1. Introduction and Literature review

1.1. Introduction:

Benzene is chemical that is often used in manufacturing . In its common form , benzene is a liquid that is clear , slightly sweet smelling, and highly combustible. Benzene is frequently used in manufacting rubber, paint, plastics, resins, drug, pesticides, synthetics and other products. It is also present in gasoline and tobacco smoke. (Verschueren, 1983)

A knowncarcinogen, benzene can be harmful to those exposed to it over an extended period of time. Benzene exposure is most likely to occur among workers in facilities that use the chemical in their products; in addition benzene can enter the environment through spills, accidental release. Volcanic eruptions and forest fires. It evaporates quickly in air and is partially soluble in water.(Verschueren, 1983).

Pollution can be defined as the introduction of contaminants into a natural environment , leading to instability , disorder, harm , discomfort and stress to the ecosystem , the physical systems or living organisms . Pollutants included chemicals such as petroleum hydrocarbons, heavy metals, pesticides etc. there contaminants can lead to system stress, (Prasad,*et al,* 2013).Previous studies have demonstrated increased oxidative stress IN subjects exposed to environmental air pollution through the assessment of oxidative damage to DNA, lipids or proteins,(Singh,*et al,* 2008) for example, free radicals may induce peroxidation of arachidonic acid generating isoprostanes(Gracowski,*et al*. 2002). Urinary orplasma isoprostaneshave been found to to be related to smoking and exposure to environmental tobacco smoke. (Dietrich ,*et al*. 2003).

occupational exposure to combustion products including petroleum have been associated with increased oxidative stress biomarkers in some, but not all studies exposure to petroleum hydrocarbons could be from gasoline fumes at the pumps , spilled oils , chemicals used at home and work e.g: pesticides some amount may leak from underground storage tanks and enter the ground waters . some of the petroleum fractions are released into the soil contaminating the plants grown in such soil. (Ujowundu C.O, *et al*. 2011)

It was started under normal physiological condition ,animals maintained a balance between generation and neutralization of reactive oxygen species (ROS). (Valko M.D, *et al*. 2007).however,when organisms are subjected to petroleum compounds, the rate of production of (ROS) in cells get increased along with hydrogen peroxide , hypochlorous acid (HCIO) and free radicals including hydroxyl radical (OH) and superoxide anion (O2-) . oxidative stress have been implicated in a variety of pathological conditions such as: diabetics mellitus , cancer , aging , liver damage ,and atherosclerosis etc. (Nakagawa, *et al*.2008).

Recently, it was reported that airborne particles were associated with decreased in heart rate variability. (Gold D.R, *et al*.2000).another recent paper reported nitrogen dioxide (NO^2) and PM (particulate matter with aerodynamic diameter less than 2.5 µm) were associated with defibrillator discharges due to ventricular arrhythmias In patients with implanted cardioverter defibrillators. (Peters A, *et al*.2000).

Antioxidants such as glutathione (GSH) , uric acid , ascorbate and α-tocopherol present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants. (Kelly, *et al*.1996).the antioxidants act as sacrificial substrates scavenging oxidant pollutants from the air ways and thereby

preventing oxidation of macromolecules such as: lipids , protins and carbohydrates.

The aim of this study is to estimate the effect of benzene on the antioxidant such as uric acid, totalbilirubin and albumin level in the workers of benzene station in Khartoum state.

1.2.Literature review

1.2.1Benzene

A colorless Volatile Liquid hydrocarbon present in coal tar and petroleum, used in chemical synthesis. It's use as solvent has been reduced because of it's carcinogenic properties. (Katzungand Diuritic. 2004)

1.2.2Physical properties:

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

1.2.3Chemical Properties:

Benzene is an organic chemical compound with the molecular formula C_6H_6. 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 of carbon and hydrogen atoms, benzene is classed as a hydrocarbon. (Langley A,2005)

Benzene is a colorless and highlyflammable liquid with a sweet smell. because it has high octane number, it is an important component of gasoline, comprising a few percent of its mass. (Katzungand Diuritic. 2004)

1.2.4Benzene structure:

The carbons are arranged in a hexagon, and the suggested alternating double and single bounds 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. (Langley A,2005)

1.2.5 Distribution of Benzene:

After entry into the human organism, benzene is distributed throughout the body and, owing to its lipophilic nature, accumulated preferentially in fat-rich tissues, 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. (Katzungand Diuritic. 2004)

1.2.6 Metabolism of benzene:

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

The metabolism responsible for benzene toxicity are not yet fully understood. The key toxic metabolism for cytotoxicity and the induction of leukemia are thought to be benzoquinone, benzene oxide and muconaldehde. (Katzungand Diuritic. 2004) The genotoxic activity of benzene metabolites is through to be clastogenic (causing chromosomal damage) rather than acting through point mutations. Benzoquinone and muconaldehyde are both reactive, bipolar compound known to be clastogenic and the pathways leading to their formation are favored at low concentrations in both mice and humans. (Langley A,2005)

1.2.7 Benzene exposure in the work place:

Exposure to benzene occurs by three main ways:

1. Breathing (inhalation exposure)

2. Eating and/or drinking contaminating food or water

3. Absorption through the skin (contact with skin). (Katzungand Diuritic. 2004)

People who breath in high levels of benzene may develop the following signs and symptoms within minutes to several hours.

a) Drowsiness

b) Dizziness

c) Rapid or irregular heartbeat

d) Headaches

e) Tremors

f) Confusion

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)

1.2.8 Hazard effects of exposure to benzene:

effect are dividing according to :

1. Duration time

2. Level of benzene. (Katzung and Diuritic.2004)

1.2.9 Long-term exposure to benzene:

Hematological effect (in blood forming organs), prolonged exposure to benzene can cause a serious condition where the number of circulating erythrocytes, leucocytes and reduced (pancytopenia) at this stage effects are thought to be readily reversible. However continued exposure can result in a plastic anemia or leukaemia. (Monica.C. 2009)

Time of exposure, as well as the age and preexisting medical condition of the exposure person.(Verschueren, 1983)

1.3 Albumin:

The albumins (formed from Latin: albumen "(egg) white; dried egg white") are a family of globular proteins, the most common of which is serum albumin. The albumin family consists of all proteins that are water-soluble, are moderately soluble in concentrated salt solutions, and experience heat denaturation. Albumins are commonly found in blood plasma, and are unique from other blood proteins in that they are not glycosylated. Substances containing albumins, such as egg white, are called albuminoids. A number of blood transport proteins are evolutionarily

related, including serum albumin, alpha-fetoprotein, vitamin D-binding protein and afamin. (McCrudden, Francis H. 2008).

1.3.1 Functions of Albumin

Albumin is the main protein of human blood plasma. It binds water, cations (such as Ca2+, Na+ and K+), fatty acids, hormones, bilirubin, thyroxin (T4) and pharmaceuticals (including barbiturates) – its main function is to regulate the colloidalosmotic pressure of blood. Alpha-fetoprotein (alpha-fetoglobulin) is a fetal plasma protein that binds various cations, fatty acids and bilirubin. Vitamin D-binding protein binds tovitamin D and its metabolites, as well as to fatty acids. The biological role of afamin (alpha-albumin) has not yet been characterized.(Wilcox WD,1996).

1.3.2 Structure of Albumin

The 3D structure of human serum albumin has been determined by X-ray crystallography to a resolution of 2.5 A.(Wilcox WD, 1996).

Albumin comprises three homologous domains that assemble to from a heart-shaped molecule. (Wilcox WD. 1996). Each domain is a product of two subdomains that possess common structural motifs.(Wilcox WD, 1996). The principal regions of ligand binding to human serum albumins are similar, each domain containing five or six internal disulfide bonds.(Wilcox WD, 1996).

1.3.3 Types of Albumin:

Serum albumin:

Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. The human version is

human serum albumin, and it normally constitutes about 50% of human plasma protein.(Wilcox WD, 1996).

Serum albumins are important in regulating blood volume by maintaining the oncotic pressure (also known as colloid osmotic pressure) of the blood compartment.(Wilcox WD, 1996). They also serve as carriers for molecules of low water solubility this way isolating their hydrophobic nature, including lipid solubility this way isolating their hydrophobic nature, including lipid soluble hormones, bile salts, unconjugated bilirubin, free fatty acids (Apo protein), calcium, ions (transferrin), and some drugs like warfarin, phenobutazone, clofibrate & phenytoin. For this reason, it's sometimes referred as a molecular "taxi". Competition between drugs for albumin binding sites may cause drug interaction by increasing the free fraction of one of the drugs, thereby affecting potency.(Wilcox WD, 1996)

Specific types include :

a- Human serum albumin

b- bovine serum albumin (cattle serum albumin) or BSA, often used in medical and molecular biology labs. (Wilcox WD, 1996).

1.3.4 clinical significance:

for patients with low blood volume, there is no evidence that albumin reduces mortality when compared with cheaper alternative such as normal saline, or that albumin reduces mortality in patients with burns and low albumin levels. Therefore, the Cochrane Collaboration recommends that in not be used, except in clinical trials. (De Oliveira EP, Burini RC. 2012).

a.hypoalbuminemia:

Low albumin may be caused by liver disease, nephroticsyndrom, burns, protein-losing, enteropathy, malabsorption , malnutrition, late pregnancy, artifact and malignancy. (Wilcox WD, 1996).

b. hypoalbuminemia:

High albumin is almost always caused by dehydration. some cases of retinol (Vitamin A) deficiency, the albumin level can be evaluated to high-normal values (eg, 4.9 g/dL). This is because retinol cases cells to swell with water (this is also the reason too much Vitamin A is toxic).(Katzungand Diuritic. 2004).In the lab experiments it has been shown that All-trans retinoic acid down regulates human albumin production. (Katzungand Diuritic. 2004)

1.3.6 Effect of Benzene with albumin:

Albumin is major antioxidant component of plasma. In addition, albumin might play major role of the total antioxidant capacity of plasma .(Lopez-Tinoco C , 2011). Albumin , one of the most important protein in human plasma , is able to bind to Cu^{2} tightily and withiron weakly . Copper bond to albumin is still effective in generation radicals species (Hydroxyl radicals) in the presence of hydrogen peroxide by fenton reaction . Therefore increasing it to protect human from oxidants compounds formation. (Clausen J.,2013). Albumin and others , an index

or marker of glomerular disease through raised in Benzene workers were probably a sufficiently reliable of Benzene nephropathy .(Hong Y , 2009).

1.4Uric Acid:

Uric Acid is the chief end-product of purine catabolism in human, purine such as adenosine and guanine from the breakdown of ingested nucleic acid or from tissue destruction is converted in uric acid primary in the liver. (David, 2001), other mammals degrade uric acid to allantoin by means of the enzyme, uricase, which is lacking in primates. (DR A.C.DEB,2008).

1.4.1Metabolism

Purines, such as adenosine and guanine from the breakdown of ingested nucleic acids or from tissue destruction, are converted into uric acid, primarily in the liver. Uric Acid is transported in the plasma from the liver to kidney, where it is filtered by glomerulus. Reabsorption of 98% to 100% of the uric acid in the glomerular filtrate occurs in the proximal tubules. Small amounts of uric acid are secreted by distal tubules into the urine. This route amounts for about 70% of the daily uric acid excretion. The remainder is excreted into the GI tract and degraded by bacterial enzymes. (Michael L. Bishop, *et al*. 2005)

1.4.2Uric acid with kidney

Uric acid is transported in the plasma from the liver to the kidney, where it is filtered by the glomerulus. Reabsorption of 98-100 % of uric acid in the glomerular filtrate occurs. In the proximal tubules small amount of uric acid are secreted by distal tubule into the urine, this uric acid accounts for about 70% of daily uric acid excretion and the remainder is excreted into GIT and degraded by bacterial enzymes.(David, 2001).

1.4.3 Disease correlation with uric acid

Three major disease are associated with elevated plasma uric acid concentration :

- Gout

- Increased catabolism of nucleic acid

- Renal disease (David, 2001).

Gout Is a disease found primary in men and usually is first diagnosed between 30 and 40 years of age. Patient have pain and inflammation of joints caused by precipitation of sodium urate. patient with gout are highly susceptible to development of renal calculi. But not all patient. In women, hypouricemia develop with post menopausal life. (Burtis, 2008)

1.4.4 Hyperuricaemia

Many factors contribute to the hyperuricaemia, including: genetics, insulin resistance, hypertension, renal insufficiency, obesity, diet, use of diuretics and consumption of alcoholic beverages. (Sam Z Sun, *et al*. 2010)

Cause of hyperuricaemia can be classified into three functional types (Yamamoto T, *et al*.2008) :

(I) Increase production of uric acid :

The type case with the type of uric acid overproduction are :

- Hypoxanthine guanine phosphoribosy1 transferase deficiency (due to HPRT gene abnormality)

- Excessive consumption of purine - rich die.

- Cytolysis induced by chemotherapy of blood neoplasm.

(II) Decreased excretion of uric acid :

Those with the type of under excretion are :

- Family juvenile hyperuricaemia nephropathy (due to uromodulin gene abnormality).

- Abrupt body weight loss (due to low calorie diet). (Yamamoto T, *et al*. 2008)

(III) Mix type:

Those with the mixed type are :

- Glucose 6-phosphatase deficiency (due to glucose 6-phosphatase gene abnormality)

- Excessive consumption of alcohol beverages.

1.4.5 Low uric acid:

Cause of low uric acid

Low dietary zinc intakes cause lower uric acid levels. This effect can be even more pronounced in women taking oral contraceptive medication, Xanthine oxidase is an Fe-Mo enzyme, so people with Fe deficiency [the most common cause of anemia in young women] or Mo deficiency can experience hypouricemia and Xanthine oxidase loses it's function and gains ascorbase function when some of the Fe atoms inn XO are replaced with Gu atoms. Accordingly, people with high Cu/Fe can experience hypouricemia and vitamin C deficiency, resulting in oxidative damage since estrogen increases the half life of Cu, women with very high estrogen estrogen levels and intense blood loss during menstruation are likely to have Cu/Fe and present with hypouricemia. (Garg,*et al*.2005)

1.4.6Oxidative stress

Uric Acid may be maker of oxidative stress, and may have a potential therapeutic role as an antioxidant. On the other hand, like other strong reducing substances such as ascorbate, uric acid can also act as a prooxidant, particularly at elevated levels. Thus, it is unclear whether elevated levels of uric acid in diseases associated with oxidative stress such as stroke and antherosclerosis are a productive response or primary cause. (Proctor,2008) .

1.4.7Effect of Benzene with Uric acid

Uric acid is major antioxidant components of plasma it was reported that uric acid might be a consistent and reliable biomarkers of significant exposure to benzene and lead (Ball G.V,2002).the pathophysiology by which benzene exposure causes

elevation in uric acid level is thought to be due to damage tubules which causes retention of uric acid. (Ball ,2002).

1.5Bilirubin:

1.5.1Bilirubin Metabolism:

Bilirubin is an end product of haem metabolism, the iron in haem is reutilized but the tetrapyrrole ring is degraded to bilirubin. Approximately 250-300mg is produced each day in reticuloendothelial system. 80% arising from the breakdown of older erythrocytes. A small amount of bilirubin (15%) often called "shunt bilirubin" is formed directly in the bone marrow, mostly as a by-product of hemoglobin synthesis, and some is derived from breakdown of hemoprotein-myoglobin and cytochromes.

Bilirubin is then transported to the smooth endoplasmic reticulum where it undergoes conjugation; principally with glucuronic acid to form diglucuronide this process is catalyzed by enzyme bilirubin-uridyl diphosphate glucuronyl transferase. It is further metabolism can be divided into four steps:

(Praveen K, M, C, 1991)

1- Transfer into the liver cell from the hepatic sinusoidal blood.

2- Concentration in the liver cell.

3- Conjugation to two molecules of glucuronic acid and

4- Secretion as diglucuronide into the bile canaliculi.(Praveen , 1991)

Unconjugated bilirubin is transported in plasma bound to protein mainly albumin (Laker M.F, 1996). Under normal circumstances this binding is tight although hydrogen ions, fatty acids and some drugs.E.g. salicylates and sulphonamides may compete for the same binding sites.

Owing to its solubility characteristics, unconjugated bilirubin is not filiterd by the renal glomeruli and in health bilirubin is not detectable in the urine. Bilirubin reflects an increase in the plasma concentration of conjugated bilirubin, and is always pathological.Conjugated bilirubin is water-soluble and is secreted into biliary canaliculi, eventually reaching the duodenum by ducts of biliary system where the diglucuronide is spilt and bilirubin converted by bacterial action in the small intestine into urobilinogens. Some urobilinogen is absorbed from the gut into portal blood; hepatic uptake of this is incomplete, and a small quantity reaches the systemic circulation and is excreted in the urine.

Most of the urobilinogen in the gut is oxidized in the colon to a brown pigment, urobilin, which is excreted in the feces.

Some 300 mg of bilirubin is produced daily but the healthy liver can metabolize and excrete ten times this amount. The measurement of plasma bilirubin concentration is thus an insensitive test of liver function. The bilirubin normally present in plasma is mainly unconjugated type. (William J.M, 1992).

1.5.2 Jaundice:

Jaundice or icterus comprises a yellow discoloration of the skin and sclera produce by accumulation of bilirubin in the tissue and interstitial fluids. Jaundice generally appears first in the sclera in values of 35-50 micromole/L. the intensity of jaundice depends on many factors. Including the level of hyperbilirubinemia,which may

result from excessive bilirubin production, a failure of bilirubin transport across the liver or biliary obstruction. A consideration of the mechanisms of jaundice involves an understanding of formation, transportation, metabolism and excretion of bilirubin.

The specific pathophysiologic mechanisms that cause jaundice:-

1-excess production of bilirubin.When an excessive amount of bilirubin is presented to the liver for metabolism, such as occurring in unconjugated hyperbilirubinemia. Here the serum bilirubin level rarely exceeds 5 mg/dl because the normal liver is capable of handling most of the overload.

2-reduced hepatic uptake of bilirubin.

3-impaired excretion of conjugated bilirubin. The secretion of conjugated bilirubin may be impaired at the level of the liver cell membrane, within the bile canaliculi or at the level within the excretory duct system. Depending on the level of the derangement, disorders of bilirubin secretion of excretion are usually divided into intrahepatic and extrahepatic causes of cholestasis, various drugs may cause intrahepatic cholestasis, acute hepatitis and cirrhosis may act several levels. (Praveen K, M, C, 1991).

1.5.3Analysis of bilirubin:

The most frequently performed biochemical tests are measurement plasma bilirubin, protein, and measurement of the amino transferase, alkaline phosphatese and gamma glutamyletransferase in the plasma. In addition to the other serological tests were performed for specific auto antibodies and detection of bile pigments in urine and stool.

Several methods were performed for bilirubin estimation and revealed two types of bilirubin. (Arai N, *et al*.1997). The faction that produced a color aqueous solution in the van den Bergh method was described as direct bilirubin, whereas the fraction that produced a color only after alcohol was added was called indirect bilirubin. For many years, results of bilirubin determinations were reported as direct of indirect. This terminology is now outdated. Since 1956, it has been known that the direct bilirubin reaction is given by the diglucuronide of bilirubin or conjugated bilirubin, which is water soluble. The indirect reaction, however, is given by unconjugated bilirubin, which is water-insoluble but dissolve in alcohol to couple with the diazo reagent. Direct and indirect bilirubin should be reported as conjugated and unconjugated, respectively. Most commonly, conjugated and total bilirubin are reported.(Sherwood, L, 1997). Unconjugated bilirubin may be determined by subtracting conjugated bilirubin from total bilirubin.

Unfortunately, no single method for the determination of bilirubin will meet all the requirements of the clinical laboratory. The sources of error in this technique are turbidity, hemolysis, and yellow lipochrome pigments. Malloy or Jendrassik-grof method is suitable. The Jendrassik-Grof is slightly more complex but has advantages over the Evelyn-Malloy method because, it is more sensitive, has minimal turbidity, relatively serum blank not affected by hemoglobin up to 750 mg/dl mos clinical laboratories use either the Evelyn-Mallory or Jendrassik-Grof method.(Arai N, *et al*., 1997).

1.5.4 Effect of Benzene with total bilirubin:

A raised total bilirubin was reported among diesel engine workers who were exposed to high pressure resistant lubricants containing lead

naphthanate .(Seema T, 2013) . they blamed the icrease in total bilirubin to the poor compliance of the workers resulting in skin exposure , where benzene absorption might have occurred through the lung . many conturies have reduced or eliminated the use of lead additives in motor gasoline due to the health consequences of lead exposure as well as to the introduction of catalytic converter , but in other counters leaded gasoline remains the normal , and still acts as major source of lead exposure .(Kelly F.J, 2003).

1.6 Rationale

The benzene usually enters the body in one of three ways: skin contact, consumption of contaminated water or food and inhalation. inside the body , benzene enters the bloodstream and is carried into bone marrow and fatty tissues. Eventually it passes through the liver, where it is broken down. As result, harmful metabolites are formed. Some of the health problems caused by benzene exposure are due to the presence of metabolites in the body. Benzene cause harmful effects on the bonemarrow and can cause a decrease in red blood cells, leading to anemia. It can also cause excessive bleeding and can affect the immune system, increasing the chance for infections.

The seriousness of poisoning caused by benzene depends on the amount , route,and length of time of exposure , as well as the age and preexisting medical condition of the exposed person.

There is no studies had conducted to determine the effect of benzene on the blood antioxidant in Sudan ,therefore this study conducted to evaluate the plasma anti-oxidant(uric acid , total bilirubin and albumin) levels in the workers of benzene station in Khartoum state in order to investigate, develop, follw up and furtherprevention .

1.7 Objectives:

1.5.1General objectives:

To assess the plasma anti-oxidant levels (uric acid, total bilirubin and albumin) in benzene station workers.

1.5.2 Specific objectives:

1.To estimate the plasma levels of antioxidant (uric acid , total bilirubin and albumin) in case and control group.

2.To find correlation of plasma antioxidant levels(uric acid , total bilirubin and albumin) with duration of work.

3. To find correlation of plasma antioxidant levels(uric acid , total bilirubin and albumin) with age .

2.Materialand Methods

2.1 Materials:

2.1.1study design:

This is analytical case – control study

2.1.2 study area and period:-

This study was done in Khartoum state during the period of January 2015 to April2015.

2..1.3study population:

The targeting group for this study was Sudanese benzene station workers.

2.1.4 samplesize:

The study covered 80 individuals , 50 samples from gasoline station workers as (case) group and the rest were healthy persons (control group).

2.1.5 inclusionand exclusion criteria:

Test group: benzene station workers .

Control group: healthy subjects .

Subjects with renal or heart disease, gout and liver disease were excluded.

2.1.6ethicalconsiderations:

* The aim and benefits of this study were explained to the participants * an informed consent was obtained from each participant.

* Health education was provided to each participant.

2.1.7data collection and analysis:

1. interview with questionnaire an interview with questionnaire

The clinical data was used for each participant in this study.

2. blood samples and collection from each patients as well as contrall subject using disposable syringe. Blood samples were collected in heparin containers. Lipemre sample were cleared by centrifugation >hemolysis samples were dis qualitied> specimen of about lml heparinized plasma were preserved at 20c prior to processing.

3. biomedical measurements and instruments used Colton 70%alcohon containers and mindary automation device .

2.1.8statistical analyses:

Data analy ted by using apss 17.0 computer programs .

2.2method:

2.2.1Uric acid albumin and total bilirubinestimation:

Serum (uric acid , total bilirubin , albumin) was determined by mindary instrument according to the regent manufactures instruction .

** determination of Uric acid ** determination of Total bilirubin .

**determination of Albumin :see Appendix .

2.2.2quality control:

The accuracy of all methods used in this study controlled by commercially prepared control sera.

3. Results

This study was conducted on 50 workers of petroleum station as case 30 subject from community control group.

In this study test group composed of workers which they work at least years and maximums 27 years.

-Table (3-1) showed there was significant increase in the means of plasma levels of uric acid, total bilirubin and albumin(6.75±0.70) (0.886±0.25) (5.62±0.45) when compare to controlgroup (5.13±1.23) (0.353±0.183) (3.90±0.40)respectively.

Figure (3-1) there was positive significant correlation between plasma level of Uric acid and duration (r: 0.910, p value: 0.000).

Figure (3-2) there was positive significant correlation between plasma level of TotalBilirubin and duration (r: 0.963, p value: 0.00) .

Figure (3-3) there was positive significant correlation between plasma level of Albumin and duration (r=0.968 , pvalue=0.000).

Figure (3-4) there was positive significant correlation between plasma level of Uric acid and age (r: 0.629, p value: 0.000).

Figure (3-5) there was positive significant correlation between plasma level of Total Bilirubin and age (r: 0.577, p value: 0.000).

Figure (3-6) there was positive significant correlation between plasma level of Albumin and age (r: 0.602, p value: 0.000).

Table (3-1) Comparison of means±SDof plasma levels of Albumin, Uric acid and Total Bilirubin (mg/dl) on the Benzene station workers and control group.

Variable	Test group n:50	Control group n:30	P Value
Albumin	5.62±0.45	3.90±0.40	0.000
Uric acid	6.75±0.70	5.13±1.23	0.000
Total bilirubin	0.886±0.25	0.353±0.183	0.00

**The Table shows the (mean±SD) and The probability (P).

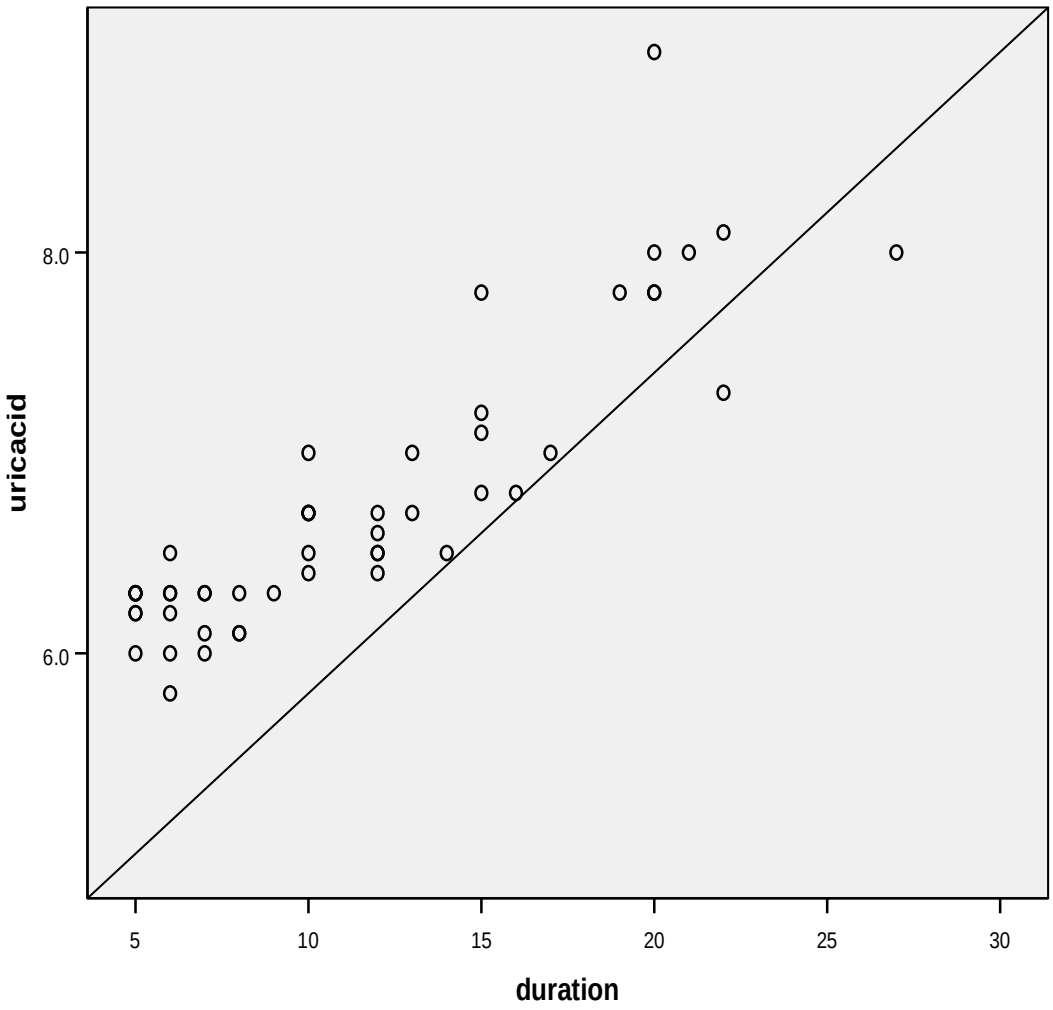

Figure (3-1) correlation between plasma level of Uric acid and duration (r: 0.910, p value: 0.000)

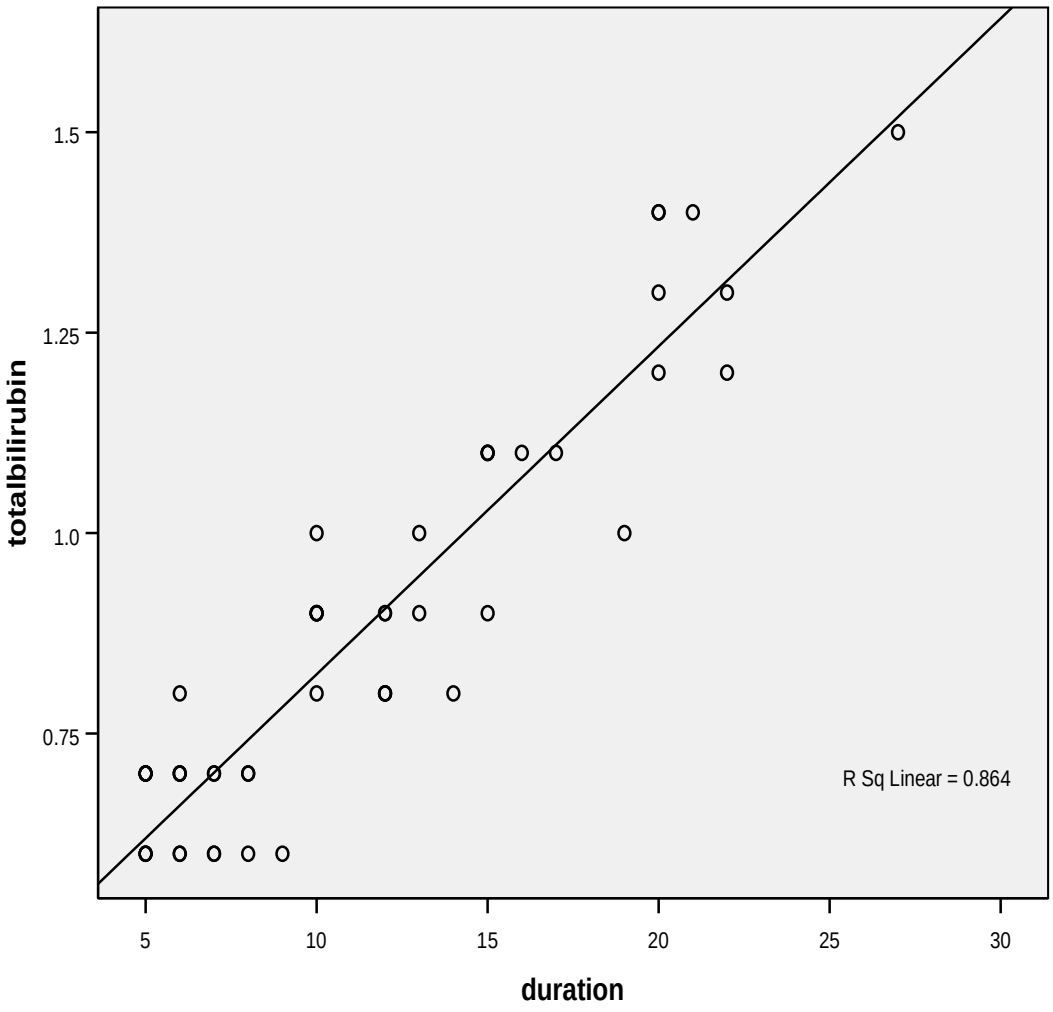

Figure (3-2)correlation between plasma level of Total Bilirubin and duration (r: 0.963, p value: 0.00) .

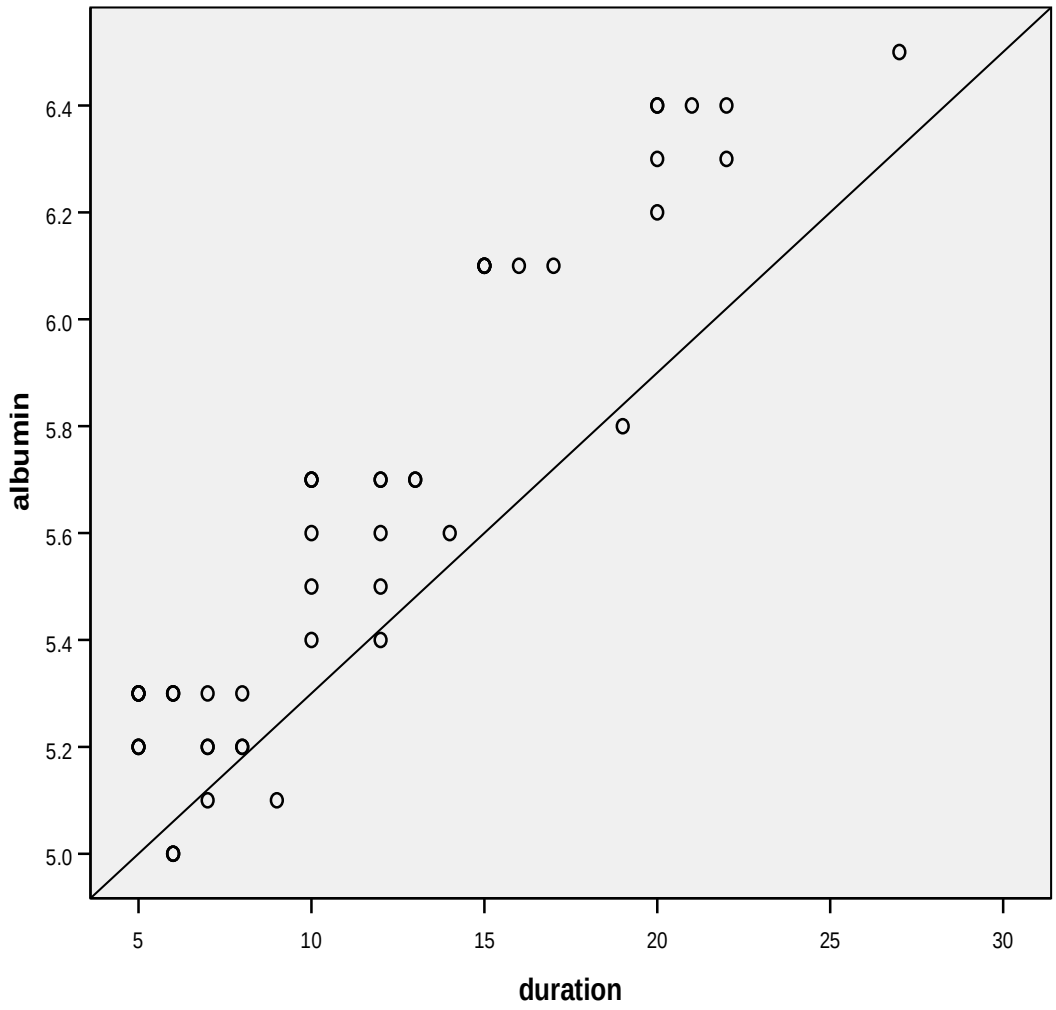

Figure (3-3)correlation between plasma level of Albumin and duration (r=0.968 , pvalue=0.000).

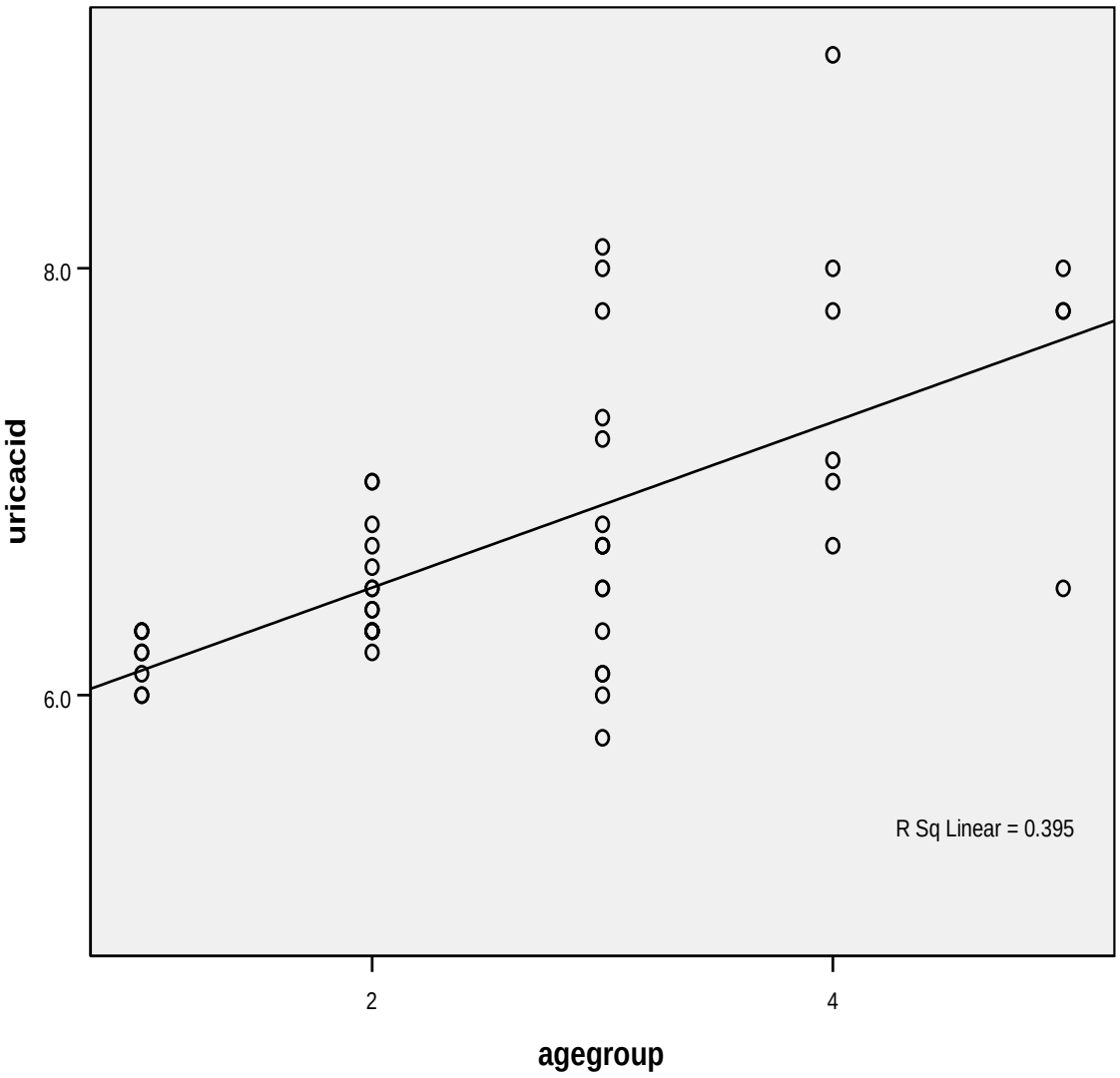

Figure (3-4)correlation between plasma level of Uric acid and age (r: 0.629, p value: 0.000).

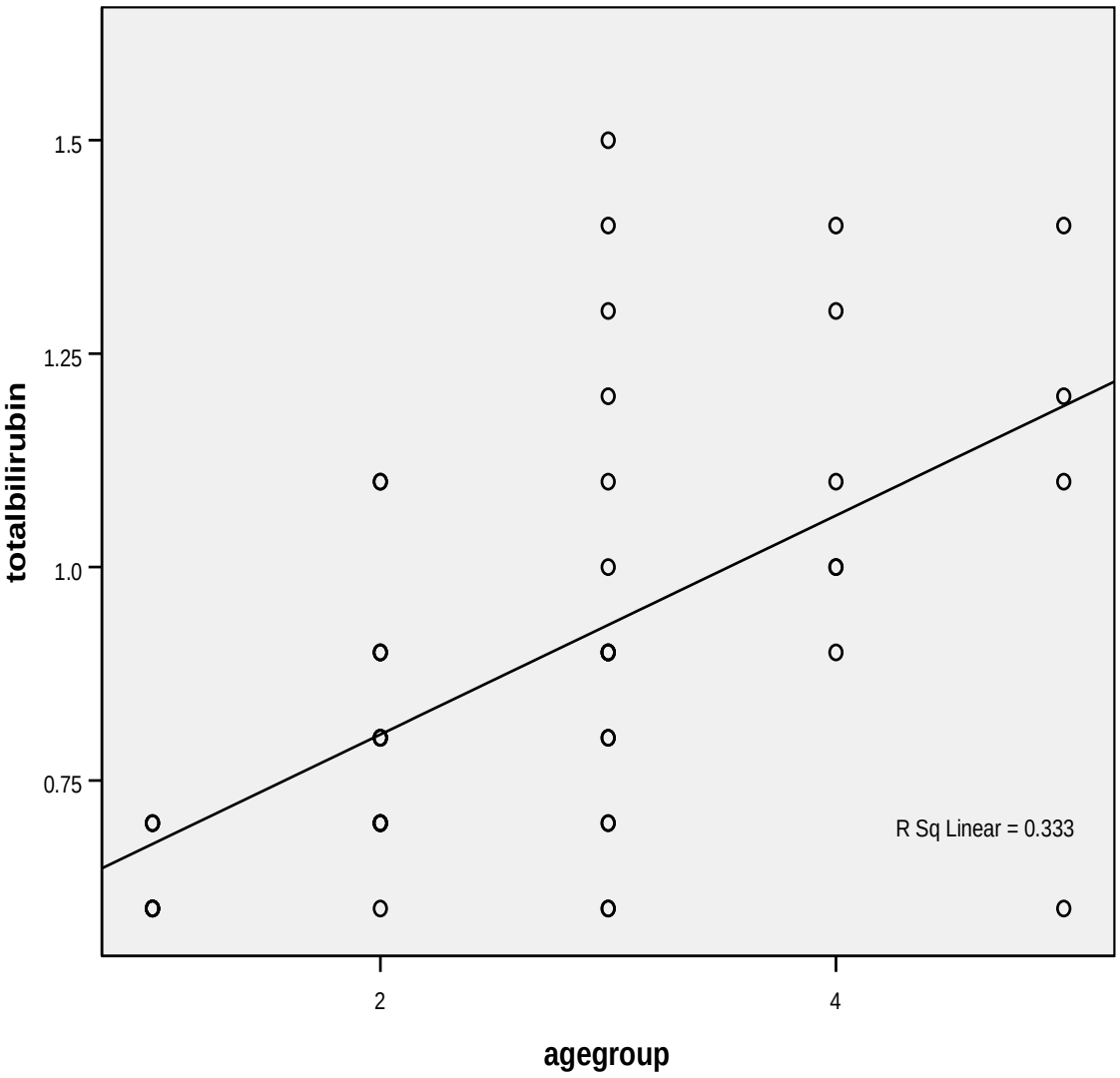

Figure (3-5)correlation between plasma level of Total Bilirubin and age (r: 0.577, p value: 0.000).

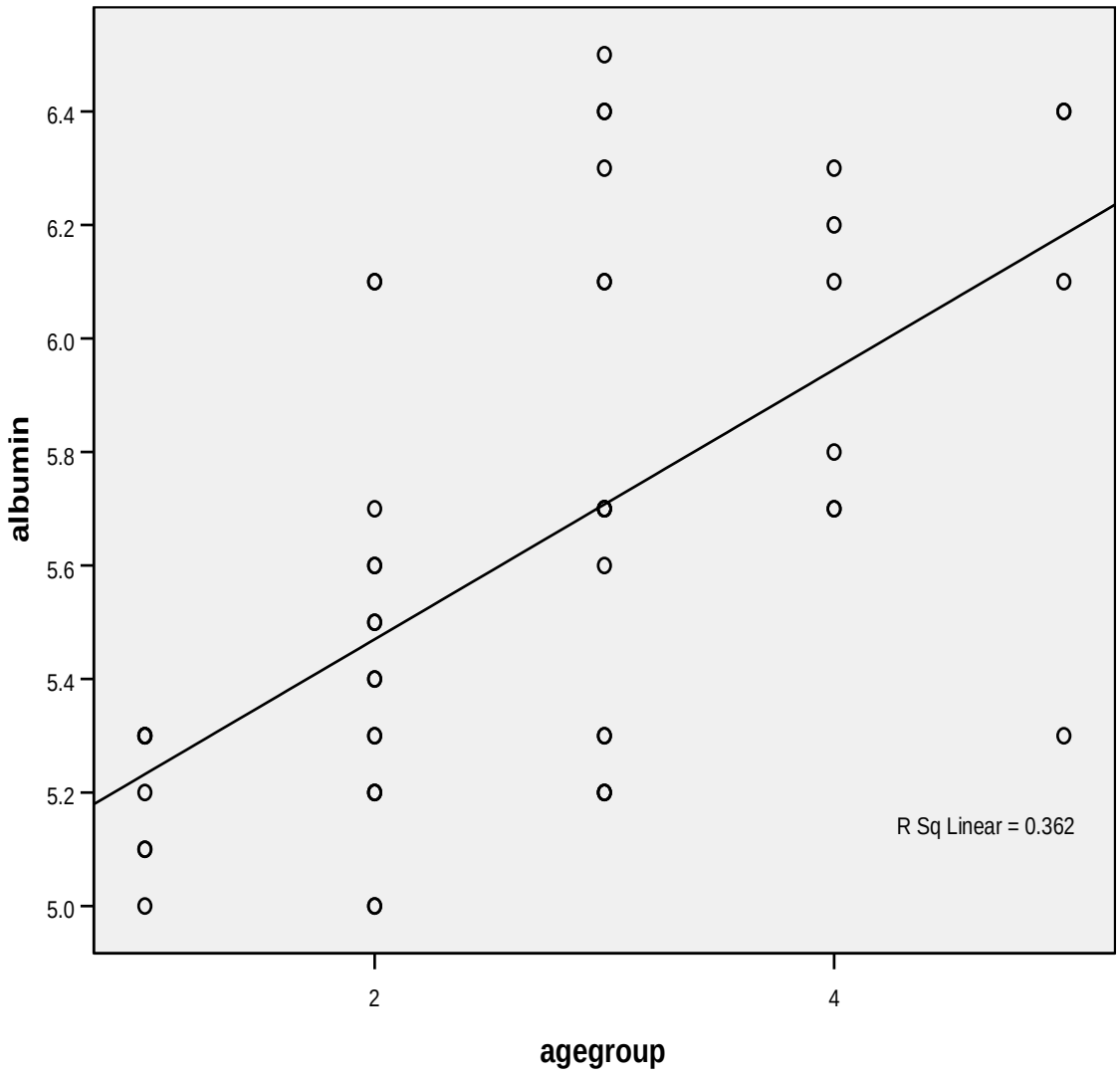

Figure (3-6)correlation between plasma level of Albumin and age (r: 0.602, p value: 0.000).

4. Discussion, Conclusions, and Recommendation

4.1 Discussions:

In normal physiological condition animals maintained abalancebetween generation and neutralization of reactive oxygen species (ROS). (Valko M.D, *et al*. 2007).however,when organisms are subjected to petroleum compounds such as benzene , the rate of production of (ROS) in cells get increased along with hydrogen peroxide , hypochlorous acid (HCIO) and free radicals including hydroxyl radical (OH) and superoxide anion (O2-) . oxidative stress have been implicated in a variety of pathological conditions such as: diabetics mellitus , cancer , aging , liver damage ,and atherosclerosis etc. (Nakagawa, *et al*.2008).

-In this study workers at benzene station has a significant increase in the means levels of plasma uric acid, total bilirubin and albumin (when compare to control group) (p=0.000, p=0.00, p=0.000) respectively. This is agree with other studies which illustrated that in benzene exposure has been associated with increase in the overall formation of anti-oxidant, albumin(p=0.0001), uric acid (P=0.014) Total Bilirubin (p=0.029) (GeorgievaT,2012)

-The plasma level of uric acid showed positive significantcorrelation with increasing the period of petroleum station workers (r=0.910, p=0.000) this finding agrees with other observations found earlier it was reported that uric acid is anendoantiaxidant , thus raising urate level might in part be an antioxidant response to protect against the provident effect of benzene.(r=0.80 ,p=0.001).

-Plasma level of Total bilirubin was showed positive significant correlation with increasing the period of benzene station workers (r: 0.963, p value: 0.00) . and

there is significant differences among the groups. This finding was in a good agreement with research(r=0.965, p=0.000), (Kelle M,1999).

-Plasma level of albumin in benzene station workers show positive significant correlation with increasing the period of benzene station workers (r= 0.968, pvalue=0.000).it was agree with other previous study had done. (hong Y, 2009).

AlsoThe plasma level of uric acid, total bilirubin and albumin showed positive significant correlation with age (r=0.629, p=0.000) (r=0.577, p=0.000) (r=0.602, p=0.000) respectively.

That's mean of the plasma antioxidant (Uric acid, Total Bilirubin and albumin) were increased in the Benzene station workers and were effected by Duration and Age.

Antioxidants such as glutathione (GSH), uric acid, ascorbate, total bilirubim albuminand α-tocopherol present in epithelial lining fluid (ELF) may protect the airways from oxidant injury induced by exposure to air pollutants. (Kelly, *et al.*1996). the antioxidants act as sacrificial substrates scavenging oxidant pollutants from the air ways and thereby preventing oxidation of macromolecules such as: lipids , protins and carbohydrates.

4.2. Conclusion:

From the result of this study is concluded that:

1. The means of plasma levels of uric acid, total bilirubin and albumin are significantly increase when compare to helthy group.
2. The levels of plasma uric acid, total bilirubin and albumin show significant correlation with duration.
3. The levels of plasma uric acid, total bilirubin and albumin show significant correlation with age.

4.3 Recommendations:

This study recommended:

1. Using protective cloths and use protective tools for workers in benzene station

2. Determine the limit period of workers .

3. Do regular monitor and follow-upof workers .

4. Do medical examination for workers regularly and save result .

5. we should assment other parameters to evaluate the effect of benzene .

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