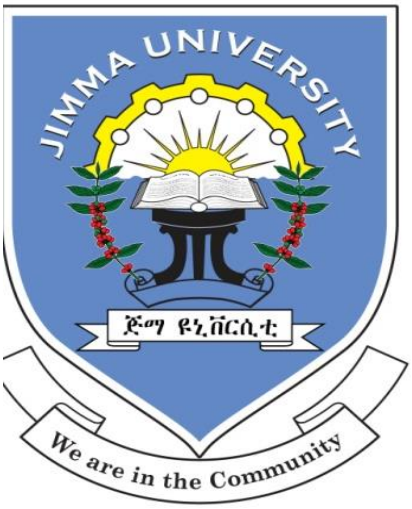
ASSESSMENT OF HEMATOLOGICAL PARAMETERS AMONG GAS-STATION WORKERS EXPOSED TO GASOLINE AT HOSSANA TOWN, SOUTH WEST ETHIOPIA: A COMPARATIVE CROSS-SECTIONAL STUDY



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A Research thesis Submitted to School of Medical Laboratory Sciences Jimma University, In Partial Fulfilment of the Requirements for Masters of Science in Clinical Laboratory Science specialty in Hematology and Immunohematology.

JIMMA UNIVERSITY

FACULTY OF HEALTH SCIENCES

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Abstract

Background: Petrol station workers are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction during vehicle refueling. The adverse health effects of gasoline exposure may be primarily related to impairment of the hematopoietic system with bone marrow depression. On top of controversial association between hematological parameters and gasoline exposures, there is paucity of data regarding the topic in the study area.

Objective: To assess the hematological parameters among gasoline exposed workers at gas station as compared to non-exposed controls in Hossana Town, South West Ethiopia from May 1 to June 15, 2020.

Method: Institutional based comparative cross-sectional study was conducted by involving 60 individuals working in gasoline stations and 120 non-exposed age and gender matched individuals. Socio-demographic, occupational and other related data of study participants were collected by structured questionnaire via face to face interview. Four milliter of venous blood sample was collected to perform hematological analysis and to examine peripheral blood morphology. Data were analyzed by SPSS version 20. Independent sample T-test, Mann-Whitney U tests, one way ANOVA and Correlation Coefficient analysis were performed. P-value<0.05 was considered as statistically significant.

Results: A total of 180 subjects (gasoline exposed, n=60 and non-exposed, n=120) were included. The majority of the workers 66.7% did not use any kind of personal protective equipment. The mean ±SD value of gasoline exposed subjects had significant reduction in RBC ($\times 10^6$ per µl) count (4.87±0.62 versus 5.08±0.41, P=0.007) and the median (IQR) of Hgb (g/dl) level (14.38(1.7) versus15.38 (1.3), p=0.001) compared with the non-exposed subjects. On the other hand, significant increases were found in median (IQR) value of absolute eosinophil count (0.3(0.57) versus 0.2(0.3), p=0.01) exposed subjects compared with the non-exposed. The peripheral blood film examination reveals 81.7% Normocytic normochromic picture and 11.7% macrocytosis in exposed group. **Conclusion:** Long-term exposure to gasoline at gas station has deleterious effect on the RBC, Hgb and Hct as compared to non-exposed subject. Therefore, protective measure should be implemented by the concerned body to minimize exposures to gasoline. **Key words**: Gasoline, Benzene, Hematological parameters, Hossana**,** Ethiopia

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Acronyms and Abbreviation

CBC	Complete Blood Count
CSA	Central Statistical Agency
DNA	Deoxyribo Nucleic Acid
EDHS	Ethiopian Demographic and Health Survey
HGB	Hemoglobin
HCT	Hematocrit
HSCs	Hematopoietic Stem Cells
K_2EDTA	Di-Potassium Ethylene Diamine Tetra Acetic Acid
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MPV	Mean Platelet Volume
ROS	Reactive Oxygen Species
SOPs	Standard operating procedures
SPSS	Statistical Package for Social Science
VOCs	Volatile Organic Compounds
WUNEMMRTH.	Wachemo University Nigist Eleni Mohammed Memorial and teaching Referral Hospital.

CHAPTER ONE: INTRODUCTION

1.1. Background

Hematopoiesis refers to the commitment and differentiation processes that leads to the formation of all blood cells from multipotent hematopoietic stem cells (HSC) in the bone marrow. But it can also occur in other tissues such as the liver and spleen. HSC have two major characteristic: ability to differentiate into all hematopoietic lineages and retain their self-renewal capacity [1]. Blood is the only fluid tissue in the body that delivers necessary substances to the body's cells such as nutrients and oxygen. It governs vital functions of the body like respiration, circulation, excretion, osmotic balance and transport of metabolic substance. Blood is composed of two components, namely; Plasma (55%) and formed elements (45%). Plasma contains 91-92% of water and 8-9% dissolved solids, while the formed elements are three main types namely; Red Blood Cells, White Blood Cells, and Platelets [2].

The hematopoietic system is the most significantly affected target tissue following exposure to gasoline in humans. Hematological parameters are probably the more rapid and detectable variations under stress conditions like gasoline exposure for assessing the health condition [3]. Benzene exposure is one of the main health concerns for occupations with risk of exposure to volatile solvents such as petrol pump workers. Human exposure to benzene is associated with multiple adverse health effects primarily related to impairment of the hematopoietic system with bone marrow depression including pancytopenia, hence aplastic anaemia and an increased risk of developing cancer (acute myeloblasticleukemia). There is also morphological effect on red blood cells [4]. Petrol filling workers are exposed to petrol carcinogenic agent through inhalation of vapors, contamination of particles during eating and drinking, and skin contact with particles from petrol vapors

Gasoline is one of the most widely used industrial chemical agents and important pollutant compound present in both occupational and general environment. Individuals are frequently exposed to pollutants in both outdoor and indoor environments. However, the major route of exposure is inhalation by workers during production and distribution of the fuel and certain people have a greater risk of exposure to gasoline vapors; these include filling station workers, service station attendants, drivers of gasoline trucks, and refinery workers and they are more likely to come down with blood related abnormalities [5].

It is commonly used as fuel for internal combustion engines, machines and is also used as a thinner, decorative agent, and industrial solvent. Aromatic compounds which include: benzene, xylene, toluene, and ethylene benzene are the most dangerous element of gasoline [6]. Some of these aromatic compounds are known to be highly toxic or carcinogenic to humans [7, 8]. 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 [9].

Benzene is an aromatic hydrocarbon that is a natural component of crude oil and natural gas. It is colourless and highly inflammable liquid with a sweet smell. The effects of systemic benzene exposure can cause acute and chronic clinical disorders associated with the risk of blood abnormalities, including leukaemia, lymphoma and chromosomal aberrations [10]. Communities surrounding petroleum refineries have important health risks due to the probability of being exposed to elevated levels of benzene and other toxic chemicals. Benzene-induced toxicity involves several mechanisms, for instance oxidative stress, DNA damage, disruption of the cell cycle and apoptosis. In addition, immune dysfunction has been hypothesized to synergise with benzene toxicity as benzene may interfere with cellular, humoral and innate immunity [11].

Metabolism of aliphatic and aromatic hydrocarbons which are the major components of petroleum and petroleum products result in generation of free radical species in various tissues. Free radicals are known to alter erythrocyte membrane as well as other cell membrane as a consequence of oxidative stress. Observed reduction in the RBC count may be attributed to cytotoxic effect of compound present in the petrol. Oxidative stress induced by petroleum products could possibly have accounted for susceptibility of red cell membrane to oxidative attack giving way to hemolysis [12].

The production of benzene metabolites, largely takes place in the liver, is followed by their transport to the bone marrow. Benzene is metabolized into primary metabolite phenol by the liver enzyme cytochrome P4502E1 (CYP2E1) through the benzene oxide intermediate. It is subsequently metabolized by CYP2E1 to hydroquinone (HQ). HQ is transported to the bone marrow and oxidized to several metabolites which can accumulate in the bone marrow where they are further bio activated by myeloperoxidases and other heme-protein

peroxidases to reactive semiquinones, quinines, benzochinones, which lead to the formation of reactive oxygen species (ROS). These reactive oxygen species can affect signal transduction cascades by altering the activities of certain protein kinases and transcription factor [13]. Finally benzene associated with many of bone marrow failure and hematological malignancies like, Acute Myeloid Leukaemia, Aplastic anemia, myelodydplastic syndrome, acute lymphoblastic leukaemia and chronic myeloid leukaemia [14]. Therefore aim of this study was to assess the hematological parameters among gasoline exposed workers at gas station in Hossana town.

1.2 Statement of the problem

As World Health Organization (WHO) reported in 2010, Human exposure to gasoline has been associated with a wide range of short-term and long-term adverse health effects and diseases [15]. Short-term exposure to benzene can cause drowsiness, dizziness, unconsciousness and death at very high level. Long-term benzene exposure is associated with progressive depression of bone marrow function, leading to a reduction in the number of circulating red blood cells, white blood cells and hematological malignancies and its harmful impacts depends on the amount, route and length of time of exposure, as well as the age and pre-existing medical condition of the exposed person [13, 16].

Continued exposure of benzene causes disturbance in the metabolism of oxidation of fatty acids and essential amino acids (lysine, phenylalanine and tyrosine) in bone marrow cells and lead to permanent suppression of bone marrow functioning, accompanied by reduction in the formation of blood cells [17]. 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 [18]. Different metabolic product of petroleum hydrocarbons metabolism like quinine or reactive metabolites that bind to macromolecules like DNA, histone, tubulin and topoisomeresII have potential to cause 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 [19].

Long-term exposure to petrol can lead to some toxic effect on the hemoglobin synthesis. It interferes in the process of red blood cell (RBC) proliferation, which causes adverse effects on heme synthesis and produce changes in the RBC membrane and make it susceptible to hemolysis, interferes with replication thereby macrocytic anemia [12].

Toxic effects of benzene metabolites with prolonged exposure, cause a significant change in blood parameters; it could cause anemia, make the body vulnerable to infections, cause thrombocytosis and Thrombocythemia (restricted blood flow due to platelet formation) or decrease in number of leucocytes (leucopenia) and other blood disorders such as leukaemia a condition that can cause severe ill health and death [20, 21].

Gasoline exposure causes the increase of total malignant cells and produces a major change on the measured hematological parameters and megakaryocytic bone marrow [22]. Benzene suppresses the cell cycle by p53-mediated over expression of p21, a cyclin-dependent kinase inhibitor, resulting not simply in suppression of hematopoiesis but rather in a dynamic change of hematopoiesis during and after benzene exposure [23].

While several studies pointed to the risk of occupational exposure to gasoline on hematological profile, in different literatures, there is controversial association between hematological parameters and gasoline exposure, and also as far as my knowledge, there are no studies regarding this title in the study area. Therefore, the aim of this study was to assess hematological parameters among gasoline exposed workers at gas station in Hossana Town, South West Ethiopia.

1.3 Significance of the study

This study will provide a scientific evidence about hematological parameters among gasoline exposed workers and will benefit study participants by early detection of hematological abnormality, if any, for appropriate interventions. It also helps them to be aware of the risk of their occupational exposure on their hematological parameters and take appropriate safety measures. It also assists to know current state of hematological profile of gasoline exposed workers, to prevent further complication and death. More over this study gives a baseline data for further investigation of hematological parameters on gasoline exposed workers in gas station. Administrators of the gas stations as well as Hossana town administration will also be benefited to take appropriate safety measures to other similar workers. The finding of this study may contribute to the strengthening of occupational safety policy at a larger scale in the country.

CHAPTER TWO: LITERATURE REVIEW

Gasoline is one of a main hazardous substance to human beings and associated with various health effects. Petrol station attendant workers are chronically exposed to petroleum derivatives primarily through inhalation of the volatile organic compounds during vehicle refuelling. Human exposure to gasoline is associated with multiple adverse health effects including pancytopenia, hence aplastic anemia and an increased chance of developing cancer (acute myeloblastic leukaemia), chromosomal aberrations [10].

A cross-sectional study conducted in a total of thirty eight (38) subjects, twenty four gasoline exposed workers and forty (14) controls at petrol stations in Babil Province/Iraq in 2017, no significant differences were found in the means of RBC counts, Hb, Hct%, MCV, MCH, MCHC, RDW, WBC counts, differential counts of WBC, PLT counts between workers exposed to benzene (filling workers) and non-exposed (office's workers). However, Hb and Hct% were significantly increased in smoker workers as compared to non-smokers at petrol stations, while the total number of platelets (PLT) was significantly reduced. There were no significant associations between the duration of benzene exposure, personal age and all measured hematological parameters. This study concluded that benzene exposure has a potential to induce hematological parameters among smoking people working at filling stations [24].

Another cross-sectional survey study was conducted in 2011 in Iraq Baghdad city with total of 292 individuals between age20-55 years, 146 petrol filling workers and 146 individuals who already work in station as overseers as control. 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 significantly reduced as compared to control. Out of six potential risk factors, smoking status, drinking status, type of work, duration of employment, previous job and duration of residency. Only smoking habit found to be significantly associated with the presence of white blood cell changes as compared with petrol filling workers who had no such risk factors. The study concluded that, the hematological indices may be useful in detection of early hematological changes among workers exposed to benzene vapor [25].

A case–control study conducted in Kermanshah City, Iran, in 2018. Total of 160 participants were involved among them, 80 participants worked in a gas station for at least 4 years and 80 age and sex matched control. A significant reduction in total leukocyte count was found in exposed group compared to non-exposed group. However, there was a significant increase in neutrophil in exposed group compared with non-exposed group. This study concludes that long-term exposure to petrol fumes has deleterious effect on white blood cells [26]. Another survey conducted in Khozestan province, Iran, on 495 individuals including those exposed and non-exposed. The hematological findings of 99 persons were compared with those of 396 individuals from the general population by age and sex. 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. They concluded that the association between exposure to natural gas and hematological changes is apparent [27].

According to cross sectional study conducted in Surat in India, 2016. A total of sixty (60) of study subjects aged 20-50 years. Thirty (30) healthy petrol pump workers working for ≥1 year in the station and thirty (30) healthy age and sex matched control group. RBC counts, Hematocrit (HCT) and concentration of Hb were significantly higher in study group than in the control group. Platelet count was lower in study group than in the control group. Red blood cell indices such as MCV) and MCH) were not statistically significant [28].

According to cross-sectional study 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 200 exposed and 200 reference subjects. The results of blood tests showed that the means of RDW were higher in the exposed group than in the reference group and HCT parameters were significantly lower while the other parameters were within the normal range. This study concluded hematotoxicity is a major outcome [29].

According to study conducted in petrol pump attendants in Pune, India in 2015 with total of 30 healthy males working in gasoline station and control group. There was a statistically significant lower value of Hb content, RBC count and PCV in test group where as MCV and MCH were

significantly higher in the test group compared to control group. This study suggest that long term exposures to gasoline leads to a decrease in hemoglobin level, decrease in RBC count, decrease in PCV and macrocytic anemia [12]. Another study conducted in different petrol stations in India, 2016 with ninety exposed groups and 30 control groups. Only Hb was significantly lower among the cases compared with controls. The level of other biochemical parameters like TLC and RBC count was similar between the two groups. [30].

Another cross-sectional study was done from November 2015 to June 2016 among petrol pump workers in India This study consist 30 adult male volunteers of age group 25-40 years working in petrol pump stations for more than 5 years and 30 control of same age group who did not have the history of exposure to petroleum products. The study showed a statistically significant reduction in RBC count, Hb concentration and TLC count among study group when compared with control group. Thus, the study concluded that chronic exposure to petrol fumes has toxic effect on haematological parameters leading to bone marrow depression [31].

According to study conducted in Dehradun region total 150 petroleum attendant were taken 50 petroleum attendant were having work experience greater than one year. This study found significant decrease in Hgb, RBC, Hct, MCHC and platelets with respect to years of exposure, other parameters such as TLC, MCV, and MCH were varying insignificantly with respect to years of working experience in petrol pumps. All the parameters were within normal limits. This concludes that chronic exposure to petroleum fumes has adverse effects on human hematopoietic system, leading to bone marrow depression and resultant pancytopenia [32].

A comparative cross-sectional study was conducted in Mansoura City Egypt in 2017. The study includes twenty five participants worked at least for one year at gasoline stations and other 25 control groups worked away from any source of gasoline. Results Obtained from CBC showed that a significant decreases in all blood indices including; RBCs, Hb, HCT, MCV and MCH, as well as total and diff WBCs counts, except neutrophils, which were significantly increased in gasoline exposed workers compared to unexposed subjects [33].

According to retrospective study conducted in British with total of 1422 study subjects in 2014. The study found benzene exposed subjects had significantly higher levels of WBC (× 103 per μl)

counts, Hemoglobin (g/dL) Hematocrit (%) and platelet (× 103 per µl) counts compared with the unexposed subjects. This study concludes that exposure to benzene may be a higher risk of developing blood related disorders [34].

According to case-control study conducted in petrol station attendants in 2015 with total of hundred (100) study subjects, fifty exposed and fifty(50) Age and gender matched non-exposed individuals were monitored as controls. This study show that mean value of Hct, RBC, WBC count, MCH and MCHC was significantly lower among exposed subjects compared to non-exposed controls and observe significant positive correlation between age of exposure with anaemia and leucopenia respectively. This study concludes that occupational exposure to petroleum product has a significant effect on some hematological parameters [35].

According to a cross sectional descriptive study conducted in Sudan, in 2014- 2015 on fifty (50) workers at fuel stations to determine any alterations in hematological parameters among workers at fifty fuel stations. The study found high prevalence of abnormalities: 50% had low hemoglobin levels, 60% low RBC counts; Hct readings showed 24% with reduced values and for MCV 92% were reduced. Half of the participants showed microcytic cytology. This 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 [38].

Several studies shows that there were no significant differences among workers exposed to benzene and not exposed individuals in terms of RBC counts, Hb, MCV, MCH, MCHC, WBC counts, differential counts of WBC and PLT counts [24]. Another studies reported that RBC count and HCT value were significantly higher among the exposed subjects compared with the control group [27, 33]. On the other hand, different studies reported that WBC count, RBC count, PLT counts, Hb, HCT and MCH of the exposed group are significantly lower compared with control groups [31, 34,35]. Taken together, most of the studies 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 study in study area in particular. This study will investigate the effect of occupational exposure on hematological parameters among gasoline exposed workers at gas station.

CHAPTER THREE: OBJECTIVES

3.1 General objective

To assess the hematological parameters among gasoline exposed workers at gas station as compared to non-exposed controls in Hossana Town, South West Ethiopia from May 1 to June 15, 2020.

3.2 Specific objective

- To compare hematological parameters between exposed and non-exposed individuals.
- To determine prevalence of hematological abnormality among exposed group.
- To assess associated factors of hematological abnormality among gasoline exposed group.
- To compare hematological parameters based on duration of exposure among exposed group

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study area

The study was conducted in Hossana town gas station workers who are exposed to gasoline. The town is the administrative centre of the Hadiya Zone in Southern Regional State, located 232km away from the capital city of Ethiopia, Addis Ababa and 157km from regional city of Hawassa with total population of 31,701. It has a latitude and longitude of 7°33′N 37°51′E with an elevation of 2177 meters above sea level [CSA 2007]. There are 65 gasoline exposed workers in five gas station of hosanna town. This information obtained from municipality of town Governorates. The laboratory test was conducted at Wachemo University Nigist Eleni Mohammed Memorial teaching and Referral Hospital (WUNEMMRH) which is located in Hosanna town.

4.2 Study design and period

Institutional based comparative cross-sectional study was conducted in Hossana town gasoline station workers from May1 to June 15, 2020.

4.3 Population

4.3.1 Source population

All adult individuals work in five gas stations in Hossana town and Wachamo University Nigist Elein Mohammed memorial teaching and referral hospital staffs(WUNEMMRTH) were used as the source population for exposed and non-exposed control group, respectively.

4.3.2 Study population

All adult workers who work at the gas stations with exposure of six months, and above were included in this study [37]. Age and gender matched non-exposed controls subjects from WUNEMMRTH staffs.

4.4 Sample size determination and sampling technique.

4.4.1 Sample size determination

According to the rule of thumb that has been recommended by van Voorhis and Morgan, 30 participants per group are mandatory to detect real differences between groups, which could lead to about 80% power [38]. Therefore, 180 study subjects which comprise 60 gas station workers as exposed group and 120 controls from WUNEMMRTH staffs.

4. 4.2. Sampling technique

A total 65 gasoline station workers are working in four sub-stations in Hossana town. Of these 65 workers, 60 subjects were fulfilled the inclusion criteria and 60 of them were included in the study. Convenient sampling technique was applied for control groups.

4.5 Variables

4.5.1 Dependent variable

Hematological parameters

4.5.2 Independent variables

- Socio-demographic variables (age, gender, marital status, and educational level)
- Behavioural characteristics/Life style (Smoking habits, alcohol-consumption, chat chewing, dietary habit and taking showers at the workplace.)
- Duration of exposure per year, hour per day and days per week.
- Protective equipment measure in use.

4.6 Inclusion and exclusion criteria

4.6.1 Inclusion criteria.

Adult individuals, who have worked at least six months and above at gas station and who were voluntary to participate in the study were eligible for the exposed group and age and gender matched individuals with exposed group and worked away from any source of gasoline were eligible for control group.

4.6.2 Exclusion criteria common to both groups

- Study participants that have chronic diseases (cardiovascular, hematological malignancies), liver and renal disease, Individuals on medication affecting blood cell count (E.g. Erythropoietin therapy, haematin factors) and individuals with blood disorder like anemia and history of previous blood transfusion in the last 3 months were excluded by face to face interview.
- Pregnant women were excluded from study by doing immunochromatic urine HCG test.
- Attendants working in their current position for less than six month.

4.7 Data collection procedure

There are five legal gasoline stations registered in Hossana town in the year 2018-2020 GC. This information was obtained from personal communication with municipalities of town Governorates. The stations are distributed in the four sub-stations as follows: yetababarut (1), asirasemint (1), Mobil (2), qalisha (1). Prior to data collection, all participants were formally informed regarding to importance of this research on the general health and safety of all workers in gasoline stations and hence consents were obtained. Out of 65 gasoline station workers, a total of 60 workers gave a blood sample for analysis. Two of the workers were not volunteers to participate, and the two were excluded due to cardiovascular disease and one pregnancy case. Age and sex matched 120 control participants were selected from WUNEMMTRH staff.

4.7.1 Socio-demographic, behavioural and other related data collection

Data on socio-demographic characteristics, occupational and other related data were collected using a structured questionnaire via face to face interview. Socio-demographic data like age, gender, educational status and occupational data (duration of exposure, daily duty hour and personal protective equipment) and behavioural data like smoking habit, alcohol consumption, dietary habit, taking shower and chat chewing were collected by structured questionnaire interviews which was conducted by the data collector in Hossana town from May 1, to June 15, 2020 (**Annex III)**.

4.7.2 Study participant recruitment and Laboratory data

Eligible participants were requested for their consent after explaining the purpose, benefit and risk of the study by data collectors (**Annex I**). Written consent was obtained from each participant. Consented participants were interviewed using structured questionnaire about socio-demographic, occupational and other related data. For laboratory data, 4ml of venous blood sample was collected in ethylene diaminetertaacetic acid (EDTA) tube by laboratory technologist from each study participants for hematological parameter analysis and blood film preparation. All samples received in the laboratory were checked for quality, and labelled by code numbers. A complete blood count (CBC) tests including RBC count, Hb and HCT levels, red cell indices, Platelet, mean platelet volume(MPV), total and differential counts of white blood cells (WBCs) were analyzed using the BECKMAN COULTER (UniCel® DxH 800, USA) analyzer. Peripheral blood film was prepared from all participants for investigation of red blood cell morphology, white blood cell abnormality and platelets abnormalities. A wedge method of thin blood film preparation was used. After drying the film, Wright stains were added and examined by using light microscope **(Annex IV)**. Approximately five gram of pea sized stool specimen were collected from each study participant of gas station workers and preserved by formalin (10%) in 0.85% saline and wet mount stool examination test was done for detection of intestinal parasites **(Annex IV)**.

Laboratory results feedback

All hematological abnormal results and intestinal parasite positive results were reported to the clinician for proper treatment.

4.8. Data Quality Assurance

All reagents were checked for their expiry date and prepared according to the manufacturer's instructions. The training was given for data collectors to apply standard operational diagnostic procedures to ensure the quality of test. The questionnaire was adopted from other similar studies after review of related literature and prepared in English. It was translated into Amharic then, Hadiyisa version by language linguistics. Finally it was translated back into English to check for consistency. All samples were analyzed in one laboratory (WUNEMMTRH central Laboratory) with the same hematology analyzer and the same trained professionals.

All laboratory tests were done by following the standard operation procedures (SOPs) and manufacturer instructions (**Annex IV**). For automated hematology analyzer, backgrounds check initial, repeated analysis of randomly selected specimens to see reproducibility; randomly selected specimens (high, normal, and low) were checked by other similar hematology analyzer and as a part of laboratory protocol hospital laboratory evaluates instrument performance using whole blood quality control material. Wright stain was filtered every day using filter paper and also stored at locked cabinets away from moisture and sunlight. The data collection, application of standard procedure, accuracy of test results was checked by principal investigator.

4.9 Operational definition

Anemia: defined according to WHO cut-off value as a Hgb concentration of less than 12 g/dL in women and less than 13 g/dL in men [42].

Leukopenia: Defined as the total white blood count less than $4.0x10^3$/ µl for both sex [52].

Thrombocytopenia: Platelet count less than $150x10^3$/ µl for both sex [52].

Exposed group: a person who have worked in petrol station at least six month and above as gasoline station workers.

Control group: a group of people without a condition of interest (

Gas-station attendants: are workers at a full-service filling station who perform service, pumping fuel, cleaning windshields, and checking vehicle oil levels.

Hematological parameters: Are parameters such as RBC parameters including; RBC count, Hgb, HCT. RBC indices such as, MCV, MCH, MCHC, RDW and WBC parameters including total WBC counts, differential and absolute number of WBC, platelet count and mean platelet volume.

4.10. Data processing and analysis.

After checking data for completeness, missing values, and coding of the questionnaires, the data were entered into Epi-data version 3.1(Epi-Data, Odense, Denmark), and were exported into Statistical Package for Social Science (SPSS) version 20(SPSS, Chicago, USA) statistical software for windows. To avoid any effects of ambient temperatures on the samples, collected blood samples were always stored in cold conditions using a wet-ice box until the time of measurement. Prior to performing an appropriate type of analysis, all data were tested for normality by a Shapiro-Wilk test.

Hematological parameters were compared between gasoline exposed workers and non-exposed controls using independent-samples t-test for normally distributed data and Mann-Whitney U-test for non-normally distributed data. The one-way ANOVA test was used for analysis of variance for average hematological parameters according to duration of exposure. To measure correlation of hematological parameters with duration of exposure and personal age, Pearson correlation coefficient was used for normally distributed data and Spearman correlation coefficient for non-normally distributed data. Logistic regression analysis was conducted to assess the association of predictors and outcome variables. The results were summarized in mean ± standard deviation (SD), median, and interquartile range (IQR). The results were presented in tables and texts. P-value <0.05 was considered as statistically significant for all analysis.

Age, duration of exposure and daily duty hour was categorized based on previous study [30]. HGB concentration was adjusted for altitudes and smoking as per recommended by WHO standard guide line [39].

4.11 Ethical consideration.

Ethical clearance was obtained from the Institutional review board of Institute of Health, Jimma University. Letter of cooperation was written to both Hossana town municipalities and WUNEMMRH to obtain approval. The purpose, benefit and procedure of the study were clearly explained to the study participants **(Annex I)** and written informed consent was obtained from the study participants and those willing to participate were included. All of the study group were informed that their response was kept confidential through anonymity. The specimens collected from the participants were analyzed only for the intended purposes. Those study participants who have the abnormal laboratory test result during the laboratory testing process were referred to the clinician for proper treatment, and management according to their specific disease.

4.11 Dissemination of the results.

The findings will be presented to Jimma University School of Medical Laboratory Science and provided for Hossana town administration to take appropriate measure. Finally, it will be submitted to medical journal for publication.

CHAPTER FIVE: RESULT

5.1. Socio-demographic characteristics of study participants

This study included a total of 180 study participants. Out of 180 study subjects, 60 individuals were gasoline exposed workers and 120 individuals were non-exposed control groups. The study subject's socio-demographics data are shown in Table 1.The age of the gasoline exposed workers was between 20-45 years, with mean ± SD of 30±5.23. Out of the 60 exposed subjects, 93.3 %(n=56) were male and 6.7% (n=4) were female. Ninety two (76.7%) subjects were male in the non-exposed control group.

Table 1: Socio-demographic characteristics of the study group at Hossana town, SNNPR, South Ethiopia, from May to June 2020 (n=180).

Variables	**Gasoline station workers** (n=60)	**Control group** (n=120)	***Chi-square test***
Gender			
Male n (%)	56(93.3%)	92(76.7%)	p=0.006
Female n (%)	4(6.7%)	28(23.3%)	
Age group in years			
20-24	15(25%)	30(25%)	
25-29	33(55%)	58(48.3%)	
30-39	11(18.3%)	30(25%)	p=0.7
≥40	1(1.7%)	2(1.7%)	
Mean±SD	30±5.23	31±5.49	
Marital status			
Single	38(63.3%)	54(45%)	
Married	20(23.3%)	62(51.7%)	p=0.5
Divorced	1(1.7%)	1(0.8%)	
Widowed	1(1.7%)	3(2.5%)	
Educational status			
no formal education	13(21.7%)	--	
read and write	13(21.7%)	--	
primary	22(36.7%)	3(2.5%)	p=0.01
secondary	8(13.3%)	1(0.8%)	
college/university	4(6.7%)	116(96.7%)	

Abbreviation, SD: Standard deviation

5.2. Duration of work at gas station

More than one third of the gasoline station workers 36.6 %(n=22) were found to have worked in the gasoline station for 2-5 years. Eighteen (30 %) of workers had worked for only year in the station. The average exposure time was 3.63 ± 2.04 years with an average of 6.77±0.18 hour/day. Thirty five (58.3%) of workers were worked 7-10 hour per day (Table2).

Table 2: Socio-demographic characteristics of the study group at Hossana town, SNNPR, South Ethiopia, from May to June 2020 (n=180).

Variables	**Gasoline station workers (n=60)**	
Duration of work in years	**Frequency**	**Percentage**
1 year	18	30
2-5 years	22	36.7
>5 years	20	33.3
Mean±SD	3.63±2.04	
Working hours / day		
3-6	25	41.7
7-10	35	58.3
Mean±SD	6.77±0.18	
Working hour per week		
Mean±SD	47.3±1.3	

Abbreviation, SD: Standard deviation

5.3. Personal characteristics of individuals in gasoline exposed workers

Personal characteristics of the gasoline station workers are illustrated in Table 3. One third 20 (33.3%) of the exposed groups have habit of smoking cigarette. From these 33.3% (n=20) of smokers, 55 % (n=11) of them smokes more than 10 pieces of cigarette per day. Fourteen (23.3%) of the exposed group were alcohol drinkers. More than half 57% (n=8) of alcohol drinkers drinks greater than 1060ml (3 bottles) of alcohol per day. During time of the data collection, 30% (n=18) of participants had habit of consumption of red meat and 66.7 %(n=40) had habit of drinking coffee or tea immediately after meal.

Table 3: Behavioural and related characteristics of gasoline exposed workers at Hossana town, SNNPR, South Ethiopia, from May-June 2020 (n=60).

Characteristics	Categories	frequency	Percentage
Smoking status	No	40	67.3
	Yes	20	33.3
Number cigarette smoke per day	≤10	9	45
	>10	11	55
Alcohol consumption	No	46	76.3
	Yes	14	23.3
volume of alcohol consumed per day	≤355ml	3	21.4
	710-1060ml	3	21.4
	>1060ml	8	57.1
chat chewing	No	51	85
	Yes	9	15
habit of consuming fruit/green vegetable	No	16	26.7
	Yes	44	73.3
Habit of consumption of red meat	No	42	70
	Yes	18	30
drinking coffee immediately after meal	No	20	33.3
	Yes	40	66.7
Intestinal parasite	No	52	86.7
	Yes	8	13.3
	hookworm	4	50
	A.lumbericoide	1	12.5
	E. histolytica/ dispar	1	12.5
	others	2	25

Other: E. vermicularis and T. trichiura

5.4. Protective measures in use among gasoline station workers

The majority of the workers 66.7% (n=40) did not use any kind of personal PPE. From PPE users, 90% (n=18), 15% (n=3), 10% (n=2), and 35 %(n=7) workers had use glove, hat, face mask, and special shoes respectively. Regarding to activities of gasoline station workers in working place, 35(58.5%) of the gasoline station workers had a habit of chewing gum and 25 % (n=45) of workers do not eat, not drink, and chew gum during working time in the station. The main reason for not using safety equipments was lack of the protective equipments to the workers as 61.7% (n=37) of them responded that safety equipments were not provided (Table 4).

Table 4: Protective measures in use among gasoline station workers at Hossana town, SNNPR, South Ethiopia, from May-June 2020 (n=60).

Protective measure in use		
		Frequency (%)
PPE	No	40(66.7)
	Yes	20(33.3)
Glove	No	2(10)
	Yes	18(90)
Hat	No	17(85)
	Yes	3(15)
Face mask	No	18(90)
	Yes	2(10)
Special shoes	No	13(65)
	Yes	7(35)
Showering at work place	No	46(76.7)
	Yes	14(23.3)
Eating at work place	No	53(88.3)
	Yes	7(11.7)
Drinking at work place	No	57(95)
	Yes	3(5)
chewing gum at work place	No	35(58.3)
	Yes	25(41.7)
Never	No	45(75)
	Yes	15(25)

Reason for not using protective equipments		
Not provided	37	61.7%
Not comfortable	10	16.7%
Not necessary	8	13.3%
Carelessness	5	8.3%

Abbreviation: PPE, personal protective equipment, Never means study subjects not eat, drink and not chew gum at work place.

5.5. Hematological parameters of gasoline exposed and non–exposed control groups

The mean±SD or median (IQR) of the hematological parameters were compared between gasoline exposed groups and control groups of study participants are presented in Table 5. Accordingly statistically significant differences were found in the median (IQR) of Hgb (p=0.001) and the mean±SD value of RBC count (p=0.007) between exposed subjects and control group. The median (IQR) value of absolute eosinophil count were significantly higher among exposed subjects as compared to control subjects (p=0.01) respectively. The levels of other hematological parameters like MCHC, RDW and absolute monocyte count were not significant different between the groups.

Table 5: complete blood count picture of the study group at Hossana town, SNNPR, South Ethiopia, from May-June 2020 (n=180).

Parameters	**Exposed group (n=60)**	**Control group (n=120)**	**P-value**
WBC($\times 10^3/\mu l$)$^{\alpha}$	7.31±2.39	7.0±1.98	0.39
RBC($\times 10^6/\mu l$)α	4.87±0.62	5.08±0.41	0.007
HGB (g/dl)	14.38(1.7)	15.4(1.3)	0.001
HCT%	45.7(5.8)	47.7(6.5)	0.04
MCV(fl)$^{\alpha}$	89.08±4.23	89.3±3.59	0.74
MCH (pg)	29.2 (2.07)	29.3(1.6)	0.192
MCHC(g/dl)	32.9(1.17)	32.9(1)	0.82
RDW%$^{\alpha}$	13.804±0.65	13.8±0.7	0.62
PLT ($x10^3/\mu l$)$^{\alpha}$	264.67±73.19	272.61±62.0	0.44
MPV(fl)$^{\alpha}$	7.903±0.75	7.83±0.6	0.78
Neutrophil absolute	4.2(3.85)	4.3(2.5)	0.58
Lymphocyte absolute	1.85(1.17)	1.7±(1)	0.57
Monocyte absolute	0.5(0.2)	0.5(0.2)	0.16
Eosinophil absolute	0.3(0.57)	0.2±(0.3)	0.01
Basophil absolute	0.00(0.1)	0.00(0.1)	0.79
Neutrophil relative	58.4(23.6)	63.6(19)	0.66
Lymphocyte relative$^{\alpha}$	24.362±9.38	27.0±7.7	0.04
Monocyte relative	6.6(2.95)	6.65(3)	0.61
Eosinophil relative	3.4(6.92)	2.45(3.7)	0.09
Basophil relative	0.5(0.57)	0.6±0.5	0.17

P-*value, Independent sample t-test=α* :(Mean±SD), ***Mann-Whitney U test= not labelled as α:*** **median (IQR)**

5.6. Comparison of hematological parameters based on duration of exposure among exposed gasoline exposed workers

The mean value of MCV (p=0.04) were significantly decrease with respect to duration of working experience. The mean value of RBC count at (p=0.2) and, MPV (p=0.37) were not significant difference with respect to duration of working experience. Other parameters were varying non-significantly with respect to years of working experience in gasoline station workers (Table 6).

Table 6: Comparison of change in hematological parameters based on duration of exposure among gasoline exposed workers , one way ANOVA analysis at Hosanna town, SNNPR, South Ethiopia, from May-June 2020 (n=60).

Parameters	Duration of exposure in year	Mean±SD	P-value
WBC($\times 10^3/\mu l$)	1	7.16±2.32	0.8
	2-5	7.5±2.31	
	>5	7.2±2.6	
RBC($\times 10^6/\mu l$)	1	5.09±0.65	0.2
	2-5	4.8±0.5	
	>5	4.75±0.66	
MCV(fl)	1	90±2.94	0.04
	2-5	89.4±4.6	
	>5	87.9±3.0	
RDW%	1	13.67±0.65	0.58
	2-5	13.83±0.5	
	>5	13.88±0.78	
LYM%	1	26.23±9.9	0.31
	2-5	27.65±8.2	
	>5	21.46±9.6	
PLT($\times 10^3/\mu l$)	1	266.5±51	0.5
	2-5	279.6±79	
	>5	253±88	
MPV(fl)	1	8.1±0.78	0.37
	2-5	7.79±0.69	
	>5	7.8±0.79	

WBC: white blood cell, LYM%: relative lymphocyte, RBC: red blood cell: MCV: mean cell volume, RDW: red cell distribution width, MPV, mean platelet volume.

5.7. Comparison of hematological parameters between smokers and non-smokers in exposed group

A number of smokers who work at gasoline stations were 20 subjects compared to 40 in non-smokers. Most of the tested parameters were not significantly different among both smokers and non-smokers. However, Hgb (p=0.02), Hct (p=0.001) and absolute eosinophil count (p=0.04) were shown to be significantly increased in the smoker workers compared to non-smoker workers (Table 7).

Table 7: Comparison of some hematological profile between smokers and non-smokers in gasoline exposed groups in gas station at Hosanna town, SNNPR, South Ethiopia, from May-June 2020 (n=60).

Parameters	Smokers at gasoline exposed group (n=20)	Non –Smokers at gasoline exposed group (n=40)	P-value
WBC($\times10^3$/µl)	8.12±2.6	6.9±2.16	0.06
RBC($\times10^3$/µl)	4.94±0.72	4.899±0.55	0.97
Hgb(g/dl)	15.06±1.99	14.04±1.4	0.02
Hct%	49.1±7.1	43.95±4.1	0.001
MCV(fl)	89.6±3.63	88.8±4.52	0.5
MCH(pg)	29.35±2	29.1±2.35	0.5
MCHC(g/dl)	32.9±1	32.85±1.27	0.8
RDW%	13.82±0.74	13.79±0.61	0.85
lymphocyte%	24.35±8.70	24.36±9.81	0.99
MPV(fl)	7.87±0.76	7.91±0.75	0.83
PLT (x10^3/µl)	252±80.7	274±71.69	0.27
Neutrophil	5.5±3.85	4.1±1.97	0.16
Lymphocyte	2±1.07	1.65±1.1	0.07
Monocyte	0.5±0.37	0.5±0.2	0.5
Eosinophil	0.5±0.7	0.2±0.5	0.04
Basophil	0.00±0.1	0.00±0.1	0.75

Independent sample t-test: WBC, RBC, MCV, RDW, PLT, MPV and lymphocyte percentage.
***Mann-Whitney U*-test:** Hgb, Hct, MCH, MCHC, absolute neutrophil, lymphocyte, monocyte, eosinophil, Basophil

5.8. The Correlation of hematological parameters with duration of exposure and age for the exposed group

As described in Table 7, there was a negative correlation between Hct and MPV with duration of exposure (r= -0.11, p= 0.4, r= -0.14, p=0.27) respectively. Hgb and MCV had positive correlation (r = 0.11, p = 0.12, r=0.1, p=0.14) with age. There was a negative correlation of MCHC with years of exposure and age of participants. Rest of parameters did not show any correlation with duration of exposure and age of participants.

Table 8: Correlation of hematological indices with duration of exposure and age for the exposed group at Hosanna town, SNNPR, South Ethiopia, from May-June 2020 (n=60).

Parameters	**Duration of exposure in year**		**Age of exposed group**	
	r	P- value	r	P-value
WBC($\times 10^3/\mu l$)	0.044	0.737	0.070	0.353
RBC($\times 10^3/\mu l$)	-0.05	0.705	0.088	0.242
Hgb	-0.076	0.56	0.11	0.12
Hct	-0.11	0.4	0.15	0.43
MCV	-0.032	0.01	0.1	0.14
MCH	-0.04	0.002	0.06	0.39
MCHC	-0.13	0.32	-0.11	0.12
RDW%	0.054	0.68	-0.13	0.7
PLT ($x10^3/\mu l$)	0.012	0.92	-0.013	0.86
MPV	-0.14	0.27	-0.05	0.46
Neutrophil absolute	0.002	0.12	0.02	0.7
Lymphocyte absolute	0.006	0.96	0.03	0.61
Monocyte absolute	0.085	0.5	0.06	0.42
Eosinophil absolute	0.074	0.57	0.03	0.63
Basophil absolute	0.071	0.6	0.03	0.63

Pearson correlation-test was applied for WBC, RBC, MCV, RDW, PLT and MPV
Spearman rho correlation -test was used for rest of parameters.

5.9. Prevalence of hematological abnormality (leucopenia, anemia and thrombocytopenia) among gasoline exposed groups.

The prevalence of leucopenia, anemia and thrombocytopenia among gasoline exposed workers was 6.6 % (n=4), 11.7 %(n=7) and 10 %(n=6) respectively. Among those exposed 8.3 %(n=5) had leukocytosis. There were no thrombocytosis found in gasoline exposed gas station workers.

5.10. Peripheral blood morphology examination results

All gasoline exposed gas station workers were examined for peripheral morphology**.** Of the examined peripheral blood films from the exposed group, 81.7% (n=49) of the result were Normocytic normochromic picture, 11.7 %(n=7) had macrocytic oval shaped red cells with increasing corresponding MCV value and 6.6 %(n=4) participant had microcytic red cells with reduced MCV value (66 fl).

5.11 Associated factors and hematological abnormality among exposed group

In Bivarate analysis of the association between hematological abnormality and associated factors, there were no significant association between hematological abnormality and associated factors among gasoline exposed groups. And no variable was candidate for multivariate analysis.

CHAPTER SIX: Discussion

Human exposure to benzene is associated with multiple adverse health effects primarily related to impairment of the hematopoietic system with bone marrow depression including pancytopenia, hence aplastic anaemia and an increased risk of developing cancer. However, the precise mechanism of benzene induced toxic effects is not fully understood. Therefore, this study was conducted to assess the changes in the hematological parameters among gasoline exposed workers at gas station in Hossana Town, SNNPR, South Ethiopia. Current study showed that more than half of gasoline stations workers (55%) were between 25 and 29 years of age and a large proportion of gas workers have spent between 1– 8 years on this job. and were usually worked in stations for many hours (3-10) per day.

In the present study, the majority of participants (66.7%) were found to be working without use of any proper personal protective equipment (PPE). Use of PPE was found to be poor, with only twenty (33.3%) of the gasoline station workers using it, of which seven used special shoes (boots), eighteen gloves, two face mask, and three a hat. This study is similar with study conducted in Nigeria petrol station attendants, showed that greater than half of study participants did not use any kind of PPE [32]. A possible reason for not usage PPE may be lack of adequate information from the employers about the hazards involved in working at a petrol station and lack of provision of the PPE by owners of the gasoline station.

The results of current study showed that RBC, Hgb, and Hct count were significantly decreased in exposed subjects compared to non-exposed control subjects. Results of this study were in agreement with different studies which explained that decreased in Hgb and RBC count could be attributed to shortened life span of RBC by the metabolic end product of free radicals produced during benzene metabolism. These free radicals can alter the erythrocyte membrane and heme protein synthesis in bone marrow [12, 17, and 31]. And also decline of these parameters is due to hematotoxic effect of benzene that causes the impairment of the haemopoetic system with bone marrow depression leading to pancytopenia (a general depression of erythrocytes, leucocytes and platelet production [20]. These findings are inconsistent with results from one study done in Texas City in 2014, which reported that Hgb and Hct levels were elevated in benzene exposed subjects compared with the unexposed subjects [35]. This difference could be explained by the

idea that individual variation in genetic susceptibility to benzene toxicity caused by a series of in genes that control cell adhesion as well as DNA stability and repair [25].

The results of present study were in contrast with study, performed in Iraq on 38 workers of benzene exposed and non-exposed controls. There were no significant differences in the mean value of RBC counts, Hb, Hct%, MCV, MCH, MCHC, RDW, WBC counts, differential counts of WBC, PLT counts between workers exposed to benzene (filling workers) and non-exposed [24]. This might be due to difference in duration of exposure and daily duty hour of the participants and difference in small sample size of study.

The red cell indices of present findings are in accordance with that of a study, conducted in Surat city on 30 workers of petrol filling station with ≥1 year's duration of exposure and 30 controls show that there were no statistical significant difference of RBC indices such as MCV and MCH between exposed and non-exposed to benzene which is similar to present study [28] and inconsistent with study done Pune, India, MCV and MCH were significantly higher in the test group compared to non-exposed control group[12]. This inconsistency might be difference in length of exposure and small size of pervious study.

There was no statistically significant difference in platelet count between gasoline exposed and non-exposed control subjects in a study done in Bhubaneswar [11] which is consistent with our findings. In contrast to present findings, platelet count was increased non-significantly in benzene exposed subjects in study done in British in Texas City [27, 35]. This might be small sample size used in present study and difference in amount and duration of exposure.

Results of current study showed that there was non-significant increase of white blood cells (WBC) count in gasoline exposed subjects as compared to non exposed groups. This result is in accordance with study conducted in Nigeria. This could be explained by different studies, high rate of infection in exposed groups due to immunosuppressant effect of toxic petrol products, which in turn leads to an increase in WBC [43] and inconsistent with study conducted India showed non-significant decrease of WBC between gasoline exposed and unexposed groups [30]. This might be difference in length of exposure time of study participants

In this study red blood cell (RBC) and mean platelet volume(MPV) were insignificantly decrease with respect to duration of exposure, other parameters such as platelet count(PLT), relative lymphocyte count and RDW were varying insignificantly with respect to duration of working experience in gasoline station workers. This finding is in agreement with study conducted in Dehradun region, where petrol pump workers with longer exposure time experienced more adverse effects on hematological parameters [33].

The results obtained from current study indicated that a significant increased level of Hgb and Hct in workers at filling stations among cigarette smokers. These findings are in agreement with a previous study conducted in Iraq and Baghdad city [24, 25]. The elevation of Hgb and Hct levels observed in the smokers group could be partially attributed to the synergistic effect of smoking with benzene exposure. Another reason is that smoking is known cause of increase Hgb concentration that is believed to be mediated by exposure of carbon monoxide (CO). CO binds to Hgb to form carboxyhemoglobin, an inactive form of Hgb having no oxygen carrying capacity. Carboxyhemoglobin also shifts the Hgb dissociation curve in the left side, resulting in a reduction in ability of Hb to deliver oxygen to the tissue [44]. Significant increase in Hct in gas station smoker is believed that CO from the tobacco smoke leads to an increase in permeability of the capillaries which decreases the volume of plasma, which finally mimics the condition of polycythemia share of the RBC in the blood volume, which is reflected also through increased values of Hct [45].

Results of present study showed that, neutrophil count was non-significantly decreased in gasoline exposed workers compared to unexposed subjects. This study was inconsistent with study conducted in Mansoura City Egypt in 2017 which was neutrophil count increased in gasoline exposed workers compared to unexposed subjects [34]. This differene might be small sample size of previous study and also difference in level of gasoline exposure.

Lack of findings any significant correlations between duration of gasoline exposure, personal age with the measured hematological parameters in our study are in line with the findings of study conducted in Iraq [24]. The absence of such correlations could be explained by the idea that the workers with different exposure periods are still having an effective compensatory system to cope with possible changes that might be found during the exposed levels and thus ending up

with good homeostasis [46**]**. On the other hand, our result was contrary to study conducted in Nigeria [36]. Variation in the sample size and duration of exposure might be the possible reason for the discrepancies.

The finding of present study showed that prevalence of hematological abnormalities such as: leucopenia (6.6%), anemia (11.7%) and thrombocytopenia (10%) respectively among gasoline exposed group. The hematopoietic system is highly sensitive to most of the volatile organic solvents reaching the blood. The solvents and air pollutants may interfere in the process of RBC proliferation, which causes adverse effects on heme synthesis and the life expectancy of RBCs **[**29]. Another reason is that toxic effects of benzene metabolites with prolonged exposure, cause a suppression of bone marrow results change in blood parameters; it could cause anemia, or (leucopenia) or thrombocytopenia [12,13]. Prevalence of anemia in this study among those gasoline exposed workers were (11.7%) which is in line with EDHS report of 2012 of which is 11% of men aged between 15-49 were anaemic [47]. And lower than the EDHS report of 2016: 15% of men aged between 15-49 were anaemic [48]. The possible reason for this discrepancy might be due to variation in duration of exposure.

Regarding to peripheral blood film of the study participants showed (6.6%)microcytic red cells with reduced MCV value gasoline exposed workers, which is inconsistent with study conducted in Sudan, from 2014- 2015 reported regarding cell morphology 50% of the participants showed a microcytic picture [38]. This inconsistency might be difference in duration of exposure and sample size of the study. Current study showed that (11.7%) macrocytosis of red cell which is in line with this finding FirouzkouhiM, *et al* [49] and study done in Nigeria [50] reported macrocytosis. The possible cause of macrocytosis was a defect or delay in DNA synthesis that interferes with cellular proliferation and maturation can lead to large` erythrocytes. In addition to that toxic component of petrol can affect erythropoiesis, produce changes in the RBC membrane and make it susceptible to hemolysis, interferes with replication and thereby macrocytic anemia [12]. Present study showed that (81.7%) Normocytic-picture. This morphological variation might be because of method and/or equipment, performance difference in the study.

Strength and Limitation

Strength of study

- Nature of comparative study design.
- Peripheral blood film morphology examination was done.
- Analysis of the laboratory results was carried out using, the same haematology analyzer and the same trained professionals.

Limitation

This study has several limitations. A cross-sectional nature of study design allows only hypothesis-generating, and not causality to be investigated. Thus, it is difficult to infer a causality using such a study design because the clinical outcomes were measured at one time point after exposure to gasoline. Another important limitation of this study has been the lack of baseline data of the study subjects prior to their gasoline exposure for the comparison of hematological profile of the gasoline station workers when they first started work as gasoline station workers. On the other hand, intestinal parasites were not detected by concentration techniques. Current study was conducted among small sample size; this might reduce the statistical power of the study. In present study, we measure blood parameters in gasoline station workers exposed to a mixture of different chemicals. We did not measure the specific chemicals to which gasoline station workers and nor did define the effect of a single chemical due to lack of method to analyze and failed to produce comparison with hematological indices. Exposure levels were not assessed due to lack of method to measure. It would be better to assess types of their occupational chemical exposure and their effect on health in future studies.

Chapter seven: Conclusion and recommendation

7.1. Conclusion.

From the results of present study, we concluded that occupational exposure to gasoline and its product cause alteration of some hematological parameters. These hematological alterations include decreased RBC, Hgb, Hct count and increased absolute eosinophil count in gasoline exposed subjects compared with unexposed subjects. Gasoline exposure has a potential effect to induce hematological alterations among smoking people working at gasoline stations. It has been observed that smoker workers at gasoline stations had significantly higher levels of Hb and Hct%, but decreased levels of total number of PLT compared to their levels in non-smokers. Therefore, this study concluded that the association between exposure to gasoline and hematological changes is apparent.

7.2. Recommendation

Based on this study finding, the following recommendations are forwarded:

- The availability and regular use of personal protective equipment like (Glove, face mask, special shoes or boots) at work to minimize workplace gasoline exposure should be encouraged.
- A medical observation, including prior to employment and periodic medical check up of hematological profile before development of chronic impairment should be done..
- Further long-term prospective studies with larger sample size of gasoline worker should be needed to get a more comprehensive picture of long term effects of gasoline exposure and identify the specific types of chemicals and define the effect of a single chemical to which the gasoline station workers are exposed.

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ANNEXES

Annex-I information sheet (English version)

Title of the research project: Assessment of hematological parameters among gasoline exposed workers at gas station in Hossana Town, South West Ethiopia.

Name of principal investigator: Tamirat Ersino kebamo

Name of organization: Jimma University School of Medical Laboratory Sciences.

Introduction: You are being invited as study participant of this study conducted by MSc candidate, from Jimma University. Your participation is voluntarily. The research team was includes one principal investigator, two advisors from Jimma University. Please take as much time as you need to listen information sheet. The study has been approved by ethical review committee of institute of health Jimma University.

Purpose of the research project: We are asking you to take part in this study because we was assess the effect of gasoline exposure on hematological parameters among gasoline station workers in South West Ethiopia, SNNPR in Hossana town from May 1 to June 15, 2020.

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

Procedures and what will be expected from you for participation: In order to conduct the indicated study at Hosanna gas station, you are invited to take part in this study. 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, 4 ml blood sample, urine for HCG and stool sample will be collected from you by specimen collectors.

Incentives and compensation; You will not receive any payment for your participation in this study 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

The information obtained during this study was kept confidential. Disclosure of any of the data to third parties other than those allowed in the informed consent was not permitted. Records will be remained confidential. To maintain confidentiality, the investigator will be kept records in locked cabinets and the results of the tests will be coded to prevent identification of the volunteers.

Right to refuse or withdraw: free to withdraw from the study at any time and will not discriminated in any form of health services due to refusal.

Agreement

After 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 question about this study, you can contact the following principal investigator and advisors for further information.

Mr Tamirat Ersino **(BSc)**, Phone Tel: +251-919-675172, Email:tamiratersino128@gmail.com

Dr Tilahun Yemane (**MD, MSc**) Tel: +251-917-804067, Email: yemanetilahun@yahoo.com

Mr Girum Tesfaye, (**MSc, PhD candidate**) Tel: +251920274035, Email:girumtesfaye12@gmail.com

Information sheet Hadiyisa version

Sorophii qoxoi woshi. Hossana beeroo makhin gazaha inkiro qorri mannanene xiggi celluwwa yo'oi hofechi qaxomaa xanqoo laimma.

Wonii sarayanchi: Tamirat Ersino

Haramuki kitaphii mini: Jimmi Yunvursitee/Labratoorii Losanni Mine

Kuhi woshi kitabi gudukoki hanani kuramuki sorrobane bikkokik manina ihukuyyaa lulomanemii kahi sorrobimane hasisokii luwuwwaa kuriminate . kaahi sorobane bikokii mani hundemi ixxi hasenina ihoisa chakinsammo.

Sorophii woni woshii ; Hossani beeroo makihin gazaha inkiro qorri mannanene xiggi celluwwa yo'oi hofechi qaxomaa laimma.

Sorophii fintouwaa

Sorrobane bikokimina itamuki manisi woroni yokii woshuwaa xigga masinomisa kullamo.

- Oddimi 10 dakikina xamicha xaminomisa
- xigga oddimii masina monomisa.

Kuhi wixuki xiggi oddimi wachaami yunvursitena nigisti ellinii mohammad sawyimi hospitalena wonhi labratorena baxamo.

Sorophii dangoii daffii

Xigga massakamare hofi qaxii xissi machesamoisa kullamo.xigga oddimii xoxolakosine masinamo.

Sidesena xanokii luwuwwa

Kahi sorobane bikamichine sidamoki luwuwuwi khis dinatih ihuki muli luwi beisa xaxihinsa kullamo. Kahi sorobane bikokii manene xiqqi hofechi celluwane heehulas /sidamulas errii fayaomina hakimichii beyo maramoisa isinomo.

Sorophii maxamii woshuwaa

Ayyi kaa sorobane sidamukii woshuwii daphakosine disinomo. Ayyi kaa sorobane sidamukii woshuwii ka sorrobina yakaa uwamukii anan xiggina afurohani ihukuyaa kaha soroba baxokii maninse mulli kenii la'ena xanoyoo. kaa sorophi bikaneka sidamukii woshaa bikkanchi furrmane'e itamukissa kurubellasesnse chakinsomoyo. kuhi sorobi sansawe'e woshii ihukisam ku kitabii erri higgi chakishanehe bikaneka manomato kuroni firoissa kuramakoo.

Sabimmik oddimi ae'imi urmii xanomisa kurimaa

kaa sorobane bikoki manii hundimi hundomissinemi ixii amanene hassa ittaha bikoissa kullamo. sorobanse hasukii amanene itukii belasii firim xanamoisa kullamo. kaa sorobane bikimaa sabimine illagene mahi luwamii hogobeisa fayomii quxone mahi dangoimi affoo beisaa kuramakoo

xammichi yollassi

ka sorobane ayyi xamichi yolassi kanii woronii yooki silki xigi teimi emailena xamimaa xanamoisa chakinisamoo

Tamirat Ersino፡xigi +251-919-675172, imalii :tamiratersino128@gmail.com

Dr. Tilaahun Yamana:xigi+251-917-804067, imalii: yemanetilahun@yahoo.com

Abachii Giruumi Tasfayee: xigi +251-920-274035: imalii: girumtesfaye12@gmail.com

Amharic version

የጥናቱ ርዕስ፡በሆሳዕና ከተማ ዉስጥ ባሉት ማድያ ነዳጅ በሚቀዱት ሰዎች ለይ የደም ህዋስ ፒሮፋይል ማወቅ

የዋና ታመራማሪ ስም: ታምራት ኤርስኖ

የተቋሙ ስም፡-ጅማ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል

ይህ የመረጃ ቅጽ የተዘጋጀዉ ከላይ በተጠቀሰው ጥናት ለሚሳተፉት ታሳታፊዎች ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሄዳቸው ስለፈለግንለቸው ጉዳዮች እና ስለጥናቱ ጠቅላላ ማብራሪያ ለመስጠት ነዉ። በመሆኑ በጥናቱ የሚሳተፉት በራሷዎ ፍላጐት ብቻ መሆኑን በትህትና እንገልፃለን።

የጥናቱ ዓላማ፤ በሆሳዕና ከተማ ዉስጥ ባሉት ማድያ ነዳጅ በሚቀዱት ሰዎች ለይ የደም ህዋሳት ፒሮፋይል ማወቅ

ስጋትና ጉዳት

በአጠቃላይ ናሙና በሚወሰድበት ጊዜ ሊያጋጥም የሚችል አደጋ እንደማይኖር ግን ናሙና ሲወሳድ ትንሽ የህማም ስሜት ልኖር ይችላል። ነገር ግን የከፋ ችግር ስያገጥም የሕክምና እርዳታ ይደራግሎታል።

ጥቅሞች

በዚህ ጥናት ውስጥ በመሳተፎ በጥሬ ገንዘብ የሚደረግ ክፍያ እንደማይኖር ተነገግሮናል። የጥናቱ ተሳታፊዎች በምርመራ pani(የምያስደነግጥ) ዉጤት ከተገኘቦት ለበለጠ ህክምና እና እንክብካቤ ወደ ህክምናችው እንድሄዱ ይደረግሎታል።

የጥናቱ ምስጢራዊነት

ማንኛዉም በጥናቱ የተገኙ መረጃዎች ምስጥራዊነቱ የተጠበቀ ነዉ:: የጥናቱ መረጃዎች በሙሉ የተቀመጡት ለጥናቱ ተብሎ በሚሰጠው ስውር ቁጥር ሲሆን ጥናቱን ከሚያስከሄዱት ባለሙያዎች በስተቀር ማንም ሊያወቅ አይችልም።የጥናቱ ተሳታፊ ማንነት በሚገልጥ መልኩ የተዘጋጃውን መረጃ የጥናቱ ተሳታፊ በፊርማዉ የተረጋገጠ ፍቃድ ሳይሰጥ ይፋ አይደርግም:። ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢወጣ ወይንም በሚድያ ቢነገር የጥናቱ ተሳታፊ ስም በምንም መልኩ አይጠቀስም።

ያለመቀበል ወይም ጥሎ የመዉጣት መብት

በዚህ ጥናት ዉስጥ የሚኖሮዎት ተሳትፎ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ እንደሆነ የተገለፀላቸዉ ሲሆን በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብታቸዉ ሙሉ በሙሉ የተጠበቀ እንደሆነ ተገልፆላቸዋል:: በጥናቱ ባለመሳተፋቸዉ ወይም ከጥናት በመገለላቸዉ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታ ላይ ተፅዕኖ እንደማይኖረዉ በግልፅ ተነግሮናል።

ጥያቄ ካለወት

ስለ ጥናቱ ማንኛዉም ጥያቄ ወይም ቅሬታ ስኖራቸዉ የሚከተሉትን ስልኮች ወይም ኢሜል አድራሻ በመጠቀም የጥናቱን ባለቤቶች ማነጋገር እንደሚችሉ ተገልፆላቸዋል።

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Annex II: Consent form in English Version

Participants name _____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with "Assessment of hematological parameters among gasoline exposed workers in gas station in Hosanna town" I have been informed specimen will be taken and there will be interview. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

Agree_____ Not agree_____

Therefore I give my consent freely for my participation in this study.

Name of participant: _____Signature:_____

Name of investigator: _____

Signature_____

Date_____

Hadiyisa version consent form

Bikanchi anani xiggi _____

Bikanchi summi_____

kanni hanane summi chakisumi bikanchi Hossani beeroo makihin gazaha inkiro qorri mannanene xiggi celluwwa yo'oi hofechi qaxomaa laimmina gudukii sorrobane anii laomanine

machesomi saggarine ihokii woshuwaa kuramako. Fayaaomii woshuwaa xammkamisa xiggaa mahi dangimi afonii masakamisa chakisamako. Oddimii masakamii woshuwaa hundami maxamisane amdakamisa chakisamako. Kaa sorobane bikimm urimaa shigigumlas ayii amanenem sabimaa xanomisa kuramakoo kaka isimina mahi luwamii hoggomi beisaa danamisa chakisamko.

Itaamomo_____ _ittamumoyyo _____

Bikanchi summi _____furma'i---------- balii ----------

Sorobanchi summi ---------- furma'i ---------- balii ----------

Amharic version consent form

የተሳታፊው ልዩ መለያ ቁጥር _____

የተሳታፊው ስም_____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በሆሳዕና ከተማ በተለያዩ የነዳጅ ማደያ የሚሰሩ ሰራተኞች በቤንዚን ምክንያት በደማቸው በተለያዩ የደም ዓይነቶች ላይ የሚያመጣውን ጉዳት ለማወቅ በታሰበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ አግኝቻለሁ። የህክምና መረጃና ናሙና ምንም አይነት ጉዳት በማያደርስ መልኩ እንደሚወሰድ ተረድቻለሁ። በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጢር እንደሚያዙ ተነግሮኛል። እንድሁም የሚጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ መብት እንዳለኝ ተነግሮኛል። ከጥናቱ በማንቸውም ወቅት ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንን በማድረጌ አሁንም ሆነ ወደ ፊት የሚያገኝበትን የህክምና አገልግሎቶች እንደማይጎድልብኝ ተነግሮኛል።

እስማማለሁ _____አልስማማም _____

የተሳታፊ ስም _____ፊርማ ---------- ቀን ----------

የተመራማሪ ስም_____

QUESTIONARIES DESIGN

An English language questionnaire was adopted from other similar studies by principal investigator, translated to Amharic and then to Hadiyisa version. Finally, it translated back into English to check for consistency. An interview was done after taking their consent. All interviews were conduct face to face by data collectors. 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 and open ended questions. The questionnaire includes several areas of questions such as socio-demographic characteristic (age, marital status and education), work duration, and personal protective equipment in use. And also Workers was asked to provide information on personal hygiene practices and behavioural characteristics such as: smoking, Alcohol drinking, chat chewing, dietary habit and chewing gum.

Annex III: Structured Questionnaire

This questionnaire was organized for the research project in partial Fulfillment of the Requirements for Masters of Science in Clinical Laboratory Science specialty in Haematology and Immunohematology. It is intended to assess hematological parameters among gasoline exposed workers at gas station in hosanna town**.**

Respondent's identification code:_____ phone number_____

Part I. Socio-demographic characteristics of study participants

1. Sex: _____
2. Age (Years):_____
3. Marital status: A. single B. married C. Divorced D. Widowed
4. Level of education: A. no formal education B. read & write C. Primary D. Secondary School E. college/ university

Part II. Work experience and duration of exposure.

5. What is your role in the station: A. Mechanics B. Gas Fueling C. Cashier D. other specify_____.
6. How many hours you work at this station per day……. Days per week.....
7. How long have you worked in the gas station? (Years)_____
8. Do you use personal protective equipment in working station?
   A. No B. Yes
   Which type of personal protective equipment you use in working station?

8.1 Glove: A. No B. Yes

8.2 Hat A. No B. Yes .

8.3 Face/respiratory mask A. No B. Yes .

8.4 Special shoes (boot). A. No B. Yes

8.5 If you have not use any of equipments listed above what is the reason?

A. Not provided B. Not-comfortable C. Not necessary D. Carelessness

Part III: Behavioural characteristics

9. During working time in the station are you doing the following?
10. Drinking A. No B. Yes
11. Eating A. No B. Yes
12. Chewing gum A. No B. Yes
13. never

11. Do you smoke tobacco/cigarette? A. No B. Yes

12. If your answer is yes to question Number 10, how many pieces of cigarettes on average do you smoke per day? _____

13. Do you drink alcohol? A. No B. Yes

14. If your answer is yes to question number 12, how many bottle of alcohol on average do you drink per day? _____

15. Do you chew chat? A. No B. Yes.

16. Do you take a shower at work site before going home? A. No B. Yes

17. Do you consume fruit and green vegetable? A. No B. Yes

18 Do you consume red meat? A. No B. Yes

19. Do you have habit of drinking coffee or tea immediately after meal?

A. No B. Yes

Thank you very much for your participation!!!

Annex-VIII: Questionnaire Amharic version

መጠይቅ

መለያ ቁጥር_____ ስልክ ቁጥር_____

1. ፆታ A. ወንድ B. ሴት
2. ዕድሜ _____
3. የጋብቻ ሁኔታ A. ያገባ/ች B. ያላገባ/ች C. ባሊ/ምስት የሞተ/ች D ያተፋታ/ች
4. የትምርት ደረጃ

   A. ያልተማረ B ማንበብና መፃፍ C. የመጀመረያ ደረጀ D. ከ 9-12 ክፍል E. ዲፕሎማ/ ዲግሪና ከዛ በላይ
5. ቢዚህ ስራ ቦታ የለበዎት የስራ ምድብ ምንድ ነዉ? A. ቤንዚን መምላት B. ማካኒክስ C. ገንዘብ ያዥ D. ሌላ ከሆነ ይግለፁ_____
6. በዚ ስራ ውስጥ ለስንት ጊዜ ወይም ዓመት ያህል ሰርተዋል?_____
7. በስራ ምድብዎ በቀን ስንት ሳዓት ይሰራሉ?_____
8 የመከላከያ እቃዎች ስራ በሚሰሩበት ጊዜ ይጠቀማሉ? 1. አዎ 2. አይደለም

   የሚጠቀሙ ከሆና ምን ምን?
9 ባርኔጣ 1. አዎን 2. አይደለም
10 የፊት መሸፈኛ 1. አዎን 2. አይደለም
11 ሽፉን ጫማ 1. አዎ 2. አይደለም
12 የእግር ቦቲ አዎ 2. አይደለም
13 ከለይ በቁጥር 9-12 የተጠቃሱትን የመከላከያ እቃዎችን የማይጠቀሙ ከሆነ ምክንያትዎ ምንድ ነዉ?

   1. አቅርቦት የለም 2. ስለማይመች 3. ኣስፈላጊ ስላልሆነ 4. ግድየለሽነት
14 ስራ በሚሰሩበት ቦታ ቀጥሎ ያሉትን ነገሮችን ይጠቀማሉ?

   10.1. ምግብ በስራ ቦታ መመገብ 1. አዎ 2. አይደለም

   10.2. ማስቲካ ማኘክ 1. አዎ አይደለም

   10.3 መጠጥ ይጠጡሉ 1. አዎ 2. አይደለም

   10.4. በፉውም ምንም አልጠቃምም
15 ሲጋራ የጨሰሉ 1. አዎ 2. አይደለም
16 ለጥያቄ 15.መልስዎ አዎን ከሆነ ባአማካይ በቀን በቁጥር ስንት ስጋራ የጨሰሉ?_____

17 የሚያሰክረ መጠጥ ይጠጠሉ? 1. አዎ 2. አይደለም

18 ለጥያቄ 17.መልስዎ አዎን ከሆነ አማካይ በቀን በቁጥር ስንት ጠርሙዛ ይጠጠሉ _____

19 ጨት ይቅማሉ 1. አዎ 2. አይደለም

20 ወደ ቤትዎ ከመሄዶዎ በፊት ሻወር ይወስዳሉ? 1. አዎ 2 አይደለም

21 አሮንጋደ ቅጣላ ቅጣል ምግቦች ይማጋባሉ? 1. አዎ 2. አይደለም

22 ጥሬ ስጋት ይማጋባሉ? 1. አዎ 2. አይደለም

23 ምግብ ኢንዳተጠቀሙ ቶሎ ሻይ/ ቡና የማጣጠት ልማድ አላቦት 1. አዎ 2. አይደለም

Questionnaire Hadiyisa version

Annannaxi xigi_____

Kihii dabachine marokih doolouwwi mato xaxehe.

Bon beyone yooki xamichuwaa kinuwiina agukisinehe dabacha kitabehe

shootii I: hechii qanqikuwi xammichuwaa.

1. Ummur womi hinchone_____
2. Alibacha_____
3. Mini isata A. Issamo B. isumoyoo
4. Losani gaballi A. Losani bee B. Kitabima sorobima xanomo C. (1-8) D. Lami losani gabala oddimi preparatoree E dipilomi imehe

shootii II: Baximine ammanine ammaxamako xammichuwa.

5. kiih baxi maha. A. Mekanika B. Gaza inkirima C. Sitiba ayima D. Mulleki yollas_____
6. ballane teim santane meei saata baxo_____
7. kaa baxo meei aganna/hinicho baxaha_____

Baxi beyyone oracho egelim muta

8.1 Baxi beyone gilaza isioto A. Eyaa B. aeae

8.2 Baxi beyone qobe isioto A. Eyaa B. aeae

8. 3 Baxi beyone sane ifiso A. Eyaa B. aeae

8.4 Baxi beyone ananni kobe A. Eyaa B. aeae

8.5. Kaa kuramuki gaga egelim muta isitobeeki mahina? A. bee bikina B. Mako bee bikina C. Hasisobee bikina D. cawwa

9. Baxi ammane kanni woron yoo luwwa issito A. Agimma B. Itimma C. Macitkia icimma D gaza tuximma E. Horem.

10. Cigara/tamba wrisito? A. Eyaa B. aeae.

11. chata icooho A. Eyaa B. aeae.

12. Xamichi xig 16 yoo sawwite eyya yitako las ballane meei cigara wirisito_____

13. Dimbiso ago agoho A. Eyaa B. aeaei

14. Xamichi xig 13 yoo sawwite eyya yitako las ballane meei xarmuza ago_____

15. . Baxo gulita mine elelena gasita oracho anshaito A. Eyaa B. aeaeie.

16. Dubi kasha teim fraree awaxitakamo. A. Eyaa B. aeaeie

17. Kashari maara itakamo. A. Eyaa B. aeaeie

18. Hurbata itaka lasone qaqisome buna teim shahe agakamo A. Eyaa B. aeaeie

Galatomoo!!!

Annex IV: Laboratory procedure

SOP for blood collection

Materials and reagents

- Glove
- 70% alcohol
- Tourniquet
- Test tube with EDTA anticoagulant
- Gauze pads or cotton
- Marker
- Rack
- Band Aid

Procedure for drawing blood [refer: 51]

1. All necessary the necessary materials were assembled.
2. The right patient were identified and allowed to sit
3. Tourniquet was applied.
4. Venipuncture site were selected.
5. Selected site were cleaned with70% alcohol.
6. The selected vein were anchored with thumb
7. The needle was inserted into the vein at a 15–30 degree angle with the bevel faced up and collect sample, the tourniquet was released.
8. Needle was withdrawed from the vein
9. The needle was placed into waste disposal
10. Test tube was filled, mixed well
11. Test tube was labeled.

A. Complete blood count.

Principle

A suspension of blood cells is passed through a small orifice simultaneously with an electric current. The individual blood cells passing through the orifice introduce an impedance (electrical resistance) change in the orifice determined by the size of the cell. The change in electrical resistance introduces the voltage pulses. The number of pulses generated indicates the number of particles that pass through the aperture. The amplitude of each pulse is essentially proportional to the particle volume (refer: Beckman Coulter (UniCel® DxH 800**)**. Other parameters are derived as follows:-

.MCV: The average volume of individual erythrocytes derived from the RBC histogram, multiplied by a calibration factor. Expressed in femtoliter (fl).

Hct: The relative volume of packed erythrocytes to whole blood. Calculated by using Hct (%) = (RBC X MCV)/10.

MCH: The weight of Hgb in the average erythrocyte. Calculated by using MCH= (pg)(Hgb/RBC) x 10

MCHC: The average weight of hemoglobulin in a measured dilution. Calculated by using MCHC (g/dL) = (Hgb/Hct) x 100

RDW: The size distribution spread of the erythrocyte population derived from the RBC histogram. Expressed as coefficient of variation (%)

RDW-SD: The size distribution spread of the erythrocyte population derived from the RBC histogram. Expressed as a standard deviation in fL.

MPV: The average volume of individual platelets derived from the Plt histogram Expressed in fL.

HGB measurements

HGB is determined by the photometric method. Transmittance of light at 525 nm through a lysed WBC solution in the Hgb cuvette, compared to the transmittance of the same light through a reagent blank. The system converts this ratio to the Hgb value using a calibration factor. Weight (mass) of Hgb determined from the degree of absorbance found through photo current transmittance expressed in g/dL. Hgb (g/dL) = [constant X log10 (Reference %T/Sample %T)].

Beckman Coulter (unicel® DxH 800) Reagents

Coulter DxH Diluent

Act as a conductive solution. Used for rinsing agent on all DxH System.

Coulter DxH Cell lyes: It is an erythrocyte lytic agent for quantative determination of Hgb, enumeration of NRBC, and counting and sizing of leukocyte.

Coulter DxH Diff Pack: Differential lyse and used as stabilizer (preservative).

Coulter DxH Retic Pack: It clear RBC and stain reticulocyte

Coulter DxH Cleaner. Used as a cleaning

Specimen type: EDTA anticoagulated whole blood

QUALITY CONTROL

Types of Controls used for Beckman DxH-800

A. **Latrol control:** no levels are found(one tube)
   Used for alignment of flow cell and it control flow rate of cell.
B. **6-cell control:** have three levels (level 1-3). It controls complete blood count, differential and NRBC.
C. **Retic control:** control retics
D. **BF control:** control body fluid

Acceptable Background Counts

parameter	count
WBC	≤0.05 x 103/µl
RBC	≤0.005 x 10$_6$/µl
HGB	≤0.1 g/dL
PLT	≤3 x 103/µL

Source: Beckman Coulter (UniCel® DxH 800) Operating manual

Beckman Coulter (UniCel® DxH 800) Whole Blood Reference Ranges

parameter	units	Overall		
		Mean	95% Confidence Low Limit	95% Confidence High Limit
WBC	x10^3/µl	6.3	3.6	11.2
RBC	x10^6/µl	4.52	3.73	5.50
HGB	g/dl	13.4	11.4	15.9
HCT	%	39	33.3	47.7
MCV	fL	86.4	73.7	95.5
MCH	pg	29.6	24.3	33.2
MCHC	g/dl	34.2	32.5	35.8
RDW	%	13.8	12.3	17.0
RDW-SD	fL	41.4	37.1	47.8
PLT	x10^3/µl	257	159	386
MPV	fL	9.2	7.5	11.2
NE	%	58.5	43.3	76.6
LY	%	29.6	16	43.5
MO	%	8.3	4.5	12.5
EO	%	2.8	0.6	7.9
BA	%	0.7	0.2	1.4
NE #	x10^3/µl	3.7	1.8	7.8
LY#	x10^3/µl	1.8	1.0	3.0
MO#	x10^3/µl	0.5	0.3	1.0
EO#	x10^3/µl	0.2	0.0	0.5
BA#	x10^3/µl	0.0	0.0	0.1

B. Preparation of thin blood film

Materials and Reagents

- Clean microscope slides
- Blood sample
- Pipette
- pencil
- Gloves
- Waste and sharps disposal containers

Procedures

- Place a small drop of well mixed EDTA blood 1.0 cm far from the end of the glass slide
- The spreading slide is placed in front of the drop of blood at an angle of about 30^{o}-40^{o} to the slide and then is moved back to make contact with the drop
- The drop will spread out quickly along the line of contact of the spreader with the slide
- The spreader is advanced with a smooth steady motion so that a thin film of blood is spread over the slide
- Allow the smear to air-dry
- Label with identification card number.

Wright staining and examination

Principle:

Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors.

Procedure

- Air-dried smear was placed up on a staining rack
- The smear was covered with undiluted filtered stain and leave for 1 minute
- Add equal volume of distilled water (i.e., the same number of drops as the stain)
- The stain was mixed by blowing until a metallic sheen appears.
- The diluted stain was allowed to for 3-5 minutes
- the stain was washed with running tap water
- Back of the slide was cleaned and standed in a rack for the smear to dry.
- Gross morphology was examined by 40x and use the 100x objective for studying the fine details of the cell morphology.

Storage and Shelf life

Wright's Stain should be stored at room temperature and protected from light. Under these conditions it has a shelf life of 52 weeks from the date of manufacture.

Quality control: Wright stain was filtered every day by using filter paper and also storied at locked cabinets away from moisture and sunlight

***Source**: Laboratory working manual*

Annex V- Laboratory Result Reporting Format for CBC and diff count

Date_____

Age_____ Gender_____ ID No_____

Test	Result
RBC	10^6/uL
HGB	g/dl
HCT	%
MCV	fl
MCH	Pg
MCHC	g/dl
RDW	%
WBC	10^3/uL
Neutrophil	%
lymphocyte	%
monocyte	%
eosinophil	%
basophil	%
neutrophil	10^3/uL
lymphocyte	10^3/uL
monocyte	10^3/uL
eosinophil	10^3/uL
basophil	10^3/uL
platelet	10^3/uL
MPV	fl

Peripheral Blood Film form Reporting Format

Date _____Age_____ Gender_____ Identification card No_____

WBC Series_____

_____

RBC Series _____

_____

PLT Series_____

_____

Possible conclusion _____

HCG Test

Principle of the test

- Most of the urine pregnancy test kits are based on lateral-flow technology. Most of them qualitatively detect the presence of hCG in urine specimen at the sensitivity of 25 mIU/mL. The test uses two lines to indicate results. The test line utilizes a combination of antibodies including a monoclonal hCG antibody to selectively detect elevated levels of hCG. The control line is composed of goat polyclonal antibodies and colloidal gold particles.
- The sample migrates via capillary action along the membrane to react with the colored conjugate. Positive samples react with the specific antibody-hCG-colored conjugate to form a colored line at the test line region of the membrane. Absence of this colored line suggests a negative result. A colored line will always appear in the control line region if the tests have been performed properly.

Procedure of the test

1. the Pregnancy Test Strip and urine sample were allowed to reach room temperature (15-30°C) before opening the foil pouch.

2. Pregnancy Test Strip removed from the pouch and use it as soon as possible.

3. Placed the test device on clean and level surface. Hold the dropper vertically and transfer 3 full drops to the specimen well and start the timer. Avoid air bubble formation.

4. Wait for the colored line(s) to appear. Read the result after 5 minutes. Do not read the result after 15 minutes.

Interpretation

- Positive: Two colored lines appear. One line should be in the Control region (C) and another line should be in the Test region (T). This means there is a strong possibility that patient is pregnant.
- Negative: One line should be in the Control region (C) and no line appears in the Test region (T).

Quality control: check the expiry date of strip**.**

Stool Examination

- **Principle**: wet mount made from patient stool specimens and can be examined under low and high power for the presence of parasites.
- **Reagents and equipment:**

✓ Cover slips
✓ Glass slides
✓ Gloves
✓ Microscopes
✓ Normal Saline (85%)
✓ Pipettes
✓ applasticstick

Microscopic examination

- **Procedure:**

1. Apply the patient's sample to a small area on a clean microscope slide.
2. Immediately before the specimen dries, add 2 drops of saline with a pipette. Mix with a pipette tip.
3. Cover the specimen with a cover slip.
4. Examine the specimen with the low power objective
5. Examine the entire cover slip
6. Ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

- **Quality control:** Check the saline. It should be clear with no visible signs of contamination.

Formalin (10%) in 0.85% saline used for preservation of parasite morphology. It is recommended that 1 part of stool be mixed with 3 parts of formalin/formalin-saline preservative for the storage of bulk specimens. This preservative is a good overall fixative and will fix both ova and cysts although it only preserves the internal morphology of the cysts for up to 6 months.

Intestinal parasite result reporting format

Test	result
Stool examination	

Annex VI: - Declaration Form

Assurance of Principal Investigator

I, the undersigned, hereby declare that this MSc thesis is my original work, has not been presented for any degree in Jimma University or any other institutions of higher learning in Ethiopia. I also declare the duly acknowledgement of all material sources used for this thesis work.

Name of the Student: Tamirat Ersino kebamo (BSc)

Signature _____Date of submission____/____/_____

APPROVAL OF THE ADVISORS

This thesis has been approved by the supervision of University advisors:

Name of the first advisor: Dr. Tilahun Yemane (MD, MSC)

Signature._____ Date____/____/_____

Name of the Second advisor: Mr. Girum Tesfaye (BSc, MSc)

Signature_____ Date____/____/__

Examiner

Name_____Signature_____

Name_____Signature_____