

## **2. Materials and methods**

### **2.1 Study design:**

It is Case control- hospital base study.

### **2.2 Study area:**

The study was done in IBN SINA Hospital, Khartoum state, Sudan.

### **2.3 Study period:**

The study was carried during the period from March and June 2014.

### **2.4 Target population:**

Sudanese patients with chronic renal failure (males and females).

### **2.5 Inclusion criteria:**

Inclusion criteria people whom have chronic renal failure

### **Exclusion criteria:**

Exclusion criteria people who have gout, hypertension and DM and bone diseases.

### **2.6 Sample size:**

50 patients with chronic renal failure and 20 control.

### **2.7 Ethical consideration:**

- \* Permission of this study was obtained from the local authorities in the area of the study.
- \* The objectives of the study were explained to all individuals participating in the study.
- \* An informed consent was obtained from each participant in the study.

### **2.8 Sampling:**

2.5ml of venous blood collected in plain container immediately centrifuge at 3000 rpm for 5 minutes to separate serum for investigation of uric acid, calcium and phosphate and stored at -21<sup>0</sup>C until used.

### **2.9 Method:**

Mindary BS 200 analyzer was used to measure and report the serum uric acid, calcium and phosphate. This analyzer is fully automated computerized and include: photometric measuring system analytical processing unit, screen and printer.

### \* Analytical unit operating principle:

The general sequences of events are:

- The sample disk rotates the appropriate sample to the sample probe.
- The sample probe aspirates sample for testing.
- The sample is delivered into the reaction cell, the reagent probe adds up the reagents in separate dispense cycle.
- Incubation occurs as the reaction cell is measured in the incubation bath below the reaction disk, reaction cell rotate through the photometer light path and measurement is taken.

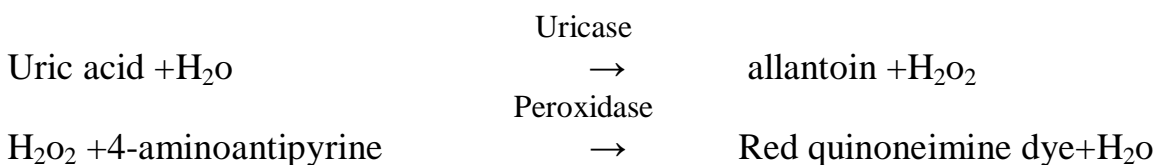
### 2.9.1 Uric acid:

Estimation of Uric Acid :( Uricase-POD) method

#### Principle:

Uricase converts uric acid to allantoin and hydrogen peroxide, the hydrogen peroxide formed further reacts with phenolic compound and 4-amino antipyrine by catalytic action of peroxidase to form a red colored quinoneimine dye complex.

Intensity of the color formed is directly proportional to the amount of uric acid present in the sample. (Ramink, 2006).



#### 2.9.1.2 Reagent composition:

Uric Acid reagent: 4-AAP 1.7mmol/L, Uricase 750 U/L

Peroxidase 10000 U/L,phosphate buffer70 mmol/L.

#### 2.9.1.3 Reagent Preparation:

Ready for use.

#### 2.9.1.4 Reagent stability:

The reagent is stable up to stated expiry date when stored at 2-8 °C and protected from light.

#### **2.9.1.5 Procedure**

200µL from Reagent and 4µL from sample to the reaction disk automatically by the analyzer.

#### **2.9.1.6 Calculations**

The analyte concentration of each sample was calculated automatically by the analyzer the result then appeared directly on the screen of the machine at the end of the reaction between the reagents and sample.

Linearity: the test is linear up to a uric acid concentration of 20mg/dl.

Reference intervals:

Male 3.6-8.2 mg/dl

Female 2.3-6.1mg/dl(Thomas, 1998)

#### **2.9.1.6 Quality control**

Serum controls with known normal and abnormal uric acid values were

Run routinely to monitor the validity of the reaction. These controls were

Run at least with every working shift in which uric acid determinations are

Performed.

### **2.9.2 Calcium:**

Estimation of calcium by Aresnazo III method:

#### **Principle:**

Calcium in the sample reacts with Aresnazo III produce blue colored complex whose intensity is apparitional to the calcium concentration. (Lorentz, 1982).

#### **2.9.2.1 Reagents composition:**

##### **1. Reagent**

Aresnazo III	0.12mmol/L
Phosphate buffer	50 mmol/L

8-Hydroxyquinoline-5-sulfonic acid

5mmol/L

### **2.9.2.2 Reagent Preparation:**

Ready to use.

### **2.9.2.3 Reagent stability:**

The reagents are stable up to stated expiry date when stored at 2-8 °C.

### **2.9.2.4 Procedure:**

250µL from Reagent and 3µL from sample to the reaction disk automatically by the analyzer.

### **2.9.2.5 Calculations:**

The analyte concentration of each sample was calculated automatically by the analyzer the result then appeared directly on the screen of the machine at the end of the reaction between the reagents and sample.

Linearity: the test is linear up to calcium concentration of 20mg/dl

Normal values:

Serum calcium 8.8-10.6 mg/dl.(Thomas, 1998).

## **2.9.3 Phosphorus:**

Estimation of phosphorus by spectrophotometer method:

### **Principle:**

Inorganic phosphorus in the sample reacts with ammonium molybdate in acidic media to give phosphomolybdate complex that can measure spectrophotometer at 340 nm. (Gamst, *et al.*, 1980).

### **2.9.3.1 Reagents composition:**

1. Reagent

Sulfuric acid

0.5 mol/L

Ammonium molybdate

0.3mmol/L

### **2.9.3.2 Reagent Preparation:**

Reagent provided ready to use

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### **2.9.3.3 Reagent stability:**

The reagents are stable up to stated expiry date when stored at 2-8 °C.

### **2.9.3.4 Procedure:**

300µL from Reagent and 3µL from sample to the reaction disk automatically by the analyzer.

### **2.9.3.5 Calculations:**

The analyte concentration of each sample was calculated automatically by the analyzer the result then appeared directly on the screen of the machine at the end of the reaction between the reagents and sample.

Linearity: the test is linear up to phosphorus concentration of 20mg/dl if higher dilute samples.

Normal values:

Adults: 2.5 -4.5 mg/dL

Children: 4-7mg/dL (Thomas, 1998)

### **2.10 Quality control:**

It is recommended that each run contain a normal control with assayed values, the use of this material checks both instrument and reagent functions together.

### **2.11 Statistical method:**

SPSS package was used for the analysis of the result