine disorder characterized by persistent hyperglycemia resulting from the defects in insulin secretion in the β-cells of pancreas and desensitization of insulin receptors for insulin. The chronic elevation in the levels of fasting and postprandial blood glucose levels is linked with the dysfunction and consequent failure of vital organs such as eyes, kidneys, heart and blood vessels. Several pathogenic processes have been involved in the development and progression of diabetes and its secondary complications. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency (**American Diabetes Association, 2018**).

Diabetes presents a large social, financial and health system burden across the world. It was reported that in 2017, there were about 451 million (age 18-99 years) people with diabetes worldwide. These figures were expected to increase to 693 million by 2045. It was speculated that almost half of all people (49.7%) living with diabetes are undiagnosed. Moreover, there were an estimated 374 million people with impaired glucose tolerance (IGT) and it was projected that almost 21.3 million live births to women were affected by some form of hyperglycemia in pregnancy (**Guariguata et al., 2014; Nanditha et al., 2016**).

In both type 1 and type 2 diabetes, various genetic and environmental factors can result in the progressive loss of β-cell mass and/or function that manifests clinically as hyperglycemia. As the hyperglycemia predominates, individuals with all forms of diabetes are at risk for developing secondary complications, although rates of progression may differ. Thus, the identification of individualized therapies for the treatment of diabetes will require better characterization of many pathways leading to β-cell dysfunction (**Butler et al., 2003; Tomita, 2010; Skyler et al., 2017**).

Materials and Methodology

Subjects and Sample Collection: 90 volunteers of whom 45 of them are diabetes and 45 of them are normal persons with different age group as 20 - 40, 40 – 60, and above 60. In each group with 15 members are taken for study. A study of renal function test between the normal persons and diabetes patients is done. The different age group are assigned as 20 – 40 as A, 40 – 60 as B and above 60 as C throughout the report.

Group Number	Different Age Group	Number Of Samples
Group A	A* [Normal]	15
	A** [Diabetes]	15
Group B	B* [Normal]	15
	B** [Diabetes]	15
Group C	C* [Normal]	15
	C** [Diabetes]	15

Collection of Blood: Sample for test requiring large amount of blood are obtained by vein puncture. All materials that touch the patients in the process of obtaining a blood specimen must be sterile. This means we should use sterile needles, cotton gauze and alcohol swabs. The skin must be thoroughly cleaned with some antiseptic e.g., 70% alcohol. After the skin area has been cleaned the operator must not touch the area again.

Vein Puncture: Large quantities of blood require vein puncture. The blood is usually obtained from the anticubital vein which is congested by means of tourniquet placed on the upper arm and tightened to a pressure that is just sufficient to prevent the venous return. The tourniquet is removed after the blood is collected.

Preparation of Serum: The freshly drawn blood obtained by vein puncture is placed directly into the test tube without anticoagulant. The blood is allowed to clot at room temperature and the clot is separated from the inner surface of the tube, centrifuged immediately and the supernatant obtained is serum which is removed with a rubber bulb pipette and is used for estimation.

Estimation of Glucose by Ortho Toluidine Method: Glucose in the blood was estimated by O – Toluidine method by Sasaki et al., (1972). In a series of clean and dry test tubes, labeled as S1 – S5, 0.2 to 1.0ml of working standard glucose solution was pippeted out in the concentration range of 20 – 100 microgram. 0.2ml of serum supernatant was pipetted out in test tube labeled as "T". The contents in all the tubes were made up to 1ml with distilled water. 1Ml distilled water alone serves as blank. 5Ml of o-toluidine reagent was added to all the test tubes and mixed well, closed with marble and kept in boiling water bath for 10min. The contents were cooled and bluish green colour developed was read at 640nm using red filter.

Estimation of Renal Function Test

Estimation of Urea by Diacetyl Monoxime Method: The major constituents of renal function are urea, creatinine, and micro albumin. These are the parameters to analyse the renal function test of kidney.

Principle: When urea is heated with a substance such as diacetyl monoxime, which contains two adjacent carbonyl groups, in an acid medium a yellow colour is developed, its intensity is read at 480 nm by using blue filter. A series of clean and dry test tubes were labeled as S1-S5 and 0.2 – 1.0 ml of working standard urea solution with a concentration range of 10 – 50 micro grams was pipetted out. 1.0 ml of serum supernatant was taken in a separate tube labeled as T. The volume in all the tubes was made up to 6.0 ml with distilled water. 6ml of distilled water alone serves as bland B. 0.8ml of diacetyl monoxime was added to all the tubes followed by 3.2 ml of acid mixture. The tubes were heated in a boiling water bath for 30 minutes. The tubes were cooled and the intensity of orange yellow colour developed was read at 480 nm.

Estimation of Creatinine by Jaffe's Method: Creatinine is estimated by using Jaffe's method while using jaffe's reaction in which creatinine gives an orange yellow colour with alkaline picrate solution. The intensity at which is measured at 540 nm. Creatinine is an anhydrate form of creatine. When creatine is heated with an acid with a molecule of water gives creatinine. In a series of clean and dry test tubes labeled as S1 – S5, 0.4 – 2.0ml of working standard creatinine solution was pipette out, with the concentration range of 16 – 80 micro gram of creatinine was pipetted out. 2.0 ml of serum supernatant was taken in a tube as labeled as T. The volume in all the tubes was made up to 8 ml with distilled water. 8 ml of distilled water alone served as blank B. 1.0 ml of 1% picric acid was added. Then, 1.0 ml of 10% sodium hydroxide were added to all the tubes and the tubes were incubated at room temperature for 15 minutes. The orange colour was developed whose intensity was read at 540 nm.

Estimation of Plasma Proteins And A/G Rati: This is the most commonly used method and is based on the fact that the peptide group of the protein forms a purple coloured complex with copper ion in alkaline medium. Since all proteins contains peptide bond, the protein is sparcely, contains specific there is little interference from other compounds. Some substance like urea interference

as it possess peptide bond other interfering materials are reducing sugar like glucose which interact with cuprite ion in the reagent. Globulin is precipitated from plasma by adding sodium sulphate sodium sulphate mixture which selectively precipitate the globulin from plasma protein and peptide react with alkaline copper tartarate solution to give a blue colour. The intensity of the blue colour developed was read at 540nm.

In a series of the tubes has 0.4 – 2.0, ml of standard protein. The corresponding range of standard protein as 0.9 – 4.0 mg was pipette out. 2.0 ml of globulin precipitated filtrate was taken in a tube TA. 0.1 ml of plasma was taken in a tube and 6.0 ml of saline was added to it. This tube was shaken well, 2.0 of this diluted plasma were taken in a tube labeled as TT. The volumes in all the tubes were made up to 2.5 ml with distilled water. 2.5 ml of distilled water alone served as blank. 2.0 ml of biuret reagent was added to all the tubes. The tubes were allowed to stand for 15 minutes at 37°c. The intensity of blue colour developed was read at 540 nm.

Statistical AnalysiS

All the experiments were conducted for number of samples indicated in the parenthesis and the values were expressed as mean ± SD, the modified student “t” tests were used to compare the mean of two groups.

Result and Discussion

Diabetes mellitus is a metabolic disease that causes high blood sugar. The hormone insulin changes sugar from the blood into our cells to be stored or used for energy. With diabetes, our body either doesn't make enough insulin production or can't effectively use the insulin it does make.

Table 1 indicates the level of glucose in blood among various age groups as shown. The age groups are 20 – 40, 40 – 60 and above 60. The increasing the levels of blood sugar as a marked for certain complications such as heart disease, diabetic neuropathy, nephropathy and diabetic retinopathy.

Table 1: The Level of Blood Glucose in Different Age Groups

Age Group	A (Age :20-40)	B(Age:40-60)	C(Above 60)
Normal	99±9.6	97.6±10	100±10
Diabetes	163±22.6	203.5±36.6	272.3±8.9

Table 2 depicts the level of urea, creatinine and micro-albumin on normal (group A*) and diabetes (group A**). From the age group of 20 – 40 we compared with normal all the renal function parameters shows a marked increase ($p<0.001$). The increased levels of urea and microalbumin as showed a marker for renal disorder, tubular dysfunction and glomerular nephritis.

Table 2: The Level of renal parameters in different age groups (20-40)

Age Groups	Urea	Creatinine	Micro Albumin
Group A*	17±2.4	0.7±0.25	24.9±3.6
Group A**	33.9±40.4	1.3±0.4	34.7±3.9

Table 3 shows the level of urea, creatinine and microalbumin on normal (group B*) and diabetes (group B**) under the age group of 40 – 60, when compared with normal all the renal function parameters shows a marked increase ($p < 0.001$). Increased renal function parameters are a marker for kidney failure due to renal diseases and also tubular dysfunction and microalbuminuria.

Table 3: The Level of renal parameters in different age groups (40-60)

Age Group	Urea	Creatinine	Micro Albumin
Group B*	22.1±3.2	0.7±0.24	26.2±3.1
Groupb**	38.2±5.7	1.4±0.4	38.2±7.1

Table 4 depicts the level of urea, creatinine and microalbumin on normal (group C*) and diabetes (GROUP C**) under the age group of 60 above, when compared with normal all the renal function parameters shows a marked increase ($p < 0.001$). Increased renal function parameters are marked for severe kidney failure due to tubular dysfunction damages, glomerular nephritis. So the above 60 age group people are severe in kidney disease.

Table 4: The Level of renal parameters in different age groups (above 60)

Age Group	Urea	Creatinine	Microalbumin
Group C*	25.7±2.5	0.6±0.23	27.8±1.6
Group C**	37.9±5.7	1.5±0.4	39.6±5.4

All the above graphs shows the different age group of individuals between 20 – 40, 40 – 60 and above 60 years and the group of people with blood sugar and renal parameters in normal and diabetic persons.

Diabetic nephropathy accounts for 6% of deaths in diabetic patients. Proteinuria is found around 5-10% of diabetes patients. It is the first sign, appearing around 20 years of age after the onset of adult type diabetes mellitus and appears around 14-15 years in the juvenile type. The adrenal disease may progress to Nephrotic syndrome, but it is relatively infrequent to terminal renal failure, as most patients will die of vascular disease. The appearance of proteinuria is a poor prognotic sign for diabetes mellitus (**Shahbazian, 2013**).

The kidneys are destroyed by combination of glomerulosclerosis, which may be diffuse or nodular (the kimmelstiel-wilson lesion) and vascular disease (**Remuzzi, 2002**). There is a good correlation between the development of nephropathy and diabetic retinopathy. Retinal changes precede proteinuria for several years and are invariably present in patient with proteinuria. Not all patients with retinopathy have proteinuria (**Bello, 2011**).

Diabetes mellitus may also cause renal damage leading to papillary necrosis, pyelonephritis, and acute renal failure due to fluid and electrolyte loss in diabetics' ketosis, neurogenic bladder dysfunction. (**Li,2011**)

Diabetes for many centuries was regarded as a disease of the kidneys this was the opinion of Aretaeus, the Cappadocia in the second century AD and this was still held by Erasmus Darwin in 1801. The presence of proteinuria in diabetes has been known by him and he described the association of dropsy with diabetes vacuolization of the tubular epithelium was observed by Armani (1875) and Ebstein (1881) and showed that this was due to glycogen infiltration. Kimmelstiel and Wilson (1936) were the first to attribute specific glomerular lesions to diabetes. (**Vallon., 2012, Yamamoto., 1993, Chiarelli.,2009**)

During recent years it has become clear that striking changes in the renal function are present very early in the course of diabetes, even at the diagnosis stage. These stages include an increase in glomerular filtration rate (GFR), renal plasma flow (RPF), filtration fraction (FF), glomerular permeability to macro molecules and the tubular maximal capacity for glucose absorption (TMG). The changes in GFR cannot be entirely explained by the increased glucose load which the diabetic patients handle. Because fasting serum growth hormone concentration was elevated in the newly diagnosed diabetics and it fell in parallel with GFR. Hansen and Monessen (1972) have suggested that change in renal function may be mediated by three to four fold increases in this hormone, both in the fasting state and in relation to exercise and poor diabetic control. (**Raats, 2000., Stout,1994., Stamler,1993., Molitch,2010**)

Fractionation and analysis of the glomeruli from human diabetic kidney has shown that glomeruli are larger and heavier and contain increased quantities of hydroxyl proline associated with basement membrane collagen. Micro albuminuria detected by a very sensitive radioimmunoassay technique is also increased during the stress of excess in diabetic of short duration when compared with non diabetics. It is increased to some extent in mild diabetes and those of long duration but without clinical proteinuria being diagnosed. It can be decreased by accurate control of the blood glucose. (**Subramanian, 2011., Tsujimoto.,2014., Elsayed., 2008.)**

The significance of these alterations of renal function early in diabetic is unknown. The functional importance of the universal basement membrane thickening occurring in the long term diabetic is also unknown.

Diabetes mellitus is an important disorder of the carbohydrate metabolism. It is hereditary. The people under the age group of 20 – 42 have no severe diabetes. In the middle age group 40-60; people are with moderate increase in glucose level and also in renal parameter, so they are less prone to renal disorders. Obesity is the common problem that is seen in the age group of 40-60, these people are more prone to severe diabetes, renal disorder and kidney failure.

The prevalence of persons under this age group has severe renal parameters and also in glucose, so the risk factor is seen in renal parameters. Under ageing processes people above 60 have severe diabetes and are more prone to renal disorders, tubular dysfunction, and glomerular nephritis.

Conclusion

The principle of management of hypertension in the diabetic patient resembles those in a hypertensive individual without diabetes. These are additional problems and hence different classes of antihypertensive drugs are considered in diabetic patients. Individuals with both hypertensive and diabetes are at high risk for both vascular and renal diseases.

Acknowledgements: The authors grateful to The Principal & Head of the Department of Biochemistry, DG Vaishnav College, Chennai – 106 for providing the laboratory facilities to carry out this work.

Author's Contributions: All the Authors have equal contributions in designing, executing and preparation of manuscript.

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