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Research Article

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Biochemical screening of diabetic nephropathy patients in Mani Hospital, Thiruthuraipoondi

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ABSTRACT

Diabetic Nephropathy is a metabolic disorder associated with the Diabetes mellitus. People suffering from diabetes are prone to nephropathy conditions. The present aim of our study is to find out the biochemical parameters such as Glucose, Urea, Creatinine, Uric acid and Ketone bodies. These parameters were analyzed in Sulphonyl Urea, Biguanide treated patients. From this study it was concluded that the above hypoglycemic agents drugs shows the maximum effect in restoring these patients to normal condition.

Key words: Biguanide, Creatinine, Ketone bodies, Sulfonyl urea, Urea, Uric acid

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INTRODUCTION

Diabetes mellitus is a clinical syndrome caused by an absolute or relative deficiency of insulin. Diabetic mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and insulin action. The chronic hypoglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs especially the eyes , kidneys, nerves , heart, and blood vessels [1].Overall kidney damage rarely occurs in the first 10 years of diabetes, and usually 15 to 25 years will pass before kidney failure occurs [2].

Classification

Diabetes Association as well as the WHO have recommended altering the classification to define four main subtypes of diabetes mellitus [3].

Type I (Insulin dependent diabetes mellitus (IDDM), Juvenile onset diabetes) [4].

Type II Non insulin dependent diabetes mellitus (NIDDM), Adult onset of diabetes[5].

Type III include genetic defects of beta cell function and insulin action as well as disease of the exocrine pancreas [6].

Type IV diabetes mellitus is gestational diabetes [7].

Diabetic nephropathy
Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Nephropathy is the leading cause of chronic renal failure. Diabetic nephropathy takes many years to develop. In some people, the filtering function of the kidneys is actually higher than normal in the first few years of their diabetes [8].

Diabetic nephropathy is associated with proteinuria, nephrotic syndrome, progressive renal failure and hypertension [9].

Diabetic complications
- Hypertension
- Poor metabolic control
- Smoking
- Obesity
- Hyperlipidemia. [10].

Urea
Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys urea is filtered out of blood by glomerulli and is partially being reabsorbed with water [11].

Blood urea nitrogen (BUN)
Blood tests for Blood urea nitrogen (BUN) and creatinine are the simplest way to monitor kidney function. These substances are normal metabolic waste products that are excreted by the kidneys. Urea is a byproduct of protein breakdown. A blood test can be done to measure the amount of urea nitrogen in the blood. In kidney disease, these substances are not excreted normally, and so they accumulate in the body thus causing an increase in blood levels of urea.

The normal level of Blood Urea Nitrogen is 7-20 mg/dL [12].

Creatinine
Creatinine is a breakdown product of creatinine phosphate in muscle, and is usually produced at a constant rate by the body depending on muscle mass [7].Creatinine is a commonly used as a measure of kidney function. The normal creatinine clearance test valve is 110-150ml/min in male and in female it is 100-130ml/min [13].

The normal level of creatinine in males - 0.8 to 1.4 mg/dL.
Normal value of creatinine in females-0.6 to 1.2mg/dl [14].

Uric acid
Uric acid is the primary end product of purine metabolism and excreted in the urine. It is derived from purines arising from the catabolism of dietary and endogenous nucleic acid increased catabolism dysfunction of one of the shunt pathways which leads to increased urate production [15].Normally uric acid present in blood in concentration range 0.15- 0.45 mmol/ L and excreted in urine in 1.19-2.98 mmol/ day [16]

Ketone bodies
ketone bodies are acetone, acetoacetic acid, and beta-hydroxybutyric acid . Ketone bodies are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney [17].This process termed ketogenesis occurs in liver. The ketone bodies are utilized as fuel by extrahepatic tissues such as the heart and kidney [18].

Diabetic Ketoacidosis
The condition may be caused by cessation of insulin therapy infection, Vomiting, emotional stress, trauma and myocardial infarction [19].

Chronic kidney disease:
Chronic kidney disease (CKD) is one of the major public health problems [20].In the United States there are approximately twenty six million adults having non-dialysis dependent kidney disease [21].

EXPERIMENTAL SECTION

In the present study blood samples were collected from 40 normal patients and 60 diabetic patients from Mani hospital, Thiruthuraipoondi, Thiruvarur(D.t),Tamil nadu.

Sample Collection

A total of 100 blood samples were collected from hyperglycemic patients from Mani hospital, Thiruthuraipoondi, Thiruvarur (D.T). Five milliliters (5ml) of venous blood was collected at 8.00 hour every morning after overnight fast. The blood was dispensed into plane dry glass test tubes. Serums were isolated by centrifuging in a laboratory centrifuge at 2000g for 3 minutes immediately after blood clotting and retraction at room temperature. The serums were refrigerated at 4^{o}c.

Estimation of glucose

The glucose was estimated by Glucose Oxidase method [22].To a series of aliquots, added sample ,standard ,enzyme to blank, standard with enzyme amount as 1.5 ml respectively. Mix well incubates at 37 C for 10 minutes at room temperature (15-30 C)for 30 minutes. Now add 1.5 ml of distilled water in each tube respectively.

Estimation of Urea:

The urea was estimated by Diacetyl Monoxime method [23].To a series of test tubes added 2.5 ml of working standard , 0.01 ml of standard was added to standard tubes.0.25 ml of diacetyl monoxime reagent was added to blank and standard. Mix well and keep the tubes in the boiling water exactly for 10 minutes. Cool immediately under running water for 5 minutes and measure the colour intensity within 10 minutes.

Estimation of creatinine:

The creatinine was estimated by Jaffe ‘s method [24].To a set of test tubes 100µl of standard was taken in standard test tube.100 µl of sample was taken as test. Mix well and read initial absorbance (A1) 20 Second after mixing and final absorbance (A2) 80 seconds after mixing.

Estimation of uric acid:

The uric acid was estimated by Phosphotungstic acid method [25].To a set of aliquotes added 1ml of working to blank, Standard, test respectively.0.025ml of test was added to sample alone.0.025 ml of standard was added to standard tubes Mix well and placed in boling water bath for 30 minutes. The colour developed was read at 660nm.

Estimation of ketone bodies:

The ketone bodies was estimated by Rothers method[26].Saturated 5ml of urine with ammonium sulphate crystals added to 5 drops of freshly prepared 3% sodium nitroprusside and 10 drops of conc. Nitric acid and pink colour was developed.

RESULTS

The present study was carried out to analyse the various biochemical parameters in normal patients and diabetic patients. Totally 100 patients were selected for this study. 60 diabetes patients and 40 normal patients were studied.

EXPERIMENTAL DESIGN:

Group I : Patients of age 25-40 years
Group I: Patients of age 40-50 years
Group I: Patients of age 50-60 years
Group I: Patients of age 60-70 years

The table 1 , shows the levels of serum glucose of normal and different aged diabetic persons. The level of glucose of fasting and post prandial in diabetes and diabetes treated with standard drug

S. NO	GROUP	FBS(mg/dl)	PPBS(mg/dl)	Glucose normal values
1	Group I	112± 2.23	1.72±2.82	80-120mg/dl
2	Group II	157± 5.91	2.30±2.82	
3	Group III	204±6.48	2.50±3.60	
4	Group IV	207±9.11	2.77±7.07	

The table 2 , shows the level of urea and creatinine in normal patients and diabetic patients treated with standard drug

S.NO	GROUP	UREA (mg/ dl)	NORMAL VALUES OF UREA	CREATININE (mg/dl)
1	Group I	30.1± 3.200	15-45 mg/ dl	2.6±8.685
2	Group II	33.6± 2.126		2.8±0.583
3	Group III	50.6± o.827		3.46±0.694
4	Group IV	58.9±1.195		4.1± 0.701

The table 3 , shows the levels of plasma uric acid in normal and different used diabetic patients

S.NO	GROUP	URICACID (mg/ dl)	NORMAL LEVEL OF URIC ACID
1	Group I	5.18± 0.8	3.0-7.0 mg/dl
2	Group II	5.23± 0.3	
3	Group III	5.48± 1.2	
4	Group IV	5.33±1.0	

The table 4 , shows the level of urine ketone bodies (Acetoacetate) in normal and different used diabetic patients

S.NO	GROUP	ACETOACTATE	BETA HYDROXY BUTRIC ACID	ACETONE
1	Group I	0.2±0.141	6±2.449	9±1.414
2	Group II	1.2±1.392	8±2.228	14±2.828
3	Group III	2±1.414	9± 3	17±2
4	Group IV	7±3.605	50±7.07	23.3±7.21

CONCLUSION

In table I, reveals about the level f blood glucose in in diabetic and non diabetic patients. Blood glucose was normal in group I,Where as group 1I, group III, group IV shows increased levels of blood glucose. This shows that blood glucose is directly correlated to diabetes.

In table II , Shows about the level of plasma urea and creatinine
In table III , Shows about the level of uric acid
In table IV , Shows about the level of ketone bodies

In sulfonylurea treated diabetic patients sugar level decreased than without treatment diabetic patients. Sulfonylureas cause hypoglycaemia by stimulating insulin release from pancreatic β- cell plasma membrane, causing closure of adenosine triphosphate (ATP) sensitive potassium channels, leading to depolarization of the cell membrane.

The term biguanide refers to a group of oral type 2 diabetes drugs that work by preventing the production of glucose in the liver, improving the body's sensitivity towards insulin and reducing the amount of sugar absorbed by the intestine. Metformin is the only biguanide available on the market. The only available biguanide medication is metformin, which is commonly used as a first-line treatment for type 2 diabetes.Type2 diabetics who are unable to control their blood sugars through diet and exercise [27].

Uric acid was used as a marker of renal damage but recent observational studies have raised the possibility that uric acid may have a contributory role in the development of CKD [28].The role of uric acid as an independent risk factor for the development of new-onset CKD has been supported by several studies.

Diabetic ketoacidosis arises because of a lack of insulin in the body. The lack of insulin and corresponding elevation of glucagon leads to increased release of glucose by the liver from glycogen glycogenolysis and also through gluconeogenesis. High glucose levels spill over into the urine, taking water and solutes along with it in a process known as osmotic diuresis. This leads to polyuria, dehydration, and compensatory thirst and polydipsia. The absence of insulin also leads to the release of free fatty acids from adipose tissue (lipolysis), which are converted, again in the liver, into ketone bodies (acetoacetate and β-hydroxybutyrate). β-Hydroxybutyrate can serve as an energy source in the absence of insulin-mediated glucose delivery, and is a protective mechanism in case of starvation [29].

In our study, various biochemical parameter were investigated is increasing rapidly in most parts of the world. Diabetic patients showed increased level of fasting and post prandial glucose, urea, creatinine, uric acid, ketone bodies than the normal healthy patients.
High creatinine levels observed in diabetic patients may be due to impaired function of the nephrons. High urea level in diabetes mellitus patients could be attributed to a fall in the filtering capacity of the kidney thus leading to accumulation of waste products within the system[30].

Metformin is generally suitable for most people with type 2 diabetes as a first line of medication if lifestyle changes have no sufficiently lowered blood glucose levels by reducing the liver's blood glucose raising effect, metformin helps to lower blood glucose levels through the day. Rather than stimulating the release of insulin, metformin increases the body's sensitivity to insulin and therefore has benefits for weight management. The detailed study of this project will be carried out in future.

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