

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

**Assessment of plasma UreaCreatinine and Uric  
acid levels among Sudanese Cigarette Smokers  
(study in Khartoum) State**

قويم مستويات البويينا، معدل ترشيح الدم وحمض اليوريك في مصل المدخنين السودانيين-  
دراسة حالة في ولاية الخرطوم

**Desertion submitted in partial fulfillment for the requirements  
for master Degree in clinical chemistry**

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# بسم الله الرحمن الرحيم

قال الله تعالى :

((ونرفع درجات من نشاء وفوق كل ذي علم عليم ))

صدق الله العظيم

سورة يوسف(الايه 76)

# ***Dedication***

***To my family, my fiancé....With love***

**Wala .....**

## **Acknowledgements**

Thanks first and last to (ALLAH) who enabled me to conduct this study by the grace of him and give me strength and patience, jewel immense debt and respect to my supervisor Dr: NuhaAlgaili Abu Baker for her continuous supervisor, patience, wisdom, critical comments, invaluable sound advice and careful guidance.

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## **Abstract**

This is a case-control study, conducted to determine the effect of cigarettes smoking on urea, creatinine and uric acid levels in Sudanese male cigarette smokers in a period from March to May 2015.

Eighty samples were collected from cigarette smokers without diseases that may affect levels of urea, creatinine and uric acid. And fifty non-smokers people as control were informed about study and informed consent for participation was obtained. 2.5 ml of venous blood was collected in heparin containers and investigated for urea, creatinnine and uric acid levels using semi-automated mindery analyzer. statistical package for social science (SPSS) computer program version 16 was used for data analysis.

The study results showed a significant elevation in Urea and Creatinine levels in cigarette smokers when compared to non-cigarette smokers.

Urea ( $29.2 \pm 6.7$  versus  $25.4 \pm 5.9$  mg/dl, p.value= 0.00).

Creatinine ( $1.05 \pm 0.233$  versus  $0.76 \pm 0.28$  mg/dl, p.value =0.00).

The study results showed a significant decreased in Uric acid level in cigarette smokers when compared to non-cigarette smokers.

( $5.06 \pm 1.1$  versus  $5.6 \pm 0.81$  mg/dl, p.value =0.01).

Person correlation showed that there was a significant positive correlation between the duration of cigarette smokers and the levels of Urea ( $r=0.63$ , p.value=0.00) and Creatinine ( $r=0.51$ , p.value=0.00).

Also there was a significant negative correlation between the duration of cigarette smokers and the level of Uric acid ( $r=0.39$ , p.value=0.00).

There was no obvious significant correlation between the number of cigarettes per day and the levels of Urea, Creatinine and Uric acid.

Urea ( $r=0.031$ ,  $p.value=0.79$ ), Creatinine ( $r=0.02$ ,  $p.value=0.84$ ) and Uric acid ( $r= -0.009$ ,  $p.value=0.94$ ).

This study concluded that the plasma Urea and creatinine levels are significantly increased in Sudanese smokers, while the plasma Uric acid level is significantly decreased. Also it was found that the plasma Urea, creatinine and Uric acid levels affected by age and duration of smoking, and not affected by number of cigarette per day.

## مستخلص الدراسة

اجريت هذه الدراسة التوقعية لقياس مدى تأثير تدخين السجائر على مستويات البولينا، الكرياتينين وحمض اليوريك لدى المدخنين السودانيين في الفترة ما بين مارس حتى مايو ٢٠١٥.

ثمانون عينة اخذت من مدخنين للسجائر الاصحاء الذين لا يعانون من مرض يمكن ان يؤثر على معدل البولينا، الكرياتينين وحمض اليوريك مع خمسون عينة اخذت من غير المدخنين كمجموعة تحكم (مجموعة ضابطة) تم اخبارهم بغرض الدراسة واخذ موافقتهم.

تم اخذ عينة 2.5 مل من الدم وتم قياس مستويات البولينا، الكرياتينين و حمض اليوريك باستخدام (جهاز ميندري شبه اتوماتيكي للتحليل) وبرنامج الحزمة الإحصائية للعلوم الاجتماعية لمعالجة البيانات.

توصلت نتائج الدراسة الى أن مستوى البولينا والكرياتينين يزداد في مجموعة المدخنين مقارنة بغير المدخنين.

"المتوسط  $\pm$  الانحراف المعياري عند المدخنين مقارنة بغير المدخنين"

البولينا (٢٩,٧  $\pm$  ٦,٧ ملجم/ديسيلتر مقابل ٢٥,٤  $\pm$  ٥,٩ ملجم/ديسيلتر، القيمة المعنوية = ٠,٠٠)، الكرياتينين (١,٠٥  $\pm$  ٠,٢٣٣٣ ملجم/ديسيلتر مقابل ٠,٧٦  $\pm$  ٠,٢٨ ملجم/ديسيلتر، القيمة المعنوية = ٠,٠٠).

أيضا هذه الدراسة توصلت الى نقصان في حامض اليوريك في مجموعة المدخنين مقارنة بغير المدخنين.

حمض اليوريك (٠,٠٦  $\pm$  ١,١ ملجم/ديسيلتر مقابل ٠,٨١  $\pm$  ٥,٦ ملجم/ديسيلتر، القيمة المعنوية = ٠,٠١).

تحليل ارتباط بيرسون أظهر علاقة إيجابية ذات دلالة إحصائية بين مدة التدخين ومستويات البولينا (معامل بيرسون للارتباط = ٠,٦٣، القيمة المعنوية = ٠,٠٠) والكرياتينين (معامل بيرسون للارتباط = ٠,٥١، القيمة المعنوية = ٠,٠٠)، أيضا وجد ان هنالك علاقة سلبية ذات دلالة إحصائية بين مدة التدخين ومستوى حمض اليوريك (معامل بيرسون للارتباط = ٠,٣٩، القيمة المعنوية = ٠,٠٠).

لم تثبت الدراسة أن هنالك علاقة ذات دلالة إحصائية واضحة بين عدد مرات التدخين في اليوم مع معدلات البولينا، الكرياتينين وحمض اليوريك.

البولينا (معامل بيرسون للارتباط = ٠,٣١، القيمة المعنوية = ٠,٧٩)،

الكرياتينين (معامل بيرسون للارتباط = ٠,٠٢، القيمة المعنوية = ٠,٨٤)،

حمض اليوريك (معامل بيرسون للارتباط = -٠,٠٠٩، القيمة المعنوية = ٠,٩٤).

أظهرت نتائج هذه الدراسة أن مستوى البولينا والكرياتينين يزداد في مجموعة المدخنين مقارنة بغير المدخنين وإيضاً نقصان حمض اليوريك في مجموعة المدخنين مقارنة بغير المدخنين.

وأيضاً وجدت هذه الدراسة أن مستوى البولينا، الكرياتينين وحمض اليوريك يتأثر بالعمر ومدة التدخين، ولا تتأثر بعدد مرات التدخين في اليوم.



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### Abbreviations:

abbreviation	Full term
ATP	Adenosine Triphosphate
BUN	Blood Urea Nitrogen
Cr	Creatinine
CrCL	Creatinine Clearance
eGFR	Estimated Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
GOP	Glucose monophosphate
G6P	Glucose 6 Phosphate
JG	Juxtaglomerular
MDRD	Modification of Diet in Renal Disease
PRPP	Phosphoribosyl Pyrophosphate Synthetase



# **1. Introduction and objectives**

## **1.1Introduction:**

Cigarette smoking is a major cause of preventable morbidity and mortality. Worldwide, more than 3 million people currently die each year from cigarette smoking (Aurelio, 2005). The risk of death in the smokers measured by the number of cigarettes smoked daily, the duration of smoking, the degree of inhalation and the age of initiation (Lubin *et al*, 2007). Cigarette smoke contains over 4000 different chemicals, 400 of which are proven to be carcinogenic; it also contains various oxidants such as oxygen free radicals and volatile aldehydes which are probably the major causes of damage to biomolecules .Cigarette smoking yields chemical substances with high cytotoxic potentials. Cigarette smoke consists of many chemicals, including nicotine, tar with its many carcinogens, and gaseous compounds including carbon monoxide (Benowitz *et al.*, 2007).

Theeffect of it in kidney through rising level of serum cadmium and lead resulting in glomerular dysfunction (Satarug S *et al*; 2004).

Some earlier studies found increase inplasma Urea and creatinine levelsconducted in al Mosul population (adnan; 2010), and decrease Uric acid level(Bassam*et al*; 2008).And those studies have been reported primarily fromal mosul, while no study of the effects of the cigarette smoking on plasmaUrea,creatinine and uric acid levelsfor persons living in Sudan country had performed or published. Thus, this study was conducted to assessplasma Urea,creatinine and uric acid levels among Sudanese male smokers.

## **1.2 Rationale:**

Every year's hundreds of thousands of people around the world die from disease caused by cigarette smoking. Cigarette smoking has much serious effect on human body and cause different diseases such as cardiac disease, lung cancer, gastrointestinal, immune and metabolic syndrome. It contains numerous harmful substances, Nicotine is one of these substances that may be acquired through active and passive smoking; it travelling rapidly in the blood stream and it metabolize by various pathways kidney is one of them. Carbon monoxide binding to hemoglobin in red blood cells; it responsible for high toxicity effect and also disrupt the antioxidant defense mechanism in human body which cause oxidative damage to kidney, liver, lung, brain and heart .

Some studies have demonstrated increase plasma urea and creatinine level, and decreased plasma uric acid level in smoker compared to non smoker.

This study was conducted to assess the level of plasma urea, creatinine and uric acid in Sudanese smokers and to find if there is relationship between duration of smoking, age and number of cigarette smoked per day.

### **1.3 Objectives:**

#### **1.3.1 General objective:**

To assess urea, creatinine and uric acid levels among Sudanese smokers in Khartoum state.

#### **1.3.2 Specific objectives:**

1. To estimate the urea, creatinine and uric acid levels among male cigarettes smokers in comparison to non smokers.
2. To correlate between urea, creatinine, uric acid and duration of smoking, number of cigarette per day and age of smokers.

## **2. Literate review**

### **2.1 Smoking:**

#### **2.1.1 Definition:**

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small, round cylinder called a "cigarette". Smoking is primarily practiced as a route of administration for recreational drug use because the combustion of the dried plant leaves vaporizes and delivers active substances into the lungs where they are rapidly absorbed into the bloodstream and reach bodily tissue. In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologically active alkaloid nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs. In some cultures, smoking is also carried out as a part of various rituals, where participants use it to help induce trance-like states that, they believe, can lead them to "spiritual enlightenment" (West *et al* ,2007).

Cigarettes are primarily industrially manufactured but also can be hand-rolled from loose tobacco and rolling paper. Other smoking implements include pipes, cigars, bidis, hookahs, vaporizers, and bongs. Smoking-related diseases have been shown to kill approximately half of long term smokers when

compared to average mortality rates faced by non-smokers. A 2007 report states that, each year, about 4.9 million people worldwide die as a result of smoking (West *et al* ,2007).

Smoking is one of the most common forms of recreational drug use. Tobacco smoking is the most popular form, being practiced by over one billion people globally, of whom the majority are in the developing world (Tobacco fact sheet belong to WHO,2014). Less common drugs for smoking include cannabis and opium. Some of the substances are classified as hard narcotics, like heroin, but the use of these is very limited as they are usually not commercially available.

The history of smoking can be dated to as early as 5000 BC, and has been recorded in many different cultures across the world. Early smoking evolved in association with religious ceremonies; as offerings to deities, in cleansing rituals or to allow shamans and priests to alter their minds for purposes of divination or spiritual enlightenment. After the European exploration and conquest of the Americas, the practice of smoking tobacco quickly spread to the rest of the world. In regions like India and Sub-Saharan Africa, it merged with existing practices of smoking (mostly of cannabis). In Europe, it introduced a new type of social activity and a form of drug intake which previously had been unknown (Tobacco fact sheet belong to WHO ,2014)

Perception surrounding smoking has varied over time and from one place to another; holy and sinful, sophisticated and vulgar, a panacea and deadly health hazard. In the 20th century smoking come to be viewed in a decidedly negative light, especially in Western countries. This is due to smoking tobacco being among the leading causes of many diseases such as lung cancer, heart attacks, erectile dysfunction, and birth

defects(Tobacco fact sheet belong to WHO ,2014). The health hazards of smoking have caused many countries to institute high taxes on tobacco products, run ads to discourage use, limit ads that promote use, and provide help with quitting for those who do smoke (Tobacco fact sheet belong to WHO ,2014).

### **2.1.2 Physiology of smoking:**

Inhaling the vaporized gas form of substances into the lungs is a quick and very effective way of delivering drugs into the bloodstream (as the gas diffuses directly into the pulmonary vein, then into the heart and from there to the brain) and affects the user within less than a second of the first inhalation. The lungs consist of several million tiny bulbs called alveoli that altogether have an area of over 70 m<sup>2</sup> (about the area of a tennis court). This can be used to administer useful medical as well as recreational drugs such as aerosols, consisting of tiny droplets of a medication, or as gas produced by burning plant material with a psychoactive substance or pure forms of the substance itself. Not all drugs can be smoked, for example the sulphate derivative that is most commonly inhaled through the nose, though purer free base forms of substances can, but often require considerable skill in administering the drug properly. The method is also somewhat inefficient since not all of the smoke will be inhaled (Leslie Iverson, 2004).The inhaled substances trigger chemical reactions in nerve endings in the brain due to being similar to naturally occurring substances such as endorphins and dopamine, which are associated with sensations of pleasure. The result is what is usually referred to as a "high" that ranges between the mild stimulus caused by nicotine to the intense euphoria caused by heroin, cocaine and methamphetamines(Leslie Iverson, 2004).

Inhaling smoke into the lungs, no matter the substance, has adverse effects on one's health. The incomplete combustion produced by burning plant material, like tobacco or cannabis, produces carbon monoxide, which impairs the ability of blood to carry oxygen when inhaled into the lungs. There are several other toxic compounds in tobacco that constitute serious health hazards to long-term smokers from a whole range of causes; vascular abnormalities such as stenosis, lung cancer, heart attacks, strokes, impotence, low birth weight of infants born by smoking mothers. 8% of long-term smokers develop the characteristic set of facial changes known to doctors as smoker's face (Model D, 1985).

### **2.1.3 Component of cigarette smoke:**

More than 4000 different chemicals have been identified in cigarette smoke. Most of us have a very basic idea that these chemicals can be harmful to health and that the mechanisms whereby this complex mixture of toxins contained in tobacco smoke leads to specific diseases are complex (Jonathan foulds, 2004).

The simplest categorization of the components if cigarette smoking identifies 3 major components: tar, nicotine, and carbon-monoxide (CO). Tar is the black sticky mass that coats the lungs and the airways. There are many hundreds of different chemicals within the tar, some of which have been shown to be carcinogenic in animals and/or humans. The deposition of particles of tar in the lungs and upper airways leads to the blocking of airways and to serious breathing problems, including Chronic Obstructive Pulmonary Disease .The toxic chemicals also cause inflammation and reduce the elasticity of the lungs and hence the ability to inhale and exhale normally. The carbon-monoxide in smoke replaces oxygen in the hemoglobin (a component of blood), adversely affecting

oxygen transport and energy supply, and requiring the heart to do more work to supply the same amount of oxygen to the body. A large number of smoke constituents, and particularly components of the gaseous phase of the tobacco smoke, cause immunologic responses and inflammation in the cells. This causes increased stickiness of the blood which increases the risk of clots (Jonathan foulds, 2004). These processes increase the likelihood of a heart attack, stroke or other problems with the cardiovascular system. Irritants such as nitric oxide cause hypersecretion of mucus and substances such as acrolein, acetone and acetaldehyde cause damage to the small hair-like strands that line the airways (cilia). This damage to the cilia impairs the ability of the cilia to clear mucus, causing breathing difficulties. Years of smoking and daily coating of the lungs and airways in tar leads to irreversible lung damage and ultimately death. Acute nicotine (critical for the development of addiction), increases heart rate, blood pressure and causes peripheral vasoconstriction (i.e. impairs peripheral circulation and thus exacerbates Reynauds' Disease and erectile dysfunction). However, studies of smokeless tobacco users (who have high nicotine exposure like smokers, but without the smoke) compared with smokers, suggest that most of the cardiovascular problems are not caused by nicotine. It therefore appears that it is the chromogenic effects of tobacco smoke exposure (primarily oxidant gases), combined with reduced oxygen supply (carbon monoxide) and increased myocardial oxygen demand (nicotine) that cause the cardiovascular harms from smoking. Include Formaldehyde, Acetaldehyde, Acetone, Acrolein, Propionaldehyde, Crotonaldehyde, Methyl-Ethyl-Ketone, Butyraldehyde, Hydroquinone, Resorcinol, Catechol, Phenol, Cresol (m+p and o), 3- and 4-aminobiphenyl, 1- and 2-aminonaphthene, o-toluidine, o-anisidine, NO, Benzene, Toluene (Jonathan foulds, 2004).



#### **2.1.4 Health effects and regulation:**

Smoking can damage every part of the body. Smoking is one of the leading causes of preventable death globally. In the United States about 500,000 deaths per year are attributed to smoking-related diseases and a recent study estimated that as much as 1/3 of China's male population will have significantly shortened life-spans due to smoking. Male and female smokers lose an average of 13.2 and 14.5 years of life, respectively (Morbetal, 2002). At least half of all life long smokers die earlier as a result of smoking (Doll R *et al*; 2004), (Thun MJ *etal*,1995) The risk of dying from lung cancer before age 85 is 22.1% for a male smoker and 11.9% for a female current smoker, in the absence of competing causes of death. The corresponding estimates for lifelong nonsmokers are a 1.1% probability of dying from lung cancer before age 85 for a man of European descent, and a 0.8% probability for a woman (Thun MJ *et al*,2008)Smoking one cigarette a day results in a risk of heart disease that is halfway between that of a smoker and a non-smoker. The non-linear dose response relationship is explained by smoking's effect on platelet aggregation (Law *et al*, 1997).

Among the diseases that can be caused by smoking are vascular stenosis, lung cancer, heart attacks (Nyboe J *et al*,1989) and chronic obstructive pulmonary disease (Devereux G, 2006) Smoking during pregnancy may cause ADHD to a fetus (Braun JM *et al*;2006).

Smoking is a risk factor in Alzheimer's disease (Cataldo JK *et al*,2010) While smoking more than 15 cigarettes per day has been shown to worsen the symptoms of Crohn's disease (Cosnes J *et al*,1999), smoking has been shown to actually lower the prevalence of ulcerative colitis (Calkins BM; 1989), (Lakatos PL *et al*,2007).

## **2.2 Urea:**

### **2.2.1 Biochemistry of urea :**

Urea constitutes nearly half the non-protein nitrogen substance in blood. It is synthesized in liver from  $\text{CO}_2$  and ammonia during passage of the filtrate through the renal tubules (Michael L Bishop, 2010). The amount reabsorbed depends on urine flow rate and level of hydration. Small amounts of urea (<10% of the total) are excreted ammonia arising from the deamination of amino acids by means of the ornithine or Krebs-Henseleit cycle (Michael L Bishop; 2010). Urea constitutes the major excretory product of protein metabolism. Following synthesis in the liver, urea is carried in the blood to the kidney, where it is readily filtered from the plasma by glomerulus. Most of the urea in the glomerular filtrate is excreted in the urine, although up to 40% is reabsorbed by passive transport through the gastrointestinal (GI) tract and skin. The level of urea in the plasma is governed by renal function and perfusion, the protein content of the diet, and the amount of protein catabolism. The term blood urea nitrogen (BUN) is used extensively when referring to urea measurement because historical assays for urea were based on nitrogen measurement (Michael L Bishop, 2010).

### **2.2.2 Physiology of urea :**

Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances or produced from catabolism of muscle protein are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy (Sakami *et al*, 1963). The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle. The first step in the conversion of amino acids from protein into metabolic waste

in the liver is removal of the alpha-amino nitrogen, which results in ammonia. Because ammonia is toxic, it is excreted immediately by fish, converted into uric acid by birds, and converted into urea by mammals (Imperial College London Retrieved 2015, "Urea").

Ammonia ( $\text{NH}_3$ ) is a common by product of the metabolism of nitrogenous compounds. Ammonia is smaller more volatile and more mobile than urea. If allowed to accumulate, ammonia would raise the pH in cells to toxic levels. Therefore many organisms convert ammonia to urea, even though this synthesis has a net energy cost. Being practically neutral and highly soluble in water, urea is a safe vehicle for the body to transport and excrete excess nitrogen. Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is then dissolved into the blood (in the reference range of 2.5 to 6.7 mmol/liter) and further transported and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat.

In water, the amine groups undergo slow displacement by water molecules, producing ammonia, ammonium ion, and bicarbonate ion. For this reason, old, stale urine has a stronger odor than fresh urine.

### **2.2.3Disease correlation with urea :**

**2.2.3.1Uraemia** can be translated as "urea in the blood". Urea is one of the primary components of urine. It can be defined as an excess of amino acid and protein metabolism end products, such as urea and creatinine, in the blood that would be normally excreted in the urine. *The Uremic Syndrome* can be defined as the terminal clinical manifestation of kidney failure “also called renal failure” (Michael L Bishop, 2010).It is the signs, symptoms and results from laboratory tests which result from inadequate excretory, regulatory and endocrine function of the kidneys (Burtiset *al*, 2006).Both *uremia* and *the uremic syndrome* have been used interchangeably to define a very high plasma urea concentration that is the result of renal failure (Michael L Bishop, 2010).The latter definition will be used for the rest of the article.

**2.2.3.2Azotemia**is another word that refers to high levels of urea, but is used primarily when the abnormality can be measured chemically but is not yet so severe as to produce symptoms. Uremia is the pathological manifestations of severe azotemia (Michael L Bishop; 2010).

There is no specific time for the onset of uremia for people with progressive loss of kidney function. People with kidney function below 50% (i.e. a glomerular filtration rate [GFR] between 50 and 60 mL) and 30 years of age may have uremia to a degree. This means an estimated 8 million people in the United States with a GFR of less than 60 mL have uremic symptoms (Meyeret *al*;2007). The symptoms, such as fatigue, can be very vague, making the diagnosis of impaired renal functiondifficult. Treatment would be to perform dialysis or a renal transplant (Meyer *et al*, 2007).

### 2.2.3.2.1 Types of Azotemia :

Azotemia has three classifications, depending on its causative origin, but all three types share a few common features. All forms of azotemia are characterized by a decrease in the glomerular filtration rate (GFR) of the kidneys and increases in blood urea nitrogen (BUN) and serum creatinine concentrations. The BUN-to-creatinine ratio (BUN:Cr) is a useful measure in determining the type of azotemia. A normal BUN:Cr is equal to 15 (Goljan *et al*, 2007).

**Prerenal azotemia** is caused by a decrease in blood flow (hypoperfusion) to the kidneys. However, there is no inherent kidney disease. It can occur following hemorrhage, shock, volume depletion, congestive heart failure, adrenal insufficiency, and narrowing of the renal artery among other things (Kumar *et al*, 2005).

The BUN:Cr in prerenal azotemia is greater than 20. The reason for this lies in the mechanism of filtration of BUN and creatinine. Renal Plasma Flow (RPF) is decreased due to hypo perfusion which results in a proportional decrease in GFR. In turn, the decreased flow and pressure to the kidney will be sensed by baroreceptors in the Juxtaglomerular (JG) Cells of the afferent arteriole. If the decrease in blood pressure is systemic (rather than occlusion of the renal artery) baroreceptors in the carotid sinus and aortic arch will be stimulated. This leads to sympathetic nerve activation, resulting in renin secretion through  $\beta_1$ -receptors. Constriction of the afferent arterioles causes a decrease in the intraglomerular pressure, reducing GFR proportionally. Renin is the main effector of the juxtaglomerular baroreceptors. Renin is secreted from granules in the JG cells, and once in the blood stream, it acts as a protease

to convert angiotensinogen to angiotensin I, which is converted by angiotensin converting enzyme, to angiotensin II, which, in turn, stimulates aldosterone release. Increased aldosterone levels results in salt and water absorption in the distal collecting tubule (Goljanet *al*, 2007).

A decrease in volume or pressure is a nonosmotic stimulus for antidiuretic hormone production in the hypothalamus, which exerts its effect in the medullary collecting duct for water reabsorption. Through unknown mechanisms, activation of the sympathetic nervous system leads to enhanced proximal tubular reabsorption of salt and water, as well as urea (BUN), calcium, uric acid, and bicarbonate. The net result of these 4 mechanisms of salt and water retention is decreased output and decreased urinary excretion of sodium ( $< 20$  mEq/L). The increased reabsorption of Na leads to increased water and urea reabsorption from the proximal tubules of the kidney back into the blood. In contrast, creatinine is actually secreted in the proximal tubule. This generally leads to a BUN:Cr ratio  $> 20$  and a fractional excretion of Na of  $< 1\%$  and an elevated urine osmolarity (Goljanet *al*;2007). the level of protein metabolism also causes prerenal changes in blood urea connection. a high –protein diet, or increased protein catabolism such as occurs fever, major illness, stress, corticosteroid therapy, and gastrointestinal hemorrhage, may increase urea levels. levels will be decreased protein synthesis, such as late pregnancy and infancy (Michael L Bishop, 2010).

**Primary renal azotemia** (acute renal failure) typically leads to uremia. It is an intrinsic disease of the kidney, generally the result of renal parenchymal damage. Causes include renal failure, glomerulonephritis, acute tubular necrosis, or any other kind of renal disease (Goljanet *al*, 2007). The BUN:Cr in renal azotemia is less than

15. In cases of renal disease, glomerular filtration rate decreases, so nothing gets filtered as well as it normally would. However, in addition to not being normally filtered, what urea does get filtered is not reabsorbed by the proximal tubule as it normally would be. This results in higher levels of urea in the blood and lower levels of urea in the urine as compared to creatinine. Creatinine filtration decreases, leading to a higher amount of creatinine in the blood. Third spacing of fluids such as peritonitis, osmotic diuresis, or low aldosterone states such as Addison's Disease (Goljan *et al*, 2007).

**Postrenal azotemia:** Blockage of urine flow in an area below the kidneys results in postrenal azotemia. It can be caused by congenital abnormalities such as vesicoureteral reflux, blockage of the ureters by kidney stones, pregnancy, compression of the ureters by cancer, prostatic hyperplasia, or blockage of the urethra by kidney or bladder stones (Kumar *et al*, 2005). Like in prerenal azotemia, there is no inherent renal disease. The increased resistance to urine flow can cause back up into the kidneys, leading to hydronephrosis (Goljan *et al*, 2007).

The BUN:Cr in postrenal azotemia is initially  $>15$ . Over time the BUN:Cr will decrease due to tubule epithelial damage. The increased nephron tubular pressure causes increased reabsorption of urea, elevating it abnormally relative to creatinine (Goljan *et al*, 2007).

### **Hypouremia:**

The major causes of decreased plasma urea levels include decreased protein intake and severe liver.

### 2.2.3.2.2Diagnosis of uremia :

A detailed and accurate history and physical will help determine if uremia is acute or chronic. In the cases of acute uremia, causes may be identified and eliminated, leading to higher chance for recovery of normal renal function, if treated correctly (ABrentAperet *al*, 2015).

**Blood tests:**Primary tests performed for the diagnosis of uremia are basic metabolic panel with serum calcium and phosphorus to evaluate the GFR, blood urea nitrogen and creatinine as well as serum potassium, phosphate, calcium and sodium levels. Principal abnormality is very low (<30) GFR. Uremia will demonstrate elevation of both urea and creatinine, likely *et alas* well as likely depressed calcium levels. As a basic work up a physician will also evaluate for anemia and thyroid and parathyroid functions. Chronic anemia may be an ominous sign of established renal failure. The thyroid and parathyroid panels will help work up any symptoms of fatigue, as well as determine calcium abnormalities as they relate to uremia vs longstanding or unrelated illness of calcium metabolism (ABrentAperet *al*, 2015).

**Urine tests:**A 24-hour urine collection for determination of creatinine clearance may be an alternative, although not a very accurate test due to the collection procedure. Another laboratory test that should be considered is urinalysis with microscopic examination for the presence of protein, casts, blood and pH (ABrentAperet *al*, 2015).

**Radioisotope tests:**The "gold-standard" for determining GFR is iothalamate clearance. However, it may be cost-prohibitive and time-consuming. Clinical laboratories generally calculate the GFR with Modification of Diet in Renal Disease (MDRD) formula or the Cockcroft-Gault formula (ABrentAperet *al*, 2015).



**Other:** In addition, coagulation studies may indicate prolonged bleeding time with otherwise normal values (Meyer *et al*, 2007).

#### **2.2.3.2.3 Treatment:**

Prompt treatment of some causes of azotemia can result in restoration of kidney function; delayed treatment may result in permanent loss of renal function. Treatment may include hemodialysis or peritoneal dialysis, medications to increase cardiac output and increase blood pressure, and the treatment of the condition that caused the azotemia (Goljanet *al*, 2007).

#### **2.2.6 Relation between Urea level and cigarette smoking:**

There are elevated serum cadmium and lead in smokers resulting in glomerular dysfunction which lead to accumulation of urea in blood so from this cigarette smoking elevated serum urea.

#### **2.3 Creatinine:**

A chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter out most of the creatinine and dispose of it in the urine (Karet *al*, 2011).

Although it is a waste, creatinine serves a vital diagnostic function. Creatinine has been found to be a fairly reliable indicator of kidney function. As the kidneys become impaired the creatinine will rise. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys, sometimes even before a patient reports any

symptoms. It is for this reason that standard blood and urine tests routinely check the amount of creatinine in the blood (Deng *et al*, 2007).

### **2.3.1 Biosynthesis of creatinine:**

Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced (Deng *et al*, 2007) via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply).

Creatine is synthesized primarily in the liver from the methylation of glycocyamine (guanidino acetate, synthesized in the kidney from the amino acids arginine and glycine) by S-adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain, where, through phosphorylation, it becomes the high-energy compound phosphocreatine (Taylor *et al*, 1989). During the reaction, creatine and phosphocreatine are catalyzed by creatine kinase, and a spontaneous conversion to creatinine may occur (Allen PJ, 2012).

Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration, but also by proximal tubular secretion. Little or no tubular reabsorption of creatinine occurs. If the filtration in the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which correlates with the glomerular filtration rate (GFR). Blood creatinine levels may also be used alone to calculate the estimated GFR (eGFR).

The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the CrCl rate will overestimate the GFR because hypersecretion of creatinine by the proximal tubules will account for a larger fraction of the total creatinine cleared (Shemesh O *et al*, 1985). Ketoacids, cimetidine, and trimethoprim reduce creatinine tubular secretion and, therefore, increase the accuracy of the GFR estimate, in particular in severe renal dysfunction. (In the absence of secretion, creatinine behaves like inulin.)

An alternate estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. BUN-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a prerenal problem such as volume depletion.

Each day, 1-2% of muscle creatine is converted to creatinine (Taylor;1989). Men tend to have higher levels of creatinine than women because, in general, they have a greater mass of skeletal muscle. Increased dietary intake of creatine or eating a lot of protein (like meat) can increase daily creatinine excretion (Taylor, 1989).

### **2.3.2 Diagnostic use of creatinine:**

Creatinine is a breakdown product of muscle metabolism. The main route of creatinine excretion is through the kidneys, where it is filtered by the glomerulus and also secreted by the proximal tubule.

In a healthy kidney, there is little or no tubular reabsorption of creatinine. Creatinine is therefore a useful indicator of renal health because it is excreted in the urine as an unchanged and easily measured by-product of

muscle metabolism. In a healthy kidney, little or no creatinine is reabsorbed, whereas in kidney disease, the creatinine concentration in the blood may increase. The creatinine concentration in the urine and blood can therefore be used to calculate the rate at which the kidney is clearing creatinine – the creatinine clearance (CrCl) rate. This CrCl rate is correlated with the glomerular filtration rate (GFR), which is important in the clinical assessment of renal function (Ananyamandal, 2015).

The ways in which creatinine is used to assess kidney health are described in more detail below.

- Serum creatinine – This is a measurement of the blood creatinine level, which provides a simple way of checking kidney function. However, the serum creatinine concentration only increases in cases of severe nephron dysfunction, meaning the test is not suitable for detecting early-stage kidney disease. When applied to women, the reference range for serum creatinine is 0.5 to 1.0 mg/dl (45-90  $\mu$ mol/l), while for men the range is 0.7 to 1.2 mg/dl (60-110  $\mu$ mol/l) (Ananyamandal, 2015).
- A more accurate way of assessing kidney function is to calculate the estimated glomerular filtration rate (eGFR). The creatinine levels in the blood and urine can be used to calculate the rate at which creatinine is being cleared by the kidney. This is referred to as the CrCl rate. In turn, the CrCl can be used to calculate the eGFR.
- An eGFR can also be calculated using the serum creatinine level alone, using the following variables:
  - Gender
  - Age

- Weight
- Race

GFR is a useful indicator of kidney function, although calculations using the CrCl rate will give an overestimation of the GFR in cases of severe renal dysfunction because the proximal tubule secretes excess amounts of creatinine, thereby increasing the overall amount of total creatinine cleared. Drugs that can be used to minimize this excess secretion and therefore the accuracy of the eGFR include cimetidine and trimethoprim.

An alternative to using CrCl rate and eGFR to indicate renal function is to interpret the plasma concentration of creatinine along with the blood urea level. A test called the BUN (blood urea nitrogen)-to-creatinine ratio is also used as a measure of kidney health, with BUN rising the more kidney function decreases (Ananyamandal, 2015).

### **2.3.3 Relation between Creatinine level and Cigarette smoking:**

Kidney is one of organs that are adversely influenced by smoking so cigarette smoking will lead to elevated serum creatinine compared with non-smokers.

## **2.4 Uric acid**

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula  $C_5H_4N_4O_3$ . It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides. High blood concentrations of uric acid can lead to gout. The chemical is associated

with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones (McCrudden *et al*, 2008).

Uric acid was first isolated from kidney stones in 1776 by Scheele (Scheele *et al*, 1776). As far as laboratory synthesis is concerned, in 1882, Ivan Horbaczewski claimed to have prepared uric acid by melting urea hydrogen peroxide with glycine, trichlorolactic acid, and its amide. Soon after, repetition by Eduard Hoffmann shows that this preparation with glycine gives no trace of uric acid, but trichlorolacetamide produces some uric acid. Thus, Hoffmann was the first to synthesize uric acid (Behrend *et al*, 1925).

#### **2.4.1 Solubility of uric acid :**

In general, the water solubility of uric acid and its alkali metal and alkaline earth salts is rather low. All these salts exhibit greater solubility in hot water than cold, allowing for easy recrystallization. This low solubility is significant for the etiology of gout. The solubility of the acid and its salts in ethanol is very low or negligible. In ethanol water mixtures, the solubilities are somewhere between the end values for pure ethanol and pure water (McCrudden *et al*, 2008).

#### **2.4.2 Biology of uric acid:**

The enzyme xanthine oxidase makes uric acid from xanthine and hypoxanthine, which in turn are produced from other purines. Xanthine oxidase is a large enzyme whose active site consists of the metal molybdenum bound to sulfur and oxygen (Hille *et al*, 2005). Within cells, xanthine oxidase can exist as xanthine dehydrogenase and xanthine oxidoreductase, which has also been purified from bovine milk and spleen

extracts (Hori *et al*,1992) Uric acid is released in hypoxic conditions (Baillie *et al*,2007).

In humans and higher primates, uric acid is the final oxidation (breakdown) product of purine metabolism and is excreted in urine. In most other mammals, the enzyme uricase further oxidizes uric acid to allantoin (Angstadt *et al*,1997). The loss of uricase in higher primates parallels the similar loss of the ability to synthesize ascorbic acid, leading to the suggestion that urate may partially substitute for ascorbate in such species (Proctor P ,1970). Both uric acid and ascorbic acid are strong reducing agents (electron donors) and potent antioxidants. In humans, over half the antioxidant capacity of blood plasma comes from uric acid (Maxwell *et al*,1997).

The Dalmatian dog has a genetic defect in uric acid uptake by the liver and kidneys, resulting in decreased conversion to allantoin, so this breed excretes uric acid, and not allantoin, in the urine (Friedman *et al*,1948).

In birds and reptiles, and in some desert dwelling mammals (e.g., the kangaroo rat), uric acid also is the end-product of purine metabolism, but it is excreted in feces as a dry mass. This involves a complex metabolic pathway that is energetically costly in comparison to processing of other nitrogenous wastes such as urea (from urea cycle) or ammonia, but has the advantages of reducing water loss and, hence, reducing the need for water (Hazard *et al*,2004).

In humans, *about* 70% of daily uric acid disposal occurs via the kidneys, and in 5-25% of humans, impaired renal (kidney) excretion leads to hyperuricemia (Vitart V *et al*,2008).

### **2.4.3 Physiology of uric acid:**

Purines, 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 liver to the kidney, where it is filtered by the glomerulus. Reabsorption of 98% to 100% of the uric acid from the glomerular filtrate occurs in the proximal tubules. Small amount of uric acid is secreted by distal tubules into urine. Renal excretion accounts for about 70% uric acid elimination; the remainder pass into the gastrointestinal tract and is degraded by bacterial enzyme (Michael L Bishop, 2010).

Nearly all of the uric acid in plasma is present as mono sodium urate. At the pH of plasma (pH=7), urate is relatively insoluble at concentration greater than 6.8mg/dl, the plasma is saturated. As a result, urate crystals may form and precipitate in the tissue. In acidic urine (pH<5.75), Uric acid is the predominant species and uric acid crystals may form.

The daily synthesis rate of uric acid is approximately 400mg. Source contribute another 300mg. In men consuming a purine free diet, the total body pool of exchangeable urate is estimated at 1200mg. In women it is estimated to be 600mg. By contrast, patient with gout arthritis and tissue deposition of urate may have urate, pools as large as 18,000 to 30,000mg. Over production of uric acid may result from increased synthesis of purine precursors (Michael L Bishop, 2010).

### **2.4.4 Clinical application of uric acid:**

Uric acid is measured to assess inherited disorders of purine metabolism; to confirm diagnosis and monitor treatment of gout, to assist in the diagnosis of renal calculi, and to prevent uric acid nephropathy during



chemotherapeutic treatment and detect kidney dysfunction (Michael L Bishop, 2010).

### **2.4.5 Hyperuricaemia:**

Hyperuricaemia is a level of uric acid in the blood that is abnormally high. In humans, the upper end of the range is  $360\mu\text{mol/l}$  ( $6\text{mg/dl}$ ) for women and  $400\mu\text{mol/l}$  ( $6.8\text{mg/dl}$ ) for men (Philip D Mayne, 1996).

Factor that may contribute to hyperuricemia:

1. Increase rate of urate formation :
  - Increase synthesis of purines.
  - Increased turnover of nucleic acid.
2. Reduced rate of excretion:

Increase synthesis due to impaired feedback control causing primary hyperuricaemia whereas abnormalities of the other three steps are causes of the secondary hyperuricaemia (Philip D Mayne, 1996).

### **Primary hyperuricaemia:**

1. inherited disorders of purine metabolism are associated with significant increase in physiological uric acid concentrations. Lesch – Nyhan syndrome is an x linked genetic disorder caused by the complete deficiency of hypoxanthine guanine phosphoribosyltransferase. "HGPRT, EC2.4.2.8" an important enzyme in biosynthesis of purine. Lack of this enzyme prevents the realization of purine bases in the nucleotide salvage pathway and results in increased de novo synthesis of purine nucleotides and high plasma and urine concentration of uric acid. Neurologic syndrome, mental retardation and self mutilation characterize this extremely rare disease (Philip D Mayne, 1996).

2. Mutation in the first enzyme in the purine synthesis pathway (phosphoribosyl pyrophosphate synthetase )(PRPP synthetase)also caused elevated uric acid concentration (Philip D Mayne, 1996).

### **Secondary hyperuricaemia:**

1. Glucose 6 phosphatase deficiency :

increase uric acid concentration is found secondary to glycogen storage disease (deficiency of glucose -6-phosphatase)and fructose intolerance (deficiency of fructose -1-phosphate alolase).

The tendency to hyperuricemia in these patient may be related directly to the inability to convert glucose 6 phosphate (G6P)to glucose mono GOP is available for metabolism through intracellular pathways including the pentose-phosphate pathway,thus increasing ribose phosphate synthesis.This may accelerate the first step in purine synthesis with consequent urate over production lactic acid may reduce renal urate excretion.

2. Increased catabolism of nucleic acid :

Another common cause of elevated plasma uric acid concentration is increased metabolism of cell nuclei as occur in patient on chemotherapy for such proliferative disease as leukemia,lymphoma,multiple myeloma and polycythemia.Monitoring uric acid concentration in these patient is important to avoid nephrotoxicity (Philip D Mayne, 1996).

3. Chronic renal disease :

Cause increase uric acid concentration because filtration and secretion are impaired.

However uric acid not useful as an indicator of renal function because many factor affected its plasma concentration .

Reduced excretion of urate due to:

- i. Renal glomerular dysfunction .
- ii. Thiazide diuretic: Although hyperuricaemia is relatively common during diuretic treatment clinical gout is rare complication .it may however be precipitated in those patient with gout tendency.
- iii. Prolonged metabolic acidosis (Philip D Mayne, 1996).

#### **2.4.6 Hypouricemia:**

Hypouricemia is a level of uric acid in blood serum that is below normal. In humans, the normal range of this blood component has a lower threshold set variously in the range of 2mg/dl to 4mg/dl, while the upper threshold is 530  $\mu\text{mol/l}$  (6mg/dl) for women and 619  $\mu\text{mol/l}$  (7mg/dl) for men.

Hypouricemia result of treatment of hyperuricemia it is unimportant finding associated with proximal renal tubules in which reabsorption of urate is reduced. It is also unimportant finding in some patient receiving parenteral nutrition.

#### **2.4.7 Relation between Uric acid level and cigarette smoking:**

Cigarette smoke contains superoxide and reactive nitrogen species that readily react with various biomolecules which cause oxidative damage to endothelial cells which result in nitric oxide shortage ,therefore imbalance between oxidant and antioxidant occur and uric acid is one of antioxidant so it will be decrease.

### **3. Materials and Methods**

#### **3.1 Material**

**3.1.1 Study approach:** a quantitative methods were used to measure plasma urea, creatinin and uric acid levels in Sudanese in cigarette smokers in Khartoum state, during the period from April to May 2015.

**3.1.2 Study design:** this is case control study.

**3.1.3 Study area:** Khartoum state.

**3.1.4 Target population:** eighty smokers were enrolled as test group and fifty non smokers as control group were enrolled in this study according to inclusion and exclusion criteria.

**Inclusion Criteria:** Cigarettes male Smokers in Khartoum state .

**Exclusion criteria:** Cigarettes male Smokers who have renal disease, alcohol abuse, joint disease, cardiac disease, gout and hypertension were excluded.

**3.1.5 Ethical consideration** The aim and benefits of this study were explained to participations and an informed consent was obtained from each participation.

**3.1.6 Data collection and analysis:** (Appendix I)

**3.1.6.1 interview a questionnaire:** was used for each participant in this study.

#### 3.1.6.2. Blood samples collection:

After informed consent, local antiseptic 70% ethanol was used to clean the skin. Venous blood (2.5mls) were taken from each participant by standard procedures divided into one container in heparin anti-coagulant container ( for plasma urea, creatinine and uric acid) and then centrifuged at 3000 rpm for 3 minutes and obtained plasma for BUN, creatinine and uric acid the plasma was separated in a pandov tube and kept in refrigater until used.

#### 3.1.7 Biochemical measurement and instruments used:

Mindery-semi automated were used for estimation Urea, creatinine and uric acid.

### 3.2 Methods:

#### 3.2.1 Measurement of blood urea:

**3.2.1.1 Principle:** Urease catalyzed conversion of urea to ammonium in modified Berthelot reaction .the ammonium ions react with mixture of salicylate, hypochlorite ,nitro prusside to yield a blue–green dye (indo phenol).the intensity of this dye is proportional to the concentration of urea in sample

**3.2.1.2. Reagent composition:** (see appendix II).

**3.2.1.3 Procedure:** (see appendix II).

**3.2.1.4 Calculation:**(see appendix II)

### **3.2.2 Measurement of plasma creatinin:**

**3.2.2.1 Principle :** Creatinine in sample react with picrate in alkaline medium forming a colored complex (Jaffe" method ). The absorbance of the color produced measured at wave length 492nm,is directly proportional to creatinine concentration in the sample.

**3.2.2.2 Reagent composition:** (see appendix III).

**3.2.2.3 Procedure:** (see appendix III).

**3.2.2.4 Calculation:**(see appendix III).

### **3.2.3 Measurement of plasma uric acid:**

**3.2.3.1 Principle:** Uricase oxidize uric acid to allantoin and carbon dioxide and hydrogen peroxide by peroxidase break down into water and oxygen which accepted by oxygen acceptor para-aminophenazol to give quinoneimine pink color which absorbed at filter( 520+2)nm.the color produced proportion to uric acid in specimen .

**3.2.3.2 Reagent composition:**(see appendix IV).

**3.2.3.3 Procedure:**(see appendix IV).

**3.2.3.4 Calculation :**(see appendix IV).

**3.3 Quality control:** The precision and accuracy of all methods in this study were checked each time batch was analyzed by including commercially prepared control sera.

**3.4. Statistical analysis:** Statistical package for social science (spss version 16) computer software was used for data analysis.

The mean and standard deviation of plasma level of Urea, creatinine and uric acid was calculated and t-test was used for comparison (significant level was set at  $p < 0.05$ ).

Linear regression analysis was used to assess correlation between the duration, number of cigarette per day, Age) and the plasma level of Urea, creatinine and uric acid and result presented in the form of tables and figures.

## 4. Results

The levels of biochemical parameter of plasma urea, creatinine and uric acid in cigarette smokers compared with non-cigarette smokers, presented as follow:

Table (4.1) represents the mean of the levels of plasma urea and creatinine (mg/dl) in cigarette smokers and control subjects were significantly increase in cigarette smokerscomparsion with control group.

Urea (mean  $\pm$ sD: 30.0 $\pm$ 6.8 versus 25.4 $\pm$ 5.9 mg/dl. P.value =0.000).Creatinine (mean  $\pm$ sD: 1.1  $\pm$ 0.23 versus 0.7  $\pm$  0.2 mg/dl. P.value =0.000).

There was significant decrease between the mean of the level of plasma uric acid in cigarette and control group. (mean  $\pm$ sD : 5.06  $\pm$ 1.1 versus 5.6 $\pm$ 0.80 mg/dl p. value =0.01).

Figure(4.1) shows that correlation between age and urea level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.29$  p.value =0.03 (significant positive correlation).

Figure (4.2)shows that correlation between number of cigarette and urea level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.031$  p.value =0.79 (No correlation).

Figure (4.3) shows that correlation between duration of smoking and urea level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.63$  p.value 0.00 (significant positive correlation).

Figure (4.4) shows that correlation between age and creatinine level (mg/dl) among Sudanese smokers.Scatter plot showing  $r=0.27$  p.value 0.07 (significant positive correlation).



Figure (4.5) shows that correlation between duration of smoking and creatinine level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.51$  p.value 0.00 (significant positive correlation).

Figure (4.6) shows that correlation between number of cigarette and creatinine level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.02$  p.value 0.84 (No correlation).

Figure (4.7) shows that correlation between age and uric acid level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.22$  p.value 0.02 (significant negative correlation).

Figure (4.8) shows that correlation between duration of smoking and uric acid level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.39$  p.value 0.00 (significant negative correlation).

Figure (4.9) shows that correlation between number of cigarette and uric acid level (mg/dl) among Sudanese smokers. Scatter plot showing  $r=0.009$  p.value 0.94 (No correlation)

**Table (4.1):** The comparison of Urea, creatinine and uric acid levels (mg/dl) in Sudanese smokers and control group.

Parameter	Smokers	Non smokers	P value
-----------	---------	-------------	---------

Urea(mg/dl)	29.24+6.753	25.43+5.900	0.000
creatinine(mg/dl)	1.054+.2339	.760+.2860	0.000
Uric acid(mg/dl)	5.066+1.0682	5.620+.8062	0.01

\*Data represent mean  $\pm$  SD, P.value  $\leq 0.05$  Considered significant.

\*\* Independent sample T test was used for comparison, value considered significant at level  $\leq 0.05$ .

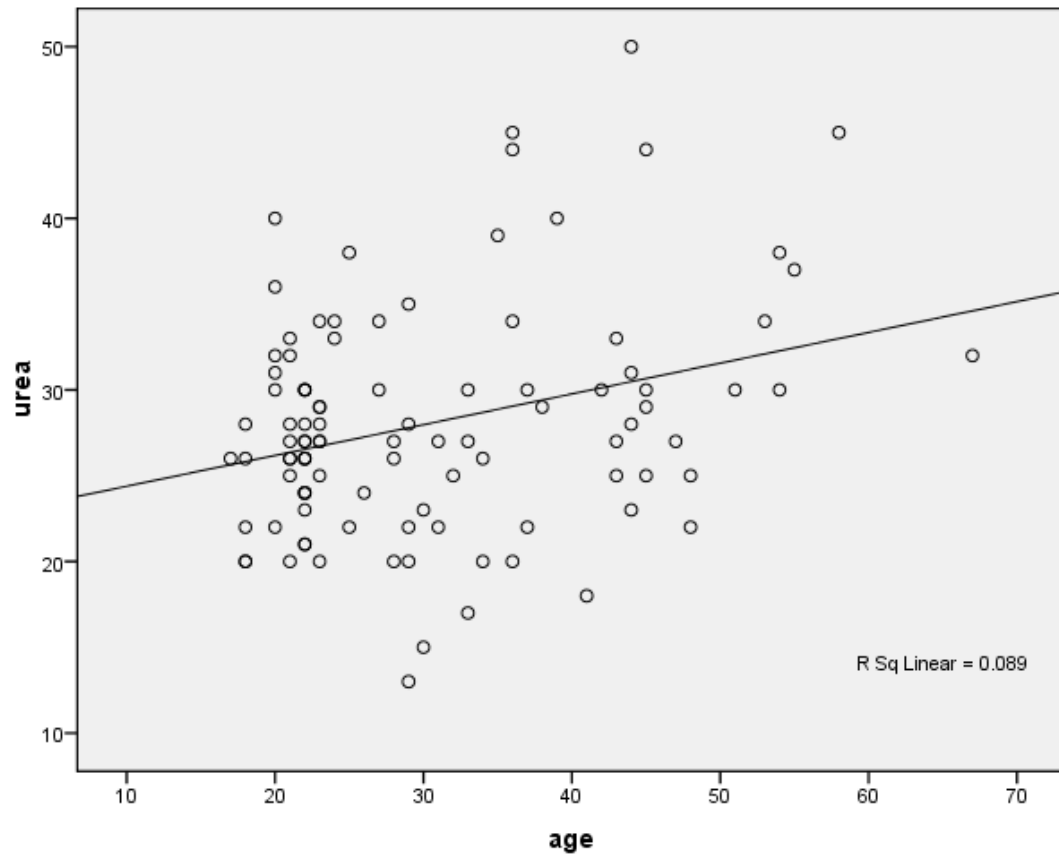


Figure (4.1): Scatter plot of age and urea among Sudanese smokers.  
( $p=0.03$ ,  $r=0.29$ )

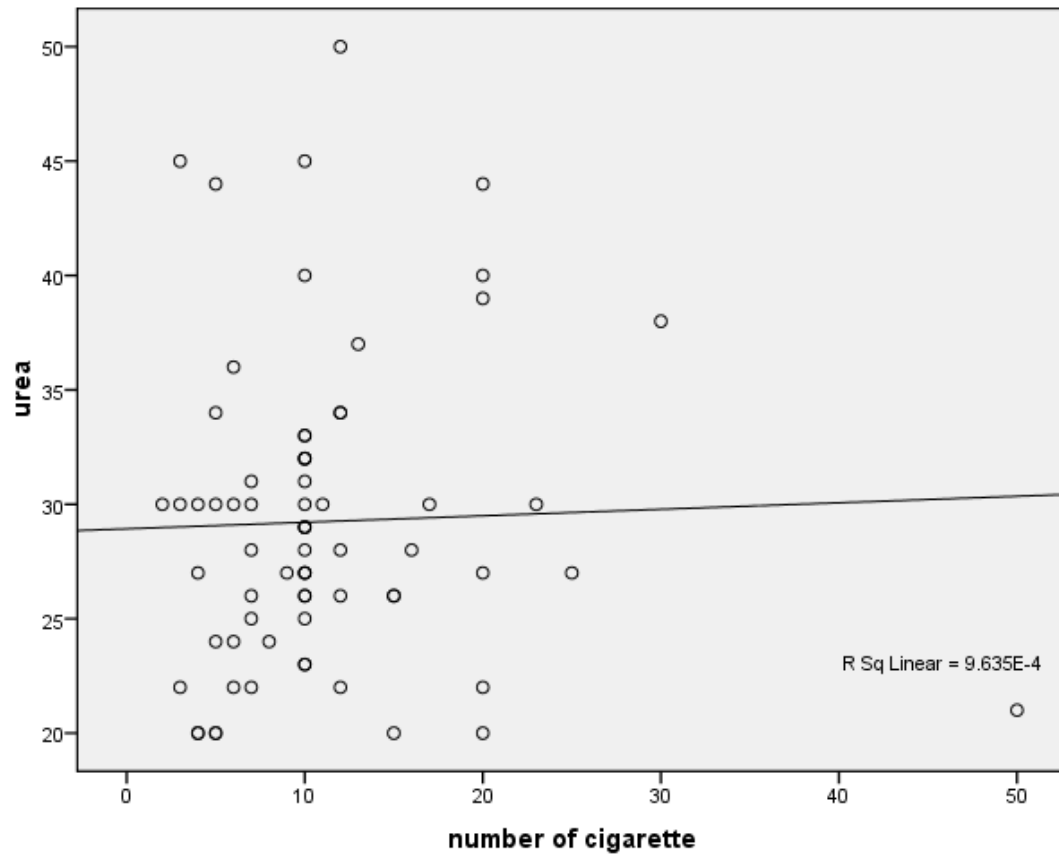


Figure (4.2): Scatter plot of number of cigarette and urea among Sudanese smokers.

( $p=0.79$ ,  $r=0.031$ )

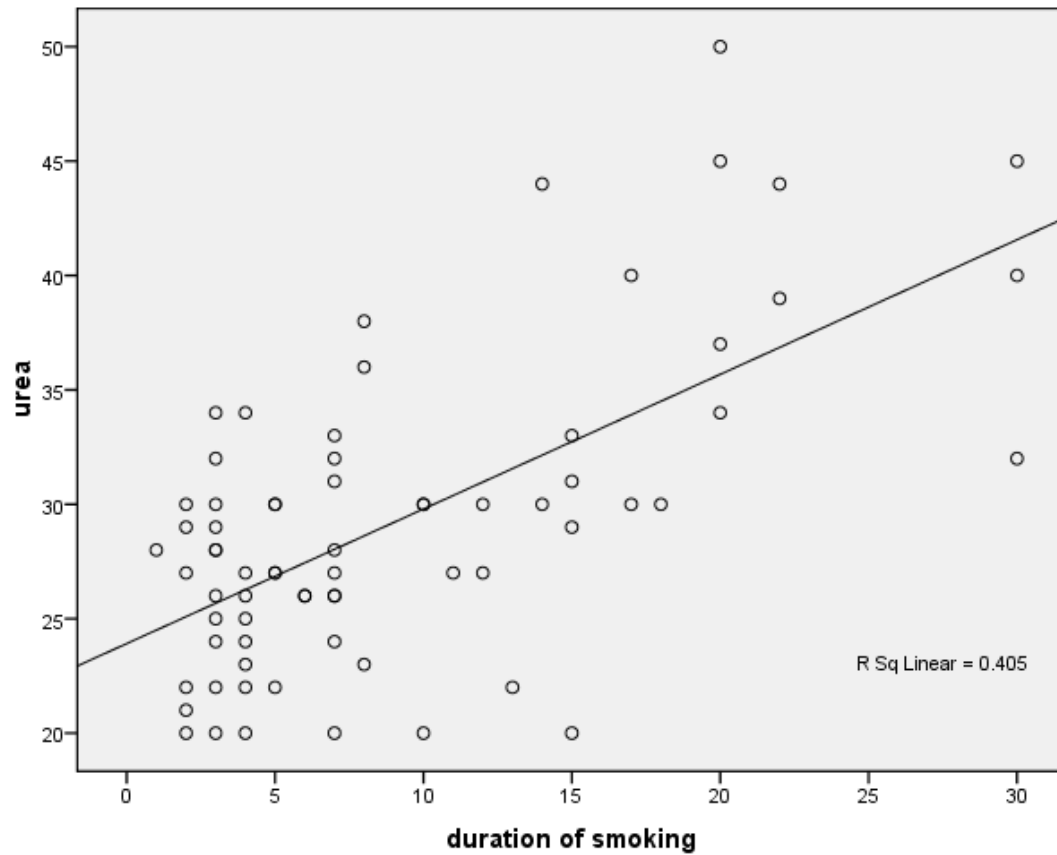


Figure (4.3):Scatter plot of urea level and duration of smoking among Sudanese smokers.

( $p=0.00$ ,  $r=0.63$ )

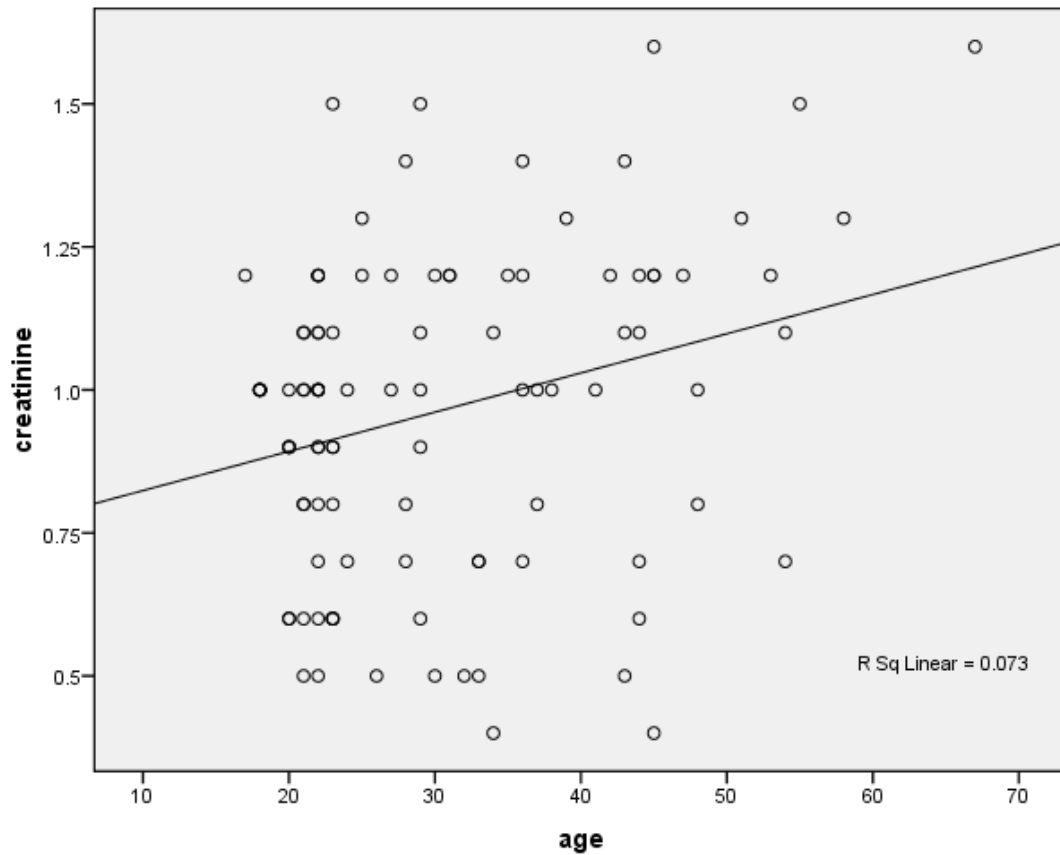


Figure (4.4): Scatter plot of age and creatinine among Sudanese smokers.  
( $p=0.07$ ,  $r=0.27$ )

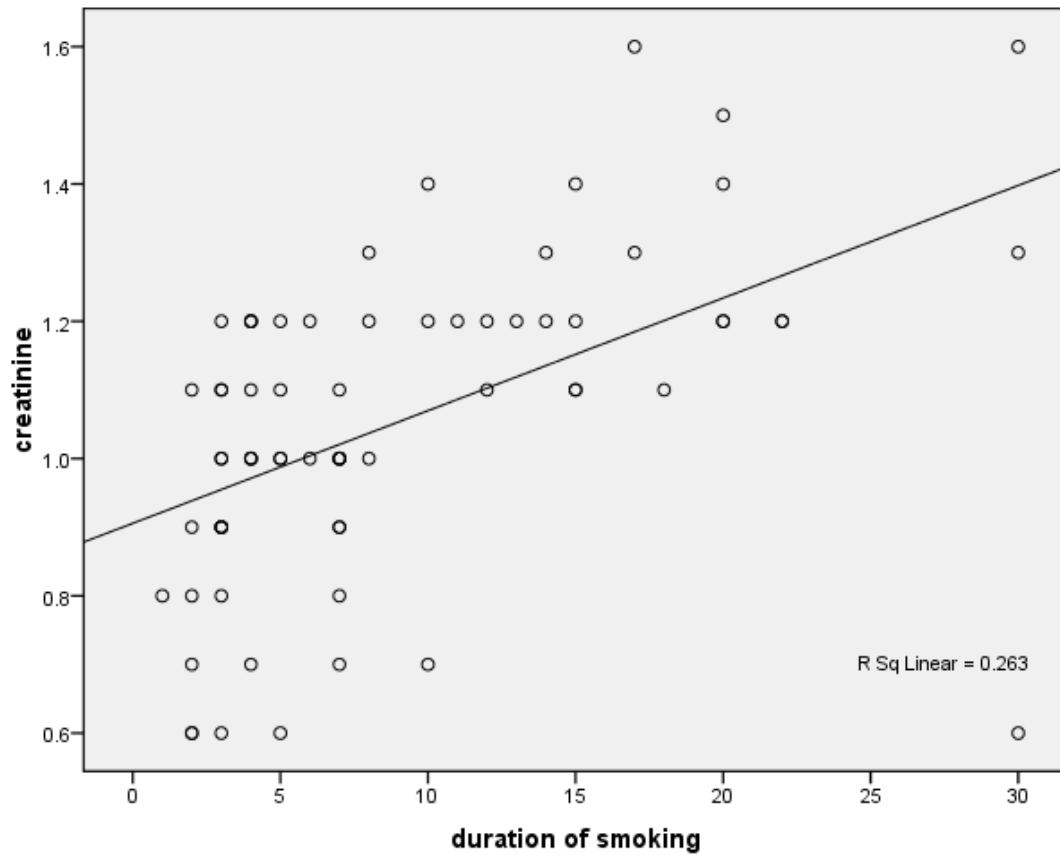


Figure (4.5): Scatter plot of creatinine level and duration of smoking among Sudanese smokers.

( $p=0.00$  , $r =0.51$ )

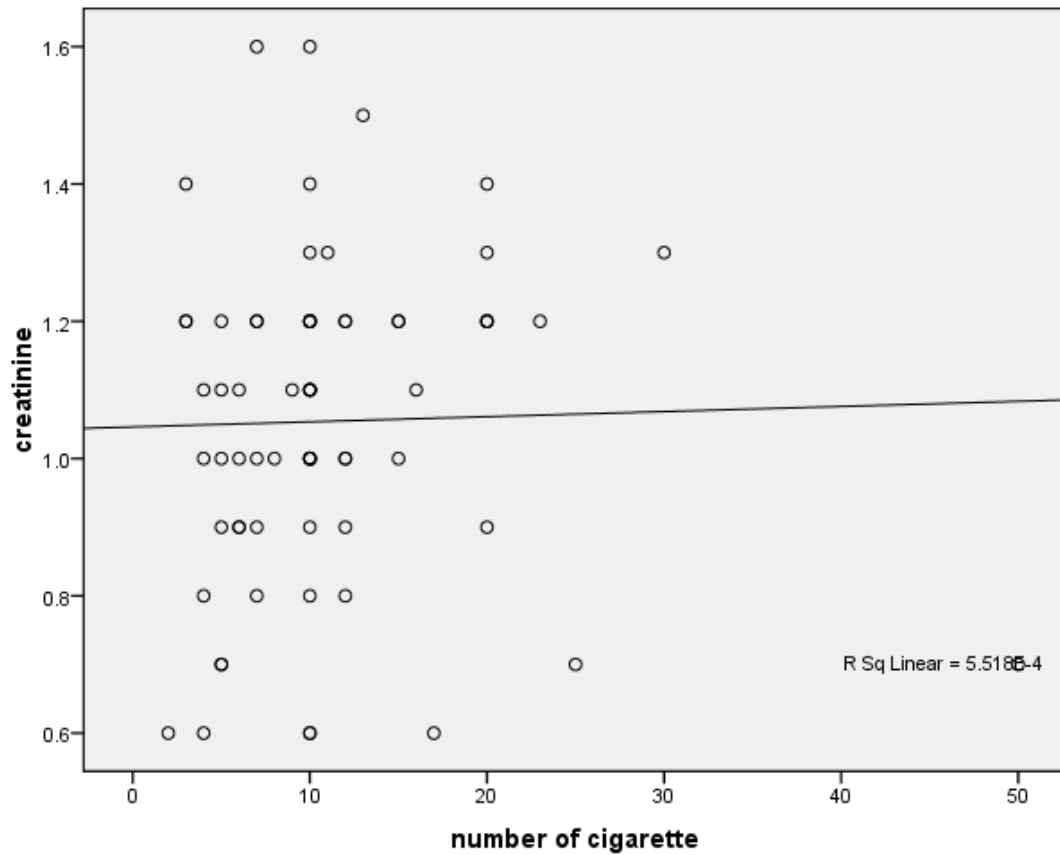


Figure (4.6): Scatter plot of number of cigarette and creatinine among Sudanese smokers.

( $P=0.48$  ,  $r=0.02$ )



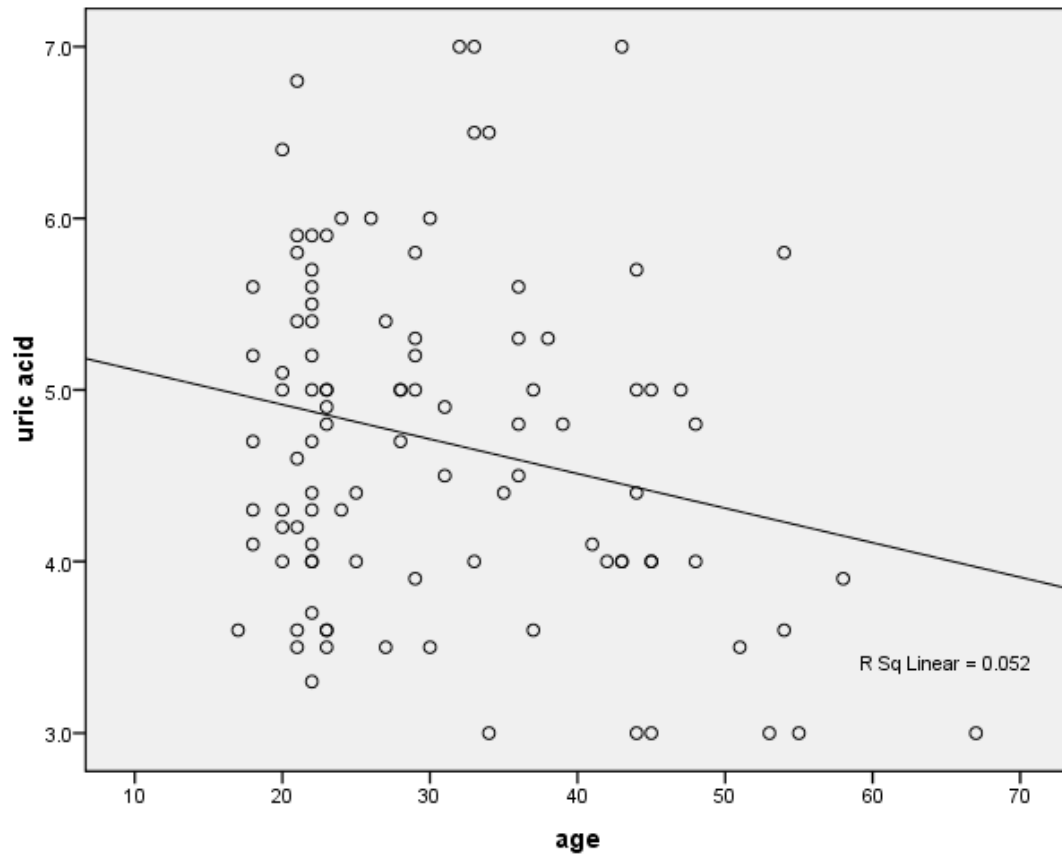


Figure (4.7): Scatter plot age and uric acid levels among Sudanese smokers.

( $p=0.02$  , $r=0.22$ )

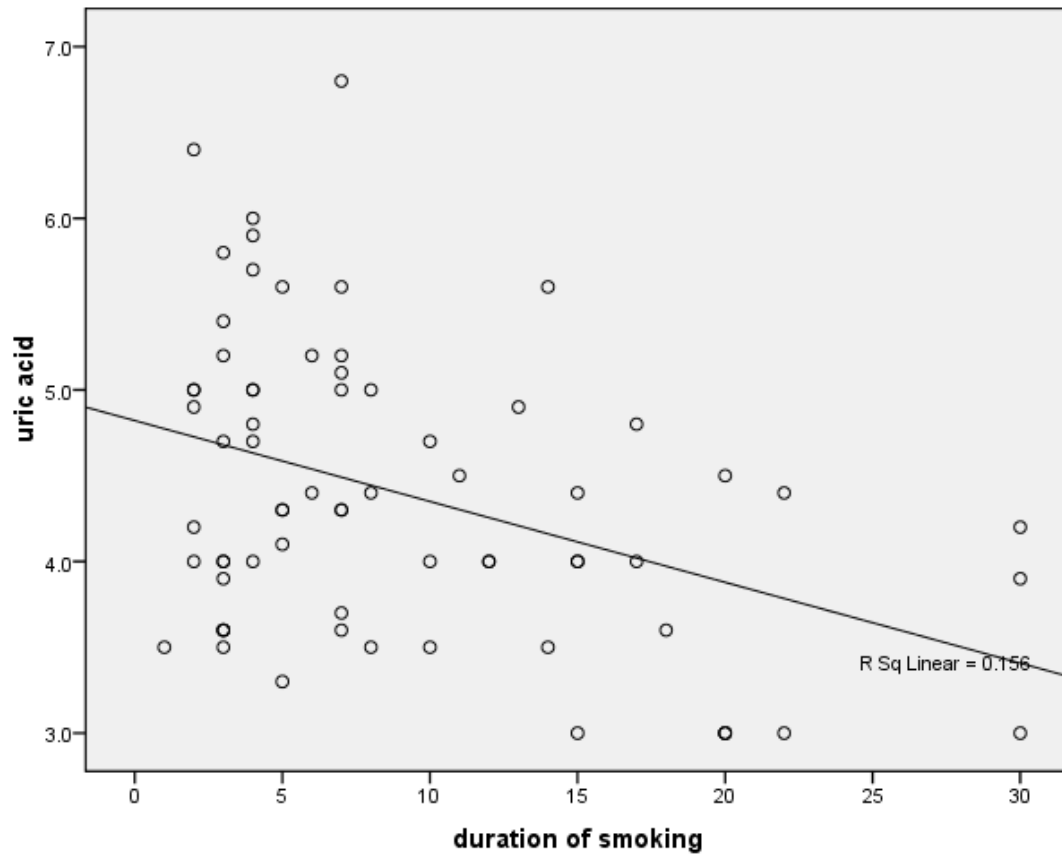


Figure (4.8): Scatter plot of uric acid level and duration of smoking among Sudanese smokers.

( $p=0.00$ ,  $r=0.39$ )

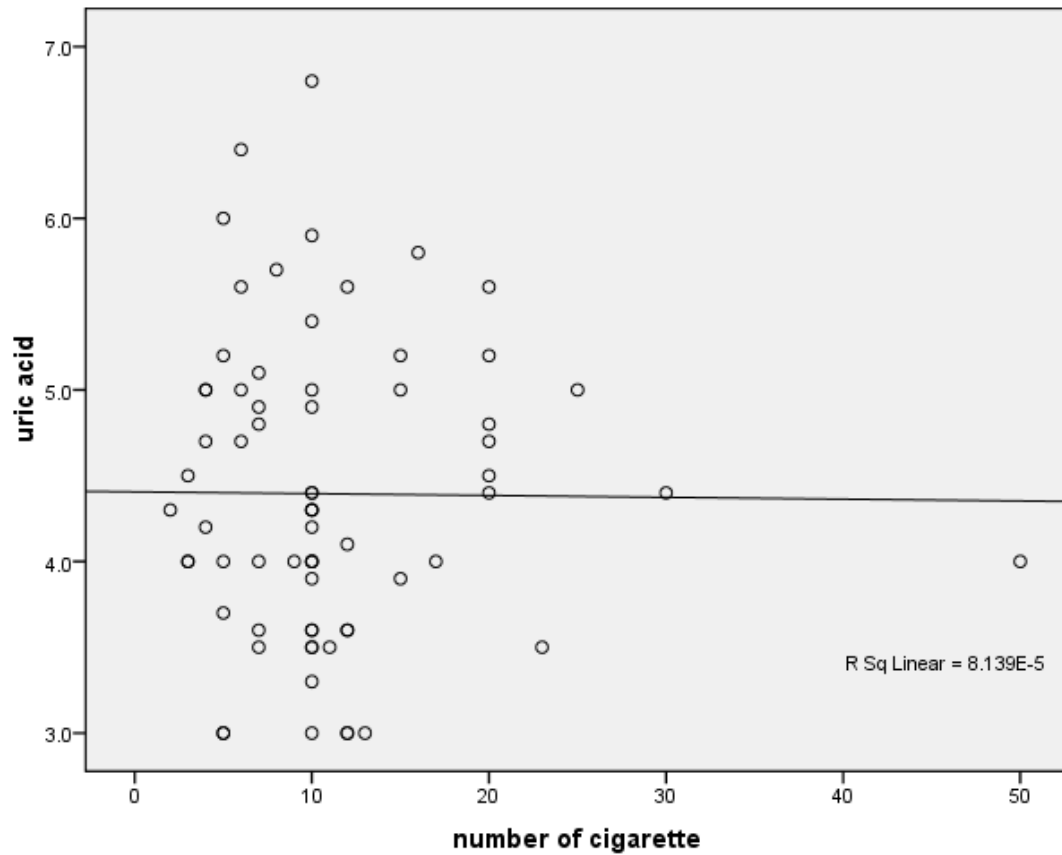


Figure (4.9): Scatter plot of number of cigarette and uric acid among Sudanese smokers.

( $p=0.94$  , $r=0.009$ )

## 5. Discussion, conclusion and recommendation

### 5.1 Discussion

There are many evidences that smoking is harmful and lead to death. Worldwide the effects of smoking are estimated to kill about 3million per year (Tsuchiya *et al*, 2002).

This study conducted to study the effect of smoking on the levels of urea, creatinine and uric acid in male.

The results obtained from this study indicated that, there were significant increasing of urea and creatinine in the blood of smokers when compared with control .Urea ( $29.2 \pm 6.7$  versus  $25.4 \pm 5.9$ mg/dl, p.value= 0.000). Creatinine ( $1.05 \pm 0.233$  versus  $0.76 \pm 0.28$  mg/dl, p.value =0.000).

This result was in agreement with finds done by Adnan which showed that there were significantly increased in the levels of urea and creatinine in smokers compared with non-smokers subject (Adnan., 2010). The cause of these results can be explained as cigarette smoking increase renovascular resistance that lead to a significant fall in glomerular filtration rate (GFR). The result agrees with another result which find confirmed that the decrease in GFR will lead to increase of urea reabsorption. (RitzEet *al* ,1998).This result agreed with previous study which found that smoking is an established risk factor for arteriosclerosis,including the renal arteries.It is also associated with arteriolar hyalinosis and thickens of small arteries in kidney and various other organs (Auerbach; 1968; Black *et al*, 1983; Tracy *et al* ,1994).

The result disagreed with result carried by (Chan-Yeung ,1981) which found that smoking was significantly associated with lower level of urea (blood urea nitrogen),but there was no significant with creatinine level.

In this study the comparison of level of uric acid between case and control showed that, there was significant decreasing of level of uric acid in smokers compared with control ( $5.06 \pm 1.1$  versus  $5.6 \pm 0.81$ mg/dl p.value =0.01), this result was in agreement with previous study carried by (Bassam *et al*; 2008)which found that serum uric acid was significantly lower in smokers. The finding of this study showed that there was a significant positive correlation between age and urea,creatinine levels.The result was in agreement with result carried by (Chan-Yeung;m1981) which found that increasing age was significantly associated with higher Blood urea nitrogen (BUN) and creatinine levels.The finding of this study showed that,there was significant negative correlation between age and uric acid in smokers,this study disagreed with previous study carried by (Bassam *et al*, 2008)which found that no significant difference in age and uric acid level in smokers. Result in this study showed that, there was significant positive correlation between duration of smoking and the levels of urea and creatinine. This result agrees with previous study carried by (Adnan, 2010) which found the effect of smoking on renal function did show significantly bad effects particularly above 10 years. In this study there was significant negative correlation between duration of smoking and uric acid level. This finding agreed with previous study which found that was significant negative correlation of duration between smoking and uric acid level (Bassam *et al*, 2008). Also in this study as appeared in Figures (4.2, 4.6 and 4.9 ), which showed no correlation between number of cigarette smoked per day and the level of urea,creatinine and uric acid. This result is similar to result carried by

many authors (Lauweryset *al*, 1979; Tozawaet *al*, 2002; Bassamet *al*, 2008), which found there were no correlation between average number of cigarette per/day and serum uric acid. This result disagreed with result carried by (Adnan, 2010), which found that smoking of 20 cigarettes per /day results in inhalation of approximately 3.6-6.0 µg of cadmium (Cd) ,which cumulative nephrotoxicant .The nephrotoxicity of Cd results in change in proximal tubular function, characterized by an increase excreation of beta 2-microglobulin and giving rise to classical tubular proteinuria and in aglomerular dysfunction evidenced by increase microglobulin and creatinine in plasma.

## **5.2 Conclusion**

From the results and findings in this study, it is concluded that:

- 1 Plasma Urea and creatinine levels are significantly increased in Sudanese smokers.
- 2 Plasma Uric acid level is significantly decreased in Sudanese smokers.
- 3 Plasma Urea, creatinine and Uric acid levels affected by age and duration of smoking, and not affected by number of cigarette per day.

### **5.3Recommendation**

- 1 Urea, creatinine and uric acid should be regularly monitor in the blood of heavy smokers.
- 2 Health education for the community to know the hazard and complication of cigarette smoking.
- 3 The government must play an obvious role in the war against cigarette smoking.



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**Appendix (1):**

***Sudan University Of Science and Technology***

***College of Graduated Studies***

***Questionnaire***

***Master Degree***

TOPIC:

This is the very secret information and will using for scientific research

Agree(    )                      Disagree(    )

1-name:

2-Gender:

Male(    )                      Female(    )

3- Age /year:

4-Any disease:

5-Any drug used :

6-Are you smoker:

Yes(    )                      No(    )

7-Duration of smoking:

8-Number of cigarette /day:

Exclusion Criteria :

Alcohol consumption

Diabetes Mellitus

Hypertension

Gout

Renal disease

Heart disease

Joint disease

Investigations :

Urea.....mg/dl

Creatinine.....mg/dl

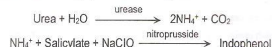
Uric acid.....mg/dl

## Appendix (2):

COD 11536 4 x 50 mL	COD 11537 2 x 250 mL
STORE AT 2-8°C	
Reagents for measurement of urea concentration Only for <i>in vitro</i> use in the clinical laboratory	

### PRINCIPLE OF THE METHOD

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry<sup>1,2,3</sup>.



### CONTENTS

	COD 11536	COD 11537
A1. Reagent	2 x 48 mL	1 x 240 mL
A2. Reagent	2 x 2 mL	1 x 10 mL
B. Reagent	2 x 50 mL	1 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

### COMPOSITION

- A1. Reagent: Sodium salicylate 62 mmol/L, sodium nitroprusside 3.4 mmol/L, phosphate buffer 20 mmol/L, pH 6.9.
- A2. Reagent: Urease > 500 U/mL
- B. Reagent: Sodium hypochlorite 7 mmol/L, sodium hydroxide 150 mmol/L.
- Irritant (X): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.
- S. Glucose/Urea/Creatinine Standard: Glucose 100 mg/dL, urea 50 mg/dL (8.3 mmol/L, BUN 23.3 mg/dL), creatinine 2 mg/dL. Aqueous primary standard.

### STORAGE

- Store at 2-8°C.
- Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.
- Indications of deterioration:
- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.250 at 600 nm (1 cm cuvette).
  - Standard: Presence of particulate material, turbidity.

### REAGENT PREPARATION

- Reagent (B) and Standard (S) are provided ready to use.
- Reagent (A): Transfer the contents of one Reagent A2 vial into a Reagent A1 bottle (Note 1). Mix thoroughly. Other volumes can be prepared in the proportion: 1 mL Reagent A2 → 24 mL Reagent A1. Stable for 2 months at 2-8°C (Note 2).

### ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyser, spectrophotometer or photometer able to read at 600 ± 20 nm.

### SAMPLES

- Serum, plasma or urine collected by standard procedures. Dilute urine 1/50 with distilled water before measurement.
- Urea in serum or plasma is stable for 7 days at 2-8°C. Heparin is recommended as anticoagulant.
- Urea in urine is stable for 3 days at room temperature if microbial growth is prevented.

### PROCEDURE

- Bring the Reagents to room temperature.
- Pipette into labelled test tubes:

	Blank	Standard	Sample
Urea Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

- Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.

- Pipette:

	Reagent (B)		
	1.0 mL	1.0 mL	1.0 mL

- Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.

- Read the absorbance (A) of the Standard and the Sample at 600 nm against the Blank. The colour is stable for at least 2 hours.

### CALCULATIONS

The urea concentration in the sample is calculated using the following general formula:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}$$

### UREA/BUN - COLOR



### UREA/BUN - COLOR UREASE/SALICYLATE

If the Urea Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
A Sample	x 50 = mg/dL urea x 23.3 = mg/dL BUN	x 2500 = mg/dL urea x 1165 = mg/dL BUN
A Standard	x 8.3 = mmol/L urea	x 415 = mmol/L urea

### REFERENCE VALUES

Serum and plasma: 15-39 mg/dL urea = 7-18 mg/dL BUN = 2.5-6.5 mmol/L urea. Concentrations in the neonatal period are lower, and in adults over 60 years of age are higher than in adults. Concentrations also tend to be slightly higher in males than in females.

Urine: 26-43 g/24-h urea = 12-20 g/24 h BUN = 428-714 mmol/24-h urea

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

### QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042), level II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

### METROLOGICAL CHARACTERISTICS

- Detection limit 1.3 mg/dL urea = 0.60 mg/dL BUN = 0.21 mmol/L urea
- Linearity limit: 300 mg/dL = 140 mg/dL BUN = 50 mmol/L urea. For higher values dilute sample 1/5 with distilled water and repeat measurement.
- Repeatability (within run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	1.6 %	20
86 mg/dL = 14.2 mmol/L	0.8 %	20

- Reproducibility (run to run):

Mean urea concentration	CV	n
26 mg/dL = 4.3 mmol/L	2.4 %	25
86 mg/dL = 14.2 mmol/L	1.3 %	25

- Sensitivity: 8.6 mA·dL/mg = 0.143 mA·L/mmol

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

- Interferences: Lipemia (triglycerides 10 g/L) and bilirubin (20 mg/dL) do not interfere. Hemolysis (hemoglobin 2 g/L) and elevated ammonia interfere. Other drugs and substances, may interfere<sup>5</sup>.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

### DIAGNOSTIC CHARACTERISTICS

Urea is synthesized in the liver as a by-product of the deamination of amino acids. Its elimination in the urine represents the major route for nitrogen excretion.

Elevated urea concentration in plasma is found as a result of a high-protein diet, increased protein catabolism, after a gastrointestinal hemorrhage, mild dehydration, shock and heart failure or treatment with glucocorticoids (pre-renal uremia)<sup>4,6</sup>.

Post-renal uremia is caused by conditions that obstruct urine outflow: nephrolithiasis, tumor or prostatic hypertrophy. The usefulness of urea as an indicator of renal function is limited by the variability of its plasma concentration as a result of nonrenal factors<sup>4,6</sup>.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

### NOTES

- It is advisable to wash the Reagent A2 vial with a small volume of the prepared mixture in order to completely rinse the vial and avoid any losses.
- The stability of Reagent A may be drastically reduced when it is not stored at 2-8°C.
- Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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## Appendix (3):

COD 11802 2 x 50 mL	COD 11502 4 x 50 mL	COD 11542 1 x 1 L
STORE AT 15-30°C		
Reagents for measurement of creatinine concentration Only for <i>in vitro</i> use in the clinical laboratory		

### CREATININE



CREATININE  
JAFFÉ

#### PRINCIPLE OF THE METHOD

Creatinine in the sample reacts with picric acid in alkaline medium forming a coloured complex (Jaffé method). The complex formation rate is measured in a short period to avoid interferences<sup>1,2</sup>. Serum and plasma samples contain proteins that react in a non specific way; nevertheless, the results can be corrected subtracting a fixed value. The use of this correction is known as the Jaffé method compensated<sup>3,4</sup>.

#### CONTENTS

	COD 11802	COD 11502	COD 11542
A. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
B. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

#### COMPOSITION

A. Reagent. Sodium hydroxide 0.4 mol/L, detergent.

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

B. Reagent. Picric acid 25 mmol/L.

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL, creatinine 2 mg/dL (177 µmol/L). Aqueous primary standard.

#### STORAGE

Store at 15-30°C.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

#### REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Mix equal volumes of Reagent A and Reagent B. Mix thoroughly. Stable for 1 month at 2-8°C.

#### ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

#### SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/50 with distilled water before measurement. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants. Creatinine in samples is stable for 24 hours at 2-8°C.

#### PROCEDURE

- Bring the Working Reagent and the photometer to 37°C.
- Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Standard (S) or Sample	0.1 mL

- Mix and insert cuvette into the photometer. Start stopwatch.
- Record the absorbance at 500 nm after 30 seconds (A<sub>1</sub>) and after 90 seconds (A<sub>2</sub>).

#### CALCULATIONS

The creatinine concentration in the sample is calculated using the following general formula (Note 2):

$$\frac{(A_2 - A_1) \text{ Sample}}{(A_2 - A_1) \text{ Standard}} \times C \text{ Standard} \times \text{Sample dilution factor} - \text{Corrective Factor}^{4,5,6} = C \text{ sample}$$

If the Creatinine Standard provided has been used to calibrate (Note 3):

	Serum and plasma		Urine
	Jaffé non compensated	Jaffé compensated	
$\frac{(A_2 - A_1) \text{ Sample}}{(A_2 - A_1) \text{ Standard}}$	x 2] = mg/dL	x 2] - 0.37 = mg/dL	x 100] = mg/dL
	x 177] = µmol/L	x 177] - 33 = µmol/L	x 8840] = µmol/L

#### REFERENCE VALUES

Serum and plasma<sup>7</sup>:

Method	Jaffé non compensated	Jaffé compensated
Men	0.9 - 1.3 mg/dL = 80 - 115 µmol/L	0.7 - 1.2 mg/dL = 62 - 106 µmol/L
Women	0.6 - 1.1 mg/dL = 53 - 97 µmol/L	0.5 - 0.9 mg/dL = 44 - 80 µmol/L

Urine<sup>8</sup>:

Men: 14 - 26 mg/kg/24-h = 124 - 230 µmol/kg/24-h  
Women: 11 - 20 mg/kg/24-h = 97 - 177 µmol/kg/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

#### QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

#### METROLOGICAL CHARACTERISTICS

- Detection limit: 0.03 mg/dL creatinine = 2.65 µmol/L creatinine
- Linearity limit: 20 mg/dL = 1768 µmol/L creatinine. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	2.9 %	20
5.3 mg/dL = 468 µmol/L	1.3 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	3.9 %	25
5.3 mg/dL = 468 µmol/L	2.9 %	25

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

- Interferences: Hemoglobin (10 g/L), bilirubin (10 mg/dL), protein and ketonic bodies do not interfere. Lipemia (triglycerides > 2 g/L) may interfere. High concentration of reducing compounds may interfere. Other drugs and substances may interfere<sup>9</sup>.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

#### DIAGNOSTIC CHARACTERISTICS

Creatinine is a catabolic end product of creatine (or phosphocreatine). The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomerulus (small amounts are reabsorbed and are also secreted by the renal tubules).

Creatinine measurement is used almost exclusively in the assessment of kidney function (impaired renal perfusion, loss of functioning nephrons) and in the monitoring renal dialysis<sup>4,8</sup>.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

#### NOTES

- These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
- For measurement in serum or plasma with the Jaffé method compensated, introduce the corrective value for the reaction of nonspecific proteins as a constant factor subtracted from the concentration value obtained<sup>5,6</sup>.
- Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

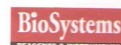
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## Appendix (4):

COD 11821 1 x 50 mL	COD 11521 1 x 200 mL	COD 11522 1 x 500 mL	COD 11540 1 x 1 L
STORE AT 2-8°C			
Reagents for measurement of uric acid concentration Only for <i>in vitro</i> use in the clinical laboratory			

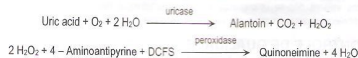
### URIC ACID



URIC ACID  
URICASE/PEROXIDASE

#### PRINCIPLE OF THE METHOD

Uric acid in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry<sup>1,2</sup>.



#### CONTENTS

	COD 11821	COD 11521	COD 11522	COD 11540
A. Reagent	1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

#### COMPOSITION

A. Reagent: Phosphate 100 mmol/L, detergent 1.5 g/L, dichlorophenolsulfonate 4 mmol/L, uricase > 0.12 U/mL, ascorbate oxidase > 5 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.8.

S. Uric Acid Standard: Uric acid 6 mg/dL (357 µmol/L). Aqueous primary standard.

#### STORAGE

Store at 2-8°C.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 520 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

#### REAGENT PREPARATION

Reagent and Standard are provided ready to use.

#### ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37°C
- Analyzer, spectrophotometer or photometer able to read at 520 ± 10 nm

#### SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute urine 1/10 with distilled water before measurement.

Uric acid in serum or plasma is stable for 7 days at 2-8°C. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Uric acid in urine is stable for 4 days at room temperature if pH is adjusted to > 8 with NaOH. Do not refrigerate.

#### PROCEDURE

- Bring the Reagent to room temperature.
- Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Distilled water	25 µL	—	—
Uric Acid Standard (S)	—	25 µL	—
Sample	—	—	25 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

- Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
- Measure the absorbance (A) of the Standard and the Sample at 520 nm against the Blank. The colour is stable for at least 30 minutes.

#### CALCULATIONS

The uric acid concentration in the sample is calculated using the following general formula:

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} = C_{\text{Sample}}$$

If the Uric Acid Standard provided has been used to calibrate (Note 2):

	Serum and plasma	Urine
$\frac{A_{\text{Sample}}}{A_{\text{Standard}}}$	$\times 6 = \text{mg/dL uric acid}$ $\times 357 = \mu\text{mol/L uric acid}$	$\times 60 = \text{mg/dL uric acid}$ $\times 3570 = \mu\text{mol/L uric acid}$

#### REFERENCE VALUES

Serum and plasma<sup>3</sup>

Men: 3.5-7.2 mg/dL = 210-420 µmol/L

Women: 2.6-6.0 mg/dL = 150-350 µmol/L

Urine<sup>3</sup>

250-750 mg/24-h = 1.5-4.5 mmol/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

#### QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042), level II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

#### METROLOGICAL CHARACTERISTICS

- Detection limit: 0.02 mg/dL = 1.19 µmol/L
- Linearity limit: 25 mg/dL = 1487 µmol/L. For higher values dilute sample 1/5 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	CV	n
5.00 mg/dL = 298 µmol/L	0.4 %	20
8.22 mg/dL = 489 µmol/L	0.5 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
5.00 mg/dL = 298 µmol/L	2.1 %	25
8.22 mg/dL = 489 µmol/L	1.9 %	25

- Sensitivity: 33.3 mA·dL/mg = 0.66 mA·L/µmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (2 g/L), bilirubin (2.5 mg/dL) and lipemia interfere. Other drugs and substances may interfere<sup>4</sup>.

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

#### DIAGNOSTIC CHARACTERISTICS

In humans, uric acid is the major product of the catabolism of the purine bases which are obtained partly from the diet and partly from *in vivo* synthesis.

Increased uric acid concentration in serum and urine maybe attributable to an overproduction of urate (increased purine synthesis) or to a defective elimination of urate<sup>5</sup>.

Hyperuricemia is commonly associated with gout, decreased renal function, dehydration, myeloproliferative disorders, and other conditions not well known<sup>1,5</sup>.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

#### NOTES

- These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.
- Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analysers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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