ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of hematological parameters among gasoline exposed workers at gas station in Mekelle City, Tigray Region, Northern Ethiopia.

By: GebreTeklu (BSc)

Advisors: Aster Tsegaye (MSc, PhD)

MikiasNegash (MSc, PhD candidate)

A research thesis submitted to the Department of Medical Laboratory Sciences, School of Allied Health Science, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology)

June, 2018

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

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

Signed by the Examining Committee:

External Examiner______________________Signature ___________Date ____________

Internal Examiner_______________________ Signature ___________Date ___________

Advisor________________________________Signature ___________Date ___________

Advisor________________________________Signature ___________Date ___________

____

Chairman of the Department or Graduate Program Coordinator

Acknowledgement

It is impossible to convey, in a couple of sentences, my gratitude to many people for helping me to learn and whose cooperation make this work possible.

All praises and glory are due to GOD for all the bounty and support granted to me. My heartful gratitude goes to my Advisor Dr. Aster Tsegaye, Addis Ababa University Ethiopia, for her initiating and planning of this work and for her valuable scientific advices. She stands with me step by step and she is very keen to show me everything right. And I will like to thanks for Co-Advisor Mr. Mikyas Negash (MSc, PhD follow) for his advice throughout completion of the proposal to the thesis. He helps me in selecting the topic and reading the proposal and thesis result and made the suitable correction and comments.

I would like to extend my gratitude to Addis Ababa University, College of Health Science and Department of Medical Laboratory Science for giving me the opportunity to develop this thesis project and financial support. Administrative office of Ayder comprehensive specialized hospital (ACSH) and central laboratory workers are also gratefully acknowledged for permitting and supporting this study in analysis of hematological indices. My sincere also goes to the study participants for their kind collabration in filling questionnaire and donating biologic samples which allowed further insights on gasoline exposure.

Last but not least, I want to thank my beloved father and mother, thank you for always believing in me and loving me unconditionally, without your support and love I would not be here today.

Table of content

Acknowledgement II

List of Tables VI

List of figures VII

Abbreviations VIII

Abstract X

1. Introduction 1

1.1. Background 1

1.2. Statement of the problem 4

1.3. Significance of the study 5

2. Literature review 6

2.1. Conceptual frame work 12

3. Objectives 13

3.1. General objective: 13

3.2. Specific objective: 13

4. Hypothesis 13

5. Materials and Methods 14

5.1. Study Setting 14

5.2. Study Design and Period 14

5.3. Population 14

5.3.1. Source population 14

5.3.2. StudyPopulation 14

5.4. Inclusion and Exclusion Criteria 15

5.4.1. Inclusion criteria 15

5.4.2. Exclusion criteria 15
5.5. Study Variables 15
5.5.1. Dependent variables 15
5.5.2. Independentvariables 15
5.6. Measurement and Data collection 16
5.6.1. Data collection procedure/technique 16
5.6.2. Data collection procedure 16
5.6.4. Blood sampling and processing 16
5.6.5. Laboratory analysis 17
5.7. Data Quality Assurance 18
5.8. Data analysis and interpretation: 19
5.9. Ethical considerations 19
5.10. Dissemination of results 20
5.11. Definition of terms 20
6. Work flows 21
7. Result 22
7.1. Sociodemographic characteristics of the study participant 22
7.1.2. Work duration, working hours and awairness related to gasoline exposure 23
7.1.3. Knowledge of workers regarding routes of gasoline entry into the body, gasoline health effects and gasoline as an environment pollutant 25
7.2. Hematological parameters profile of gasoline exposed and control groups 27
7.2.1. Hematological parameter and effects of exposure to gasoline with duration of exposure 29
7.2.2. The correlation of hematological indicies with year of exposure, working hour and age of the exposed group at Mekelle City 31
7.3. Peripheral blood morphology examination results 31
8. Discussion 32
9. Conclusion and Recommendation 35

9.1. Conclusion 35

9.2. Recommendation 35

10. Strength and Limitation 37

10.1. Strength of the study 37

10.2. Limitation of the study 37

11. References 38

12. Annexes 43

Annex-I; Participants' information sheet (English version) 43

Annex-II; Information sheet Amharic version 45

Annex III-Information sheet Tigrigna version 47

Annex-IV: Consent Form (study participants) - English version 49

Annex-V; Consent form Amharic version 50

Annex VI-Consent form Tigrigna version 51

Annex VII; Structured Questionnaire for Gasoline exposed Study participants 53

Annex-VIII; Questionnaire Amharic version 56

Annex-IX; Questionnaire Tigrigna version 58

Annex-X; standard operating procedures 60

Annex XI-Declaration 67

List of Tables

Table-1: Socio-demographic and occupational profile of the study group at gas station Mekelle City, Tigray Rigion, Northern Ethiopia, from January-April 2018 (n=120). 22

Table-2: Risk factors asociated with gasoline exposure of study groups at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43). 24

Table-3: Knowledge of workers regarding gasoline among the exposed group at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43) 25

Table-4: Protective measures in use among gasoline station workers at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43). 26

Table-5: complete blood count picture of the study group at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=120). 28

Table 6: Comparison of the hematological indices with duration of exposure in exposed groups post hoc analysis at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43) 30

Table 7: Correlation of hematological indicies with year of exposure, working hour, and age for the exposed group at Mekelle City (n=43) 31

List of figures

Figure-1.Simplified metabolic scheme for benzene showing site specific metabolism from liver to bone marrow 3
Figure 2; Conceptual frame work 12
Figure 3; work flow 21
Figure 4; Educational status for the study population at gas station in Mekelle city (n=43) 23
Figure 5: Reasonf for not using safety equipments by gasoline station workers at Mekelle City (n=43) 26

Abbreviations

AAU	Addis Ababa University
ALC	Absolute Lymphocyte Count
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
AM	Automobile Mechanics
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BTX	Benzene, Toluene and Xylene
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
CSA	Central Statistical Agency
DNA	Deoxyribo Nucleic Acid
GST	Glutathione S-Transferase
HGB	Hemoglobin
HCT	Hematocrit
HQ	Hydroquinone
HSCs	Hemopoietic Stem Cells
K_2EDTA	Di-Potassium Ethylene Diamine Tetra Acetic Acid
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
mEH	Microsomal Epoxide Hydrolase
MPO	Myeloperoxidase
MPV	Mean Platelet Volume
MTBE	Methyl Tert-Butyl Ether
PAH	Polycyclic Aromatic Hydrocarbons
PAS	Periodic Acid Schiff

PCV	Packed Cell Volume
PMS	Premium Motor Spirit
PPM	Parts Per Million
PSA	Petrol Station Attendant
PST	Phenol Sulfo-transferase
QC	Quality Controls
RBC	Red Blood Cell
RDW	Red Blood Cell Distribution Width
ROS	Reactive Oxygen Species
SD	Standard Deviation
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
TLC	Total Leukocyte Count
UDPGT	Uridine Diphosphate Glucuronosyltransferase
VOCs	Volatile Organic Compounds
WBC	White Blood Cells

Abstract

Background: Petrol station attendants are chronically exposed to gasolineof the volatile fraction during vehicle refueling. The adverse health effects of gasoline exposure may be related to impairment of the hemopoietic system with bone marrow depression and an increased risk of developing cancer.

Objective: To assese the hematological parameters among gasoline exposed workers at gas station in Mekelle City, Tigray Region, Northern Ethiopia.

Method: A cross sectional study was carried on 43 individuals working in 9 petrol filling stations and 77 age and sex matched non exposed controls. Socio-demographic and exposure duration of workers were collected using structured questionnaire and observation checklist. Hematological analysis was performed using Sysmex XP -300 and peripheral blood morphology was determined. Data were entered and analyzed using SPSS version 23.

Results: 28/43 (65.1%) of exposed individuals and 49/77 (63.6%) of controls were males. The average exposure time was 5.19 ± 4.38 years with an average workig hour of 11.74 ± 1.89 hours/day. The mean RBC count (10^{12}/L), HCT (%), HGB (g/dl) and platelets count (10^{9}/l) of the exposed group were significantly lower (4.88±0.573, 43.29±3.71, 15.04±1.33 and 248.95±58.19) compared with controls (5.35±0.533, 44.95±3.10, 15.59±1.26 and 292.45±62.17) at ($P<0.05$) respectively. The MCH (pg) (30.48±2.06 vs 29.52±1.66) and MCHC (g/l) (34.83±0.988 vs 34.32±0.927) were significantly higher in the exposed compared with controls ($P<0.05$). HCT, RBC, HGB and platelets count were significantly decreased with increased year of exposure ($P<0.05$). The peripheral blood film examination reveals basophillic stippling and macrocytosis.

Conclusion: In this study long term exposure to gasoline at gas station has deleterious effect on the RBC indices and platelet. Significant negative correlation was noted between long term exposures to gasoline and a decrease in HGB, HCT and platelet count. Thus, protective measure should be implemented by the concerned ministries with labour union to minimize exposures to gasoline fuel.

Key words; gasoline, petrol, hematological parameters, petroleum

1. Introduction

1.1. Background

Blood as specialized body fluid has many functions like respiration, circulation, excretion, osmotic balance and transport of metabolic substance. And it delivers necessary substances to the body's cells such as nutrients, gases, minerals, metabolic products and hormones between different organs [1]. Hematopoiesis is the development of all mature blood cell lineages that emerge from multipotent hematopoietic stem cells (HSC) in the bone marrow. The human hematopoietic system produces around 10^{12} cells every day. HSC have the ability to differentiate into all hematopoietic lineages but also retain their self-renewal capacity [2]. Blood is composed of two components, namely; Plasma (55%) and Blood cells (45%). Plasma contains 91-92% of water and 8-9% solids, while the blood cells are of three main types namely; Red Blood Cells (RBC) or Erythrocytes, White Blood Cells (WBC) or Leukocytes, and Platelets or Thrombocytes [3]. Other blood parameters include packed cell volume (PCV), Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration. Blood parameters are probably the more rapid and detectable variations under stress conditions like gasoline exposure for assessing the health condition [4].

Exposures to several chemicals are implicated in the derangement of hematological profile. The adverse health effects of gasoline exposure may be primarily related to impairment of the hemopoietic system with bone marrow depression, including pancytopenia, hence aplastic anemia and an increased risk of developing cancer (acute myeloblasticleukemia). Morphological effect on red blood cells (RBCs) like microcytosis also occurs [5].

The use of gasoline in the industries and homes has rapidly increased in the recent times. In the course of usage, individuals are frequently exposed to pollutants from it in both outdoor and indoor environments. However, the major route of exposure is inhalation by workers during production and distribution of the fuel, contamination with diet and by the general public during refueling at service stations [6]. Gasoline, one of the fractionated products of crude oil, is widely used as fuels for automobiles and some electricity generating machines. It is known to be a very volatile liquid, with several organic and inorganic constituents. Gasoline vapor may be derived from direct evaporation of liquid gasoline [7].

Gasoline, or petrol, is also a volatile liquid with a complex mixture of aliphatic and aromatic hydrocarbons. It is commonly used as fuel for internal combustion engines and is also used as a thinner, decorative agent, and industrial solvent. Some of its constituents are known to be highly toxic or carcinogenic to humans [8, 9]. Many of the toxicological effects associated with the exposure to gasoline can be attributed to specific components of gasoline, such as benzene, toluene, ethylene and xylene, which are also known as volatile organic compounds (VOCs) [10,11].

Occupational exposure to hazardous chemicals has been associated with hematological abnormalities. Petrol attendants and other workers at fuel-filling stations are at risk of gasoline exposure and for the development of cancers [12]. Exposure to gasoline for long periods may lead to permanent suppression of bone marrow functioning, accompanied by reduction in the formation of new blood cells in a condition known as aplastic anemia [13]. Exposures experienced by such workers include the polycyclic aromatic hydrocarbons (PAH) and other volatile organic compounds. These constituents present an important concern for their carcinogenic potential [14]. Benzene is a known carcinogen primarily affecting the hematopoeticsystem. The effects of systemic benzene exposure can cause acute and chronic clinical disorders, of the cardiovascular, respiratory, neurological, gastrointestinal, liver, renal, dermatology, local effects, and immunological, metabolic and allergic reactions [15].

The above mentioned disorders are believed to be caused by toxic Benzene metabolites. Benzene is metabolized in the liver to its primary metabolite phenol by cytochrome P4502E1 (CYP2E1) through the benzene oxide intermediate. It is subsequently metabolized by CYP2E1 to hydroquinone (HQ) [16, 17]. HQ is transported to the bone marrow and oxidized to benzochinones, which eventually releases reactive oxygen species (ROS) damaging hematopoietic cells [16, 18] as shown in Figure 1 [30], therefore chronic exposure to benzene is believed to be associated with many of bone marrow failure and hematological malignancies like, Acute Myeloid Leukemia (AML), Aplastic anemia myelodydplastic syndrome, acute lymphoblastic leukemia and chronic myeloid leukemia [19, 20]

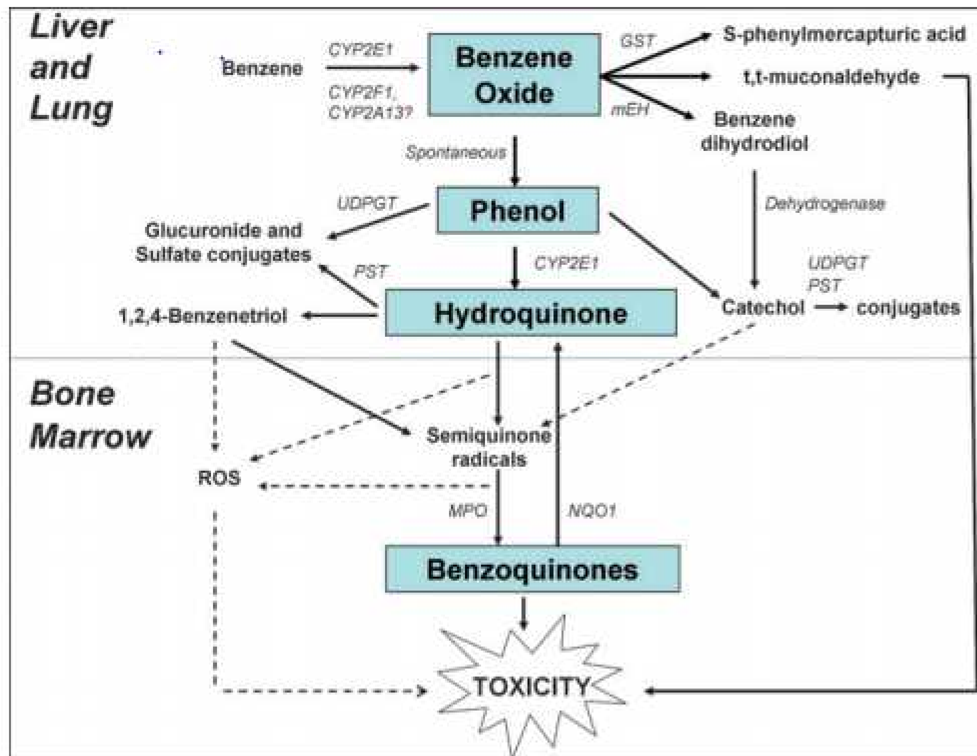

Figure-1.Simplified metabolic scheme for benzene showing site specific metabolism from liver to bone marrow including major pathways and metabolizing enzymes leading to toxicity.*GST, glutathione-S-transferase; mEH, microsomal epoxide hydrolase; MPO, myeloperoxidase; PST, phenol sulfotransferase; UDPGT, uridine diphosphate glucuronosyltransferase, ROS, reactive oxygen species,*

1.2. Statement of the problem

Chronic exposure to benzene, which is the main ingredient of gasoline, is believed to be associated with many of bone marrow failure and hematological malignancies [19, 20]. Gasoline is a complex manufactured mixture that does not exist naturally in the environment which is produced from petroleum in the refining process. Typically, gasoline contains more than 150 chemicals including benzene, toluene, xylene, and sometimes lead. Most people can begin to smell gasoline at 0.25 parts of gasoline per million parts of air (ppm) [21]. Several authors reported that toxicity of gasoline comes mainly from benzene metabolites [22, 23]. According to existing evidences, petroleum hydrocarbons have the potential to cause Deoxyribo Nucleic Acid (DNA) damage in gasoline exposed individuals and exposure to petrol vapor induce genotoxic effects, confirming that the gasoline station workers have a high risk of cancer due to their daily occupational exposure [24, 25].

Once gasoline is inhaled, benzene as main ingredient of gasoline, enter the lung then passed to the blood stream from which it goes to the liver, where three main phenolic metabolites of benzene are released including; transient phenol and accumulated hydroquinone and catechol in relatively high concentrations [26]. Benzene is a lipophilic agent, so its metabolites go directly to fatty tissues such as, bone marrow where actual toxic species are generated [27]. In recent years, in South Africa, this risk has increased with the increased concentration of benzene in motor vehicle fuels, particularly with the removal of lead as an anti-knock agent [14].

It has been shown that genetic polymorphisms of xenobiotic metabolizing enzymes may modulate the susceptibility of individuals to toxic compounds. Among these enzymes is glutathione S-transferase (GST) superfamily which plays an important role in detoxification of various toxicants. The GSTs have been divided into a number of subclasses , μ, , and [28]. There are well-defined genetic polymorphisms in the expression of GSTM1 (a member of class μ) and GSTT1 (a member of class) enzymes with nonfunctional null-alleles named GSTM1-0 and GSTT1-0, respectively. Previous studies have shown that the homozygocity for these null-alleles is associated with the absence of the corresponding enzyme activity [28]. It is reported that these enzymes involve in detoxification of several toxins including some of the compounds present in gasoline [29]. Evidence is provided also for wide toxic effects of benzene metabolites with prolonged exposure including; pancytopenia (decrease in all blood contents counts) and

leucopenia (decrease in number of leucocytes) [31] and other blood disorders such as leukemia [32].

Studies conducted in the animal models and human beings suggested that long term exposure to petrol has a deleterious effect on blood cells [32]. Long-term experimental carcinogenicity studies of the effects of gasoline, and major gasoline aromatics on Sprague–Dawley rats showed that all the tested materials were found to increase the total number of malignant and observe for hematological changes, and cause a significant changes on the parameters [33]. Benzene suppresses the cell cycle by p53-mediated overexpression of p21, a cyclin-dependent kinase inhibitor, resulting not simply in suppression of hemopoiesis but rather in a dynamic change of hemopoiesis during and after benzene exposure [34]. While several studies pointed to the risk of occupational exposure to gasoline on hematological profiles, there are no published studies in this regard in the country which this study tries to address.

1.3. Significance of the study

The study will benefit study participants by early detection of hematological abnormality, if any, for appropriate interventions. It also helps them to be aware the risk of their occupational exposure on their hematological parameters and take appropriate safety measures. Administrators of the gas stations as well as and Mekele city administration will also be benefitted to take appropriate safety measures to other related workers. The finding will contribute its share to the strengthening of occupational safety policy at a larger scale in the country.

2. Literature review

Gasoline exposure is one of the main health concerns for occupations with risk of exposure to volatile solvents such as in petrol pump workers. Human exposure to gasoline is associated with multiple adverse health effects including pancytopenia, hence aplastic anemia and an increased risk of developing cancer (acute myeloblastic leukemia) and Morphological effect on RBCs (microcytosis) also occurs [35]. Benzene is one of the most broadly used chemicals in the synthesis of various polymers, resins and synthetic fibers. Furthermore, benzene is a common component of gasoline [36].

Complete blood count (CBC) is one of the most important hematological tests. Complete blood count (CBC) provides valuable information about the quantity of the different types of cells in the blood. The test might help in diagnosing several hematological disorders e.g. anemia, certain cancers, kidney problems, and allergic reactions [31].

A cross-sectional study was done in Shanghai, China in 2010 in five factories that used benzene in their production processes to determine the hematotoxic effects of benzene exposure involving 928 workers on twelve peripheral blood effects. Specifically, they observed statistically significant decrements of RBC count and HGB at ($p < 0.001$) with a ($p < 0.001$) increase in MCV. They also observed small, but clear signals for total platelet count and MPV reductions ($p < 0.001$) per log of benzene concentration. There were 55 workers anemic, showing RBC values lower than normal ranges, 31 were defined as having macrocytosis by showing high MCV values, 69 had an abnormally low reading for HGB and 16 were having thrombocytopenia with low platelet readings. This study has shown that absolute neutrophil counts as well as reduced MPV may be a sensitive blood parameter for benzene exposure. Among the three types of blood elements (WBCs, RBCs and platelets), stronger effects on peripheral blood was observed for red cell indices. [37].

A survey was conducted on individuals from polluted areas of Khozestan province, Iran, in 2002 on 495 individuals including those exposed and unexposed. In the present report, the hematological findings of 99 persons from polluted areas of MIS were compared with those of 396 individuals from the general population of MIS matched by age (±3 years) and sex. The results showed that, absolute mean number of RBC, HCT (%), level of HGB, and absolute

number of platelets were significantly higher among the exposed subjects compared with the control group. However, the absolute mean numbers of WBC, lymphocytes, and neutrophils were significantly decreased in the exposed group compared with the control. This research concluded that the association between exposure to natural gas and hematological changes is apparent [38].

Cross-sectional studies were conducted in 2004 and 2009 recruiting male gasoline station workers in Pathumwan District, Bangkok to compare workers' hematological indices and biochemical change in 2009 to that in 2004. Between these years, use of methyl tert-butyl ether (MTBE) in gasoline was banned and use of gasohol increased. Data were collected from 44 male employees at 9 gas stations and 83 male from 11 stations in 2004 and 2009 respectively. In multiple linear regression models, no white cell-related endpoint differed significantly between 2004 and 2009. The same was true for HGB level, HCT and RBC volume. Modeled prevalence of abnormal red cell structure (morphology) was significantly lower in 2009 than 2004 (OR=0.34, 95% CI 0.12 to 0.95, p=0.040). Modeled prevalence of low HGB did not differ significantly between the two study years. The current study showed a limited tendency toward improvement of gasoline station workers' health between 2004 and 2009. Removal of MTBE in 2006 may have contributed to this improvement, although this cannot be asserted with confidence [39].

A cross-sectional study was conducted in Shiraz, Iran, in 2011 to assess changes in the level of hematological parameters of petrol station workers exposed to toxic components of unleaded petrol in 400 subjects (200 exposed and 200 reference subjects). All participants underwent complete blood counts and white blood cell differential tests. The results of blood tests showed that the means of RDW were higher in the exposed group than in the reference group (13.3 ± 1.2 12.8 ± 1.7 p=0.003), and HCT parameters were significantly lower (44.2 ± 4.3 45.3 ± 4.1 p=0.009) while the other parameters were within the normal range. The results of this study indicated that exposure of private petrol station employees to BTX did not exceed the current threshold levels for these chemicals. Though under the existing conditions (in terms of intensity and duration of exposure) overt hematotoxicity is unlikely to be a major outcome, possible long-term pathological consequence and ramification of the subtle, subclinical, statistically significant changes in HCT and RDW values require and deserve further investigation [40].

A cross sectional survey was carried out recruiting 292 individuals, 146 petrol filling workers and 146 individuals who already work in station as overseers as control in Baghdad city and they were investigated for the effect of gasoline on hematological indice. Of examined 292 individuals, 146 petrol filling workers (all of them) were found with hematopoietic changes. The Hb, RBC count, WBC count levels in petrol filling station workers were significantely reduced compaire to control and results of the subjects were, (12.5±1.5, 13.8 ±0.3g/dl, p= 0.0001), (4.3±1.9, 4.7±0.3x1012/L, p = 0.0125) and (5.5±1.7, 6.4±1.0 x10^9/L, p= 0.0001) respectively. Out of six potential risk factors, only one (smoking habit) found to be significantly associated with the presence of white blood cell changes ($p<0.05$) as compared with petrol filling workers who had no such risk factors. The study concluded that, although no cases of blood disorders were detected but blood involvement in petrol stations workers is still possible and should be given full attention in medical surveillance of workers [41].

A case-control study done in different petrol stations in Lucknow, Uttar Pradesh, India, 90 male petrol pump workers (exposed group) and 30 non-exposed groups were involved. Venous blood sample was collected for the assessment of Hb, RBC, WBC, and TLC. The finding showed that Hb was significantly (13.78±1.38 vs 15.02±1.17; p=0.001) lower among the cases compared with controls. The level of other biochemical parameters like TLC (8.37±1.78 vs 8.47±2.84; p= 0.77) and RBC (4.58±0.56 vs 4.79±0.50; p= 0.06) count was similar between the two groups. The study suggests petrol station workers could be under risk of hematological abnormalities [42].

Another cross-sectional study was conducted for a period of 8 months from November 2015 to June 2016 among petrol pump workers in and around Nandyal, Andhra Pradesh. This study was carried out in 30 adult male volunteers of age group 25-40 years working in petrol pump stations for more than 5 years. Control group consisted of 30 male adults of same age group who did not have the history of exposure to petroleum products. Blood samples were collected and analyzed for RBC, TLC, platelet count and HB concentrations.The study showed a statistically significant reduction in RBC count (4.29±0.383 vs 5.21±0.336, p=0.001), Hb concentration (13.06±0.634 vs 15.36±0.730, p=0.001) and TLC count (8466.6±1908.4 vs 7180.8±1393.4p=0.0042) among study group when compared with control group [$P<0.05$]. Thus, the study concluded that chronic exposure to petrol fumes has toxic effect on haematological parameters leading to bone marrow depression [43].

A case-control study (2012) was conducted in Zabol, Iran to explore the effect of gasoline on blood, kidney and liver parameters of unregulated gasoline traders. Complete blood count, blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were tested. The hemoglobin, hematocrit, mean corpuscular hemoglobin concentration, BUN, AST, ALT, ALP levels were higher in the exposed group. However, the Cr level and platelete count were significantly lower in the exposed group compared to the non-exposed ones. Although the findings of the current study were not clinically significant, they indicate important changes that require a longitudinal study to find the impacts of prolonged exposure to gasoline vapors at risk populations. However, the results of the present study are limited to the blood, kidney and liver tests through a blood sample. Exposure assessment was based on participants' reports, which is inaccurate [44].

A similar study done in Hyderabad, India (2008) on a group consisted of 42 healthy, non-smoker petrol filling workers to investigate whether chronic exposure to solvents like benzene and carbon monoxide in petrol filling workers had effect on blood parameters. Participants were; aged 19-50 years with occupational exposure duration from 2-15 years.The result showed that during the early period of exposure (1-5 years & 5-10 years), the average Hb concentrations and RBC count were unchanged while comparing with the controls .But as the year of exposure increases more than 10 years there was statically significant increase ($p<0.05$) in the concentration of Hb (g/dl) from 14.537±0.8501 at 1-5 year, 14.56±0.8912 at 5-10 years and 16.16±1.15 at 10-15 years when compared to controls which was 14.483±0.828. And the RBC (millions/mm^3) count was 5.103±0.400 at 1-5 years, 5.403±0.166 at 5-10 years and 5.373±1.15 at 10-15 years of exposure than controls 4.836±0.254. The platelet count was observed a statistical significant decrease in workers with more than 10 years of exposure ($p=0.009$). White blood cell count except eosinophils and platelets were significantly lower in workers compared to controls. The result obtained from the study concluded that there is a significant toxic effect of solvents and air pollutants on workers exposed for longer duration [45].

Another cross-sectional study done (2015) in petrol pump attendants in Pune, India to investigate the effect of long term exposure to petrol fumes on Hb content, RBC count, PCV, MCV and MCH and on TLC, differential WBC count.The test group consisted of healthy adult male aged

25-45 years working in the petrol pump for more than 5 years. Results of the study showed a statistically significant reduction in the Hb (g/dl) (13.73 ± 1.10 vs 15.56 ± 0.63), PCV (%) (42.69 ± 3.86 vs 48 ± 1.55) and RBC count (millions/mm^3) (4.52 ± 0.58 vs 5.53 ±0.34) at $p < 0.001$ of the test group compared to control group respectively. Whereas MCV and MCH were significantly higher in the test group compaired to control at $p<0.001$. A statistically significant reduction in the total WBC and lymphocyte percentage at $p = 0.001$ and an increase in neutrophil $p = 0.001$ were recorded. The peripheral blood smear reveals, 31 workers had basopillic stipling. Hence, from the study they concluded that, reduction in the RBC count, PCV, total WBC count and lymphocyte count could be due to toxic effect of components of petrol on hemopoietic cells in the bone marrow as well as increased hemolysis [46, 47].

A cross sectional study carried out in Ekpoma, Edo State, Nigeria, seeks to evaluate the consequences of exposure to premium motor spirit (PMS) fumes on some hematological parameters of Automobile Mechanics and Petrol Station Attendants from a total of 150 participants. 50 were petrol station attendant (PSA), 50 automobile mechanics (AM) and 50 apparently healthy individuals. The result showed that, the Hb (g/dl) level and PCV (l/l) were significantly lower amongst the PSA compared to the AM and with the apparently healthy individuals with (12.6±1.0, 13.4±0.5, 14.4±1.9, p=0.00) and (0.38±0.03, 0.40±0.02, 0.43±0.04, p = 0.001) respectively. The total WBC counts, Neutrophile counts and lymphocyte counts (x10^9/l) of the fuel attendants, automobile mechanics were higher than the apparently healthy participants with (7.55±0.74 vs 7.42±1.43 vs 4.46±1.18), (5.29±0.92vs 5.31±1.51 vs 2.62±0.68) and (2.19 ±1.53vs 1.82±0.39 vs 1.75±0.57) respectivelyat (p=0.001). This study suggested increased exposure to petrol fumes among automobile mechanics, petrol station attendant, is highly toxic and are potential damaging agents to the hematopoietic stem cell and could cause anemia [48].

A case-control study was done in Sokoto, North Western, Nigeria, designed to determine the effect that occupational exposure to unleaded Premium Motor Sprit (PMS) on hematological parameters of 100 petrol station attendants. Results of the study showed the Hb, PCV, RBC, WBC, MCH and MCHC were significantly lower among exposed compared to non-exposed controls (p 0.05). Participants who were exposed to Premium Motor Spirit for 2 years had significantly ($p < 0.001$) lower Hb (g/dl), PCV (%), RBC (x10^{12}/L), WBC (x10^9/L) MCH (pg) and MCHC (g/dl) compared to those exposed for < 2 years and unexposed controls. The

prevalence of anemia (hemoglobin <13.5 g/dl) was significantly higher among participants exposed to PMS for 2 years (70%) compared to those exposed for < 2 years (24%) and unexposed controls (0%) (p=0.001). They observed a significant positive correlation between length of exposure and anemia and leucopenia (r=0.68 and r=0.45 respectively, p=0.01) [49].

According to a cross sectional descriptive study done in Kosti and Rabak cities, White Nile State, Sudan, in 2014- 2015 to determine any alterations in hematological parameters among workers at fifty fuel stations,the following results were obtained. The study found high prevalence of abnormalities: 50% had low hemoglobin levels, 60% low RBC counts (although 30% were higher than normal), hematocrit readings showed 24% with reduced values and for MCV 92% were reduced. Half of the participants showed microcytic cytology. The study concluded that there are abnormalities in hematological parameters among fuel stations workers, particularly in Hb and RBC indices, as well as lymphocytosis and neutropenia. Workers at fuel stations should be protected from exposure to benzene by training with the equipment to minimizing leakages and spillages and by wearing protective devices, such as masks and goggles [50].

A comparative cross-sectional study (2015) was conducted in Mansoura City Egypt on 102 petrol station attendants and a matched group of healthy 102 male service and office workers at the Faculty of Medicine, Mansoura University as controls. The results of blood picture, were compared between both groups and showed that, RBC (x 10^{12}/L), Hb (g/dl), and HCT (%) level (4.8 ± 0.4, 13.9 ± 1.3, 39.7 ± 3.4) at (p 0.001) were significantly lower in petrol station attendants than the comparison group (5.3 ± 0.5, 15.2 ± 1.2, 43.7 ± 3.6) at (p 0.001). All other blood picture parameters showed non-significant difference between both groups. It concluded that some laboratory parameters among petrol station attendants showed changes that could be attributed to workplace exposure and should be given attention at pre-employment and periodic medical examination [51].

Taken together, most of the studies reviewed above pointed to the toxic effect of gasoline exposure on hematological parameters. This being the case, as far as my literature search goes; there is no published study in Ethiopia and the study area in particular. This necessitates the need to investigate the effect of occupational exposure on hematological profile of gas station workers.

2.1. Conceptual frame work

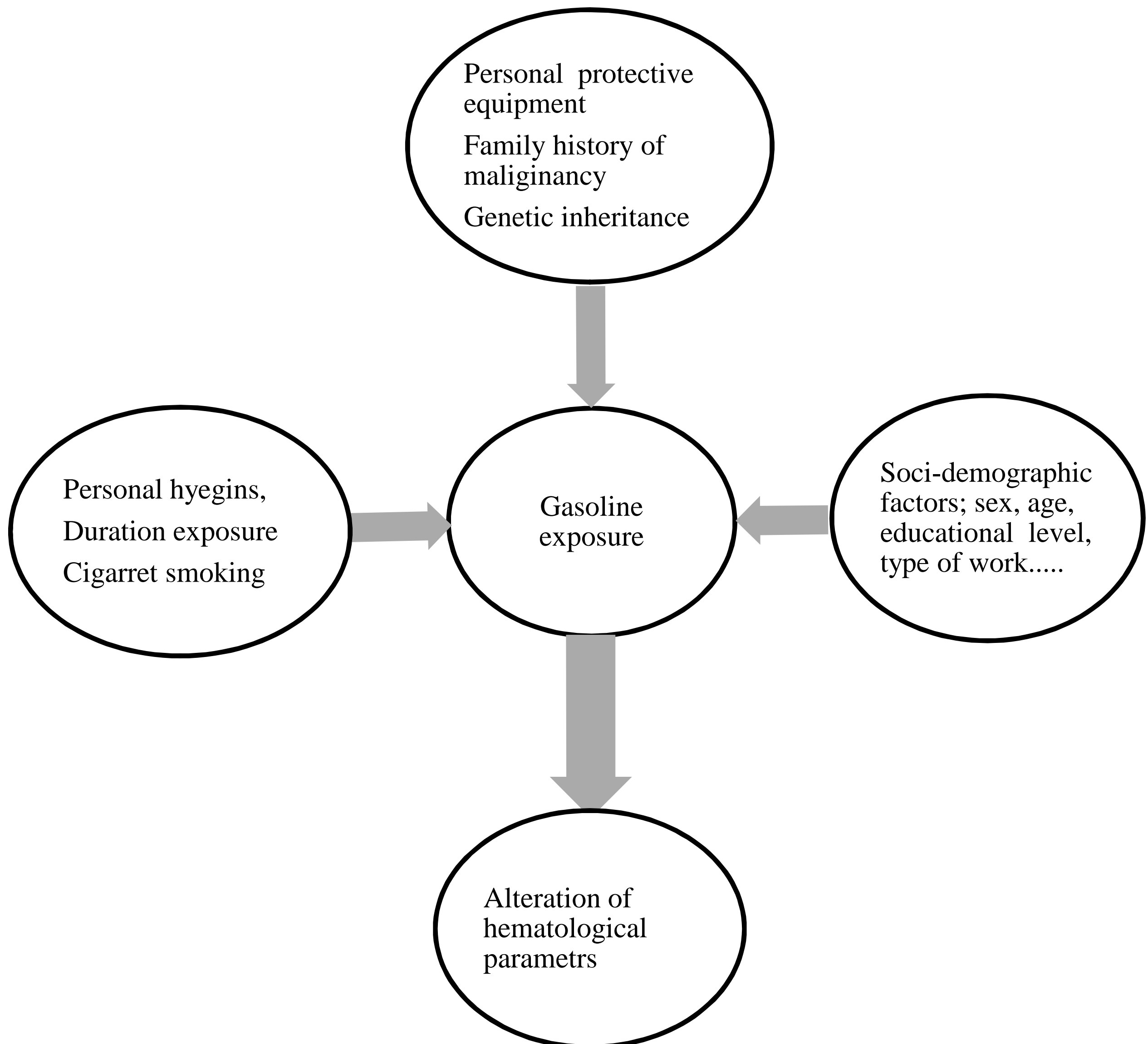

Figure 2; Conceptual frame work

3. Objectives

3.1. General objective:

To assess the hematological parameters among gasoline exposed workers at gas station in Mekelle City, Tigray Region, Northern Ethiopia.

3.2. Specific objective:

- To determine complete blood count (CBC) profile and peripheral morphology of the exposed workers as compared with controls.
- To identify associated risk factors on the hematological parameters to gasoline exposed in gas station workers.

4. Hypothesis

H_o-gasoline exposure in gas station workers has no significant effects on hematological parameters.

5. Materials and Methods

5.1. Study Setting

The study was carried out at Mekelle City Gas Station workers who are exposed to gasoline and its products. Mekelle is Capital City of Tigray Regional State and is located in the Northern part of Ethiopia, at 783km from the Capital City of Ethiopia, Addis Ababa. It was established in 13th century. Mekelle City is administratively divided in to seven sub-ciyies as follw: Semien, Kedamay Weyane, Hawelti, Ayder, Hadnet, Adi Haqi and Quiha. According to projetced Central Statistical Agency of Ethiopia (CSA), of 2013, the town of Mekelle City has a total of population of 307,307 people, of whom 155,497 are men and 151,810 women [52]. The samples collected from exposed and non-exposed individuals were analyzed at Ayder Comprehensive Specialized Hospital (ACSH). The hospital is last referral hospital of the region that is found in Ayder sub-City, Mekelle City (capital city of Tigray Region) in Northern Ethiopia. Originally the Ayder Comprehensive Specialized hospital was built to host/accommodate around 8 million in population in its catchment areas of the Tigray, Afar and South-eastern parts of the Amhara Regional States with total patient flow of above 100,000 per year and to host above 500 beds.The hospital has a well organized hematology laboratory.

5.2. Study Design and Period

A comparative cross sectional study at Mekelle City was done. The study period was from January/2018 - April/2018.

5.3. Population

5.3.1. Source population

All adult male and female who work in gas station at Mekelle City were accounted as source population for the study.

5.3.2. StudyPopulation

All adult workers who work at the gas station at Mekelle City with exposure of six months and above and fulfilling the eligibility criteria were accounted as study population. Apparently healthy age and sex matched controls were voluntarily recruited from Mekelle University Medicial Laboratory department staffs and graduate students.

5.4. Inclusion and Exclusion Criteria

5.4.1. Inclusion criteria

Adult males and females aged 18-60 years, who have worked at least six months and above at gas station and who were voluntary to participate in the study were eligible for inclusion.

5.4.2. Exclusion criteria

Participant with the following data were excluded from the study; any acute infection during blood collection, past history of chronic diseases, individuals on medication affecting blood cell count and individuals with already blood disorder, oil workers and mechanics were the exclusion critera.

5.5. Study Variables

5.5.1. Dependent variables

Hematological parameters are dependent variables;

- ✓ RBC parameters
- ✓ WBC parameters
- ✓ Platelet parameters
- ✓ Peripheral blood morphology

5.5.2. Independentvariables

- ✓ Socio-demographic variables; (age, marital status, educational level...)
- ✓ Smoking habits
- ✓ Duration of exposure
- ✓ protective equipment measure in use

5.6. Measurement and Data collection

5.6.1. Data collection procedure/technique

The estimated number of legal gasoline stations registered in Mekell city in the year 2017-18 was 13 (personal communication with municipalities of Mekelle city Governorates). The stations are distributed in the five sub-cities as follows: Semen (8), Kedamay Weyane (1), Ayder (1), Hadnet (1) and Quiha (2). Out of these 13 gas stations, 9 of them were giving fueling of gas for the automobiles. But the remaining was non-functional due to different reasons; the three are newly opened (less than six months) during data collection and the other one was temporarily closed due to road construction. In the functional gas stations, there were a total of 49 gas fueling attendant. A total of 43 adult male and female aged 18-60 years ; working at least for six months at those gas stations volunteered to participate in the study were all recruited. Age and sex matched control participants working away from any source of gasoline exposure from Mekelle University, Ayder Comprhensive Specialized Hospital Department of Medical Laboratory staffs and graduate students, were recruited in this study by using a non-probability convenient sampling method.

5.6.2. Data collection procedure

Data were collected over three month's through questionnaire interviews which were conducted by the principal investigator. The questionnaire was mainly based on multiple-choice and yes or no questions focusing on socio-demographic data, age, gender, job duration (years), working time (hour/day) and knowledge on gasoline exposure, utilization of protective methods, and health status, smoking and getting any medications.

Blood was collected from participants who complete the questionnaire and who agreed to give blood. After collection, blood analysis was done for the hematological parametres using sysmex XP-300 a three differential hematology analyzer and peripheral blood morphology was examined in three stare laboratory.

5.6.4. Blood sampling and processing

Out of 49 gasoline station workers, a total of 43 workers gave a blood sample for analysis. Four of the workers were not volunteers to participate, and the two were excluded due to acute infection and pregnancy cases. Controls who gave blood sample were 77 participants selected

from Mekelle Universty MSc students and Mekelle Universty department of loboratory workers population who almost have no history of being exposed to gasoline and matching the study group in age, sex and residence. Blood samples were collected by the principal investigator from the antecubital area of the workers and controls hands after cleaning with 70% ethanol. About 5 ml of blood was collected using vacutainer tube containing, Ethylene Dieamine Tetra Acetic Acid (K_2EDTA) by standard venous-puncture method from each of the individual willing to participate in this study during their routine work hours. As soon as the sample is collected and labeled, it was transported to Ayder Comprehensive Specialized Hospital Central Laboratory to be analyzed. During collection of venous blood, four smears were prepared for each participant using fresh whole blood for peripheral blood morphology examination.

5.6.5. Laboratory analysis

5.6.5.1. Complete blood count (CBC)

A complete blood count (CBC) tests including RBC count, Hb and Hct levels, Platelet, MCV, MCH, MCHC, mean platelet volume, and red blood cell distribution width (RDW), total and differential counts of white blood cells (WBCs) were analyzed using the 3-part hematological auto analyzer (Sysmex XP-300, symex corporation, Kobe, Japan) with in a 4 hour blood collection. Sysmex XP-300 performs rapid and accurate analysis of a 20-parameter CBC, including a 3-part WBC differential with an Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC).

5.6.5.2. Peripheral blood morphology

Peripheral blood smears were prepared from all participants for investigation of red blood cell morphology, white blood cell abnormality and platelets abnormalities.

5.6.5.3. Peripheral blood film preparation and examination

A properly prepared blood film is essential to accurate assessment of cellular morphology. A variety of methods are available for preparing and staining blood films, but in this study a wedge method of thin blood film preparation was used because it is a convenient and commonly used technique for making peripheral blood films. The blood films were fixed to the glass slide by the methanol in the stain. After drying the film, Giemsa stains was added to stain the cells. The stained and air dried slides were examined using a microscope. Examination of the blood film is

a multistep process. Initially, the film was examined with a scan of the slide using the 10x or low-power objective. This step is necessary to assess the overall quality of the film, including abnormal distribution of RBCs, suggesting the presence of rouleaux or autoagglutination, and/or the presence of a disproportionate number of large nucleated cells such as monocytes or neutrophils at the edges of the film. The next step is evaluation of WBC, RBC and platelet. This was done in the same area of the film using the 100x oil immersion objective [53].

5.7. Data Quality Assurance

The quality of any research depends on the quality of data that are used as input for that research. Therefore the quality of the blood and the participant information were ensured by collecting and processing using a standard operating procedure.

Pre-analytic

- The blood was collected from the participants groups using the right tube following standard operating procedure.
- The blood sample container was labeled with participant’s unique code to minimize errors.
- The quality of the collected samples was checked like hemolysis, clot, correct volume etc.
- Site assessment and pre-test of data collection were done prior to data collection and the collected data from the study goup was checked for completeness.
- Qualities of the information that are collected by questionnaire from the participants were ensured by principal investigator.

Analytic

- The reliability of the study finding especially the analytical part was guaranteed by implementing Quality Controls (QC) sample for the complete blood count and peripheral morphology through the whole processes of laboratory works.
- The manufacturer and laboratory SOP for analytical phase were followed.

Post analytic

- The results of the Complete Blood Count (CBC) and peripheral morphology were registered with correct value and units.
- Data were entered using double entry method to trace data entry errors which has strong negative effect on study results and conclusions.
- All data were properly documented

In short pre-analytical, analytical and post-analytical stages of quality assurance for Complete Blood Count (CBC) and peripheral morphology were strictly followed.

5.8. Data analysis and interpretation:

Data was entered and analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Tables and graphs were used for description of data. The cut-off points for different parameters were defined according to the laboratory standard defined by the manufacturer of instructions. Data analyses was carried by overviewing field questionnaire, coding of questionnaire, choosing data entry mode and data entry, data cleaning, defining and re-coding of certain variables.

The statistical tests of significance were used depending on the nature of data as follows:

The one-way ANOVA test was used for analysis of variance for average hematologicl parametrs as quantitative dependant variable by qualitative variables such as the relationship between hematologicl parametrs by duration of work, age groups, and levels of education and so on. The multiple comparisons were made using the Post hoc test for the duration of exposure.The independent-samples t-test procedure was used to compare means of quantitative variables between gas station workers and controls hematological parameters. Pearson Correlation Coefficient was used to assess measured parameters with years of exposure, working hour and age. The results in all the above mentioned procedures were accepted as statistical significant when the P-value was less than 5% ($P < 0.05$) at 95% confidence intervals.

5.9. Ethical considerations

The study was conducted after approval by the research and ethics committee of department of Medical Laboratory Sciences of Addis Ababa University. The research committee is an

authorized professional body for giving permission to researchers to conduct their studies with ethical concern in the area. An official letter of request was sent to Mekelle University, Ayder Comprehensive Specialized Hospital to obtain approval to carry out hematological analysis in the central laboratories. Gasoline station workers were given an explanation about the purpose of the study and assurance about the confidentiality of the information and that the participation is purely voluntary. Participants were given the hematologic tests result later on.

5.10. Dissemination of results

After conducting the research, the results of the study will be presented to Department of Medical Laboratory Sciences, College of Health Science, AAU and other stakeholders. Moreover, the output of the results will be given to study sites. The results of the study will be submitted to peer review journals for publication.

5.11. Definition of terms

Gasoline –is a transparent, petroleum derivatives liquid that is used primarily as a fuel in internal combustion engine. It consists mostly of organic compounds obtained by the fractional distillation of petroleum, enhanced with variety derivatives.

Benzene- is an important organic chemical compound with the chemical formula C6H6. As it contains only carbon and hydrogen atoms, benzene is classed as a hydrocarbon and it is the main component of gasoline.

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

Hematological parameters – are parameters such as RBC parameters including; RBC count, HB, HCT, RBC indices like MCV, MCH MCHC and RDW; WBC parameters including total WBC counts, differential and absolute number of the five type of WBC and platelet parameters including total platelet count, MPV, PDW. Values outside normal ranges are diagnostic for disorders, including cancer, immune diseases, and cardiovascular diseases.

Petroleum – is a complex mixture of hydrocarbons naturally occurring yellow- to -black liquid, gaseous or solid form that occurs in the earth. The term is often restricted to the liquid form commonly called crude oil.

6. Work flows

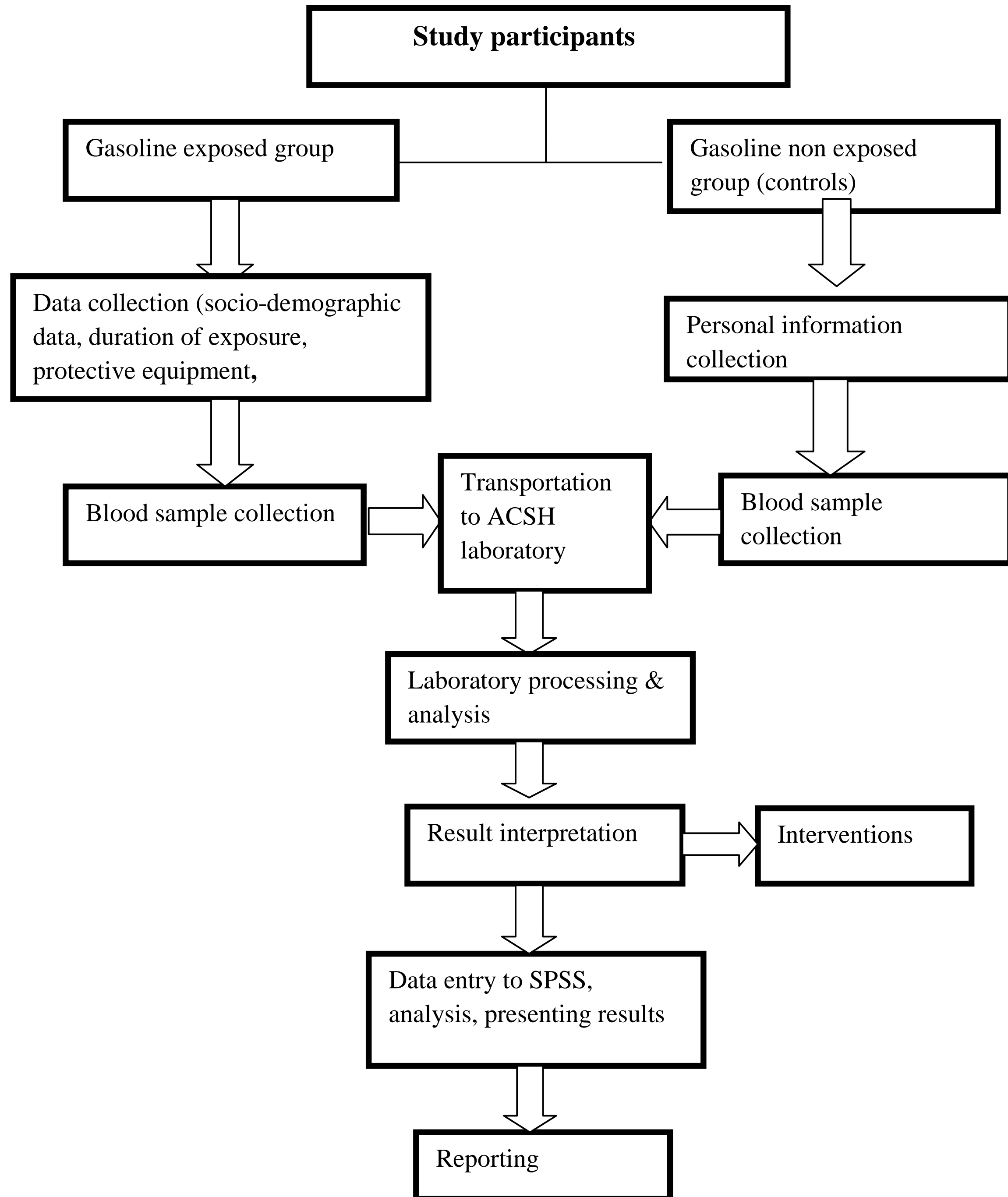

Figure 3; work flow

7. Result

7.1. Sociodemographic characteristics of the study participant

In this study a total of 120 patricipants were entrolled where the exposed group consisted of 43 individuals and the control group consisted of 77 indivduals.There was no statistically significant age differences between the exposed group (mean age±SD=30.09±8.49) and the control group (mean age±SD=29.06±6.07; P=0.444). The age of the participants was between 19-54 years. Table 1, summarizes the personal profiles of study population. The gas station workers were found to be married 21 (48.8%) and 22 (51.2%) were single and the educational status of the study population showed that 31 (72.1%) had finished secondary and preparatory school (Figure 4).

Table-1: Socio-demographic characterstics of the study group at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-April 2018 (n=120).

Variables	Gas station attendant (n=43)	Control group (n=77)	Test of significance
Gender			
Male n (%)	28 (65.1%)	49(63.6%)	^{2} = 0.008, p = 0.540
Female n (%)	15 (34.9%)	28 (36.4%)	
Age in year n (%)			
19-26	17 (39.5%)	29 (37.7%)	
27-34	16 (37.2%)	37 (48.1%)	
35-44	6 (14%)	9 (11.7%)	
>45	4 (9.3%)	2 (2.6%)	
Mean ±SD	30.09 ± 8.49	29.06 ± 6.07	P=0.444

Abbreviations: SD, standard deviation

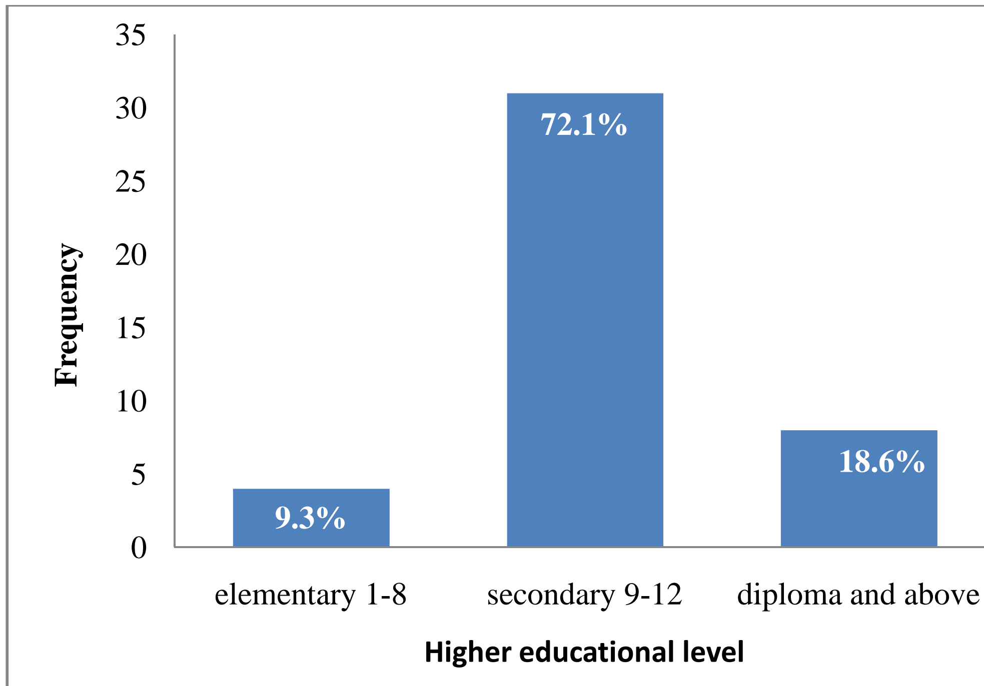

Figure 4; Educational status for the study population at gas station in Mekelle city (n=43)

7.1.2. Work duration, working hours and awairness related to gasoline exposure

The average exposure time was 5.188 ± 4.38 years (minimum 8 months and maximum 16 years) with an average of 11.74 ± 1.89 hours/day. The working hour was more than 12 hours/day among 58.1% and less than 11 hours/ day among 41.9%. The highest number of the workers 17 (39.5%) worked in the gasoline station for less than two years, whereas 14 (32.6 %) and 12 (27.9%) of them worked for more than 8 and 3-7 years, respectively. It is worth mentioning that, all interviewed workers had no history of previous job related gasoline exposure (table 2).

The number of workers who mentioned not eating, not chewing gum and not sucking fuel by mouth were 23 (53.5%), 31 (72.1%), 35 (81.4%), respectively. But all the study population had no history of smoking cigarrets. Moreover, while 18 (41.9%) workers drank milk often and 8 (18.5%) sometimes, ten (23%) of the workers took shower at work place (table 2).

Table-2: Asociated risk factors with gasoline exposure of study groups at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43).

Confounding factors	**Gas station attendant (n=43)**	
Year of expoure	**No**	**%**
<= 2 years	17	39.5
3-7 years	12	27.9
>=8 years	14	32.6
Mean±SD	5.17±4.39	
Daily exposure hour mean±SD	11.16±2.08	
Smoking		
Yes	0	0
No	43	100
Eating		
Yes	20	46.5
No	23	53.5
Chewing gum		
Yes	12	27.9
No	31	72.1
Fuel sucking by mouth		
Yes	8	18.6
No	35	81.4
Taking shower at work place		
Yes	10	23.3
No	33	76.7

SD, standard deviation: CI, confidence interval:

7.1.3. Knowledge of workers regarding routes of gasoline entry into the body, gasoline health effects and gasoline as an environment pollutant

Regarding possible routes of gasoline entry into the body, 39 (90.7%) workers mentioned that inhalation is the route of entry, followed by 28 (65.1%) who reported that skin is the route of entry, and 23 (53.5%) who claimed that mouth is the route of entry of gasoline into the body. A total of 39 (90.7%) workers had knowledge about the health effects of gasoline on human health. It was also found that 34 (79.1%) knew that gasoline is an environmental pollutant (Table 3).

Table-3: Knowledge of workers regarding gasoline among the exposed group at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43)

Gasoline as enviroment pollutant	**N**	**%**
Yes	34	79.1
No	9	20.9
Effect of gasoline exposure on Health		
Yes	39	90.7
No	4	9.3
Through inhalation		
Yes	39	90.7
No	4	9.3
Through skin		
Yes	28	65.1
No	15	34.9
Through mouth		
Yes	23	53.5
No	20	46.5

In general, the protective measures during work in the station were poorly followed. The highest number of workers (n=37, 86.0%) always wore complete dressing (overall gown) (24, 55.8%) wore special shoes or boots always and the lowest number, wore hat sometimes (n=5, 11.62%), face mask sometimes (n=2, 4.7%), and glove sometimes (n=1, 2.3%) table 4.

As displayed in figure 5 the main reason for not using safety equipments was lack of the protective equipements to the workers as 30 (69.77%) of them responded that safety equipments were not provided.

Table-4: Protective measures in use among gasoline station workers at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43).

Protective measure in use					
	Always	Usually	Often	Sometimes	Never
Glove n (%)	-	-	-	1 (2.3%)	42 (97.7%)
Hat n (%)	-	-	2 (4.7%)	3 (7%)	38 (88.4%)
Face mask n (%)	-	-	2 (4.7%)	-	41 (95.3%)
Shoes n (%)	24 (55.8%)	13 (30.2%)	4 (9.3%)	2 (4.7%)	-
Overall n (%)	37 (86%)	6 (14%)	-	-	-

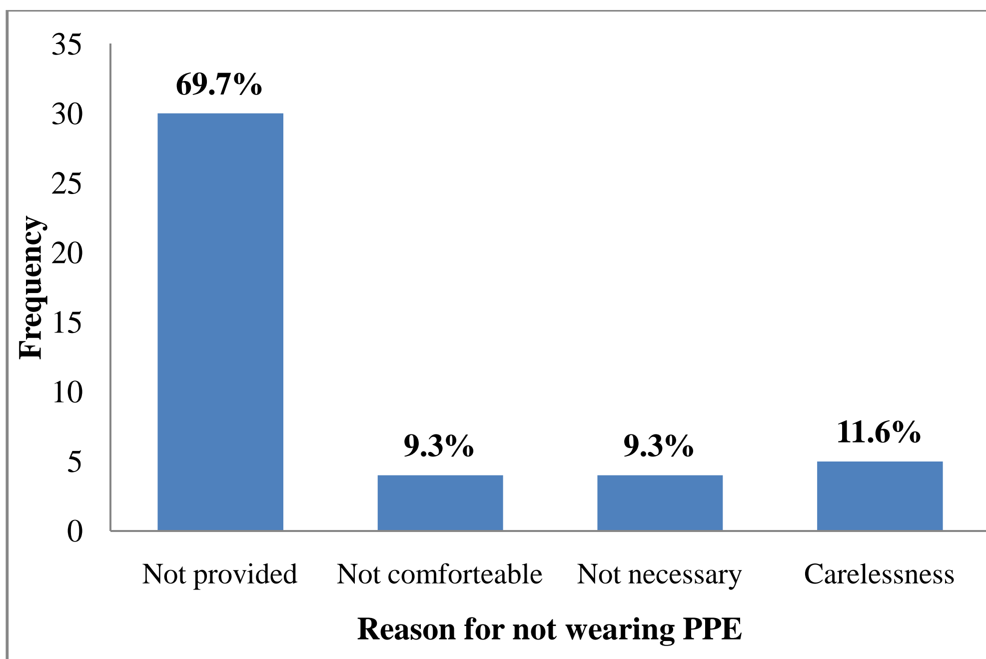

Figure 5: Reasonf for not using safety equipments by gasoline station workers at Mekelle City (n=43)

The assessment also revealed that neither of the worker attended training courses nor they had health professionals visited their station. In addition, no worker had heard about case of gasoline poisoning during their work in the gasoline station. And none of the workers were diagnosed or treated for anemia and leukomia and also niether of them took any medication or treatment drugs.

7.2. Hematological parameters profile of gasoline exposed and control groups

The means and standard deviations of hematological indices of exposed and unexposed groups are presented in Table 5. These results showed that the hematological markers, in both study groups were within the normal ranges.The absolute mean number of RBC (10^{12}/L), percentages of hematocrit (%) at ($p<0.0001$), level of hemoglobin (g/dl) at ($p<0.027$) and absolute mean number of platelets (10^{9}/l) at ($p<0.0001$) were significantly lower among the exposed subjects compared with the control group. The mean cell hemoglobin (pg) and mean cell hemoglobin concentration (g/dl) were significantly higher in the exposed participants compared with the control group at ($p=0.006$). The levels of other hematological parametrs like WBC count (10^{9}/l), nuetrophill percentage, lymphocyte percentage were similar between the groups.

Table-5: complete blood count picture of the study group at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=120).

Parametrs	**Exposed group(n=43) (mean±SD)**	**Control group(n=77) (mean±SD)**	**95% confidence interval of the defference**		
			Lower	Upper	p-value
RBC ($\times 10^{12}$/L)	4.88± 0.573	5.35 ±0.533	-0.674	-0.261	<0.001
HGB (g/dl)	15.04 ±1.33	15.59± 1.25	-1.033	-0.063	<0.027
HCT (%)	43.29 ±3.71	44.95± 3.10	-2.919	-0.403	<0.010
MCV (fl)	87.37 ±4.98	85.87 ±4.36	0.238	3.226	0.090
MCH (pg)	30.48 ±2.06	29.52 ±1.66	0.313	1.686	0.006
MCHC (g/dl)	34.83 ±0.99	34.32 ±0.92	0.153	0.873	0.006
RDW (%)	12.87 0.59	12.95± 0.84	-0.419	0.253	0.626
WBC ($\times 10^{9}$/L)	6.32 ±1.73	6.44± 1.38	-0.692	0.452	0.679
LYM (%)	36.02± 11.20	34.71± 10.04	-2.644	5.254	0.514
NUETR (%)	52.14± 12.05	54.72 ±10.44	-6.739	1.589	0.223
Lyphocyte ($\times 10^{9}$/l)	2.16 ±0.59	2.16± 0.58	-0.224	0.219	0.980
Nuetrophil ($\times 10^{9}$/l)	3.37 ± 1.46	3.60 ±1.31	-0.745	0.289	0.384
Platelete ($\times 10^{9}$/L)	248.95± 58.19	292.45± 62.17	-66.418	-20.583	<0.001
MPV (fl)	10.74 ±1.21	10.96 ±1.78	-3.492	3.054	0.895

HGB, hemoglobin: HCT, hematocrit: RBC, red blood cell: MCV, mean cell volume: MCH, mean cell hemoglobine: MCHC, mean cell hemoglobine concentration: WBC, white blood cell: RDW, red cell distribution width: LYM, lymphocyte: NUETRO, neutrophill: MPV, mean platelet volume, SD, standard deviation: g/dl, gram per decilitter: fl, fomtolitter: pg, pico gram

7.2.1. Hematological parameter and effects of exposure to gasoline with duration of exposure

The self-reported duration of exposure relates with the measured hematological indices shows that, HCT levels (p=0.003), RBC count (p<0.001), HGB concentration (p=0.001), platelete count (p<0.001), MPV value (p=0.005), Lymphocyte percent (p=0.028) and Nuetrophil percent (p=0.027) in the studied group were decreased as the number of reported years of exposure increased. Eventhough they were not statistically significant, the WBC count and other hematological indices like MCV, MCH and MCHC were increased.

Most participants exposed for longer than eight years had significantly lower values of haemoglobin (g/dl) (p< 0.001), PCV (%) (p < 0.001), RBC (10^{12}/l) (p<0.001) and platelte (10^{9}/l) (p<0.001) compared to those exposed for 2 years, respectively. And most subjects exposed longer than eight years have lower value of HGB, RBC, HCT and pletelete compared to those exposed 3-7 years (p<0.010). The average value of RBC, HGB, HCT and platelete were similar with indivduals exposed for < 2 and 3-7 years (Table 6).

Table 6: Comparison of the hematological indices with duration of exposure in exposed groups post hoc analysis at Mekelle City, Tigray Rigion, Northern Ethiopia, from January-Aprile 2018 (n=43)

Year of exposure	**Parameters**	**p-value**
	RBC(10^{12}/l) (mean±SD)	
<=2 year vs 3-7 year	5.17±0.395 vs 5.07±0.579	0.587
<=2 year vs >=8 year	5.17±0.395 vs 4.37±0.402	<0.0001
3-7 year vs >=8year	5.07±0.579 vs 4.37±0.402	<0.0001
	HGB(g/dl)	
<=2 year vs 3-7 year	15.72±1.08 vs 15.25±1.55	0.271
<=2 year vs >=8 year	15.72±1.08 vs 14.03±0.70	<0.0001
3-7 year vs >=8year	15.25±1.55 vs 14.03±0.70	0.010
	HCT (%)	
<=2 year vs 3-7 year	45.15±2.94 vs 43.55±4.86	0.202
<=2 year vs >=8 year	45.15±2.94 vs 40.80±1.59	<0.001
3-7 year vs >=8year	43.55±4.86 vs 40.80±1.59	0.041
	WBC (10^9/L)	
<=2 year vs 3-7 year	5.90±1.28 vs 6.11±1.65	0.744
<=2 year vs >=8 year	5.90±1.28 vs 7.01±2.13	0.078
3-7 year vs >=8year	6.11±1.65 vs 7.01±2.13	0.187
	LYM (%)	
<=2 year vs 3-7 year	37.47±9.84 vs 41.07±12.32	0.366
<=2 year vs >=8 year	37.47±9.84 vs 29.91±9.54	0.053
3-7 year vs >=8year	41.07±12.32 vs 29.91±9.54	0.010
	NUE (%)	
<=2 year vs 3-7 year	50.80±10.47 vs 46.49±13.7	0.318
<=2 year vs >=8 year	50.80±10.47 vs 58.63±9.83	0.062
3-7 year vs >=8year	46.49±13.7 vs 58.63±9.83	0.009
	Patelete (10^9/l)	
<=2 year vs 3-7 year	267.05±54.74 vs 280.16±39.07	0.472
<=2 year vs >=8 year	267.05±54.74 vs 200.21±45.54	<0.0001
3-7 year vs >=8year	280.16±39.07 vs 200.21±45.54	<0.0001

HGB, hemoglobin: HCT, hematocrit: RBC, red blood cell: WBC, white blood cell: LYM, lymphocyte: NUETRO, neutrophill: CI, confidence interval: g/dl, gram per decilitter:

7.2.2. The correlation of hematological indicies with year of exposure, working hour and age of the exposed group at Mekelle City

As illistrated inTable 7, there was a negative correlation between RBC, HGB, HCT and Platelet with duration of exposure (r = -0.619, p<0.001, r = -0.581, p<0.001, r = -0.524, p <0.001, r = -0.499, p = 0.001) respectively. Absolute number of nuetrophil was positively correlated with duration (r = 0.337, p = 0.027).There was also a negative correlation of RBC (r = -0.418, p = 0.005), HGB (r = -0.368, p = 0.015) and platelete (r = -0.330, p = 0.030) with age.

Table 7: Correlation of hematological indices with year of exposure, working hour, and age for the exposed group at Mekelle City (n=43)

Parameters	Year of exposure		Working hour		Age of exposed	
	r	p-value	r	p-value	r	p-value
RBC (10^9/l)	-0.619	**<0.001**	0.292	0.057	-0.418	**0.005**
HGB (g/dl)	-0.581	**<0.001**	0.266	0.085	-0.368	**0.015**
HCT (%)	-0.524	**0.000**	0.332	0.129	-0.270	0.080
MCV (fl)	0.152	0.330	-0.195	0.209	0.172	0.269
MCH (pg)	0.044	0.779	-0.273	0.076	0.050	0.750
WBC (10^9/l)	0.268	0.082	0.017	0.912	0.166	0.289
Lymphocyte(10^9/l)	-0.041	0.794	-0.054	0.730	-0.022	0.890
Nuetrophill (10^9/l)	0.337	**0.027**	0.000	1.000	0.154	0.324
Platelete (10^9/l)	-0.499	**0.001**	0.160	0.307	-0.330	**0.030**
MPV(fl)	0.222	0.153	0.242	0.117	0.113	0.470

HGB, hemoglobin: HCT, hematocrit: RBC, red blood cell: MCV, mean cell volume: MCH, mean cell hemoglobine: WBC, white blood cell: MPV, mean platelet volume, CI, confidence interval: g/dl, gram per decilitter: fl, fomtolitter

7.3. Peripheral blood morphology examination results

Of the examined peripheral blood films from the exposed group, 37 (79.1%) of the result were normal, 4 (9.3%) had macrocytosis with increasing corrossponding MCV value, 4 (9.3%) had basopillic stipling inclusions and one participant had microcytic red cells with reduced MCV value (77.1 fl).

8. Discussion

This study aimed to assess the effect of gasoline and gasoline products exposure on hematological parameters among fuel station workers as compared with controls in Mekelle City, Tigray Region. It also tried to identify risk factors associated with gasoline product exposure in gasoline station workers and the relationship between durtion of gasoline exposure and hematological parameters. The findings are pointing toward the fact that gasoline and its content have an advers effects on the hematological functioning with a longer period of exposure.

The observed statistically significant decrease in the measured parameters such as red blood cell (RBC), haemoglobin (HGB) concentration, hematocrit (HCT) and platelet (PLT) count compaired to control may be due to several toxic effects arising from the gasoline and its products. Benzene, one of the main constituents of gasoline, is a well known systemic toxicant in humans at any concentration and a cause of aplastic anemia. It is hematotoxic and depresses the bone marrow, leading to pancytopenia (a general depression of erythrocytes (red blood cells), leucocytes (white blood cells) and thrombocytes (platelets) [13, 15, 16, 17, 30, and 31]. These studies demonstrate that benzene is indeed a haematotoxicant.

The results of the present study showed that the mean hemoglobin concentration, mean RBCs count, mean hematocrit value, platelet count of petrol station workers were significantly lower than those of the comparison group. Decreased in hemoglobin content and RBC count could be attributed to shortened life span of RBC as well as impairment of heme synthesis by the metabolic end product of free radicals of benzene and other aliphatic hydrocarbon constituent of gasoline. These free radicals can alter the erythrocyte membrane and heme protein synthesis in bone marrow [26, 27]. This result is similar with the study conducted in hematological assessment of gasoline exposure among petrol filling workers by Schnatter AR *et al* [37], Nair DS *et al* [47, 48], Neghab M *et al* [36], Kamal A *et al* [39], Ali A *et al* [42], Elderdery AY *et al* [51] and Abdrabouh A *et al* [52]. A decrease in RBC count can lead to decrease in PCV. But the hematocrit, RBC count, hemoglobin and platelet count were not consistent with those reports which described the hematological changes of individuals occupationally exposed to gasoline were significantely high, like by Saadat M *et al* [38], and by Firouzkouhi M *et al* [45]. Reduction

in the value of RBC, HCT and HGB content as reported in this study is suggestive of anemia a condition which agrees with the report of Uko EK *et al* [50].

The present study indicated that MPV value, RDW value, and mean white blood cells (WBCs) count, were lower among petrol station attendants with non-significant difference between both groups which is similar to finding of Saadat M *et al* [38]. Eventhough leukocyte counts were within the normal range, they showed non-significant decrease as compared to controls. This might be due to small exposure time of most of the participants. Another study conducted in Shiraz Iran reported that hematocrit value was significantly decreased which agree with the current results and the study reported the RDW was significantly increased which is not consistent, Neghab M *et al* [41]. In this study, mean MCH and MCHC value were significantly higher in petrol station attendants than comparison group while mean corpuscular volume (MCV) was slightly increased in exposed groups with statistically non-significant difference between them. This findings is in agreement with another study by Firouzkouhi M *et al* [45] and by Nair DS*et al* [47] as they observed statistically significant increase in MCH and MCHC value ($p < 0.001$) and that of current study was also statistically significant ($p = 0.006$). Even though the MCV value is not significantly higher in the exposed group ($p=0.090$), it shows a similar increase with result reported by Schnatter AR *et al* [37]. The increase in MCV, MCH and MCHC in this study can be due to macrocytosis induced by benzene, because benzene is an ingredient of petrol. It has been established that toxic constituents of petroleum such as benzene and lead are activated in the bone marrow, where the substances exert cytotoxic effect that could be mediated through destruction in DNA function. A defect in DNA synthesis that interferes with cellular proliferation and maturation can lead to large` erythrocytes [24]. There were neither increase nor decrease in mean absolute number of lymphocyte and nuetrophill of gasoline exposed compaired to control ($p =0.980$ and $p = 0.384$) respectively. And this result is different with result reported by Bedekar NY*et al* [48] and Saadat M *et al* [38], where they observed a decrease in lymphocyte and nuetrophill count ($p <0.05$). This difference might be due to short exposure duration of most participants in the present study.

And this study indicates that total RBC count, hemoglobin concentration and HCT value were decreased in workers with longer periods of exposure as the duration of exposure increase from less than 2 year to more than 8 years ($p<0.001$). The results of this study were different from the

studies conducted by Uzma N *et al* [46], which showed that during the early period of exposure (1-5 years & 5-10 years), the average Hb, HCT and RBC count were unchanged, which were similar with the present study, but as the year of exposure increases to more than 10 years there was statistically significant increase ($p<0.05$) in the concentration of Hb, RBC count and HCT value. Platelets count of the present study was significantly decreased as year of exposure increase from two year to eight year and is consistent with previous study Uzma N *et al* [46]. But the decrease in total RBC count, hemoglobin concentration and HCT value in workers with longer periods of exposure as the duration of exposure increase from less than 2 year to more than 8 years were consistent to study conducted by Uko EK *et al* [50]. In the present study the reported WBC count was unchanged as year of exposure increase but it was decreased in the study done by Uko EK *et al* [50].

The peripheral blood smear of the study participants showed (9.3%) basophilic stippling and (9.3%) macrocytosis. In line with this finding Firouzkouhi M *et al* [45] and Uko EK *et al* [50] reported basopilic stippling and macrocytosis respectively from the gasoline exposed attendants but is not consistent to Elderdery AY *et al* [51] which reported regarding cell morphology 50% of the participants showed a microcytic picture and a quarter (26%) show normocytic picture. This inconsistency maight be because of method and/or equipement, performance and the gasuline constituent difference in the study.

9. Conclusion and Recommendation

9.1. Conclusion

In conculusion, the present study has shown that occupational exposure to gasoline and its constituent product have a significant effect on some hematological parameters. And also this study showed that long term exposure to petrol fumes might have deleterious effects on human hematopoietic system leading to bone marrow depression. It has been observed that chronic ingestion of gasoline might result in a significant reduction in RBC count, Hb concentration and total platelete count, as the hematopoietic system is the major target of chalenge. It is, therefore, concluded that gasoline and some of its product are highly toxic and damaging to hematopoietic system.

9.2. Recommendation

The study showed quite large numbers of the workers are not using protective devices. Thus, the concerned ministries with labor unions and medical occupational and environmental health professionals are recommended to develop and enforce strict regulations, instructions, guidelines of occupational health and safety to protect workers.

Occupationally exposed workers to gasoline and its products should be provided with appropriate personal protective equipment and respiratory protection devices like (gawons, mask, hand gloves and goggles) to reduce the effect of exposure.

The gasoline station owners should provide hygiene facilities in their stations such as; change area, showers, and eating facilities. These measures can potentially reduce the hematological effects of occupational exposure to gasoline and benzene.

Occupationally chronic exposed workers should have periodic medical examinations to include the evaluation of their hematological profile and measurement of blood benzene and blood lead levels. In this regard, Ministry of health is recommended to avail blood benzene and blood lead level analyser automachine. Further research is recommended to include other gas station workers with large sample size and to measure the blood benzene level and blood lead level of these occupatiionally gasolines exposed workers.

10. Strength and Limitation

10.1. Strength of the study

- ✓ The study is the first of its kind in our country in the assessments of hematological parameters to gasoline at gas station.
- ✓ Assessment/analysis of the parameteres was carried out using the same anticoagulant and hematology analyzer.

10.2. Limitation of the study

- ✓ The number of exposed subjects and controls individuals included in this study was 43 and 77 respectively. Larger participant and surveillance over a longer period of years (> 5 years) may have been more informative.
- ✓ The blood benzene concentration and blood lead level tests were not measured due to lack of method to analyze and failed to produce comparison with hematological indices.
- ✓ The current study did not have baseline information on the hematological profile of the petrol station attendants when they first started work as petrol station attendants. Workers were tested once and categorized subjects based on their years of exposure. A better design would have been to test the subjects at the time they first started work as petrol station attendants, and then again at serial intervals to see change on hematological indices. However, non-exposed controls were used for comparison to minimize this limitation.

11. References

1. Baynes WJ, Dominiczak HM. Oxygen and life. *Medical Biochemistry*. Second Edition. Philadelphia: Elsevier Mosby Ltd. 2005: 497-506.
2. Doulatov S, Notta F, LaurentiE, and Dick JK. Hematopoiesis: a human perspective.*Cell Stem Cell*. 2012; 10(2):120–36.
3. Sembulingam P, Sembulingam K. *Essentials of Medical Physiology*: 6thedition. Vol 6. India: Jaypee brothers, 2012: 56-98.
4. Hymavathi V, Rao LM. Effect of sub lethal concentrations of lead on the hematology and biochemical constituents of Channapunctatus. *Bull .Pur. Appl. Sci;* 2000: 19A: 1-5.
5. Mandiracioglu A, Akgur S, Kocabiyik N, Sener U. Evaluation of neuropsychological symptoms and exposure to benzene, toluene and xylene among two different furniture worker groups in Izmir. *Toxicology and idustrial health*. 2011; 27(9):802-9.
6. Wixtrom RN, Brown SL. Individual and population exposures to gasoline. *J Expo Anal Environ Epidemiol*. 1992; 2(3): 23-78.
7. Carballo M, Nigro ML, Fraga I, Gadano A. Ethylene oxide: cytogenetic and biochemical studies in persons occupationally exposed. *Environ Mole Mutagen.* 1994; 23(8): 7-12.
8. Mehlman MA. Dangerous properties of petroleum refining products: carcinogenicity of motor fuels (gasoline). *Teratogenesis, Carcinogenesis, and Mutagenesis*. 1990; 10(5):399–408.
9. Caprino L, Togna GI. Potential health effects of gasoline and its constituents: A review of current literature (1990-1997) on toxicological data. *Environmental Health Perspectives*. 1998; 106(3):115.
10. Kirchstetter TW, Singer BC, Harley RA, Kendall GR, Traverse M. Impact of California reformulated gasoline on motor vehicle emissions and Volatile organic compound speciation and reactivity. *Environ Scie Technology*. 1999; 33(2):329–336.
11. Madhoun A, Ahmed W, Ramli NA, Yahaya AS, Yasuf MD, Fitri NF, et al. A Framework for Monitoring and Modeling of BTEX in Various Development Statuses in Penang, Malaysia. Report USM (MY): ICENV; 2008; Available from: http://eprints.usm.my/13180/1/a_ framework_for_monitoring.pdf. Accessed on September 22, 2017.

12. World Health Organization/International Agency for Research on Cancer (2000) Monographs on the evaluation of carcinogenic risks to humans, vol 77. IARC, Lyon, France:https://monographs.iarc.fr/ENG/Monographs/vol77/mono77.pdf. Accessed on December 5, 2017.
13. Okonkwo COJ, Ehileboh AD, Nwobodo E, Dike CC. The effects of acute gasoline vapour inhalation on some hematological indices of albino Wistar rats. *Journal of Acute Disease*. 2016; 5(2):123–5.
14. Graboski M. An analysis of alternatives for unleaded petrol additives for South Africa, report for the United Nations Environment Program. 2003 May. Available from: http://www.unep.org/transport/pcfv/PDF/ PubGraboskiReport.pdf. Date Accessed 8 Oct 2017.
15. Cocheo V (2008).Polluting agents and sources of urban air pollution.*International Agency for Research on Cancer (IARC)*, Annali dell'lstituto superiore di sanita.2000; 36(3):267- 74.
16. World Health Organization (2000) Benzene. In: *Air Quality Guidelines for Europe*, 3rd edn. WHO Regional Office for Europe, Copenhagen, pp 62–66.
17. Kim SY, Choi JK, Cho YH, Chung EJ, Paek D, Chung HW. Chromosomal aberrations in workers exposed to low levels of benzene: association with genetic polymorphisms. *Pharmacogenet and Genomics. 2*004; 14(7):453–63.
18 Ross D. The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues. *Journal of Toxicol and Environment Health Part A*. 2000; 61(5–6):357–72.
19. Smith MT. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. *Environmental Health Perspectives.* 1996; 104 (suppl 6):1219.
20. US Department of Health and Human Services. The health consequences of involuntary exposure to tobacco smoke: a report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health. 2006 Jun 27; 709.
21. Harper C, Liccione JJ. Toxicological Profile for Gasoline. *Agency for Toxic Substances and Disease Registry (ATSDR)*. June 1995.

22. Mitri S, Fonseca AS, Otero UB, Tabalipa MM, Moreira JC,Sarcinelli PD. Metabolic Polymorphisms and Clinical Findings Related to Benzene Poisoning Detected in Exposed Brazilian Gas Station Workers. *International journal of environmental research and public health.* 2015; 12(7):8434-47.
23. Carbonari D, Chiarella P, Mansi A, Pigini D, Iavicoli S, Tranfo G. Biomarkers of susceptibility following benzene exposure: influence of genetic polymorphisms on benzene metabolism and health effects. *Biomarkers in Medicine*. 2016; 10(2):145-63.
24. Khisroon M, Gul A, Khan A, Ali N, Zaidi F, Rasheed SB, Ahmadullah et al. Comet assay based DNA evaluation of fuel filling stations and automobile workshops workers from Khyber Pakhtunkhwa province, Pakistan. *Journal of Occupational Medicine and Toxicology.* 2015; 10(1): 10-27.
25. Beceren A, Akdemir N, Omurtag GZ, Tatlıpınar ME, arda S. DNA Damage in Gasoline Station Workers Caused by Occupational Exposure to Petrol Vapour in Turkey. *ACTA PharmaceuticaSciencia*. 2016; 54(1):53-62.
26. Knutsen JS, Kerger BD, Finley B, Paustenbach DJ. A calibrated human PBPK model for benzene inhalation with urinary bladder and bone marrow compartments.*Risk Analysis.* 2013; 33(7):1237-51.
27. Sun R, Zhang J, Yin L and Pu Y. Investigation into Variation of Endogenous Metabolites in Bone Marrow Cells and Plasma in C3H/He Mice Exposed to Benzene. *International journal of molecular sciences.* 2014; 15(3): 4994-5010.
28. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, et al. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochemical Juornal.*1994; 300 (1): 271–6.
29. Sorsa M, Osterman-Gokar S, Peltonen K, Saarikoski ST, Sram R. Assessment of exposure to butadiene in the process industry.*Toxicology.* 1996; 113(1-3): 77–83.
30. Smith MT. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. *Environmental Health Perspective*. 1996; 104 (6): 1219–1225.
31. Li K, Jing Y,Yang C, Liu S, ZhaoY, He X. Increased leukemia associated gene expression in benzene-exposed workers. *Scientific Reports*. 2014; 4:5369.
32. Seriki SA, Adebayo OF, Shafe MO, Bivan ZK. Effects of Gasoline Poisoning on Some Hematological Parameters. *Annalas Hematologic Oncology*. 2016; 3(4): 1087-1095.

33. Smith MT. Advances in understanding benzene health effects and susceptibility. *Annual Review of Public Health.* 2010; 31:133-48.
34. Abubakar BM, Abdullah WZ, Sulaiman SA, Ang BS. The Effects of Exposure to Petrol Vapourson growth, haematological parameters and oxidative markers in Sprague-Dawley male rats. *The Malaysian journal of medical sciences: MJMS.*2015; 22(1): 23.
35. Yoon BI, Hirabayashia Y, Kawasaki Y, Kodamaa Y, Kanekoa T, Kim DY, Inoue T. Mechanism of action of benzene toxicity: Cell cycle suppression in hemopoietic progenitor cells (CFU-GM). *Experimental Hematology.*2001; 29 (3): 278–285.
36. Hameed FR, Abd-Alhusein A, Salim A, Hussein M. Effect of benzene on some haematological parameters of oil station workers. *Ibn Al-Haitham journalfor Pure & Appllied Science.*2009; 22(4); 26-32.
37. Schnatter AR, Kerzic PJ, Zhouc Y, Chena M, Nicolichd MJ, Lavellea K, et al. Peripheral blood effects in benzene-exposed workers. *Chemico-Biological Interactions*. 2010; 184 (1-2): 174–181.
38. Saadat M, Bahaoddini A. Hematological changes due to chronic exposure to natural gasleakage in polluted areas of Masjid-i-Sulaiman (Khozestan province, Iran). *Ecotoxicology and Environmental Safety*. 2004; 58 (2): 273–6.
39. Tunsaringkarn T, Soogarun S, Rungsiyothin A, Zapuang K, Chapman RS. Health Status of Gasoline Station Workers in Pathumwan Area, Bangkok, Thailand, In 2004 and 2009.Journal of Health Research. *2011;* 25(1): 15-19.
40. Neghab M, Hosseinzadeh K, Hassanzadeah J. Hematological study of petrol station workers exposed to unleaded petrol. *Toxicological & Environmental Chemistry.* 2014; 96(6): 951-61.
41. Ali A, Sahb A. Hematological assessment of gasoline exposure among petrol filling workers in Baghdad. *Journal of the Faculty of Medicine*. 2011; 53(4): 396-400.
42. Shilpi GS, Dar MS, Baghel A, Singh A, Sonam M. Cytogenetic Biomonitoring among Petrol Filling Station Workers; a hematological and micronucleus study. *International Journal of Contemporary Medical Research*. 2016; 3(7):2060-2063.
43. Lavanya M, Rajanand G, Kumar ZN. A study of blood cell count in petrol pumps workers. *Journal of Evolution of Medical and Dental Sciences*. 2016; 5(89): 6611-4.

44. Firouzkouhi M, Abdollahimohammad A, Babaiepur-Diveshali M, Firouzkouhi A, Shaikh M. Effects of Gasoline on Blood, Kidney and Liver Parameters of Unregulated Gasoline Traders. *Der Pharmacia Lettre*. 2016; 8(8):58-61.
45. Uzma N, Salar KM, Kumar S, Aziz N, David MA, Reddy VD. Impact of Organic Solvents and Environmental Pollutants on the Physiological Function in Petrol Filling Workers. *International Journal of Environmental Research and Public health* 2008; 5(3): 139-46.
46. Nair DS, Bedekar MY, Agrawal MJ. Deleterious Effects of Petrol Fumes on Erythrocytes. *International Journal of Health Sciences and Research (IJHSR).* 2015; 5(9):237-41.
47. Bedekar MY, Nair DS, Agrawal MJ. Toxic effect of petrol fumes on white blood corpuscles. *Annals of Applied Bio-Sciences.* 2015; 2(3):57-59.
48. Emelike OF, Okhuakhua OJ, Momodu MM, Ogunsuyi OE, Ezimah AC. Some Haematological Parameters of Petrol Attendants and Automobile Mechanics in Ekpoma, Edo State, Nigeria. *School of Journal and Applied Medical Science (SJAMS).* 2015; 3(3):1400-03.
49. Uko EK, Erhabor O, Bashiru GA, Isaac IZ, Abdulrahaman Y, Aghedo F, et al. Some Hematological Indices Among Petrol Station Attendants Occupationally Exposed To Premium Motor Spirit In Sokoto, North Western, Nigeria. *Journal of International Research in Medical and Pharmaceutical Sciences.* 2015; 4(2): 53-58.
50. Elderdery AY, Elnour AM, Ali NY, Elsaken AA, AbdElgabar RE, Elbashier RA, et al. Alterations in hematological parameters among workers of fuel stations in White Nile State, Sudan. *International Journal of Biomedical and Advance Research*. 2015; 6(11): 780-784.
51. Abdrabouh A, El-Wakf A, Hagras A, Elgharieb A. Health Assessment Approach for Evaluating Hematologic and Immune Toxicity of Prolonged Gasoline Inhalation in Fuel Station Workers at Mansoura City, Egypt. *Journal of Appllied Environmental and Biological Science*. 2017; 7(8):30-36.
52. Federal Democratic Republic of Ethiopia Central Statistics Agency. Population projection of Ethiopia for all regions at wereda level from 2014-2017. 2013; Addis Ababa.
53. Keohane EM, Smith LJ, Walenga JM. Rodak's hematology: clinical principles and applications. 5th ed. St. Louis: Elsevier Health Sciences; 2016.

12. Annexes

Annex-I; Participants' information sheet (English version)

Principal investigator; GebreTeklu Embay

Addis Ababa University College of Health Science

Title of the research project: Assessment of hematological parameters among gasoline exposed at gas station workers; community based study, in Mekelle City, Tigray Region, Northern Ethiopia.

Introduction: You are being invited as study participant of this study conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research team will include two principal investigators, four advisor from Addis Ababa University. Please take as much time as you need to read or to listen the information sheet. The study has been approved by Addis Ababa University Medical Laboratory Science department research ethics committee.

Purpose of the research project: We are asking you to take part in this study because we will assess the effect of gasoline and gasoline products exposure on hematological parameters among fuel station attendants/workers in Northern Ethiopia, Tigray Region, Mekelle City.

Risks associated with the study: there will be a minor discomfort during blood specimen collection. During collection of blood specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professional. If any serious risk happened, appropriate medical care will be provided to you.

Procedures and what will be expected from you for participation: in order to conduct the indicated study at Mekelle City Gas station, you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose only. After consent, 5 ml blood specimen will be collected from you by specimen collectors.

Incentives and compensation; You will not receive any payment for your participation in this research project as compensation. But you will get your results for free and if there is any change in your result it will be interpreted and communicated with physicians. However, this study will

benefit at large those individuals like you who are exposed to gasoline to take appropriate safety measures.

Confidentiality of your information: we respect your privacy and confidentiality. The information will collect from you as part of the study will kept in a locked cabinet, or be protected by a password on the computer only accessible to the personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Participation and withdrawal from the study: the participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequence. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory results for free.

Agreement

After reading and listening about the study procedures and other related issues done in the study, you will be kindly requested to put your signature of agreement. Your signature indicates that your participation is purely voluntary.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

GebreTekluEmbaye (BSc) Tel: +251914155120 E-mail: gebreteklu30@gmail.com

Aster Tsegaye (MSc, PhD) Tel: +251911696085 E-mail: tsegayeaster@yahoo.com

Mikias Negash (MSc, PhD follow) Tel: +251913724956 Email: mikias2@gmail.com

Annex-II; Information sheet Amharic version

የተሳታፊዎችፍቃድመተማመኛቅፅ

ጥናት የሚያካይደው ሰው ስም: ገብረ ተክሉ እምባየ

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

መግቢያ: በአድስ አበባ ዪኒቨርስቲ ጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዛል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት፤ ከዚህ ቀጥሎ የሚገኘውን ንባብ በጥሞና ያንቡቡና ግልፅ ያለሆነሎዎትን ማንኛውምን ሃሳብ ይጠይቁ።

የጥናቱርእስ: በተለያዩ የነዳጅ ማደያ የሚሰሩ ሰራተኞች በቤንዚን ምክንያት በደማቸው በተለያዩ የደም ዓይነቶች ላይ የሚያመጣው ጉዳትመ ጠኑን ለማወቅ ነው።

የጥና ቱዓላማ: በነዳጅ ማደያ የሚሰሩ ሰዎች ተገቢውን የህክምና ክትትል ወይም ተገቢውን ምርመራ እንድያደርጉ ነው። እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል። የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ ለሙሉ በበጎፍቃደኝነት ላይ የተመሰረተ ነው። በዚህ ጥናት ለመሳተፍ ከወሰኑ በኃላ ለማቃረጥ የሚወስኑ ቢሆንም እንካዋን የሚጠቅም እንደሆነ በግምት ውስጥ ማስገባት ያስፈልጋል።

በዚህ ጥናት መሳተፍ የምያስከትላቸው ችግሮች ምንድ ናቸው?

የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ልያስከትል ይችላል። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ባላቸዉ ባለሞያ ስለሚመደብና አስፈላጊውን ጥንቃቄ ስለሚወስድ የህመም ስሜት አይኖርም።

በጥናቱ ተሳታፊ ለመሆን የሚጠበቅቦዎት ምንድ ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ 5 ሚ.ሊ የደም ናሙና እንደሚወስድና ለጥናቱ እንደሚውል መስማማት ይጠበቅቦዎታል። ከተወሰደ ናሙና ላይ የሚገኙ ለስራ አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠብቅቦታል።

በዚህ ጥናት መሳተፍ የሚያስገኘው ጥቅም ምንድ ነው ?

ይህንን ጥናት በማስተርስ ድግሪ መመሪቅያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈሎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም በጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ኖዎት።

ለጥናቱ በተወሰደ ናሙና ላይ የሚገኘውን ውጤት በነፃ ያገኛሉ። በተጨሪም በውጤቱ ላይ ለውጥ ካለው ከሀኪሞችን እንዲገናኙና እንዲመረመሩ ይደረጋል። የእርስዎ ተሳትፎ የእርስዎንና እርስዎን የመሰሉ ስራቶኞች የጤና ሁኔታንና አስፈላጊ ጥንቃቄ እንዲያደርጉ ይጠቅማል።

የህክምና መረጃ በሚስጢር ጠብቆ መቆየት የሚችለው እንዴት ነው?

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

በዚህ ጥናት ተሳታፊ የመሆኖዎ መብቶት ምንድ ናቸው

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብትዎን የተጠበቀ ነው። የደም ናሙና ያለመስጠት መብቶ የተጠበቀ ነው። ከዚህ በተጨማሪ ጥናቱን በተመለከተ ማንኛው ን አይነት ጥያቄ የመጠየቅ ወይም ገለፃ የማግኘት መብት አሎት። እርስዎ በሚሰጡን መረጃ የችግሩን ስፍት ለመከላከልና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብሎዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክባሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛምዶ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ

በሚከተለውን አድራሻ ይጠቀሙ።

ጥናቱን የሚያከህደው ሰው ስም: ገብረ ተክሉ ሞባይል; 251914155120 ኢሜል; gebreteklu3o@gmail.com

አማካሪዎች ዶ/ር አስቴርፀጋዬ ሞባይል: +251911696085: ኢሜል፤ tsegayeaster@yahoo.com

ሚኪያስ ነጋሽ ሞባይል: +251913724956፡ ኢሜል፡ mikias2@gmail.com

Annex III-Information sheet Tigrigna version

ንተሳተፍቲ ሓበሬታ መውሃቢ ቅጥዒ

ናይ መፅናዓይ ሽም: ገብረ ተኽሉ እምባየ

ናይቲ ትካል ሽም: አዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

መእተዊ: ብአድስ አበባ ዩኒቨርስቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ብማስተርስ ድግሪ ተምሃራይ ዝካየድ ናይ መመረቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኾኑ ተዓድሞም አለዉ። በጃኹም/ኽን አብዚ መፅናዕቲ ንምስታፍ ቅድሚ ምስምመፆም ፤ ካብዚ ቐፅሉ ዘሎ ንባብ ብፅሞና ምስ አንበቡ ግልፅ ዘይኾነሎም ዝኾነ ይኹን ሓሳብ ይሕተቱ ።

ናይቲ መፅናዕቲ ርእሲ፡ አብ ናይ ጋዝ ነዳዲ መዐደሊ ድርጅት ዝሰርሑን ብስርሖም ምኽንያት ብጋዝ ነዳዲ (ቤንዝን) ዝጥቅዑ ሰራሕተኛታት አብ ደሞም ውሽጢ ዘሎዉ ዋህየታት ደም ጥዕኖምን መጠኖምን ምምርማርን ምዕቃንን እዩ።

ናይቲ መፅናዕቲ ዋና ዓላማ፡-አብ ነዳዲ መዐደሊ ዝሰርሑ ሰባት አድላዪ ናይ ሕክምና ክትትል ወይ ከዓ አድላዪ ምርመራ ንኽገብሩ ምሕባር እዩ። ስለዚ ንሶም/ሰን አብዚ መፅናዕቲ ንምስታፍ ጠቓምን ምቹውን ኾይኖም ተመርፆም አለዉ። ናቶም/ናተን አብዚ መፅናዕቲ ዝህልዎም ተሳትፎ ሙሉእ ብሙሉእ ብዓርሰ-ፍቃደኝነት ዝተመስረተ እዩ ። አብዚ መፅናዕቲ ንምስታፍ ድሕሪ ምውሳኖም ምቁራፅ ዝኽእሉ እንትኾኑ እኳ ጠቓሚ ከምዝኾነ አብ ግምት ውሽጢ ምእታው የድሊ።

አብዚ መፅናዕቲ ብምስታፍ ዘሰዕቦም ፀገማት እንታይ እዮም?

ንምርመራ ዝከዉን ደም አብ ዝህብሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም አየጋጥሞምን። ነገር ግን ደም አብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉይ ኽእል እዩ። ይኹን ዳአምበር ደም ንምስብሳብ ልምዲ ብዘለዎም ባዓል ሞያታት ስለ ዝምደቡን አድላዪ ዝኾነ ጥንቃቐን ስለዝውሰድ ናይ ምሕማም ስምዕት አይህሉን።

አብዚ መፅናዕቲ ተሳታፊ ንምኻን ዘድልዩ ነገራት እንታይ እዮም?

አብዚ መፅናዕቲ ንምስታፍ እንድሕር ተስማምዖም 5 ሚ.ሊ ዝከዉን ደም ከምዝውሰድን ነቲ መፅናዕቲ ከምዝውዕል ምስምማዕ የድልዮም እዩ። ካብ ዝተወሰደ ደም ዝርከቡ ዉፅኢት ነቲ ስራሕ አግባብነት ዘለዎም ሰባት እንተተነጊሩ ዘይቃወሙ ምኻኖም ምስምማዕ የድልዮም።

አብዚ መፅናዕቲ ብምስታፍ ዝርከቡ ጥቕምታት እንታይ እዮም ?

እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቕ ፅሕፍ ከም ምኻኑ መጠን አብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቕሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቃሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት ብነፃ ይረኽቡ እዮም። ብተወሳኺ አብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኸቡን ንኽምርመሩን ይግበር እዩ። ናቶም/ተን ተሳትፎ ናቶምን/ተንን ንዐኦም/አን ዝአም ሰሉ ስራሕተኛታትናይ ናይ ጥዕና ኹነታትን ንምፍላጥን አድላዪ ጥንቃቌታትን ንኽገብሩ ይጠቅም።

ናይ ሕክምና መረዳእታ ብምስጥር ሓልኻ ምፅናሕ ዝከአል ብኸመይ እዩ?

ስለ ናቶም/ተን ዝሃቡና ዝኾነ ይኹን መረዳእታ ወይ ሓበሪታን ካብ ዝተወሰደ ደም ዝርከብ ናይ ላቦራቶሪ ውፅኢት ነቲ መፅናዕቲ ዓላማ ጥራሕ ይውዕል። ስለ ናቶም/ተን ዘሎ ዝኾነ ይኹን መረዳእታ ዝተፈለየ ናይ ሚስጢር ቃል ብዘለዎ ናይ

ኮምፒተር መረዳእታ ማህደር ውሽጢ ንክቕመጥ ይግበር እዩ። ናቶም/ተን መንነት ዝገልፁ ሓበሬታት ከም ሽም፣ ኣድራሻ ፣ስልኪ ቁፅሪን ዝኣመሰሉን ኣብዚ መፅናዕቲ ኣይካተትን።ነዚ ኣገልግሎት ጥራሕ ዝውዕል እሶም/ሰን ንምፍላጥ ዘኽእል መፍለዪ ቁፅሪ ኣብጥቕሚ ንክውዕል ክግበር እዩ።

ኣብዚ መፅናዕቲ ተሳታፊ ብምኻኖም ዘለዎም መሰላት እንታይ እዮም?

ኣብዚ መፅናዕቲን ምስታፍ ሙሉእብሙሉእ ብናቶም/ተን ፍቃድ ዝተመስረተ ብምኻኑ ኣብ ዝኾነ ይኹን ሰዓትን ቦታን ናይ ምቁራፅ ይኩን ንምርመራ ዝከዉን ደም ዘይምሃብ መሰሎም ዝተሓለወ እዩ። ካብዚ ብተወሳኺ መፅናዕቱ ብዝተመልከተ ዝኾነ ይኹን ዓይነት ሕቶ ናይ ምሕታትን ሙብርሂ ናይ ምርኻብን መሰል ኣለዎም። እሶም/ሰን ብዝህቡና መረዳእታ ናይቲ ፀገም ክብደትን ምክልኻልን ምቁፅፃርን ጠቓሚ ስለዝኾነ ንዝቕርበሎም ሕቶታት ቁኑዕ መልሲ ንክህቡና ብዝልዓለ ኣኽብሮት ንሓትት።

ሕቶ እንተልዩኒ ወይ ከዓ ፀገም እንተኣጋጥሙኒ እንታይ ክገብር ይግባእ?

ነዚ መፅናዕቲ ብዝተመልከተ ወይ ከዓ ምስዚ መፀናዕቲ ብዝተተሓሓዘ መልክዑ ንዘጋጥሙ ፀገማት ወይ ከዓ ንዝህልዎም ሕቶታት በዞም ዝስዕቡ ኣድራሻታት ይጠቐሙ።

ናይ መፅናዒይ ሽም: ገብረ ተኽሉ እምባየ ስልኪ ቑፅሪ፤ 251914155120 ኢሜል: gebreteklu3o@gmail.com

መማኽርቲ: 1) ዶ/ር ኣስቴር ፀጋዬ ስልኪ ቑፅሪ፤ +251911696085 ኢሜል፤ tsegayeaster@yahoo.com

ı) ሚኪያስ ነጋሽ ስልኪ ቑፅሪ፤ +251913724956፤ ኢሜል፡ mikias2@gmail.com

ናይ ሕክምናላቦራቶሪ ትምህርቲክፍሊ ምርምርን ሥነምግባርን ቢሮ ስልኪ ቁፅሪ: +251 11 275 5170

Annex-IV: Consent Form (study participants) - English version

Name of main researcher: GebreTeklu (BSc, MSc candidate)

Advisor: Aster Tsegaye (MSc, PhD)

MikiasNegash (MSc, PhD follow)

Funded by: Addis Ababa University

Reviewed by: DREC (AAU)

Research title: Assessment of hematological parameters among gasoline exposed at gas station workers; community based study, in Mekelle City, Tigray Region, Northern Ethiopia.

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

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

Name of Participant's obtaining the Informed Consent: ________________Sign________ address____________ ID No__________

Name of deponent _______________ Sign_______ (For participant's unable to read)

Name of counselor nurse ______________ Sign _______

Annex-V; Consent form Amharic version

ኮድ___________

የፍቃደኝነት ማረጋገጫ ቅጽ

ጥናቱን የሚያከህደው ሰው ስም፤ ገብረ ተኽሉ እምባየ

አማካሪዎች፤ 1. ዶ/ር አስቴር ፀጋዬ

2. ሚክያስ ነጋሽ

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

ስፖንሰር ያደረገው ድርጅት: አዲስ አበባ ዩኒቨርስቲ

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

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

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

እባክዎ ከተስማሙ እያንዳንዱ ሳጥን ላይ ምልክት ያድርጉ።

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

2. በዚህ ጥናት ሊኖር ስለሚችለው አደጋ እና ጥቅሞች አውቂያለሁ። ☐

3. በዚህ ጥናት ውስጥ መሳተፍየ በፍቃደኝነት መሆኑን አውቂያለሁ ። ☐

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

5. በዚህ ጥናት ውስጥ ያሉኝ ጥያቄዎች በመረጃ ሰበሳቢው ተመልሰውልኛል። ☐

6. በጥናቱ ውስጥ ለመሳተፍ ምንም ልዩ ክፍያ እንደሌለ አውቂያለሁ። ☐

7. በዚህ ጥናት ለመሳተፍ እስማማለሁ። ☐

የተሳታፊው ስም ________________________አድራሻ______________ፊርማ ______________ቀን ______________

በስምምነቱን ቅፅ ማንበብ የማይችሉ ተሳታፊዎች

የአማካሪስም_________________አድራሻ______________ፊርማ_____________________ቀን_____________________

የመረጃ ሰብሳቢ ስም _________________________ፊርማ ____________ ቀን______________________

Annex VI-Consent form Tigrigna version

ኮድ___________

ናይ ፍቓድ መረጋገፂ ቅጥዒ

ናይ መፅናዓይ ሽም: ገብረ ተኽሉ እምባየ

ኣማኸርቲ 1) ዶ/ር አስተር ፀጋዬ

2) ሚኪያስ ነጋሽ

ስፖንሰር ዝገበሮ ድርጅት: አዲስ አበባ ዩኒቨርስቲ

ፍቃድ ወሃቢ: ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ናይ ምርምርን ሥነ-ምግባርን ቢሮ

ናይቲ መፅናዕቲ ርእሲ:-አብ ነዳዲ መዐደሊን መኪና ጋራጅን ዝሰርሑን ብስርሖም ምኽንያት ብነዳዲ (ቤኒዚን) ዝጥቅዑ ሰራሕተኛታት አብ ደሞም ውሽጢ ዘሎ ናይ ዋህዬታት ደም ጥዕናምን መጠኖም ምዕቃን፤

አብዚ መፅናዕቲ ንምስታፍ ፍቓደኛ እንተኾይንኹም፤በጃኹም/ኽን እዚ ቐፅሉ ዘሎ ዓንቀፅ ብምንባብ አብቲ ናይ ስምምዕ ወረቐት ይፈርሙ።ስለዚ ምስ ተሰማምዑ አብ ሕድሕድ ሳንዱቕ ምልክት ይግበሩ።

1. ብዛዕባ እዚ መፅናዕቲ ዝተፅሓፈ ናይ ሓበሬታ ወረቐት ኣንቢበዮ ወይ ከዓ ተነቢቡለይን አብዚ መፅናዕቲ ንምስታፍ እንታይ ከምዝድለ ተረድኡኒ እዩ። ☐

2. አብዚመፅናዕቲ ክህልዩ ዝኽእሉ ፀገማትን ጥቕሞምን ፈልጠ እየ ። ☐

3. አብዚ መፅናዕቲ ንምስታፍ ብፍቓደኛነት ምኻኑ ተረድኡኒ አሎ። ☐

4. ካብዚ መፅናዕቲ አብ ዝኾነይኹን ሰዓት ምቁራፅ ከምዝኽእል ተረድኡኒ አሎ። ☐

5. አብዚ መፅናዕቲ ውሽጢ ዝነበሩኒ ሕቶታት ተመልስለይ እዩ። ☐

6. አብዚ መፅናዕቲ ውሽጢ ብምስታፈይ ምንም ዓይነት ኽፍሊት ከምዘይወሃበኒ ፈሊጠ እየ። ☐

7. አብዚ መፅናዕቲ ንምስታፍ ይስምማዕ አለኹ። ☐

ናይ ተሳታፊ/ፊት ሽም______________________አድራሻ_____________ፊርማ _____________ዕለት _____________

አብቲ ናይ ስምምዕ ዓንቀፅ ምንባብን ዘይኽእሉ ተሳተፍቲ

ናይ አማኻሪ ሽም_____________________ አድራሻ_____________ፊርማ________________ዕለት__________________

ሓበሬታ ሰቢሳቢሽም _______________________ፊርማ ___________ ዕለት____________________

Questionnaire Design

An English language questionnaire form was prepared by researcher, translated to Amharic and then to Tigrigna version. A meeting interview was done to use for filling in the questionnaire after taking their consent. All interviews were conducted face to face by one investigator I. Most questions are one of two types: the yes/no question, which offers a dichotomous choice; and the multiple choice question, which offers several fixed alternatives. The questionnaire includes several areas of questions such as socio-demographic characteristic (address, age, marital status and education), work duration, and personal protective equipment in use. Workers were asked to provide information on personal hygiene practices and habits such as: smoking, drinking, eating, chewing gum, milk consumption and taking shower at work site. Several questions related workers knowledge's were included such as routes of benzene entry into human body, and benzene as environmental pollutant.

Pilot study

Pilot study was done at three gas stations out of Mekelle City which is Wukro City for 6 workers prior to beginning of data collection to know the quality and clarity of questionnaire and to evaluate the outcome. At the end of the pilot study, a comprehensive revision to questionnaire was made and modified as necessary. The pilot subjects are not included in the study.

Annex VII; Structured Questionnaire for Gasoline exposed Study participants

Title; Assessment of hematological parameters among gasoline exposed at gas station workers in Mekelle City, Tigray Region, Northern Ethiopia.

Serial No / / / /

Date: / /2017/18

Name of facility (optional)_____________ Institution code_______________

Kifle Ketema__________ Woreda__________ Kebelle_________

Respondent's identification code: _____________________________________

Address: ____

Tel: ____

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

I. Socio-demographic characteristics

1. Sex: (1) Male (2) Female

2. Age (Years): _______________________________

3. Marital status: (1) Single (2) Married

4. What is your higher level of education?

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

II. Work experience and duration of exposure related information

5. What is your type of work?

1. Gas Fueling 2. Mechanics 3. Cashier 4. Other specify.............

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

7. How many hours you work at this station per day………. Days per week-----------

8. What was your previous job(s) and for how long? ____________________

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

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

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

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

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

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

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

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

11. During work are you doing the following?

11.1. Drinking (1) Yes (2) No

11.2. Eating (1) Yes (2) No

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

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

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

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

14. Do you take a shower at work site before going home? (1) Yes (2) No

15. How frequently do you drink milk?

(1) Always (2) Frequently (3) sometimes (4) Often (5) Never

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

(1) Yes (2) No

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

(1) Yes (2) No

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

18.1. Inhalation (1) Yes (2) No

18.2. Skin (1) Yes (2) No

18.3. Mouth (1) Yes (2) No

19. Have you been attending any training course(s) in health hazards of gasoline exposure?

(1) Yes (2) No

20. If yes for No 19, please specify the institution(s) or any other sector, which conducted the course(s)? ....

21. Have you seen or hear about gasoline poisoning or death cases?

(1) Yes (2) No

22. Have you been diagnosed before hiring to this work for any anemia or malignancy?

(1) Yes (2) No

23. If yes for number 22, what is the treatment you are taking or taken, please write the type of treatment.......................................?

Thank you very much for your participation!!

Annex-VIII; Questionnaire Amharic version

መጠይቅ

የመጥይቁ ኮድ………………የተሳታፊዎች መለያ ቁጥር…………ቀን………………እድሪስ......................ስልክ ቁጥር...............

ማሳሰብያ: እባክዎ ትክክለኛዉን የሆነዉን መልስ ያክብቡ ወይም ይፃፉ፡፡

1. ፆታ (1) ወንድ (2) ሴት

2. ዕድሜ --------------

3. የጋብቻ ሁኔታ (1) በትዳር ላይ (2) ያላገባ/ች

4. የትምርት ደረጃ

(1) ያልተማረ (2) ማንበብና መፃፍ (3) ከ 1-8 ክፍል (4) ከ 9-12 ክፍል (5) ዲፕሎማ/ ዲግሪና ከዛ በላይ

5. ቢዚህ ስራ ቦታ የለበዎት የስራ ምድብ ምንድ ነዉ?

(1) ቤንዚን መምላት (2) ማካኒክስ (3) ገንዘብ ያዥ (4) ሌላ ከሆነ ይግለፁ--------------

6. በዚ ስራ ውስጥ ለስንት ግዜ ወይም ዓመት ያህል ሰርተዋል?--------------------------

7. በስራ ምድብዎ በቀን ስንት ሳዓት ይሰራሉ?-----------------------------

8. እዚህ ስራ ከመስራትዎ በፊት ምን ይሰሩ ነበሩ? ለስንት ግዜ ያህል?-------------------------

9.የሚከተሉት የመከላከያ እቃዎች ስራ በሚሰሩበት ግዜ ምን ያህል ይጠቀማሉ?

9.1. የእጅ ጓንት (1) ሁሉ ግዜ (2) ኣዘውትሮ (3)ብዙ ግዜ (4) ኣንድ ኣንድ ግዜ (5) ፈፅሞ

9.2. ባርኔጣ (1) ሁሉ ግዜ (2) ኣዘውትሮ (3)ብዙ ግዜ (4) ኣንድ ኣንድ ግዜ (5) ፈፅሞ

9.3. የፊት መሸፈኛ (1) ሁሉ ግዜ (2) ኣዘውትሮ (3)ብዙ ግዜ (4) ኣንድ ኣንድ ግዜ (5) ፈፅሞ

9.4. ሽፉን ጫማ (1) ሁሉ ግዜ (2) ኣዘውትሮ (3)ብዙ ግዜ (4) ኣንድ ኣንድ ግዜ (5) ፈፅሞ

9.5. ገዋን (1) ሁሉ ግዜ (2) ኣዘውትሮ (3)ብዙ ግዜ (4) ኣንድ ኣንድ ግዜ (5) ፈፅሞ

10. በቁጥር 9 የተጠቀሱ የመከላከያ እቃዎች የማይጠቀሙ ከሆነ ምክንያትዎ ምንድ ነዉ?

(1) ኣቅርቦት የለም (2) ስለማይመች (3) ኣስፈላጊ ስላልሆነ (4) ግድየለሽነት (5) ሌላ ካለ ይግለፁ………..

11. በሚሰሩበት ቦታ ቀጥሎ ያሉትን ኣዘዉትሮ ይጠቀማለዎ?

11.1. ሲጋራ ማጨስ (1) ኣዎ (2) የለም

11.2. ምግብ በስራ ቦታ መመገብ (1) ኣዎ (2) የለም

11.3. ማስቲካ ማኘክ (1) ኣዎ (2) የለም

11.4. ብኣፍዎ ቤንዚን መምጠጥ (1) ኣዎ (2) የለም

12. ወደ ቤትዎ ከመሄዶዎ በፊት ሻወር ይወስዳሉ? (1) ኣዎ (2) የለም

13. ወተት በሳምንት ምን ያህል ይጠጣሉ?

(1) ሁሉ ግዜ (2) ኣዘዉትሮ (3) ብዙ ግዜ (4) ኣንዳንድ ግዜ (5) ፈፅሞ

14. ቤንዚን የኣካባቢ ብክለት ያመጣል ብሎ ያስባሉ? (1) ኣዎ (2) የለም

15. ቤንዚን ጤናን ይጎዳል ብሎ ያስባሉ? (1) ኣዎ (2) የለም

16. በተራ ቁጥር 14 ና 15 መልስዎ ኣዎ ከሆነ፣ ቤንዚን በየተኛዉ መንገድ ወደ ሰዉነት ውስጥ ሊገባ ይችላል?

16.1. በመተንፈሻ ኣካላት ወደ ውስጥ በመግባት (1) ኣዎ (2) የለም

16.2. በቆዳ (1) ኣዎ (2) የለም

16.3. በኣፍ (1) ኣዎ (2) የለም

17. ቤንዚን ስለሚያመጣው ጉዳት ላይ ስልጠና ተካፍሎ ያዉቃሉ? (1) ኣዎ (2) የለም

18. በጥያቄ ተራ ቁጥር 17 መልስዎ ኣዎ ከሆነ፤ ስልጠና የሰጠዉ ተቋም ወይም ሰዉ ይግለፁልን...............

19. ከዚህ በፊት የጤና ተቋም ወይም የጤና ባለሞያ በስራ ቦታቹ ጎብኝቶት ያውቃል?

(1)ኣዎ (2) የለም

20. በተራ ቁጥር 19 መልስዎ ኣዎ ከሆነ፤ የጎበኘዉ የጤና ተቋም ወይም ባለሞያ ስም ይፃፉ...........

21. በቤንዚን ምክንያት የታመመ ወይም የሞተ ሰዉ ያውቃሎ? (1) ኣዎ (2) የለም

22. እዚህ ስራ ከመጀመርዎ በፊት የደም ማነስ ወይም የደም ካንሰር ተይዞ ያውቃሉ?

(1) ኣዎ (2) የለም

23. ከዚህ በፊት ወይም ኣሁን የሚወስዱት መድሃኒት ኣለ? (1) ኣዎ (2) የለም

24. በቁጥር 23 መልስዎ ኣዎ ከሆነ፤ የመድሃኒት ኣይነት ይፃፉ-----------------------

Thank you very much for your participation!!

Annex-IX; Questionnaire Tigrigna version

መጠይቕ

መፍለዪ: ናይቲ ተቋም ሽም:___________________ናይቲ ተቋም ኮድ:___________________
ክፍለ ከተማ___________ ወረዳ___________ቀበሌ__________ናይ መጥይቕ ኮድ.................ናይ ተሳተፍቲ መፍለዪቁፅሪ............ዕለት.................

ናይቲ መፅናዕቲ ኣርእስቲ: ኣብ ነዳዲ መዐደሊ ዝሰርሑን ብስርሖም ምኽንያት ብነዳዲ ዝጥቅዑ ሰራሕተኛታት ኣብ ደሞም ውሽጢ ዘሎ ዝተፈላለያ ናይ ዋዬህታት ደም ጥዕናምን መጠኖመን ጠቆምቲ ምርመራታት ምዕቃን;

መተሓሳሰቢ: በጃኹም ትኽክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፅሓፉ።

1ይ ክፋል: ናይ ማሕበራዊን ነባራዊን ኹነታት ዝምልከቱ ሕቶታት

1. ፆታ (1) ተባዕታይ (2) ኣንስታይ

2. ዕድመ--------------

3. ኹነታት ሓዳር (1) በዓል ሓዳር (2) ዘይተመርዓወ/ወት

4. ናይ ትምርህርቲ ደረጃ

(1) ዘይተምሃረ (2) ምንባብን ምፅሓፍን (3) ካብ 1-8 ክፍሊ (4) ካብ 9-12 ክፍሊ (5) ዲፕሎማ/ድግሪን ልዕሉኡን

2ይ ክፋል: ምስ ኩነታት ጥዕና ዝተተሓሐዙ ነገራት

5. ናይ ስራሕ መደቦም እንታይ እዪ;

(1) ነዳዲ ምሙላእ (2) መካኒክስ (3) ገንዘብተሓዚ/ት (4) ካልእ እንተኮይኑ ይግለፁ--------------

6. ኣብዚ ስራሕ ውሽጢ ክንደይ ግዜ ዝኣክል ሰርሖም/ሐን? --------------------------

7. ኣብ ስራሕ ምድቦም ኣብ መዓልቲ ክንደይ ሰዓት ይሰርሑ/ሓ? ----------- ኣብ ሰሙን ከ ክንደይ መዓሊቲ ይሰርሑ/ሓ?---

8. ኣብዚ ስራሕ ቅድሚ ምስራሖም እንታይ ይሰርሑ/ሓ ነይሮም/ረን? ------------------- ንክንደይ ግዜ ዝኣክል?-------

9. ነዞም ስዕቦም ዘለዉ ናይ ሰውነት መከላከሊ መሳርሕታት ስራሕ ኣብ ዝሰርሕሉ ግዜ ክንደየናይ ዝኣክል ይጥቀሙ?

9.1. ናይ ኢድጓንት (1) ኹሉሻዕ (2) ሙብዛሕትኡግዜ (3) ዝተወሰኑ መዓልትታት (4) ሓሓልፉ (5) ዋላሓንቲ

9.2. ባርኔጣ (1) ኹሉሻዕ (2) ሙብዛሕትኡግዜ (3) ዝተወሰኑ መዓልትታት (4) ሓሓልፉ (5) ዋላሓንቲ

9.3. ናይ ገፂመሸፈኒ (1) ኹሉሻዕ (2) ሙብዛሕትኡግዜ (3) ዝተወሰኑ መዓልትታት (4) ሓሓልፉ (5) ዋላሓንቲ

9.4. ሽፉንጫማ (1) ኹሉሻዕ (2) ሙብዛሕትኡግዜ (3) ዝተወሰኑ መዓልትታት (4) ሓሓልፉ (5) ዋላሓንቲ

9.5. ጋዎን (1) ኹሉሻዕ (2) ሙብዛሕትኡግዜ (3) ዝተወሰኑ መዓልትታት (4) ሓሓልፉ (5) ዋላሓንቲ

10. ኣብ ቑፅሪ 9 ዝተጠቕሱ ናይ ሰውነት መከላኸሊ መሳርሕታት ዘይጥቀሙ እንተኾይኖም ምኽንያቶም/ተን እንታይ እዩ?
(1) ኣቕርቦት የለን (2) ስለዘማይምቾ (3) ኣድላዪ ስለዘይኾነ (4) ግድየለሽነት (5) ካልእ እንተልዩይግለፁ...........

11. ኣብ ዝሰርሕሉ ቦታ ነዞም ቐፅሎም ዘለዉ ብኣብዝሓ ይጥቀሙ/ማ ዶ?

  11.1. ምግቢ ኣብ ስራሕ ቦታ ምምጋብ (1) እወ (2) ኣይምገብን
  11.2. ማስቲካ ምሕያኽ (1) እወ (2) ኣይሓይክን
  11.3. ብኣፎም/ፈን ቤንዚን ምምጣጥ (1) እወ (2) ኣይመጥን

12. ሽጋራ ይስሕቡ/ባ ዶ (የጭሱ/ሳ ዶ)? (1) እወ (2) ኣያጨስን

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

14. ናብ ገዝኦም/እን ቅድሚ ምኻዶም/ደን ሻወር ይወስዱ/ዳ ዶ? (1) እወ (2) ኣይወስድን

15. ፀባ ኣብ ሰሙን ክንደይ ዝኣክል ግዜ ይሰትዩ/ያ?

  (1) ኹሉሻዕ (2) ብተደጋጋሚ (3) ሙብዛሕትኡግዜ (4) ሓሓልፉ (5) ዋላሓንቲ

16. ቤንዚን ንከባቢ ኣየረ ብኽለት የምፅእ እዩኢሎም/ለን ይሓስቡ/ባ ዶ? (1) እወ (2) ኣይሓስብን

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

21. ኣብ ተራ ቑፅሪ 17 መልሶም/ሰን እወ እንተኾይኑ፣ቤንዚን በየናይ መንገዲ ናብ ሰብነት ውሽጢ ክኣቱ ይኽእል?

  21.1.ብመተንፈሲኣካላትናብውሽጢ ብምእታው (1) እወ (2) ኣይፈልጥን

  21.2. በቆርበት (1) እወ (2) ኣይፈልጥን

  21.3. ብኣፍ (1) እወ (2) ኣይፈልጥን

22. ኣብ ቤንዚን (ነዳዲ) ዘምፅኦ ጉድኣት ዘድሀበ ስልጠና ተኻፍሎም/ለን ይፈልጡ/ጣ ዶ? (1) እወ (2) ኣይፈልጥን

23. ንሕቶ ተራ ቑፅሪ 22 መልሶም/ሰን እወ እንተኾይኑ፣ ስልጠና ዝሃበ ተቋም ወይ ከዓ ሰብ ይግለፁ/፣...............

24. ብቤንዚን ምኽንያት ዝሓመመ ወይ ከዓ ዝሞተ ሰብ ይፈልጡ/ጣ ዶ? (1) እወ (2) ኣይፈልጥን

25. ኣብዚ ስራሕ ቕድሚ ምጅማሮም/ረን ናይ ዋሕድ ደም ወይ ከዓ ናይ ደም ካንሰር ተታሕዞም ይፈልጡ/ጣ ዶ?

  (1) እወ (2) ኣይፈልጥን

27. ቕድሚ ሐዚ ወይ ከዓ ሐዚ ዝወሰድዎ ናይ መድሓኒት ዓይነትእንተልዩ ይፅሓፉ/ፋ-----------------------

Annex-X; standard operating procedures

SOP for blood collection

Equipment

- 21 gauge needle for each participant with closed vacutainer system
- Blood collection tubes for each participant
- Tourniquet
- Box of nitrile /vinyl gloves
- 70% Alcohol wipes
- Cotton balls/swabs
- Bandages
- Disposable, single use materials or equipment are to be used whenever possible
- Any reusable materials or equipment must be cleaned and disinfected with alcohol-based sanitizers before use with another participant

Safeguards /safety procedures

- A new pair of disposable latex/vinyl gloves is used with each participant.

Gloves are for single-procedure use only. Gloves should always be removed using a glove-to-glove or skin-to-skin technique which will prevent contaminating the hands.

- The use of gloves does not replace the need for hand hygiene. Hands should be properly washed before the gloves are put on and after the gloves are removed. Hand hygiene is also needed before and after the replacement of gloves during a procedure or in between tasks.

- Participants are reminded to do no heavy lifting for 24 hours.

Procedure for drawing blood

Steps 1; Assemble equipment

Collect all the equipment needed for the procedure and place it within safe pack which is simple for transport to collection site and place easy reach on a flat surface table ensuring that all the items are clearly visible.

Step 2; Identify and prepare the participants and allow to sit comfortably preferably be stretching his/her arm

Step 3; Perform hand hygiene and put on gloves

Step 4; Select the site of injection

Step 5; Apply the tourniquet

Step 6; Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol.

Step 7; insert the needle properly into the vein

Step 8; draw the required amount of blood

Step 9; Fill the laboratory sample tubes and mix properly

- When obtaining multiple tubes of blood, use evacuated tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.
- If a syringe or winged needle set is used, best practice is to place the tube into a rack before filling the tube. To prevent needle-sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of hemolytic.
- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper. DO NOT remove the stopper because it will release the vacuum.
- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of hemolysis. DO NOT recap and remove the needle.
- Before dispatch, invert the tubes containing additives for the required number of times (as specified by the local laboratory).

Step 10; Draw samples in the correct order and label the sample using unique code of participants.

Step 11; Clean contaminated surfaces and complete patient procedure.

Step 12; Prepare samples for transportation.

Step 13; Clean up spills of blood or body fluids.

SOP for haematology analyser SYSMEX –XP – 300

The Sysmex XP-300 performs a reliable analysis of 20 parameters CBC, including a 3-part WBC differential and displays analysis results in 3 histograms on the LCD screen. In addition, the analysis data can be printed on the internal/external printer. Analysis is possible in whole blood mode as well as in pre-diluted mode. For this reason, the XP-300 can also be used with a minute amount of blood (Minimum required volume: 20 µL when analyzing in pre-diluted mode).

Principle

This instrument performs blood cell count by DC detection method.

DC detection method

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The TD chamber has a minute hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

Non-cyanide hemoglobin analysis method

Non-cyanide hemoglobin analysis method utilizes the advantage of both of Oxyhemoglobin and cyanmethemoglobin. Noncyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing methemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

Blood cell discrimination circuit

WBC, RBC, and PLT are discriminated and calculated by the following blood cell discriminator.

WBC discriminator

As to WBC lower discriminator, the optimum position in 30 - 60 fL is automatically determined by the microcomputer. WBC is calculated from the particle counts more than this lower discriminator.

RBC discriminator

As to RBC lower discriminator and upper discriminator, the optimum position in 25 - 75 fL and 200 - 250 fL, respectively, are automatically determined by the microcomputer. RBC is calculated from the particle counts between this lower discriminator and upper discriminator.

Platelete discriminator

As to Platelete lower discriminator and upper discriminator, the optimum position in 2 - 6 fL and 12 - 30 fL, respectively, are automatically determined by the microcomputer. Platelete count is calculated from the particle counts between this lower discriminator and upper discriminator.

Analysis parameters

This instrument provides results for the following parameters:

- WBC: Number of leukocytes (Analysis principle: DC detection method), WBC count in 1 µL of whole blood).
- RBC: Number of erythrocytes (Analysis principle: DC detection method), RBC count in 1 µL of whole blood).
- HGB: Hemoglobin (Analysis principle: Non-cyanide hemoglobin analysis method) Concentration (gram) of hemoglobin in 1 dL of whole blood.
- HCT: Hematocrit value (Analysis principle: RBC pulse height detection method). Ratio (%) of whole RBC volume in whole blood.
- MCV: Mean RBC volume (fL) in whole blood. The volume is calculated by HCT/RBC.
- MCH: Mean hemoglobin concentration (pg) per RBC. The concentration is calculated by HGB/RBC.
- MCHC: Mean RBC hemoglobin concentration; Mean hemoglobin concentration (g/dL). The concentration is calculated by HGB/HCT.
- PLT: Platelet (Analysis principle: DC detection method). Platelet count in 1 µL of whole blood.
- LYM% (W-SCR): WBC-Small Cell Ratio, Ratio (%) of small cells (the lymphocytes) to whole WBC.
- MXD% (W-MCR): WBC-Middle Cell Ratio, Ratio (%) of middle cells (the mixed population (EO, BASO, MONO)) to whole WBC.
- NEUT% (W-LCR): WBC-Large Cell Ratio, Ratio (%) of large cells (the neutrophils) to whole WBC.
- LYM# (W-SCC): WBC-Small Cell Count; Absolute count of WBC-small cells (the lymphocytes) in 1 µL of whole blood.
- MXD# (W-MCC): WBC-Middle Cell Count; Absolute count of WBC-middle cells (the mixed population (EO, BASO, MONO)) in 1 µL of whole blood.
- NEUT# (W-LCC): WBC-Large Cell Count; Absolute count of WBC-large cells (the neutrophils) in 1 µL of whole blood.

- RDW-SD: RBC distribution width - SD The distribution width (fL) at the height of 20% from the bottom when the peak RBC particle distribution curve is taken as 100%.
- RDW-CV: RBC distribution width - CV RBC distribution width (%) calculated from the points defining 68.26% of the entire area spreading from the peak of the RBC particle distribution curve.
- PDW: Platelet distribution width. The distribution width (fL) at the height of 20% from the bottom with the peak of platelet particle distribution curve taken as 100%.
- MPV: Mean platelet volume, Mean volume of platelet (fL).
- P-LCR: Large platelet ratio Ratio (%) of large platelet volume exceeding 12 fL to the platelet volume.
- PCT: Plateletcrit Ratio (%) of platelet volume to whole WBC

Reagents;

- CELLPACK; is a diluent used to dilute aspirated analysis samples in order to measure an RBC count, WBC count, hemoglobin concentration and platelet count.
- STROMATOLYSER-WH; is a reagent used to lyse white blood cells in analysis samples in order to measure a WBC count and hemoglobin concentration.
- CELLCLEAN; is a strong alkaline detergent used to remove lyse reagents, cellular residuals and blood proteins remaining in the hydraulics of the instrument.
- EIGHTCHECK-3WP; is control blood for testing the precision and accuracy of hematology analyzers.

Throughput; Approximatly 60 whole blood specimens/hr depending on mode used.

Quality Control

The reliability of this instrument and reagents is monitored by quality control. By use of control blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

Control material

The control materials, EIGHTCHECK-3WP-N (Normal), EIGHTCHECK-3WP-L (Low level) and EIGHTCHECK-3WPH (High level) are used. These are equivalent to Low, Normal and High level.

Specimen
Specimen Type/Stability/Storage

Some anticoagulants will alter test results due to their effects on hemolysis and blood platelet agglutination. Therefore, use EDTA-2K, 3K or 2Na as the anticoagulant.

Specimens should be stored at room temperature of 18 - 26ºC or in the refrigerator of 2 - 8ºC. If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis. Otherwise correct results may not be obtained.

Equipment and apparatus

Sysmex XP-300

Procedure

Instrument Start-Up

01.8.2 . Running Controls

01.8.3. Running Patient Samples

Internal Quality Control

Control samples will be analyzed by the L-J control programs, and the data will be stored in the quality control file. At least two levels of controls should be run before analyzing the patient samples. L-J program uses the results from one analysis as one control data. In this lab we will use the L-J program.

Hematology Adult Normal Ranges

Instrument Used: SYSMEX -XP-300

PARAMETER	REFERENCE RANGE
Red Blood Cell Count (RBC) Men Women	 $4.5 – 5.5 \times 10^{12}$/l $3.8 – 4.8 \times 10^{12}$/l
Haemoglobin (Hb) Men Women	 13 – 17 g/dl 12 – 15 g/dl
Haematocrit (HCT) Men Women	 40 – 50 % 36 – 45 %
Mean Cell Volume (MCV) Men Women	 83 – 99 fl 83 – 99 fl
Mean Cell Haemoglobin (MCH) Men Women	 27 – 32 pg 27 – 32 pg

Mean Cell Haemoglobin Concentration (MCHC) Men Women	 32 – 34 g/dl 32 – 34 g/dl
Red Cell Distribution Width (RDW)	11.6 – 14.0 %
White Blood Cell Count (WBC)	4-10 x 10^9/l
Differential White Cell Count (Diff) Neutrophils Lymphocytes Monocytes Eosinophils Basophils	 40 – 80 % (2 - 7 x 10^9/l) 20 – 40 % (1 – 3 x 10^9/l) 2- 10 % (0.2 – 1.0 x 10^9/l) 1 – 6 % (0.02 – 0.5 x 10^9/l) < 1- 2 % (0.02 – 0.1 10^9/l)
Platelet Count MPV Female Male PDW	150 – 400 x 10^9/l 7.2-10.4 FL 7.5-11.5 FL 9-14 FL

Annex XI-Declaration

Assurance of Principal Investigator

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

Name of the student: GebreTeklu Embaye

Date ________________________ Signature ________________________

Approval of Advisors:

Aster Tsegaye, MSc, PhD

Date____________________________ Signature _____________________________

MikiasNegash, MSc PhD follow

Date ___________________________ Signature _________________________________