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Evaluation of Hematological Parameters among Gas station Workers in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Abigail Mesfin, entitled “**Evaluation of Hematological Parameters among Gas Station Workers In Addis Ababa Ethiopia** and submitted in partial fulfillment of the requirements for a Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology), complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Contents

Acknowledgment	iii
List of Figure.....	vii
List of Table.....	viii
Abbreviation	ix
Abstract.....	x
1. INTRODUCTION.....	1
1.1. Background	1
1.2. Statement of the problem	4
1.3. Significance of the study.....	6
2. LITERATURE REVIEW	7
3. OBJECTIVE.....	12
3.1. General Objective.....	12
3.2. Specific Objective	12
4. MATERIAL AND METHODS.....	13
4.1. Study Area.....	13
4.2. Study design and period.....	13
4.3. Population.....	13
4.3.1. Source population	13
4.3.2. Study population	13
4.4. Inclusion and exclusion criteria.....	14
4.4.1. Inclusion criteria	14
4.4.2. Exclusion criteria	14
4.5. Study variable.....	15
4.5.1. Dependent variable	15
4.5.2. Independent variable.....	15
4.6. Sample size calculation and sampling method.....	15
4.6.1. Sample size calculation.....	15
4.6.2. Sampling method	16
4.7. Measurement and data collection.....	16
4.7.1. Data collection procedure	16

4.7.1.1.	Laboratory Analysis	17
4.7.1.2.	Principle of Unice1®DxH800 Beckman-coulter automated hematology analyzer. 17	
4.7.1.3.	Principle of peripheral blood film stained by wright stain.....	17
4.8.	Data quality assurance.....	18
4.8.1.	Pre-analytical	18
4.8.2.	Analytical.....	18
4.8.3.	Post analytical	18
4.9.	Data analysis and interpretation	18
4.10.	Operational definition.....	19
4.11.	Ethical consideration	20
4.12.	Dissemination of the result	20
5.	Result.....	20
5.1.	Socio-demographic characteristic of study participants.....	21
5.2.	Occupational information of study participants.....	22
5.3.	Clinical data of study participants.....	24
5.4.	Comparison of hematological parameters between exposed and control group.....	25
5.5.	Comparison between hematological parameters and year of exposure in the subgroups of gas station workers.....	27
5.6.	Correlation analysis of hematological parameters with year of exposure, working hour per day and working days per week	29
6.	Discussion.....	31
7.	Strength and limitation of the study	34
7.1.	Strength	34
7.2.	Limitation.....	34
8.	Conclusion and recommendation	34
8.1.	Conclusion.....	34
8.2.	Recommendation.....	34
	REFERENCE.....	35
	ANNEXES	38
	Annex I; participants Information sheet.....	38
	A English version	38

B, Participant Information sheet Amharic version	40
Annex II ; Consent form(study participants)	42
<u>A</u> ;Informed consent form English form	42
B; Informed consent form Amharic form.....	43
Annex III; Questionnaires	44
A, Questionnaires English form	44
B; Questionnaires Amharic form.....	49
Annex IV: Standard operating procedure for blood collection	56
Annex V; standard operating procedure for UniCel® DxH 800 Beckman	57
Annex VI ; standard operating procedure for wright stain.....	62
Declaration	63

List of table

Table 1; Socio-demographic profile of the exposed and control group using t-Test and χ^2 test at Addis Ababa city, from January to March 2025 21

Table 2; Utilization of personal protective equipment among exposed group in Addis Ababa city, from January to March 2025 23

Table 3; Reasons for not to using personal protective equipment by gas station workers in Addis Ababa city, from January to March 2025 24

Table 4; Comparison of hematological profile of exposed and control group using independent t-Test at Addis Ababa city, from January to March 2025 25

Table 5; Comparison between hematological parameters and year of exposure in the subgroups of gas station workers at Addis Ababa city, from January to March 2025 27

Table 6; Correlation analysis of hematological parameters with year of exposure, working hour and working days per week among gas station workers at Addis Ababa city, from January to March 2025 29

List of Figures

Figure 1. Sign and symptom of gas station workers	24
Figure 2; Comparison between both hemoglobin and white blood cell with year of exposure....	28
Figure 3; Comparison of Neutrophil % and Neutrophil absolute count with year of exposure ...	29

Abbreviation

ALL- acute lymphocytic leukemia

AML- acute myeloid leukemia

CBC- complete blood count

CLL- chronic lymphocytic leukemia

CML- chronic myeloid leukemia

CRP- C reactive protein

CYP2E1- cytochrome enzyme P450 2E1

DNA- deoxyribonucleic acid

ESR- erythrocyte sedimentation rate

fl- femtoliters

Hb- hemoglobin

HQ- hydroquinone

IARC- international agency for research on cancer

MCH- Mean cell hemoglobin

MCHC- Mean cell hemoglobin concentration

MPV- Mean platelet volume

pg- picogram

PLT- platelet

RBC- Red blood cell

VOC- volatile organic compounds

WBC- White blood cell

WHO- world health organization

μl- Microliter

kpa- kilopascal

ABSTRACT

Background: Chronic exposure of gas station workers to hydrocarbons of gasoline particularly benzene, toluene, ethyl benzene and xylene were reported to induce bone marrow failure and impaired hematopoietic system. However hematological profile of gas station workers is not well documented in resource limited settings like Ethiopia in particular in the study area Addis Ababa.

Objective: To evaluate hematological parameters of gas station workers in Addis Ababa, Ethiopia from January to March 2025.

Methods: Comparative cross sectional study was conducted on 302 (151 exposed and 151 apparently healthy control) study subjects enrolled using convenient sampling technique. Complete blood count was performed using Unicel®DxH800 Beckman-coulter automated hematology analyzer and peripheral morphology using wright stain were used to diagnose any hematological changes in participants. Socio-demographic and duration of exposure was collected using a semi structured questionnaire and observational checklist. The statistical package for the social sciences (SPSS) version 30 was used to enter and analyze the data.

Result: From the total participants 13.2% (20/151) from exposed and 13.2% (20/151) from control group of the participants were women. Median age of participants was 36.23±9.41 and 36.08±9.41, respectively. Hemoglobin (16.39±1.43 vs 15.54±1.85; p<0.001), Mean platelet volume (9.3±1.24 vs 9.0±1.2; p=0.031), lymphocyte percentage (28.39±9.13 vs 25.40±8.8; p=0.004), absolute lymphocyte (1.69±0.59 vs 1.39±0.41; p<0.001) were significantly higher in gas station workers as compared with the control group. The highest number of exposed group, 66 (43.7%), had worked in the gas station for 2 and less years.

Conclusion; Overall, the current study findings have revealed that occupational exposure to gasoline have significant alteration on selected hematological parameters. The increased lymphocyte count and decrease hemoglobin with long term exposure suggest varied effect on different cell lineages. Decreased neutrophil and monocyte count observed were also implication of early stage hematological disturbance due to chronic benzene exposure.

Key words: *hematological parameters, Hematopoiesis, peripheral morphology, Gasoline constitute*

1. INTRODUCTION

1.1. BACKGROUND

Acute and chronic exposure to hydrocarbons of gasoline particularly benzene, toluene, ethyl benzene and xylene which are also known as volatile organic compounds (VOC) induce bone marrow failure and impaired hematopoietic system (1). The unfavorable impact of hydrocarbons of gasoline exposure also cause nausea, vomiting, hypersensitivity (allergic reaction), headache, myalgia, somnolence, mental confusion, tremors, repetitive infection, contact with epithelial tissue cause rash, swelling, redness, contact with mucosa and eye cause irritation and tissue damage, but these are rare effects (2).

Heavy metals contained in gasoline vapor inhalation extremely affect hematological parameters and provoke bone marrow hypoplasia (3). Fragments generated from gasoline are incredibly tiny in diameter and substantial in surface area, even though it can carry many huge fragments of toxic compounds such as metal and hydrocarbons on their surface. These fragments can be accumulated in substantial quantities and deeper into the lung (4). Benzene, a more carcinogenic (group 1) hydrocarbon of gasoline can enter the human body through pulmonary, gastrointestinal and cutaneous. Half of the benzene inhaled passes through the lining of the pulmonary and enters the bloodstream (5).

Benzene can be consumed through the gastrointestinal tract and proceeds to the bloodstream. Small amount of benzene enters the human body through skin and can easily enter the bloodstream. Once it is in the circulation it can travel throughout the body and is temporarily stored in liver, bone marrow and fat tissue (4, 6). Benzene can metabolize in complex processes primarily occurring in the liver which contain several steps catalyzed by enzymes mainly by cytochrome P450 enzymes (CYP_{2E1} and CYP_{2E2}) (7).

Initially benzene is oxidized to its intermediate compound called benzene oxide by CYP_{2E1} . This intermediate compound can undergo an impulsive process to form phenol or can open a spontaneous ring which forms trans-muconaldehyde and benzene dihydrodiol into catechol or hydroquinone which are extremely reactive metabolites (7).

Phase two starts when phenol, catechol and hydroquinone go through a conjugation reaction which is mandatory to make the compound water soluble and easy for excretion. Phenol, hydroquinone and catechol conjugate with sulfate and form sulfo-transferases which is a water soluble compound and easy for excretion(8). If some compounds such as hydroquinone (HQ) further oxidize into a toxic metabolite called benzoquinone inside bone marrow it can interact with DNA and proteins (9). These interactions can break DNA strands and cause point mutation with the production of reactive oxygen species (ROS) (9).

It was reported that DNA damage can be induced by HQ, PH, 1-2-4-benzenetriol in HL60 cell lines and by benzene in bone marrow cells(10). DNA exposure to benzene and its metabolites is associated with increased mitochondria DNA copy number variation and homologous chromosome recombination (9). Benzoquinone can release reactive oxygen species (ROS) which damages hematopoietic cells and bone marrow (11, 12). Trans, trans-muconaldehyde and benzoquinone can form adducts with cellular macromolecules which causes geno-toxicity and carcinogenicity.

Thus chronic benzene exposure causes bone marrow failure, hematological malignancies such as chronic myeloid, acute lymphoblastic leukemia, myelodysplastic syndrome, aplastic anemia, acute myeloid leukemia (AML) (12, 13). Alteration in hematological parameters such as leukopenia, eosinophilia, monocytopenia, increased mean corpuscular volume (macrocytosis), , thrombocytopenia, lymphocytopenia, change in neutrophil such as neutropenia, basophilic stippling, and hyper-segmentation was detected (14).

Gasoline is a blend of saturated and unsaturated hydrocarbons (hydrogen and carbon mixture). Those hydrocarbons contain alkanes, cycloalkanes, aromatics, alkenes, and butane, pentane, isopentane and volatile organic compounds like BTEX (benzene, toluene, ethylbenzene and xylenes) (15). Gasoline hydrocarbons can be found in surrounding air due to hydrocarbon evaporation from production, gasoline filling operations, commercialization and transportation. As stated by the international agency for research on cancer (IARC) benzene is a mixture that poses greatest risk to exposed individuals and can even cause cancer and is stated as group one carcinogenic agent ethyl-benzene as group two carcinogenic agent (5). Gas station workers are

exposed to gasoline carcinogenic agents through inhalation, dermal contact, contamination of molecules during drinking and eating (1).

However hematological profile of gas station workers is not well documented in resource limited settings like Ethiopia in particular in the study area Addis Ababa. In addition to the inadequacy of focused research on this topic, another significant gap lies in the lack of standardized health assessment protocol adapted for this particular occupational group and restricted awareness and training regarding occupational health and safety practice among gas station workers, which further intensifies the risk of hematological problems (11).

1.2.STATEMENT OF THE PROBLEM

Exposure to Gasoline hydrocarbons especially benzene poses significant strain on global health, affect respiratory health, pregnancy and leading to physiological and hematological alterations. Inhalation of hydrocarbons of gasoline has been linked to raise respiratory rates and apoptosis to lung tissue. In case of pregnancy benzene and its metabolites can cross placenta barrier with cord blood concentrations and potentially cause decreased fetal weight, interfere with cellular division and DNA replication, spontaneous abortions, childhood leukemia especially acute lymphoblastic leukemia (ALL) and skeletal anomalies (16). Acute exposure to gasoline hydrocarbons disturb central nerves system and cause symptoms like cause nausea, vomiting, repetitive headaches, dizziness and drowsiness. Benzene metabolites like benzoquinone produce oxidative stress leading to mitochondrial dysfunction and lipid peroxidation which cause impaired energy metabolism and cellular damage (2).

Gas station workers who are easily exposed to gasoline suffer in respiratory disorders such as irritation of lung and throat, skin irritation, neurological effect such as dizziness, cognitive impairments, and hematological changes. Increased red blood cells (RBC), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), decreased white blood cell (WBC), neutrophil, lymphocytes and monocytes, increased erythrocyte sedimentation rate (ESR), c-reactive protein (CRP) and interleukin-6 (IL-6) and increased cancer risk specially leukemia have been reported (13). In Brazil from 324 gas station workers 38.3-45.1% of them showed somnolence, headache, dizziness and tingling. Significant hematological changes such as increased leukocyte count, reduced lymphocyte count. The same result was observed in Istanbul (17), western Asia, Kuwait(18), Basra and Kermanshah (19), Iraq, Ekpoma (20), Edo state, Nigeria (21), North Africa, Tuzla (22), Egypt, Mansoura city (23).

Ethiopia has about 800 gas stations throughout the country, 126 of these stations are found in Addis Ababa, according to Addis insight. In these stations 900-2800 individuals are employed as managers, attendants, security and maintenance staff. This individual's work 5-6 days a week, 8-12 hours per day according to the station's schedule. This hour of exposure to gasoline can cause occupational hazards like bone marrow depression and hematological malignancies since the odor threshold of gasoline exposure is 0.025 ppm (24). Benzene has 12.7 kpa vapor pressure at room temperature, which makes it a highly volatile compound. Benzene in the air mostly exists

in the vapor phase. This vapor phase can stay one day to two weeks depending on the climate, environment and other pollutants (25). Addis Ababa's weather condition is 7°C at night and 22°C during the day according to the weather atlas. Benzene can remain in coldest temperature like this longer in contrast to the hottest environment (26). Thus individuals in Addis Ababa can be among the affected groups in the country.

The awareness of the effect of gasoline on hematological parameters and bone marrow is extremely dim, thus the use of personal protective equipment for protection during handling fuel is low. Even eating, drinking and showering in the workplace are also seen in some places because of lack of awareness and poor supply of the equipment needed for protection like gloves, goggles and masks (27).

Intensified use of unleaded gasoline reduces the effectiveness of diagnosis for blood or urine lead as biomarker for gasoline hydrocarbon exposure (28). This test was also not specific for gasoline lead exposure, the concentration of lead found in the urine and blood can be from other compounds (28). In addition, concentration of phenol in urine also can measure the amount of gasoline found in the human body. But this test is also not specific. Nowadays benzene poisoning blood tests are done under heavy metal testing in the United States. But this test is not found in Ethiopia. Individuals exposed to this occupational hazard in Addis Ababa may not get any diagnosis before being extremely affected by this problem. Therefore assessing the hematological and biochemical profile of such workers is essential to monitor their health and facilitate in prevention through personal protective precaution efforts.

1.3.SIGNIFICANCE OF THE STUDY

Even though gasoline hydrocarbon exposure can cause bone marrow suppression and hematological malignancies, it is neglected in developing countries. Thus, this study is significant in highlighting the issue as an occupational hazard in Addis Ababa gas station workers. Nowadays no fairly established diagnosis is developed to test exposure of gas station employees. The diagnosis is given already after the bone marrow suppression or hematological changes occur. The results of this research may notify policymakers and health authorities about the risk faced by gas station employees. This finding can be crucial for developing specific interventions, guideline for occupational health, and regulatory measures to enhance the working circumstances and health outcomes of these vulnerable employees. By evaluating hematological parameters, findings of this study will offer additional data that can be utilized for future research and health assessment, enabling comparisons over time and with different occupational groups. Additionally there are no published papers regarding this topic in Addis Ababa city. The finding of this research also helps to establish policies regarding personal protective equipment and awareness of the problem by Ethiopian ministry of labor and social affairs.

2. LITERATURE REVIEW

2.1.SIGN AND SYMPTOMS CAUSED BY GASOLINE EXPOSURE

Gasoline is used as a fuel all over the world. Even though gasoline has its own purpose, its harm cannot be neglected. Hematological changes such as increased MCHC, MCH, WBC, PLT, MCV and MPV were observed in short and long term exposed gas station employees (29). In a cross sectional study in Brazil, Rio de Janeiro study enrolled 542 participants, 324 were gas station employees and 218 control groups. The most outlined signs and symptoms in gas station workers include headache (38.3%), somnolence (45.1%), involuntary movement (25%), tingling (25.4%) and dizziness (27.5%). Furthermore gas station workers show total leukocyte count above the upper limit and lymphocyte count near to lower limit (29).

A cross sectional study performed in Basra city encompass 72 exposed groups who employed in gas station for 5 years and above and 75 control groups showed a significant reduction in all RBC (3.04 ± 0.77 vs 4.34 ± 2.14), HGB (g/dl) (9.3 ± 2.1 vs 11.8 ± 1.5), MCV (fl) (9.3 ± 2.1 vs 11.8 ± 1.5), MCH (pg) (75.02 ± 5.84 vs 86.91 ± 6.06), and MCHC (g/l) (75.02 ± 5.84 vs 86.91 ± 6.06) parameters when compared with control group. WBC, neutrophil and lymphocyte mean value decreased significantly as well (4198.1 ± 337.5 vs 6478.1 ± 381.4), (3.06 ± 1.02 vs 5.00 ± 1.388) (1.46 ± 0.8 vs 2.47 ± 1.19) respectively. This study also showed significant deference in mean monocytes between exposed and control group. While inflammatory markers such as ESR (11.80 ± 3.04 vs 8.83 ± 1.75), hs-CRP (5.87 ± 1.51 vs 1.93 ± 0.80), and IL-6 (12.21 ± 2.80 vs 5.54 ± 1.80) increased significantly in exposed group (18).

2.2. HEMATOLOGICAL ABNORMALITIES CAUSED BY GASOLINE EXPOSURE

There is no ambiguity about leukemogenic activity of benzene. Research carried out in malignancies due to occupational exposure to benzene in Istanbul shows that incidence of leukemia to benzene exposed individuals in the duration of 8 years was 13.6/100,000, Which was significantly higher than that for leukemia in the whole population in the city. In pancytopenia patients who were exposed to benzene the incidence of leukemia were 13 out of 51 patients. The dissemination of types of leukemia in the exposed and control group had a huge difference. Acute leukemia (96.1% vs 46%) and 12 malignant lymphoma cases were reported

(16). Cross sectional Study conducted in Western Asia, Kuwait indicated that exposure to petrochemical substances can be correlated with increased risk of morbidities. In their study, 460 gas station workers participated in this study. Typical morbidities were tremors (42%), skin complaint/eczema (44.1%), dizziness (9.8%), periodic headache (48.3%), allergic rhinitis and frequent sneezing (44.3%), shortness of breath (asthma) (7.6%) and eye complaint (45.2%). Gas station workers were remarkably more likely to report one or more diseases related to liver, blood or some other organs (17).

In Kosti city, Sudan Analytical cross-sectional study was carried out on 87 gas station workers and 87 control group. Mean RBCs count ($5.30 \pm 0.75 \times 10^{12}$ vs $4.97 \pm 0.58 \times 10^{12}$ $p=0.002$) MCHC ($32.67 \pm 2.12\%$ vs $31.71 \pm 1.37\%$; $p<0.001$), and lymphocyte ($44.61 \pm 8.41\%$ vs $29.19 \pm 3.98\%$; $p<0.001$) count were significantly elevated in exposed group as compared to non-occupationally exposed group respectively. Average PLT (240.78 ± 71.37 vs 262.54 ± 70.77 ; $p=0.046$), monocyte ($4.44 \pm 2.39\%$ vs $5.64 \pm 1.92\%$; $p<0.001$), PCV (43.83 ± 2.47 vs 44.95 ± 1.15 ; $p<0.001$), hemoglobin (13.11 ± 2.28 vs 14.33 ± 1.48 g/dl $p<0.001$), MCH (26.35 ± 2.01 pg vs 29.88 ± 1.98 pg; $p<0.001$), MCV (78.69 ± 4.66 fl vs 85.93 ± 6.73 fl; $p<0.001$), and neutrophil ($36.98 \pm 14.93\%$ vs $47.99 \pm 14.54\%$; $p<0.001$) count were significantly decreased in exposed group (30)

Community based cross sectional study was performed in Khon, Kaen Thailand on 20 exposed and 20 occupationally non exposed individuals. Median HCT (39.6% (33.8-50.8%)) which was outside the reference range, (40-54%) were appreciated. WBC result within reference range (4000-10,000 cells/mm³) with maximum value of 12,100 were also found. There were 12 individuals (60%) who had hematological findings outside the reference range. RBC (16.6%), WBC (16.6%), HCT (67.7%), eosinophil (41.7%), MCV (41.7%), MCH (50.0%) and MCHC (5%) were also observed (31).

In Nandyal, Andhra Pradesh, 30 volunteers with age range of 25-40 who worked in petrol pump station for greater than 5 years with comparison of non-petrol pumping workers were included in cross sectional study. Reduced RBC (4.29 ± 0.383 vs 5.21 ± 0.336 ; $p<0.0001$), HGB (13.06 ± 0.634 vs 15.36 ± 0.73 ; $p<0.001$) leucocyte (8566.6 ± 1908.4 vs 7180.0 ± 1393.4 ; $p=0.0042$) count among petrol pump station as compared to non-occupationally exposed individuals. The difference between exposed and control group in platelet count was not statistically significant ($p=0.4827$) (32).

In Kermanshah city, Iraq, case-control study was carried out on 80 exposed individuals for four years and above with 80 individuals who have no occupational exposure to gasoline. Significant leukocyte differences were observed between exposed (20.4 ± 3.50) and control group (29.94 ± 3.15). Neutrophil and lymphocyte mean value decreased significantly (4198.1 ± 337.5 vs 6478.1 ± 381.4), (3.06 ± 1.02 vs 5.00 ± 1.388) (1.46 ± 0.8 vs 2.47 ± 1.19) respectively (19).

Case-control comparative cross sectional were performed in India on 50 petrol station workers and 49 non-occupationally exposed voluntary individuals. The findings showed RBC (3.99 ± 0.86 vs 4.17 ± 0.64 ; $P=0.035$), HGB (13.19 ± 2.84 vs 13.77 ± 2.11 ; $P=0.035$), PCV (37 ± 7.7 vs 39.01 ± 5.27 ; $P=0.006$), and RDW-SD (34.6 vs 38.10 ; $p<0.001$) were significantly decreased in petrol station workers as compared to non-exposed group. In other hand WBC (12.4 vs 5.4 ; $p<0.001$), neutrophil (5.26 vs 2.77 ; $p<0.001$), lymphocyte (5.45 vs 2.05 ; $p<0.001$) and differential count (1.05 vs 0.5 ; $p<0.001$) were significantly higher in petrol station workers. No statistically significant results were observed in platelet, MPV and MCV results among petrol station workers and control group (33).

Retrospective study from 2004 to 2006 in Texas carried out on 294 elderly individuals who age 60 years old and above. 78 un-exposed and 216 exposed individuals were included. Mean age of exposed and control groups were 68.7 and 69 years old respectively. In elderly exposed individual statistically elevated mean WBC (7.7 ± 1.9 vs 6.3 ± 1.5 ; $p<0.001$) and platelet values (256.8 ± 51.6 vs 237 ± 41.9 ; $p=0.0104$) were observed (34).

Cross sectional study was carried out in shanghai, china on 928 workers who worked in five company which use benzene in their production process. Complete blood count and peripheral morphology were examined. RBC ($p<0.001$) and HGB ($p<0.001$) revealed statistically significant reduction. MPV, MCV and platelet count show significant reduction as year of exposure increases ($p<0.001$). 55 workers were anemic which reveal RBC results lower than reference range. By comparing and contrasting MPV value with peripheral morphology 31 workers have macrocytosis. Thrombocytopenia also found with decreased platelet count among 16 individuals. These studies also reveal reduced neutrophil and MPV count (35).

Case-control comparative cross sectional study which includes 150 case and 150 control group conducted in zabol, Iran showed significantly increased result in RBC (6.37 ± 1.89 vs 7.02 ± 2.07 ; $p=0.005$), HGB (15.17 ± 2.04 vs 14.33 ± 1.47 ; $p<0.001$) and HCT(44.03 ± 7.57 vs 41.9 ± 4.1 ; $p=0.003$). Even though the finding were statistically significant both the exposed and control group is in a reference range which cannot reveal severe sign and symptom (36).

A prospective cohort study conducted on 37 gas station workers who had been exposed to gasoline for more than five years was examined in North Africa, Tuzla. The data were compared to two control groups; 25 individuals who only worked in a gas station for nine months and 61 non-exposed healthy controls. Basophilic stippling and reticulocytosis was observed under peripheral smear in 25 of 35 in chronic exposed gas station workers, and 15 of 25 in acute gasoline exposed workers. In chronic and acute exposure of gasoline lower mean RBC count was seen ($4.55 \times 10^{12}/L \pm 0.21 \times 10^{12}/L$) as compared to those in the control group ($4.75 \times 10^{12}/L \pm 0.23 \times 10^{12}/L$). The least number of mean red blood cells were seen in those who had 19 years and above occupational history (22).

A comparative cross sectional study was conducted upon gas station workers (GSW) at Egypt, Mansoura city which contain 112 GSW and 102 control groups. Mean hemoglobin level and red blood cell count of gas station works were significantly low as compared to the 102 control group. Platelet and mean white blood cell (WBC) were higher among gas station workers (22).

In Ethiopia there are a few researches carried out related to exposure of gas station workers to gasoline and its hematological effect. Comparative cross sectional study carried out in northwest Ethiopia Gondar city encompass 55 exposed and 55 control groups who was selected in convenient sampling technique. Exposed groups reveal leukocytosis 14.5%, neutropenia 18.2% and leukopenia 3.6%. RBC (5.38 ± 0.57 vs 4.75 ± 0.52), HGB (15.51 ± 1.61 vs 13.77 ± 1.55), HCT ($46.6(4.5)$ vs ($42.2(6.4)$), MCHC (29.3 vs 29.2), platelet count ($258(98)$ vs $222(81)$), absolute lymphocyte count (3 vs 3.1) and RDW ($13.7 (0.9)$ vs $13.1 (1.1)$) which was significantly elevated in exposed group (37).

In eastern Ethiopia, Haramaya city, a comparative cross sectional study was conducted in 30 garage workers who were exposed to gasoline hydrocarbons and 30 control groups who are not occupationally exposed to gasoline chemicals. Occupationally exposed groups do not implement

personal protective equipment which makes this individuals exposed to gasoline hydrocarbons easily. Diastolic and systolic blood pressure and platelets shows increased result in exposed group (128.67 ± 18.14 vs 106.3 ± 9.27 mmHg) and (90.33 ± 11.29 vs 75.67 ± 5.68 mmHg) and (323.20 ± 48.82 vs 244.1 ± 47.3 cells/L) respectively. however significantly reduced results of red blood cells, hemoglobin, hematocrit and mean corpuscular volume (5.13 ± 0.83 vs 5.46 ± 0.36 cells/L), (14.89 ± 0.71 vs 15.45 ± 0.87 g/dl), ($43.98\% \pm 1.99\%$ vs $46.4\% \pm 2.32\%$) and (83.19 ± 2.93 vs 85.11 ± 3.87 fl) were seen in exposed group (27).

Cross sectional study performed in Northern Ethiopia, Mekelle city contains 43 gas station workers and 77 occasionally unexposed subjects. This exposed group has a working hour of 11.74 ± 1.89 hours/day. Reduced Mean RBC count (4.88 ± 0.573 vs $5.35 \pm 0.533 \times 10^{12}$ L), hematocrit ($43.29 \pm 3.71\%$ vs $44.95 \pm 3.10\%$), hemoglobin (15.04 ± 1.33 vs 15.59 ± 1.26) and platelets (248.95 ± 58.19 vs 295.45 ± 62.17) respectively with increased year of exposure. Peripheral blood film also demonstrates macrocytosis and basophilic stippling in 9.3% in an occupationally exposed group. MCH and MCHC (30.48 ± 2.06 pg vs 29.52 ± 1.66 pg) and (34.83 ± 0.988 g/dl vs 34.32 ± 0.927 g/d) were significantly high in exposed groups (38).

Other comparative cross sectional study was carried out in Hosanna Town, Southwest Ethiopia. This study encompassed 180 subjects, 60 gas station workers and 120 control subjects who were occupationally not exposed. The results show that the mean RBC of the exposed group was significantly reduced as compared to the control group. Median hemoglobin among occupationally exposed individuals was significantly reduced. In contrast, significantly increased eosinophil count was observed in the exposed group (39). Since variation in hematological parameters among geographically diverse population and the practice of using PPE determine the degree of effect of gasoline, evaluating the effect in Addis Ababa where no published study is found can provide useful evidence to inform appropriate intervention.

3. OBJECTIVE

3.1. General Objective

To evaluate hematological parameters of gas station workers in Addis Ababa, Ethiopia from January to March, 2025.

3.2. Specific Objectives

- To compare hematological parameters of gas station workers with individuals who are not occupationally exposed to gasoline.
- To determine correlation of duration of exposure and hematological parameters.

4. MATERIAL AND METHODS

4.1. Study Area

The study was conducted in Addis Ababa, which is the capital city of Ethiopia. This city lies at an elevation of 7,726ft located at 9°1'48''N38°44'24''E with a population of 5, 704, 000. Addis Ababa has 11 sub-cities and 126 gas stations. This study recruited gas station workers from gas stations in Arada, Gulele and Addis Ketema sub-cities. The sub-cities are conveniently selected as they are relatively close to the testing laboratory (Ethiopian Public Health Institution) which makes the time of sample transportation short.

4.2. Study design and period

Comparative cross sectional study was conducted from January to March 2025.

4.3. Population

4.3.1. Source population

All gas station workers in Addis Ababa city.

4.3.2. Study population

Gas station workers in the selected sub-cities who meet inclusion criteria and who volunteered to take part in the study.

4.3.3. Comparison groups

Age and sex matched non-exposed apparently healthy senior students and staffs from Addis Ababa University College of Health Sciences and EPHI staffs were used as a control group.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

For exposed group

- Age between 18-60.
- Free from any acute and chronic hematological problem
- Willing to participate

For control group

- Age between 18-60
- Non-gas station workers who lives in Addis Ababa
- Free from any acute and chronic hematological problem
- Willing for participation

4.4.2. Exclusion criteria

For exposed group

- Pregnancy
- feeling sick on the day of sample collection, not taking any antibiotics, or pain reliever medication
- Current use and history of drug that affect blood cell or clotting
- Gas station workers with history of alcoholism and smoking as well as those who recently started
- Workers employed for less than 6 months

For control group

- Pregnancy
- Current use and history of drug that affect blood cell or clotting
- Individuals with a history of occupational exposure to gasoline
- Individuals with any acute and chronic disease including diabetes. Liver, kidney and hematological malignancies

- feeling sick on the day of sample collection, not taking any antibiotics, or pain reliever medication
- Individual who have a history of alcoholism and smoking as well as those who recently started

4.5. Study variable

4.5.1. Dependent variable

Hematological parameters

4.5.2. Independent variable

- Age
- Sex
- Utilization of personal protective equipment
- Working hour per day
- Employment duration

4.6. Sample size calculation and sampling method

4.6.1. Sample size calculation

Sample size is calculated using sample size calculation from medical.org for comparing two means by considering Hematocrit of exposed and control group 45.7 and 47.7 respectively. Standard deviation is 5.8 and 6.5 respectively (39).

$$N = 2\sigma^2 [Z_{1-\alpha/2} + Z_{1-\beta}]^2 / (\mu_1 - \mu_2)^2$$

Where, σ is pooled standard deviation of the two groups $\sigma^2 = (S_1^2 + S_2^2)/2$

$$N = \frac{(S_1^2 + S_2^2) * [Z_{1-\alpha/2} + Z_{1-\beta}]^2}{(\mu_1 - \mu_2)^2}$$

$$N = (1.96 + 0.84)^2 * (5.8^2 + 6.5^2) / (47.7 - 45.7)^2$$

$$N = (7.84) * (33.64 + 42.25) / (2)^2$$

$$N = (7.84) * (75.89) / 4$$

N=150

N = sample size

Z1- $\alpha/2=95\%$ significance level

Z1- $\beta= 80\%$ power

M1= percent mean of 45.7 in exposed group

M2= percent mean of 47.7 in control

σ_1 = standard deviation of exposed group (5.8)

σ_2 = standard deviation of control group (6.5)

1:1 ratios are used for the exposed group. **Thus** 150 and 150 individuals were used as exposed and control groups respectively.

4.6.2. Sampling method

Non probability, convenient sampling technique was used to enroll participants.

4.7.Measurement and data collection

4.7.1. Data collection procedure

A pre-tested structured questionnaire was used to collect socio demographic data of participants. The questionnaire was written in two languages; English and Amharic. The questionnaires included data collection format for age, sex, use of personal protective equipment, smoking and drinking habit, nutritional status, working hour per day and employment duration. Gas station workers in the selected sub-cities who fulfill the eligibility criteria were approached through their office and recruited conveniently. Age and sex-matched staff and senior students were recruited in the same manner.

4.7.1.1.Laboratory Analysis

Trained laboratory technologists collected about 5 ml blood from the antecubital area of the participants using EDTA-K3 tube in the morning until 11:00 am. Participants who fulfill the inclusion criteria were welcomed for the test. All specimens were labeled in identification numbers. For the whole process standard operating procedures were used. (See Annex for detailed sample collection and analysis procedure). Specimens were analyzed within 4 hours of data collection. Result analysis, verification, reporting, interpretation and validation was performed by the principal investigator. Complete blood count was performed using UniceL®DxH800 Beckman-coulter automated hematology analyzer. Peripheral blood film was prepared to examine hematological abnormalities. All laboratory Assay was performed in EPHI.

4.7.1.2.Principle of UniceL®DxH800 Beckman-coulter automated hematology analyzer.

Coulter method

UniceL®DxH800 Beckman-coulter automated hematology analyzer count and size cell by measuring and detecting changes in electrical resistance when cells pass through small apertures. Volume, conductivity and scatter (VSC) established WBC differential technology using individual cell volume; high frequency current and light scattering technology provided cell by cell information and translated by scatter-gram, platelet and mean plots (SPM) into data plots. Electronic leukocyte volume analysis using low frequency current is also used.

4.7.1.3.Principle of peripheral blood film stained by wright stain

Peripheral blood film (PBF) reveals morphology of peripheral blood cells. Wright stain is used to stain peripheral blood film which is used to diagnose morphology of blood cells. Wright stain is a combination of basic dyes (methylene blue) and acidic dyes (eosin) which stain acidic components of DNA as blue or purple and basic component of the cell such as cytoplasm, hemoglobin and eosinophilic granule as red or orange (40).Wright stains was used to review abnormal CBC outputs, classify anemia and rule out unexpected results.

4.8.Data quality assurance

4.8.1. Pre-analytical

To guarantee quality of data, socio-demographic information was collected using pre-tested structured questionnaires and training was given to data collectors. The quality of the sample was maintained by following sample rejection criteria of EPHI hematology laboratory including, clotted sample, incorrectly labeled sample and severely hemolyzed sample.

4.8.2. Analytical

Commercially prepared low, normal and high quality control samples were used to check the accuracy and precision of the hematology analyzer. Quality of the staining solution was checked. Two slides per sample was prepared and stained using a wright stain solution. The finest slide was examined by the principal investigator and confirmed by hematologist in EPHI.

4.8.3. Post analytical

The completeness and accuracy of data was checked by the principal investigator. Data was cleaned, coded and entered correctly.

4.9.Data analysis and interpretation

After data is checked for completeness, it was entered in Excel for windows and the statistical package for the social sciences (SPSS) version 30 was used for the analysis. Independent t-test was used to compare mean hematological parameters between gas station workers and control groups. One way ANOVA was used for analysis of variance between qualitative variables such as duration of work, age, PPE usage and with hematological variables; post hoc test was performed to identify exactly which groups differ from each other. Pearson correlation coefficient was used to assess hematological parameters with age, working hour and year of exposure. Results were summarized as mean \pm standard deviation, mean, interquartile range and median. P-values less than 5% at 95% confidence interval were considered as statistically significant.

4.10. Operational definition

Classification and definition of hematological parameters are based on hematological reference intervals determined in adults at Gondar university hospital, Northwest, Ethiopia (41).

Red blood cell- $3.53-6.93 \times 10^{12}/l$ male and $3.45-6.23 \times 10^{12}/l$ for female

Hematocrit 36.2-58.6% for male and 32.1-56.6% for female

Mean cell hemoglobin concentration (MCHC) - 30.8-34.4% for male and 29.5-34.4% for female

Mean cell hemoglobin (MCH)-26.6-33.3 pg for male and 25.8-32.8 pg for female

Hemoglobin 11.5-18g/dl for male and 11-16g/dl for female

White blood cell- $3.2-8.8 \times 10^9/l$ for both genders

Absolute neutrophil, lymphocyte, mixed cell- $1.6-5.1 \times 10^9/l$, $1-3.5 \times 10^9/l$ and $0.2-1 \times 10^9/l$ respectively for both genders

Platelet- $128-432 \times 10^9/l$ for both genders

Mean cell volume (MCV)- 85-100 fl for both gender

Red cell distribution (RDW)- 12-17% for both gender

Neutrophil, lymphocyte and mixed cell %- 36-69, 22-55 and 0.2-1 respectively for both genders.

Any parameters above and below these intervals are considered as hematological changes.(42).

4.11. Ethical considerations

The study proposal was examined and approved by the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences of Addis Ababa University (DRERC). Official letter of request was sent to the gas stations to get permission and the Ethiopian Public Health Institution to get approval to carry out hematological analysis in the hematology laboratory. Informed consent was obtained from the study participants. All procedures were carried out after introduction of the purpose and goal of the study to the participants and obtaining an informed consent. Personal identification was not used to uphold confidentiality. Study was carried out in accordance with the Declaration of Helsinki.

4.12. Dissemination of the result

The Results of this study will be presented to Addis Ababa University, Department of Medical Laboratory Sciences through thesis defense and record will be repositied at Addis Ababa University library and publication office and department of medical laboratory sciences. International journals or peer-reviewed national journals will be considered for publication.

5. RESULTS

5.1. Socio-demographic characteristic of study participants

From 302 participants, 151 exposed and 151 controls were enrolled in this study. Gasoline station workers and control groups revealed no statistically significance difference in terms of age and sex ($p=0.894$, $p=0.7385$) with median age (36.23 ± 9.41 and 36.08 ± 9.41), respectively. From the total participants only 20 (13.2%) from exposed and 20(13.2%) from control group of the participants were females (Table 1).

Table 1; Socio-demographic profile of the exposed and control group using t-Test and χ^2 test at Addis Ababa city, from January to March 2025

Variables	Socio-demographic Characteristic	Exposed group		Control group percentage		Test of significance
		Frequency	percent age	frequency	Percentage	
Sex	Male	131	86.8%	131	86.8%	P=0.74 $\chi^2 =0.73$
	Female	20	13.2%	20	13.2%	
Age	19-26	29	9.6%	25	8.2%	P=0.89
	27-34	37	12.3%	42	13.9%	
	35-44	58	19.2%	57	18.9%	
	>45	27	8.9%	27	8.9%	
	Mean \pm SD	36.23 \pm 9.51	36.08 \pm 9.41			
Marital status	Single	57	18.9%	70	23.2%	
	Married	91	30.1%	79	26.2%	
	Divorced	3	1%	2	0.7%	
	Widowed	0	0%	0	0%	
Educational status	Unable to write and read	17	5.6%	0	0%	
	Primary school	24	7.9%	6	2.0%	
	Secondary school	105	34.8%	22	7.3%	
	College and above	22	7.3%	106	35.1%	

Abbreviation; SD, standard deviation

5.2. Occupational information of study participants

Table 2 summarizes the median duration of gas station workers year of exposure is 6 years (minimum 9 months and maximum 41 years). The gas station workers were working for a c, while 77.7% of the exposed group was working for 6-12 hours per day. The highest number of exposed group, 66 (43.7%), had worked in the gas station for 2 and less years, whereas 23 (15.2%) and 62 (41.1%) of them worked 3–7 and for more than 8 years, respectively. Gas station workers pour 300-50,000 liters of gasoline per day. More than half 56.9% of the exposed group had poor awareness about the impact of gasoline on human health. Majority of the exposed group (87.4%) eat and drink at the gas station. It was observed that 39.1% of gas station workers were consuming food and drink during data collection.

Category	Details
Median duration of exposure	6 years years (minimum 9 months and maximum 41 years)
Work schedule	minimum 2 days/week maximum 7 days/week
Hours worked per day	77.7% worked 6-12 hours/day
Awareness of gasoline’s impact on health	59.9% had poor awareness
Eating and drinking at station	39.1% consumed food and drink at work place

Table 2; Occupational information of study participants among exposed group in Addis Ababa city, from January to March 2025

Table 3 summarizes the utilization of personal protective equipment (PPE) among gas station workers. Glove and mask use was limited to 15 (9.9%) and 27 (17.9%) of the workers. There was a better practice of safety shoes and protective clothing (uniform), 132 (87.4%) and 133 (88.1%), respectively. When participants were asked for the reason, as displayed in Table 3, the majority declared inconvenience to wear (79, 52.3%) and not aware of the need (63, 41.7%).

Table 3; Utilization of personal protective equipment among exposed group in Addis Ababa city, from [January to March 2025](#)

Items	Response	Frequency	Percentage (%)
Glove	Yes	15	9.9
	No	136	90.1
Mask	Yes	27	17.9
	No	124	82.1
Safety shoes	Yes	132	87.4
	No	18	12.6
Protective clothing(uniform)	Yes	133	88.1
	No	18	11.9

Table 4; Reasons for not to using personal protective equipment by gas station workers in Addis Ababa city, from [January to March 2025](#)

Reasons for not to use PPE		
	Frequency	Percentage (%)
Inconvenient to wear	79	52.3
Not provided by the company	24	15.9
Not aware of the need	48	31.8

5.3.Clinical data of study participants

About 106 (70.2%) gas station workers had health related complaints. These include, Skin complaint/eczema 27 (17.9%), Dizziness 17 (11.3%), eye complaint 24 (15.9%), shortness of breath (asthma) 16(10.6%), Fatigue 19(12.6%) and Nosebleed 3. (figure 1)

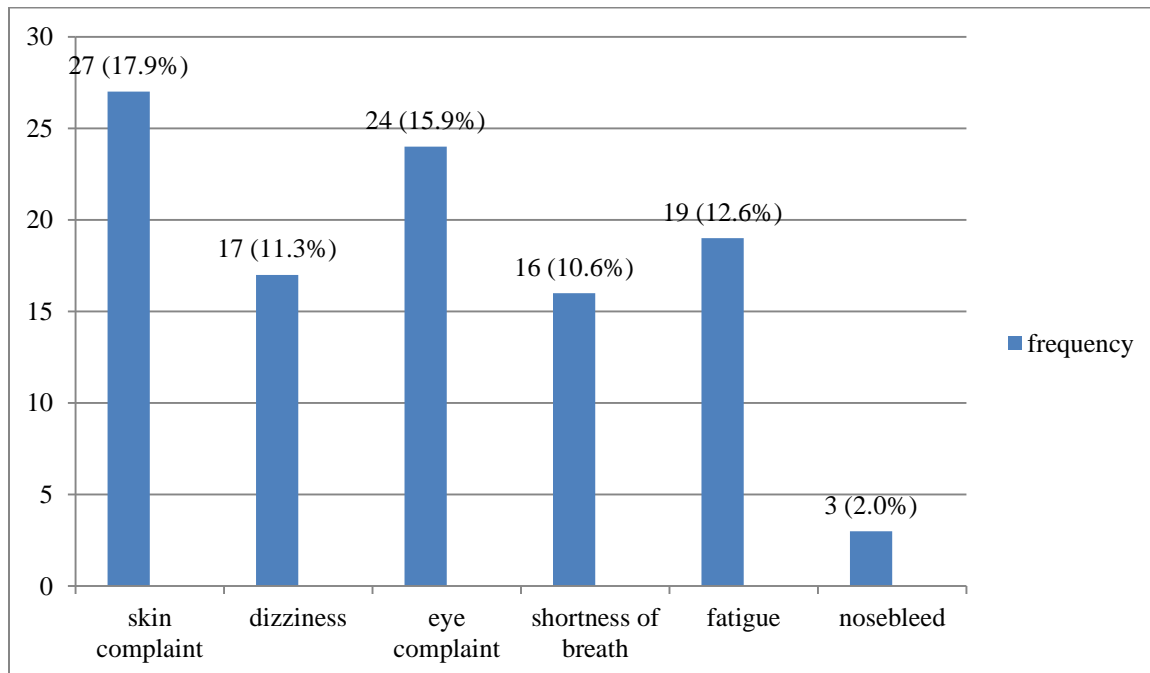


Figure 1. Sign and symptom of gas station workers

5.4. Comparison of hematological parameters between exposed and control group

Mean and standard deviation of hematological parameters of exposed and control group were presented in table five. All the hematological parameters are in reference range. RBC, WBC, HGB, HCT, MCV, MCH, MCHC, RDW-SD, PLT, MPV, lymphocyte, eosinophil and basophil showed elevated results in exposed group although absolute and percentage count of monocyte, neutrophil and RDW decrease in expose group. Hemoglobin count (16.394 ± 1.42974 vs 15.5397 ± 1.85268 ; $p < 0.001$), Mean platelet volume (9.3430 ± 1.23631 vs 9.0417 ± 1.17719 ; $p = 0.031$), lymphocyte percentage (28.3914 ± 9.13303 vs 25.3993 ± 8.8243 ; $p = 0.004$), absolute lymphocyte (1.6914 ± 0.5856 vs 1.3946 ± 0.40879 ; $p < 0.001$) were significantly higher in gas station workers as compared with the control group. (table 5)

Table 5; Comparison of hematological profile of exposed and control group using independent t-Test at Addis Ababa city, from January to March 2025.

Hematological parameters	Exposed group Mean \pm SD	Control group Mean \pm SD	95% confidence interval of the difference		
			lower	Upper	P value
WBC(*10 ⁹ /L)	6.17 \pm 1.75	5.87 \pm 1.73	-.688	.101	0.144
RBC (*10 ¹² /L)	5.33 \pm .49	5.26 \pm .52	-.194	.037	0.184
HGB (g/dl)	16.39 \pm 1.43	15.54 \pm .1.85	-1.229	-.479	.000
HCT%	47.34 \pm 4.39	46.50 \pm 4.24	-1.819	.136	0.091
MCV (fl)	88.78 \pm 5.72	88.65 \pm 5.01	-1.351	1.082	0.828
MCH (PG)	30.88 \pm 3.05	30.58 \pm 1.97	-.889	.275	0.300

MCHC%	34.74±2.08	34.47±.78	-.619	.091	0.146
RDW	13.46±1.12	13.51±.94	-.184	.286	0.669
RDW-SD	43.14±2.21	42.79±3.20	-.974	.272	0.269
PLT (*10 ⁹ /L)	259.33±67.42	247.19±60.67	-26.664	2.386	0.101
MPV	9.34±1.24	9.04±1.18	-.575	-.027	0.031
Neutrophil%	59.18±13.44	61.88±11.68	-.146	5.560	0.63
Lymphocyte%	28.39±9.133	25.39±8.82	-5.026	-.958	0.004
Monocyte%	8.23±3.86	9.31±7.25	-.232	2.398	0.106
Eosinophil%	3.83±4.53	3.26±3.44	-1.491	.332	0.212
Basophil	.62±3.93	.19±.53	-1.052	.217	0.196
Neutrophil#	3.69±1.48	3.75±1.60	-.287	.410	0.728
Lymphocyte#	1.69±.59	1.39±.40	-.411	-.182	0.000
monocyte#	.49±.269	.52±.39	-.052	.101	0.523
eosinophil#	.23±.29	.18±.23	-.104	.014	0.132
basophil#	.03±.29	.003±.02	-.076	.017	0.207

Abbreviation; **WBC;** white blood cell, **RBC;** red blood cell, **MCV;** mean corpuscular volume, **MCH;** mean corpuscular hemoglobin, **MCHC;** mean corpuscular volume concentration, **HGB;** hemoglobin, **HCT;** hematocrit, **MPV;** mean platelet volume, **PLT;** platelet, **CI;** confidence interval, g/dl; gram per deciliter, **PG;** pictogram, **fi;** femtoliter

5.5. Comparison between hematological parameters and year of exposure in the subgroups of gas station workers

Comparison of hematological parameters across different years of exposure was presented in table 6. Statistically significant result was observed in WBC ($p=0.048$) with individuals exposed for ≥ 8 years compared to those exposed for ≤ 2 years. Neutrophil percentage ($p=0.042$) and absolute neutrophil count ($p=0.017$) showed significant increase among 3-7 years and ≥ 8 years of exposure. Hemoglobin result showed statistically significant difference between individual who exposed for ≤ 2 years and ≥ 8 years. ($p=0.037$) as a year of exposure increases. The mean value of WBC, hemoglobin were not statistically significantly different among individuals exposed for ≤ 2 and 3-7 years. (Table 6)

Table 6; Comparison between hematological parameters and year of exposure in the subgroups of gas station workers at Addis Ababa city, from January to March 2025.

Year of exposure	Parameters	p value
	WBC ($\times 10^9/L$)	
≤ 2 yrs vs 3-7 yrs	5.70 \pm 1.92 vs 6.06 \pm 1.53	0.997
≤ 2 yrs vs ≥ 8 yrs	5.70 \pm 1.92 vs 6.55 \pm 1.73	0.048
3-7 yrs vs ≥ 8 yrs	6.06 \pm 1.53 vs 6.55 \pm 1.73	0.405
HGB (g/dl)		
≤ 2 yrs vs 3-7 yrs	16.74 \pm 1.29 vs 16.57 \pm 0.99	1
≤ 2 yrs vs ≥ 8 yrs	16.74 \pm 1.29 vs 16.02 \pm 1.71	0.037

3-7yrs vs >=8 yrs	16.57±0.99vs 16.02±1.71	0.125
neutrophil %		
=<2yrs vs 3-7 yrs	59.33±11.8 vs 56.06±11.79	0.556
=<2yrs vs >=8yrs	59.33±11.8 vs 61.53 ±11.12	1
3-7yrs vs >=8 yrs	56.06±11.79 vs 61.53 ±11.12	0.042
Neutrophil (*10 ⁹ /L)		
=<2yrs vs 3-7 yrs	3.48±1.76 vs 3.33±0.99	1
=<2yrs vs >=8yrs	3.48±1.76 vs 4.1 ±1.5	0.101
3-7yrs vs >=8 yrs	3.33±0.99 vs 4.1±1.5	0.017

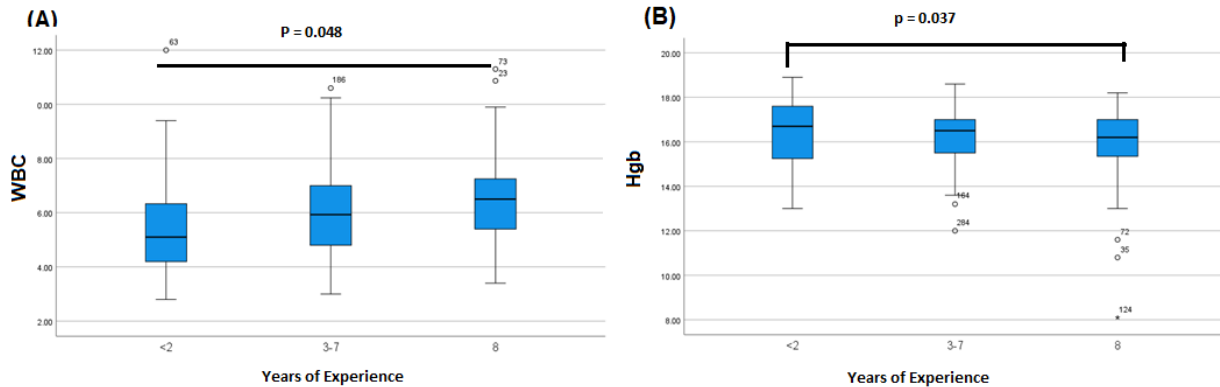


Figure 2; comparison between both hemoglobin and white blood cell with year of exposure

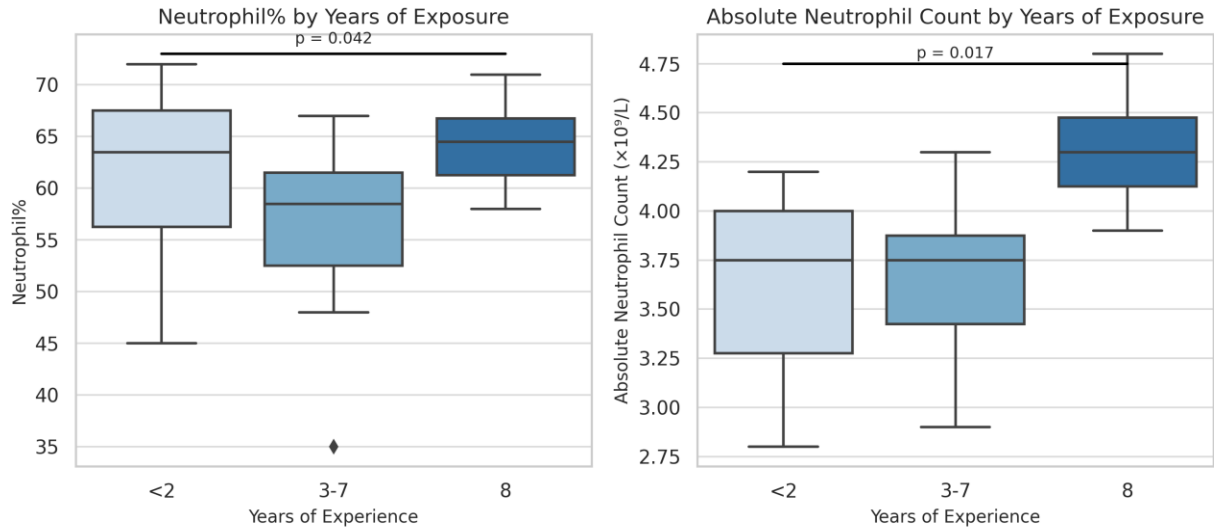


Figure 3; Comparison of Neutrophil % and Neutrophil absolute count with year of exposure

5.6. Correlation analysis of hematological parameters with year of exposure, working hour per day and working days per week

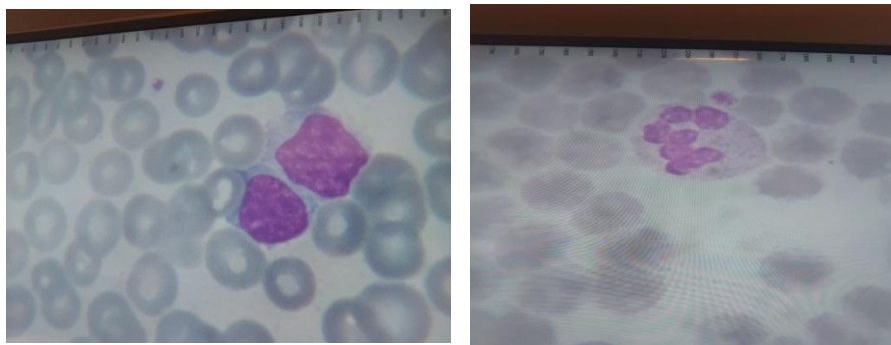
As illustrated in table 7 RBC count tend to slightly decrease as a year of exposure increases with a statistically significant result ($p=0.033$). As working hour increase hemoglobin decreases moderately with significant value ($p<0.001$). As year of exposure increases RDW-SD results increase slightly with statistically significant value ($p=0.003$). MPV slightly decreases as working days per week increases ($p=0.001$). As working hour increases monocytes tend to slightly increases ($p<0.001$). As working hour increases monocyte decrease slightly ($p<0.001$). Nevertheless, the correlation coefficient for many of these is poor with r value of less than 0.3. (Table 7)

Table 7; Correlation analysis of hematological parameters with year of exposure, working hour and working days per week among gas station workers at Addis Ababa city, from January to March 2025.

parameters	Year of exposure		Working days per week		Working hour per day	
	r	p- value	r	p- value	r	p-value
RBC	-0.171	0.033	0.014	0.867	0.098	0.238
HGB	-0.126	0.122	-0.011	0.898	-0.487	<0.001
RDW-SD	0.19	0.019	0.039	0.632	-0.243	0.003
MPV	-0.4	0.958	-0.258	0.001	-0.067	0.413
Monocyte %	0.063	0.442	-0.071	0.39	0.258	0.001
Lymphocyte count	-0.011	0.898	0.083	0.312	-0.281	<0.001

5.7. Peripheral morphology results

Among one hundred fifty one gas station attendants 3 (1.99%) exhibited echinocytosis, 12 (7.95%) showed reactive lymphocyte, band cell were observed in 13 (8.61%) of the exposed group. 5 (3.3%) hyper-segmented neutrophil, 10 (6.62%) disintegrated eosinophil and 3 dysmorphic neutrophil were detected.



6. Discussion

Acute and chronic occupational exposure to hydrocarbons of gasoline particularly benzene, toluene, ethyl benzene and xylene which are also known as volatile organic compounds (VOC) induce bone marrow suppression, impaired hematopoietic system, even cancer (1). Hence evaluating hematological parameters among gas station workers which are occupationally exposed to gasoline hydrocarbons as compared to non-exposed individuals in Addis Ababa, Ethiopia was the objective of this study. The results reveal significant changes for selected hematological parameters in line with earlier study carried out in Ethiopia, Africa and worldwide.

In current study the control and exposed group revealed no statistically significant difference in terms of age and sex ($p=0.894$, $p=0.7385$) respectively, explaining that the observed hematological changes were more likely to be caused by occupational exposure. The occupational information findings in this study showed median 6 years work experience with 6-12 hours of exposure up to 7 days per week. This high frequency occupational exposure to gasoline mirrors finding with previously reported finding from Thailand city of Khon Kaen (31).

This study reveals extensive insight into the hematological changes of gasoline station workers compared to control group. Hemoglobin were significantly elevated in control group ($p<0.001$) because of compensatory reply to hypoxia due to inhalation of volatile hydrocarbons of gasoline which was a mirror result with a finding from Egypt and Nigeria(23, 43). Although this result contradicts a finding from Gondar Ethiopia which reported decreased hemoglobin results in gas station workers (37). In current research 66 (43.7%) of gas station workers worked in the station for less than 2 years, but in the Gondar study 31 (56.3%) of the gas station workers worked in station for 5 years which shows that early exposure to gasoline elevate hemoglobin result. As working hour started to elevate hemoglobin result start to decline in mild negative correlation ($r=-0.487$, $p<0.001$) which implies cumulative burden on erythropoiesis.

Mean platelet volume ($p=0.031$), lymphocyte% ($p=0,004$) and lymphocyte absolute count ($p<0.001$) demonstrates significant difference among exposed and control groups. These findings line up with studies conducted in Mekelle, Ethiopia, and Iran (36, 38, 43). our finding also demonstrate mild elevated RBC count even though the results start to decrease after 8 years

which implies bone marrow suppression, Increased lymphocyte count due to possible immunologic response towards chronic benzene exposure were also appreciated. Systemic inflammation and oxidative stress in the context of chronic benzene metabolites exposure could modulate immune system and alter lymphocyte function and number. Eventually such chronic stimulation could lead to immune exhaustion and suppression leading to susceptibility for infection and cancers (44). The negative correlation between MPV and working days ($r=-0.258$, $p=0.001$) demonstrate platelet consumption or chronic stress-related changes in megakaryopoiesis.

Increased monocyte percentage with longer working hour ($r=0.258$, $p=0.001$) showed chronic inflammation. These results are line up with studies from Kenya where longer working hour without utilization of personal protective equipment reveal elevated hematological markers (45).

The Pearson correlation coefficient reveals poor negative relation between RBC ($r=-0.171$, $p=0.033$) and exposed year, positive relation with RDW-SD ($r=0.19$, $p=0.019$) which is consistent with a finding from Ghana, which also got the same finding as a current study which was implication of anisocytosis due to oxidative damage (46).

Decreased monocyte and neutrophil count were also observed in this study, which align with a finding from Mekele Ethiopia and Hosanna Ethiopia (38, 39). Even though the results are not statistically significant it shows early stage hematological disturbance due to chronic benzene exposure.

Despite the significance difference in lymphocyte between exposed and control group and significance difference in neutrophil among gas station attendants peripheral morphology also showed reactive lymphocyte, disintegrated eosinophil echinocytosis and hyper segmented neutrophil which is markers of oxidative damage to blood cells. This results line up with a finding from oxidative stress-related hematological changes in Brazilian petrol station workers (47).

Utilization of personal protective equipment (PPE) among exposed group was noticeably inadequate. In spite of high utilization of safety shoes 132 (82.7%) and protective clothing (uniforms) 133 (88.1%), only 9.9% and 17.9% of the exposed group use gloves and masks respectively. These results align with findings from Kenya, where utilization of PPE was also

inadequate for related reasons(45). Causes not to use PPE such as not provided by the company, inconvenient to wear and not aware of the need were comparable with researches from Brazil, where improper utilization of PPE were major contributor to raised exposure of gasoline hydrocarbons (48).

About 87.4% of the exposed group reported drinking and eating at the gas station even 39.1% of the workers observed taking action during data collection. This action more give chance for ingestion of toxic hydrocarbons of gasoline and shows a huge occupational health void. This trends line up with studies in Ethiopia, haromaya, where gas station hygiene practice were poor(27). Symptom findings like nosebleeds (2%), shortness of breath (10%), fatigue (12.6%), dizziness (11.3%), eye irritation (15.9%) and skin complaints (17.9%) were comparable with findings from studies Ghana and RJ Brazil (46, 49, 50), where exposed group revealed neurological, respiratory issue and dermatological complication.

7. Strength and limitation of the study

7.1. Strength

Large sample size with age and sex matched control inclusion

7.2. Limitation

Biomarker of oxidative stress and DNA damage were not included

Cross sectional study design is just snap shoot which cannot allow follow up over time

8. Conclusion and recommendation

8.1. Conclusion

Overall, the current study findings have revealed that occupational exposure to gasoline and its volatile hydrocarbons have significant alteration on hematological parameters. The increased lymphocyte count and decrease hemoglobin count with increased year of experience suggest early toxic effect. Decreased neutrophil and monocyte count observed were also implication of early stage hematological disturbance due to chronic benzene exposure. There for results from this study was evidence that occupation exposure to gasoline and its volatile component negatively affect hematological parameters.

8.2. Recommendation

The inadequate utilization of personal protective equipment, restricted risk awareness long hour of exposure per day and noted hematological changes underline the require for powerful occupational health policies in Ethiopia. Creating awareness for gas station works about the potential risk of gasoline exposure, avoiding consumption of food and drinks at gas stations, regular medical check-up, policies limiting hourly exposure on a daily basis, and required provision of personal protective equipment should be given top priority.

Particularly gas station workers who worked for a long period of time should get periodic diagnosis and blood benzene level examination. Additional researches are recommended to include biomarkers and incorporate biomarkers of oxidative stress and DNA damage.

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ANNEXES

Annex I; participants Information sheet

A English version

Principal investigator; Abigail Mesfin

Institution; Addis Ababa University, Collage Of Health Science, Department Of Medical Laboratory Science

Title of the project; evaluation of hematological parameters among station workers at Addis Ababa Ethiopia.

Introduction; gear participant; I am an MSC student at Addis Ababa University, school of Medical Laboratory Sciences, you are invited to participate in the study of evaluation of hematological parameters among station workers at Addis Ababa Ethiopia in 2025.

Purpose of this study; the purpose of this study is to evaluate hematological parameters among gas station attendants and compare them with non-occupationally exposed individuals at Addis Ababa Ethiopia.

Duration; duration of this study will be 2-3 months.

Procedure to be followed; Dear participants, if you agree to participate in this study, you will be asked to receive punctures to draw a 3-5 blood sample from your vein. The sample will not be used for any purpose other than the objective of this study.

Risk and Discomfort; During this process you will feel a small amount of temporary pain. Appropriate precaution will be taken during sample collection. Samples will be collected by trained health professionals.

Expected Benefit; there will be no any cash payment or benefit for participating, but we will inform you your blood test diagnosis results.

Confidentiality; All information taken for this study purpose will be kept totally confidential, information you provide for this study, such as name and identity, on the questionnaire will be changed to a confidentiality code. In any publication where the results of the study appear, the details will be spread without including your name.

Withdrawal from the study; your participation in this study will be voluntary, if participating and giving answer any question will give you discomfort you can drop your participation at any time without justification to withdraw. You have the right to obtain your result. If you have any further questions, kindly contact us in the following address;

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Email; - Abiagemesfin@gmail.com

Advisors

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DMLS Ethical review committee

Tel: 0112755170

The participant will be given a copy of this to retain for his/her records if applicable.

Thank you for your participation.

B, Participant Information sheet Amharic version
ለጥናቱ ተሳታፊዎች የመረጃና ስምምነት ቅፅ (Amharic Version)

ለጥናቱ ተሳታፊዎች የመረጃ ቅፅ ጥናቱ የሚካሄድበት ቦታ _____

የጥናቱ ርዕስ: በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ ላይ ማጥናት (evaluation of Hematological parameters in Gas Station Workers at Addis Ababa city)

የተከበሩ የጥናቱ ተሳታፊ:

በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ በሚዳስስ ጥናት ውስጥ እንዲሳተፉ ልንጠይቅዎት እንወዳልን። የጥናቱን ሁኔታ በተመለከተ ማብራሪያ እንሰጥዎታለን። እናም ጥያቄ ካለዎት ግልፅ ማድረግ እንችላለን። በዚህ ጥናት ለመሳተፍ ግዴታ የሌለበት ሲሆን ለመሳተፍ ፍቃደኛ ከሆኑ ግን ይህን ቅፅ አንብቦ በቅጹ መጨረሻ ላይ ይፈረማሉ። ይህ ጥናትና የስምምነት ቅፅ በአዲስ አበባ ዩኒቨርሲቲ ሜዲካል ላቦራቶሪ ሳይንስ ስነምግባር ገምጋሚ ኮሚቴዎች የእርስዎን መብት እንደሚያስጠብቅ

የጥናቱ ዓላማ:

የጥናቱ ዋና ዓላማ በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ ማጥናት ነው።

በዚህ ጥናት የሚሳተፉት ሰዎች:

ጋዝ ማሰራጨ ሰራተኞች, ለመሳተፍ ፍቃደኛ የሆኑ የሚወሰደው ናሙና ለዚህ ጥናት ከእርስዎ የሚፈለገውን 5 ሚሊ ሊትር የደም ናሙና ከክንድዎት በመስጠትዎ የማያሰጋ በሳይንስ በተረጋገጠና ለምንም በማያጋልጥ መልኩ ይወሰዳል። ነገር ግን ከዚህ ጥናት ውጭ ለሌላ የተለየ ዓላማ አይወጣም። በፈለጉት ጊዜ ይህን ስምምነት ሊያቃርጡ ይችላሉ። ባቋረጡ ጊዜም እርስዎን የሚመለከቱ ነገሮች ሁሉ ከላብራቶሪ የመረጃ መረብ እናስወግዳለን። ተያያዥነት አላቸው ተብለው የሚታሰቡ ጉዳዮች እርስዎ እዚህ ጥናት ላይ በመሳተፍዎ እና ከላይ የተጠቀሰውን የደም ናሙና በመስጠትዎ በጤናዎ ላይ የከፋ ችግር አያመጣም። ነገር ግን መርፌ በሚወጋበት አካባቢ ትንሽ የህመም ስሜት ሊኖር ይችላል። ይሁን እንጂ ይህም በጤና ባለሙያዎቹ እይታ ስር ስለሆነ ለከፋ ጉዳት አይዳርግም።

ጥቅማጥቅምና ክፍያ:

እርስዎ በዚህ ጥናት በመሳተፍዎ ብቻ የሚከፈለዎት ተጨማሪ ገንዘብ ክፍያ የለዉም። ነገር ግን ከሚሰጡት የደም ናሙና የሚሰራውን የላቦራቶሪ ዉጤት የማግኘት መብት አለዎት።

የመረጃዉ ምስጢራዊነትና የተሳታፊዉ መብት:

በጥናቱ የሚሳተፉት የፍቃደኝነት ማረጋገጫ ሲሰጡን ብቻ ሲሆን በፈለጉት ጊዜ ስምምነቱን ሊያቋርጡ ይችላሉ። እርስዎን የሚመለከት ሁሉም ነገር በምስጢር ይቀመጣል። የእርስዎ ስምም ከእርስዎ ከምንወስደዉ ናሙና ጋር አይገናኝም። ከእርስዎ የምንወስዉ መረጃ ለተጠቀሰዉ ዓላማ የሚዉል ሲሆን ምስጢራዊነቱን በጠበቀ ሁኔታ ለህትመት ይላካል። በጥናቱ ሲሳተፉ ግልፅ ያልሆኑ ጉዳዮች የመጠየቅ ሙሉ መብት አለዎት። እኛም የተነሱትን ጥያቄዎች ግልፅ እናደርግልዎታለን። ጥናቱን በተመለከተ ማነጋገር ቢያስፈልግዎ የጥናቱን አስተባባሪ ስምና አድራሻ እንደሚከተለዉ ነዉ።

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አማካሪዎች

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በጥናቱ ለመሳተፍ ፍቃደኛ በመሆንዎ እናመሰግናለን!!! ቃለ መጠይቁን ያካሄደዉ ሰዉ

ስም: _____

ፊርማ: _____

Annex II ; Consent form(study participants)

Informed consent form English form

I am hereby concerned to participate in the study of evaluation of hematological change in gas station workers at Addis Ababa city. I have read and fully understand the participant information sheet and have the opportunity to ask questions related to this study. To participate in this study, I agree to receive a puncture for the purpose of drawing blood for laboratory testing. I understand that this puncture will cause a small amount of temporary pain at the puncture site and the sample will not be used for another purpose. All information regarding my sample will remain completely confidential and will not be used for any other purpose than the objective of this study. I understand that I'm not obligated to participate in this study, and I can decide not to participate at any time. I have read the above document and I understand that I have agreed to participate in this study.

Name of participant; _____

Signature of participant; _____

B; Informed consent form Amharic form

የስምምነት ቅፅ

በሚቀጥለው ርዕስ ላይ የተመለከተ ጥናት ለማካሄድ ስለመሳተፍ የሚያመለክት የስምምነት ቅፅ

በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ ላይ ማጥናት (evaluation of Hematological parameters in Gas Station Workers at Addis Ababa city) ማጥናት ነዉ።

በዚህ ጥናት ውስጥ ለመሳተፍ እባክዎን የሚከተለውን ቅፅ አንብበዉ በሚከተሉት ባዶ ቦታዎች ወይም ሳጥኖች ውስጥ ምልክት ያድርጉ።

1. የጥናቱን ዓላማ ተገንዝቤአለሁ ጥናቱን የሚያካሂደውን ሰዉ ስፈልገዉ ማግኘት እንደምችል ተረድቻለሁ።

2. የእኔ የደም ናሙና ተወስዶ ለጥናቱ ዓላማ እንደሚውል ተረድቻለሁ።

3. ለጥናቱ የሚሰጡ መረጃዎች እና ከጥናቱ የሚገኙ ውጤቶች በሚስጢር እንደሚቀመጡ ተረድቻለሁ።

4. ከዚህ ጥናት ገንዘብ በተለየ መልኩ አንደማላገኝ ተረድቻለሁ ።

5. ከዚህ ጥናት በፈለግሁ ጊዜ አቋርጬ መዉጣት እንደምችልም ተረድቻለሁ።

የጥናቱ ተሳታፊ ስም: _____

ፊርማ: _____ ቀን : __/__/__

Annex III; Questionnaires

A, Questionnaires English form

Addis Ababa University
College of health science
Department of medical laboratory science

Questionnaires (exposed group) data collection on evaluation of hematological parameters in gas station works at Addis Ababa, 2025. Fill if dashed lines are provided, otherwise, encircle the number corresponding to your responses.

Questionnaires identification number _____, Date; ____/____/____

Part 1; socio-demographic characteristic

No	Questions	Response
1	Age (n years)	
2	Sex	1. Female 2. Female
3	Educational status	1. No formal education 2. Primary school 3. Secondary school 4. Above secondary school

Part 2; occupational information

4	Employment duration (in years)	
5	Do you eat or drink at work place?	1. Yes 2. No
6	Working hour	
7	Utilization of personal protective equipment	1. Face mask 2. Glove 3. Safety vast 4. All 5. None

--	--

Part 3; lifestyle and habits

No	Questions	Response
8	Do you smoke?	3. Yes 4. No
9	Do you consume alcohol?	1. Yes 2. no
10	How do you rate your current diet?	1. healthy 2. moderately healthy 3. unhealthy

Part 4; clinical data

No	Questions	Response
11	Have you ever examine your blood for the past yr?	1. yes 2. no
12	If yes were any abnormalities found in your blood?	1. yes 2. no 3. not sure
13	Do you take medication?	1. yes 2. no
14	If yes, please specify the medication?	
15	Do you have any sign and symptom of skin complaint/ eczema, dizziness, periodic	If yes which one, write any of the listed here

	headache, allergic rhinitis, frequent sneezing, shortness of breath (asthma), eye complaint?	
--	--	--

Part 5; perception of work environment

No	Questions	Response
16	Do you know the impact of gasoline in your blood?	1. yes 2. no
17	If yes, please specify	

Part 6; Checklist to collect data regarding consumption of food and drinks

No	Questions	Response
18	Are workers consuming foods or drinks at gas station during data collection?	
19	Is food or drink visibly stored in the Gas-station?	
20	Is there any evidence of food, plates and drinks being used at gas station?	

Addis Ababa University
College of health science
Department of medical laboratory science

Questionnaires (control group) data collection on evaluation of hematological parameters in gas station works at Addis Ababa, 2024. Fill if dashed lines are provided, otherwise, encircle the number corresponding to your responses.

Questionnaires identification number _____, Date; ____/ ____/____

Part 1; socio-demographic characteristic

No	Questions	Response
1	Age	
2	Sex	2. Female 2. Female
3	Educational status	5. No formal education 6. Primary school 7. Secondary school 8. Above secondary school

Part 2; occupational information

4	Working hour	
---	--------------	--

Part 3; lifestyle and habits

No	Questions	Response
5	Do you smoke?	5. Yes 6. No
6	Do you consume alcohol?	3. Yes 4. no
7	How do you rate your current diet?	4. healthy 5. moderately healthy

		6. unhealthy
--	--	--------------

Part 4; clinical data

No	Questions	Response
8	Have you ever examine your blood for the past yr?	3. yes 4. no
9	If yes were any abnormalities found in your blood?	4. yes 5. no 6. not sure
10	Do you take medication?	3. yes 4. no
11	If yes, please specify the medication	

Part 5; perception of work environment

No	Questions	Response
12	Do you think your job has any impact in your blood?	1. yes 2. no
13	If yes, please specify	

B; Questionnaires Amharic form

**አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ
የሕክምና ላቦራቶሪ ሳይንስ ት/ት ክፍል**

መጠይቆች (exposed group) በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ ላይ ማጥናት (evaluation of Hematological parameters in Gas Station Workers at Addis Ababa city) ማጥናት ነዉ።

ሶሻል-ዲሞክራሲያዊ መረጃ መሰብሰቢያ መጠይቅ፤ ከጥያቄዉ ፊትለፊት የተሰመረ ቦታ ከገኙ አስፍላጊዉን መልስዉን በጽሁፍ ያስፍሩ ፤ የመልስ አማራጮች በቁጥር መልክ ከተዘረዘሩ ግን የርስዎን መልስ የያዘውን ቁጥር ይከበቡ።

የመጠይቁ መለያ ቁጥር _____ ፣ ቀን፡ ____ / ____ / ____

ክፍል 1: የተሳታፊዎች ሶሻል-ዲሞክራሲያዊ መረጃ

ተ.ቁ	ጥያቄዎች	መልሶች
1	ዕድሜ	
2	ጾታ	1 ወንድ 2.ሴት
3	የትምህርት ደረጃ	1. መደበኛ ትምህርት የለም 2. የመጀመሪያ ደረጃ ትምህርት 3. ሁለተኛ ደረጃ ትምህርት 4. ከሁለተኛ ደረጃ ትምህርት በላይ

ክፍል 2; የሙያ መረጃ

4	በቅጥር ስንት አመት ወይም ወራት አሳልፈዋል?	
5	በሥራ ቦታ ትመገባለህ /ሺ ወይም ትጠጥለህ	1 አዎ

	/ሺ?	2. አይ
6	በቀን ስንት ሰዓት ይሰራሉ?	
7	የግል መከላከያ መሳሪያዎችን ትጠቀማለህ /ሺ?	1 የፊት mask 2 ዳንት 3 Safety vast 4 ሁሉም 5. ምንም አልጠቀምም::

ክፍል 3; የአኗኗር ዘይቤ እና ልምዶች

8	ታጨሳለህ /ሺ?	1 አዎ 2. አይ
9	አልኮል ትጠጣለህ /ሺ?	1አዎ 2. አይ
10	አሁን ያለዎትን አመጋገብ እንዴት ይገመግማሉ?	1. ጤናማ 2. በመጠኑ ጤናማ 3. ጤናማ ያልሆነ

ክፍል 4; ክሊኒካዊ መረጃ

11	ላለፈው አመት ደምህን ተመርምረህ ታውቃለህ /ሺ?	1 አዎ 2. አይ
12	አዎ ከሆነ በደምዎ	1አዎ

	ውስጥ ማናቸውም ያልተለመዱ ነገሮች ተገኝተዋል?	2አይ 3እርግጠኛ አይደሉም
13	መድሃኒት ትወስዳለህ /ሺ?	1 አዎ 2. አይ
14	አዎ ከሆነ፣ እባክዎን መድሃኒቱን ይግለጹ?	
15	የዓይን ሕመም ፣ ማዘር፣ ወቅታዊ ራስ ምታት፣ ተደጋጋሚ ማስነጠስ (አለርጂክ)፣ የትንፋሽ ማጠር(አስም)፣ ምልክቶች አለብህ /ሺ ?	አዎ ከሆነ የትኛው፣ እዚህ ይፃፉ _____

ክፍል 5; የሥራ አካባቢ ግንዛቤ

16	ነዳጅ በደምዎ ያለው ተጽእኖ ያውቃሉ?	1 አዎ 2. አይ
17	አዎ ከሆነ፣ እባክዎን ይግለጹ	

ክፍል 6; የምግብ እና መጠጦችን አጠቃቀም በተመለከተ መረጃ ለመሰብሰብ የሚረጋገጩ ዝርዝር

ተ.ቁ	ጥያቄዎች	መልሶች
18	መረጃ በሚሰጠውበት ጊዜ ሰራተኞች በነዳጅ	

	<p>ማደያ ውስጥ ምግብ ወይም መጠጥ እየበሉ ነው?</p>	
19	<p>ምግብ ወይም መጠጥ በነዳጅ ማደያ ውስጥ በግልጽ ተቀምጧል?</p>	
20	<p>በነዳጅ ማደያ ውስጥ ምግብ፣ ሳህኖች እና መጠጦች ጥቅም ላይ እንደሚውሉ የሚያሳይ ማስረጃ አለ?</p>	

አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ
የሕክምና ላቦራቶሪ ሳይንስ ት/ት ክፍል

መጠይቆች (control group) በአዲስ አበባ ከተማ በጋዝ ማሰራጨ ሰራተኞች ላይ የደም ህክምና ጤና ግምገማ ላይ ማጥናት (evaluation of Hematological parameters among Gas Station Workers at Addis Ababa city) ማጥናት ነው።

ሰሽዎ-ዲሞክራሲክ መረጃ መሰብሰቢያ መጠይቅ፤ ከጥያቄዉ ፊትለፊት የተሰመረ ቦታ ከገኙ አስፍላጊዉን መልስዉን በጽሁፍ ያስፍሩ ፤ የመልስ አማራጮች በቁጥር መልክ ከተዘረዘሩ ግን የርስዎን መልስ የያዘውን ቁጥር ይከበቡ።

የመጠይቁ መለያ ቁጥር _____ ፣ ቀን: ____/____/____

ክፍል 1: የተሳታፊዎች ሰሽዎ-ዲሞክራሲክ መረጃ

ተ.ቁ	ጥያቄዎች	መልሶች
1	ዕድሜ	
2	ጾታ	1 ወንድ 2.ሴት
3	የትምህርት ደረጃ	1. መደበኛ ትምህርት የለም 2. የመጀመሪያ ደረጃ ትምህርት 3. ሁለተኛ ደረጃ ትምህርት 4. ከሁለተኛ ደረጃ ትምህርት በላይ

ክፍል 2; የሙያ መረጃ

4	በቀን ስንት ሰዓት ይሰራሉ?	
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ክፍል 3; የአኗኗር ዘይቤ እና ልምዶች

5	ታጨሳለህ /ሺ?	1 አዎ 2. አይ
6	አልኮል ትጠጣለህ /ሺ?	1አዎ 2. አይ
7	አሁን ያለዎትን አመጋገብ እንዴት ይገመግማሉ?	1. ጤናማ 2. በመጠኑ ጤናማ 3. ጤናማ ያልሆነ

ክፍል 4; ሂማቶሎጂካል ጤና

8	ላለፈው አመት ደምህን ተመርምረህ ታውቃለህ /ሺ?	1 አዎ 2. አይ
9	አዎ ከሆነ በደምዎ ውስጥ ማናቸውም ያልተለመዱ ነገሮች ተገኝተዋል /ሺ?	1አዎ 2አይ 3እርግጠኛ አይደሉም
10	መድሃኒት ትወስዳለህ /ሺ?	1 አዎ 2. አይ
11	አዎ ከሆነ፣ እባክዎን መድሃኒቱን ይግለጹ	

ክፍል 5; የሥራ አካባቢ ግንዛቤ

12	ስራዎ በደምዎ ተጽእኖ	1 አዎ
----	---------------	------

	ይኖረዋል ብለው ያስባሉ?	2. አይ
13	አዎ ከሆነ፣ እባክዎን ይግለጹ	

Annex IV: Standard operating procedure for blood collection

Venous blood sample collection

Equipment and reagents

Tourniquet

Gloves

Alcohol wipes

Sterile disposable needles

Collection procedure

Material needed for the procedure should be assembled.

Visual inspection of both arms is performed by the phlebotomist.

Puncture sites that are free from bruises, abrasions and sites of infection should be chosen as sites of sample collection.

Once an appropriate site has been chosen a tourniquet should be applied just above the area to be punctured.

The chosen area should be cleaned inside out using alcohol wipes.

A needle with its bevel faced upward should be inserted gently into the selected site and the tourniquet will be removed as soon as blood begins flowing into the syringes.

Once the desired volume is taken, the needle and syringe should be removed from the patient's arm and patients will be informed to hold the puncture area with a cotton ball for a min to stop bleeding.

Blood withdrawn should be dispensed into a labeled tube containing EDTA.

The tube then inverted 8 times to ensure proper mixing of sample to anticoagulant present in the tube

Annex V; standard operating procedure for UniCel® DxH 800 Beckman

Principle and procedure on UniCel® DxH 800 Beckman- Coulter automated hematology Analyzer

Purpose: automated hematology analyzer for in vitro diagnostic use in screening blood in clinical laboratory.

The UniCel® DxH 800 Analyzer provides a:

Complete Blood Count (CBC),

Leukocyte 5 Part Differential (Diff),

Scope: This is applicable in all examination procedures applied using the UniCel® DxH 800 instrument in Hematology laboratory(51).

Principle

Coulter Method (impedance)

Accurately count and size cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

VCS Technology

The COULTER VCS established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity, and laser-light scatter. The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the SPM into data plots.

Volume Analysis

Electronic Leukocyte Volume Analysis using low-frequency current has been used since 1967. It has been evaluated as a possible adjunct to the differential white cell count

Conductivity Analysis

Cell walls act as conductors to high-frequency current. The current, while passing through the cell walls and each cell interior, detects differences in the insulating properties of the cell components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.

Light Scatter Analysis

Coulter's experience in flow cytometer dates back decades to Fulwyler's pioneering use of light scatter for cell analysis. Loken et al. and Jovin et al. discuss the relationship of particle size and refractivity to the angle of light scattered from a laser beam.

Clinical utility:

A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. A CBC helps to check any symptoms, such as weakness, fatigue, or bruising. It also helps to diagnose conditions, such as anemia, infection, and many other disorders. In general, the complete blood count can be done as part of routine health examination and general screening.

Reagents

COULTER® DxH Diluent

COULTER DxH Diluent is a cyanide-free, isotonic buffered saline solution. COULTER DxH Diluent dilutes the specimen, is used for rinsing SPM components between sample analyses, and provides a sheath stream to transport the sample through the flow cell.

COULTER® DxH Cell Lyse

COULTER DxH Cell Lyse is a cyanide-free CBC lytic reagent that lyses red blood cells for the white blood cell count, and works in conjunction with COULTER DxH Diluent to generate a stable hemoglobin measurement. Also used to lyse the red blood cells and 38 discriminates nucleated red blood cells from white blood cells.

COULTER® DxH Diff Pack

The COULTER DxH Diff Pack consists of the Erythrolyse™ Lytic Reagent and StabiLyse™ Preservative reagent.

The Erythrolyse Lytic Reagent is a cyanide-free lytic reagent that dilutes the blood sample, and lyses red blood cells in preparation for white blood cell measurement in the flow cell. The StabiLyse Preservative Reagent neutralizes the Diff lytic reagent and preserves the white blood cells for measurement in the flow cell. Together, Erythrolyse and StabiLyse provide the five-part differential

COULTER® DxH Retic Pack

The DxH Retic Pack consists of a reticulocyte stain reagent and a reticulocyte-clearing reagent. The reticulocyte stain reagent is a cyanide-free reagent that uses a dye to stain reticulocytes. The reticulocyte-clearing reagent is a cyanide-free reagent that stabilizes the dye-reticulum complex to enhance discrimination of reticulocytes from mature red blood cells utilizing the VCS technology.

COULTER® DxH Cleaner

DxH Cleaner is a cyanide-free, aldehyde-free cleaning agent that degrades residual materials so that they may be flushed from the system with the diluent.

Reagent stability and storage:

Stable up to expiry date and, up to 60 days after opening and store at room temperature except for cleaner it's stable up to 90 days after opening.

Equipment:

UniCel® DxH 800 hematologyAnalyzer

Printer

Test tube cassette

LIS computer

Barcode reader

Sample and container type

Collect whole blood in EDTA according to tube manufacturer's instructions and procedures in:

CLSI publication H4-A5 (for capillary)

CLSI publication H3-A6 (for venipuncture) Beckman Coulter recommends using K2 or K3 EDTA

Sample collection materials

Vacutainer needle,

Glove

Vacutainer test tube

Alcohol

Cotton

Safety precautions

Read all product safety data sheets and don't attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and 40 manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter representative.

Controls

The COULTER 6C Cell Control is an integrated control (3 level), that enables monitoring of system Performance and calibration status for all directly measured and calculated CBC and Diff parameters

Background - Daily Checks

Parameter	Limit
WBC	$\leq 0.05 \times 10^3/\mu\text{L}$
RBC	$\leq 0.005 \times 10^6/\mu\text{L}$
HGB	$\leq 0.1 \text{ g/dL}$
PLT	$\leq 3 \times 10^3/\mu\text{L}$
Diff	$\leq 100 \text{ events}$

Result interpretation

A low hemoglobin level indicates anemia. However, hemoglobin findings are even more dependent upon the total number of RBCs. In other words, for the diagnosis of anemia, the number of RBCs is as important as the hemoglobin level. In response to an acute infection, trauma, or inflammation, white blood cells release a substance called colony-stimulating factor (CSF). CSF stimulates the bone marrow to increase white blood cell production.

Annex VI ; standard operating procedure for wright stain

Blood smear preparation and staining

Smear preparation

Slides should be labeled.

A small drop of blood should be applied 1 cm above the end of the glass slide using a blood dropping device.

The drop of blood on the slide should be approached by moving from the back until it will touch the applied sample. Then the blood will be spread along the slide of the spreader.

The spreader should be held at an angle of 45 and will be spread covering one tie of the sample slide.

Allow the slide air dry.

Staining procedure

Place the slide on a staining rack

Cover the smear with a diluted wright staining solution for 1-2 min

Add equal amount of buffered water(PH 6.5) and allowed for 3 min

Wash the slide with tap water.

Bruise the back of the slide with gauze to remove dirt and remnants of staining solutions.

Allow slide air to dry.

Microscopic examination will be performed by principal investigator and assigned hematologist in EPHI

Declaration

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

Abigail Mesfin

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye(MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mikias Negash (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.