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Assessment of liver and renal function tests among gasoline exposed gas station workers in Mekelle city, Tigray region, Northern Ethiopia

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This is to certify that the thesis prepared by Tsegay Asefaw, entitled: Assessment of liver and renal function tests among gasoline exposed workers at gas station in Mekelle City, Tigray Region, Northern Ethiopia complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

ALP	alkaline phosphatase
ALT	Alanine Transeaminase
AST	Aspartate Transeaminase
CYP450	cytochrome P450
DNA	Deoxyribonucleic acid
GSH	Glutathione
IU/L	International unit per liter
LFTs	Liver function tests
mg/dL	Mill gram per deciliter
RFTs	Renal function tests
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RPM	Revolution per Minute
SOP	Standard Operating Procedure
SPSS	Statistical package for social science
Tbil	Total Bilirubin
US	United States
WHO	World health Organization

Abstract

Background: Volatile organic compounds such as gasoline and other fuels are associated with a wide variety of deleterious health effects including liver and kidney diseases. Gasoline station attendants are exposed to a mixture of hydrocarbons in fuel vapors during dispensing fuel and to the gases from vehicular exhaust. Nevertheless there is no published data on the effect of volatile organic compounds, such as gasoline, on health effect in Ethiopia in general, and in Tigray area in particular.

Objective: To assess liver and renal function tests among gasoline exposed gas station workers in Mekelle city, Tigray region, Northern Ethiopia

Method: A cross sectional comparative study was conducted from January 2018 to April 2018 at Mekelle city, Tigray region, Northern Ethiopia. A total of 90 (43 gasoline exposed and 47 controls) study participants were involved in this study by convenience sampling method. Data was collected using structured questionnaire. About 3mL blood was collected on serum separator tube. Liver and renal function tests were analyzed by Pentra C400 automated clinical chemistry analyzer. Data was analyzed using SPSS Ver23. Frequency table, percent, Student independent t-test, and Analysis of variance (ANOVA) statistical methods were employed for data analysis. P-value < 0.05 was regarded as statistically significant at 95% confidence level.

Result: The mean age of the gasoline exposed and control study participants were 30.02(±8.62) and 29.85 (±7.29) years respectively. Mean work duration of gas station workers was 5.187±4.39 years. The mean level of ALT, AST, Urea, creatinine, and uric acid was significantly higher among gasoline stations workers when compared to control study participants. There was also a significant increase in ALT, AST, Urea, creatinine and uric acid among gas stations with above 6 years exposure when compared with those exposed for ≤2 and 3-6years. The highest and lowest number of workers who worn gown and gloves at work place (42(97.7%) and (n=1, 2.3%) respectively. Majority of gasoline station workers 39(90.7%) had awareness on health impact of inhalational of gasoline. Besides to inhalation, 41(95.3%).gas station workers knew other route of exposure to gasoline has health impact

Conclusion: These findings suggest that high liver and renal parameters are associated with exposure to gasoline and it is dependent on time of exposure to gasoline.

Key word: Gasoline, Gas station, liver function Tests, kidney function tests

1. INTRODUCTION

1.1 Background

1.1.1 Liver Function Tests

The liver is the largest internal organ of the human body. It is a functionally complex organ that plays a critical biochemical role in the metabolism, digestion, detoxification, and elimination of substances from the body. The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short-term injury or disease. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with its essential functions (1).

Liver Function Tests (LFTs) are one of the most commonly requested screening blood tests. Whether for the investigation of suspected liver disease, monitoring of disease activity, or simply as 'routine' blood analysis, these tests can provide a host of information on a range of disease processes (2). Liver enzymes activities measurements are useful tests in the evaluation and treatment of patients with hepatic dysfunction. Serum level of liver enzymes increased according to the damage of the liver cell (3). These enzyme include transferase enzymes ,Aspartate amino transferase, Alanine amino transferase, and alkaline phosphatase. Alkaline phosphatase is most frequently measured indicator for liver bile ducts disease. AST and ALT enzymes frequently appear in the serum following liver cell injury or sometimes in smaller amounts from degraded cells. Elevated liver enzymes may indicate inflammation or damage to cell in the liver (4, 5). Different chemical agents, including gasoline vapor constituents, are known to be hepatotoxic (6).

1.1.2 Renal Function

Kidney is a paired organ whose functions include removing waste products from the blood and regulating the amount of fluid in the body. The basic units of the kidneys are microscopically thin structures called nephrons, which filter the blood and cause wastes to be removed in the form of urine. Together with the bladder, two ureters, and the single urethra, the kidneys make up the body's urinary system (1, 7). The kidney is critical to maintain the homeostasis of the

body through its endocrine and metabolic activities, its excretion of waste products and its reabsorption of essential compounds (8).

Frequent and heavy exposure to gasoline constituents is associated with increased risk of renal function impairment, due to their potential to either initiate kidney injury or worsen extant impaired renal function, or both (9). The markers of renal function test assess the normal functioning of kidneys. Creatinine, urea, uric acid and electrolytes are markers for routine analysis (10).

1.1.3 Gasoline Over view

Gasoline is a volatile and inflammable petroleum-derived liquid mixture primarily used for internal combustion of machines (11) . It is a complex mixture of hydrocarbons and additives, including short-chain organic compounds, light-chain volatile compounds, and heavy-chain hydrocarbons(12). The actual composition of petrol (gasoline) varies according to the source of crude oil origin, differences in process techniques and blends, between batches, and the additives required to meet particular performance specifications (13, 14).

Gasoline mainly consists of C4–C12 hydrocarbons (i.e., paraffins, naphthenes, and olefins) and substantial amount of antiknocking additives. Aromatics (i.e., benzene, toluene, xylenes, and ethylbenzen) and oxygenate compounds, such as methyl tertiary butyl ether, methanol and ethanol, are the most widely used antiknocking additives (15).

The major route of exposure pathways of gasoline are inhalation, ingestion, and skin contact. Inhalation is an important route of exposure. The more rapid absorption of gasoline is via inhalation than by the oral route and skin route. The dermal route appears to be slower than oral and inhalation routes (16). Some gasoline components are absorbed more rapidly than others. For example, Aromatic compounds (e.g; Benzene, Toluene, and Xylene) are absorbed more rapidly than other gasoline components. Because gasoline components have different metabolic pathways, the biotransformations of all its components are not addressed individually; but the metabolic pathway of benzene is defined here.

Benzene is primarily metabolized in the liver via cytochrome P450 (CYP) 2E1 to benzene oxide. Benzene oxide is in equilibrium with the intermediate benzene oxepin. Benzene oxide (or the oxepin) can undergo non-enzymatic rearrangement to phenol, hydrolysis to a dihydrodiol, ring

opening to trans, trans-muconic acid or react with glutathione to form a pre-mercapturic acid conjugate (figure1). The metabolites can undergo further metabolism by oxidation, dehydrogenation or conjugation with sulfate or glucuronic acid. For example, a primary metabolite of benzene oxidation, phenol, can undergo an additional oxidation catalyzed by CYP2E1 to hydroquinone and catechol or can be conjugated with sulfate to form phenyl sulfate. Hydroquinone and catechol can undergo further oxidation catalyzed by peroxidases to their respective quinones. These quinones, which are reactive species, can be reduced back to hydroquinone and catechol by NAD (P) H: quinone oxidoreductase (17, 18).

Activation of benzene and its reactive metabolites leads to continuous production of reactive oxygen species (ROS), which leads to lipid peroxidation and damages DNA, RNA, leading to genetic modification and alterations in the functions of important enzymes and proteins(19). Human exposure to benzene has been associated with a range of acute and long-term adverse health effects and diseases. Benzene is highly volatile, and exposure occurs mostly through inhalation(20).

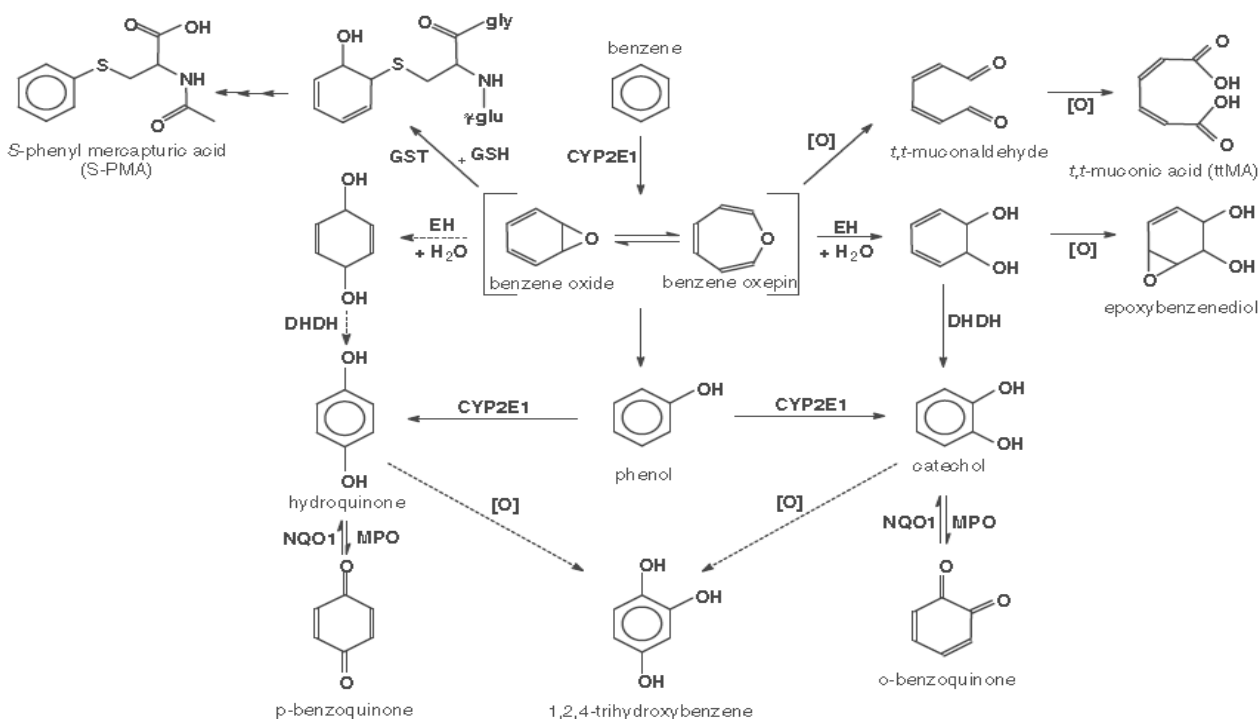


Figure 1: A schematic of liver metabolism of benzene(18)

Legend: EH, epoxide hydrolase; GSH, glutathione; GST, glutathione-S-transferase; DHDH, dihydrodiol dehydrogenase; MPO, myeloperoxidase; NQO1, NADPH quinone oxidoreductase

1.2 Statement of the problem

Gasoline stations are likely to be an important point source of carbonyl compounds and volatile organic compounds, especially emission of benzene, toluene, ethylbenzen, and xylenes (21). Volatile organic compounds such as gasoline and other fuels are associated with a wide variety of deleterious health effects including liver and kidney diseases (22). Most of the aromatics have adverse effects on human health after either chronic or acute exposure. Benzene, toluene, and xylenes are considered to be the most hazardous, predominantly benzene because of its carcinogenic potency. Exposure to these compounds may have an impact on the health of the exposed subjects (23, 24) due to free radicals, reactive oxygen species (ROS) and reactive nitrogen species (25).

Gasoline station attendants are exposed to a mixture of hydrocarbons in fuel vapours during dispensing fuel and to the gases from vehicular exhaust (26). Most of peoples have a higher risk of exposure to petroleum products vapors; these include fuel -station workers, people attendants to service station, drivers of petrol trucks (26, 27). The nature of chemical products of petrol makes them readily available in the atmosphere any time it is dispensed, especially at petrol fuel stations. People who are exposed to petrol fumes during fueling and refueling at gas stations, but the people who are working at filling station are more risky by virtue of their occupational exposure. The cytotoxic effects of petroleum products are exerted on most of body organs of humans and animals such as the liver and kidney (27-30).

Exposure to benzene over a long period of time could cause nephrotoxicity and hepatotoxicity 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 (31).

Even though, several studies pointed to the risk of occupational exposure to gasoline on liver and renal function profiles, to the best of our knowledge there is no published data which is similar with this study in Ethiopia in general in the study area in particular. Therefore, this study was aimed to assess the effect of gasoline exposure on liver (ALT, AST, ALP, total bilirubin) and renal (urea, creatinine, and uric acid) function tests in gasoline station workers.

1.3. Significance of the Study

Findings of the present study would have a great importance, since it can assist in the clinical management of liver and renal dysfunctions in gasoline exposed attendants and may give convenient ways for solving it.

Furthermore, the associated factors identified could have implications and immediate benefits for the design, targeting and implementation of gasoline exposure education and prevention.

This study will also be useful to planners, policy makers and community at large for planning, interventions, prevention and control of gasoline exposure in Ethiopia in general and specifically in the study area.

Moreover the findings of this study will serve as baseline information for the further studies.

2. Literature Review

2.1 Profile of Liver and Renal Function Tests and Associated Factors

The available studies suggest that petroleum products are toxic to liver and kidney. Petroleum fumes are ubiquitous in our environment and the common sources of contact or exposure are petrochemical industries (refineries, oils field, and filling stations) and homes (32).

A study conducted in Texas City showed significantly higher levels of mean serum creatinine (mg/dL) on benzene exposed subjects compared with the unexposed subjects. Serum level of ALP (IU/L) was also significantly elevated in the benzene exposed subjects compared with the unexposed subjects. Similarly, benzene exposed subjects had significantly higher levels of AST and ALT compared with those unexposed subjects (33).

A cross sectional study conducted in Iraq revealed that serum alkaline phosphatase and uric acid were significantly higher among the workers (34). Whereas, in a two case control studies conducted in Palestine showed that RFTs (urea, creatinine, and uric acid) and liver enzymes (AST and ALT) levels were significantly higher in the exposed group compared to control group(35, 36) .

Prolonged exposure to gasoline compounds may represent a significant risk factor for a wide spectrum of liver and renal disorders. This is supported by cross sectional studies conducted in Iraq. The result showed a significant increase in level of urea, creatinine, ALT, AST, and ALP for those worked for 6-10 years at gas station, when compared the results with the control group Liver function tests (ALT, AST, and ALP) of gasoline station attendants were significantly increase which related to the time of exposure to petroleum products for 3-6 years (27, 37).

Exposure to gasoline compounds is associated with a significantly higher risk of abnormal values of renal markers. A cross sectional study was conducted on effect of fuel inhalation in Saudi Arabia studied by Mashaal Bin-Mefrij and Suaad Alwakeel. The result shown serum creatinine and serum urea level concentrations were significantly higher in exposed group compared to controls. But, there were no significant increases seen in the mean serum concentrations of ALP and AST between exposed and control study participants (22).

According to a case control study conducted in India, liver function tests (ALT and AST) were found significantly higher among the petrol filling attendants in comparison to the control group. Whereas, level of ALP was statistically insignificant between the two groups (38). On the other hand, in a cross sectional studies conducted in Turkey and Egypt revealed that liver enzymes and renal functions were reported statistically no significant difference between exposed and control groups except for blood urea nitrogen levels (39) and ALT (26) which were significantly higher in exposed group.

In studies by El-Said et al.(24) and Gungor et al.(39), gasoline exposure leads to significant increase of ALT and AST in exposed workers when compared to control groups. In a similar manner , significant increase of liver enzymes were reported in studies in Egypt by Hagazy et al.(40) and Ibrahim et al.(41).

A case control study in Ibadan, Nigeria stated that serum AST, ALT, and ALP in the gasoline station attendants were significantly higher compared with the control. The petrol attendants that have spent 27-36 months in the petrol station show significant increase in AST, ALT, and ALP compared with other petrol attendants that have spent lesser duration in the gasoline station. The kidney function test revealed that petrol attendants show significant increase in creatinine and urea level compared with the control (14).

Nwanjo et al. in Nigeria (42) investigated that a significant increase in liver function tests and renal function tests were associated with time of exposure. LFTs and RFTs of gasoline station attendants who were worked for 6-10 years were significantly higher when compared with the control. But total bilirubin level was showed no significant change.

Significantly high levels of all liver enzymes in gasoline station workers were reported in study by Gali et al. (13) in Nigeria. Nevertheless, other study in Nigeria (43) has found no association between exposure to gasoline and liver enzymes except ALP which were significantly lower in exposed group.

A cross sectional study by Monem et al. (44) in Palestine reported that 86.7% gasoline station attendants were known that inhalation is the route of gasoline exposure. In this study knowledge of gas station attendant on effect of leaded gasoline exposure on human health and environmental pollutant were 83.8% and 84.8% respectively.

2.2 Mechanism of Gasoline-induced Liver and Renal Toxicity

Gasoline is a mixture of several hydrocarbon compounds and additives. Different metabolic pathways gasoline is reported for the different compounds in the mixture. Nevertheless, it is readily absorbed when inhaled or ingested. The active metabolites undergo further toxicokinetic processes, such as generation of ROS, oxidative tissue damage, leading to altered structure and functions, and multi-system toxicity (figure2) (45).

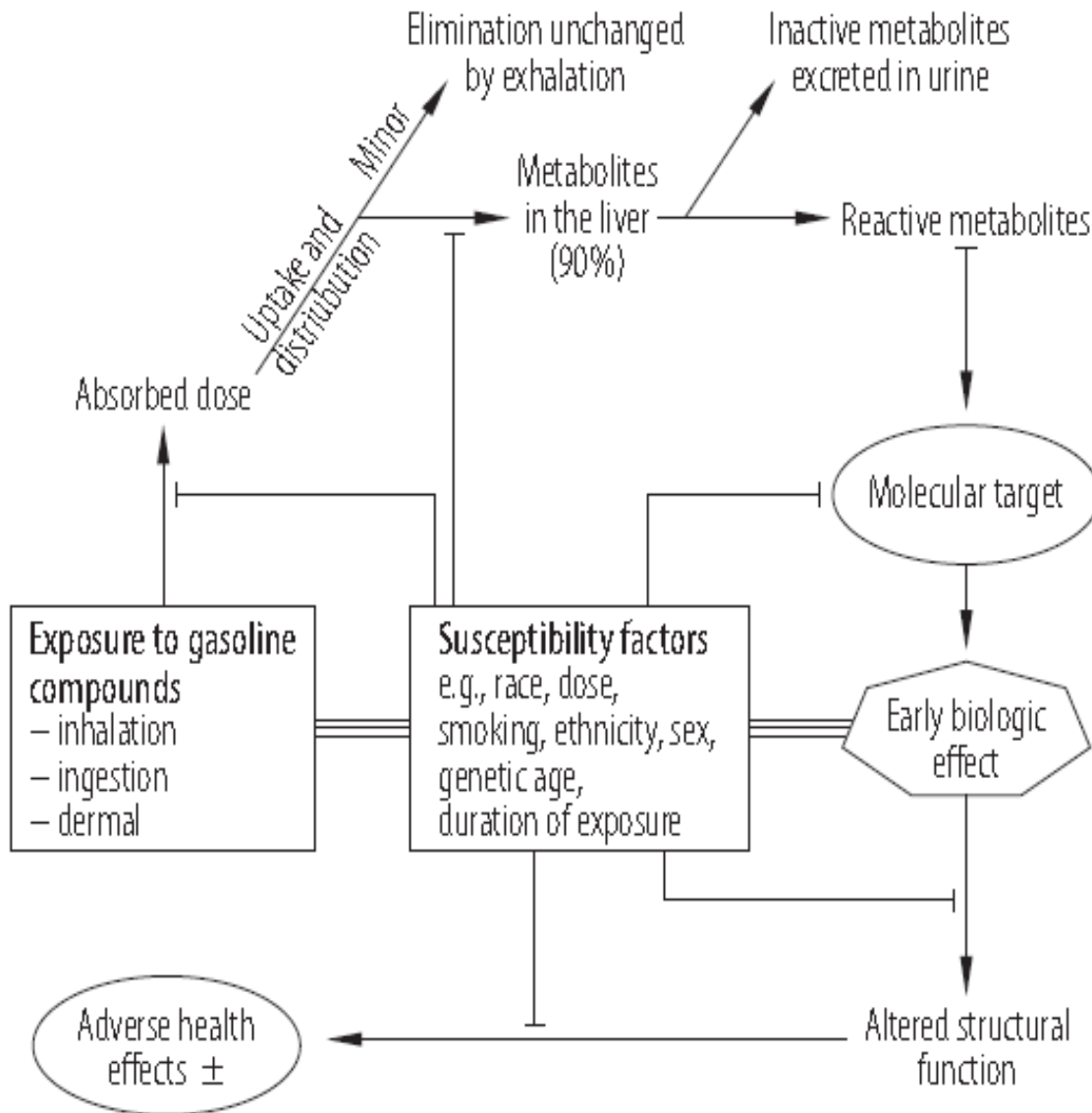


Figure 2: Toxicokinetic pathway of gasoline compounds for exposed workers, and the modulatory effects of individual's susceptibility factors (45)

Several mechanisms contribute to gasoline-induced hepatotoxicity. These include induction of cellular degeneration, downregulation of gene expression, and induction of oxidative stress. The metabolism of gasoline and its compounds is known to generate reactive metabolites (1, 2, 4-benzenetriol, benzequinone), which interact with the membrane lipids of hepatocytes to produce lipid peroxide and ROS. Reactive oxygen species and lipid peroxidation lead to damage of the biomembrane (figure3) (45).

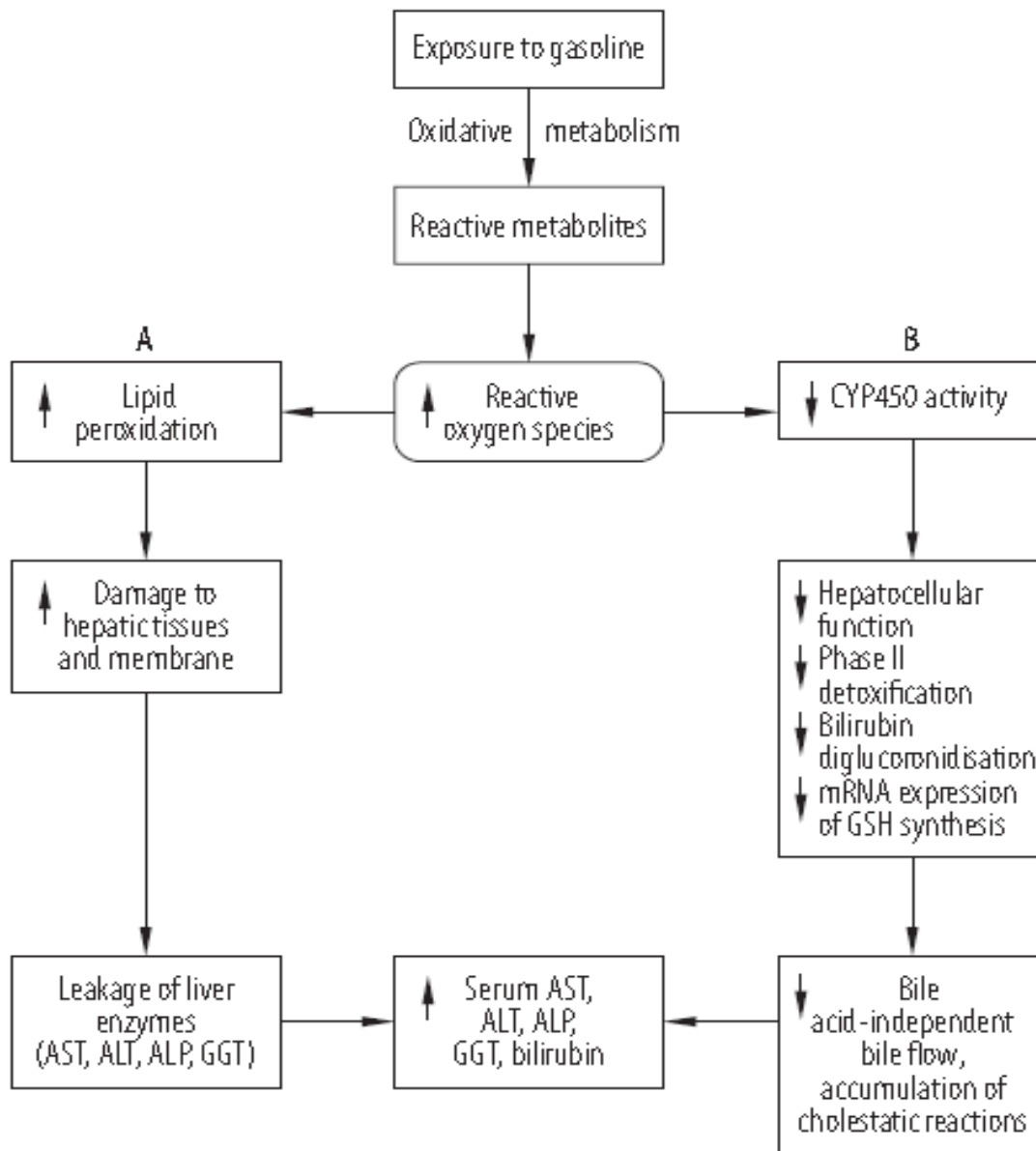


Figure 3: Mechanisms of gasoline-induced hepatotoxicity (45)

Gasoline-induced renal function impairment is based on the high lipophilicity of gasoline and induction of oxidative stress. Induction of oxidative stress by gasoline oxidative metabolites has been reported to cause disruption of the immune system. High lipophilicity of gasoline induces physiochemical damage to the glomerular and tubules, including damage to the membrane lipid bilayer and proteins. Such damage may compromise the functional integrity of the membrane, with a resultant derangement in Na⁺/K⁺/ATPase activity and membrane proteins. This may lead to a cascade of events leading to renal function impairment (Figure 4) (45).

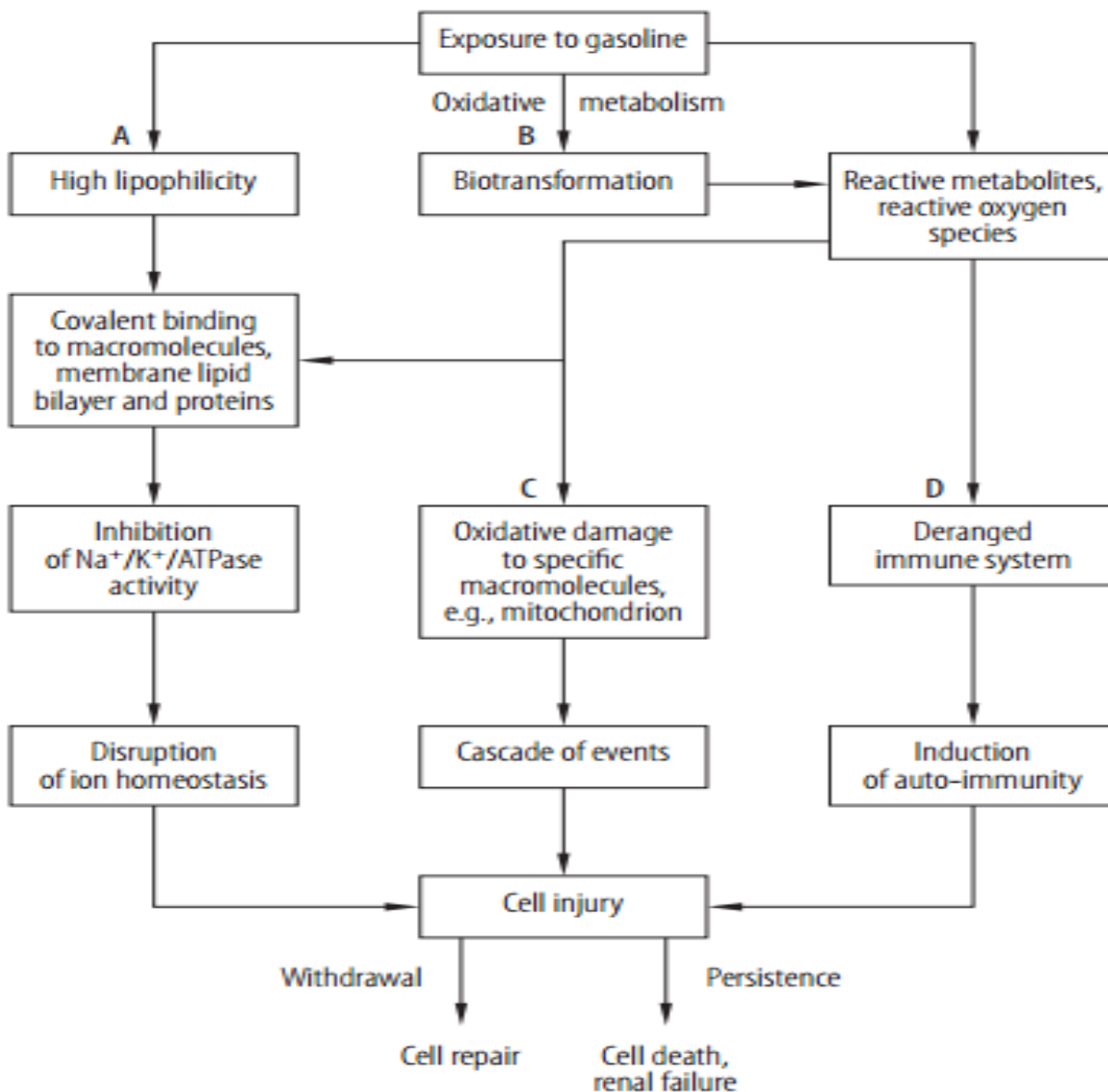


Figure 4: Pathways underlying gasoline-induced nephrotoxicity(45)

3. Objectives

3.1 General objective

To assess liver and renal function tests among gasoline exposed gas station workers in Mekelle city, Tigray, Northern Ethiopia

3.2 Specific Objective

- To assess the comparability of liver and renal parameters of gas station workers with controls.
- To assess associated factors with gasoline exposure
- To assess awareness of gasoline station workers toward gasoline exposure

4. Hypothesis

Ho- There is no significance difference of liver and renal function tests level among gasoline exposed and gasoline non exposed groups.

5. Materials and Method

5.1 Study area

The study was conducted in Mekelle city Gas station workers who are exposed to gasoline. Mekelle is capital City of Tigray Regional State and is located in the Northern part of Ethiopia, at 783 km from the capital City of Ethiopia, Addis Ababa. Mekelle has weyna-dega climatic conditions, which is administratively divided into 7 sub-Cities; namely: Ayder, Hawelti, Adi Haqi, Hadnet, Kedamay Weyane, Quiha and Semien. According to projected Central Statistical Agency of Ethiopia (2013), total population of 307,307 resides in the town. Out of those 50.6% (155,497) of total population are male and 49.1% (151, 810) of total population are female (46).

The estimated number of legal gasoline stations registered in the Mekell city in the year 2017-18 was 13 (personal communication with municipalities of Mekell city governorates). Out of these 13 gas stations, 9 of them were giving fueling of gas for the automobiles. But the remaining was non-functional due to different reasons; the three were newly opened (less than six months) during data collection and the other one was temporarily closed due to road construction.

5.2 Study design and Period

A cross sectional comparative study was conducted from January to April 2018

5.3 Population

5.3.1 Source Population

All people who were working at gas station of Mekelle city were considered as source population.

5.3.2 Study Population

Apparently health workers who were working as gasoline filler at gas station in Mekelle city.

5.4 Inclusion and Exclusion Criteria

5.4.1 Inclusion Criteria

Exposed group:

- Workers who have been working minimum of six months in the gas station
- Workers who voluntarily participated in the study and sign for consent.

Control group:

- Workers of Ayder Comprehensive Specialized Hospital who were matched with exposed participants in age and sex and not exposed to gasoline.

5.4.2 Exclusion Criteria

- Workers who had history of renal and liver health problem

5.5 Study Variables

5.5.1 Dependent Variable

- LFTs(ALT, AST, ALP, Total bilirubin)
- RFTs (Urea, creatinine, uric acid)

5.5.2 Independent Variables

- Socio demographic characteristics (age, sex)
- Duration of work exposure
- Personal protective measure
- Smoking status

5.6 Measurement and Data Collection

5.6.1 Sample Size and Sampling method

Because of small gasoline stations in Mekelle city, all volunteer gasoline station attendants were participated. From a total of 49 gasoline station attendants 43 adult male and female aged (18-60) years and age and sex matched 47 volunteer controls participants from Ayder Comprehensive Specialized Hospital staffs were included. Convenience sampling method was used in this study.

5.6.2 Data Collection Procedure

The gas station workers were interviewed with Tigrigna language translated administered questionnaire. The questionnaire mainly consists of closed and open ended questions. It was focusing on socio-demographic data, duration of employment, and awareness on gasoline exposure, protective methods, and smoking status.

5.6.3 Specimen Collection and Processing

About 4 mL blood sample was taken from each study participant at each station on routine work hours to determine liver and renal function tests. Venous blood was collected in to serum separator tube and transport to Ayder comprehensive specialized hospital laboratory. Serum sample was separated by centrifuging at 4000 RPM for 5 minute and analyzed by Pentra C400 clinical chemistry auto analyzer.

5.6.4 Laboratory Analysis

5.6.4.1 Liver Function Tests Analysis

Liver parameters including ALT, AST, ALP, and total bilirubin were analyzed in the serum. These biochemical parameters were done by spectrophotometric determination of their absorbance's using analytical grade laboratory reagent kits. The laboratory reagent kits from HORIBA ABX SAS (Parc Euromedecen-Rue du caducee, France) were used to assess the concentration of ALT, AST and ALP in the serum. All LFTs were analyzed by Pentra C400 automated clinical chemistry analyzer with its own reagent kit. All biochemical analysis for this study was according to manufactures protocol.

5.6.4.2 Renal Function Tests Analysis

Renal parameters including concentration of urea, creatinine, and uric acid were analyzed. These biochemical parameters were done by spectrophotometric determination of their absorbance's using analytical grade laboratory reagent kits. The laboratory reagent kits from HORIBA ABX SAS (Parc Euromedecen-Rue du caducee, France) were used to assess the concentration of urea, creatinine and uric acid in the serum. All RFTs were analyzed by Pentra C400 automated clinical chemistry analyzer with its own reagent kit. All biochemical analysis for this study was according to manufactures protocol.

5.7 Data Quality Assurance

5.7.1 Pre-analytical

Data was collected after questioner was translated to local language Tigrigna to make certain that the participants can understand the questions at the time of interview. Before the actual data collection questionnaire was pre tested on 10% participants. Then correction was taken and those participants were excluded from study.

Concerning sample collection, transportation and processing the principal investigator was assembled blood sample collection materials. Principal investigator was strictly followed SOP to assure that sample was collected on serum separator tube, labeling with participant identification number, allow the sample for minimum of 30 minutes to clot, transportation, and centrifuging sample on 4000RPM for 5 minutes.

5.7.2 Analytical

Before participants sample analyzed to assure the accuracy and functionality of the instrument both normal and pathological quality control for each test was done. The participants sample was analyzed after both controls were accepted.

5.7.3 Post Analytical

All data was recorded with identification of each participant, checked for completeness, and interpreted.

5.8 Data Analysis and Interpretation

Data was analyzed using SPSS Version 23. Table, percent and graphs was used for descriptive data. Independent t-test was used to determine mean difference of exposed group and control group. One way ANOVA (Analysis of variance) was used to determine duration exposure category of exposed group with liver and renal function tests. P value < 0.05 was considered statistically significant at 95% of confidence level.

5.9 Ethical Consideration

The study was conducted after ethically reviewed and approved by the Department of Medical Laboratory Science research and ethical review committee, College of Health Science, Addis Ababa University. An official letter of request was sent to Mekelle City administration to obtain approval to assess liver and renal function test among gasoline exposed gas station workers. Another official letter of request was sent to Mekelle University, Ayder Comprehensive Specialized Hospital to obtain approval to carry out liver and renal function test analysis in the central laboratories.

Gas station workers were given an explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation is voluntary based. Data

collection was carried out after receiving approval letters from administration of each gas station. Signed consent was also obtained from the study participants before administering the questionnaire. Participants were got their results free and those with abnormal findings were linked to Ayder Hospital.

5.10 Operational Definition

Benzene- is an organic chemical compound with the chemical formula C_6H_6 found in gasoline.

Fuel-station attendants – are workers at a full-service filling station who perform service, pumping fuel, cleaning windshields, and checking vehicle oil levels other than accepting payment or a person employed to refuel motor vehicles at a petrol station.

Hepatotoxicity: is toxicity to liver, bile duct, and gallbladder

Liver function Tests- are groups of blood tests that useful in the evaluation and management of patients with hepatic dysfunction. Commonly used tests to check liver function are the alanine transaminase, aspartate aminotransferase, alkaline phosphatase, and bilirubin tests.

Nephrotoxicity: is toxicity to the urinary tract system

Renal Function Tests- are groups of blood tests that useful in the evaluation and management of patients with kidney dysfunction. Some of blood tests are urea, creatinine, and uric acid.

6. Result

6.1 Socio demographic Characteristics of study participants

A total of 90 study participants comprising of 43(28 male and 15 female) gasoline station attendants and 47 (30 male and 17 female) controls were recruited for the study (figure7). The mean age (\pm SD) of the gasoline exposed and unexposed study participants were 30.02(\pm 8.62) years (range 19-54 years) and 29.85 (\pm 7.29) years (range 20-52 years) respectively. Mean of work duration in years of gas station workers was 5.187 \pm 4.39 years (ranged from 10 month to 16 years) and mean hour per day of gas station workers was 11.16 \pm 2.08 hours (range 8-14 hours). All gasoline exposed study participants were nonsmoker.

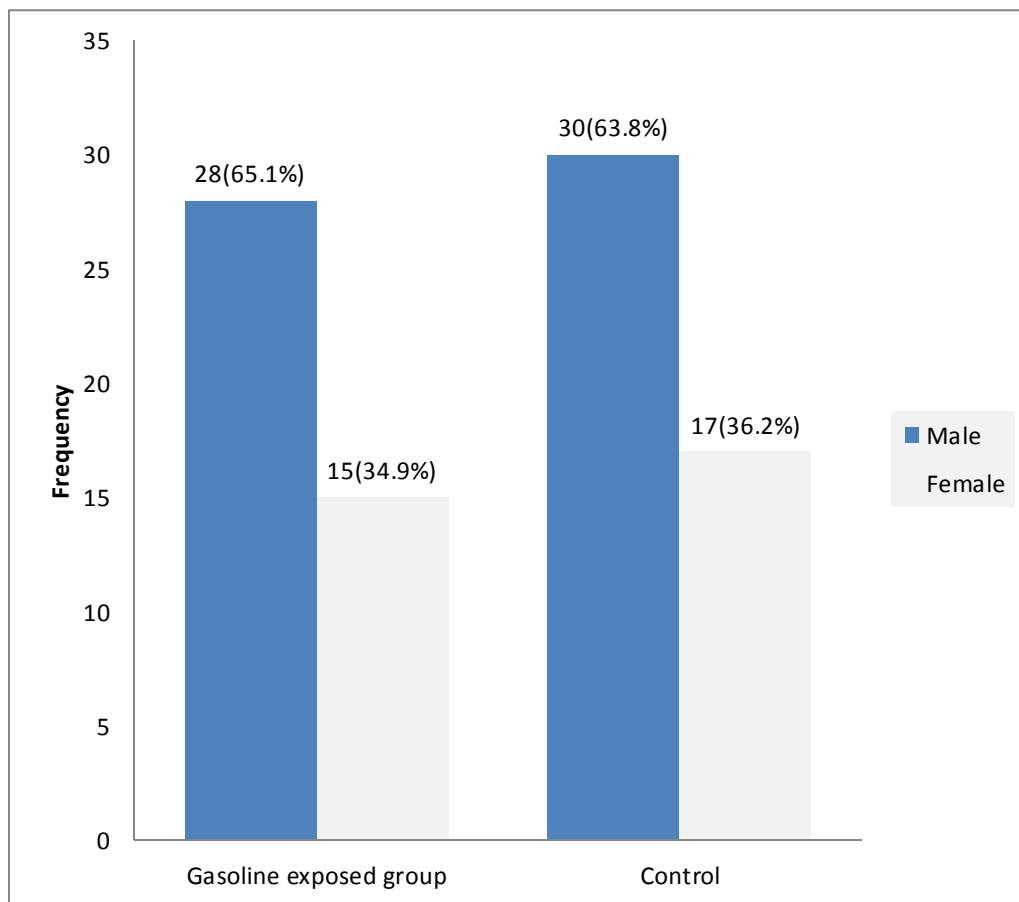


Figure 5: Distribution of study participants by sex

6.2 Comparison of Liver and Renal Parameters among gasoline exposed and control

Study participants who were exposed to gasoline experienced significantly increased mean urea, creatinine, and uric acid (mg/dL) compared with the unexposed study participants (29.82 ± 7.56 versus 20.03 ± 3.69 , $p < 0.001$, 0.91 ± 0.14 versus 0.8 ± 0.104 , $p < 0.001$, 5.6 ± 1.6 versus 4.24 ± 0.78 , $p < 0.001$) respectively. Similarly, the mean ALT and AST (IU/L) in the gasoline exposed group was significantly elevated compared with the unexposed group (24.4 ± 10.21 versus 19.06 ± 5.96 , $P = 0.003$; 26.26 ± 9.59 versus 20.05 ± 4.55 , $p < 0.001$) respectively. The mean serum concentration of ALP (U/L) was significantly lower among workers who were exposed to gasoline (73.89 ± 16.71 versus 82.91 ± 16.67 , $p = 0.012$). There was no significant difference in the mean serum concentration of total bilirubin (mg/dL) between participants working at gas station and comparison groups (0.558 ± 0.289 versus 0.497 ± 0.205 , $p = 0.251$) (table1).

Table 1: Comparison of liver and renal function tests among exposed and control study participants in Mekelle City, Tigray Region, Northern Ethiopia, January to April, 2018 (n=90)

parameter	Study group(n=43) Mean \pm SD	Control group(n=47) Mean \pm SD	p-value
ALT U/L	24.4 ± 10.2	19.06 ± 5.96	0.003
AST U/L	26.26 ± 9.59	20.05 ± 4.55	<0.001
ALP U/L	73.94 ± 16.71	82.91 ± 16.67	0.012
Tbil md/dL	0.558 ± 0.289	0.497 ± 0.205	0.251
Urea md/dL	29.82 ± 7.56	20.03 ± 3.69	<0.001
Creatinine mg/dL	0.91 ± 0.14	0.8 ± 0.104	<0.001
UA mg/dL	5.6 ± 1.6	4.24 ± 0.78	<0.001

- SD- Standard deviation, Independent-t –test is used for comparison and P- value ≤ 0.05 is considered significant.

6.3 Liver and Renal parameters and associated factors with gasoline exposure

6.3.1 Effect of Work duration on liver and renal function tests of gas station workers

Duration of exposure in year had effects on liver and renal function tests of gasoline exposed groups. The ALT, AST, Urea, and Creatinine level of gas station study participants in above 6 years period of exposure to the gasoline showed significant higher when compared with study

participants exposed for less than or equal to two and 3-6 years. No statistically significant difference was observed on ALP and total bilirubin level based on duration of exposure (table 2).

Table 2: The effect of duration of exposure on Liver and Renal function tests of gas station workers in Mekelle city, Tigray Region, Northern Ethiopia, January to April, 2018 (n=43)

Test parameter	Duration of Exposure in years		
	≤ 2 years (mean± SD)	3-6years (mean± SD)	>6years (mean± SD)
ALT(IU/L)	17.5±5.9	22.4±4.8	34.5±1.1 ^{a,b}
AST (IU/L)	20.9±5.3	24.3±4.8	34.4±11.5 ^{a,d}
ALP(IU/L)	70.9±22.4	73±10.1	78.3±12.96
Tbil (mg/dL)	0.53±0.33	0.50±0.25	0.64±0.28
Urea (mg/dL)	26.5±6.4	26.7±5.3	36.5±6.3 ^{a,b}
Creatinine (mg/dL)	0.85±0.1	0.87±0.9	1.03±0.1 ^{a,d}
Uric acid (mg/dL)	5.09±1.6	5.16±1.3	6.6±1.4 ^{c,d}
Total	17	12	14

- SD-Standard deviation. F-test (One way-Anova) with post hoc multi comparison is used to compare means.
 - a- $p < 0.001$ when >6 years compared to ≤ 2 years (One-way ANOVA/tukey post hoc test)
 - b- $p < 0.001$ when >6 years compared to 3-6 years(One-way ANOVA/tukey post hoc test)
 - c- $p < 0.05$ when >6 years compared to ≤ 2 years(One-way ANOVA/tukey post hoc test)
 - d- $p < 0.05$ when >6 years compared to 3-6 years(One-way ANOVA/tukey post hoc test)

6.3.2 Protective Measures in use by gas station workers

The highest number of workers worn overall (gown) and shoes at work place (42(97.7%) and 36(83.7%) respectively).The lowest number of workers worn gloves (n=1, 2.3%), hat (n=7, 11.6%), and respiratory mask (n=4, 9.3%) (Table3).

Table 3: protective measures in use by gasoline station workers in Mekelle city, Tigray region, Northern Ethiopia, January to April, 2018 (n=43)

Protective measures	Yes	No	Total
	Number (%)	Number (%)	
Wear glove	1(2.3%)	42(97.7%)	43
Wear hat	5(11.6%)	38(88.4%)	43
Wear respiratory mask	4(9.3%)	39(90.7%)	43
Wear shoes(Boots)	36(83.7%)	7(16.3%)	43
Wear Over all(gown)	42(97.7%)	1(2.3%)	43
Eating at work place	22(51.2%)	21(48.8%)	43
Chewing gum at work place	11(25.6%)	32(74.4%)	43
Taking shower after work	10(23.3%)	33(76.7%)	43
Sucking benzene by mouth	9(20.9%)	34(79.1%)	43

The main cause of not using safety equipment was not available 30 (69.8%) followed by carelessness 5(11.6%) (Figure 8)

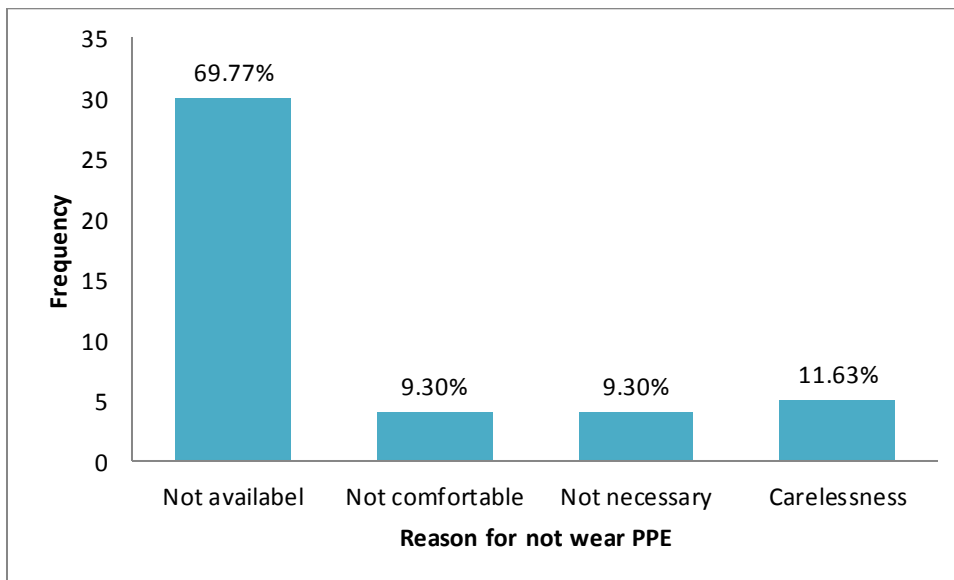


Figure 6: Cause of not using safety equipment (n=43)

6.4. Awareness of gasoline station workers toward gasoline exposure

About 39 (90.3%) workers claimed that inhalation is the route of entry, followed by 30 (69.8%) who reported that skin is the route of entry, and 24 (55.8%) who claimed that ingestion is the route of entry of gasoline into the body. A total of 41 (95.3%) and 34 (79.1%) workers knew that gasoline exposure do affect human health and that gasoline is an environmental pollutant respectively (table4).

Table 4: Awareness of gasoline station workers on route of gasoline entry into the body, health effects of gasoline exposure and gasoline as an environmental pollutant in Mekelle city, Tigray region, Northern Ethiopia, January to April,2018 (n=43)

Variable		Yes	No	Total
		N (%)	N (%)	
Route of gasoline entry to body	Inhalation	39(90.7%)	4(9.3 %)	43
	Skin	30(69.8%)	13(30.2%)	43
	Ingestion	24(55.8%)	19(44.2%)	43
Health effect of gasoline exposure		41(95.3%)	2(4.6%)	43
Gasoline as environmental pollutant		34(79.1%)	9(20.9%)	43

7. Discussion

Gasoline station workers are regularly exposed to many hazardous toxins vapors. Some of these vapors are gasoline, kerosene, and diesel, among the most risky toxin is benzene fume. Which can cause abnormal alterations in the functioning of many vital organs and they are associated with increased risk of renal and liver cancer. (35)

The findings of the present study indicate that gasoline exposure can induce significant alterations in renal and hepatic functions. Specifically; ALT, AST, Urea, creatinine, and uric acid are significantly higher among gasoline stations workers when compared to control study participants. ALP level was significantly lower among gasoline exposed subjects compared to unexposed study participants. However, total bilirubin level was statistically no significant between exposed and unexposed study participants.

The significant increase in the creatinine and urea levels of the gasoline station workers compared with the control in our study was in agreement with the findings in previous studies conducted in Nigeria, Iraq, Palestine, and Saudi Arabia (14, 22, 27, 35, 42). This may be attributed to an increase in liberating toxic metabolites, such as reactive oxygen species (ROS). Some experiments with rats indicate that exposure by inhalation to aromatic hydrocarbons can cause nephrotoxic(30). Furthermore human and other experimental studies suggest that some of chemicals can affect the renal system (30, 47-49). Study findings indicate petroleum (benzene) product chemicals can cause renal impairment(26).Serum uric acid level was also significantly increase which was in line with studies done in Iraq and Palestine (34, 35). This may be due to degradation of purines or an increase of uric acid level by either over production or inability of excretion (35).

Serum ALT and AST were significantly higher in gasoline station workers than controls study participants while ALP was significantly decreased in exposed study participants than controls. Whereas, total bilirubin had no significantly different between exposed and controls. The significantly increase in ALT and AST is in line with other studies conducted in Egypt, Nigeria, Turkey, Palestine, India, and Brazil(13, 14, 24, 35, 38, 40, 42, 50, 51).This observation may be due to the fact that hydrocarbons are a major component of petroleum products that are

metabolized in the liver by CYP450 2E1 oxidative pathways, which contribute to the production of free radicals and quinone metabolites such as phenol, hydroquinone, benzoquinone;1,2,4 benzenetriol (18). These free radicals and toxic metabolites cause lipid peroxidation and damage of hepatic cell membrane, causing the release of liver enzymes in the circulation(45).

The ALP level significantly lower in exposed subjects than controls was in line with study conducted in Nigeria by Akinosun O. M et al(43). ALP is an enzyme in the cells lining the biliary ducts of the liver. If there is an obstruction in the bile duct, ALP levels in plasma will rise (52). All the study participants considered for this study are adult; therefore, significantly low levels of ALP in petrol attendants might indicate absence of bile duct obstruction. Similar levels of total bilirubin in petrol attendants and controls might indicate that no hemolysis or liver damage occurred in the petrol attendants

In our study the effect of time exposure to petroleum products and their derivatives on liver and renal parameters of gasoline station workers was assessed. Liver function tests such as; ALT, AST level of gas station study participants in above 6 years period of exposure to the gasoline showed significant increase compared with participants exposed for less than or equal to two years and 3-6 years of work duration. These findings are in agreement with the earlier report in Nigeria and Iraq (14, 37, 42). Renal function test (urea and creatinine) level of study participants with above 6 years of work duration was also significantly higher compared with study participants within less than or equal to two years and 3-6 years of work duration. Other reports confirmed that petrol products may have some effects on kidney functions (14, 27, 42).

The higher proportion of gasoline station workers were more aware of inhalational of gasoline than other routes of exposure, health impact of gasoline, gasoline as environmental pollutant. This finding is in line with study conducted by Monem et al in Gaza Strip, Palestine(44).

8. Strengthens and Limitations of the study

8.1 Strengthens of the Study

To the best of the author's knowledge this is the first study in Ethiopia. So it will be used as baseline information for policy makers and for further study.

8.2 Limitations of the Study

The main limitation of this study was being small sample size, which was not enough to generalize the general population of gasoline station workers. Blood level of gasoline component such as; benzene, toluene, and xylene were not measured which were important to identify the magnitude of the problem of the gasoline component. Limitation of literature on awareness of gasoline station workers were also another obstacle.

9. Conclusion and Recommendation

9.1 Conclusion

Exposure to petroleum products by gasoline station workers showed increased liver and renal function test parameters. This study observed that the gasoline station workers are at risk of developing biochemical alterations in the hepatic enzymes and renal function tests. The liver and renal parameters were significantly increased with duration of exposure of gasoline station attendants. That indicates increased the probability of liver and kidney function tests among gasoline station workers with increased exposure time.

9.2 Recommendation

- Gasoline station owners are recommended to provide protective equipment includes gloves, hat, masks, shoes, and overall (gown) to gas station workers.
- Gasoline station workers are advised to take protective measure at work place includes wear protective equipment's
- Periodic medical checkup is recommended for early recognition
- Further research is recommended to include other occupations that are susceptible to cause gasoline exposure to build up a comprehensive picture of occupational gasoline exposure in Ethiopia.

10. Reference

1. Bishop ML, Fody EP, Schoeff LE. *Clinical chemistry: principles, techniques, and correlations*: Lippincott Williams & Wilkins; 2013.
2. Hall P, Cash J. What is the real function of the Liver 'function' tests? *The Ulster medical journal*. 2012;81(1):30.
3. Sipos P, Szentmihályi K, Fehér E, Abaza M, Szilágyi M, Blázovics A. Some effects of lead contamination on liver and gallbladder bile. *Acta Biol Szeged*. 2003;47(1-4):139-42.
4. American Gastroenterological Association medical position statement: evaluation of liver chemistry tests. *Gastroenterology*. 2002;123(4):1364-6.
5. Hemalatha T, UmaMaheswari T, Krithiga G, Sankaranarayanan P, Puvanakrishnan R. Enzymes in clinical medicine: an overview. *Indian journal of experimental biology*. 2013;51(10):777-88.
6. Uboh FE, Akpanabiatu MI, Atangwho IJ, Ebong PE, Umoh IB. Effect of Gasoline Vapours on Serum Lipid Profile and Oxidative Stress in Hepatocyte of Male and Female Rats. *Acta Toxicologica*. 2007;15(1).
7. Ogbekhuemen T. Kidney. *Microsoft Encanta*. 2009:1-5.
8. Li Z, Jiang L, Zhu Y, Su W, Xu C, Tao T, et al. Assessment of hepatic metabolism-dependent nephrotoxicity on an organs-on-a-chip microdevice. *Toxicology in Vitro*. 2018;46:1-8.
9. Ravnskov U. Experimental glomerulonephritis induced by hydrocarbon exposure: a systematic review. *BMC nephrology*. 2005;6(1):15.
10. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AAK, Vernekar SN. Markers of renal function tests. *North American Journal of Medical Sciences*. 2010;2(4):170-3.
11. Micyus NJ, McCurry JD, Seeley JV. Analysis of aromatic compounds in gasoline with flow-switching comprehensive two-dimensional gas chromatography. *Journal of Chromatography A*. 2005;1086(1-2):115-21.
12. Rezazadeh Azari M, Naghavi Konjin Z, Zayeri F, Salehpour S, Seyedi M. Occupational exposure of petroleum depot workers to BTEX compounds. *International Journal of Occupational and Environmental Medicine*. 2012;3(1):39-44.
13. Gali R, Daja A, Mamza Y, Ani G, Ani G. Liver enzymes and protein among petrol hawkers and petrol-pump attendants in a Nigerian population. *Adv Lab Med Int*. 2012;2:123-9.
14. Ogunneye A, Omoboyowa D, Sonibare A, Adebuseyi A, Faniran T. Hepatotoxic and nephrotoxic effects of petroleum fumes on petrol attendants in Ibadan, Nigeria. *Nigerian Journal of Basic and Applied Sciences*. 2014;22(3-4):57-62.
15. Domej W, Mitterhammer H, Stauber R, Kaufmann P, Smolle KH. Successful outcome after intravenous gasoline injection. *Journal of medical toxicology*. 2007;3(4):173-7.
16. Kinawy AA. Impact of gasoline inhalation on some neurobehavioural characteristics of male rats. *BMC physiology*. 2009;9(1):21.
17. Arnold SM, Angerer J, Boogaard PJ, Hughes MF, O'Lone RB, Robison SH, et al. The use of biomonitoring data in exposure and human health risk assessment: benzene case study. *Critical reviews in toxicology*. 2013;43(2):119-53.
18. Kim S, Vermeulen R, Waidyanatha S, Johnson BA, Lan Q, Smith MT, et al. Modeling human metabolism of benzene following occupational and environmental exposures. *Cancer Epidemiology and Prevention Biomarkers*. 2006;15(11):2246-52.

19. Malini SS, Maithily K. Analysis of oxidative stress in chronic exposure to petroleum hydrocarbons in Karnataka, India. *Asia Pacific Journal of Medical Toxicology*. 2017;6(1):6-11.
20. WHO. Exposure To Benzene: A Major Public Health Concern Preventing Disease Through Healthy Environments. 2010;20:27.
21. Kitwattanavong M, Prueksasit T, Morknoid D, Tunsaringkarn T, Siriwong W. Health risk assessment of petrol station workers in the inner city of Bangkok, Thailand, to the exposure to BTEX and carbonyl compounds by inhalation. *Human and Ecological Risk Assessment: An International Journal*. 2013;19(6):1424-39.
22. Bin-Mefrij M, Alwakeel S. The effect of fuel inhalation on the kidney and liver function and blood indices in gasoline station workers. *Advances in Natural and Applied Sciences*. 2017;11(1):45-50.
23. El Mahdy N, Radwan N, Kharoub H, El-Halawany F. Chromosomal abnormalities among petrol station workers occupationally exposed to benzene. *Brit J Appl Sci Tech*. 2015;7(5):502-13.
24. El-Said KF, El-Noueam A. Biological Monitoring of Fuel Stations Workers Occupationally Exposed to Petroleum Products. *Journal of High Institute of Public Health*. 2010;40(3):586-95.
25. Sharif NEMA, Elzein AOM, Ahmed MAWA. Trace elements disturbance and Liver toxicity in Sudanese Fuel Stations Workers. *Scholars Academic Journal of Bioscience*. 2016; 4(6):498-501
26. Abou-ElWafa HS, Albadry AA, El-Gilany A-H, Bazeed FB. Some biochemical and hematological parameters among petrol station attendants: a comparative study. *BioMed research international*. 2015.
27. Jabir MS, Taqi ZJ, Khalil OA, Abdulwaheb HE, Subree D, Ommer S, et al. Biochemical Changes in Renal Function and Plasma Protein Profile of Petrol Station Attendants in Basrah. *Engineering and Technology Journal*. 2016;34(3 Part (B)):375-80.
28. Momoh J, Oshin T. Severe hepatotoxicity and nephrotoxicity of gasoline (petrol) on some biochemical parameters in Wistar male albino rats. *American Journal of Biochemistry*. 2015;5(1):6-14.
29. Saadat M, Ansari-Lari M. Alterations of liver function test indices of filling station workers with respect of genetic polymorphisms of GSTM1 and GSTT1. *Cancer letters*. 2005;227(2):163-7.
30. Uboh F, Ufot S, Eyong E. Comparative effect of withdrawal from exposure on gasoline and diesel induced nephrotoxicity in male albino Wistar rats. *J Clin Toxicol*. 2013;3:170.
31. Soderland P, Lovekar S, Weiner DE, Brooks DR, Kaufman JS. Chronic kidney disease associated with environmental toxins and exposures. *Advances in chronic kidney disease*. 2010;17(3):254-64.
32. Awasthi G, Joshi D, Swarup A, Mandal T, Awasthi D. Epidemiological studies on Petroleum toxicity. *Int J Pharm Drug Anlys*. 2016;4:251-7.
33. D'Andrea MA, Reddy GK. Hematological and hepatic alterations in nonsmoking residents exposed to benzene following a flaring incident at the British petroleum plant in Texas City. *Environmental Health*. 2014;13(1):115.
34. Mohammed SM. Hematological, Biochemical and Blood Lead Level Profile among Gasoline Exposed Station Workers in Sulaimaniya City. *ARO-The Scientific Journal of Koya University*. 2016;2(1):6-11.

35. Aziz IA, Al Agha SZ, Shehwan O. Hematological and biochemical studies for gasoline toxicity among gasoline workers in Gaza Strip. *J Al-Aqsa Univ.* 2006;10(SE):41-9.
36. Sirdah M, Al Laham N, El Madhoun R. Possible health effects of liquefied petroleum gas on workers at filling and distribution stations of Gaza governorates. *Eastern Mediterranean Health Journal.* 2013;19(3).
37. Jabir M, A. Khalil O, J. Taqi Z, A. Hussain H. Biochemical Changes in Hepatic Function of Petrol Station Attendants in Basrah. *Journal of Al-Nahrain University.* 2016;19(4):135-8.
38. Gupta N, Vyas S, Sankhla M, Punjabi P. Biochemical assessment of the hepatic functions of the petrol pump workers of Jaipur city. *National Journal of Physiology, Pharmacy and Pharmacology.* 2017;7(10):1099.
39. Güngör OT, Bal C, Ercan M, Gündüzöz M, Tutkun L, Hocaoglu A, et al. Investigation of Biochemical and Hematological Parameters of Workers Exposed to Benzene. *Turkish Journal of Occupational/Environmental Medicine and Safety.* 2015;1(1):33-40.
40. Hegazy RM, Kamel HF. Oxidant Hepatic &/or Haem. Injury on Fuel-Station Workers Exposed to Benzene Vapor, Possible Protection of Antioxidants. *American Journal of Medicine and Medical Sciences.* 2014;4(2):35-46.
41. Ibrahim KS, Amer NM, El-dossuky EA, Emara AM, Abd AE-SM, El-Fattah EM. Hepatic Dysfunction and Immune Suppression among Egyptian Workers Occupationally Exposed to Benzene. *International Public Health Forum.* 2014;1(4):1.
42. Nwanjo H, Ojiako O. Investigation of the potential health hazards of petrol station attendants in Owerri Nigeria. *Journal of Applied Sciences and Environmental Management.* 2007;11(2).
43. Akinosun O, Arinola O, Salimonu L. Immunoglobulin classes and liver function tests in Nigerian petrol attendants. *Indian journal of occupational and environmental medicine.* 2006;10(2):58.
44. Monem HA, Adnan I, Al-Rahman HA, Maged M. Exposure of gasoline station workers to leaded gasoline in the Gaza Strip: Awareness and self-reported symptoms. *Annals of Alquds medicine.* 2010(6).
45. Ekpenyong CE, Asuquo AE. Recent advances in occupational and environmental health hazards of workers exposed to gasoline compounds. *International journal of occupational medicine and environmental health.* 2017;30(1):1-26.
46. Federal Democratic Republic of Ethiopia Central Statistics Agency. Population Projection of Ethiopia for all Regions at Woreda Level from 2014-2017. Addis Ababa: 2013.
47. Orisakwe O, Njan A, Afonne O, Orish V, Udemezie O. Investigation into the nephrotoxicity of Nigerian bonny light crude oil in albino rats. *International journal of environmental research and public health.* 2004;1(2):106-10.
48. Festus O, Dada F, Iweka F, Eyaufe A, Osagie R, Osagie E, et al. Plasma Renal Functions amongst Petrol Station Attendants in Owerri, South-East Nigeria. *International Journal of Community Research.* 2013;2(2):34-8.
49. Awadalla AH, Ahmed NA, Yagoob AYE. The effects of Petroleum Products on Renal Function among Petroleum Filling Workers Stations in EL-Obied City. *European Journal of Pharmaceutical and Medical Research(ejpmr).* 2017;4(10):395-9.
50. Eltom A, Hamd HTE. Assessment of liver Enzymes level among Sudanese Gasoline Station Workers. *Scholars Journal of Applied Medical Sciences (SJAMS).* 2017 5(3A):738-43.
51. Moro AM, Brucker N, Charão MF, Baierle M, Sauer E, Goethel G, et al. Biomonitoring of gasoline station attendants exposed to benzene: Effect of gender. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis.* 2017;813:1-9.

52. Limdi J, Hyde G. Evaluation of abnormal liver function tests. *Postgraduate medical journal*. 2003;79(932):307-12.
53. Horiba Medical. ABX Pentra C400 [cited 2017 October 16]. Available from: <http://www.horiba.com/.../clinical-chemistry/abx-pentra-400>.
54. Thapa B, Walia A. Liver function tests and their interpretation. *The Indian Journal of Pediatrics*. 2007;74(7):663-71.

Annexes

Annex -I -Participants' information sheet

A. English version

Principal investigator: Tsegay Asefaw Kahsay

Institution: Addis Ababa University College of health Science, School of Allied Health Science
Department of Medical Laboratory Science

Introduction: You are kindly invited to participate on the study conducted by Addis Ababa University College of Health Science, Department of medical Laboratory Masters student thesis.

Title of the project: Assessment of liver and renal function tests in gasoline exposed gas station workers in Mekelle city, Tigray region, North Ethiopia

Purpose of the study: The purpose of the study is to assess liver and renal function tests in gasoline exposed gas station workers and recommending tangible solutions to prevent occupational gasoline exposure.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving blood samples to assess your liver and renal function tests.

Risks and Discomfort: There will be minor discomfort or feel pain during collection of samples. During collection of samples from your hand appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be provided to you if needed.

Expected Benefit: the result of the study will be have direct benefit to you since you will be communicate with your results and the administrative and city government to take appropriate preventive action There is no any financial benefit to you. But

Confidentiality: Your name will not be written in the form and I assure you all the information you give and the laboratory findings will be kept strictly confidential and could only be accessed by the researcher.

Termination of the Study: The participation is completely voluntary. You can stop participating in the study at any time. This decision will not affect in any way yours current or future medical care in the health facility.

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B. Amharic Version

ለተሳታፊዎች መረጃ መስጫ ሰነድ

የአጥኝው ስም: ፀጋይ አሰፋው ካሕሳይ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

መግቢያ: በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል በማስተርስ ድግሪ ተማሪ

የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት፤ ከዚህ ቀጥሎ

የሚገኘውን ንባብ በጥሞና ያንቡቡና ግልፅ ያልሆነሎዎትን ማንኛውምን ሃሳብ ይጠይቁ።

የጥናቱ ዋና አላማ:- በማደያ ድርጅት የሚሰሩና በስራቸው ምክንያት በቤንዝን የሚጠቁ ሰራተኞች በደማቸው ውስጥ ያለውን የጉበት ና ኩላሊት ጤንነት ጠቋሚ ምርመራዎች መጠን በመለካት በሰራተኛው ያሳደረውን የጤና ችግር ክብደት እና የተጋላጭነቱ መንስኤዎች በመለየት የመፍትሄ ሀሳቦችን ማቅረብ ነው።

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት: ደም በሚወሰድበት ሰዓት ትንሽ ልያሳምመዎት ይችላል። ከዛ ውጪ ጥናቱ በርሶ ላይ የሚያመጣዉ ከፍተኛ ጉዳት ሆነ ለጥናቱ የሚያጠፋት ተጨማሪ ጊዜ አይኖርም።

ከጥናቱ የሚያገኙት ጥቅም : ምንም አይነት የገንዘብ ክፍያ ባይኖረውም ከጥናቱ የሚገኘው ዉጤት በማወቅ እና አስተዳደሩ በሚወስደው የመከላከያ ዘዴ ተጠቃሚ ይሆናሉ።

የህክምና መረጃ በሚሰጠር ተጠብቆ መቆየት የሚችለው እንዴት ነው?

ስለራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው። ስለእርስዎ ያለውን ማንኛውምን መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል። የርስዎን ማንነት የሚገልጡ መረጃዎች ማለትም ስም፣ አድራሻ ፣ የስልክ ቁጥር እና የመሳሰሉትን አይጨምርም። ይሉቁንም ለዚህ አገልግሎት ብቻ የሚውል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲውል ይደረጋል።

ከጥናቱ ስለ ማቋረጥ

በዚህ ጥናት ላይ መሳተፍ በእርሶ ፍቃደኝነት ላይ የተመሰረተ እና መመለስ የማይፈልጉትን ማንኛውም ጥያቄ አለመመለስ ይችላሉ። በቃለ-መጠይቁ ምቹት ካልተሰማዎት በማንኛውም ጊዜ መጠይቁን ማቋረጥ ይችላሉ።

በዚህ ጥናት ላይ ያለዎትን ጥያቄ በሚከተሉት አድራሻ በማንኛውም መጠቀም ይችላሉ።

የአጥኝው ስም: አቶ ፀጋይ አሰፋው ተንቀሳቃሽ ስልክ: +251924327946 Email: tasefaw4@gmail.com

አማካሪዎች 1) ዶ/ር ሚስጥረ ወልዴ ተንቀሳቃሽ ስልክ: +251-911699710 Email: mistire08@gmail.com

2) አበበ ኢዳአ ተንቀሳቃሽ ስልክ: +251-913855657 Email: abenegesso@gmail.com

የህክምና ላቦራቶሪ ትምህርት ክፍል የምርምር ሥነምግባር ቢሮ

ስልክ ቁጥር: +251 11 275 5170

C. Tigrigna Version

ናይ መፅናዓይ ሸም: ፀጋይ አሰፋው ካሕሳይ

ናይቲ ትካል ሸም: ኡዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

መእተዊ: ብአድስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ብማስተርስ ድግሪ ተምሃራይ ዝካየድ ናይ መመሪቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኾኑ ተዓድሞም አለዉ። በጃኹም/ኸን ኣብዚ መፅናዕቲ ንምስታፍ ቅድሚ ምስምመዎም ፤ ካብዚ ቐፅሎ ዘሎ ንባብ ብፅሖም ምስ አንበቡ ግልፅ ዘይኾነሎም ዝኾነ ይኹን ሓሳብ ይሕተቱ ።

ናይቲ መፅናዕቲ ርእሲ:- ኣብ ናይ ነዳዲ መዐደሊ ድርጅት ዝሰርሑን ብሰርሑም ምኽንያት ብጋዝ ነዳዲ (ቤንዝን) ዝጥቅዑ ሰራሕተኛታት ኣብ ደሞም ውሽጢ ዘሎ ናይ ፀላም ኩባይ (ጉብት) ኩሊትን ጥዕና ጠቆምቲ ምርመራታት መጠን ምዕቃን

ናይቲ መፅናዕቲ ዋና ዓላማ:- ኣብ ነዳዲ መዐደሊ ዝሰርሑን ብሰርሑም ምኽንያት ብቤንዝን ዝጥቅዑ ሰራሕተኛታት ኣብ ደሞም ውሽጢ ዘሎ ናይ ፀላም ኩባይን ኩላሊትን ጥዕና ጠቆምቲ ምርመራታት መጠን ብምዕቃን፣ ኣብቲ ሰራሕተኛ ዘሕደር ናይ ጥዕና ፀገም፣ኸብደት፣ ተጋላፅነትን መልዕኢኦምን ብምፍላይ ናይ መፍትሒ ሓሳባት ምቕራብ እዩ ።

ምስቲ መፅናዕቲ ተታሒዙ ዝመልእ ሳዕቤን:- ደም ኣብ ዝቐደሐሉ ሰዓት ዝተወሰነ ናይ ምሕማም ስሚዕት ክስመዎም ክኸእል እዩ። ካብኡ ወፃኢ ግን እቲ መፅናዕቲ ኣብኡም ዘሕደር ክበድ ጉዳኣት ኮነ ነቲ መፅናዕቲ ዘጥፍእዎ ግዜ የለን።

ካብቲ መፅናዕቲ ዝረከብዎ ጥቕሚ:- ኣብዚ ምርምር ብምስታፎም/ፈን ናይ ደም ምርመራ ውፅኢት ምፍላጥን ኣማሓዳሪ ብዝወሰደ ናይ መከላኸሊ ሜላታት ተጠቓሚ ክኮኑ ይክእሉ እዮም። ነገር ግን ኣብዚ ምርምር ብምስታፎም/ፈን ምንም ዓይነት ናይ ገንዘብ ክፍሊት ኣይረክቡን።

ናይ ሕክምና መረዳእታ ብምስጥር ሓልኻ ምፅናሕ ዝከኣል ብኸመይ እዩ?

ስለ ናዮም/የን ዝሃቡና ዝኾነ ይኹን መረዳእታ ወይከዓ ሓበሬታ ኾነ ካብ ዝተወሰደ ደም ዝርከብ ናይ ላቦራቶሪ ውፅኢት ነቲ መፅናዕቲ ዓላማ ጥራሕ ይውዕል። ስለ ናዮም/የን ዘሎ ዝኾነ ይኹን መረዳእታ ዝተፈለየ ናይ ሚስጢር ቃል ብዘለዎ ናይ ኮምፒተር መረዳእታ ማህደር ውሽጢ ንክቐመጥ ይግበር ። ናዮም/የን መንነት ዝገልፁ ሓበሬታት ከም ሸም፣ አድራሻ ፣ስልኪ ቁፅሪን ዝአመሰሉን ኣብዚ መፅናዕቲ ኣይካተቱን።ነዚ አገልግሎት ጥራሕ ዝውዕል እሶም/ሰን ንምፍላጥ ዝኸእል መፍለዩ ቁፅሪ ኣብጥቐሚ ንክውዕል ይግበር እዩ።

ካብቲ መፅናዕቲ ስለምቁራፅ:- ኣብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመሰረተ ኮይኑ ኣብ ማእከል ምቕራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኸእሉ/ላ እዮም/የን።

ነዚ መፅናዕቲ ብዝተመልኸተ ወይ ከዓ ምስዚ መፅናዕቲ ብዝተተሓሓዘ መልክዑ ንዘጋጥሙ ፀገማት ወይ ከዓ ንዝህልዎም ሕቶታት በዞም ዝስዕቡ አድራሻታት ይጠቐሙ።

ናይ መፅናዓይ ሸም:ፀጋይ አሰፋው ካሕሳይ ተንቀሳቓሲ ስልኪ: +251924327946 ኢ.ሜል: tasefaw4@gmail.com

መማኸርቲ 1)ዶ/ር ሚስጥረ ወልዴ ተንቀሳቓሲ ስልኪ: +251-911699710 ኢ.ሜል: mistire08@gmail.com

2) አበበ ኢዳአ ተንቀሳቓሲ ስልኪ: +251-913855657 ኢ.ሜል: abenegesso@gmail.com

ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ምርምርን ሥነምግባርን ቢሮ : ስልኪ ቁፅሪ: +251 11 275 5170

Code: _____

Annex II: Consent form

A. English version

Principal investigator: Tsegay Asefaw Kahsay

Advisors: Mistire Wolde (MSc, PhD)

Funded by: Mekelle University

Reviewed: By department research ethics committee of department of Medical Laboratory Science, Addis Ababa University

Research title: Assessment of liver and renal function tests among gasoline exposed gas station workers in Mekelle City, Tigray region, North Ethiopia.

If you agree to take part, please read this form and sign the consent sheets at the end. Please tick off every box, if you agree.

1. I have read, or it was read to me, the information sheet concerning this study and I understand what will be required of me if I take part in the study.
2. I am aware of the possible risk and benefits of this study.
3. I know that being in this study is voluntary.
4. I understand that at any time I may withdraw from this study without giving reason and without affecting my normal care.
5. My questions concerning this study have been answered by
6. I know that no special payment for being participating in the study.
7. I agree to take part in this study.

Name of participant _____ Age _____ Address _____ Signature _____ Date _____

Interviewer's name _____ Signature _____

Date of interview _____ Time started _____ Time finished _____

Principal investigator Name _____ Signature _____

B. Amharic Version

የፍቃደኝነት ማረጋገጫ ሰነድ

የአጥኝው ስም: ፀጋይ አሰፋው ካሕሳይ

አማካሪዎች 1) ዶ/ር ሚስጥረ ወልዴ

2) አበበ ኢዳኦ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

ስፖንሰር ያደረገው ድርጅት: መቐለ ዩኒቨርሲቲ

ፍቃድ ሰጪ: የህክምና ላቦራቶሪ ትምህርት ክፍል የምርምርና ሥነ-ምግባር ቢሮ

የጥናቱ ርዕስ: “ በተለያዩ የነዳጅ ማደያ የሚሰሩ ሰራተኞች በቤንዚን ምክንያት በደማቸው በተለያዩ የደም ዓይነቶች ላይ የሚያመጣው ጉዳት መጠኑን ለማወቅ ነው ።

ለመሳተፍ ከተስማሙ እባክዎ ይህን ቅጽ ያንብቡ እና በመጨረሻም የስምምነት ወረቀቶችን ይፈረሙ።

እባክዎ ከተስማሙ እያንዳንዱ ሳጥን ላይ ምልክት ያድርጉ።

1. ይህንን ጥናት በተመለከተ የተጻፈውን መረጃ አንብቤያለሁ። እና በጥናቱ ላይ ከተካፈልኩኝ ምን እንደሚጠበቅብኝ ተረድቻለሁ።

2. በዚህ ጥናት ሊኖር ስለሚችለው አደጋ እና ጥቅሞች አውቂያለሁ።

3. በዚህ ጥናት ውስጥ መሳተፌ በፍቃደኝነት መሆኑን አውቂያለሁ ።

4. በማንኛውም ጊዜ እኔ ምንም ሳልሆን እና መደበኛ እንክብካቤዬን ሳይነካ ከዚህ ጥናት ልወጣ እችላለሁ።

5. በዚህ ጥናት ውስጥ ያሉኝ ጥያቄዎች በመረጃ ሰበሰቢው ተመልሰውልኛል።

6. በጥናቱ ውስጥ ለመሳተፍ ምንም ልዩ ክፍያ እንደሌለ አውቂያለሁ።

7. በዚህ ጥናት ለመሳተፍ እስማማለሁ።

የተሳታፊው ስም _____ አድራሻ _____ ፊርማ _____ ቀን _____

በስምምነቱን ቅፅ ማንብብ የማይችሉ ተሳታፊዎች

የአማካሪ ስም _____

አድራሻ _____ ፊርማ _____ ቀን _____

የመረጃ ሰብሳቢ ስም _____ ፊርማ _____ ቀን _____

ዋና ተመራማሪ ስም _____ ፊርማ _____ ቀን _____

C. Tigrigna Version

ናይ ፍቓደኛነት መረገጫ ዓንቀፅ

ናይ መፅናዓይ ሸም፡ ፀጋይ አሰፋው ካሕሳይ

አማካርቲ 1) ዶ/ር ሚስጥረ ወልዴ

2) አበበ ኢዳኦ

ናይቲ ትካል ሸም፡ አዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ስፖንሰር ዝገበሮ ድርጅት፡ መቐለ ዩኒቨርሲቲ

ፍቓድ ወሃቢ፡ አዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ናይ ምርምርን ሥነ ምግባርን ቢሮ

ናይቲ መፅናዕቲ ርእሲ፡-አብ ነዳዲ መዐደሊ ዝሰርሑን ብስርሖም ምኽንያት ብነዳዲ (ቤኒዚን) ዝጥቅዑ ሰራሕተኛታት አብ ደምም ውሽጢ ዘሎ ናይ ፀላም ከብዲ (ጉብት)ን ኩላልትን ጥዕና ጠቆምቲ ምርመራታት መጠን ምዕቃን

አብዚ መፅናዕቲ ንምስታፍ ፍቓደኛ እንተኾይንኹም፤ በጃኹም/ኸን እዚ ቐፅሎ ዘሎ ዓንቀፅ ብምንባብ አብቲ ናይ ስምምዕ ወረቐት ይፈርሙ። ስለዚ አብ ሕድሕድ ሳንዲቕ ምልክት ይግበሩ።

1. ብዛዕባ እዚ መፅናዕቲ ዝተፀሓፈ ናይ ሓበሬታ ወረቐት አንቢብዮ ወይከዓ ተነብቡለይ አብዚ መፅናዕቲ ንምስታፍ እንታይ ከምዝድለ ተረድኡኒ እዩ።

2. አብዚ መፅናዕቲ ከህልዩ ዝኸእሉ ፀገማትን ጥቕምምን ፈልጠ እዩ።

3. አብዚ መፅናዕቲ ንምስታፍ ብፍቓደኛነት ምኻኑ ተረድኡኒ እዩ።

4. ካብዚ መፅናዕቲ አብ ዝኾነ ይኹን ሰዓት ምቁራፅ ከምዝኸእል ተረድኡኒ አሎ።

5. አብዚ መፅናዕቲ ውሽጢ ዝነበሩኒ ሕቶታት ተመልስለይ እዩ።

6. አብዚ መፅናዕቲ ውሽጢ ብምስታፈይ ምንም ዓይነት ናይ ገንዘብ ኸፍሊት ከምዘይወሃበኒ ፈልጠ እዩ።

7. አብዚ መፅናዕቲ ንምስታፍ ይስምማዕ አለኹ።

ናይ ተሳታፊ/ፊት ሸም _____ አድራሻ _____ ፊርማ _____ ዕለት _____

አብቲ ናይ ስምምዕ ዓንቀፅ ምንባብ ንዘይኸእሉ ተሳተፍቲ

ናይ አማካሪ ሸም _____ አድራሻ _____ ፊርማ _____ ዕለት _____

ሓበሬታ ሰቢሳቢ ሸም _____ ፊርማ _____ ዕለት _____

ናይ መፅናዓይ ሸም _____ ፊርማ _____ ዕለት _____

Annex III: Structured Questionnaire for Gasoline exposed Study participants

Assessment of liver and renal function tests among gasoline exposed gas station workers in Mekelle city, Tigray region, North Ethiopia

A. English Version

Identification:

Name of facility (optional)_____ Institution code_____

Kifle Ketema_____ Woreda_____ Kebelle_____ Tel: -----

Respondent's identification code: _____

Note: please encircle or write the appropriate answer on the space provided.

I. Socio-demographic characteristics

1. Sex: 1. Male 2. Female

2. Age (Years): _____

3. Marital status:

(1) Married (2) Single (3) Widowed (4) Divorced.

4. What is your level of education?

(1) Illiterate (2) reading & writing (3) elementary (4) Secondary School (5) Diploma/University and above

II. Health related associated factors

6. How long have you worked in the gas station? (Years of work).....

7. How many hours you work at this station per day.....

8. What was your previous job(s)_____ for how long_____

9. How frequency does you wear the following at your work place?

9.1 Gloves (1) Always (2) Frequently (3) Sometimes (4) Often(5) Never

9.2 Hat (1) Always (2) Frequently (3) Sometimes (4) Often (5) Never

9.3 Respirator/Mask (1) Always (2) Frequently (3) Sometimes (4) Often (5) Never

9.4 Special shoes (1) Always (2) Frequently (3) Sometimes (4) Often (5) Never

9.5 Overall (1) Always (2) Frequently (3) Sometimes (4) Often (5) Never

9.6 Other, specify.....

10. If you have not worn any of the equipment listed above what is the reason?

(1) Not provided (2) Not-comfortable (3) Not necessary (4) Carelessness (5) other, specify.....

11. During work are you doing the following?

11.1. Drinking (1) Yes (2) No

11.2. Eating (1) Yes (2) No

11.3. Chewing gum (1) Yes (2) No

11.4. Mouth sucking fuel (1) Yes (2) No

12. Do you smoke tobacco/cigarette? (1) Yes (2) No

13. If your answer to question No 12 is Yes, How many pieces of cigarettes on average do you smoke per day?

14. According to your knowledge, is gasoline an environmental pollutant?

(1) Yes (2) No

15. Do you know that, exposure to gasoline has an adverse health effect?

(1) Yes (2) No

16. According to your knowledge, by which of the following pathway do you think gasoline enters into the human body?

16.1 Inhalation (1) Yes (2) No

16.2 Skin (1) Yes (2) No

16.3 Mouth. (1) Yes (2) No

17. Do you have a history of any liver or kidney problem before hiring to this work?

(1) Yes (2) No

18. Do you take any medication? If specify_____

B. Amharic Version

መለያ: የተቋሙ ስም _____ የተቋሙ ኮድ:-----የተሳታፊዎች መለያ ቁጥር _____

ክፍለ ከተማ _____ ወረዳ _____ ቀበሌ _____ ስልክ:-----

የጥናቱ ርዕስ: “በነዳጅ ማድያና ድርጅት የሚሰሩና በስራቸው ምክንያት በነዳጅ የሚጠቁ ሰራተኞች በደማቸው ውስጥ ያለውን የጉበትና የኩላሊት ጤንነት ጠቋሚም ርመራዎች መጠን መለካት”

ማሳሰቢያ: እባክዎ ትክክለኛውን የሆነውን መልስ ያክብቡ ወይም ይጻፉ።

መጠይቅ አንድ: ማህበራዊ ተጨባጭ ሁኔታዎች

- 1. ያታ (1) ወንድ (2) ሴት
- 2. ዕድሜ-----
- 3. የጋብቻ ሁኔታ (1) በትዳር ላይ (2) ያላገባ/ች (3) የተፋታ/ች (4) በሞት ምክንያት የተለያዩ
- 4. የትምርት ደረጃ
 - (1) ያልተማረ (2) ማንበብና መጻፍ (3) ከ 1-8 ክፍል (4) ከ 9-12 ክፍል (5) ዲፕሎማ/ ዲግሪና ከዛ በላይ

መጠይቅ ሁለት: ከጤና ጋር ተያያዥነቶች

- 6. በዚህ ስራ ውስጥ ለስንት ጊዜ ያህል ሰርተዋል?-----
- 7. በስራ ምድብዎ በቀን ስንት ሰዓት ይሰራሉ?-----በሳምንት ስንት ቀን ይሰራሉ?-----
- 8. እዚህ ስራ ከመስራትዎ በፊት ምን ይሰሩ ነበሩ?-----ለስንት ጊዜ ያህል?-----
- 9. የሚከተሉት የሰውነት መከላከያ እቃዎች ስራ በሚሰሩበት ጊዜ ምን ያህል ይጠቀማሉ?
 - 9.1. የእጅጓንት (1) ሁሉ ጊዜ (2) አዘውትሮ (3) ለተወሰኑ ቀናቶች (4) አንድ አንድ ጊዜ (5) ፊፅሞ
 - 9.2. ባርኔጣ (1) ሁሉ ጊዜ (2) አዘውትሮ (3) ለተወሰኑ ቀናቶች (4) አንድ አንድ ጊዜ (5) ፊፅሞ
 - 9.3. የፊት መሸፈኛ (1) ሁሉ ጊዜ (2) አዘውትሮ (3) ለተወሰኑ ቀናቶች (4) አንድ አንድ ጊዜ (5) ፊፅሞ
 - 9.4. ሽፋን ጫማ (1) ሁሉ ጊዜ (2) አዘውትሮ (3) ለተወሰኑ ቀናቶች (4) አንድ አንድ ጊዜ (5) ፊፅሞ
 - 9.5. ጋዎን (1) ሁሉ ጊዜ (2) አዘውትሮ (3) ለተወሰኑ ቀናቶች (4) አንድ አንድ ጊዜ (5) ፊፅሞ
 - 9.6. ሌላ ካለ ይጻፉ-----

10. በቁጥር 9 የተጠቀሱ የመከላከያ እቃዎች የማይጠቀሙ ከሆነ ምክንያትዎ ምንድነው?
(1) አቅርቦትየለም(2) ስለማይመች(3) አስፈላጊስላልሆነ(4) ግድየለሽነት (5) ሌላካለይግለፁ.....

11. በሚሰሩበት ቦታ ቀጥሎ ያሉትን አዘውትሮ ይጠቀማሉ?
11.1. ምግብ በስራ ቦታ መመገብ (1) አዎ (2) አልበላም
11.2. ማስቲካ ማንኸ (1) አዎ (2) አላኝክም
11.3. በአፍ ያሉ ንዚን መምጠጥ (1) አዎ (2) አልመጥም

12. ስጋራ ይስባሉ (ያጨሳሉ)? (1) አዎ (2) አላጨስም

13. ለጥያቄ ቁጥር 12 መልስዎ አዎ ከሆነ, በቀን በአማካኝ ስንት ፓክ(pack) ስጋራ ይጠቀማሉ?

14. ቤንዚን የአካባቢ በክለት ያመጣል ብሎው ያስባሉ?
(1) አዎ (2) አላስብም

15. ቤንዚን ጤናን ይጎዳል ብሎው ያስባሉ?
(1) አዎ (2) አላስብም

16. በተራ ቁጥር 20 መልስዎ አዎ ከሆነ፣ ቤንዚን በየተኛው መንገድ ወደ ሰውነት ውስጥ ሊገባ ይችላል?

16.1 በመተንፈሻ አካላት ወደ ውስጥ በመግባት (1) አዎ (2) አላስብም

16.2 በቆዳ (1) አዎ (2) አላስብም

16.3 በአፍ (1) አዎ (2) አላስብም

17. እዚህ ስራ ከመጀመርዎ በፊት የኩላሊት ወይም የጉበት በሽታ ተይዘው ያውቃሉ?

(1) አዎ (2) አላውቅም

18. ከዚህ በፊት ወይም አሁን የሚወስዱት የመድኃኒት አይነት ካለ ይጻፉ-----

ስለትብብርዎ እና መሰግናለን!

C. Tigrigna Version

መፍለዩ፡ ናይቲ ትካል ሽም፡_____ ናይቲ ትካል ኮድ፡_____ ናይ ተሳተፍቲ መፍለዩ ቁፅሪ

ክፍለ ከተማ _____ ወረዳ _____ ቀበሌ _____ ስልኪ፡ -----

ናይቲ መፅናዕቲ ኣርእስቲ፡ ኣብ ኣዲ መዐደሊ ዝሰርሑን ብስርሖም ምኽንያት ብኣዲ ዝጥቅዑ ስራሕተኛታት ኣብ ደሞም ውሽጢ ዘሎ ናይ ፀላም ከብዲ(ጉበት) ንኩላልትን ጥዕና ጠቆምቲ ምርመራታት መጠን ምዕቃን

መተሓሳስቢ፡ በጃኹም ትኽክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፅሓፉ።

1ይ ክፋል፡ ናይ ማሕበራዊን ነባራዊን ኹነታት ዝምልከቱ ሕቶታት

- 1. ሆታ (1) ተባዕታይ (2) ኣንስታይ
- 2. ዕድመ-----
- 3. ኹነታት ሓዳር (1) በዓል ሓዳር (2) ዘይተመርዓው/ወት (3) ዝፈትሐ//ሐት (4) ብሞት ምኽንያት ዝተፈላለዩ
- 4. ናይ ትምርህርት ደረጃ
(1) ዘይተምሃረ(2) ምንባብን ምፅሓፍን(3) ካብ 1-8 ክፍል(4) ካብ 9-12 ክፍል(5) ዲፕሎማ/ድግሪን ልዕሉኡን

2ይ ክፋል፡ ምስ ኩነታት ጥዕና ዝተተሓሓዙ ነገራት

- 6. ኣብ ዚሰራሕውሽጢ ክንደይ ግዜ ዝኣክል ሰርሖ/ሐን?-----
- 7. ኣብ ስራሕ ምድብ ምዓልቲ ክንደይ ሰዓት ይሰርሖ/ሐን?----- ኣብ ሰሙን ክክንደይ መዓሊቲ ይሰርሖ/ሐን?-----
- 8. ኣብ ዚሰራሕቲ ምስራሕ ምእንታይ ይሰርሖ/ሐን ይሮም/ረን?----- ንክንደይ ግዜ ዝኣክል?-----
- 9. ነዘም ስዕብም ዘለዉ ናይ ሰውነት መከላከሊ መሳርሕታት ስራሕ ኣብ ዝሰርሖ ግዜ ክንደይ ገና ይኣክል ይጥቀሙ?
- 9.1. ናይ ኢድንገት (1) ኹሉሻዕ (2) መብዛሕትኡ ግዜ (3) ዝተወሰኑ መዓልታት (4) ሓሓልፉ (5) ዋላ ኣንቲ
- 9.2. ባርኔጣ (1) ኹሉሻዕ (2) መብዛሕትኡ ግዜ (3) ዝተወሰኑ መዓልታት (4) ሓሓልፉ (5) ዋላ ኣንቲ
- 9.3. ናይ ገዢ መሸፈኒ (1) ኹሉሻዕ (2) መብዛሕትኡ ግዜ (3) ዝተወሰኑ መዓልታት (4) ሓሓልፉ (5) ዋላ ኣንቲ
- 9.4. ሽፉንጫማ (1) ኹሉሻዕ (2) መብዛሕትኡ ግዜ (3) ዝተወሰኑ መዓልታት (4) ሓሓልፉ (5) ዋላ ኣንቲ
- 9.5. ጋዎን (1) ኹሉሻዕ (2) መብዛሕትኡ ግዜ (3) ዝተወሰኑ መዓልታት (4) ሓሓልፉ (5) ዋላ ኣንቲ
- 9.6. ካልእ እንተ ልዩ ይፅሓፉ/ፋ-----

10. ኡብ ቕፅፅ 9 ዝተጠቐሱናይ ሰውነት መከላኸሊ መሳርሕታት ዘይጥቀሙ እንተኾይኖም ኸንያቶም/ተንእንታይ እዩ?

(1) አቕርቦት የለን(2) ስለዘማይምቶ(3) አድላዩ ስለዘይኾነ(4) ግድየለሽነት (5) ካልእ እንተልዩይግለፁ.....

11. አብ ዝሰርሕሉብ ታንዘም ቕፅሎም ዘለው ብአብ ዝሓይጥቀሙ/ማ ዶ?

11.1. ምግብ ኡብ ስራሕብ ታምምጋብ (1) እወ (2) አይምገብን

11.2. ማስቲካም ሕያኽ (1) እወ (2) አይሓይክን

11.3. ብአፎም/ፈንቤን ዘንምምጣጥ (1) እወ (2) አይመጥን

12. ሽጋራ ይስሕቡ/ባዶ(የጭሱ/ሳ ዶ)? (1) እወ (2) አያጨስን

13. ንሕቶ ቕፅፅ 12 መልሶም/ሰን እወ እንተኾይኑ ብመዓልቲ ብማአኽላይ ክንደይ ፓክ (pack) ሽጋራ ይጥቀሙ/ማ?

14. ቤንዚን ከባቢ አየር ብኸለት የምፅእ እዩ ኢሎም/ለን ይሓስቡ/ባ ዶ?

(1) እወ (2) አይሓስብን

15. ቤንዚን ንጥዕና ይጎድእ እዩ ኢሎም/ለን ይሓስቡ/ባ ዶ? (1) እወ (2) አይሓስብን

16. ኡብ ተራ ቕፅፅ 20 መልሶም/ሰን እወ እንተኾይኑ ቤንዚን በየናይ መንገዲ ናብ ሰብነት ውሽጢ ክኣቲ ይኽእል?

16.1 ብመተንፈሲ ኣካላት ናብ ውሽጢ ብምእታው (1) እወ (2) አይሓስብን

16.2 በቆርቦት (1) እወ (2) አይሓስብን

16.3 ብአፍ (1) እወ (2) አይሓስብን

17. ኡብዚ ስራሕ ቕድሚ ምጅማሮም/ረን ናይ ኩላሊት ወይከዓ ገብት(ፀላም ከብዲ) ሕማም ተታሕዘም ይፈልጡ/ጣ ዶ?

(1) እወ (2) አይፈልጥን

18. ቕድሚ ሓዚ ወይከዓ ሓዚ ዝወስድዎ ናይ መድሓኒት ዓይነት እንተልዩ ይፅሓፉ/ፋ-----

ስለ ዝተሓባበሩና ነመስግን!

Annex IV. Standard Operating Procedure

SOP for blood collection

Equipment

- 21 gauge needle for each participant
- Blood collection tubes (serum separator tube)
- Tourniquet
- Box of nitrile/vinyl gloves
- 70% alcohol
- Cotton

Laboratory Blood sample collection procedure and processing

1. Assemble blood collection materials.
2. Identify and prepare the patient.
3. Label tubes with the client's name/identification number.
4. Wear the rubber gloves and make the patient a comfortable position
5. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
6. Using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture
7. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 3ml in serum separator tube
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe container.

11. Leave for 30 to 45 min. to clot the blood

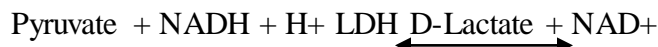
12. Centrifuge at 4000 rpm for 5 minutes and Serum will separate

Principle of Liver and Renal function tests

1. Liver function Tests

1.1 Alanine amino transeaminase

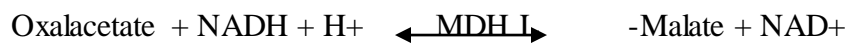
ALT, a cytosolic enzyme is found in its highest concentrations in the liver and is more specific to the liver. It participates in gluconeogenesis by catalysing the transfer of amino groups from alanine to ketoglutaric acid to produce pyruvic acid. It is an excellent marker of hepatocellular injury (52). The method will be used for ALT measurement in this study is Colorimetry using IFCC (International Federation of Clinical Chemistry) modified method without pyridoxal phosphate (53).



(ALT = Alanine Aminotransferase, LDH = Lactate Dehydrogenase)

1.2 Aspartate amino transeaminase

AST is present in cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells. It is less sensitive and specific for the liver. AST participates in gluconeogenesis by catalysing the transfer of amino groups from aspartic acid to ketoglutaric acid to produce oxaloacetic acid (52). It is present in both the mitochondria and cytosol of hepatocyte. About 80% of AST activity in human liver is contributed by the mitochondrial isoenzyme, whereas most of the circulating AST activity in normal people is derived from the cytosolic isoenzyme (54). The method used for AST measurement in this study is Colorimetry using IFCC (International Federation of Clinical Chemistry) modified method without pyridoxal phosphate(53).

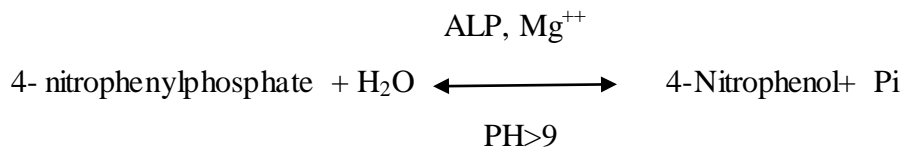


(AST = Aspartate Aminotransferase, MDH = Malate Dehydrogenase)

1.3 Alkaline phosphatase

ALP originates mainly from two sources: liver and bone. The enzymes may be present in a variety of other tissues namely intestine, kidney, placenta, and leucocytes. The elevation may be physiological or pathological. The physiological role of these enzymes is not entirely clear but production increases in tissues undergoing metabolic stimulation (52). Alkaline phosphatase from the liver, bone and kidney are thought to be from the same gene but that from intestine and placenta are derived from different genes. Highest levels of alkaline phosphatase occur in cholestatic disorders. Elevations occur as a result of both intrahepatic and extrahepatic obstruction to bile flow and the degree of elevation does not help to distinguish between the two (54).

It was analyzed by Kinetic photometric test, according to the International Federation of Clinical Chemistry (IFCC) method. Alkaline phosphatase catalyze the hydrolysis of 4-nitrophenylphosphate (4-NPP) with the formation of free 4-nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate – group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4- nitrophenol, proportional to the activity of ALP present in the sample (53).



1.4 Total Bilirubin

Bilirubin is a breakdown product of hemoglobin. Free, unconjugated bilirubin is extremely apolar and nearly insoluble in water, thus forming a complex with albumin for the transport in the blood from the spleen to the liver. In the liver, bilirubin is conjugated with glucuronic acid and the resulting water soluble bilirubin glucuronides are excreted via the bile ducts. Hyperbilirubinemia can be caused by increased Bilirubin production due to hemolysis (pre-hepatic jaundice), by parenchymal damages of the liver (intrahepatic jaundice) or by occlusion of bile ducts (post-hepatic jaundice). A chronic congenital (predominantly unconjugated) hyperbilirubinemia called Gilbert's syndrome is quite frequent in the population.

A specific mixture of detergents enables a safe determination of the total Bilirubin(53).

2 Renal Function Tests

2.1. Creatinine

Creatinine, formed in the muscle, is a product of the degradation of creatine phosphate, a high energy storage component. Creatininaemia is quite constant (contrary to ureamia), it mainly depends of the muscular mass. It is not very modified by food diet, age, sex or exercise. Creatininaemia is an excellent reflection of renal function, however, the serum creatinine level don't increase as long as the renal function has not decreased of at least 50%.

In the Jaffe reaction, creatinine reacts with picric acid in an alkaline environment to generate an orange-red product. At an alkaline pH, creatinine reacts with picrate to form Janovsky complex.

The rate of increase in absorbance at 510 nm due to the formation of creatinine picrate complex is directly proportional to the creatinine concentration present in the sample (53).

Creatinine + alkaline picrate $\xrightarrow{\text{creatinine}}$ picrate complex

2.2. Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent. In renal diseases urea concentrations are elevated when the glomerular filtration rate is markedly reduced and when the protein intake is higher than 200 g/ day(53) .

Urease - GLDH: enzymatic UV test

Urea + 2H₂O $\xrightarrow{\text{Urease}}$ 2NH₄ + + 2 HCO₃ -

2-Oxoglutarate + NH₄ + + NADH $\xrightarrow{\text{GLDH}}$ L-Glutamate + NAD⁺ + H₂O

(GLDH = Glutamate dehydrogenase)

2.3 Uric acid

Uric acid is the final product of endogenic and exogenic (food origin) purine catabolism (adenosine and guanidine). This transformation takes place mainly in the liver. Approximately 75% of uric acid is eliminated by kidneys; the rest is released in the gastro-intestinal tractus where it will be degraded by the intestinal flora. Seric hypouricaemia is more unusual. This decrease can be observed in different cases as: defect of renal elimination (Fanconi syndrome)

Uric acid was analyzed by using Trinder method (53).

Adult Reference Ranges for LFT &RFT

Instrument used: ABX Pentra C400

S.no	Parameter	Reference		unit
		Men	Women	
1	ALT	≤ 45 (37°C)	≤ 34 (37°C)	U/L
2	AST	< 35 (37°C)	< 31 (37°C)	U/L
3	ALP	20 - 50 years: 53 – 128 > 60 years: 56 - 119	20 - 50 years: 42 – 98 > 60 years: 53 - 141	U/L
4	Total Bilirubin	0.1 - 1.2	0.1 - 1.2	mg/dl
5	Urea	< 50 years:19 – 44 > 50 years: 18 - 55	< 50 years: 15 – 40 > 50 years 21 - 43	mg/dL
6	Creatinine	0.8- 1.3	0.6 - 1.2	mg/dL
7	Uric acid	3.5 - 7.2	2.6 - 6	mg/dL

Declaration

Assurance of Principal Investigator

Assurance of Principal Investigator
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I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the student: Tsegay Asefaw Kahsay
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Date _____ Signature _____

Approval of Advisors:

Mistire Welde, MSc, PhD

Date _____ Signature _____

Abebe Edao, MSc

Date _____ Signature _____