Sudan University of Science and Technology

Collage of Medical Laboratory Science

Clinical Chemistry Department

Assessment of Uric Acid Level as Antioxidant in Serum of Type 2 Diabetes Mellitus Patients

تقييم مستوي حمض اليوريك كمضاد للأكسدة في مصل الدم لدي المصابين بالنوع الثاني من مرض السكري

A dissertation submitted in partial fulfillment of for the requirmentof B.Sc (Honor) degree in medical laboratory science (clinical chemistry)

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بسم الله الرحمن الرحيم

قال تعالي:

لاَ(يُكَلِّفُ اللّهُ نَفْساً إِلاَّ وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لاَ تُؤَاخِذْنَا إِن نَّسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلاَ تَحْمِلْ عَلَيْنَا إِصْراً كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِن قَبْلِنَا رَبَّنَا وَلاَ تُحَمِّلْنَا مَا لاَ طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنتَ مَوْلاَنَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِين)

صدق الله العظيم

(سورة البقرة الآية 286)

Dedication

To

Our fathers

Our mothers

Sisters and brothers

To

Our teachers and especially our supervisor

To

Our colleagues whom help us

To

All people, whom we love, respect and appreciate.

Acknowledgements

Thank you our God, for giving us the ability and courage to bring this research to light. Our great thank to the soul of Dr. Mohammed Abd Elraheem our supervisor, who started with us this research from the zero level. He was the one who directed us to this important topic, so we are really grateful.

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Abstract

Diabetes mellitus is usually irreversible and although patients have a reasonably normal life style, its late complication result in life expectancy and major health

Cost.

This study was conducted essentially to assess the level of serum uric acid among Sudanese with type 2 Diabetes Mellitus as anti oxidant.

Forty blood sample were collected from diabetic patients with type 2 diabetes mellitus, their age range from (40-75) years, and twenty healthy volunteers as control group for the comparison. uric acid was estimated enzymatic ally by colorimeter ,then the data was analyzed statically by (SPSS) computer software .

The results of study found there is no significant difference in the serum uric acid level between study and control groups.

There is weak positive correlation between duration of disease and level of serum uric acid .

المستخلص

مرض السكري مرض دائم والمرضي يعيشون حياة طبيعية لكن لديه مضاعفات متأخرة تزيد التكلفة وتهدد الحياة

هذه الدراسة اجريت لتقدير مستوى حمض اليوريك عند المرضى السودانيين المصابين بالسكري النوع الثاني .

تم جمع عينات الدم من اربعين من المرضى المصابين بداء السكر من النوع الثاني وكانت ذات اعمار ما بين (40-75)وعشرين متطوعا من الاصحاء متشابهين في الاعمار كمجموعة للمقارنة . وقمنا بقياس حض ليوريك في مصل الدم بطريقة الانزيمية بواسطة جهاز الكلروميتر تم تحليل البيانات احصائيا.

لاحظت الدراسة ان ليس هنالك فرق ذات دلالة احصائية في مستوي حمض اليوريك (كمضاد للأكسدة) في المرضي المصابين بالنوع الثاني من السكري عند مقارنة مع مجموعة التحكم.

ولوحظ ايضا من خلال الدراسة ان هنالك علاقة بين مستوى حمض اليوريك ومدة المرض .

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Chapter One

Introduction and Literature Review

1 Introduction and Literature Review

1.1 Diabetes mellitus

Diabetes mellitus is group of metabolic disorder of carbohydrate metabolism in which glucose is underutilize, produce hyperglycemia and relative insulin deficiency, resistance or both. It affects more than 120 million by the year 2020. Diabetes is usually irreversible and although patients have reasonably normal life style, its late complication result in reduce life expectancy and major health costs (David *et al.*, 2005).

Diabetes is widely recognized as one of the leading causes death. The rapid increase in diabetes parallels the increase in obesity and overweight. Recent information indicates that 5.5% in northern state of Sudan and 8.6% in Khartoum state have diabetes and the numbers are expected to rise (Elbagir *et al.*, 2008).

1.1.1 Types of diabetes mellitus

In 1979, the national diabetes data group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: type1 insulin dependent diabetes mellitus (IDDM) and type2 Non insulin dependent diabetes mellitus (NIDDM).

Therefore the WHO guideline recommends the following categories of diabetes: type1 diabetes, type 2 diabetes, other specific type and Gestational diabetes mellitus (GDM) (Bishop *et al.*, 2010).

1.1.1.1 Type 1 diabetes

Type 1 Diabetes is characterized by inappropriate hyperglycemia primarily a result of pancreatic islet Beta cell destruction and a

tendency to ketoacidosis. Type 1 Diabetes mellitus is a result of cellular - mediated auto immune destruction of the pancreas, causing an absolute deficiency of insulin secretion .upper limit of 110 mg/dl on the fasting plasma glucose is designated as the upper limit of normal blood glucose. Type 1constitutes only 10% to 20% OF all cases of diabetes and commonly occur in child hood and adolescence .this disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with genetic predisposition and causes the immune destruction of Beta cells of pancreas and, therefore, a decrease production of insulin.

Characteristics of Type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency .this diabetic type is genetically related. One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia: islet cell auto anti bodies, insulin auto antibodies, and Glutamic acid decarboxylase auto anti bodies, and tyrosine phosphatase IA-2and IA-2B auto antibodies (Bishop *et al*., 2010).

Signs and symptoms include polydipsia (excessive thirst), polyphagia (increase food intake), polyuria (excessive urine production), rapid weight loss, hyperventilation, mental confusion, and possible loss of consciousness (due to increase glucose to brain). Complications include microvascullar problems such as nephropathy, neuropathy, and retinopathy. Increased heart disease is also found in patients with diabetes (Bishop *et al*., 2010).

1.1.1.2 Type 2 diabetes

Type2 diabetes mellitus is characterized by hyperglycemia as a result of an individual's resistance to insulin with an insulin secretary

defect. This resistance result in relative, not an absolute insulin deficiency.Type2 constitutes the majority of the diabetes cases. Most patients in this type are obese or have an increased percentage of body fat distribution in the abnormal region. This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an increase in age, obesity, and lack of physical exercise. Characteristic usually include adult onset of the disease and milder symptoms than in type1, with ketoacidosis seldom occurring. However, these patients are more likely to go into a hyperosmoler coma and are at an increased risk of developing macro vascular and micro vascular complications (Bishop *et al*., 2010).

1.1.1.3 Other specific type of diabetes

Other specific type of diabetes are associated with certain conditions (secondary), including genetic defect of Beta-cell function or insulin action, pancreatic disease, disease of endocrine origin, drug or chemical induced insulin receptor abnormalities, and certain genetic syndromes. The characteristics and prognosis of this form of diabetes depend on primary disorder (Bishop *et al*., 2010).

1.1.1.4 Gestational Diabetes Mellitus (GDM)

GDM is any degree of glucose intolerance with onset or first recognition during pregnancy. Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal post partum. However, this disease is associated with increased prenatal complications and an increased risk for development of diabetes in later years. Infants born to mothers with diabetes are at increased risk for respiratory distress syndrome,

hypocalcaemia, and hyperbilirubinemia. Fetal insulin secretion is stimulated in the neonate of mother with diabetes. However, when the infant is born and the umbilical cord is severed, the infant's over supply of glucose is abruptly terminated, causing sever hypoglycemia (Bishop *et al*., 2010).

1.1.2 Insulin

Insulin is primary hormone responsible for the entry of glucose into the cell it is synthesized by the Beta cell of islets of langerhans in the pancreas. When these cells defect an increase in body glucose, they release of insulin causes an increased movement of glucose into the cells and increased glucose metabolism. Insulin is normally released when glucose levels are high and is not released when glucose levels are decreased. It decreases plasma glucose levels by increasing the transport entry of glucose in muscle and a dispose tissue by way of non specific receptors. It also regulates glucose by increasing glycogen sis, lipogenesis and glycolysis and inhibiting glycogenolysis. Insulin is the only hormone that decreases glucose levels and can be referred to as a hypoglycemic agent (Bishop *et al*., 2010).

1.2 Uric acid

Uric acid is the products of catabolism of the purine nucleic acids. Although it is filtered by the glomreulus and secreted by the distal tubules into the urine, most uric acid is reabsorbed in the proximal tubules and reused. Uric acid is relatively in soluble in plasma, at high concentration, can be deposited in the joints and tissue, causing pain full inflammation. The range of plasma urate in men and women is, increasing slightly with age. There is a urate binding

globulin. the plasma urate level is little affected by variation in the purine content of the diet and present a steady state almost wholly reabsorbed, there is also some intestine destruction (Bishop *et al.*, 2010).

1.2.1 Function of uric acid

Uric acid, or more correctly (at physiological ph values), it is mono anion urate is traditionally considered to be metabolically inert end – produce of purine metabolism in man without any physiology value .

However this ubiquitous compound has proven to be selective anti oxidant, capable especially of reaction with hydroxyl radical and hypo chlorous acid, itself being converted to innocuous product (allantoina, allanotdate, glyoxylate, uria oxalate).

There is now evidence for such processes not only in vitro and in isolated organ, but also in the human lung in vivo. urate may also serve as an oxidase substrate for enzyme cyclooxygenase. as showing for the coronary system, major site of production of urate is the microvascullar endothelium, and there is generally anet release of urate from human myocardium in vivo. in isolated organ preparation urate protect against re perfusion damage induced by activated granulocytes, cell known to produce variety of radical an oxidant (Bern hard and Becker, 1993).

Intriguingly ,urate prevent in activation of endothelial enzyme (cyclooxygenaes , angiotensin converting enzyme) and preserves the ability of endothelium of mediate vascular dilation in the face of oxidative stress, suggesting particular relationship between site of urate formation and need for biologically potent radical scavenger and anti oxidant (Bern hard and Becker, 1993).

1.2.2Physiology of uric acid

Purine, such as adenosine and guanine from the breakdown of ingested nucleic acid or from tissue destruction, are converted into uric acid, primarily in the liver. Uric acid is transported in the plasma from the liver to the kidney, where its filtered by the glomerular. Reabsorption of 98% to 100% of uric acid from the glomerular filtrate occurs in the proximal tubules. Small amounts of uric acid are secreted by the distal tubules into urine. Renal excretion accounts for about 70% of uric acid elimination, the remainder passes into the gastrointestinal tract and is degraded by bacterial enzymes (Bishop *et al*., 2010).

Nearly all of the uric acid in plasma is present as monosodium urate. At the ph of plasma (ph 7), urate is relatively insoluble, at concentration greater than 6.8 mg\dl, the plasma is saturated. As a result, urate crystal may form and precipitate in the tissue. In acidic urine (ph <5.75), uric acid is the predominant species and uric acid crystal may form (Bishop *et al*., 2010).

1.2.3 Clinical significant of uric acid

1.2.3.1 Hyperuricemia

Hyperuricemia is a result of over production of uric acid, although Hyperuricemia may be exacerbated by purine –rich diet, drug, and alcohol. Plasma uric acid concentration in affected individual is usually greater than 6.0 mg\dl. Patients with gout are very susceptible to the development of renal calculi, although not all people with high serum urate concentration develop this complication. In women, urate concentration rises after menopause. Postmenopausal women may develop Hyperuricemia and gout. In

severe cases, deposit of crystalline uric acid and urate called tophi form in tissue, causing deformities (Bishop *et al*., 2010).

Another common cause of elevated plasma uric acid concentration is increased metabolism of cell nuclei, as occurs in patients on chemotherapy for such proliferative disease as leukemia, lymphoma, multiple myeloma, and polycythemia. Monitoring uric acid concentration in these patients is important to avoid nephrotoxicity. Chronic renal disease causes increased uric acid concentration because filtration and secretion are impaired. However, uric acid is not useful as indicator of renal function because many other factors affect its plasma concentration. Patients with hemolytic or megaloblastic anemia may exhibit elevated uric acid concentration. Hyperuricemia is a common feature of toxemia of pregnancy (preeclampsia) and lactic acidosis, presumably as a result of competition for binding sites in the renal tubules. increased urate concentration may be found following ingestion of a diet rich in purine (Liver, kidney, sweetbread, shellfish) or as a result of increased tissue catabolism due to inadequate dietary intake (starvation) (Bishop *et al*., 2010).

1.2.3.2 Hypouricemia

Hypouricemia is less common than Hyperuricemia and is usually secondary to sever liver disease or defective tubular reabsorption, as in fanconi syndrome. Hypouricemia can be caused by chemotherapy with 6-mercaptopurine or azathioprine, inhibitors of de novo purine synthesis, and as a result of overtreatment with Allopurinol (Bishop *et al*., 2010).

1.2.3.3 Gout

Gout (also known as podagra when it involves the big toe) is a medical condition usually characterized by recurrent attacks of acute inflammatory arthritis: a red, tender, hot, swollen joint. The metatarsal –phalangeal joint at the base of the big toe is the most commonly affected (approximately 50% of cases). However, it may also present as tophi, kidney stones, or urate nephropathy. It is caused by elevated level of uric acid in the blood which crystallize and deposited in joints, tendon, and surrounding tissues (Schlesinger, 2010).

1.2.3.3.1Causes of gout

Hyperuricemia is the underlying cause of gout. This can occur for a number of reasons, including diet, genetic predisposition, or under excretion of urate, the salts of uric acid. Renal under excretion of uric acid is the primary cause of Hyperuricemia in about 90% of cases, while overproduction is the cause in less than 10%. About 10% of people with Hyperuricemia develop gout at some point in their lifetimes. The risk, however, varies depending on the degree of Hyperuricemia. When levels are between 415 and 530 mol\l (7and 8.9 mg\dl), the risk is 0.5 % per year, while in those with a level greater than 535 mol\l (9mg\dl), the risk is 4.5 per year (Richette et al., 2010).

1.2.4 Correlation between diabetes mellitus and uric acid

Serum uric acid correlation concentration is higher in patient with established coronary heart disease compared with healthy control Hyperuricemia is also associated with elevation in blood triglyceride and cholesterol concentration, no correlation between Hyperuricemia

and diabetes mellitus, the negative correlation between serum uric acid concentration and fasting blood glucose may reflect the role of Hyperuricemia in the genesis of oxidative stress in the diabetic patients (Sood, 2009).

1.3 Objectives

1.3.1 General objective

To assess serum uric acid level in diabetic patients (Type2) in Jaber Abo Elez center for diabetes mellitus.

1.3.2 Specific objectives

1. To determine the level of serum uric acid in diabetic patients compared to control group.
2. To measure the relationship between serum uric acid level and sex of patients.
3. To correlate between serum uric acid level and duration of diabetes.

Chapter Two

Materials and Methods

2 Materials and Methods

2.1 Materials

2.1.1 Study design

This is descriptive, cross-sectional study, carried out during the period from March to Augusts 2014.

2.1.2 Study area

The study was conducted in Jaber Abo Elez center for diabetes mellitus.

2.1.3 Study population

Patients with type 2 diabetes mellitus were enrolled in this study as case group and volunteer non diabetic as control group.

2.1.4 Inclusion criteria

Patients with type2 diabetes mellitus were included in this study.

2.1.5 Exclusion criteria

Patients with type 1 diabetes mellitus, patients with type 2 diabetes mellitus that suffering of gout and acute or chronic renal failure were excluded.

2.1.6 Data collection and clinical assessment

Specially designed questionnaires and interviews with the patients were made. Doctor conducted the clinical examination and assessment.

2.1.7 Sample size

Forty patients with type2 diabetes as test group and twenty apparently healthy individuals as control group.

2.1.8 Sampling

About 5 ml of venous blood were collected by using sterile disposable syringes and poured into plane containers then centrifuge to obtain serum which used to measure uric acid.

2.1.9 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 this study.

2.2 Methods

2.2.1Estimation of uric acid

Principle of the reaction:

Determination of uric acid by reaction with uricase, the formed H_2O_2 reacts under catalysis of peroxides with 3,5-di-chloro hydroxyl benzene sulfuric acid (DCHBS)and 4 aminophenazone (PAP) to give red violet quinonamine dye as indicator.

Procedure:

	Blank	Standard	Sample
WR(ml)	1.0	1.0	1.0
Standard(µL)	_	25	_
Sample(µL)	_	_	25

Mix and incubate for 5 min as 37C then read absorbencies at 520nm.

Calculation:

Concentration of uric acid was calculated using the following formula:

A sample/A standard×concentration of standard

Reference value:

Male:3.6-7.7mg/dl

Female:2.5-6.8mg/dl

2.2.2 Statistical analysis

Data was analyzed by using statistical package for social science (SPSS) computer system program. The means and standard deviation (SD) of serum levels of uric acid were calculated and t-test was used for comparison (significant at P-value $\leq$ 0.05). Linear regression analysis was used to assess correlation between duration and serum uric acid level .

2.2.3 Quality Control

Control sera are recommended to monitor the performance of assay procedure:SPINTROL H Normal and pathologic(Ref.1002120 and 1002210).

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems .

Each laboratory should establish its own quality control scheme and corrective actions if controls do not meet the acceptable tolerances.

Chapter Three

Results

3. Results

Forty patients with type2 diabetes were involved in this study. The samples (serum) were collected from patients. The serum uric acid of those patients was estimated and data analyzed statically using SPSS computer system program. The result obtain were as fallow:

Table (3.1) shows insignificant difference between the mean of serum uric acid level of patients compared to control values were (5.27±1.35) mg/dl and (5.14±1.1) mg/dl respectively, *P*-value = 0.6.

Table (3.2) shows insignificant difference between the mean of serum uric acid level of male compared to female (5.2±2.1) mg/dl and (5.3±1.5) mg/dl respectively, *P*-value = 0.7.

Figure (3.1) Scatter plot shows insignificant weak positive correlation between the duration of diabetes and level of serum uric acid (r = 0.16, *P*-value = 0.3).

Table (3.1) comparison the means of serum uric acid level of diabetic patients to control group.

	Diabetic patients no = 40	Control group no = 20	*P*-value
Serum uric acid (mg/dl)	5.27 ± 1.35	5.14 ± 1.1	0.6

Table (3.2) comparison the means of serum uric acid level of males to females.

	males no = 27	females no = 13	*P*-value
Serum uric acid (mg/dl)	5.2 ± 1.2	5.3 ± 1.5	0.7

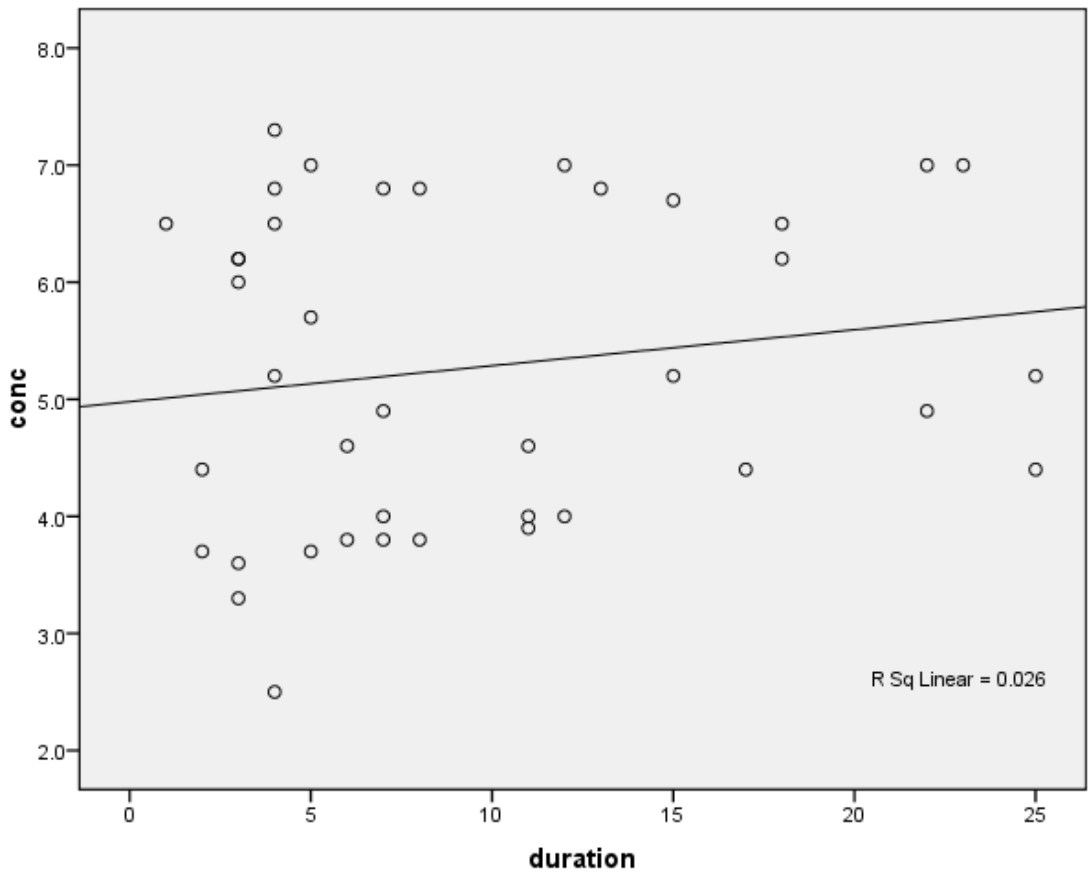

Figure (3-1) A scatter plotted the correlation between duration of diabetes and level of serum uric acid of diabetic patients (r = 0.16 , *P*-value = 0.3).

Chapter Four

Discussion , Conclusions and Recommendations

4 Discussion, Conclusions And Recommendations

4.1 Discussion

Diabetes mellitus affects more than 120 million by the year 2020. Diabetes mellitus affects the normal biochemical status of human body and lead to complication over period of time. Diabetes mellitus for example disrobe urea, creatinine, uric acid and lipids. When uric acid disrobed affect oxidative stress and increase incidence of cardiac disease.

The study was conducted on 40 patients with type2 diabetes as test group and 20 healthy volunteers as control group.

The results of the present study provided experimental evidence that the mean of serum uric acid level found no significant difference between level of uric acid in diabetic patients and control subjects with *P*-value more than 0.05. These results discrepancy with those obtained by study done by Isam who state that significant decrease in level of serum uric acid in diabetic patients when compared with control subjects (Isam, 2007). in addition to that there is no significant difference between level of uric acid in males when compared with females with *P*-value more than 0.05.

There is weak positive correlation between level of uric acid and duration of diseases.

4.2 Conclusions

From the results of this study it is conclude that:

1. Uric acid level remain in normal values in diabetic patients .

2. The level of uric acid is high in males than females.

3.There is weak positive correlation between level of uric acid and duration of dieses.

4.3 Recommendations

1. To prevent oxidative damage cause by free radical in diabetic patients, patient are advised to eat diet containing high antioxidant but not eat diet containing high purine.
2. Monitoring the glycemic control to prevent hyperglycemic which lead to auto-oxidant of glucose.
3. More investigation for diabetic patients must be done regularly such as serum uric acid, microalbuminuria, creatinince clearance, lipid profile and urine analysis.
4. further study should be done to measure serum uric acid level and glucose level to assess relationship between them.

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Appendices

Questionnaire

Sudan university of science and technology

Collage of Medical laboratory science

Department of Clinical Chemistry

Assessment of serum uric acid as anti oxidant among Sudanese patients diabetes type2 in Khartoum state

Name:…….

Number:……..

Date:……..

Age:…….

Sex:……

Duration of diabetic:……..

Type of treatment:……..

Do you suffering from the following disease?

Gout Yes( )

No( )

Renal disease Yes( )

No( )

Result:

Serum uric acid……mg/dl