بسم الله الرحمن الرحيم

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Liver Function Test among Petrol Workers

Athesis submitted in partial fulfillment for the requirement of MSc degree in medical laboratory sciences (clinical chemistry)

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

(وَقُل رَبِّ زِدْنِي عِلْمًا)

صدق الله العظيم

{طه:114}

Dedication

I dedicate this research to my father, mother sisters, family, friends and teachers, who taught us to think, understand and express

Acknowledgments

First I wish to thank Allah for granting me the Confidence and Success to complete this study. I would like to express my sincere gratitude and honest appreciation tomyi supervisor Dr Abdelwahab Abdeen. My thanks are extended to my colleagues in the Clinical Chemistry Department, Faculty of Medical Laboratory Science, shendi University. Great thanks for my brothers who helped meand made my work wonderful for the better. Thanks for my friends, Last thanks for everyone helped me my research.

List of Abbreviations

Abbreviation	Words
AH	Alcoholic hepatitis
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
DILIN	Drugs induced liver injury
DMSO	Dimethylsulfoxid
HAV	Hepatitis A virus
IU/L	International unite/liter
PT	Prothrombin time
R.P.M	Round Per Minutes
SPSS	Statistical package for social sciences

Abstract

This study was carried out in River Nile state Sudan working in petrol station gain without considering long term of exposure, could there be such effects on them. 80 subjects participated in the study individuals. Blood sample was collected from all participants and evaluated to liver function test (Total and direct bilirubin, Enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein and albumin were determined), obtained results were analyzed statistically by using SPSS. Student t- test among the groups and controls are statistically significant at $p < 0.05$.

This study shows normal total and direct bilirubin, and no effect on AST, ALT, albumin and protein levels in petrol worker when compared with the controls, because P.values were > 0.05. Only alkaline phosphatase was statistically significant elevated when compared to controls, the mean of ALP was 58.8 IU/L in test group, and 36.0 IU/L in control group with p.value of 0.000**.**

المستخلص

اجريت هذه الدراسة في ولاية نهر النيل السودان لدراسة تأثير ابخرة البترول على وظائف الكبد في عمال محطة الوقود

اخذت 80 عينه 50 من عمال الوقود و30 كمجموعه ضابطه لم يتعرضوا لأبخرة البترول, تم أخذ عينات دم من كل المشاركين واجريت اختبارات وظائف الكبد للمجموعتين (بيلروبين كلي- بيلروبين مباشر-بروتين كلي –البيومين-وانزيمات الكبد)

ومن ثم تم تحليل النتائج احصائيا باستخدام الحزم الاخصائية SPSS واثبتت الدراسة ليس هناك تأثير على البيلروبين الكلي، البيلروبين المباشر، البروتين الكلي، الألبومين، انزيمات الكبد (AST, ALT). لان القيم الاحتمالية كانت اكثر من 0.05

فقط يوجد تأثير على انزيم الكبد (ALP) لان متوسط قيمة ALP في عينة الاختبار كانت 58.8 وحدة عالمية لكل لتر وفي العينة الضابطة كانت 36.0 وحدة عالمية لكل لتر وكانت القيمة الاحتمالية P.value 0.000.

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Chapter one

Introduction

Rationale

Objective

1-1 Introduction

The liver is a vital organ acting as a processing center of the major metabolic activities in the body. It performs complex synthetic, excretory and detoxification functions. Unfortunately, it is continuously exposed to various noxious factors affecting its vital function. Examples of these offending agents include viral infections, alcohol, drugs and many ingested or inhaled chemical toxins.

The resulting liver cells damage is manifested clinically as jaundice, bleeding tendency and other signs and symptoms of liver failure or hypo-function, and chemically as deranged liver function tests. This usually reflects as high serum bilirubin, low albumin, and elevated liver enzymes. Petroleum fumes are among the known causes of liver toxicity. Petroleum is a mixture of aliphatic and aromatic hydrocarbons containing oxidating agents as benzens, toluen and xylen, in addition to heavy metals as lead. These compounds are converted into free radicals or activated metabolites during their oxidation in the liver cells. These metabolites attach different intracellular components and organelles leading to liver cells injury, necrosis and reduced functional capacity.

Many studies conducted on individuals exposed to inhalation of petroleum fumes concluded that the long-term exposure to these substances could have adverse effects on their liver function. Plasma aspartate and alanine amino transferase (AST and ALT), alkaline phosphatase and serum bilirubin in one study group of petrol station workers were reported to be significantly increased compared to the control subjects. Furthermore, the duration of exposure can affect the degree of liver damage. [1]

1.2 Rational

The toxicity of petroleum fuel is a major problem among the petrol workers; it may affect many body functions including the liver.

The liver function need to be evaluated on those individuals to detect the effect of petroleum fuel on liver functional capacity.

This study is designed to assess the effects of the exposure to petrol fumes on certain liver function tests among fuel station workers in the River Nile state, Sudan.

1-3 Objectives

1-3-1 General objective

To evaluate the effect of petroleum fumes on liver functions test among petrol station workers.

1-3-2 Specific objectives

1- To estimate the liver enzymes of petrol workers.

2- To evaluate the effect of duration of exposure on the liver function.

Chapter Tow

Literature review

2. Literature review

2.1 Normal structure and function of the liver

The liver is a large and complex organ weighing approximately 1.2 -1.5 kg in the healthy adults. It is located beneath and is attached to the diaphragm. It is divided unequally into lobes by the ligament with the right lobe being approximately six times larger than left lobe. [2]

2-1-2 Blood supply and venous draining of the liver

Unlike most organs which have a single blood supply the liver is an extremely vascular organ that receives as blood supply from two sources the hepatic artery and the portal vein. The liver drains its wasted blood through the hepatic vein into the superior vena cava. [2]

2-1-3 Normal liver histology:

Liver is divided into hexagonal lobules centered on a central vein. At the periphery of the lobule are portal tracts containing a branch of hepatic artery, portal vain and bile canal functionally, liver can be divided into three zones forming an acinus. Zone 1 (periportal with the highest oxygenation) zone 3 is located around central veins where oxygenation is poor. Zone 2 is located in between (mid zone 1) liver parenchyma is arranged in cards of cuboidal cells divided by blood sinusoids. [3, 4]

2-2Normal liver functions

The liver performs four major functions;

2-2-1Excretory function

One of the most important function of the liver is the processing and excreting of endogenous and exogenous substances into the bile or urine such as major hem waste product, bilirubin. [2]

2-2-2 Synthetic function:

The liver has extensive synthetic capacity .it is responsible for synthesizing many biological compounds inducing carbohydrates, lipid and proteins, in addition to vitamins. [2]

2-2-3 Detoxification and drugs metabolism

Every substance that is absorbed in gastrointestinal tract must first pass through the liver. It can allow important substances to reach the systemic circulation and serve as barrier to prevent toxic or harmful substances to reaching the systemic circulation. In addition, liver is the major site for most of drugs processing, activation and deactivation. [2]

2-2-3:-Storage function:

Liver is the main storage organ for glycogen, iron, cooper and some vitamins especially A, D, and B12. [3, 4]

2-3: Liver function test;

2-3-1 Indications of liver function test

The various uses of liver function test include:

1. They are noninvasive yet sensitive screening modality for liver dysfunction.
2. They are helpful to recognize the pattern of disease.
3. They are helpful to assess the severity and predict the outcome of certain diseases.
4. They are helpful in the follow up of certain liver diseases and also in evaluating response to therapy.

2.3.2 Limitations of liver function tests

1. Lack sensitivity: the liver function tests may be normal in certain liver diseases.
2. Lack specificity: - they are not specific for any particular disease.

Thus, we see that liver function test have certain advantages as well as limitations at the same time .thus it is important to view them keeping the clinical profile of the patient in mind.

2-3-2-1 Serum and urinary bilirubin

Bilirubin is an endogenous anion derived from hemoglobin degradation from the RBCs. Its chemical structure is $C_{33}H_{36}N_4O_6$. It is conjugated and secreted by the liver into the bile canal. Serum bilirubin level of 17umoL/L suggests liver disease.

2.3.2.1.1. Types of bilirubin:

The classification of bilirubin into direct and indirect bilirubin is based on the original Van der Bergh method of measuring bilirubin.

1. Total bilirubin:-this is measured as the amount which reacts in 30 minutes after addition of alcohol. Normal range is 0.2-0.9 mg/dl (2-15μmol/L).
2. Direct Bilirubin: This is the water-soluble fraction. This is measured by the reaction with diazotized sulfanilic acid in 1 minute and Normal range 0.3mg/dl(5.1μmol/ L)
3. Indirect bilirubin:-this fraction is calculated by difference of the total and direct bilirubin and is measure of unconjugated fraction of bilirubin.

2.3.2.1.2 Diagnostic value of bilirubin level

Bilirubin in body is a careful balance between productions and removal of the pigment. Hyperbilirubinemia is a good indicator of reduced hepatic excretory function. Serum bilirubin levels more than 17μmol/L suggest underlying liver disease. Increased unconjugated bilirubin: results from over production/ impaired uptake or conjugation of the pigment. While increased conjugated bilirubin implies impaired intra hepatic excretion of conjugated bilirubin from hepatocytes to bile ducts or bile obstruction as in surgical or obstructive jaundice.

2-3-2-2Serum protein:

The liver is the major source of most the serum proteins .The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the α and β globulins.

Albumin is quantitively the most important protein in plasma synthesized by the liver and is useful indictor of hepatic function. Because the half-life of albumin in serum is as long as 20 days. The serum albumin level is not a reliable indicator of hepatic protein synthesis in acute liver disease. Albumin synthesis is affected not only in the liver disease but also by nutrition status, hormonal balance and osmotic pressure liver is only site of synthesis of albumin Normal serum values range from 3.5-4.5g/dL.

2-3-2-3 Prothrombin time (PT)

The liver is the major site of synthesis of many blood coagulation proteins as fibrinogen, prothrombin factor 7 and others. Clotting is the end results of a complex series of enzymatic reaction that involves at least 13 factors activating each other in three pathways: common, intrinsic and extrinsic pathways.

Prothrombin time (Pt) is a lab test used routinely to assess the integrity of the extrinsic pathway of coagulation. Its normal control value is 11 seconds. High readings indicate reduced liver synthesis of the extrinsic pathways factors.

The prolonged PT is not specific for liver disease and is seen in various deficiencies of coagulation factors [5,6,7]

2-3-2-4 Liver enzymes:

Liver enzymes play an important role in the assessment of liver function because injury to the liver resulting cytolysis or necrosis will cause the release of enzymes into circulation .enzymes also play important role in differentiating hepatocellular (functional) from obstructive.(mechanical) liver

disease. The most clinically useful include the amino transferases (alanine amino transferase (ALT) and aspartate aminotransferase (AST) the alkaline phosphate (ALP) and gamma glutamyle transferase (GGT) and lactate dehydrogenase.

ALT is Found mainly in the liver (lesser amounts in skeletal muscle and kidney) where AST is widely distributed in equal amounts in heart, skeletal and liver, making ALT a more liver specific marker than AST regard less the serum activity of both transaminases rises rapidly in almost all disease of the liver. Because AST and ALT are present in other tissues besides the liver elevation in these enzymes may be a result of other organ dysfunction or failure. The ALP family of enzymes are zinc metalloenzymes that are widely distributed in all tissues however highest activity is seen in the liver, bone, intestine, kidney, and placenta.

The clinical utility of ALP lies in it is ability to differentiate hepatobiliary disease from osteogenic bone disease.

In the liver the enzyme is localized to the micro villi of the canaliculi and therefore, serves as great marker of extra hepatic biliary obstruction. [8,910]

2-4Liver pathology

2-4-1 Inflammatory conditions (hepatitis)

2.4.1.1viralhepatitis:

Viral hepatitis is inflammation on liver parenchyma caused by different vial agents, mainly the hepatitis viruses A, B, C, D and E. in addition to other verses. It can take acute or chronic forms.

Hepatitis A virus (HAV) is an RNA virus belonging to Picornaviridae family. It's transmitted through orofecal rout by contaminated food. [11.12,] It is usually an acute self limiting infection with no chronic form Hepatitis B virus is a

DNA virus of hepadanavdea family. It is transmitted parenterally through blood transfusion, by sexual contact and from mother to the newborn. [13, 14]

Hepatitis E virus is an RNA calicivirus. Infections worldwide are from faecal oral transmission .patient to patient transmission is rare. [15, 16]

Hepatitis D (Delta) virus .is the most sever from of viral hepatitis in humans. The hepatitis D virus is a defective RNA virus which requires the B virus (HBV) surface antigen for complete replication and transmission. [17, 18]

Hepatitis C virus is an RNA virus belonging to Flaviviridae family. It is transmitted parenteraly by blood transfusion, drugs injections, and sexual contact and by contaminated medical and non medical sharp equipment. [19, 20]

2-4-1-2 Alcoholic liver disease:

Alcohol is major cause of liver damage. It can cause alcoholic hepatitis, alcoholic steatosis and liver cirrhosis. Alcoholic hepatitis (AH) is inflammation of liver parancnchyma and occurs in about 40%of alcoholic characterized by liver cell swelling, Mallory bodies and neuophilic infiltration. Steatosis or fatty liver is deposition of fats inside the liver cells, clinically present with hepatomegaly. Cirrhosis is complete loss of liver architecture due to fibrous septation and nodule formation. It present with jaundice, portal hypertension and liver failure. [21, 22, 23]

2-4-1-3 Drugs induced liver injury (DILIN):

Drugs are an important cause of liver injury. More than 1000 drugs, toxins, and herbs have been reported to cause DILIN. the injury can be does dependent , non dose dependent or idisycaratic .pattern of injury include direct toxic reaction with elevated liver enzymes, allergic hepatitis , cholestatic reaction or chronic granulomatous reaction or chronic granulomatous inflammation. Examples of potentially hepatotoxic drugs include

antimicrobials (Rifampicin, INH and ketoconazole) analgesies (paraetamoi, declofenac, pyracetam) and anti convulsants (phyntoin, carboamazepine) [24, 25]

2-4-4 Toxins induced liver injury:-

Liver injury may follow the inhalation, ingestion, or contact with of a number of and chemical agent .these include industrial toxin e.g.; carbon tetra chloride, trichloroethylene, xylin, chloroform and toluene. The main mechanisms of liver damage in toxins exposure are inflammation, dysfunction of cytochrome P 450, mitochondrial dysfunction and oxidative stress. Clinical presentation of occupational liver disease may be acute /sub acute or chronic but is often insidious, include jaundice, elevated liver enzyme and frank liver failure in severe cases. [26]

2-4-2:-Liver cell necrosis:

Necrosis is death of group of cells in living organisms .it is the end result of various forms of tissue injury.

Necrosis is a common finding in acute and chronic liver disease it can take many forms.

Focal necrosis is seen viral hepatitis .zonal necrosis in ischemic and drug induced hepatitis, it involves particular acinar zone, as cetriloular necrosis of binding necrosis (central – central, portal - portal necrosis.

Confluent necrosis is involvement 0f multiple lobules and is seen in chemical injury and fulminant viral hepatitis. [27]

2.5 Petroleum toxic effects:

2.5 .1Chemical and physical properties of petroleum:

Petrol (gasoline) is a complex mixture of aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons (N, S, O_2, vanadium and nickel) derived from blending fractions of crude oil. It is commonly used as fuel for internal combustion engines (cars) and in industries. Many of the

petroleum components are highly volatile and flammable. A toxic effect of gasoline is attributed to these volatile organic compounds such as benzene, toluene, ethylene and xylene. [28]

2.5 .2 General toxicity of petroleum:

Exposure to petroleum and its products constitute health hazards. These manifest as nervous system damage, blood disorders (including anemia, leukemia), renal damage, hepatic dysfunction and intoxication leading to serious psychotic problems, anesthetic effects and other body damage. [29]

Petroleum hydrocarbons and other related carbon containing compounds are converted into free radicals or activated metabolites during their oxidation in the body especially hepatic and renal cells that react with some cellular components such as membrane lipids to produce lipid peroxidation products they may also react with enzymes and cause inactivation through protein oxidation.

2.5.3 Effects of petroleum on the liver:

Liver is the main organ affected by toxins in the body. Toxic constituents of petroleum fumes, like benzene, lead and volatile nitrate are metabolized in liver and cause a dose and time dependent increase in Cytochrome P450 monooxygenases and reduced Glutathione-S- transferases and other related oxidative substances in the liver .long term exposure to inhaled petrol products can lead to liver cells necrosis and typical toxin induced hepatitis, with deranged liver function test results. [30]

2.5.4 Effects of petroleum on the liver test

Studies conducted on petroleum stations workers showed significant increase in liver enzymes (AST, ALT, ALP) and other liver parameters in the test population compared with the control.[31] Furthermore, increase in the duration of service in the petroleum station which is equivalent to the period

of exposure of individual participant to the petrol fumes increase the level of liver function parameters AST, ALT, ALP and total bilirubin) of the subject [32]

2.6 Previous study

Study conducted in Nigeria by Gali RM, Daja A, Mamza YP, Ani GI to estimate liver function test in petrol workers. Twenty (25%) were petrol hawkers, thirty-five (35%) petrol attendants and forty (40%) apparently healthy individuals. Enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein and albumin were determined. Mean age of the subjects was 27.09 ± 6.75.Subjects between the ages of 21- 30 years were 62% while 7% of the subjects were between the ages of 41-50 years, among the entire participant 93 (93%) were male while 7 (7%) were female. Student t- test among the groups and controls are statistically significant at $p < 0.05$. This study shows high levels of all the liver enzymes, and low albumin levels in petrol hawkers compared with the controls, whereas in petrol-pump attendants only alkaline phosphatase is elevated when compared to controls. [33]

Chapter Three

Materials and Methods

3- Materials and methods

3.1 Study design:

This is a cross sectional, case control study conducted to determine the effects of inhalation of petrol fumes on liver function of petrol stations workers.

3-2 Study area:

The study was conducted in the River Nile state – Sudan. The state is located in the northern part of the Sudan.

3.3. Sample size:

Eighty specimens were collected based on non probability convenience sampling technique. (50) of the specimens were collected from the proposed cases and (30)were selected as controls.

3.4 Study population:

The study was conducted among the workers of petrol stations in the River Nile state. These stations are distributed in 7 towns; namely Aldamar, Atbara, Barbar, Shendi, Almatma, Abohamad, and Koji total number of stations was70 approximately surveyed. The average number of workers exposed to petroleum in each station was average 4-8. So the estimated number of the population form which the sample was extracted is 50.

3-5 Selection criteria:

3-5-1 Inclusion criteria:

Petrol stations workers as test group and healthy adult individuals as control.

3-5-2 Exclusion criteria:

Individuals in both cases and control groups excluded from the .study were those who ha d significant medical history e.g. hepatitis of liver diseases.

3-6 Collection of blood samples:

From each individuals, use disposable syringes and take ‘ 5 ml of blood were drown using standard procedure and placed2 ml of blood sample in heparinized container then centerifuged to obtain serum and used to estimate total bilirubin ,direct bilirubin, total protein and serum albumin, and 3ml was placed in plain container , this blood samples were allowed to colt at non temperature and then centrifuged at 4000 rpm to obtain serum and used to estimate liver enzymes(AST, ALT, ALP)

3.7 Methods

3-7-1 Estimation of serum bilirubin:

Principles:

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically of the two fractions presents in serum ,bilirubin glucuromide and free bilirubin loosely bound to albumin ,only the former reacts directly in aqueous solution (bilirubin direct),while free bilirubin requires solubilization with dimethylsulfoxid (DMSO)to reacte (bilirubin indirect).in the determination of indirect is also determined the result correspond to total bilirubin .the intensity of the color formed is proportional to the bilirubin concentration in the sample.

Reagents were mixed and then incubated exactly for 5 minutes at 15-25^{0}c and the result was read by mindary BA-88A.

Reference values:-

Bilirubin total up to 1.10 mg/dL=18.81umol/L

Bilirubin direct up to 0.25 mg/dL=4.27umol/L

3-7-2 Estimation of serum (total) protein:

Principle:

Protein in the samples reacts with copper (11) ion in alkaline medium forming a colored complex.

Reference values: - 64-83g/l

3-7-3 Estimation of serum Albumin:

Principle:

Albumin in the presence of bromocresol green at slightly acid pH produces a colour change of the indicator from yellow –green to green blue. The intensity of the color formed is proportional to the albumin concentration in the sample.

Reference value: - 3-5 to 5.0 g/dl.

3-7-4 Aspartate amino transferees (AST/GOT):-

Principle:-

Aspartate aminotranseferase:9(AST OR GOT)catalyzes the transferees of the of (Astor Got)catalyzes the transferees of the amino group from aspartate to 2 oxoglutarate forming oxaloacetate and glutamate .the catalytic concentration is determined from the rate of decrease of NADH ,measured at 340 nm by means of the malate dehdrogenenase (MDH)coupled reaction .

Aspartate +2 - 0x0glutarate$\xrightarrow{AST}$ Oxaloacetate +Glutamate

Oxaloacetate +NADH+H^+ $\xrightarrow{\text{MDH}}$ MALATE+NAD^+

Reference values:-

Without pyr-p up to 40 U/L =0.67ukat/L

With pyrp up to 50 u/L =0.83 ukat/L.

3-7-5 Alanine amino transferase (ALT or GPT):

Catalyzes the transfer of amino group from alanine to 2 oxoglutarate, forming pyruvate and glutamate, the catalytic concentration is determine from the rate decrease of NADH measured at 340 nm, by means of the lactate dehydrogenase (LDH)coupled reaction

Alanine +2- Oxoglutrate$\xrightarrow{ALT}$Pyruvate +glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$Lactate +NAD^+

Reference values:- at 37C^{0}without py-p up to 41u/L = 0.68ukat/L

With pyp-p up to 65u/L = 1.08ukat/L

3-7-6 Alkaline phosphatase:-

Principle:-

Alkaline phosphate (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrohpenylphosphate to 2-amino2 methyl-1-propanol (AMP) liberating 4 nitrophenol .the catalytic concentration is determined from the rate of 4 nitrophenol formation .measured at 405 nm .

4 - Nitrophenylphosphate +AMP$\xrightarrow{ALP}$AMP- phosphate +4- Nitro phenol.

. **Reference values:-** At 37C^0

Up to 115 u/L =1.92ukat/L for men

And 105 u/L =1.75 U/L=1.75 ukat/L for women.

3-8 Data collection:-

Questionnaire designed containing relevant question and filled by direct interview with petrol workers.

3-9 Ethical consideration:-

Before questionnaires were administered to any eligible petrol workers, the latter was provided with consent from to sign or thumb print after the study was explained to them in detail.

3-10 Statistical analysis:-

Statistical analysis was performed with statistical package for social sciences (SPSS) software version 11.5 in all cases, $p \leq 0.05$was considered statistically significant.

Chapter Four

Results

4- Results

Table (4-1) Demographic information of the subjects studied:

Demography number	Number
Pump attendant	50
Control	30
Social activity	
Alcohol	0
Liver disease	0
Other disease	0
Long term medication	0

Table 4- 2 Distribution of subject according to work duration group:

Duration/ month	Frequency	Percentage %	Mean of ALPU/L
1 – 12	17	34	0.48
13 – 24	14	28	0.16
25 – 36	11	22	0.12
37 – 48	6	12	0.04
49 – 60	2	4	0.2
Total	50	100.0	

Table 4-3 mean of serum total bilirubin in test and control group

Study groups	Frequency	Mean of S. bilirubin Mg/dl	p.value
Test	50	0.42	0.182
Control	30	0.35	

Table 4-4 mean of serum direct bilirubin in test and control group

Study groups	Frequency	Mean of S. D bilirubin Mg/dl	p.value
Test	50	0.40	0.161
Control	30	0.34	

Table 4-5 mean of serum total protein in test and control group

Study groups	Frequency	Mean of S protein g/dl	p.value
Test	50	7.04	0.157
Control	30	7.41	

Table 4-6 mean of serum albumin in test and control group

Study groups	Frequency	Mean of S. albumin g/dl	p.value
Test	50	4.35	0.146
Control	30	4.1	

Table 4-7 mean of serum AST in test and control group

Study groups	Frequency	Mean of S. AST u/l	p.value
Test	50	16.24	0.747
Control	30	15.97	

Table 4-8 Comparison mean of serum ALT in test and control group

Study groups	Frequency	Mean of S. ALT u/l	p.value
Test	50	18.2	0.069
Control	30	15.7	

Table 4-9 Mean of serum ALP in test and control group

Study groups	Frequency	Mean of S. ALP u/l	p.value
Test	50	58.8	0.000
Control	30	36.0	

Chapter five

Discussion

Conclusion

Recommendations

5.1Discussion

This study was conducted to assess the level exposure to this product and the possible damage caused to the liver among petroleum workers.

A total of 80 subjects were recruited for this study, all subjects are male, and the demographic information of the study shows the subject revealed that 0 were alcoholic, medications taken by the subjects as the long term revealed that 0. Past history which shows that fifty (50) of subjects were pump attendants, thirty (30) were used as controls. The social activity of liver disease reveled that as 0

There was a significant increase in the levels of plasma liver enzyme alkaline phosphatase; mean of S.ALP in test group was 58.8 IU/L when compared with control group 36.0 IU/L with P.value of 0.000 that means there was significant effect of petroleum inhation on epithelial cells of bile canals. And there was no significant difference in other liver enzymes such as aspartate aminotransferase, alanine aminotransferase, and other liver function tests: total and direct bilirubin in petrol worker when compared with control subjects, because the P.value was > 0.05. There was also no significant difference noticed in both protein and albumin levels when compared with controls group.

5.2Conclusion

On the basis of the result of this study, it be concluded that;

- Petrol has effect on petrol worker and the severity of effect according to duration of exposure.
- Significant effect of petrol inhalation on liver enzyme ALP.
- No significant effect of petrol inhalation on other liver parameters.

5.3Recommendations

By the end of this study, we recommend:

- Petrol workers should be advised to undergo liver function test, routinely to avoid developing to liver failure.
- Further studies should be done with large sample size to identify the more details of the effect of petrol exposure on liver function test.
- Advise them to use mask to protect them from inhalation the petroleum elements

Chapter Six

References

Appendixes

6.1References

1. Ogunneye AL, Omoboyowa DA, Sonibare AL, Adebusuyi AJ, Faniran TP. (Hepatotoxic and Nephrotoxic Effects of petroleum Fumes on Petrol Attendants in Ibadan, Nigeria). Nig .J. Basic APPL. Sci, 2014; 22(3); 57-62.

2. Michael L. Bishop, Edward P.FODY, Larry E. Schoeff, clinical chemistry principles, procedures and correlation, part (1) 5th edition, chapter 22, Lippincott Williams and Wilkirs, United State of America, 2005. PP (477-480).

3. Rapaport AM: the structural and functional units of the human liver. Micrivasc Res 6:212, 1993.

4. Jungermann K, kietzmann T: Zonation of paranchyman and non parenchymal metabolism in liver. ANNu Rev Nut 16:179-203, 1996.

5. Daniel SP, Marshall MK. Evaluation of the liver: laboratory tests. Schiff's diseases of the liver, 8th edn.USA; JB Lippincott publications, 1999; 205-239.

6. Rosen HR, Keefe EB. Evaluation of abnormal liver enzymes, use of liver tests and the serology of viral hepatitis: Liver disease, diagnosis and management. 1st ed. New York; Churchill living stone publishers, 2000; 24-35.

7. Sherlock S. Assessment of liver function Disease of liver and biliary system: Sheila Sherlock, 10th ed, London; Blackwell science ltd, 1997; 17-32.

8. Friedman SF, Martin P, Munoz JS. Laboratory evaluation of the patient with liver disease. Hepatology, a textbook of liver disease. Philadelphia; Saunders publication, 2003; 1: 661-709.

9. Rosalki SB, Mcintyre N. Biochemical investigations in the management of liver disease. Oxford textbook of clinical hepatology, 2nd ed. New York; Oxford university press, 1999; 503-521.

10. American Gastroenterological association. American gastroenterological association medical position statement: Evaluation of liver chemistry tests. Gastroenterology 2002; 123: 1364-1366.

11. Armado Leon La, de Almeida AJ, de Paula VS et al. Longitudinal Study of Hepatitis A Infection by Saliva Sampling: The Kinetics of HAV Markers in Saliva Revealed the Application of Saliva Tests for Hepatitis A Study. PLOS One 2015; 10(12).

12. Centers for Disease Control and Prevention. Viral Hepatitis Surveillance – United States 2010. Hepatitis A virus, http://www.cdc.gov/ hepatitis/ Statistics/ index.htm, accessed 26 January 2013.

13. Alexopoulou A, Karayiannis P. HBeAg negative variants and their role in the natural history of chronic hepatitis B virus infection. World J Gastroenterology. 2014; 20:7644-52.

14. Amini-Bavil-Olyaee S, Maes P, Van Ranst M, Pourkarim MR. Providing strong evidence of nosocomial outbreak of hepatitis B virus infection. J Hosp Infect. 2012; 80(3):269-70.

15. Alter HJ, Purcell RH, Shih JW, et al. Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. N Engl J Med 1989; 321:1494-500.

16. Alter MJ, Kruszon-Moran D, Nainan OV, et al.The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999; 341:556-62.

17. Abravanel F, Mansuy JM, Huynh A, et al. Low risk of hepatitis E virus reactivation after haematopoietic stem cell transplantation. J ClinVirol. 2012; 152-5.

18. Adlhoch C, Avellon A, Baylis SA, et al. Hepatitis E virus: Assessment of the epidemiological situation in humans in Europe, 2014/15. J ClinVirol. 2016; 82:9-16.
19. Baumert TF, Yang C, Schurmann P, et al. Hepatitis B virus mutations associated with fulminant hepatitis induce apoptosis in primary Tupaia hepatocytes. Hepatology 2005; 41(2):247-56.
20. Belloni L, Allweiss, L, Guerrieri F, et al. IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNAminichromosome. J Clin Invest. 2012: 122(2):529-37.
21. Burra P, Lucey MR. Liver transplantation in alcoholic patients. Transpl Int 2005; 18:491-498. Cabré E, Rodríguez-Iglesias P, Caballería J, et al. Short- and long-term outcome of severe alcohol-induced hepatitis treated with steroids or enteral nutrition: a multicenter randomized trial. Hepatology 2000; 32:36-42.
22. Christensen E, Gluud C. Glucocorticosteroids are not effective in alcoholic hepatitis. Am J Gastroenterol 1999; 94:3065-3066
23. Clot P, Bellomo G, Tabone M, et al. Detection of antibodies against proteins modified by hydroxyethyl free radicals in patients with alcoholic cirrhosis. Gastroenterology 1995;108:201-207.
24. stirnimann G kessebohmK ,LAUTERBURG B .liver injury caused by drugs:update.swiss Med wkly 2010:140:w13080.
25. Ramachandran R, kakars.Histological patterns in drug-induced liver disease .J clinpathol 2009:62:481.
26. Franco G ,Fonte R, candura F .Hepatotoxicity of industrial toxins.Br j ind.
27. Aggarwal S, Fiel MI, Schiano TD. Obliterative portal venopathy: a clinical and histopathological review. Dig Dis Sci 2013; 58:2767-76.

28. Chilcott, R.P. Petrol toxicological overview, (2007). 2:1-1.
29. Aryanpur biological effects of petroluim 1979: Nwanjo and ojiako, 2007.
30. Smith, T, J, Hammand, S. K, and Wond, O. Health effects of gasoline exposure, 1: Exposure assessment for US. Distribution workers. Environmental. Health perspective, 1993.101:13-21.
31. Ogunney AL, Omoboyowa DA, SonibareAL, Adebusuyi AJ, Faniran TP. (Hepatotoxic and Nephrotoxic Effects of petroleum fumes on petrol Attendants in Ibadan, Nigeria)'Nig. basic APPL.sci, 2014:22(3):57-62.
32. Kapil Soni , Rakeshkumar impact of petroleum fumes on liver and kidney functioning of petroleum filling attendants working in south Haryana, indiaejpmr, 2016,3(8)569-573.
33. Mandiracioglu, A., Akgur, S., Kocabiyik, N. and Sener, U. 2011.
Evaluation of neuropsychological symptoms and exposure to benzene, toluene and xylene among two different furniture worker groups in Izmir. Toxicol Ind Health, 27(9):802-809.

6.2 Appendix

6-2-1Questionnaire

بسم الله الرحمن الرحيم

Effect of Petroleum Fuel Fumes Exposure on Selected Liver Function Tests among Petroleum Stations Workers in River Nile State, Sudan

Name: ……..

Age: under 18 years ☐ from 18-29years ☐ from 30-39 years ☐

From years 40 and above ☐

Exposure to petrol steam:

1-Appointed time you work in petroleum station: ……..…

2-daily time you work in the station:

Less than 4 hours☐ from 5-8 hours ☐ from 9-12 hours☐

More than 13 hours☐

3- Are you near the petroleum station during alternation?

Always ☐ sometime ☐ seldom ☐

4-whats your job in the station:

Employer's distribution fuel ☐ director ☐ other ☐

Pathological and treatment date:

1-Do you have jaundice before? Yes ☐ No ☐

2- Do you drinking alcohol? Yes ☐ No ☐

3-Do you have taken long-term medicine? Yes ☐ No ☐

If the answer yes be explicit the kind: ……

4-At this time do you have any disease in liver? Yes ☐ No ☐

5- Do you newly have check of liver function? Yes ☐ No ☐

If the answers yes what it is? ……

6- Do you suffer from health problem now? Yes ☐ No ☐

7- Do you use any protector during deal with petroleum steam? Yes ☐

No ☐

Laboratory examination result:

1- Total Bilirubin ……

2- Direct Bilirubin……..

3- Total Protein……

4- Serum Albumin ……

5- AST……

6- ALT……

7- ALP……..

6-2-3 Data sheets

6-2-4 Ethical approval

Ethical consideration was taken verbally. This study posed no physical risk to participants though an interview of (10) minutes, might have been convenient to some participants. It is a convenient study, thus neither the participants name nor his institution in use in any of the study materials and each participant was assigned a unique identification number. Collected data will be secured in a computer protected by password.