# 1- Introduction and Literature review

## 1.1 Introduction

Human exposure to benzene has been associated with a range of acute and long-term adverse health effects and diseases . Exposure can occur occupationally and domestically as a result of the ubiquitous use of benzene-containing petroleum products, including motor fuels and solvents. Active and passive exposure to tobacco smoke is also a significant source of exposure. Benzene is highly volatile, and exposure occurs mostly through inhalation . Public health actions are needed to reduce the exposure of both workers and the general population to benzene (IARK ,1987).

Benzene is one of the fractionated products of crude oil. The indispensability of this product in our daily life cannot be over emphasized. It widely used as fuel for automobiles and some electricity generating machines. Liquid benzene is known to be very volatile, with several organic and inorganic constituents. Benzene for instance, is reported to contain about 300 different hydrocarbon fractions, most of which are volatile and may evaporate if left exposed, to constitute ubiquitous chemical pollutants in the immediate environment (Zahlsen and Tri-Tugaswati , 1993). A greater percentage of the automobile user and those residing at/and around refueling stations and traffic-congested area may directly or indirectly be exposed to these pollutants in their environments. However, those that are occupationally exposed tend to be at a greater risk of exposure (Smith *et al*., 1993; carballo *et al* ., 1995) .

Most often, much consideration is not given to possible health hazard that might be associated with exposure to constituents of benzene vapours

released into the environment. It has been reported that a higher concentration of unsaturated aromatic hydrocarbons and a lower concentration of the saturated fractions accumulate in the blood of humans and animals equally exposed to petroleum vapour (Zahlsen *et al*., 1993). Based on this report, the potential harmful effect associated with chronic or sub-chronic exposure to benzene vapour should be the concern of the general public and scientific community.

Other adverse effects associated with exposure to petroleum vapours have been reported in both the experimental animals and humans(Wixtron and Brown, 1992 ;Smith *et al*.,1993; Tilbury *et al*.,1993).

The chemical pollutants from petroleum product vapours may be metabolically transformed into various metabolites in the body (Hu and Wells,1994).Some of these metabolites may be very reactive, interacting in various ways with the metabolizing and excreting tissues (mainly the liver and kidneys) to elicit toxic effect (Macfarland *et al*., 1984; Page and cause cellular injury, hence, damage to the tissues). Once the renal tissues are damaged, the overall functionality of the kidneys may be compromised.

The kidney function may be assessed from the level of some electrolytes and metabolites (such as creatinine, urea and blood urea nitrogen ) in the plasma (Nwankwo *et al*.,2006; Atangwho *et al*., Crook ,2007).Renal dysfunction may be caused by several diseased conditions and exposure to certain reactive or toxic metabolites (Chatterjea and shinde,2002; jimoh and Odutuga, 2004; Crook 2007). Renal dysfunction of any kind effects all parts of the nephron to some extent, although sometimes, either glomerular or tubular dysfunction is predominant. The net effect of renal disease on plasma and urine depends on the proportion of glomeruli to

tubules affected and on the number of nephrons involved. In this study, comparative changes in urea and creatinine associated with exposure of male worker at benzene station were assessed.

## 1.2 Renal system

### 1.2.1 Functional anatomy of the kidney:

The kidneys are paired, bean shape organs located retroperitoneal on either side of the spinal column. Macroscopically a fibrous capsule of connective tissue encloses each kidney. When discerned an outer region called the cortex and an inner region called the medulla. The pelvis can also be seen. The bilateral ureters are thick wall canals, which connecting the kidneys to the urinary bladder (Mario Barac; 2004) .

### Gross structure of the kidney

Cortex, medulla, pyramids, renal calyxes and pelvis, ureter. Gross size and weight (300-400g) of kidneys (about 0.5%of body weight )in human.

Each kidney contains about million nephrons and each nephron in made up of five functional segment:

**1.** Glomerulus: capillary tuft surrounded by the expanded of renal tubular known as Bowman's capsule.

**2.** The proximal convoluted tubule located in the cortex.

**3.** The loop of Henle: comprises of the thin descending limb which comprise of a region that is thin and then thick.

**4.** The distal convoluted tubules located in the cortex.

**5.** The collecting duct: formed by two or more distal convoluted tubules as they pass back down through the cortex and the medulla to collect the urine that drains from each nephron

The nephron is the basic unit of renal structure and function (Bishop *et al*: 2005) .

## 1.2.2. Renal physiology:

the kidney have four major functions: the control of extra cellular fluid volume and composition, hydrogen iron homestasis, excretion of waste products of metabolism and hormone production. The best simple test of overall renal function is the plasma creatinine concentration. The presence of proteinuria is a sensitive, although not specific, indicator of damage to the kidney. Other biochemical test, urea, uric acid and electrolytes (sodium, potassium, calcium and phosphorus) (William *et al;* 2005).

There are three basic renal processes:-

**1.** Glomerular filtration.

**2.** Tubular reabsorption.

**3.** Tubular secretion (Bishop *et al*;2005).

acute renal failure (ARF) is a life-threatening condition in which there is potentially reversible deterioration in renal function. In chronic renal failure (CRF), renal function is irreversibly lost; patients eventually require transplantation or long term dialysis. The nephritic syndrome comprises protinuria , hypoprotinaemia and oedema. (William *et al;* 2005).

The formation of urinary calculi is essentially the result of supersaturation of the urine. Disorders of renal tubular function can lead to decrease excretion of substance that is excreted by tubules like hydrogen ions, or to increase excretion of substance that are normally reabsorbed like glucose (William *et al*; 2005).

## 1.3Blood urea:

urea constitutes nearly half NPN substances in the blood. It is synthesized in the liver from CO2 and the ammonia arising from the deamination of amino acid by mean the or Krebs Henseleit cycle. Urea constitute 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 the glomerular filtrate excreted in the urine, although up to 40% is reabsorbed by passive diffusion during passage of the filtrate through proximal tubules. The amount reabsorbed depends on the urine flow rate and level of hydration. Small amounts of urea (<10% of the total )are excreted through the gastrointestinal tract and the 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 blood urea nitrogen (BUN pronounced "B-U-N")test is a measure of the amount of nitrogen in the blood in the form of urea, and a measurement of renal function. Urea is by –product from metabolism of proteins by liver and is removed from the blood by the kidneys. The term blood urea nitrogen (BUN) is used extensively when referring to urea measurement because historical assays for urea were based on nitrogen measurement.(Bishop, *et al;* 2005).

### 1.3.1 Chemical structure of urea:

Its organic compound with a chemical formula CO(NH2)2. The molecule has two –NH2 group joined by carbonyl(C=O) functional group(Bishop, *et al*;2005).

Urea serves an important role in the metabolism of nitrogen _containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It's solid, colorless, and odorless (although the ammonia which it gives off in the presence of water, including water vapor in the air, has a strong odor). It's highly soluble in water and non toxic. Dissolve in water it is neither acidic nor alkaline. The body uses it in many processes, most notably nitrogen excretion. Urea is widely used in fertilizers as a convenient source of nitrogen. Urea is also an important raw material for the chemical in industry.(Matthews, *et al* ;2006).

### 1.3.2. History of urea:

Urea was first discovered in urine in 1727 by the Dutch scientist "Herman Boerhaave" ,through this discovery is often attribute to the French chemist Hilaire Rouelle in 1828 , the german chemist Friedrich Wohler obtained urea by treating silver isocyanate with ammonium chloride (oh; 2006).

### 1.3.3. Physiology of urea:

Urea is synthesized in the body of many organism as part of urea cycle either from the oxidation of amino acids or from ammonia. In the cycle of amino groups donated by ammonia and L-aspartate are converted to urea while L-ornithine, citrulline, L-argininosuccinate, and L-arginie act as intermediates(coomes; 2006).

Urea production occurs in liver and is regulated by N-acetyle glutamate. Urea is found dissolved in blood (in the reference range of 2.5 to 6.7 mmol/liter) and is 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 (Lamb, *et al*;2005).

Amino acid from ingested food which are not used for the synthesis of proteins and other biological substances are oxidized by the body , yielding urea and carbon dioxide, as an alternative source of energy

The oxidation pathway starts with the removal of the amino group by a transaminase ; the amino group is then fed into urea cycle. Ammonia (NH3) is another common by product of the metabolism of nitrogenous compound. Ammonia is smaller, more volatile and more mobile than urea. If allowed to accumulate, ammonia would raise the PH in cells to toxic level. Therefore many organisms convert ammonia to urea, even though this synthesis has a net energy cost. Being practically neutral and excrete excess nitrogen. In water, the amine groups undergo slow displacement by water molecules, producing ammonia and carbonate anion. For this reason, old stale urine has a stronger odor than fresh urine (Lamb, *et al* 2005).

## 1.3.4.Disease correlations:

An elevated level of urea in blood called "azotemia". Very high level of plasma urea accompanied by renal failure is called uremia or uremic syndrome . which is fatal if it is not treated by dialysis . condition causing elevations of plasma urea are classified according to cause into three categories ; prerenal , renal . post renal ( Kessler & siekmann; 1999).

### 1.3.4.1. Prerenal hyperuremia:

Is caused by reduced renal blood flow. Reduction in blood flow delivers less urea to the kidney and therefore less urea is filtered . causative factors include : congestive heart failure, shock, hemorrhage, and any other factors that result in a marked decrease in blood volume. A high protein diet or increased protein catabolism may increase urea levels. Level will be decreased during period of low protein intake or increased protein synthesis (first; 2003).

### 1.3.4.2. Renal hyperuremia:

Decreased renal function causes an increase in plasma urea concentration due to compromised urea excretion. Renal cause of an elevated urea include; acute and chronic renal failure, glomeruler nephritis, tubular necrosis and other intrinsic renal diseases (Kaplan; 2003).

### 1.3.4.3.Post renal hyper-uremia:

This is due to obstruction to urine flow anywhere in urinary tract by renal stones, tumor of bladder or prostate or servers infections (Kleinman & Lorenz; 2003). A pregnant woman may experience an increase in kidney and ureter size. The GFR commonly increase by 50%, returning to the normal around 20 week post-partum (Kleinman & Lorenz; 2003).

## 1.4. Plasma creatinine:

Creatinine (M.W. 113 Da.) is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine. Creatine phosphate is used as energy source in skeletal muscle contraction (Apps, Cohen & Steel. 1992).

Creatinine is synthesized in the kidneys, liver and pancreas by two enzymatically mediated reactions. In the first, transmidation of arginine and glycine forms guandinoacetic acid. In the second reaction, methylation of guandinoacetic acid occurs with S-adenosylmethionine as the methyl donor. Creatinine is then in blood to the other organs ,such as muscles and brain, where it is phosphorylated to phosphocreatinine; a high energy compound (oh.2006). lntercrconversion of phosphocreatine and creatine is aparticular feature of the metabolic (thought to be 1&-2%day) spontaneously and irreversibly converts to its anhydride waste product creatinine. The amount of creatinine produced each day is relatively constant and is related to the muscle mass. In health ,the concentration of creatinine in the blood stream is also relatively constant. Creatinine is present in all body fluids and secretion , and is freely filtered by the glomerulus . Although it is not reabsorbed to any great extent by the renal tubules , there is a small but significant tubular secretion . Creatinine production also decreases as the circulating level of creatinine increases; several mechanisms for this have been proposed, including: feedback inhibition of production of creatinine, reconversion of creatinine to creatine and conversion to other metabolites (Lamb, Newman & price.2005).

## 1.4.1. The factors which affect serum creatinine:

### 1.4.1.1. Muscle mass:-

Creatinine is formed as a result of the non-enzymatic dehydration of muscle creatine .Synthesis of creatine is performed primarily in the liver, and selectively uptaken by muscles. Because creatine is taken up by muscles,muscle mass also an important consideration in determining

creatine pool and ultimately serum creatinine. Age and gender also determine muscle mass(Grade, *et al*.2004).

### 1.4.1.2.Gender:

Men have a larger muscle mass than women, which accounts for a higher level of serum creatinine in women (Grade, *et al*.2004).

### 1.4.1.3.Age:

The muscle mass decreases with aging, which corresponding decrease in level serum of serum creatinine(Grade, *et al*.2004).

### 1.4.1.4.Race:

Serum creatinine concentrations are significantly higher in black compared with nonblack haemodialysis patients: these differences are not readily explained by differences in nutritional status or body composition. Black individuals have long been noted to have higher serum creatinine levels than white individuals that may be independent of kidney functions. In fact, the reason of these racial differences in S.Cr is unknown, and it raises several questions, including whether a given S.Cr concentration might have different clinical implications among different racial groups Determinate of S.Cr concentration include not only the GFR but also the rate of creatinine generation and tubular secretion, the dietary absorption of creatine, certain medications, and interlaboratory and intraindividual variability (Grade, *et al*.2004).

The documented differences in the body composition among black and white individuals lending support to the contention that racial disparities in SCr are related to increased creatinine generation in the black individuals as a result of increased muscle mass (Grade, *et al*:2004)

## 1.4.2. Problems associated with increased blood creatinine:-

Increased blood creatinine is usually a sign of the problems with the kidney function (Levey, *et al*;2003).

### 1.4.2.1. Acute renal (kidney) failure (ARF):-

renal failure means that the kidneys are not performing their function of removing wastes from the blood. Acute renal failure may produce no symptoms until the level of waste products in blood becomes dangerously high. Acute renal failure is characterized by a rapid lose of renal function, with retention of many metabolic product (e.g. Urea and creatinine) (Myers, *et al* 2006).

(ARF) is conventionally divided into three categories, according to whether renal function impairment is related to a decrease in renal blood flow (pre-renal). Other definition :acute renal failure (ARF) is defined as abrupt decrease in renal function sufficient to result inretention of nitrogenous waste (e.g. blood urea nitrogen (BUN) and cretinine )in body. Although there is no consensus regarding the magnitude of elevation of SCr and BUN sufficient to ascribe a diagnosis of ARF. The relationship between decreasing glomerular filtration rate (GFR) and rising SCr concentration individuals with abnormal basal SCr. Thus in individuals with abnormal basal SCr, significant decreases in GFR are after associated with either slight or modest increase in SCr concentrations (Levey, *et al*; 2007)

### 1.4.2.2.Chronic renal failure (Bright's disease):-

Chronic renal failure main a failure, developing over many years, of the kidneys to control wastes in the blood. The commonest cause of chronic renal failure is diabetes mellitus (DM). with the most cause of CRF the

kidney can not fulfill most of its normal function until the disease has destroyed 80%-90% of the organ. As a result symptoms occur very late in the disease. Even then, it can be months or year before wastes in the blood accumulate to dangerous level (Pincus, *et al*;2006).

Once renal function is chronically impaired, the dysfunction ultimately progress to end stage renal disease (ESRD) even if the cause of the original renal insult is removed. For example, patients with glomerulonephritis may recover from the initial disease with only a modest decrease in renal function , but renal failure is likely to progress. One possible reason is when the functioning nephrons decreses, the remaining nephrons have an increased workload (i.e. filteration in each of remaining nephrons (single-nephron GFR) is increased. The result is cause of in case of increased intraglomerular pressure (Mitchum;2002).

**In both chronic and acute renal failure:-** at the start of kidney failure, the blood creatinine level tends to increase slowly over time. This can take months or more often, many years. However , when the kidneys have almost completely failed, the blood creatinine level rises more rapidly. Patients will probably feel unwell when their creatinine level gets to more than about 500Mm/l (micromole per liter). Equivalent to about 10% of normal kidney function (Kramer &Luke; 2007).

### 1.4.2.3.Urinary tract obstruction:-

The very low GFR during the period of almost complete urinary tract obstruction causes creatinine to accumulate in the plasma Relief of the obstruction allowed GFR to return to near-normal levels(Chen, et al; 2004).

## Kidney stones (renal calculi):

Kidney stones (nephrolithiasis) are common : 10% of men and 5% of women will have a kidney stone at same time of their lives.Stones occur when substances are present in the urine in amounts that cannot be held in solution. The excess amount of crystals which is gradually grows to form a stone (Mitchum;2002).

## 1.4.2.4.Acute tubular necrosis:

Acute tubular necrosis is a kidney disorder involving damage to the tubular cells of the kidneys, resulting in acute kidney failure. Acute tubular necrosis (ATN) is caused by lack of oxygen to the kidney tissue (ischemia of the kidneys).The internal structure of the kidneys, particularly the tissues of the kidney tubules, become damaged or destroyed. ATN is one of the most common structural changes that may lead to acute renal failure. The serum creatinine level may increase (Kramer & Luke; 2007).

## 1.4.2.5.Glomerulonephritis:

Glomerulonephrities is a type of kidney disease that damages your kidneys ability to remove waste and excess fluids. Also called glomerular disease, glomerulonephritis can by acute (a sudden attack of inflammation),or chronic (coming on gradually). If glomerulonephritis occurs on its own, it is known as primary glomerulonephritis. If another disease, such as lupus or diabetes, is the cause, it is called secondary glomerulonephritis .Treatment depends on the type of glomerulonephritis you have. Glomerulonephritis (an inflammation of the glomeruli) can damage the kidneys so that lose their filtering ability,

allowing dangerous levels of fluid and waste to accumulate in the body (Mitchum; 2002)

### 1.4.2.6.Diabetic nephropathy:

Diabetic nephropathy is kidney disease or damage that results as a complication of diabetes. Uncontrolled high blood sugar leads to the development of kidney damage. Especially when high blood pressure is also present. The serum creatinine level will increase as kidney damage gets worse (Amcrican Diabetes Association;2002)

### 1.4.2.7. High blood pressure (HBP):

HBP or hypertension means high pressure (tension) in the arteries (systolic blood pressure>/= 140mmHg or diastolic blood pressure >/=90 mmHg). Untreated hypertension increase the risk of non-fatal and fatal cornery artery disease stroke, congestive heart failure and renal disease (the kidney damage can be the cause or the result of hypertension)(Bennett, *et al*; 1995).

An elevated level of serum creatinine indicate damage to kidney (caused by hypertension). Hypertension is a systemic condition that can cause damage to many different parts of the body. The kidney has a key role in the maintenance of normal blood pressure and many drugs used to treat hypertension act through the kidneys or through rennin-angiotensin system. Most renal disease can cause hypertension and hypertension itself can cause renal damage. Hypertension is important because it can directly damage the heart, the kidneys and the eyes because it major risk factor for vascular disease (Emancipator; 1999).

## 1.5Benzene:-

### 1.5.1.Definition

A colorless volatile liquid hydrocarbon present in coal tar and petroleum, used in chemical synthesis. Its use as a solvent has been reduced because of its carcinogenic properties.(langely A, 2005).

### 1.5.2.Physical properties

Benzene is clear, non-corrosive and highly flammable liquid, which is colorless and has strong sweet odor with relative high milting poient. (langely A, 2005)

### 1.5.3Chemical properties.

Benzene is an organic chemical compound with the moleculer formula C6H6. Its molecule is composed of 6 carbon atoms joined in a ring .with 1 hydrogen atom attached to each carbon atom. Because its molecules contain only carbone and hydrogen atom , benzene is classed as hydrocarbon.(langely A, 2005).

Benzene is a colorless and highly flammable liquid with a sweet smell, because it has a high octane number , it is an important component of gasoline. Comprising a few percent of its mass. (langely A,2005).

### 1.5.4.Benzene structure:-

The carbons are arranged in a hexagon , and suggest alternating double and single bonds between them. Each carbon atom has a hydrogen attached to it . this diagram is often simplified by leaving out all the carbon and hydrogen atoms.(langely A, 2005).

## 1.5.5. Metabolism of benzene

Qualitatively , the metabolism and elimination of benzene appear to be similar in humans and laboratory animals . benzene id metabolized mainly in the liver but also in other tissue , such as the bone marrow. (langely A,2005).

The metabolites responsible for benzene toxicity are not yet fully understood. The key toxic metabolites for cytotoxicity and the the induction of leukemia are thought to be benzoquinone, benzene oxide and muconaldehyde. The genotoxic activity of benzene metabolites is thought to be clastogenic (causing chromosomal damage) rather than acting through point mutation. Benzoquinone and muconaldehyde are both reactive, bipolar compound known to be clastogenic and the pathways leading to their formation are favored at low concentration in both mice and humans.(langely A, 2005).

## 1.5.6.Distribution of benzene:

After entry into human organism, benzene is distributed throughout the body and, owing to its lipophilic nature, accumulates preferentially in fat-rich tissue, especially fat and bone marrow. In humans, benzene crosses the blood-brain barrier and the placenta and can be found in the brain and umbilical cord blood in quantities greater than or equal to those present in maternal blood.(langely A, 2005).

## 1.5.7.Benzene exposure in the work place:-

Exposure to benzene occurs by three pathways

1. Breathing (inhalation exposure).

2.Eating and/or drinking contaminated food or water.

3.absorption through the skin (contact with skin).(Katzung and Diuretic,2004).

**People who breath in high level of benzene may develop the following signs and symptoms within minute to several hours:**

a) Drowsiness.

b) Dizziness.

c) Rapid or irregular heartbeat.

d) Tremors.

e) Confusion.

f) Headaches.

g) Unconsciousness.

h) Death (at very high levels) (verschueren,1983)

**Eating foods or drinking beverages containing high levels of benzene can cause the following symptoms within minutes to several hours:**

a) Vomiting.

b) Irritation of the stomach.

c) Dizziness.

d) Sleepiness.

e) Convulsions.

f) Rapid or irregular heartbeat.

g) Death (at very high levels).

If person vomits because of swallowing foods or beverages containing benzene , the vomit could be sucked into the lungs and cause breathing problems and coughing. Direct exposure of the eyes, skin, or lungs to benzene can cause tissue injury and irritation. Showing these signs and symptoms does not necessarily mean that a person has been exposed to benzene .(verschueren, 1983).

Benzene exposure is most dangerous when it occurs over a long period of time or when the concentration of benzene to which a person is exposed is very high. Contact with low to moderate levels of benzene for a short time can cause headaches, vomiting, disorientation, shakiness, elevated heart rate and loss of consciousness. Very high level of exposure can be fatal. People who work with benzene or who are exposed to it over a long period of time are at the highest risk for developing benzene –related illnesses (long-term exposure mean exposure of a year or more).(verschueren, 1983).

## 1.5.8. Hazard effect of exposure to benzene:-

Health effect are divided according to:

1.duration time.

2.level of benzene. (Katzung and Diuritic, 2004).

## 1.6. Rationale:

Exposure to benzene over a long period of time could cause nephrotoxicity in motor machines occupationally exposed to them. The seriousness of poisoning caused by benzene depends on the amount , rout , and length of time of exposure, as well as the age and preexisting medical condition of the exposed person.

There are no enough published data carried out in Sudan. That shows effect of benzene on renal function among petroleum station workers, there for I done this study to explain this effect.

This study done to determine the effect of benzene on creatinine and urea and express their harmful on health of worker to do suitable protection.

## 1.7 OBJECTIVES

### 1.7.1. GENERAL OBJECTIVES:-

-To assess the level of urea and creatinine in Sudanese benzene station at Khartoum state.

### 1.7.2.SPECIFIC OBJECTIVES:-

-To measure and compare the urea and creatinine level in Sudanese benzene station workers and health individual.

-To find out correlation of urea and cretinine with duration of benzene exposure in Sudanese benzene station workers.

-To find out correlation of urea and creatinin with age of benzene station workers .

# 2.Materials and method

## 2.1.Study area and period:-

The study carried out from benzene station worker at Khartoum state during the period from February to May 2015 .

## 2.2.Target population and sample size:

The study covered 100 individual randomly selected from whole population with different age. 50 were worker at benzene station and directly exposed to benzene & other 50 were not worker from community (control group).

## 2.3 Selection criteria

### 2.3.1.Inclusion criteria:

-Test group: workers at benzene station

-Control group: individual from community not worker at benzene station

### 2.3.2. Exclusion criteria:

-Test group: excluded workers that working at station less than 5 years, and excluded workers have a renal disease.

-Control group: excluded individual have a renal disease.

## 2.4 Ethical consideration

The aim & benefits of this study were explained to the participant .

An informed consent was obtained from each participant .

Health education was provided to each participant.

## 2.5.Data collection and analysis

### 2.5.1. Interview with questionnaire

An interview with questionnaire to obtained the clinical data was used for each participant in this study .

### 2.5.2.Blood sample & collection

Blood sample "5ml" were collected from subject to study group (test & control) after fulfillment of questionnaire, using disposable syringe and sprit for sterilization the area of collection.

The collected blood is drawn in heparin containers were centrifuged at 4000rpm to obtained plasma.

Hemolyzed & lipamic sample were rejected & excluded from the study.

Specimen of about" 1ml" heparinized plasma were preserved at -20 c prior to processing.

## 2.6.Biomedical measurement & instrument used:

Cotton ,70%alcohol , disposable syringes , tourniquet , heparin containers , test tubes ,atomic absorption spectrophotometer device , automatic pipette (10-100), automatic pipette(1000) and distilled water.

## 2.6.1.Estimation of urea :

## By Berthelot reaction method :

### 1. principle :

See appendix I .

### 2.Prosedure:

Three test tubes were labeled Test, STD and Blank. 1.0 ml of urease reagent was added in each tube, 0.01ml of serum was added to Test tube, 0.01ml 0f 50mg/dl of urea standard was added in STD tube. The above reagents were mixed well and incubated for 5 minutes at room temperature, and then 1.0ml of sodium hypochlorite reagent was added in each tube, then mixed thoroughly and kept at the room temperature for 10 minutes then read at 580nm , against blank reagent.

### 3. Reagent: (see appendix I)

### 4. Reference value: 10-50mg/dl .

## 2.6.2.Estimation of creatinine

## By jaffe reaction kinetic method

### 1. principle:

See appendix II.

### 2. procedure:

Two test tubes were labeled Test and STD**,**

1) 1.0ml of alkaline picrate reagent was added in Test tube.

2) 0.1ml of serum was added into Test tube.

3) Mixed well and started stop watch immediately.

4) Absorbance was read after 30 seconds .

5) absorbance was read after 90 seconds.

6) The same steps (1,2,3,4 and 5) were done using standard instead of serum .

7) Delta absorbance of every two readings was calculated.

### 3. Reagent : (see appendix II)

### 4. reference value (male):- **0.4- 1.4 mg/dl.**

## 2.7.Quality control:

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

## 2.8.Statistical analysis:

Statistical package for social science (SPSS version 14.0 ) computer software was used for data analysis . the mean and standard deviation of the plasma urea & creatinine were calculated. T-test was used for comparison (significant level was set at $p \leq 0.05$).

Person correlation analysis was used to asses relationship between duration of work at station and plasma level of urea and creatinine, the result presented in form of tables and figures.

# 3.Result

## 3.1 Result and analysis:

This study was conducted on 50 worker at benzene station as case group, 50 subject from community as control group(not work at stations) . Age of the test group was matched with the control group .

In this study the test group working at station at least 5 years .

Table (3-1) comparison of means of age in the test group and control group.

Table (3-2)comparison of means of plasma levels of urea and creatinine (mg/dl) of the workers at benzene station (test group) and control group. (P=0.000), (P=0.000) respectively.

According to correlation analysis:

Figure (3-1) A scatter plot show a significant positive correlation between plasma level of urea and duration work (r= 0.711 , P .value= 0.000 ). In this scatter show about 14% from workers have increased in urea.

Figure (3-2)A scatter plot show a significant positive correlation between plasma level of creatinine and duration work (r= 0.777 , P .value=0.000). In this scatter show about 18% from workers have increased in creatinine.

Figure (3-3) A scatter plot show a significant positive correlation between plasma level of urea and age (r=0.531 , P .value=0.000 ).

Figure (3-4)A scatter plot show a significant positive correlation between plasma level of creatinine and age (r=0.481 , P. value=0.000 ).

**Table (3-1)** Comparison of means of age in the test group and control group.

| Type | Mean | Stander deviation |
|---|---|---|
| Workers at station | 2.64 | 1.139 |
| not worker at station | 2.58 | 1.126 |

**Table (3-2)** Comparison of means and SD of plasma levels of urea and creatinine (mg/dl) of the workers at benzene station (test group) and control group.

| Variable | Test group | Control group | P. value |
|---|---|---|---|
| urea | 36.02± 12.203 | 23.16± 5.582 | 0.000 |
| creatinine | 1.298± 0.475 | 0.926± 0.330 | 0.000 |

The table show the mean ± SD & the probability (P).

The value ≤ was considered significant.

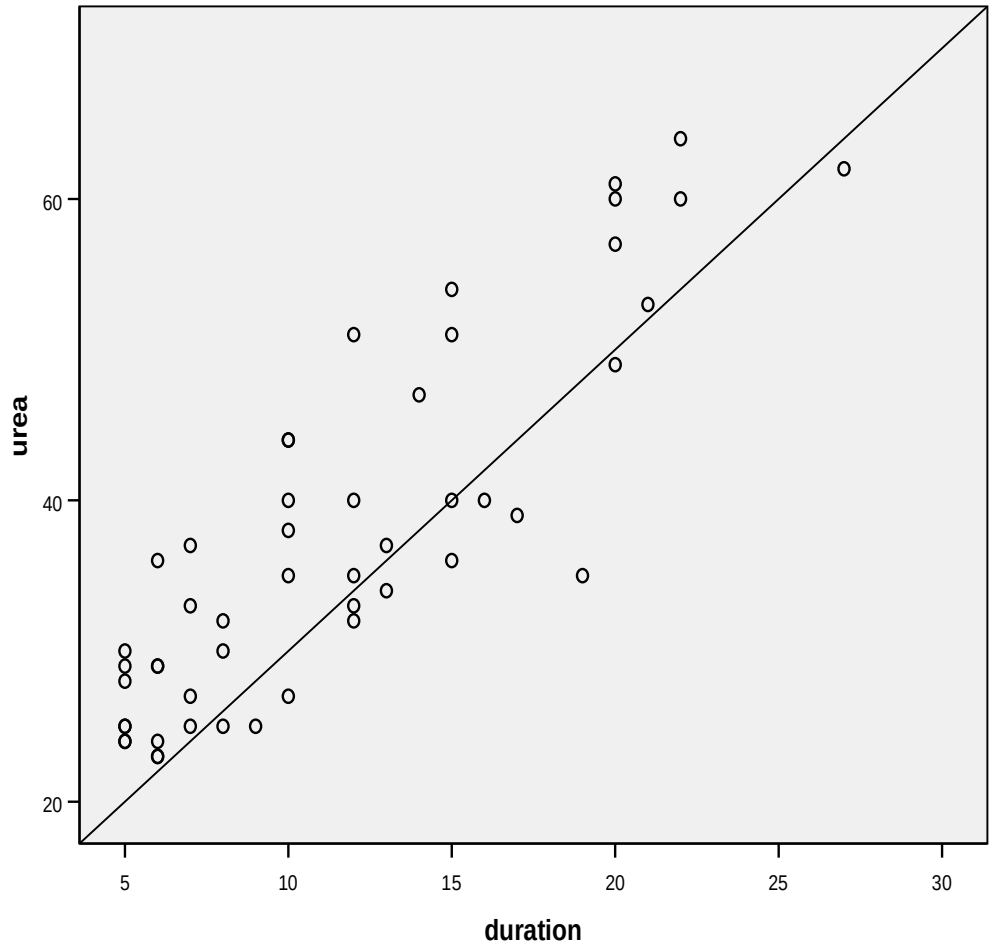


**Figure (3-1)** : The relationship between plasma level of urea and duration work.

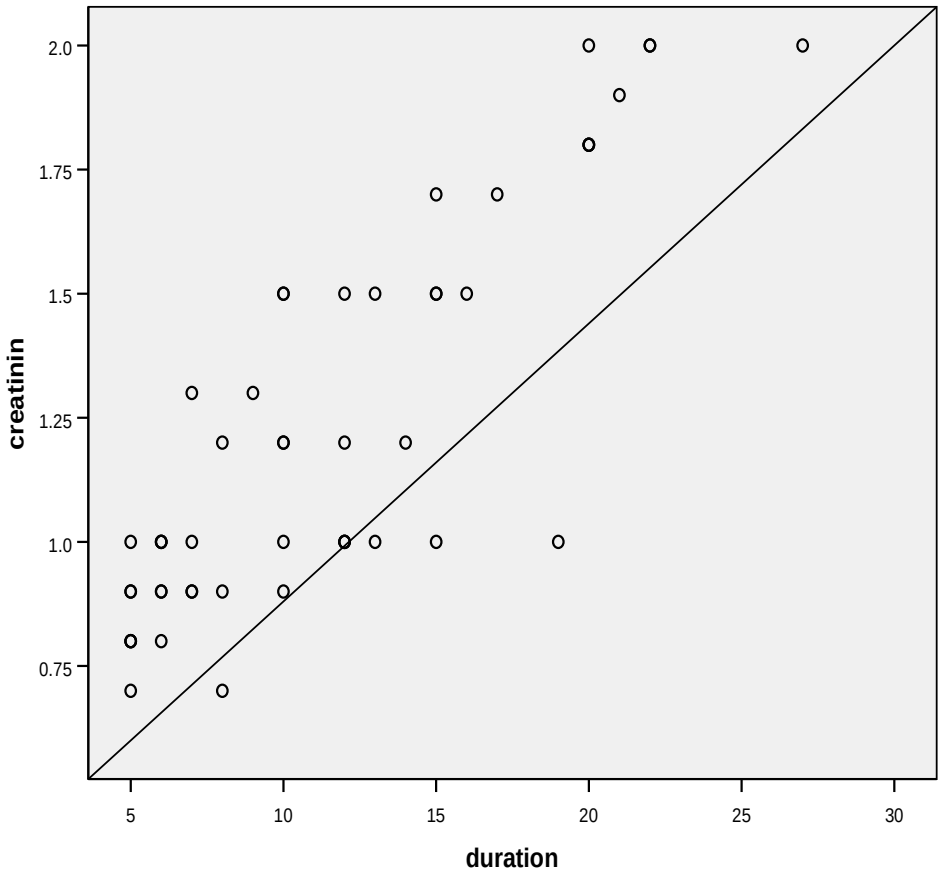


**Figure (3-2)** : The relationship between plasma level of creatinine and duration work.

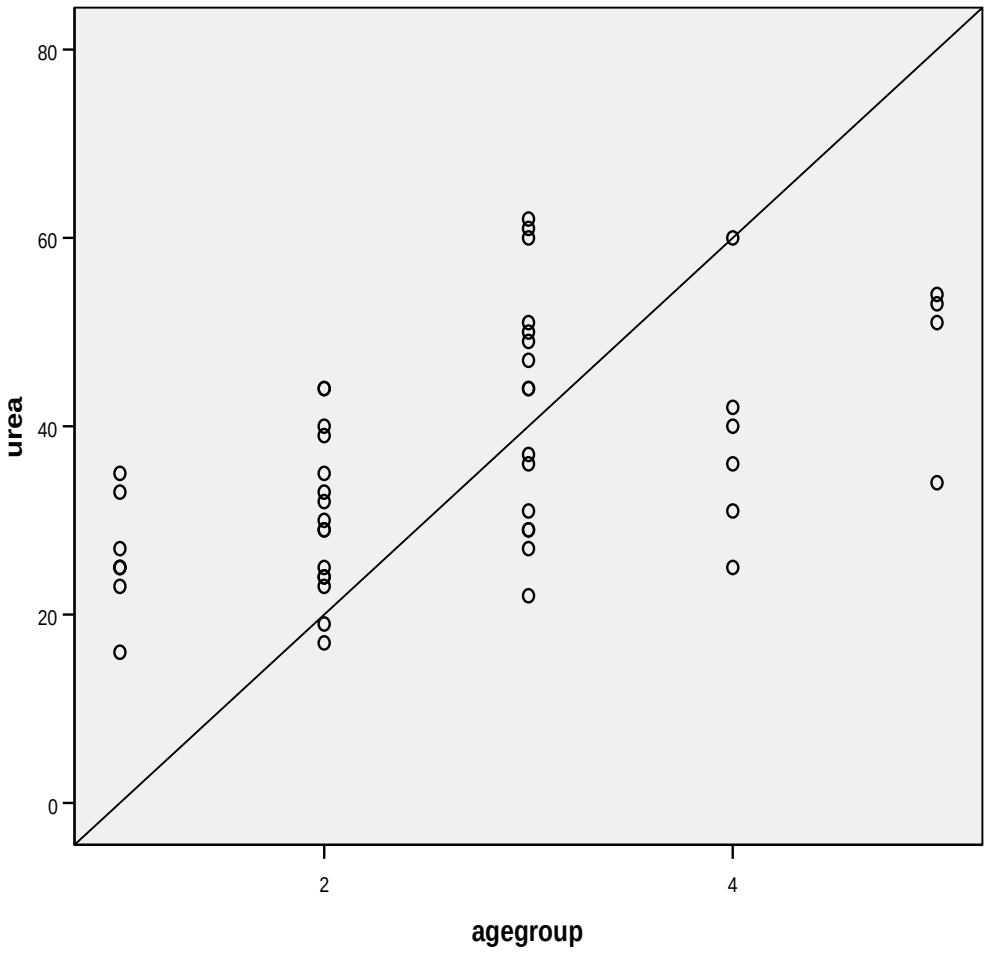


**Figure (3-3):** The relationship between plasma level of urea and age .

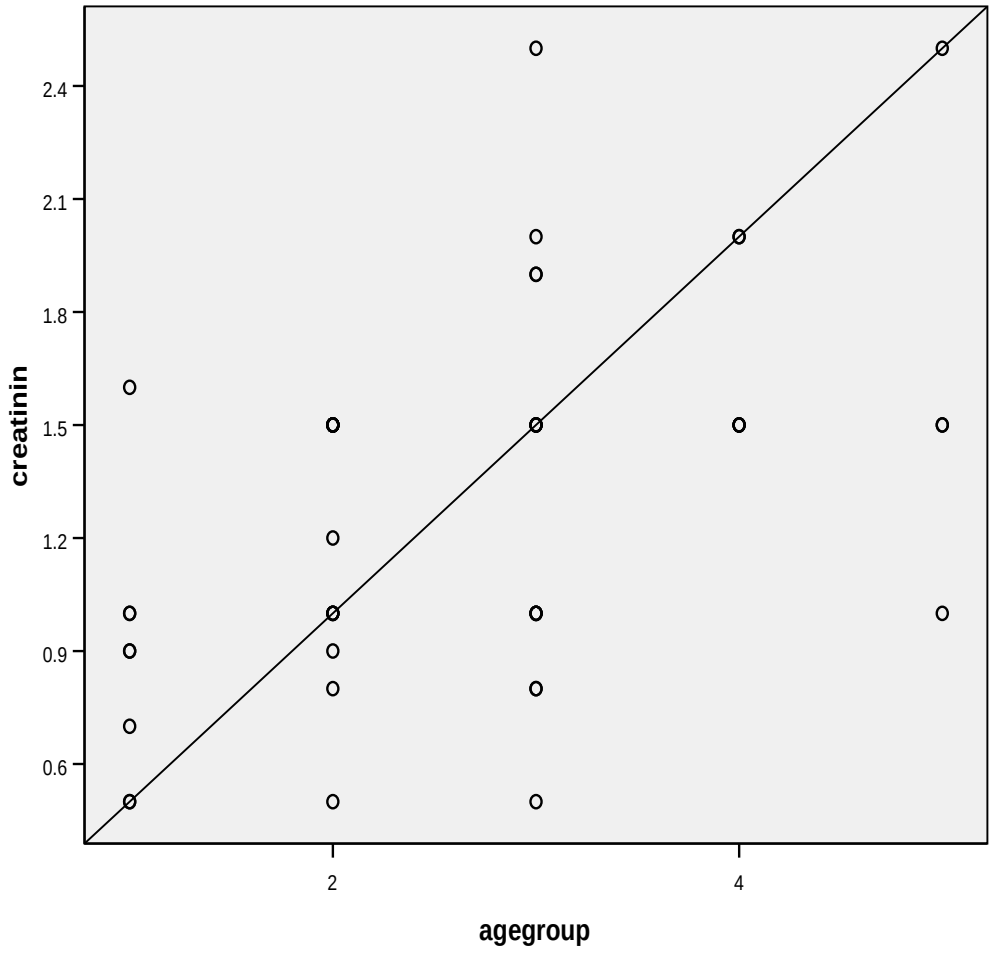


**Figure (3-4):** The relationship between plasma level of creatinine and age.

# 4.Discussion , Conclusions and Recommendation

## 4.1. Discussion:

The kidney maintains constant extracellular environment by its involvement in the excretion of such catabolites as urea and creatinine and regulation of water and electrolyte balance. Abnormal concentration of these catabolites and some electrolytes in the plasma or serum is a clear indication of renal function impairment (Nwankwo *et al;* 2006, Crook 2007; Gidado *et al;* 2001, Zanna *et al;* 2008). Impairment of the renal functions may be caused by exposure to different nephrotoxic substances, in addition to certain diseased conditions. For instance, exposure to lead from automobile exhaust is reported to be a risk factor for nephrotoxicity (Mortada *et al;* 2001).

Renal function impairment manifests in a variety of different clinical presentations, some of which may be asymptomatic. The renal function impairment with asymptomatic presentations can only be detected by routine laboratory examination.

This study has been carried out in 50 benzene station workers their range from (20-65 ) years old and the duration in work ranges from 5-27 years, this people work for 12 hours /day. The one hundred blood samples were collected (50 samples from workers in benzene station and 50 samples from control 'from community' ). In Khartoum state in the period from February to May 2015.

Table (3-2) In this study the workers at benzene station has a significant increase in the mean levels of urea and creatinine when compared to control group, p(0.000 ,0.000).

This was agreed and confirm the observation of( Bartimaeus & Jabcos (2003) who showed that considerable exposure to petrol or its product over a long period of time could cause nephrotoxicity

Figure(3-1) Plasma levels of urea in benzene station workers show a positive significant correlation of plasma level with work duration (r=0.711 , p=0.000) , the result of the level of plasma of urea in benzene station workers affected by duration, this increase due to increasing of heavy metal from pollution.

Figure (3-2) plasma levels of creatinine in benzene station workers show a positive significant correlation of plasma levels with work duration (r=0.777, P .value =0.000). The increasing of creatinine due to found chronic leads exposure decrease the glomerular filtration rate (GFR) with subsequent renal tubular fibrosis , renal atrophy and disturbances of the renal function.( Rokho *et al*; 1996).

Figure (3-3) plasma levels of urea in benzene station workers show a positive significant correlation of plasma level with age (r=0.531 ,

P .value = 0.000).This result agreed and confirm with William and Marshall.

Figure (3-4) plasma levels of creatinine in benzene station workers show a positive significant correlation of plasma level with age (r=0.481 ,

P .value = 0.000). This result agreed and confirm with William and Marshall.

## 4.2.CONCLUSION:

This study concluded:

-there is significant effect from inhalation of benzene product on renal function (urea and cratinine) show increasing in this parameter.

-observed association between exposure to benzene and increasing at in level at urea and creafinin by increasing duration.

- there is significant increase in plasma level of urea and creatinine with increasing age .

## 4.3. Recommendation:-

This study recommended:

-Using protective cloths to work in benzene station.

-Determine the limit period for workers.

-Do regular monitor and follow up for workers.

-Use protective tools for workers in benzene station.

-Do medical examination for workers  regularly and save the result.

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