

1. Introduction and literature review

1.1. Glucose

Glucose is primary energy source for the human .glucose is derived from the diet, through the digestion of dietary carbohydrate from body stores (glycogen) and from the endogenous synthesis of glucose from non carbohydrate sources (amino acid, glycerol, lactate).Metabolism of carbohydrate begins in the mouth, where salivary amylase hydrolyze starch to form the disaccharide maltose products (dextrin). pancreatic amylase complete digestion of intermediate produce maltose .In intestinal mucosa ,maltose and any ingested disaccharides (lactose ,sucrose) are hydrolyzed by enzymes to form monosaccharides (glucose, galactose, fructose). The monosacchrides are absorbed into the blood stream and transported the liver via the portal circulation .liver enzymes covert the non glucose monosaccharides to glucose ,after that glucose reacted with adenosine triphosphate (ATP) forming glucose-6-phosphate(G6P).Further glucose metabolism proceeds according to the body requirements and may follow three possible pathway :immediate energy production (to ATP)by complete conversion to CO₂ and water ;storage as glycogen in the liver or as triglyceride in adipose tissue or conversion to ketoacid, amino acid ,or protein.⁽¹⁾

1.1.1. Regulation of blood glucose concentration

Various terms are used to describe general processes in carbohydrate metabolism, glycolysis: refer to anaerobic conversion of glucose to pyruvate or lactate and glycogenesis: process of glycogen formation from glucose and glycogenolysis: break down of glycogen to form glucose and

gluconeogenesis: formation of glucose from non carbohydrate source(amino acid, lactate ,glycerol)and tricarboxylic acid (TCA):cycle and electron transport system .Aerobic phase of glucose metabolism with in mitochondria of the cell and hexose monophosphate pathway(HMP): alternate pathway for glucose oxidation.⁽¹⁾

1.1.2. Hormones that effects blood glucose concentration

Control of blood glucose is under tow major hormones: insulin and glucagon.

- I. Insulin: is the primary hormone responsible for the entry of glucose in to the cell .Synthesized by the β -cells of islets of langerhans in the pancreas .Insulin is the only hormone that decrease glucose levels by increase the transport entry of glucose in muscle and adipose tissue by way of nonspecific receptor .It also regulates glucose by increasing glycogenesis, lipogenesis and glycolysis and inhibiting glycogenolysis. So referred to as hypoglycemic agent.⁽²⁾
- II. Glucagon: is the primary hormone responsible for increasing glucose level .It is synthesized by the α -cells of islets of langerhans in the pancreas and released during stress and fasting states glucagon act by increasing plasma glucose levels by glycogenolysis in the liver and increase in gluconeogenesis . It can be referred to as a hyperglycemic agent.⁽²⁾
- III. Epinephrine: produced by the adrenal medulla released during times of stress .Increases plasma glucose by inhibiting insulin secretion, increasing glycogenolysis and promoting lipolysis⁽²⁾ .

- IV. Cortisol: are released from the adrenal cortex on stimulation by adrenocorticotrophic hormone (ACTH). Cortisol increase plasma glucose by decreasing intestinal entry into the cell and increase gluconeogenesis liver glycogen, and lipolysis.⁽²⁾
- V. Growth hormone: increases plasma glucose into the cells and increase glycolysis.⁽²⁾
- VI. Thyroxine: that increase plasma glucose level by increase glycogenolysis, gluconeogenesis and intestinal absorption of glucose.⁽²⁾
- VII. Somatostatin: produced by the δ cells of islets of langerhans of the pancreas, increase plasma glucose levels by inhibiting of insulin ,glucagons ,growth hormone and other endocrine hormones.⁽²⁾
- VIII. Counter regulatory hormones :serve as substrate provides enhancing breakdown of glycogen to glucose changes in blood glucose as well as of other stimuli will trigger the appropriate hormone response.⁽³⁾
- IX. Human placental lactogen (hpl):also know as a human chorionic stomatomammatotrophin (hcs) is a polypeptides with anti insulin in activity that is secreted by the placenta.⁽³⁾
- X. Somatomedins :are the family of small peptide hormone that mediate the growth promatine action of human growth on the skeleton and other tissue they also have non suppressible insulin like activity NSILA somatomedin a somatomedin C and other IGFSC insulin like growth factors have been isolate.

Pancreatic polypeptide: found in pp cells located chiefly in islets in the posterior portion of the head of the pancreas.⁽³⁾

1.2. Diabetes mellitus

Is actually a group of metabolic disease characterized by hyperglycemia resulting from defect in insulin secretion, action or both.

1.2.1. Classification of diabetes mellitus

1.2.1.1. Type one diabetes mellitus

Is a result of cellular-mediated autoimmune destruction of the β -cells of the pancreas, causing an absolute deficiency of insulin secretion .Type one constitutes only 10%to20% of all cases of diabetes and commonly occurs in childhood and adolescence .This disease is usually initiated by an environmental factor or infection in individuals with a genetic predisposition .Characterized of type one diabetes include abrupt onset ,insulin dependence and ketosis tendency.⁽²⁾

Treatment of the patient is dependent on exogenous insulin administration sustain life and prevent diabetic ketoacidosis.⁽⁴⁾

1.2.1.2. Type tow diabetes

Characterized by hyperglycemia as a result of an individual resistance to insulin with an insulin secretory defect .This resistance result in a relative not an absolute insulin deficiency, type tow constitutes the majority of the diabetes cases .Most patients in this type are obese or have an increase body fat, risk increase with an increase in age and lack of physical exercise .Adult onset with ketoacidosis seldom occurring.⁽²⁾

Treatment: patient may be controlled by diet, exercise, oral hypoglycemic agent or may require insulin administration.⁽⁴⁾

1.2.1.3. Other specific types of diabetes

Are associated with certain conditions including genetic defects of β -cell function or insulin action ,pancreatic disease, disease of endocrine origin ,drug or chemical induced insulin receptor or abnormalities and certain genetic syndromes.⁽²⁾

1.2.1.4. Gestational diabetes mellitus

Any degree of glucose intolerance recognition during pregnancy .Causes include metabolic and hormonal changes, this disease is associated with increase perinatal complication and an increase risk for the development of diabetes in later years.⁽²⁾

1.2.2. Acute metabolic complication of diabetes mellitus

1.2.2.1. Diabetic ketoacidosis

Is more severe form of metabolic complication .It may be precipitated by infection or by vomiting .In the absence of insulin there is increased lipid and protein break down enhanced hepatic glyconeogenesis and impaired glucose entry in to cells.

Clinical consequences of diabetic ketoacidosis are due to:

- Hyperglycaemia causing plasma hyperosmolality
- Metabolic acidosis
- Glucoseuria

Plasma glucose concentration is usually in the range 20 to 40 mmol/l but may be considerably higher.

Hyperglycaemia causes glycosuria and hence an osmotic diuresis. Water and electrolyte loss due to vomiting, common in this syndrome increases fluid depletion. There may be haemoconcentration and reduction of the glomerular filtration rate enough to cause uremia due to renal circulatory insufficiency. The extracellular hyperosmolality causes a shift of water out of the cellular compartment and severe cellular dehydration occurs. Loss of water from cerebral cells is probably the reason for the confusion and coma. Thus, there is both cellular and extracellular volume depletion.

The rate of lipolysis is increased because of decreased insulin activity more free fatty acids are produced than can be metabolized by peripheral tissue. They are either converted to ketones by liver or of less immediate clinical importance, incorporated as endogenous triglycerides into VLDL, sometimes causing hyperlipidaemia.

Hydrogen ions produced with ketones other than acetone, are buffered by plasma bicarbonate. When their rate of production exceeds the rate of bicarbonate generation the plasma bicarbonate falls. Hydrogen ion secretion causes a fall in urinary pH.

The deep sighing respiration (Kussmaul respiration) and the odor of acetone on the breath are classical features of diabetic ketoacidosis.

Plasma potassium concentration may be raised, secondarily to the metabolic acidosis before treatment is started because of failure of glucose entry into cells in the absence of insulin and because of low

GFR so cause hyperkalaemia. There is a total body deficit due to increased urinary potassium loss in the presence of an osmotic diuresis. During treatment plasma potassium concentration may fall as potassium re-enters cells, sometime causing sever hypokalaemia.

Plasma sodium concentration may be low or low normal at presentation, partly because of the osmotic effect of the high extracellular glucose concentration which draws from the cells and dilutes the sodium. In the presence of very high plasma glucose concentration, a normal or raised plasma sodium concentration is suggestive of significant water depletion.

If there is sever hyperlipidaemia the possibility of pseudohyponatraemia must be considered. If plasma sodium concentrations rise rapidly the patient may remain confused or even comatose as long as the plasma osmolality remains significantly rise, despite a satisfactory fall in plasma glucose concentration. This may also occur if is osmolar or stronger saline solutions are given in appropriately.

Associated biochemical findings may include:-

- Hypophosphataemia as plasma phosphate concentration parallel those of potassium.
- Plasma and urinary amylase activities may be markedly elevated and in the presence of abdominal pain mimicking an acute abdomen do not necessarily indicate acute pancreatitis.⁽⁴⁾

1.2.2.2. Hyperosmolal non-ketotic coma

Hypesosmolal coma or precoma is usually confined to a condition in which there is marked hyperglycaemia but not detectable ketoacidosis. The reason

is not clear. It has been suggested that insulin activity is sufficient to suppress lipolysis but insufficient hepatic glyconeogenesis or to facilitate glucose transport into cell.

This condition sudden onset, commoner in older patient .Plasma glucose concentration may exceed 50mmol/l. Hypernatraemia due to predominante water loss is more commonly found than in ketoacidosis and aggravates the plasma hyperosmolality.⁽⁴⁾

1.2.2.3. Other causes of coma in patient with diabetes mellitus

Patient with diabetes mellitus may present in coma or in confused state due to:

Hypoglycaemia is most commonly caused by accidental over-administration of insulin or sulphonylureas.

Cerebrovascular accidents.⁽⁴⁾

Long termed diabetic complications

IDDM and NIDDM are both associated with several forms of long-termed complications ,also known as diabetic tissue damage .Includes arrange of microvascular complications(e.g retinopathy, nephropathy) ,macrovascular complications(ischemic heart disease peripheral vascular disease).

Macrovascular disease

The term macrovascular disease encompasses those diabetic complication mediated via accelerated atherosclerosis occurring in larg to medium- sized arteries but excluding terminal arteries and arteriols. Although microvascular

disease and some other complications may be more characteristic of diabetes, macrovascular disease accounts for most of the excess mortality associated with the condition.

A case can be made that diabetic macrovascular disease is related to but not identical with atherosclerosis occurring in non-diabetic patients. In diabetic macrovascular disease, the age of onset is earlier and the ratio of the prevalence of vascular disease in men and women much more equal. Differences in the rank order of the strength of associations of risk markers in diabetic and non-diabetic populations suggest some differences in a etiology(smoking is the main risk factor in non-diabetic populations, but hypertension seems relatively more important in diabetic groups).The well-documented higher mortality rate from myocardial infarction in diabetes mellitus (compared to non-diabetic patients)may be related to widespread involvement of middle-sized and small arteries in diabetes.

Microvascular disease

Microvascular disease means vascular disease occurring in the smallest arteries, arteriols and capillaries. Microvascular disease includes: Diabetic nephropathy.⁽⁵⁾

Diabetes nephropathy is a major cause of premature death in patients with diabetes, with deaths related to cardiovascular disease as well as renal failure .it occurs in about 30% of patient with type 1 DM, and 25% of patient with type 2 [more in certain ethnic groups].The earliest detectable abnormality is microalbuminuria .

Normal urinary albumin excretion is $<20 \mu\text{g}/\text{min}$ [$<30 \text{ mg}/24\text{h}$] constitute microalbuminuria .

This progresses through clinical proteinuria [$>200\mu\text{g}/\text{min}; 300\text{mg}/24\text{h}$] with gradually declining filtration rate and increasing plasma creatinine concentration to frank uremia. Hyperlipidaemia and hypertension are frequently present.⁽⁵⁾

Retinopathy may lead to blindness because of vitreous haemorrhage from proliferating retinal vessels. And maculopathy as a result of exudates from

Neuropathy may become evident as diarrhea, postural hypotension, impotence, neurogenic bladder and neuropathic foot ulcers due to microangiopathy of nerve blood vessels and abnormal glucose metabolism in nerve cells.

Approximately 60% of diabetic patients die of vascular disease and 35% of coronary heart disease .Blindness is 25 times and chronic renal failure 17 times more common in the diabetic.

There is increasing evidence that tight glycaemic control delays the one of these sequelae.⁽⁷⁾

Kidney disease

Diabetic renal damage most commonly presents as hypertension or albuminuria in clinic patients under regular review .late presentation include chronic renal failure, nephrotic syndrome ,nephrogenic diabetes insipidus , renal tubular acidosis or toxicity from renal excreted drugs.

Gout has a recognized associate of diabetes .there is no consensus as to the cause of association but alterations in renal excretion of urate.⁽⁶⁾

1.2.3. Biochemical changes during Diabetes Mellitus

Most of the metabolic changes of diabetes mellitus are consequence of insulin deficiency.

Hyperglycaemia is by definition, an invariable finding. If plasma glucose concentrations exceed about 11mm/l and renal function is normal, there will be glycosuria. High urinary glucose concentration produces an osmotic diuresis and therefore polyuria. Cerebral cellular dehydration due to hyperosmolality, secondary to hyperglycaemia, causes thirst (polydipsia). A prolonged osmotic diuresis may cause excessive urinary electrolyte loss. These classical symptoms of diabetes mellitus are only present in advanced cases.

Abnormalities in lipid metabolism may be secondary to insulin deficiency. Lipolysis is enhanced and plasma free fatty acid concentration rise. In the liver free fatty acid are converted to acetyl coA and ketones, or are re-esterified to form endogenous triglycerides and incorporated in to VLDL, the latter accumulation in plasma because lipoprotein lipase, which is necessary for VLDL catabolism, requires insulin for optimal activity. If insulin deficiency is severe there may also be chylomicronaemia. The rate of cholesterol synthesis is also increased, with an associated increase in plasma LDL concentration.

Increased breakdown of protein may cause muscle wasting.⁽⁴⁾

Fatty change in the liver is common with poorly controlled diabetes and frequently causes elevation of liver alkaline phosphatase activities in plasma and less frequently of other liver enzymes.

Generalized osteopenia is associated with diabetes mellitus. No specific defect in calcium or phosphate metabolism has been identified and the basis of the association is unclear. Of particular importance is local osteolysis, with loss of bone mineral in the foot. The inactivity induced by diabetic foot ulcers may accelerate the osteopenia. Either local or generalized osteopenia may result in elevated circulating bone alkaline phosphatase.

Low concentration of circulating magnesium has been reported during therapy for ketoacidosis. Hypomagnesaemia may be more common in outpatient with diabetes mellitus than in non-diabetic subject.

Hyponatraemia may arise through several mechanisms in diabetes mellitus. Artefactual hyponatraemia may arise from the hyperlipidaemia of ketoacidosis or poor glycaemic control. Compensatory hyponatraemia is often seen in poorly controlled diabetes, as an osmoregulatory response to high glucose concentrations.⁽⁵⁾

1.3. Urea

Catabolism of proteins and amino acids results in the formation urea, which is predominantly cleared from the body by the kidney.⁽³⁾

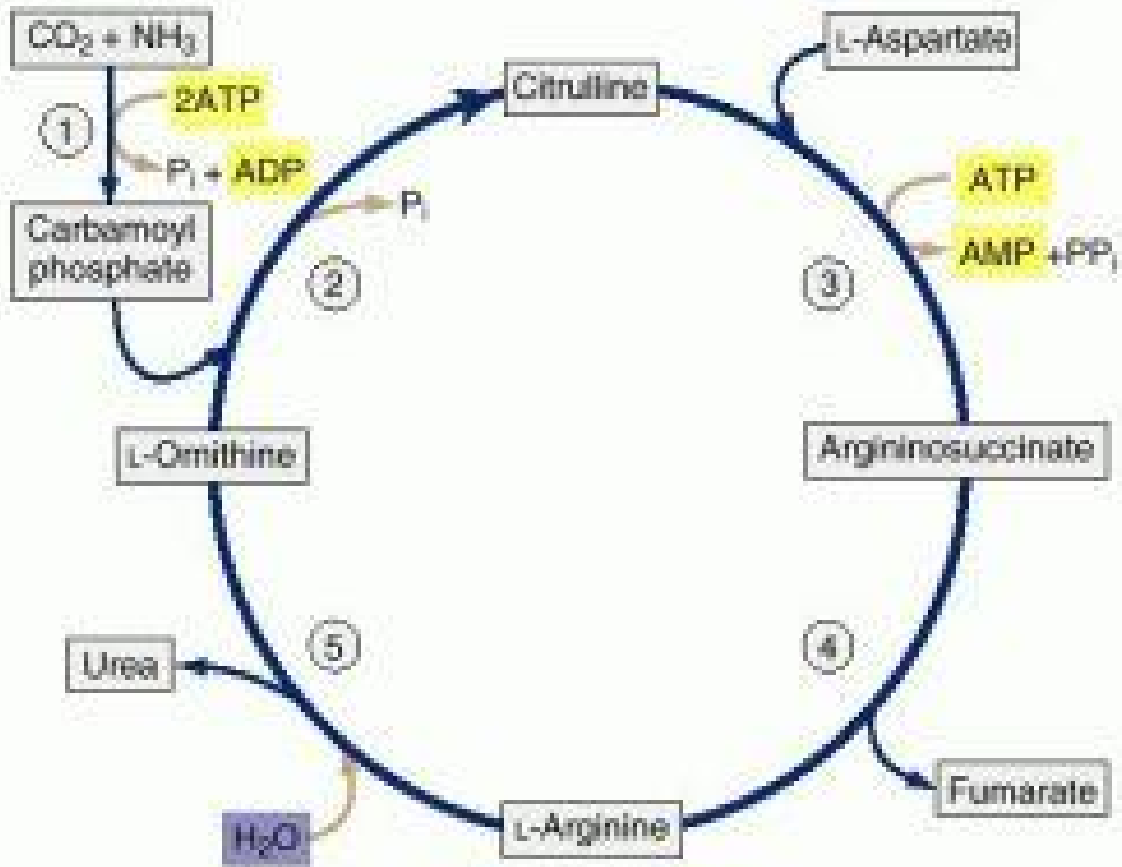
Biochemistry and physiology

Urea makes up the majority (>75%) of the non protein waste products. It is

Filtered at glomeruli and about (40-60) %of filtered urea is reabsorbed in collecting ducts. Therefore, impaired glomerular filtration results in retention of urea and its concentration rises.⁽⁸⁾

The biosynthesis of urea from amino acid nitrogen-derived ammonia is carried out exclusively by hepatic enzymes of the urea cycle. During the process of protein catabolism, amino acid nitrogen converted to urea in the liver by the action of the so called urea cycle enzymes.

More than 90% of urea is excreted through the kidneys, with losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction .consequently ,kidney disease is associated with accumulation of urea in blood .An increase in plasma urea concentration characterizes the uremic state .Urea is neither actively reabsorbed nor secreted by the tubules but is filtered freely by the glomeruli .In a normal kidney ,40%to70%of the highly diffusible urea moves passively out of the renal tubule and into the interstitium, ultimately to reenter plasma. The back diffusion of is also dependent on urine flow rate, with less entering the interstitium in high flow states (e.g pregnancy) and vice versa. Consequently, urea clearance generally underestimates GFR. In ESRD, osmotic dieresis in the remaining functional nephrons limits the back diffusion of urea so that urea clearance approaches inulin.⁽³⁾



KEY TO ENZYMES (Circled Numbers)

1. Carbamoyl-phosphate synthase (ammonia)
2. Omithine carbamoyltransferase
3. Argininosuccinate synthase
4. Argininosuccinate lyase
5. Arginase

Figure 1.1: Urea cycle

1.3.1. Abnormal plasma urea

1.3.1.1. Increase plasma urea concentration

Condition causing increase plasma urea is classified according to the cause into three main categories:-

Pre renal

- I. Congestive heart failure.
- II. Shock.
- III. Hemorrhage.
- IV. Dehydration.
- V. Amount of protein metabolism.
- VI. Other factors resulting in significant decrease in blood volume.
- VII. High protein diet or high protein catabolism.

Renal

- I. Acute and chronic renal failure
- II. Glomerular nephritis
- III. Tubular necrosis

Post renal

- I. Obstruction of urinary tract by renal calculi

II. Tumors of the bladder or prostate

III. Severe infection

1.3.1.2. Decrease plasma urea concentration

Due to:

II. Low protein intake

III. Severe liver disease

IV. Late pregnancy and in infancy

V. Severe vomiting and diarrhea

VI. Starvation

1.4. Creatinine

Is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine .⁽³⁾

Creatinine is a small compound readily filtered by the glomerulus, and unlike urea, is not reabsorbed by the tubules and collecting ducts. Elevated serum creatinine concentration is a more sensitive indicator of glomerular damage than serum urea.⁽⁸⁾

Biochemistry and physiology

Creatine is synthesized in the liver, kidney, and pancreas by two enzymatically mediated reactions. In the first transamidation of arginine and

glycin forms guanidinoacetic acid .In second reaction, methylation of guanidinoacetic acid occurs with S-adenosylmethionine as the methyl donor.

Creatine is then transported in blood to other organ as muscle and brain, where it is phosphorylated to phosphocreatine, a high energy compound.

Interconversion of phosphocreatine and creatine is a particular feature of the metabolic processes of muscle contraction .A proportion of the free creatine in muscle (thought to be between 1%and2%day) spontaneously and irreversibly converts to it anhydride waste product creatinine. Thus the amount of creatinine produced each day is relatively constant and is related to the muscle mass. In health, the concentration of creatinine in the bloodstream also is relatively constant.

However, depending on the individual meat intake, diet may influence the value. creatinine present in all body fluids and secretion ,and is freely filtered by the glomerulus. Although it is not reabsorbed to any great extent by the renal tubules ,there is a small but significant tubular secretion .Creatinine production also decrease as the circulating level of creatinine increases, several mechanism for this have been proposed including feedback inhibition of production of creatine, reconversion of creatinine to creatine and conversion to other metabolisms

The elderly and young children normally have lower creatinine level as result of reduced muscle mass .this may potentially mask renal disease in patients of these age group.⁽³⁾

1.4.1. Interfering factors

A diet high in meat content can cause transient elevations of serum creatinine. Drug that may increase creatinine value include aminoglycosides, cimetidine, heavy-metal chemotherapeutic agent and other nephrotoxic drugs such cephalosporins.⁽²⁾

1.4.2. Abnormal creatinine level

- I. Glomerulonephritis
- II. Pyelonephritis
- III. Acute tubular necrosis
- IV. Urinary tract obstruction
- V. Reduced renal blood flow
- VI. Rhabdomyolysis
- VII. Diabetic nephropathy
- VIII. Nephritis
- IX. Acromegaly
- X. Giantism

1.4.3. Decrease level

- I. Debilitation
- II. Decreased muscle mass

III. Starvation

IV. Wasting disease

V. After surgery

VI. Patient treated with corticosteroids

VII. Pregnancy

1.5. Objectives

1.5.1. General objective

To estimate plasma urea and plasma creatinine in Sudanese Type 2 Diabetic Patients

1.5.2. Specific objectives

1-To estimate plasma urea and plasma creatinine levels in Sudanese Type 2 Diabetic Patients.

3-To correlate plasma urea level and plasma creatinine level with diabetes mellitus.

2. Materials and Methods

2.1. Materials:

2.1.1. Study design:

This is a descriptive analytical comparative study.

2.1.2. Study area:

The study was conducted in long standing diabetic patient

2.1.3. Study population:

This study included 30 diabetic patients who attended zenam hospital and 20 apparently healthy individuals as control group was conducted during the period from February 2014to May 2014.

Inclusion Criteria:

Patients with diabetes mellitus who not suffer congestive heart failure, hemorrhage, rhabdomyolysis and tumor were included.

Exclusion criteria: Patients without diabetes mellitus or with diabetes mellitus and have already congestive heart failure, hemorrhage, rhabdomyolysis and tumor were excluded.

2.1.4. Samples:

About 2.5ml of venous blood were collected from each patient. The samples collected under aseptic conditions and placed in sterile heparinize containers, and after clotting centrifuged for 3 minutes at 3000 RPM to

obtain plasma, then obtained plasma were kept at -20c till the time of analysis.

2.1.5 Ethical consideration:

Patients who voluntarily accepted to participate in the study were included.

2.1.6. Equipments:

- Colorimeter, model JENWAY.
- Centrifuge
- Sterile heparinize containers
- Disposable syringes
- 70% alcohol
- Tourniquets
- Cotton
- Micropipettes (automatic pipettes)
- Graduated pipettes

2.1.7. Reagents:

a) Reagents of urea enzymatic, liquid, colorimetric method for estimation of urea concentrations.

They are supplied by Crescent diagnostic company, and reagent, composed of:

1. Enzyme concentrate
2. Sodium salicylate, sodium nitroprusside and phosphate buffer
3. Urea standard with concentration of 50mg/dl (8.3mmol/l).

When combined as working reagent contain the following:

Phosphate buffer (PH 6.9) 20mmol, urease>500U/ml, sodium salicylate 62mmol/l, sodium nitroprusside 3.4mmol/l stabilizers and preservatives (appendix III).

b) Reagents of creatinine, colorimetric method for estimation of creatinine concentrations.

They are supplied by Crescent diagnostics company, and working reagent, which composed of:

Picric acid 25mmol/l, sodium hydroxide 0.4mol/l, detergent

creatinine standard with concentration of 2mg/dl or 177 μ mol/l.

2.1.8. Data analysis:

Data was analyzed by using the SPSS computer program

2.2. Methodology:

(1). Estimation of urea concentration using the enzymatic (urease) method: ^(appendix II)

Principle of method:

Urease enzyme catalyses the conversion of urea in to carbon dioxide and ammonia, which react with salicylate and alkaline hypochlorite in presence of nitroprusside (catalyst increase rate of reaction) to give indophenol which dissociated with alkaline solution to give (indophenol) blue to green color, which can be measured calorimetrically at 600nm the color directly proportional to the concentration of urea in the sample.

Procedure:

Pipette into tube		Blank	Standard	Sample
Sample	ml	—	—	0.01
Standard	ml	—	0.01	—
Reagent(A)	ml	1.0	1.0	1.0

Then mix and incubate the tubes for 10 minutes at 25 °C or for 5 minutes at 37°C.

Pipette

Reagent(B)	ml	1.0	1.0	1.0
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Mix and incubate the tube for 10 minutes at 25 °C or for 5 minutes at 37°C and read the absorbance of standard and sample at 600nm against the blank, the color is stable for at least 2 hours.

Calculation:

The Urea concentration (mg/dl) = (A s/A Std) × Concentration of standard (C) mg/dl

Sample Urea concentration mmol/l= urea concentration mg/dl×10 / molecular weight of urea (60).

Reference values:

15 – 39 mg/dl (2.5 – 6.5mmol/l) .

(2) Estimation of creatinine concentration using kinetic method: (appendix iii)**Principle of method:**

Creatinine was thought to be react with alkaline picrate with in one minute, after additional of sample to working reagent, while pseudocreatinine (ketone bodies, Aceto acetate) react during first 30 second and other pseudocreatinine react with working reagent after 90 minutes of additional of sample(after reaction finished).

Procedure:

Working reagent	1.0ml
Standard or sample	0.1ml

Then mix, and insert cuvatte in to the calorimeter start stop watch. Then record the absorbance at 500nm after 30 seconds (A1) and after 90 seconds (A2) against the distilled water.

Calculation:

Creatinine concentration (mg/dl) = (A2-A1) sample/ (A2-A1) std ×
concentration of standard (mg/dl)

To convert mg/dl to $\mu\text{mol/l}$ calculates as follow:

Creatinine concentration $\mu\text{mol/l}$ = creatinine concentration mg/dl $\times 10 \times 1000$
/ molecular weight of creatinine (113)

Reference value:

Men = 0.9-1.3mg/dl (80-115 $\mu\text{mol/l}$)

Women = 0.6-1.1mg/dl (53-97 $\mu\text{mol/l}$).

3. Result

Fifty individual samples were enrolled in this study (30 of them were type 2 diabetic patient samples and 20 were apparently healthy individuals samples) their age ranged from (30-70 years).

To estimate urea by enzymatic method and creatinine by kinetic method and the result after using SPSS for statistical analysis of urea and creatinine concentration is shown in table 3.1 and figure 3.1.

Table 3.1: Result of diabetic patient group versus control group.

Variable	Mean \pm SD Control group n=20	Mean \pm SD diabetic patient group n=30	p.value
Plasma creatinine conc. (mg/dl)	0.835 \pm 0.266	0.873 \pm 0.208	0.571
Plasma urea conc. (mg/dl)	26.65 \pm 6.45	30.97 \pm 5.95	0.019

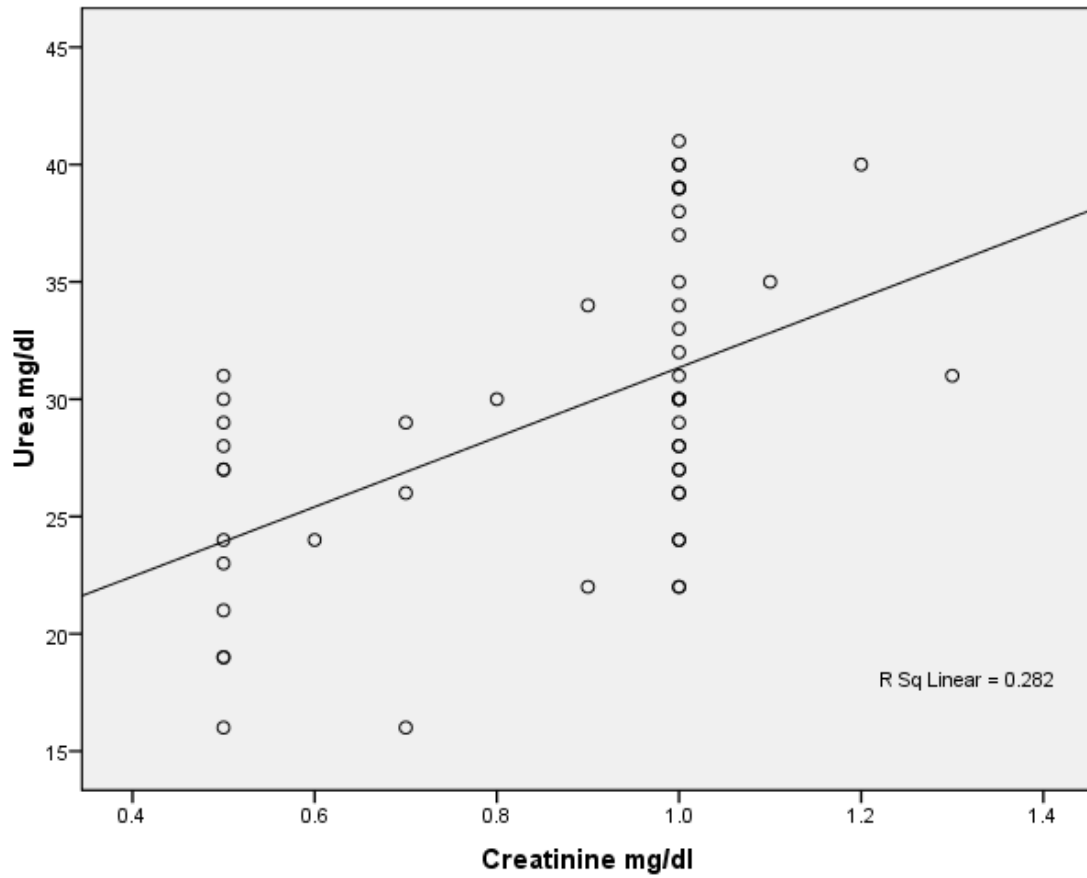


Figure 3.1: Correlation between plasma creatinine concentration and plasma urea concentration ($r=0.531$, P .value = 0.000).

4. Discussion, conclusions and recommendations

4.1. Discussion

Diabetes mellitus is a clinical syndrome associated with and abnormally high plasma glucose concentration, either when fasting or after ingestion of carbohydrate, and is often accompanied by the present of glucose in the urine from which the name of the condition is derived .It may result from by diminished insulin production by β -cells of the islets of langerhans, but this is not always the cause and hence diabetes is of heterogeneous origin.⁽⁹⁾

The present study aimed to estimate plasma urea and creatinine in diabetic patients type 2 by using enzymatic method and kinetic method respectively (30 diabetic patients type2 versus 20 apparently healthy were enrolled).

Statistical analysis by using SPSS showed that there is no significant difference in plasma creatinine in type2 diabetic patients (mean=0.873 \pm SD=0.208) mg/dl, when compared to control group (mean0.835 \pm SD=0.266)mg/dl, and p.value : 0.571 this result disagreed with the results of study done by Vemon et al in which (p.value:0.05)⁽¹⁰⁾.

And Statistical analysis showed that there is significant difference in plasma urea in type2 diabetic patient (mean=30.97 \pm SD=5.95)mg/dl, when compared to control group (mean=26.65 \pm SD=6.45)mg/dl, and p.value: 0.019 this result agreed with the results of a study done by Vemon et al in which (p.value:0.001)⁽¹⁰⁾.

This study showed that there is a significant positive correlation between the level of plasma creatinine and plasma urea in diabetic patient type2 (r=0.531, p.value=0.000).

4.2. Conclusion

From this study it is concluded that there is no difference in the level of plasma creatinine in type 2 diabetic patients and control group, and the level of plasma urea increased in type 2 diabetic patients.

4.3. Recommendation

1-To obtain reliable results it is recommended to increase sample size in further study.

2- It is recommended also to evaluate creatinine clearance of type 2 diabetic patients in addition to urea and creatinine estimation in plasma.

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Appendix I
Questionnaire

A) General information:

History of disease that affect urea and creatinine levels:

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B) Laboratory investigation:

Urea concentration (mg/dl)

Creatinine concentration (mg/dl)