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Assessment of Liver and Renal Function Tests among Gasoline-Exposed Gas station workers in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Halima Abdulkadir, entitled: "Assessment of Liver and Renal Function Tests Among Gasoline-Exposed Gas Station Workers in Addis Ababa, Ethiopia" and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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"Indeed, Allah is the best of planners" (Quran 8:30)

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Abbreviations

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
BUN	Blood Urea Nitrogen
CKD	Chronic Kidney Disease
Cr	Creatinine
EPA	Environmental Protection Agency
GGT	Gamma-Glutamyl Transferase
GSA	Gas Station Attendants
GSW	Gas Station Workers
LFTs	Liver Function Tests
NIOSH	National Institute for Occupational Safety and Health
NOEG	Non-Occupationally Exposed Group
PPE	Personal Protective Equipment
RFT	Renal Function Test
UA	Uric Acid
UG	Unleaded Gasoline
VOC	Volatile Organic Compounds
PAH	Polycyclic Aromatic Hydrocarbons

Abstract

Background: Gasoline station workers are at a higher predisposition to exposure by benzene, toluene, ethylbenzene, and xylene, which can adversely affect liver and renal functions. However, there is a scarcity of wide-ranging studies focusing on the specific impacts of occupational gasoline exposure in the Ethiopian context.

Objective: To assess Liver and renal function tests among gasoline exposed gas station workers compared to unexposed controls from February 2025 to May 2025 in Addis Ababa, Ethiopia

Methods: Comparative cross-sectional study was conducted among gas station workers versus non – gasoline exposed control group from February 2025 to May 2025 in Addis Ababa, Ethiopia. It comprised of 146 gasoline exposed workers and 146 apparently healthy control groups. Based on a structured questionnaire, each study participants' socio-demographic data was collected and afterward, five milliliters of blood sample was drawn into serum separator tubes. The samples were then transported to Ethiopian Public Health Institute's laboratory for sample processing. Cobas c311 was used to measure the analytes. SPSS version 26 was used for statistical analyses – descriptive statistics to summarize the participant characteristics, independent t-tests, Mann Whitney U, One-way ANOVA tests, Kruskal Wallis test and multivariable logistic regression were employed to compare results among the groups.

Result: From 292 participants, 146 Gas station attendants and 146 controls that were enrolled in this study. Gasoline Exposed workers showed significantly higher median ALT (12.4 vs. 7.8 U/L; $p < 0.001$), GGT (31 vs. 18 U/L; $p < 0.001$), ALP (64.0 vs. 61.0 U/L; $p = 0.045$), TBIL (0.37 vs. 0.30 mg/dL; $p = 0.005$), mean AST (19.77 vs. 18.44 U/L; $p = 0.015$), UA (5.91 vs. 5.16 mg/dL; $p < 0.001$), and Creatinine (0.90 vs. 0.81 mg/dL; $p < 0.001$), but no differences in DBIL or Urea. Employment >6 years increased GGT, TBIL, and DBIL; >8 hours/day and >6000 L/day elevated Creatinine, UA, and ALP

Conclusion: Gasoline exposure impairs liver and renal function, likely due to benzene-induced oxidative stress. Overall, this thesis highlights the need for improved workplace safety practices, regular health checkups, and targeted interventions to reduce gasoline-related health risks

Key Words: Gasoline station worker, liver function test, renal function test, occupational exposure

1. Introduction

1.1 Background

Gasoline stations, commonly known as gas stations are essential facilities in urban and rural settings, providing fuel to millions of vehicles daily. The primary operation of a gasoline station involves storage and dispensing of gasoline; a volatile, flammable substance composed of over 500 hydrocarbons, but the primary toxic compounds are light-chain volatile aromatic hydrocarbons (VOC) specifically benzene, toluene, ethylbenzene, and xylene, collectively known as BTEX (1). Workers are potentially exposed to these compounds intentionally or accidentally through inhalation, ingestion, or dermal contact. Inhalation is the primary route of occupational exposure, while dermal absorption may also occur in workers who handle gasoline directly without protection (2,3).

While gasoline exposure poses inherent health risks to all workers, several factors determine whether harmful health effects will occur and what the type and severity of those health effects will be. The potential health effects are influenced by several factors- the amount (dose) of exposure, the length of time (duration) one is exposed, and the route of exposure (4). Individual characteristics, such as age, sex, lifestyle, nutritional status, genetic predispositions, pre-existing health conditions and additionally, the presence of other chemicals in the environment that may potentially compound the toxic effects of gasoline can all play a role in health outcomes (5).

Given these factors, exposure to gasoline may have acute or chronic effects. The acute effects of gasoline exposure include headaches, dizziness, respiratory irritation, and confusion(6). While chronic exposure can lead to wide range of health issues affecting various organs and systems in the body including hematological disorders, respiratory issues, reproductive impairments, immunological dysfunction, dermatological complications, renal disorders and central nervous system disorders. Hepatotoxicity, genotoxicity, and carcinogenicity are also significant risks (7).

Among the various organs and systems affected by gasoline exposure, the liver and kidneys are vulnerable due to their central roles in detoxification and waste filtration(8). For this reason, liver and renal function were chosen as the focal point of this study to gauge the impact of occupational gasoline exposure. Both nephrotoxicity and hepatotoxicity arise through similar mechanisms of oxidative stress and cellular damage(7).

The kidney's ability to filter blood and excrete substances becomes compromised due to chronic gasoline exposure, leading to renal dysfunction or disease (7,9). The mechanism behind gasoline-induced kidney damage involves two key factors: The first one is that gasoline's lipophilic nature allows it to penetrate cell membranes, leading to direct damage to the glomeruli and renal tubules(10,11). The other factor is the excessive reactive oxygen species produced by the oxidative stress that occurs due to gasoline metabolites, this overwhelms the body's antioxidant defenses. This oxidative stress can impair immune function by affecting T-cells and even inducing autoimmune responses, where the immune system mistakenly attacks the body's own cells. The result is increased resistance in the blood vessels – post glomerular resistance and a reduced glomerular filtration rate (7).

As the liver is directly involved in metabolizing BTEX compounds found in gasoline, hepatotoxicity is a potential adverse outcome of chronic gasoline exposure. It unfolds through a sequence of harmful processes where reactive oxygen species (ROS) damage the liver cells(7). The liver metabolizes hydrocarbons primarily through the cytochrome P450 enzyme system(12,13). While this process detoxifies many compounds, it can also convert them into reactive metabolites, which directly interact with the lipid membranes of liver cells to cause lipid peroxidation. This damage leads to the breakdown of these membranes, causing the liver cells to malfunction and leak their contents(14). To defend against these harmful effects, the liver relies on antioxidant enzymes - glutathione peroxidase, catalase and superoxide dismutase (13). However, these antioxidants levels drop significantly in people exposed to gasoline. This allows ROS to build up unchecked, resulting in continuous damage to liver cells(7,15).

Given the essential roles of the liver and kidneys in detoxification and the potential for gasoline-induced damage to these organs, it is crucial to monitor the health of individuals regularly exposed to gasoline vapors, such as gas station workers. Although existing studies have highlighted the dangers of BTEX exposure, there remains a need for localized research to understand the extent of liver and kidney impairment in specific populations. Therefore, this study aims to assess the liver and renal function of gasoline-exposed workers in Addis Ababa, Ethiopia.

1.2 Statement of the Problem

The International Labour Organization estimated 2.93 million people die each year from work-related accidents and diseases. This includes nearly 2 million deaths attributed to fatal work-related diseases(16). The 2022 U.S. Bureau of Labor Statistics data on the oil and gas extraction industry shows a notable number of fatalities due to toxic exposures, fires, and explosions(17). The health effects associated with BTEX exposure are influenced by both the concentration levels and the duration of exposure, encompassing both carcinogenic and non-carcinogenic outcomes(2). Benzene is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC)(18). Other components, such as toluene and xylene, are categorized as Group 2B carcinogens, also indicating possible carcinogenicity based on animal studies (6).

Gasoline-related health conditions, such as pulmonary injury, central nervous system depression, ventricular fibrillation, contact dermatitis, liver disease, renal tubular dysfunction, and respiratory disorders reduce quality of life(7,19). Occupational exposure's adverse health effects due to gasoline and its volatile components have been widely studied across various regions. In Europe and North America, studies show gasoline-related occupational exposure has been linked to elevated rates of cancers, liver damage, and kidney dysfunction(20–22). For example, a Canadian case-control study found that a higher risk of kidney cancer (OR = 1.51, 95% CI = 1.23–1.86) was associated with exposure to both gasoline/diesel exhausts (21). And a cohort study also found significantly raised levels of ALP and AST among workers exposed to benzene from a refinery flaring incident in Texas, USA (23).

In Asia and South America, the health impacts of gasoline exposure among workers have shown similar trends. A study in Brazil, in observed higher levels of liver enzymes (AST and ALT) among gasoline station attendants compared to controls, suggesting early hepatic effects from benzene exposure(24).Furthermore, studies in Iran and Iraq noted significant imbalances in liver and kidney function due to prolonged exposure to gasoline vapors(25,26). Studies in African countries have also highlighted the negative health effects of gasoline exposure. In Egypt, workers at fuel stations exhibited increased levels of liver enzymes and kidney function markers, including ALT, AST, urea, and creatinine(27). In Sudan, a study conducted in Khartoum revealed elevated liver and kidney markers among gasoline workers, reinforcing the findings observed in Egypt(28). And Nigeria being one of the largest oil producers in Africa, contributes various studies that have reported significant imbalances in liver and kidney function due to prolonged exposure to gasoline vapors (29–32).

In Ethiopia, without wide-ranging studies any health interventions would be based on assumptions that may not hold true locally as the true burden of disease among gas station workers remains unknown, leaving gasoline exposed workers to health risks. This research is vital to assess the prevalence of liver and renal diseases in this group, determine the impact of gasoline exposure, and inform public health interventions aimed at protecting workers from long-term harm.

1.3. Significance of the study

The study holds relevance as it will provide insights into the health risks faced by gasoline station workers in Addis Ababa due to chronic exposure to gasoline, with a focus on the effects on the liver and kidney function.

The findings will benefit gasoline station workers by raising awareness about the potential long-term health impacts of their occupational exposure, promoting personal protective equipment and preventive measures, and encouraging regular health check-ups. Furthermore, the results will offer essential evidence for policymakers and stakeholders, guiding the development of improved occupational health standards and safety regulations to better protect gasoline station workers.

Overall, the research will contribute to the scientific community by expanding the existing body of knowledge on gasoline exposure in rapidly developing cities like Addis Ababa, Ethiopia.

2. Literature Review

This literature review seeks to examine the current body of research related to the effects of gasoline exposure on human health, with a focus on liver and renal diseases among gas station workers by reviewing studies from across the world.

2.1. Liver function tests in gasoline-exposed workers

Liver function tests are routinely used to evaluate hepatic health and detect signs of liver cell damage, particularly the enzymes alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and gamma-glutamyl transferase which are sensitive indicators of hepatic and hepatobiliary damage(33). Additionally, bilirubin levels, another marker of liver function, are measured to assess the liver's ability to metabolize and clear waste products. Gasoline exposure has been associated with elevated ALT, AST, ALP, and GGT levels in several international studies as damaged liver cells release them into the bloodstream (14,33).

Mark A. D'Andrea and G. Kesava Reddy conducted a cohort study on subjects exposed to over 7,711 kg of benzene at the British Petroleum refinery in Texas, USA due to a 40 - day flaring incident from June 2010 to October 2012. The group included 100 benzene-exposed and 100 unexposed subjects; the results exhibited that the benzene - exposed subjects had significantly elevated levels of ALP, 121.2 ± 73.7 IU/L vs. 65.4 ± 23.6 IU/L, $p = 0.000$ and AST, 23.4 ± 11.8 IU/L vs. 19.5 ± 8.9 IU/L, $p = 0.0089$) compared to unexposed group(34).

Moro et al. in 2017 conducted a comparative cross-sectional study in Brazil on gas station workers that constituted twenty women and twenty men versus twenty men and twenty women with no history of exposure to benzene as the control group. The study found higher ALT and AST activities in male gasoline station attendants compared to both female attendants and control males, with statistically significant differences ($p < 0.05$). Additionally, male controls showed reduced AST activity compared to female controls ($p < 0.05$). While all liver function tests were within reference range, significant gender changes were observed in urea, creatinine, and uric acid levels within the gas station workers and the control groups ($p < 0.05$). Although all values remained within normal ranges the findings suggest a budding early hepatic effects from exposure to benzene vapors among gasoline station attendants (24).

A cohort study conducted by Marja-Liisa Lindbohm, et al from the Finnish Institute of Occupational Health and the Finnish Cancer Registry examined the association between occupational exposure to solvents, gasoline vapors, and the risk of liver cancer over a 24-year period (1971–1995). Economically active Finns born between 1906 - 1945 were tracked. 2,474 incident cases of primary liver cancer were through the Finnish Cancer Registry. Among men, 19% (n = 327) of liver cancer cases were in occupations with potential exposure to organic solvents, while 5% (n = 41) of female cases had similar exposure. Gasoline vapor exposure showed a slight risk increase (RR 1.24, 95% CI 0.70–2.20). The risk for hepatocellular carcinoma, followed similar trends (20).

In 2012, a cross-sectional survey conducted by Tunsaringkarn Tanasorn, Zapaung Kalaya, and Rugsiyothin Anusorn, across 11 gasoline stations in the Pathumwan, central Bangkok, Thailand assessed the effects of BTEX exposure on hepatic biomarkers. 80 men and 25 women gasoline station workers were included. The average levels of AST, ALT and ALP were 26.0 U/L, 30.9 U/L, and 71.8 U/L, respectively(35).

In a 2023 comparative analysis study conducted by Chan Pattama Polyong and Anamai Thetkathuek from Thailand, liver functions were assessed among 100 fuel dispenser employees and 100 non-fuel dispenser employees. The study found that 9.0% and 8.0% of workers exhibited abnormal SGPT and SGOT levels, respectively, indicating liver function abnormalities. Workers who worked more than 8 hours per day had a significantly higher risk of abnormal SGPT (OR = 2.789; 95% CI: 1.003, 7.761) (36).

In a case-control study conducted by Rahul et al. (2014-2015) in Jaipur city, two groups of 40 nonsmoker, nonalcoholic males aged 20-40 years were compared. The study group consisted of fuel filling attendants who worked at least 8 hours per day for over 3 years at various petrol pumps, while the control group were not occupationally exposed to petroleum fumes. The researchers observed that while liver enzymes were within their respective reference ranges, they were significantly higher among the petrol filling attendants compared to the control group, except for ALP, which, although elevated in attendants, did not reach statistical significance. Notably, 25% of attendants with more than 10 years of exposure exhibited elevated levels of GPT(37).

In a comparative cross-sectional study conducted by Olufunsho Awodele, Ademola A. Sulayman, and Alade Akintonwa in 2014 in Lagos, Nigeria. 25 tanker drivers, who had worked for a minimum of 5 years, were selected, along with 15 matched controls who had no continuous hydrocarbon exposure. The results showed significant increases ($p \leq 0.05$) in (ALT) (31.14±13.72 vs. 22.38±9.89), (AST), and ALP (84.04±21.89 vs. 62.04±23.33) in the exposed group compared to the controls. (30).

Nwanjo and Ojiako (2007) conducted a cross-sectional study in Owerri Metropolis, Nigeria, recruiting 20 petrol attendants aged between 16 and 38 years, who were directly exposed to petrol vapors during their duties. A control group of 20 apparently healthy individuals not engaged in activities involving petrol exposure were also recruited. The study found a significant increase ($p < 0.05$) in the activities of alkaline phosphatase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) among those exposed to petrol vapors for 6–10 years. (29)

Akinosun et al. in 2006 conducted a cross-sectional study in Ibadan Metropolis, Nigeria, involving 51 Nigerian males aged between 20 and 40 years. The study included 29 petrol attendants, and 22 age- and sex-matched controls selected from the University College Hospital. The researchers assessed various liver function tests and found that all tests were similar between the petrol attendants and the controls, except for significantly lower levels of alkaline phosphatase (ALP) in the petrol attendants ($P = 0.02$)(38).

In a 2014 cross-sectional study by Masoud Neghab, Kiamars Hosseinzadeh, and Jafar Hassanzadeh, the health impact of exposure to unleaded petrol were studied among 200 petrol station employees compared to 200 unexposed employees in Shiraz, Iran. The study found that exposed individuals had significantly higher levels of direct bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)(26).

A case-control study conducted by Mohammadreza Firouzkouhi, et al in Zabol, Iran, from January to April 2012, examined gasoline exposure among 300 men, with 150 individuals working as unregulated gasoline traders (case group) and a control group, 150 people, did trade neither gasoline nor work in the oil industry. The study revealed significantly higher levels of aspartate aminotransferase (AST) ($P < 0.001$), alanine aminotransferase (ALT) ($P < 0.001$), and alkaline phosphatase (ALP) (ORP < 0.001) in the exposed group compared to controls(25).

In a comparative cross-sectional study conducted by Hatem Abdel Moniem Ahmed in Riyadh City, Saudi Arabia, from March to June 2019, the health effects of gasoline exposure were assessed among the exposed group that included 35 gas station employees, who were compared to 35 healthy male controls. The study revealed significant increases in liver enzymes - Alanine transaminase and Aspartate aminotransferase in the exposed group compared to the control group. These changes were associated with increased lead levels among the gas station employees(39)

In a case-control study by Hegazy and Kamel (2014) in Makkah, Saudi Arabia, 60 male fuel station workers aged 20-50 years were divided into three groups: 20 workers with no occupational exposure to benzene (control), 20 workers exposed to benzene for five years or more and treated with vitamin A (10,000 IU daily for three months), and 20 workers exposed to benzene but without vitamin A treatment. The study assessed liver enzymes (AST, ALT), The results showed elevated liver enzyme levels (AST: P = 0.0001, ALT: P = 0.004). Following vitamin A treatment both AST and ALT levels significantly decreased (P = 0.004 and P = 0.014, respectively)(40).

A 2006 study by Abdel Azia et al. in the Gaza Strip involved 80 workers exposed to leaded gasoline and a control group of 18 healthy workers to assess the biochemical effects of exposure. ALT activity increased from 28.6 u/l in the control to a 23.43% rise in the fifth group, while serum AST increased from 27.4 u/l to a 27.37% rise in the fifth group. Lastly, alkaline phosphatase levels rose from 80.4 u/l in the control group, with a 15.67% increase noted in the fifth group(41)

A comparative cross-sectional study conducted from January 1, 2015, to March 31, 2015by Abou-El Wafa et al. at Mansoura University, Egypt, compared the health status of 102 petrol station attendants was to a matched group of 102 healthy male service and office workers. The researchers evaluated liver enzymes, serum albumin, and total protein levels. Results indicated that while most parameters showed no statistically significant differences between the two groups, alanine aminotransferase levels were significantly higher in petrol station attendants(42).

In a cross-sectional case-control study by Marwa A. Hasb Elnabi and colleagues conducted from April 2019 to March 2020 in Sohag governorate, Egypt 100 male fuel station workers were compared to 50 non-fuel station workers. The study revealed statistically significant differences in the levels of ALT and AST between the exposed group and the controls. Smoking had a notable impact on AST levels in the exposed group. Additionally, there was a positive correlation between liver function tests, and the ages of the fuel station workers. While there was a positive correlation between ALT levels and duration of work, no significant differences were found in bilirubin levels, or creatinine levels concerning the duration of work at fuel stations(27).

A comparative cross-sectional study conducted in Sana'a, Yemen, by Ali Alhaj et al (2017-2018), compared 109 petrol station workers exposed to petrol for at least six hours daily for six months or more with 109 non-exposed office clerks from the University of Science and Technology. The results showed that the mean serum ALP levels were significantly higher among petrol station workers compared to office clerks. However, no significant differences were observed in serum ALT and AST levels between the two groups.(43).

A cross-sectional case-control study by Abdelgadir Eltom and Hajir Taj Elsir Hamd was conducted between February and March 2015, involving 50 gasoline station workers and 50 age-matched controls (ages 17-55) in Sudan. The results showed a significant increase in the mean serum levels of AST, ALT, and ALP in the exposed group compared to the controls, with P values of 0.001, 0.01, and 0.001, respectively. There was also a significant positive correlation between age and serum AST (P=0.0001, r=0.67), ALT (P=0.0001, r=0.45), and ALP (P=0.02, r=0.23). Similarly, a positive correlation was found between the duration of work at gasoline stations and serum AST (P=0.04, r=0.28), ALT (P=0.045, r=0.28), and ALP (P=0.001, r=0.44)(28).

In a comparative cross-sectional study conducted by Tsegay Asefaw et al from January to April 2018 in Mekelle, Ethiopia, 43 gasoline station workers who had been exposed to gasoline were compared to 47 healthy control participants. The results showed significantly increased mean levels of ALT and AST in gasoline station workers compared to the control group. In contrast, ALP levels were significantly lower among the exposed workers ($p = 0.012$). The study further revealed that workers exposed to gasoline for more than six years exhibited significantly higher levels of ALT and AST compared to those with less than six years of exposure(44)

2.2. Renal function test in gasoline-exposed workers

In diagnosing kidney function, serum creatinine, urea, and uric acid tests are commonly used to assess how well the kidneys are filtering waste from the blood(45). These markers tend to rise when the kidneys' filtration capacity is compromised, a condition observed in individuals exposed to nephrotoxic substances like BTEX (7). By monitoring these key markers, healthcare providers can detect signs of nephrotoxicity, offering a means to prevent further progression of disease in gasoline-exposed workers. The following are studies done around the world assessing renal function test among BTEX exposed individuals.

A Canadian population-based case–control study conducted by Cheryl E. et al used data from the National Enhanced Cancer Surveillance System (NECSS) from 1994–1997 to investigate the association between engine exhaust exposure and kidney cancer. Of the 712 kidney cancer cases, 372 (52%) had exposure to both gasoline and diesel exhausts at some point, compared to 984 (40%) of the controls. Workers exposed to engine exhausts had a higher likelihood of kidney cancer than those never exposed (OR diesel = 1.23, 95% CI = 0.99–1.53; OR gasoline = 1.51, 95% CI = 1.23–1.86). A dose–response relationship was observed between gasoline exhaust exposure and kidney cancer, with significant trends for both highest attained and cumulative exposure ($P < 0.0001$). Men with high cumulative exposure to both gasoline and diesel exhaust had a 76% increased odds of developing kidney cancer (95% CI = 1.27–2.43)(21).

In a cross-sectional survey conducted by Tunsaringkasrn et al. (2009) in Pathumwan District, Bangkok, blood samples were collected from 110 gasoline workers (80 men and 30 women) at 11 gasoline stations, along with samples from 10 office workers (6 men and 4 women) for control purposes. The study found that blood urea nitrogen (BUN) and creatinine, levels were significantly higher in men compared to women ($p < 0.001$ for BUN and creatinine). The results indicated that while BUN was within the normal range, the elevated creatinine levels suggested potential risks for kidney dysfunctions among gasoline workers, particularly in female workers(46).

In a cross-sectional survey conducted by Tunsaringkarn Tanasorn, Zapaung Kalaya, and Rugsiyothin Anusorn in 2012, 105 gasoline station workers (80 men and 25 women) from 11 gasoline stations in the Pathumwan area, central Bangkok, Thailand, were assessed for the effects of BTEX exposure on kidney function. The average levels of blood urea nitrogen (BUN) and creatinine 11.8 mg% and 1.0 mg% respectively(35).

In a 2023 comparative analysis study conducted by Chan Pattama Polyong and Anamai Thetkathuek from Thailand, kidney functions were assessed among 100 fuel dispenser employees and 100 non-fuel dispenser employees. Kidney function abnormalities were observed in 5.5% of workers for BUN and 11.5% for creatinine, though no correlation was found between BTEX exposure and kidney function abnormalities(36).

In a comparative cross-sectional study conducted by Mahmood et al. (2013) in Sulaimani City, Kurdistan, the plasma protein profile and renal function of 74 male petrol-filling workers were assessed and compared to values from 27 sex- and age-matched controls. The results showed significantly elevated levels of serum urea and creatinine among the petrol-filling workers. However, no correlation between these elevated levels and the duration of exposure to petrol was reported(47).

A comparative cross-sectional study by Egbuonu, A, Nkwazema, D., Aloh, G. S. (2016) from Calabar metropolis, south-south Nigeria aimed to compare the renal and cellular function status in participants, aged between 18 and 35, included asymptomatic male workers from 10 petroleum depots that do not provide personal protective equipment and male University of Calabar students (for 2 to 4 years) who are largely inclined to vegetable and seafood diets. The serum concentrations for urea (4.27 ± 1.28 mmol/L), creatinine (117.35 ± 7.87 µmol/L), uric acid (0.44 ± 0.07 mmol/L), and urea ratio (0.04 ± 0.81) in the exposed group were significantly higher than those in the control group (3.19 ± 0.84 mmol/L, 107.65 ± 8.17 µmol/L, 0.31 ± 0.05 mmol/L, and 0.03 ± 1.32, respectively) (31).

In a comparative cross-sectional study conducted by Olufunsho Awodele, Ademola A. Sulayman, and Alade Akintonwa in 2014 in Lagos, Nigeria. 25 tanker drivers, who had worked for a minimum of 5 years, were selected, along with 15 matched controls who had no continuous hydrocarbon exposure showed significant increases in creatinine and urea were observed in the petroleum tanker drivers (30).

Nwanjo and Ojiako (2007) conducted a cross-sectional study in Owerri Metropolis, Nigeria, involving 20 petrol attendants aged between 16 and 38 years, who were directly exposed to petrol vapors during their duties. A control group of 20 apparently healthy individuals not engaged in activities involving petrol exposure were also recruited. The study found a significant increase ($p < 0.05$) in the concentrations of serum urea, creatinine, and urinary protein in the exposed group compared to the controls (29).

In a cross-sectional analytic study conducted during the dry season from January to April 2012 by Adamu et al. in Gombe, Nigeria; the serum levels of uric acid, urea, and creatinine were compared between 90 roadside gasoline dispensers and 90 matched controls. The results showed that plasma uric acid levels were significantly higher in the exposed group (5.35 ± 0.9 mg/dL) compared to the control group (4.48 ± 0.9 mg/dL, $P = 0.001$). However, urea and creatinine levels did not differ significantly between the two groups. Additionally, a significant positive correlation was found between the duration of exposure to gasoline and uric acid levels ($r = 0.63$, $p < 0.001$(32).

In a 2014 cross-sectional study by Masoud Neghab, Kiamars Hosseinzadeh, and Jafar Hassanzadeh; the health impacts of exposure to unleaded petrol were examined among 200 petrol station employees compared to 200 unexposed employees in Shiraz, Iran. The study found that exposed individuals had significantly higher levels of blood urea, and plasma creatinine compared to the unexposed group. (26).

A case-control study conducted by Mohammadreza Firouzkouhi, et al in Zabol, Iran, from January to April 2012, examined gasoline exposure among 300 men, with 150 individuals working as unregulated gasoline traders (case group) and a control group, 150 people, did trade neither gasoline nor work in the oil industry. The study revealed significantly higher levels of blood urea nitrogen ($P = 0.008$) in the exposed group compared to controls. Conversely, the exposed group had lower creatinine (25).

Jabir et al. (2016) conducted a comparative cross-sectional study involving 56 individuals, including 36 petrol station workers in Basrah, Iraq, who were exposed to petrol products during their duties, and 20 healthy controls. The study revealed a significant increase in blood urea and blood creatinine among workers with 6 to 10 years of exposure when compared to the control group. The results indicate that exposure to petroleum products can cause an imbalance in renal function, and these effects are directly related to the duration of exposure(48).

In a comparative cross-sectional study conducted by Hatem Abdel Moniem Ahmed in Riyadh City, Saudi Arabia, from March 2019 to June 2019; the health effects of gasoline exposure were assessed among the exposed group that included 35 gas station employees, who were compared to 35 healthy male controls. The study revealed significant increases creatinine levels in the exposed group compared to the control group. These changes were associated with increased lead levels among the gas station employees(39).

In a comparative cross-sectional study conducted by Mutasim M. Khalafalla et al in 2021, 63 participants were enrolled, consisting of 44 gasoline station workers as the exposed group and 19 women as the non-exposed group in Makkah City, Saudi Arabia. The study found no significant effect of gasoline exposure on blood creatinine levels ($p > 0.05$)(49) .

A study by Abdel Azia et al. (2006) in the Gaza Strip involved 80 workers exposed to leaded gasoline and a control group of 18 healthy workers to assess the biochemical effects of exposure. The mean serum urea level in the control group was 27.4 mg/dl, while it increased to 30.0, 33.3, 36.0, 38.3, and 43.0 mg/dl in the exposed groups. Serum creatinine levels rose from 0.9 mg/dl in the control group to 1.0, 1.1, 1.2, 1.3, and 1.5 mg/dl. Uric acid concentrations also increased from 3.7 mg/dl in the control group to 4.0, 4.3, 4.6, 5.0, and 5.2 mg/dl(41)

In a comparative cross-sectional study conducted by Abou-El Wafa et al. at Mansoura University, Egypt, the health status of 102 petrol station attendants was compared to a matched group of 102 healthy male service and office workers. The study was carried out from January to March 2015. The researchers evaluated renal functions and results indicated that the parameters showed no statistically significant differences between the two groups (42).

In a cross-sectional case-control study by Marwa A. Hasb Elnabi and colleagues conducted from April 2019 to March 2020 in Sohag governorate, Egypt 100 male fuel station workers were compared to 50 non-fuel station workers. The study revealed statistically significant differences in the levels of urea between the exposed group and the controls. Smoking had a notable impact creatinine and urea levels in the exposed group. Additionally, there was a positive correlation between kidney function tests, and the ages of the fuel station workers (27).

In a comparative cross-sectional study conducted in Sana'a, Yemen, by Ali Alhaj et al (2017-2018), 109 petrol station workers exposed to petrol for at least six hours daily for six months or more were compared with 109 non-exposed office clerks from the University of Science and Technology. Although the mean concentrations of creatinine and urea were higher among the office clerks, only creatinine levels were significantly elevated in this group (43).

In a quantitative descriptive, cross-sectional study by Awadalla et al. (2016) during December 2015 and August 2016 in El-Obied, North Kordofan State, Sudan, 67 individuals were examined, including 37 petrol station attendants and 30 age- and sex-matched control group. The study, conducted between, found that plasma levels of creatinine, urea and uric acid were significantly higher among the test group compared to controls. The study also revealed a significant positive correlation between the levels of urea with the duration of exposure (in years) among the petrol station workers ($P \leq 0.05$) (50).

In a comparative cross-sectional study conducted by Tsegay Asefaw et al from January to April 2018 in Mekelle, Ethiopia, 43 gasoline station workers who had been exposed to gasoline were compared to 47 healthy control participants. The results showed significantly increased mean levels of urea, creatinine, and uric acid in gasoline station workers compared to the control group. The study further revealed that workers exposed to gasoline for more than six years exhibited significantly higher levels of urea, creatinine, and uric acid compared to those with less than six years of exposure (44)

2.3 Summary and Gaps in Literature

The reviewed literature provides evidence that occupational exposure to gasoline, particularly BTEX compounds, is associated with adverse effects on liver and renal function. However, some gaps remain. There is inconsistency in the biomarkers assessed across studies, most studies do not explore correlations between specific exposure factors — such as duration of work, hours per day, or PPE use— and biochemical outcomes, limiting insight into dose-response relationships. And despite the extensiveness of international data, there's only one study from Ethiopia that was conducted in Mekelle. There is a lack of data from Addis Ababa, Ethiopia's capital and most industrialized urban center, where gasoline usage are higher— potentially increasing exposure levels. These gaps underline the need for locally relevant studies in Addis Ababa that not only assess liver and renal function among gasoline-exposed workers but also investigate how specific exposure characteristics contribute to health risks.

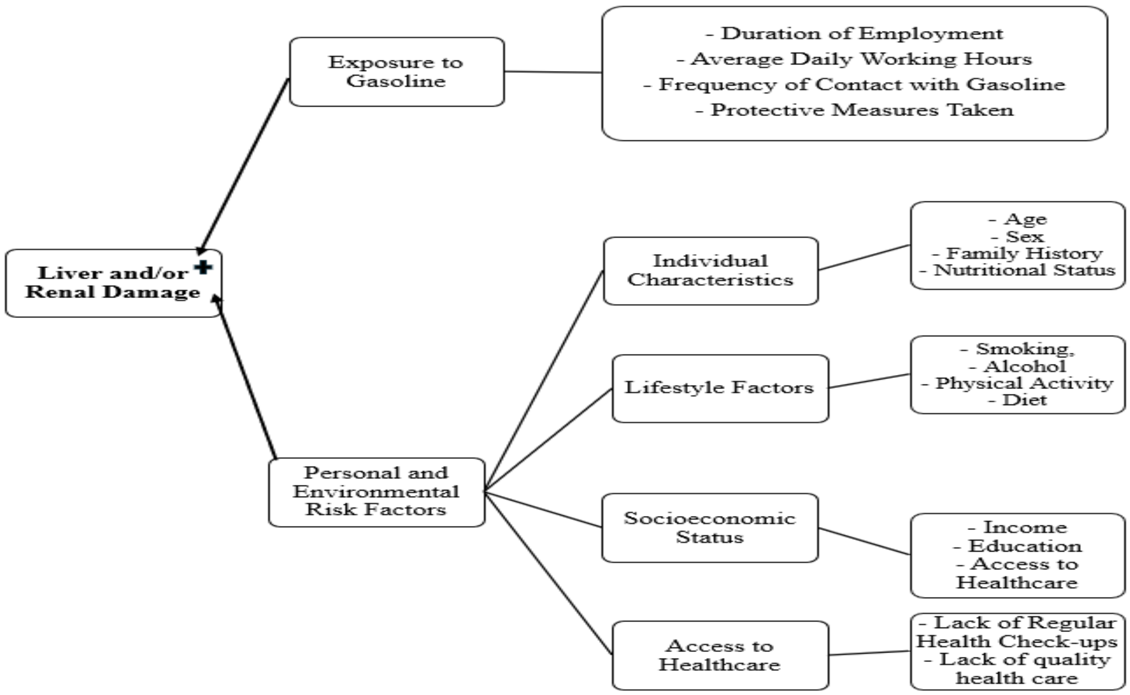


Figure 1 – Conceptual Framework

3. Objectives

3.1 General Objective

To assess liver function tests and renal function tests among gasoline exposed gas station workers in Addis Ababa, Ethiopia from February 2025 to May 2025

3.2 Specific Objectives

- To assess the levels of liver function tests among gas station workers versus non gasoline exposed groups.
- To assess the levels of renal function tests among gas station workers versus non gasoline exposed groups.
- To identify the associated factors associated with abnormal liver and renal function tests among gasoline-exposed workers

4. Hypothesis

(H_0): There is no significant difference in liver and renal function test results between gasoline-exposed gas station workers and unexposed control participants in Addis Ababa, Ethiopia.

5. Materials and Methods

5.1 Study area

The study was carried out across multiple gas stations situated in Ethiopia's capital city - Addis Ababa. Addis Ababa lies at an elevation of 2,355 meters and is located at 9°N Latitude and 38°E longitude at the geographic center of the country (51) According to projected Central Statistical Agency of Ethiopia (2024), a total population of 4,030,000 people reside in Addis Ababa. Of which 1,900,000 is projected to be male and the 2,130,000females(52). Addis Ababa serves as the administrative, diplomatic and commercial hub of Ethiopia – playing a pivotal role in national development. The estimated number of legal gasoline stations registered under the Ministry of Trade and Regional Integration in Addis Ababa as of April 2023 was 153, out of these 126 were actively providing fuel services during the study period(53).

5.2 Study Design and Period

A comparative cross-sectional study was conducted between February 2025 – May 2025 to assess liver and renal function tests among gasoline-exposed gas station workers in Addis Ababa, Ethiopia.

5.3 Population

5.3.1 Source population

- Exposed group: All gas station attendants working in gasoline stations across Addis Ababa, Ethiopia.
- Control group: All apparently healthy adults living in Addis Ababa who had no known occupational exposure to gasoline or other petroleum products

5.3.2 Study population

- Exposed group: All volunteer gas station workers in Addis Ababa, Ethiopia who met the inclusion criteria and consented to participate in the study.
- Control group: Apparently healthy individuals from Addis Ababa University—specifically employees and students— in Addis Ababa, Ethiopia that are not

occupationally exposed to gasoline who met the inclusion criteria and consented to participate in the study (matched by age and sex).

5.4 Inclusion and exclusion criteria:

Case Group: Gasoline-Exposed Workers

Inclusion Criteria:

- Currently employed as a gas station worker.
- 18 years old and above.

Control Group: Non-Exposed Individuals

Inclusion Criteria:

- Not employed in any occupation related to gasoline.
- 18 years old and above.
- No confirmed history of liver or renal disease.

Exclusion Criteria for Both Groups

- Individuals with a history of liver disease or chronic kidney disease
- Participants that were on medications known to affect liver or renal function
- Individuals with significant alcohol/Khat consumption
- Individuals with comorbidities affecting liver or kidney function were excluded to minimize confounding effects
- Individuals working in gas station for a period of less than 6 months

5.5 Study Variables

5.5.1 Dependent Variables

1. Liver Function Tests:

- Alanine Aminotransferase
- Aspartate Aminotransferase
- Alkaline Phosphatase
- Gamma-Glutamyl Transferase
- Total and Direct Bilirubin

2. Renal Function Tests:
    - Serum Creatinine
    - Urea
    - Uric acid

5.5.2 Independent Variables

- Occupational exposure factors - Duration of Employment at Gas Station, Average Daily Working Hours, Days worked per week, Average litter of gas they handle per day
- Protective Measures Taken- Gloves, Mask, Protective Clothing, Safety Shoe
- (Sociodemographic characteristics) – age, sex, residence and education.
- Occupational health awareness and training
- Lifestyle and Habits –eating and drinking at the gas station

5.6. Sample size calculation and Sampling method

5.6.1 Sample size calculation

The sample size for comparing two independent groups with continuous outcomes such as biochemical markers is determined using the two means formula. (44)

$$n_1 = n_2 = \frac{(Z_{1-\alpha/2}+Z_\beta)^2 * (\sigma_1^2+\sigma_2^2)}{(\mu_1-\mu_2)^2}$$

- $Z_{1-\alpha/2}$ = 1.96 for 95% confidence (α = 0.05)

- Z_β = 0.84 for 80% power
- μ_1 = 24.4, σ_1=10.2 → Mean and SD of ALT in exposed group
- μ_2 = 19.06, σ_2=5.96 → Mean and SD in control group

Plug the values into the formula:

$\sigma_1^2 + \sigma_2^2 = (10.2)^2 + (5.96)^2 =$

$(\sigma_1^2 + \sigma_2^2) = 104.04 + 35.52 = 139.56$

$(\mu_1 - \mu_2)^2 = (24.4 - 19.06)^2 = 5.34^2$

$$n = \frac{(1.96 + 0.84)^2 * 261.1456}{5.34^2}$$

$$= \frac{(2.8)^2 \times 139.56}{28.5156}$$

$$= \frac{1,094.11}{28.5156}$$

$= 38.7 \approx 39$

5.6.2. Sampling Method

Convenient sampling technique was used to recruit volunteer gas station workers and age/sex matched control participants. A larger sample size than 38 per group was used to enhance generalizability and allow for subgroup analysis. Additionally, since this study also takes place in Ethiopia, the population characteristics and environmental exposures are likely to be comparable, making the Tigray data a suitable reference for sample size estimation(44).

5.7. Measurement and Data collection

5.7.1. Data collection tools

Initially, potential participants were informed about the study's objectives. The volunteer participants who gave consent were then interviewed to assess eligibility by identifying exclusion criteria. Those who met inclusion criteria were enrolled in the study. Following with a structured questionnaire to gather information on demographic characteristics, lifestyle factors and occupational exposure details. After the interview a trained personnel under aseptic techniques collected 5ml of blood sample using a serum separator tube.

5.7.2. Sample collection

Approximately 5 ml venous blood sample was collected from volunteer participants by using sterile, disposable Vacutainer blood collection system with serum separator tubes. The drawn sample was appropriately labeled with participant identification code. The blood sample was then transported using a triple packaging system to the Ethiopian Public Health Institute for analysis. Upon arrival the blood sample was spun at 4000rpm for 5 minutes in a centrifuge. The separated serum was then poured into 1.5 - 2 ml Nunc tubes and were stored at -80°C deep freezer until laboratory analysis. The chemistry analysis was carried out utilizing Cobas c311, in strict accordance with the laboratory's established Standard Operating Procedures (SOPs).

5.7.3. Principles of Laboratory analysis

The LFT and RFT analytes were analyzed by utilizing cobas c311 analyzer series. The cobas c311 analyzer series is fully automated, uses serum/plasma, urine, CSF, supernatant and whole blood sample types, performs in vitro quantitative and qualitative tests on a wide range of analytes and performs photometric assays and ion-selective electrode measurements. The cobas c311 measures the absorbance of reaction solutions intermittently with a photometer during reaction time. The reaction disk rotates 3 turns in 24 seconds. During this time, the absorbance is measured and stored for all of reaction cuvettes that go across the optical path of the photometer. The computer uses the available assay parameter information to select the wavelengths and the times at which a reaction mixture's absorbance is read, and results are calculated; it keeps tracks of which test is being performed in each reaction cell. The computer then uses this tracking ability and the programmed instructions to obtain test results (54). The principles of the LFT and RFT are listed below.

Alkaline Phosphatase - The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al. In 2011 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) recommended a reference procedure for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc at 37 °C. This assay follows the recommendations of the IFCC but was optimized for performance and stability.

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{ALP} \text{phosphate} + \text{p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 450 nm and had a validated measuring range of 5-1200 U/L.

Alanine Aminotransferase - This assay follows the recommendations of the IFCC but was optimized for performance and stability. ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+ .

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{ALT} \text{pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{LDH} \text{L-lactate} + NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm and had a validated measuring range of 5-700 U/L.

Aspartate Aminotransferase- This assay follows the recommendations of the IFCC for kinetic UV method but was optimized for performance and stability. AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm and had a validated measuring range of 5 to 700 U/L.

$$\text{L-Aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H+} \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD+}$$

γ-Glutamyl transferase – In 1969, Szasz published the first kinetic procedure for GGT in serum using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor. In order to circumvent the poor solubility of γ-glutamyl-p-nitroanilide, Persijn and van der Slik investigated various derivatives and found the water-soluble substrate L-γ-glutamyl-3-carboxy-4-nitroanilide to be a better choice. In 2002, the IFCC recommended the standardized method for determining GGT including optimization of substrate concentrations, employment of NaOH, glycylglycine buffer and sample start. The GGT liquid reagent follows the formulation recommendation according to Szasz, but was optimized for performance and stability.

γ-glutamyl transferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine

$$\text{L-}\gamma\text{-glutamyl-3-carboxy-4-nitroanilide} + \text{glycylglycine} \xrightarrow{\text{GGT}} \text{L-}\gamma\text{-glutamyl-glycylglycine} + \text{5-amino-2-nitrobenzoate}$$

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance photometrically at 415 nm and had a validated measuring range of 3-1200 U/L.

Creatinine – Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests. This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. The color intensity of the quinone imine chromogen measured at 546 nm in the measuring range of 0.06-30.5 mg/dL is directly proportional to the creatinine concentration in the reaction mixture.

$$\text{creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{creatine}$$

$$\text{creatine} + H_2O \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea}$$

$$\text{sarcosine} + O_2 + H_2O \xrightarrow{\text{SOD}} \text{glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{2,4,6-triiodo-3-hydroxybenzoic acid} \xrightarrow{\text{POD}} \text{quinone imine chromogen} + H_2O + HI$$

Urea – employs kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2H_2O \xrightarrow{\text{UREASE}} 2\,NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + NADH \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically at 340 nm and had a validated measuring range of 3.0-240 mg/dL.

Uric Acid – The assay is slight modification of the colorimetric method developed by Town et al. (The sample is initially incubated with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system any ascorbic acid present in the sample is eliminated in the preliminary reaction; this prevents any ascorbic acid interference with the subsequent POD indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.) In this reaction however, the peroxide reacts in the presence of peroxidase (POD), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), and 4-aminophenazone to form a quinone-diimine dye.

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$\text{Uric acid} + 2\ H_2O + O_2 \xrightarrow{\text{URICASE}} \text{allantoin} + CO_2 + H_2O_2$$

In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye.

$$2\ H_2O_2 + H^+ + TOOS^a + \text{4-aminophenazone} \xrightarrow{\text{PEROXIDASE}} \text{quinone-diimine dye} + 4\ H_2O$$

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance at 546 nm and had a validated measuring range of 0.2-25.0 mg/dL.

Total bilirubin - Colorimetric diazo method is utilized **-** in the presence of a suitable solubilizing agent, Total bilirubin is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium.

$$\text{Bilirubin} + \text{3,5-DPD} \xrightarrow{\text{ACID}} \text{azobilirubin}$$

The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically at 546 nm and had a validated measuring range of 0.15-35.1 mg/dL.

Direct Bilirubin - the Jendrassik-Grof procedure utilizes acidified sodium nitrite which produces nitrous acid, which then reacts with sulfanilic acid (in acidic solution) to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of azobilirubin. In the direct bilirubin assay, only conjugated bilirubin is converted by the diazotized sulfanilic acid.

The intensity of the red color of azobilirubin is measured photometrically at 570 nm in the measuring range of 0.2-10.0 mg/dL and is proportional to the direct (conjugated) bilirubin concentration.

5.8. Data Quality Assurance

5.8.1 Pre analytical

Data was collected after the questionnaire was translated into the local language to ensure the participants clearly understood the questions during the interview. Standard operating procedures for sample collection were established and strictly followed to minimize contamination and ensure consistency. Trained phlebotomists collected venous blood samples using aseptic techniques. Each sample was labelled immediately after collection with a clear labeling system to avoid mix-ups and ensure traceability of samples. Proper storage conditions were maintained during the transportation of samples to the laboratory to preserve their integrity.

5.8.2 Analytical

Standard operating procedures (SOPs) were strictly followed for all measured parameters. Before running participant samples, the performance of the COBAS c311 was verified using both normal and pathological quality control materials to ensure analytical reliability and accuracy.

5.8.3 Post analytical

Cobas c311 generated printout were used as primary data source. All the test results were thoroughly examined and verified prior to data entry. The verified data were then carefully entered into SPSS version 26 and stored for subsequent statistical analysis.

5.9. Data analysis and interpretation

Data was analyzed using SPSS version 26. Descriptive statistics (mean, median, standard deviation) have been used to summarize the demographic and exposure characteristics of the study population. The Shapiro-wilks test assessed normality in continuous variables. Comparative analyses, utilizing independent t-tests and Mann-Whitney U, assessed the impact of gasoline exposure on liver and renal function between case and control groups. one-way ANOVA and Kruskal-Wallis assessed liver and renal function within case groups. Multivariable linear regression evaluated the relationship between independent variables and dependent variables. P value < 0.05 was considered statistically significant.

5.10. Operational definitions

- Gasoline Exposure: Occupational exposure to gasoline. This includes workers who have been employed for at least six months in positions where they handle gasoline.
- Liver Function Tests: measured by levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma-Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Direct Bilirubin and Total Bilirubin

- Renal Function Tests: Measured by serum creatinine, Urea and Uric Acid.
- Control Group: Individuals without occupational exposure to gasoline, matched for age and gender with the exposed group.

5.11. Ethical considerations

Ethical approval was attained from the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Science, College of Health Science of Addis Ababa University. Participants in the study were communicated the purpose of the research, and participants were asked for their written informed consent. Those willing to participate were included in the study. Participation in the study was voluntary, and withdrawal was accepted. Individual consent was obtained before the collection of clinical data and blood samples. To ensure confidentiality, participant data was linked to a study code number. Confidentiality has been ensured, and unauthorized people have not had access to the data.

5.12. Dissemination of the result

The findings of this study will be documented in a formal report and presented to the Department of Medical Laboratory Sciences, Addis Ababa University. The outcomes of the research will be shared with the international community at conferences and publication in a peer reviewed journal

6. Results

6.1 Socio-demographic characteristics of study participants

A total of 292 participants were enrolled in this study which included a gasoline exposed group of 146 individuals and a control group consisting of 146 individuals. The exposed group mean age ± SD is 35.73±9.09 and the control group mean age ± SD=35.59±9.02. The proportion of male and females is identical in both groups (87% Male and 13% Female). Educational status, however, showed a significant difference between the groups; 71.2% of cases have secondary education, while majority of the controls (69.2%) have a bachelor's degree or higher. (Table 1)

Table 1: Socio-demographic characteristics of Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

Variables	**Classifications**	**Cases (n=146)**	**Controls (n=146)**	P-Value
		Frequency (%)	**Frequency (%)**	
Age group	18 - 24	16 (11.0)	15 (10.3)	0.972
	25 - 34	50 (34.2)	52 (35.6)	
	35 - 44	57 (39.0)	54 (37.0)	
	>45	23 (15.8)	25 (17.1)	
	Mean ± SD	35.73 ± 9.09	35.59 ± 9.02	
Sex	Male	127 (87.0)	127 (87.0)	1.000
	Female	19 (13.0)	19 (13.0)	
Marital status	Single	59 (40.4)	72 (49.3)	0.126
	Married	87(59.6)	74(50.7)	
Educational status	Bachelor's degree or higher	21 (14.4)	101 (69.2)	**< 0.001**
	Primary	21(14.4)	6 (4.1)	
	Secondary	104 (71.2)	39 (26.7)	

6.2. Occupational characteristics of gas station workers

6.2.1. Work pattern and exposure intensity

As shown in table 2 below many gas station workers reported high levels of occupational exposure. About 43% of workers reported to pump between 2500 – 6000 liters of gas per day, while 30.8% of workers reported pumping over 6000 liters of gas. Regarding their years of employment at various gas stations, 34.9% had worked for less than 3 years, 32.2% for 4 – 8 years and 32.2 % for more than 9 years. Most workers (61.6%) reported working more than 8 hours daily, and half (50.0%) worked for 5 - 6 days a week. Additionally, 17.8% of workers lived less than 1 kilometers from the gas station they're employed at and 46.6% lived within 1 -5 Kilometer of their workplace.

Table 2: Work Pattern and Exposure Intensity among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

Variables	**Classifications**	**Cases (n=146)**
		Frequency (%)
Duration of employment years	< 3 Years	51 (34.9)
	4 – 8 Years	48 (32.9)
	> 9 Years	47 (32.2)
Gas pumped per Day (in liters)	< 2500	38 (26.0)
	2501 – 5999	63 (43.2)
	> 6000	45 (30.8)
Working hours Per day	≤ 8 Hours	56 (38.4)
	>8 Hours	90 (61.6)
Working days Per week	< 5 Days	58 (39.7)
	5 – 6 Days	73 (50.0)
	7 Days	15 (10.3)
Residence distance From station	< 1 Km	26 (17.8)
	1–5 Km	68 (46.6)
	> 5 Km	52 (35.6)

6.2.2. Utilization of Personal Protective Equipment

The use of Personal Protective Equipment among the workers varied considerably. While a high proportion of them wore safety shoes 129/146 (88.4 %) and protective clothing 130/146 (89%), only 17.1% (25/146) used masks and 9.6% (14/146) wore gloves during fuel pumping as shown in figure 2 below.

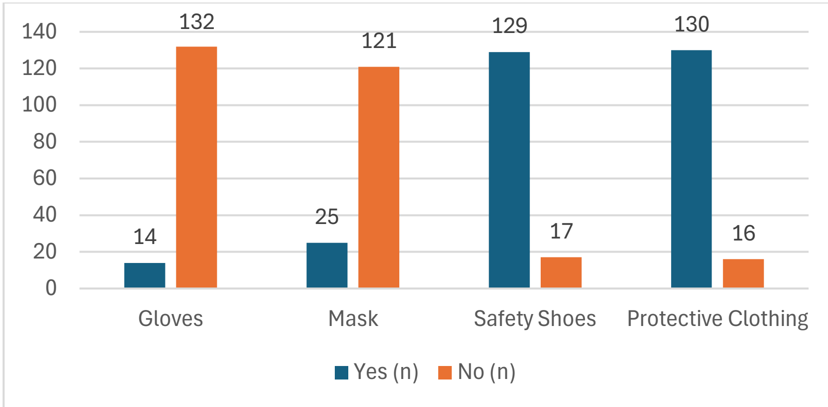


Figure 2 - Use of Personal Protective Equipment among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

The most frequently cited reason for not using personal protective equipment was that it was inconvenient to wear 69/146 (47.3%), 30.1% (44/146) reported that they were not aware of the need to use PPE and 12.3% (18/146) stated that PPE was not provided by their employers. And the rest of the 15/146 (10.3%) claimed that they used PPE properly and consistently as indicated in Fig 3

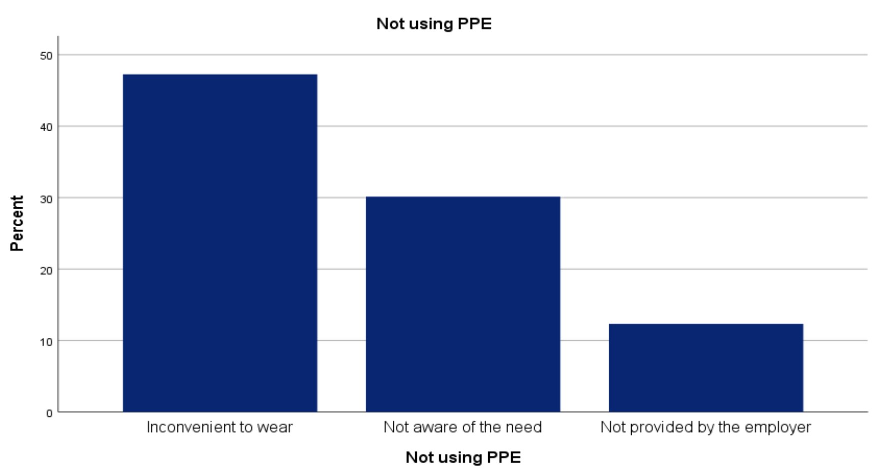


Figure 3 : Justification for not using personal protective equipment among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

6.2.3. Awareness and workplace behavior

Figure 4 below shows many of the workers 91/146 (62.3%) were aware of the health risks associated with gasoline exposure and 95/146 (65.1%) reported receiving occupational safety and health training. However, 128/146 (87.7%) consumed food and drinks at their workplace, and 57/146 (39%) were observed eating and drinking during data collection period.

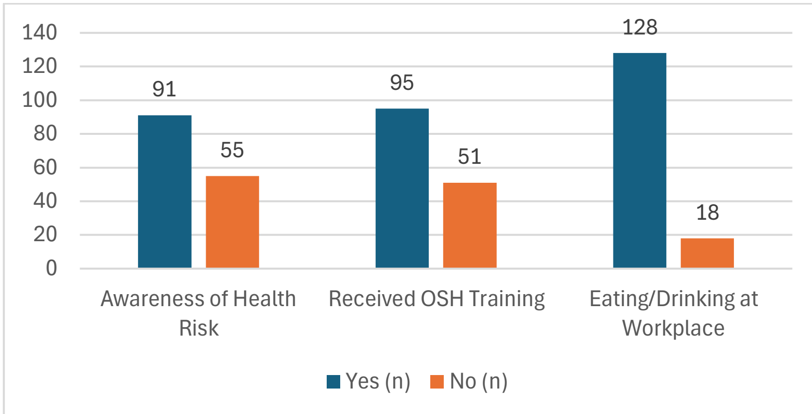


Figure 4 - Awareness and Workplace Practices among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

6.3. Comparison of mean and median liver and renal function tests among cases and control groups

The mean AST, uric acid and creatinine level was significantly higher among gasoline exposed workers compared to controls. The median ALT, GGT, ALP and Total bilirubin level was significantly higher among gasoline exposed workers as opposed to the controls. However, there were no statistically significant differences observed in the urea and direct bilirubin levels as indicated in table 3.

Table 3 - Comparison of Liver and Renal Function Parameters Between Gasoline-Exposed Workers and Controls Addis Ababa, Ethiopia from Feb-May 2025

Parameter	**Cases (n=146)** Mean ± SD	**Controls (n=146)** Mean ± SD	p-value
AST (U/L)	19.77 ± 4.87	18.44 ± 4.41	**0.015**
UA (mg/dL)	5.91 ± 1.53	5.16 ± 1.28	**<0.001**
Creatinine (mg/dL)	0.90 ± 0.16	0.81 ± 0.12	**<0.001**
	Median (IQR)	Median (IQR)	
Urea (mg/dL)	23.8 (18.2–26.4)	22.6 17.8–26.6)	0.398
ALT (U/L)	12.4 (8.9–16.5)	7.8 (5.3–10.9)	**<0.001**
ALP (U/L)	64.0 (55.0–74.0)	61.0 (48.0–73.0)	**0.045**

GGT (U/L)	31.0 (17.0–55.0)	18.0 (13.0–25.0)	**<0.001**
Total Bilirubin (mg/dL)	0.37 (0.267–0.449)	0.30 (0.222–0.427)	**0.005**
Direct Bilirubin (mg/dL)	0.118 (0.096–0.136)	0.108 (0.085–0.134)	0.075

Note: Parameters with normal distribution are presented as mean ± standard deviation and analyzed using independent t-test. Parameters with non-normal distribution are presented as median (interquartile range) and analyzed using Mann-Whitney U test.

6.4 Comparison of mean/median liver and renal function test levels across occupational exposure groups

6.4.1 Effect of duration of employment years on liver and renal function tests of gas station workers

A statistically significant difference in GGT, total bilirubin and direct bilirubin levels was observed among the different exposure duration groups. However, no statistically significant differences were found in the rest of the parameters across the exposure groups. For GGT, the < 3 years group was significantly different from both the 4–8 years (p = 0.020) and > 9 years groups (p = 0.014). For Total Bilirubin and Direct Bilirubin, the 4–8 years group was significantly different from the < 3 years group (p = 0.017) (p = 0.046) respectively. as shown in table 4.

Table 4 - Effect of duration of exposure on Liver and Renal function tests among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

Parameter	**< 3 years (n = 51)**	**4–8 years (n = 48)**	**> 9 years (n = 47)**	p-value
AST (U/L) Mean ± SD	19.12 ± 5.02	21.14 ± 4.45	19.08 ± 4.92	0.058
Uric Acid (mg/dL) Mean ± SD	5.61 ± 1.42	6.26 ± 1.63	5.88 ± 1.50	0.141
Creatinine (mg/dL) Mean ± SD	0.898 ± 0.143	0.945 ± 0.174	0.868 ± 0.152	0.089
ALT (U/L) Median (IQR)	11.9 (8.3 – 16.3)	12.9 (10.1 – 19.0)	12.2 (7.7 – 15.5)	0.189
ALP (U/L) Median (IQR)	64.0 (52.0 – 73.0)	64.0 (56.5 – 81.8)	64.0 (58.0 – 78.0)	0.537

GGT (U/L) Median (IQR)	19.0 (14.0 – 37.0)[a,b]	36.0 (22.0 – 50.3)	37.0 (20.0 – 67.0)	**0.006**
Total Bilirubin (mg/dL) Median (IQR)	0.406 (0.331 –0.501)[a]	0.325 (0.247 – 0.398)	0.350 (0.246 – 0.436)	**0.011**
Urea (mg/dL) Median (IQR)	22.7 (19.6 – 28.8)	23.6 (17.8 – 25.1)	24.2 (18.0 – 27.1)	0.947
Direct Bilirubin (mg/dL) Median (IQR)	0.121(0.105 -0.151)[a]	0.108 (0.089 – 0.132)	0.116 (0.090 – 0.138)	**0.044**

*Superscripts indicate significant pairwise differences between exposure duration groups based on post hoc Bonferroni-adjusted tests following Kruskal-Wallis tests: [a] significant difference between <3 years and 4–8 years. [b] significant difference between <3 years and >9 years.

Note: Parameters with normal distribution are presented as mean ± standard deviation and analyzed using ANOVA. Parameters with non-normal distribution are presented as median (interquartile range) and analyzed using Kruskal-Wallis tests.

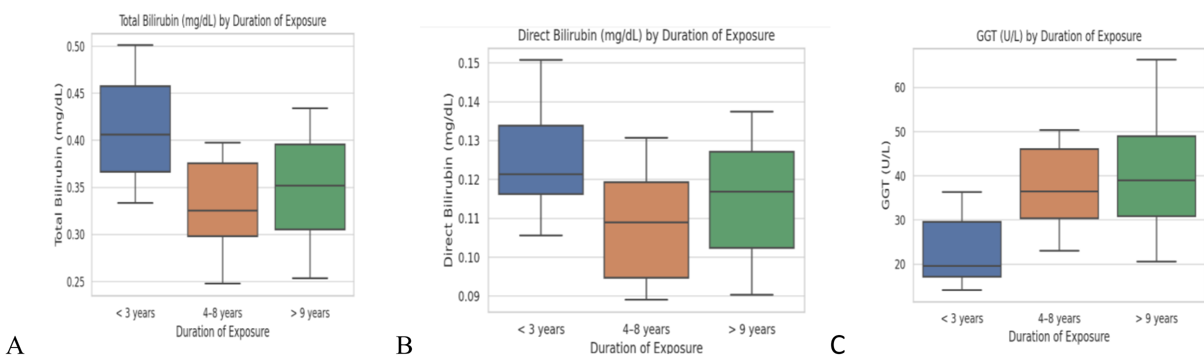


Figure 5 – Median/Mean comparison of Liver and Renal Function Parameters by effect of duration of exposure on among Gasoline Station Workers at Addis Ababa, Ethiopia from Feb-May 2025

6.4.2 Effect of workdays per week on liver and renal function tests of gas station workers

A statistically significant difference was found in direct bilirubin levels (p=0.033) and uric acid levels (p=0.046) among the different exposure duration groups but no significant pairwise differences by days of occupational exposure per week. (Table 5)

Table 5 - Comparison of Liver and Renal Function Tests by days worked per week among Gasoline Station workers at Addis Ababa, Ethiopia from Feb-May 2025

Parameter	**< 5 days (n = 58)**	**5–6 days (n = 73)**	**7 days (n = 15)**	p-value
AST (U/L) Mean ± SD	19.46 ± 5.51	19.89 ± 4.17	20.40 ± 5.61	0.770
Uric Acid (mg/dL) Mean ± SD	6.23 ± 1.63	5.80 ± 1.38	5.21 ± 1.58	**0.046**
Creatinine (mg/dL) Mean ± SD	0.936 ± 0.148	0.885 ± 0.160	0.867 ± 0.184	0.121
ALT (U/L) Median (IQR)	12.7 (8.3 – 17.5)	11.7 (8.8 – 15.7)	12.6 (11.8 – 16.2)	0.631
ALP (U/L) Median (IQR)	64.0 (54.0 – 74.0)	64.0 (56.0 – 74.0)	67.0 (48.5 – 80.0)	0.926
GGT (U/L) Median (IQR)	39.0 (17.0 – 74.0)	30.0 (19.0 – 48.0)	19.0 (15.5 – 35.0)	0.167
Total Bilirubin (mg/dL) Median (IQR)	0.384 (0.294 – 0.489)	0.370 (0.257 – 0.454)	0.314 (0.244 – 0.370)	0.074
Urea (mg/dL) Median (IQR)	23.4 (18.6 – 25.9)	23.1 (18.1 – 26.4)	23.9 (19.9 – 29.2)	0.709
Direct Bilirubin (mg/dL) Median (IQR)	0.124 (0.102 – 0.149)	0.117 (0.092 – 0.133)	0.101 (0.087 – 0.120)	**0.033**

Note: Parameters with normal distribution are presented as mean ± standard deviation and analyzed using ANOVA. Parameters with non-normal distribution are presented as median (interquartile range) and analyzed using Kruskal-Wallis tests.

6.4.3 Effect of daily working hours on liver and renal function tests of gas station workers

Statistically significant differences were observed in creatinine (0.93 ± 0.15 mg/dL vs 0.87± 0.16 mg/dL, $p = 0.037$), total bilirubin (0.33 (0.24–0.43) vs 0.37 (0.29–0.48) p=0.034) and direct bilirubin (0.10 (0.09–0.13) vs 0.12 (0.10–0.15) p=0.019) levels, with higher median values in the >8 hours group compared to the ≤8 hours group. No statistically significant differences were found in the rest. (Figure 6)

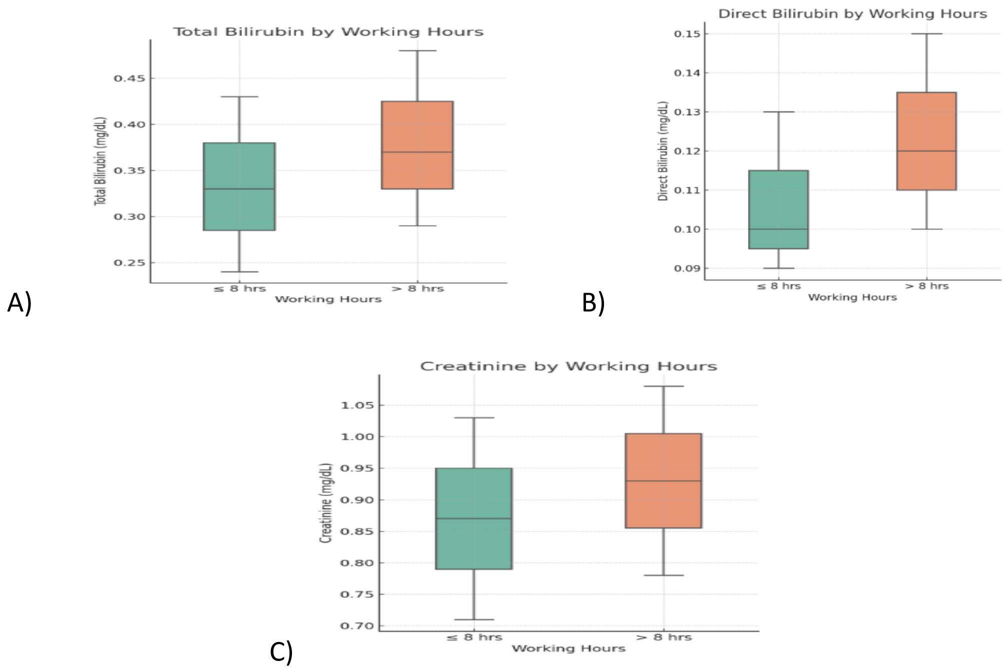


Figure 6 – Median/Mean Comparison of Liver and Renal Function Parameters between Gasoline Station workers' daily working hours at Addis Ababa, Ethiopia from Feb-May 2025

6.4.4 Effect of daily average gasoline dispensing volume on liver and renal function tests of gas station workers

Statistically significant differences were observed for uric acid, creatinine, and ALP. Post-hoc analysis revealed that uric acid levels were significantly higher in workers exposed to >6000 L/day compared to those exposed 2501–5999 L/day (p = 0.031), Creatinine was significantly elevated in the >6000 L/day group compared to the <2500 L/day group (p = 0.021), and ALP levels were also significantly higher in the >6000 L/day group compared to the <2500 L/day group (p = 0.046), with no statistically significant difference between the intermediate group and either of the two. No statistically significant differences were found in the rest of the parameters across the exposure groups. (Table 6)

Table 6 - Comparison of Liver and Renal Function Tests across daily gasoline dispensing categories among Gasoline Station workers at Addis Ababa, Ethiopia from Feb-May 2025

Parameter	**<2500 L/day (n=38)**	**2501–5999 L/day (n=63)**	**>6000 L/day (n=45)**	p-value
AST (U/L) Mean ± SD	18.466 ± 5.302	20.276 ± 4.510	20.167 ± 4.874	0.157
Uric Acid (mg/dL)	5.695 ± 1.839	5.676 ± 1.304[a]	6.424 ± 1.432	**0.024**

Mean ± SD				
Creatinine (mg/dL) Mean ± SD	0.850 ± 0.158[b]	0.909 ± 0.155	0.942 ± 0.155	**0.027**
ALT (U/L) Median (IQR)	11.9 (8.5–16.3)	12.700 (9.9–15.8)	11.900 (8.5–18.6)	0.781
ALP (U/L) Median (IQR)	60.0 (49.0–71.0)[c]	64.000 (56.500–70.500)	70.0 (60.0–82.0)	**0.040**
GGT (U/L) Median (IQR)	21.0 (14.0–51.0)	31.000 (18.5–56.5)	37.000 (19.0–52.0)	0.061
Total Bilirubin (mg/dL) Median (IQR)	0.355 (0.267–0.409)	0.373 (0.231–0.488)	0.370 (0.283–0.421)	0.870
Urea (mg/dL) Median (IQR)	22.8 (21.1–25.6)	23.9 (18.20–29.35)	22.9 (17.6–25.2)	0.482
Direct Bilirubin (mg/dL) Median (IQR)	0.118 (0.095–0.131)	0.118 (0.090–0.149)	0.118 (0.100–0.132)	0.914

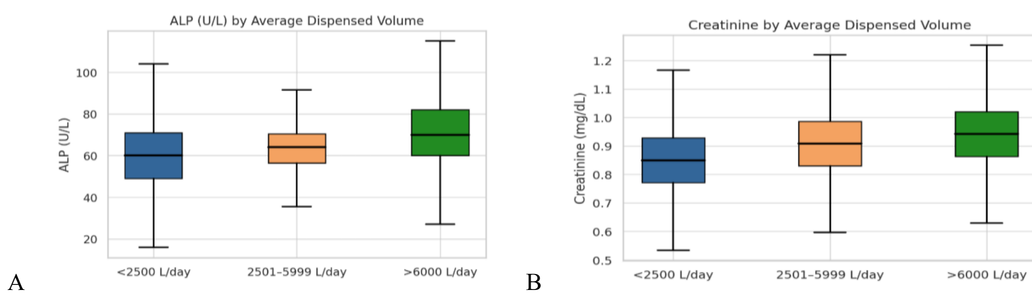
Superscripts denote significant pair wise differences at

[a]: Significant difference between 2501–5999 L/day and >6000 L/day (Uric Acid, p=0.031, Tukey HSD).

[b]: Significant difference between <2500 L/day and >6000 L/day (Creatinine, p=0.021, Tukey HSD). And

[c]: Significant difference between <2500 L/day and >6000 L/day (ALP, p=0.046, Bonferroni-adjusted Kruskal-Wallis pairwise).

Note: Parameters with normal distribution are presented as mean ± standard deviation and analyzed using independent t-test. Parameters with non-normal distribution are presented as median (interquartile range) and analyzed using Mann-Whitney U test.



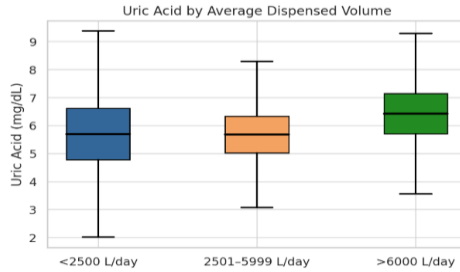


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Figure 7 - Median/Mean comparison of Liver and Renal Function Parameters by daily gasoline dispensing categories among Gasoline Station workers at Addis Ababa, Ethiopia from Feb-May 2025

6.5 Liver and Renal function levels and associated factors with gasoline exposure

Sex was a significant predictor across multiple variables - ALT, AST, Total Bilirubin, Direct Bilirubin, Uric Acid, Creatinine, and GGT. Duration of employment also indicates that longer employment increased urea levels. Higher working hours per day were also associated with higher values of creatinine levels. Days worked per week significantly affected AST levels. Safety-trained workers for occupational health and safety showed lower urea levels. Eating and drinking at the gas station significantly increased creatinine levels. (Table 7)

Table 7 - Regression Analysis of Demographic and Occupational Predictors on Liver and Renal Function Test Outcomes among Gasoline Station workers at Addis Ababa, Ethiopia from Feb-May 2025

Predictor	ALT β (95% CI); p-value	AST β (95% CI); p-value	ALP β (95% CI); p-value	TBIL β (95% CI); p-value	DBIL β (95% CI); p-value	Urea β (95% CI); p-value	UA β (95% CI); p-value	Creatinine β (95% CI); p-value	GGT β (95% CI); p-value
Age	0.056 (–0.094, 0.206); 0.462	0.076 (–0.043, 0.196); 0.210	0.092 (–0.367, 0.550); 0.693	–0.003 (–0.007, 0.001); 0.146	–0.001 (–0.001, 0.000); 0.245	–0.156 (–0.305, –0.006); **0.041**	0.034 (–0.004, 0.071); **0.050**	0.002 (–0.001, 0.005); 0.262	0.533 (–0.206, 1.272); 0.156
Sex (1 = male, ref: female)	5.40 (2.50, 8.30);**<0.001**	4.294 (1.976, 6.613);**<0.001**	7.235 (–1.626, 16.095); 0.109	0.109 (0.034, 0.185); **0.005**	0.027 (0.010, 0.044); **0.002**	1.986 (–0.903, 4.874); 0.176	1.410 (0.683, 2.137); **<0.001**	0.254 (0.189, 0.318);**<0.001**	24.612 (10.323, 38.900);**<0.001**

Liters/day	-5.142×10^{-7} (–, –); 0.995	0.000 (0.000, 0.000); 0.464	0.000 (0.000, 0.001); 0.428	0.000 (0.000, 0.000); 0.967	0.000 (0.000, 0.000); 0.739	0.000 (0.000, 0.000); 0.434	0.000 (0.000, 0.000); 0.691	0.000 (0.000, 0.000); 0.704	0.000 (–0.001, 0.000); 0.275
Duration of employment	–0.058 (–0.238, 0.123); 0.527	–0.092 (–0.236, 0.053); 0.210	0.019 (–0.533, 0.571); 0.945	0.000 (–0.005, 0.004); 0.855	0.000 (–0.001, 0.001); 0.612	0.215 (0.035, 0.395); **0.019**	–0.034 (–0.080, 0.011); 0.137	–0.004 (–0.008, 0.000); 0.052	–0.028 (–0.918, 0.862); 0.951
Working hours/day	0.088 (–0.101, 0.277); 0.357	0.106 (–0.046, 0.257); 0.169	0.206 (–0.372, 0.783); 0.482	–0.001 (–0.006, 0.004); 0.711	0.000 (–0.001, 0.001); 0.797	–0.043 (–0.231, 0.146); 0.655	0.031 (–0.016, 0.079); 0.191	0.005 (0.001, 0.009); **0.018**	0.673 (–0.258, 1.604); 0.155
Days/week	0.241 (–0.651, 1.132); 0.594	0.719 (0.006, 1.433); **0.048**	0.922 (–1.804, 3.648); 0.505	–0.012 (–0.035, 0.011); 0.317	–0.005 (–0.010, 0.000); 0.061	0.004 (–0.885, 0.892); 0.994	–0.005 (–0.229, 0.218); 0.963	–0.001 (–0.021, 0.018); 0.892	–0.308 (–4.704, 4.087); 0.890
Glove use (1 = yes, ref: no)	–0.796 (–4.700, 3.109); 0.687	–0.819 (–3.942, 2.303); 0.605	–3.971 (–15.904, 7.962); 0.511	0.016 (–0.086, 0.118); 0.762	0.006 (–0.017, 0.028); 0.628	4.356 (0.356, 8.356); 0.056	0.314 (–0.665, 1.293); 0.527	–0.036 (–0.122, 0.051); 0.416	1.720 (–17.523, 20.963); 0.860
Mask use (1 = yes, ref: no)	2.232 (–0.806, 5.270); 0.148	1.688 (–0.742, 4.118); 0.172	1.739 (–7.546, 11.025); 0.712	–0.071 (–0.151, 0.008); 0.078	–0.003 (–0.020, 0.015); 0.748	–2.878 (–5.905, 0.148); 0.062	–0.431 (–1.193, 0.331); 0.265	0.036 (–0.032, 0.103); 0.296	12.923 (–2.050, 27.896); 0.090
Safety shoes (1 = yes, ref: no)	3.686 (–3.162, 10.534); 0.289	3.514 (–1.963, 8.991); 0.207	–9.000 (–29.931, 11.931); 0.397	–0.093 (–0.272, 0.086); 0.308	–0.016 (–0.056, 0.024); 0.423	5.545 (–1.278, 12.368); 0.110	0.140 (–1.577, 1.858); 0.872	–0.021 (–0.173, 0.130); 0.784	25.074 (–8.678, 58.825); 0.144
Protective clothing (1 = yes, ref: no)	–2.337 (–9.278, 4.604); 0.507	–3.632 (–9.183, 1.919); 0.198	10.970 (–10.246, 32.185); 0.308	0.085 (–0.096, 0.266); 0.355	0.015 (–0.025, 0.055); 0.462	–3.478 (–10.394, 3.437); 0.322	–0.744 (–2.485, 0.997); 0.399	0.027 (–0.127, 0.181); 0.728	–32.395 (–66.606, 1.816); 0.063

Awareness of risks (1 = yes, ref: no)	–1.167 (–3.146, 0.813); 0.246	1.183 (–0.400, 2.766); 0.142	5.237 (–0.813, 11.288); 0.089	0.013 (–0.039, 0.065); 0.614	0.008 (–0.003, 0.020); 0.149	–0.614 (–2.587, 1.358); 0.539	0.231 (–0.266, 0.727); 0.360	0.037 (–0.007, 0.080); 0.101	0.186 (–9.571, 9.942); 0.970
Safety Training (1 = yes, ref: no)	0.381 (–1.709, 2.470); 0.719	0.273 (–1.398, 1.944); 0.747	–3.549 (–9.935, 2.838); 0.274	–0.002 (–0.056, 0.053); 0.946	–0.001 (–0.013, 0.011); 0.829	–3.812 (–5.894, –1.731); **<0.001**	0.019 (–0.505, 0.543); 0.944	–0.024 (–0.070, 0.022); 0.305	2.180 (–8.119, 12.479); 0.676
Eating/ drinking at station (1 = yes, ref: no)	0.847 (–1.227, 2.922); 0.420	–0.435 (–2.094, 1.225); 0.605	–3.056 (–9.397, 3.285); 0.342	0.015 (–0.040, 0.069); 0.596	0.008 (–0.004, 0.020); 0.201	1.114 (–0.953, 3.181); 0.288	0.267 (–0.254, 0.787); 0.312	0.067 (0.021, 0.113); **0.005**	3.219 (–7.006, 13.444); 0.534

7. Discussion

The primary aim of this study was to assess the effect of gasoline exposure on liver and renal function tests among gas station workers as compared to unexposed controls in Addis Ababa, Ethiopia. The study demonstrated that all liver and renal function tests measured were higher in gasoline exposed workers compared to controls - but the urea and total bilirubin levels showed no statistical significance (p>0.005). Multiple linear regression analysis also showed sex, duration of employment, working hours, food and drink consumption at place work and occupational safety training were significant predictors of certain liver and kidney tests.

The levels of ALT and AST were significantly higher in gasoline-exposed workers than controls, with median ALT at 12.4 (8.9–16.5) U/L vs. 7.8 (5.3–10.9) U/L (p < 0.001) and mean AST at 19.77 ± 4.87 U/L vs. 18.44 ± 4.41 U/L (p = 0.015). These findings are in line with studies from Egypt(27), Nigeria(30), Iran(26), Ethiopia(44), Palestine(41) and Brazil(24). The increase in ALT and AST levels of the exposed groups as compared to control could be attributed to hydrocarbons in gasoline, metabolized in the liver by CYP450 oxidative pathways, producing free radicals and toxic metabolites that cause lipid peroxidation and hepatic cell membrane damage, releasing enzymes into circulation(2).

No significant differences were found for ALT or AST across employment duration (p = 0.189 and 0.058), working hours/day (p = 0.639 and 0.528), or gasoline volume (p = 0.781 and 0.157), but AST increased with days/week (p = 0.048). The lack of association with most exposure metrics may indicate that mild hepatocellular injury, as reflected by ALT and AST, does not accumulate linearly with exposure duration or intensity in this population

Median ALP was slightly higher in exposed workers (64.0 [55.0–74.0] U/L) compared to controls (61.0 [48.0–73.0] U/L; p = 0.045). This finding is in line with studies from USA(23), Sudan(28), Iran(55) and Yemen(43). The discrepancy with studies in Ethiopia(44) and Nigeria(38) may reflect differences in exposure levels.

ALP was elevated in workers dispensing >6000 L/day (70.0 [60.0–82.0] U/L vs. 60.0 [49.0–71.0] U/L; p = 0.040), but not across employment duration (p = 0.537), days/week (p = 0.926), or hours/day (p = 0.094). This suggests that handling large volumes of gasoline each day may have a more immediate impact on ALP, possibly due to higher short-term exposure to harmful fumes. The lack of association with long-term exposure measures could mean that ALP is more affected by daily exposure levels than by how long or how often someone has been working.

Median total bilirubin was significantly higher in exposed workers (0.37 [0.267–0.449] mg/dL) compared to controls (0.30 [0.222–0.427] mg/dL; $p = 0.005$), but Direct Bilirubin showed no significant difference (0.118 [0.096–0.136] mg/dL vs. 0.108 [0.085–0.134] mg/dL; $p = 0.075$). The direct bilirubin increase aligns with a study Egypt (27) and Iran (56) (even if not statistically significant). Total bilirubin was not significantly altered in the Ethiopian study(44). Although median total bilirubin was significantly higher in exposed workers, bilirubin levels are generally less sensitive indicators of early liver injury. Therefore, inconsistencies across studies may be due to the limited sensitivity of bilirubin as a biomarker and the extent of exposure-related liver impairment.

TBIL and DBIL were elevated in workers with 4–8 years vs. <3 years employment ($p = 0.018$ and 0.044) and >8 hours/day ($p = 0.034$ and 0.019), with DBIL decreasing with fewer days/week ($p = 0.033$). These findings suggest that both the duration and intensity of exposure to gasoline may affect liver function over time aligning with reports from Nigeria (30) and Iraq(48). Longer working years and extended daily shifts may lead to gradual buildup of toxic effects, while fewer working days could allow for partial recovery or reduced exposure.

The significant increase in mean creatinine levels among gasoline-exposed workers (0.90 ± 0.16 mg/dL) compared to controls (0.81 ± 0.12 mg/dL; $p < 0.001$) was in agreement with several findings from studies conducted in Ethiopia(44), Sudan(50), Saudi Arabia(39), Iran(55), Iraq(57), Palestine(41), and Yemen(49). This pattern supports evidence that prolonged exposure to petroleum hydrocarbons may impair renal function, potentially through mechanisms involving oxidative stress, tubular toxicity, and glomerular damage(2,7).

Creatinine levels were also higher in workers with >8 hours/day exposure (0.93 ± 0.15 mg/dL vs. 0.87 ± 0.16 mg/dL; $p = 0.037$) and those dispensing >6000 L/day (0.942 ± 0.155 mg/dL vs. 0.850 ± 0.158 mg/dL; $p = 0.027$). These findings suggest that both the duration and intensity of daily exposure may contribute to early renal changes. Multiple regression showed associations with sex ($\beta = 0.254$; $p < 0.001$), working hours/day ($\beta = 0.005$; $p = 0.018$), eating/drinking at the station ($\beta = 0.067$; $p = 0.005$). The association with eating/drinking at the workplace may indicate ingestion of contaminants, while prolonged exposure time could lead to cumulative nephrotoxic effects from inhaled or dermally absorbed hydrocarbons.

In contrast, median urea levels didn't have statistical significant difference between exposed (23.8 [18.2–26.4] mg/dL) and control groups (22.6 [17.8–26.6] mg/dL; p = 0.398), differing from Egbuonu's Nigerian study (31) and Kurdistan (47), who reported elevated urea in exposed workers. The lack of urea differences may reflect a lower exposure levels dietary protein intake, or timing of sample collection in this study group. Regression analysis indicated urea increased with employment duration and decreased with safety training (β = -3.812; p < 0.001). These findings suggest that chronic exposure to gasoline over time may lead to renal stress, affecting tubular function. However, the protective association with safety training implies that education and awareness about proper handling practices may help reduce exposure and mitigate kidney-related effects. A higher proportion of gas station workers (62.3%) were aware of the health risks associated with gasoline exposure, and 65.1% received safety training, consistent with (41) in the Palestine study.

Uric Acid levels were significantly higher in gasoline-exposed workers (5.91 ± 1.53 mg/dL) compared to controls (5.16 ± 1.28 mg/dL; p < 0.001), consistent with studies in Nigeria(32) and Ethiopia (44). Although uric acid is not a primary renal biomarker, its elevation in exposed individuals may serve as a complementary indicator of altered renal dysfunction. It may reflect early renal dysfunction or increased oxidative stress, both of which have been associated with hydrocarbon exposure (7).

UA was also elevated in workers dispensing >6000 L/day (6.424 ± 1.432 mg/dL vs. 5.676 ± 1.304 mg/dL; p = 0.024) and varied across days/week (p = 0.046), with regression linking higher UA to sex (β = 1.410; p < 0.001). These findings align with Abdel Aziz (41), who reported increased uric acid with gasoline exposure. The elevation may result from degradation of purines or reduced excretion due to renal tubular stress from benzene metabolites ((44)). Higher gasoline volumes likely amplify exposure, increasing UA production, while the sex effect may reflect exposure differences in our predominantly male case group (87%).

8. Strength and Limitation

8.1. Strength

- Inclusion of both liver and renal function tests provided a broader understanding of systemic effects related to gasoline exposure.
- Participants were recruited from multiple gas stations across Addis Ababa which increased the representativeness of the results.
- Large sample size which had age and sex matched controls, improved comparability.
- The study contributes scarce local data on occupational gasoline exposure effects in an Ethiopian context, filling a critical knowledge gap.

8.2. Limitation

- Blood levels of specific gasoline compounds such as benzene, toluene, and xylene were not measured, preventing precise quantification of internal exposure.
- Environmental levels of VOCs at the gas stations were not assessed, which limits exposure context.
- Low female representation among participants restricted analysis of sex specific effects.
- The cross-sectional design does not establish causal relationships or allow for follow-up of changes over time.

9. Conclusion and Recommendation

9.1. Conclusion

This study assessed the effect of occupational gasoline exposure on liver and renal function tests among gas station workers compared to a non-exposed control group in Addis Ababa, Ethiopia. The results revealed that several of the liver and renal function tests were elevated among the gas station workers. Several factors, including sex, duration of employment, daily working hours, safety training, and the practice of consuming food or drinks at the gas station, were significantly associated with changes in liver and renal biomarkers. Overall, this research highlights the need for improved workplace safety practices, regular health checkups, and targeted interventions to reduce gasoline-related health risks.

9.2. Recommendation

- Implementation of occupational health and safety training for gasoline station workers, with emphasis on the health hazards of gasoline exposure and proper use of PPE.
- Ensure consistent and adequate provision of personal protective equipment (PPE) by employers and enforce the usage.
- Discourage eating or drinking at the workplace
- Establish regular medical check-ups for gasoline-exposed workers
- Conduct future studies with longitudinal study design, integrating direct measurement of blood BTEX levels and environmental VOC monitoring for more accurate exposure assessment.

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Annex

Annex I: Participants' Information sheet/ English version

Principal Investigator: Halima Abdulkadir

Institution: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Science

Title of the Project: Assessment of Liver and Renal Function Tests Among Gasoline-Exposed Gas Station Workers in Addis Ababa, Ethiopia, 2024

Dear study participants, I am an MSc student at Addis Ababa University, School of Medical Laboratory Sciences. You are invited to participate in a study assessing the liver and renal function tests among gasoline-exposed gas station workers in Addis Ababa, Ethiopia, 2024.

Purpose of the Study: The purpose of this study is to assess liver and renal function among gasoline-exposed gas station workers and compare these values with those of individuals who are not occupationally exposed to gasoline in Addis Ababa, Ethiopia.

Duration: The duration of this study will be approximately 2–3 months.

Procedures to be Followed: Dear study participant, if you agree to participate in this study, a sample of 5 ml of your blood will be collected to assess your liver and renal function. The sample collection will be done by trained health professionals using standard precautions.

Risks and Discomfort: The only risk associated with participating in this study is minor discomfort or pain during the blood sample collection. All samples will be collected by trained professionals following strict safety protocols to minimize any risks or discomfort.

Expected Benefit: There will be no financial compensation for participating. However, if your test results show any significant abnormalities, you will be informed for further diagnosis and treatment. We will also notify you if any follow-up medical action is recommended based on your results.

Confidentiality: All information collected during this study will be kept confidential. Your identity will be protected by replacing your name with a confidentiality code. Only authorized personnel will have access to the study data for analysis purposes.

Withdrawal from the Study: Your participation in this study is voluntary. You may withdraw from the study at any time, without providing a reason, and without any negative consequences. You are free to refuse to answer any question or stop participating if you feel uncomfortable. You may also request your laboratory results at any time during the study.

If you have any questions about this study, please contact us at the following address:

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DMLS Ethical Review Committee:

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Annex I:ለተሳታፊዎችመረጃመስጭያሰነድ / የአማርኛ ቅጂ

ዋናመርማሪ *ሀሊማ አብዱልቃድር*

ተቋም፡- አዲስ አበባ ዩኒቨርሲቲ ፣የጤና ሳይንስ ኮሌጅ ፣ የሕክምና ላቦራቶሪ ትምህርት ክፍል

የጥናቱ ርእስ፡- ቤንዚን የተጋለጡ የነዳጅ ማደያ ሠራተኞች ላይ. የጉበት እና የኩላሊት ምርመራ ጥናት በአዲስ አበባ፣ ኢትዮጵያ 2024

መግቢያ፡ውድ የጥናት ተሳታፊዎች በቤንዚን ለተጋለጡ የነዳጅ ማደያ ሰራተኞች የጉበት እና የኩላሊት ምርመራ ጥናት በሚገመግም ጥናት ላይ እንድትሳተፉ ተጋብዘዋል ።ይህ ጥናት በአዲስ አበባ ዩኒቨርሲቲ ላቦራቶሪ ትምህርት ክፍል የጥናት ና የሰነምግባር ኮሚቴ የጸደቀዉ ጥናት መሆኑን መግለፅ እንወዳለን።በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት ላይ የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብት ኣሎት።

የጥናቱ ዓላማ፡-የዚህ ጥናት ዓላማ በቤንዚን የተጋለጡ የነዳጅ ማደያ ሠራተኞችን የጉበት እና የኩላሊት ምርመራ ለመገምገም እና እነዚህን እሴቶች በአዲስ አበባ ኢትዮጵያ ውስጥ በሥራ ላይ ለቤንዚን ካልተጋለጡ ግለሰቦች ጋር በማነፃፀር ነው።

የጥናት የቆይታ ጊዜ: ከ2-3 ወራት ይሆናል።

የጥናቱ ሂደቶች፡-ውድ የጥናት ተሳታፊ ፣በዚህ ጥናት ለመሳተፍ ከተስማሙ፣ የጉበት እና የኩላሊት ስራዎን ለመገምገም 5 ሚ.ሊ ደም ናሙና ይሰበሰባል። የናሙና ማሰባሰቡ መደበኛ ጥንቃቄዎችን በመጠቀም በሰለጠኑ የጤና ባለሙያዎች ይከናወናል።

ስጋቶች እና ምቾት: በዚህ ጥናት ውስጥ ከመሳተፍ ጋር ተያይዞ ያለው ብቸኛው አደጋ በደም ናሙና በሚሰበሰብበት ጊዜ ትንሽ ህመም ነው. ማንኛውንም አደጋዎች ወይም ምቾት ለመቀነስ ጥብቅ የደህንነት ፕሮቶኮሎችን በመከተል ሁሉም ናሙናዎች በሰለጠኑ ባለሙያዎች ይሰበሰባሉ።

ከጥናቱ የሚያገኙት ጥቅም ለመሳተፍ የገንዘብ ማካካሻ አይኖርም።ነገር ግን፣የምርመራ ውጤት ጉልህ የሆኑ ያልተለመዱ ነገሮችን ካሳየ ለበለጠምርመራ እና ህክምና ይነገረዎታል።እንዲሁም በውጤቶችዎ ላይ በመመርኮዝ ማንኛውም የክትትል የሕክምና እርምጃ የሚመከር ከሆነ እናሳውቅዎታለን።

ሚስጥራዊነት፡በዚህ ጥናት ወቅት የሚሰበሰቡት መረጃዎች በሙሉ በሚስጥር ይቀመጣሉ።ማንነትዎ ስምዎን በሚስጥር ኮድ በመተካት ይጠበቃል። የተፈቀደላቸው ሰዎች ብቻ የጥናት መረጃን ለትንተና ዓላማዎች ማግኘት ይችላሉ።

ስለዚህ ጥናት ማንኛውም አይነት ጥያቄ ካሎት፣ እባክዎን በሚከተለው አድራሻ ያግኙን።

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Annex II: Consent Form (study participants)

English Version

Principal Investigator: Halima Abdulkadir

Research Title: Assessment of Liver and Renal Function Tests Among Gasoline-Exposed Gas Station Workers in Addis Ababa, Ethiopia, 2024

I hereby agree to participate in the study of **Assessment of Liver and Renal Function Tests Among Gasoline-Exposed Gas Station Workers in Addis Ababa, Ethiopia, 2024**. I have read and fully understand the participant information sheet and have had the opportunity to ask questions related to this study. To participate in this study, I agree to receive punctures for the purpose of drawing blood for laboratory testing. I understand that these punctures will cause a small amount of temporary discomfort at the puncture site.

I understand that the sample will not be used for any other purpose. All information regarding my sample will remain completely confidential and will not be used for any other purpose than the objective of this study. I understand that I am not obligated to participate in this study, and I can decide not to participate at any time. I understand that this study does not place me at any greater medical risk than is customary with the tests that I am receiving, nor does it interfere with the medical care that I am entitled to. I have read the above document, and I understand that I have agreed to participate in this study.

Name of Participant: ___________

Age: ___

Address: ___________

Signature: _____

Date: _______

Interviewer's Name: ___________________________________

Signature: ___________________

Annex II: የፍቃደኝነት ማረጋገጫ ቅጽ/ የአማርኛ ቅጂ

ዋና መርማሪ *ሀሊማ አብዱልቃድር*

***ተቋም*፡-** አዲስ አበባ ዩኒቨርሲቲ ፣የጤና ሳይንስ ኮሌጅ ፣የሕክምና ላቦራቶሪ ትምህርት ክፍል

የጥናቱ ርእስ:-ቤንዚን የተጋለጡ የነዳጅ ማደያ ሠራተኞች ላይ የጉበት እና የኩላሊት ምርመራ ጥናት በአዲስ አበባ፣ ኢትዮጵያ 2024

እኔ ይህን ሰነድ አንብቢያለሁ ወይም ይህ ሰነድ በሚገባኝ ቋንቋ ተነቦልኝ በውስጡ የተገለፁት ከጥናቱ የሚገኘው ጥቅም፣ የጥናቱ ሂደት እና ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት በሚገባ ተረድቼዋለሁ።በማንኛውም ጊዜ እራሴን ከጥናቱ የማግለል መብቴ የተጠበቀ መሆኑን አውቃለሁ። ስለ ጥናቱ ተጨማሪ ጥያቄ የመጠየቅ እድል ነበረኝ የተሰጠኝም መልስ አጥጋቢ ነበር። እዚ ጥናት ላይ በመሳተፌ የሚሰጠኝ ምንም ጥቅምም ሆነ ክፍያ እንደሌለ አውቃለሁ።በራሴ ፈቃደኝነት እዚህ ጥናት ለይ ለመሳተፍ መስማማቴን በፊርማየ ኣጋግጣለሁ።

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Annex III – structured questionnaire

Questionnaire for the Assessment of Liver and Renal Function Tests Among Gasoline-Exposed Gas Station Workers (Case)

Section 1: Demographic Information

1. Age: ________
2. Gender:
   - ☐ Male
   - ☐ Female
3. Educational Level:
   - ☐ No formal education
   - ☐ Primary
   - ☐ Secondary
   - ☐ College/University
4. Marital Status:
   - ☐ Single
   - ☐ Married
   - ☐ Divorced
   - ☐ Widowed

Section 2: Occupational Exposure

5. Job Position (Tasks Performed):
   - ☐ Fuel filling

- ☐ Vehicle maintenance
- ☐ Cashiering
- ☐ Supervising
- ☐ Other (please specify): _____________

6. How many years have you worked at a gas station? ________ years (Duration of Exposure)
7. How many hours do you work per day? ________ hours
8. How many days per week do you work? ________ days
9. Do you handle gasoline directly during your work?
    - ☐ Yes
    - ☐ No
10. On average, how many liters of gasoline do you handle per day? ________ liters
11. How often are you exposed to gasoline fumes?

- ☐ Always
- ☐ Most of the time
- ☐ Sometimes
- ☐ Rarely
- ☐ Never

12. Proximity to gasoline pumps or storage (distance from your work area): _______ meters

Section 3: Use of Personal Protective Equipment (PPE)

13. Do you use any PPE during your work? (Select all that apply)

- ☐ Mask
- ☐ Gloves
- ☐ Protective clothing
- ☐ Eye protection
- ☐ None

14. How often do you wear PPE at work?

- ☐ Always
- ☐ Most of the time
- ☐ Sometimes
- ☐ Rarely
- ☐ Never

15. If you do not use PPE regularly, please indicate the reason(s):

- ☐ Not provided by employer
- ☐ Inconvenient to wear
- ☐ Not aware of the need
- ☐ Other (please specify): _____________

Section 4: Health and Medical History

16. Have you been diagnosed with any of the following conditions? (Select all that apply)

- ☐ Liver disease

- ☐Kidney disease
- ☐ Respiratory issues
- ☐ Hypertension
- ☐ Diabetes
- ☐ None

17. Do you experience any of the following symptoms regularly? (Select all that apply)

- ☐ Fatigue
- ☐ Dark urine
- ☐ Abdominal pain
- ☐ Jaundice (yellowing of the skin or eyes)
- ☐ Nausea/Vomiting
- ☐ Shortness of breath
- ☐ Headaches
- ☐ Skin irritation
- ☐ Swelling in hands/feet
- ☐ None

Section 5: Lifestyle Factors

18. Do you smoke?

- ☐ Yes

- ☐ No

19. Do you consume alcohol?

- ☐ Yes
- ☐ No

20. How often do you exercise?

- ☐ Daily
- ☐ 1-3 times a week
- ☐ Occasionally
- ☐ Never

21. What type of water do you usually drink?

- ☐ Bottled water
- ☐ Tap water
- ☐ Filtered water
- ☐ Other (please specify): ____________

22. How many hours of sleep do you get per night? ______ hours

Section 6: Awareness and Workplace Environment

23. Are you aware of the potential health risks associated with gasoline exposure?

- ☐ Yes
- ☐ No

24. Have you received any occupational safety and health training related to gasoline exposure?

- ☐ Yes
- ☐ No

25. Do you think your workplace provides adequate ventilation to reduce gasoline fume exposure?

- ☐ Yes
- ☐ No
- ☐ Not sure

Questionnaire for the Assessment of Liver and Renal Function Tests Among Non-Exposed Workers (Control Group)

Section 1: Demographic Information

1. Age: ________
2. Gender:
    - ☐ Male
    - ☐ Female
    - ☐ Other
3. Educational Level:
    - ☐ No formal education
    - ☐ Primary
    - ☐ Secondary
    - ☐ College/University
4. Marital Status:
    - ☐ Single
    - ☐ Married
    - ☐ Divorced
    - ☐ Widowed

Section 2: Occupation

5. What is your current job or profession? ______________________
6. How many years have you worked in this job? ________ years
7. How many hours do you work per day? ________ hours
8. How many days per week do you work? ________ days

Section 3: Health and Medical History

9. Have you been diagnosed with any of the following conditions? (Select all that apply)
    - ☐ Liver disease
    - ☐Kidney disease
    - ☐ Respiratory issues
    - ☐ Hypertension
    - ☐ Diabetes
    - ☐ None
10. Do you experience any of the following symptoms regularly? (Select all that apply)

- ☐ Fatigue
- ☐ Dark urine
- ☐ Abdominal pain
- ☐ Jaundice (yellowing of the skin or eyes)
- ☐ Nausea/Vomiting
- ☐ Shortness of breath
- ☐ Headaches
- ☐ Skin irritation
- ☐ Swelling in hands/feet
- ☐ None

Section 4: Lifestyle Factors

11. Do you smoke?

- ☐ Yes
- ☐ No

12. Do you consume alcohol?

- ☐ Yes

- ☐ No

13. How often do you exercise?

- ☐ Daily
- ☐ 1-3 times a week
- ☐ Occasionally
- ☐ Never

14. What type of water do you usually drink?

- ☐ Bottled water
- ☐ Tap water
- ☐ Filtered water
- ☐ Other (please specify): ____________

15. How many hours of sleep do you get per night? ______ hours

Section 5: Awareness of Health Risks

16. Are you aware of any occupational health risks related to your job?

- ☐ Yes
- ☐ No
- ☐ Not sure

17. Have you received any occupational health and safety training related to your work?

- ☐ Yes
- ☐ No

Annex V: cobas c311 analyzer series

The **cobas** c311 analyzer seriesis fully automated, modular, computerized, performs in vitro quantitative and qualitative tests on a wide range of analytes and performs photometric assays and ion-selective electrode measurements.

The **cobas** c311module comprises a photometric unit and an ISE unit. It is equipped with a photometer to measure the absorbencies of the reaction mixtures in the reaction cells. The module adopts the entire reaction monitoring system, which measures the absorbance of reaction solution intermittently during reaction time. The reaction disk rotates 3 turns in 24 seconds. During this time, the absorbance is measured and stored for all of reaction cuvettes that go across the optical path of the photometer. For each reaction cuvette, water blank (absorbance 0) is measured, and then photometry is performed about every 8 seconds, or 70 times in 10 minutes.

Step Action

1. Start
2. The instrument resets the mechanisms and then starts rinsing the reaction cell. In a single cycle (6.0 s), the reaction disk rotates by 60 reaction cells, stops temporarily, and rotates by a distance of 59 reaction cells. Therefore, the reaction disk rotates 3 turns minus 4 reaction cells in 4 cycles (24.0 s)
3. Using the rinsing nozzle **a** in the rinsing mechanism, reaction solution is aspirated, and the reaction cell is rinsed with deionized water.
   - Then, after 4 cycles, the reaction cell is washed with rinsing nozzle **b** in the rinsing mechanism using Cell Wash Solution I / NaOH-D.
   - Then, after 4 cycles, the reaction cell is washed with rinsing nozzle **c** in the rinsing mechanism using Cell wash Solution II / Acid Wash.
   - Using the rinsing nozzle **d** and the rinsing nozzle **e**, the reaction cell is rinsed again with deionized water.
   - After deionized water is aspirated with nozzle **f**, rinse water is discharged with nozzle **g**.
4. Then water blank is measured 3 times. If the water blank value differs by 0.1 or more from the cell blank value, that cell is not used for analysis.
5. After the water blank measurement, the water is aspirated (using rinsing nozzles **h** and **i**) and the cells are dried and ready for measurement.

6. Sampling is carried out in the order starting from the sample with the test that takes the longest reaction time in order to shorten the time needed for completion of data output.
7. Reagents R1, R2, and R3 are usable, and added at the determined positions and time points (0, 1.5 and 5 minutes), respectively. After one of the reagents R1, R2, and R3 has been added, the liquid in the cuvette is mixed at the corresponding mixing position by means of the ultrasonic mixer.
8. Sampling is carried out every 6 s (1 cycle), and measurement is performed once in every full turn, that is 70 times in 10 minutes. On completion of measurement, the concentration is calculated by using the absorbance at the specified measuring point.
9. The instrument discharges the reaction solution with the rinsing mechanism and conducts washing with detergent and rinsing with water.
10. Then the instrument goes into standby.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Halima Abdulkadir (B.Sc.)

Signature: ___________________

Date of submission: ___________________

This thesis has been submitted with our approval as advisors.

Advisor: Mistire Wolde (PhD, Associate Professor)

Signature: _____________________

Date: _____________________

Place: Addis Ababa, Ethiopia.

Advisor: Mekdes Alem

Signature: ____________________

Date: ____________________

Place: Addis Ababa, Ethiopia.