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ORIGINAL RESEARCH

Hematological Parameters of Gasoline Station Workers at Hosanna Town, Southwest Ethiopia: A Comparative Cross-Sectional Study

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Background: Human exposure to benzene is associated with many adverse health effects. It is mainly related to impairment of the hematopoietic system and bone marrow suppression, causing abnormalities in hematological parameters. However, the reports obtained from different studies are contradictory, and there are little data regarding the hematological parameters of gas station workers in the study area. Therefore, this study aimed to evaluate the hematological parameters of gas station workers in Hosanna town, southwest Ethiopia, from May 01 to June 15, 2020.

Methods: A comparative cross-sectional study was conducted by involving 180 (60 gas-stations workers and 120 controls) participants. Socio-demographic and related data of the study participants were collected using a pre-tested structured questionnaire through face-to-face-interviews. All phases of quality assurance were maintained, and hematological parameters were determined using Uni-Cel DxH 800 automated hematological analyzer. Independent sample *T*-test, Mann–Whitney *U*-test, and one-way ANOVA were used for data analysis. Statistical significance was declared at P<0.05.

Results: Statistically significant difference was observed in hematological parameters of gasoline-workers and control groups. The mean of red blood cell count among gasoline-workers was significantly reduced as compared to control groups (p=0.007). In addition, the median of hemoglobin levels among gasoline-workers was significantly decreased as compared to the control groups (p=0.001). In contrast, a significant increase was observed in median of absolute eosinophil count among the gasoline-workers as compared to control groups (p=0.01). The mean of mean cell volume was significantly decreased with respect to the duration of work experience (p=0.04).

Conclusion: In this study, a statistically significant difference was observed in some hematological parameters of gas station workers compared to the control group. Therefore, medical observation and periodic medical check-ups of the hematological profile should be considered to prevent the development of medical complications.

Keywords: benzene, Ethiopia, gasoline workers, hematological parameters, Hossana

Introduction

Hematopoiesis refers to the commitment and differentiation processes that lead to the formation of blood cells from multipotent hematopoietic stem cells (HSCs) in the bone marrow. Extramedullary hematopoiesis can also occur in other tissues such as the liver and spleen. The ability to differentiate into all hematopoietic lineages and maintain their self-renewal capacity is a key characteristic of stem cells.[1,2] Various factors can affect the formation of blood cells in target tissues. These factors encompass metabolic abnormalities, infections, inflammation, as well as exposure to gasoline.[3–5] Under stressful conditions, such as exposure to gasoline, hematological parameters are likely to undergo rapid and easily detectable variations, making them valuable for assessing one's health status.[6]

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Human exposure to benzene has been associated with a variety of adverse health effects, primarily related to impairment of the hematopoietic system with bone marrow failure, including pancytopenia and aplastic anemia, increased risk of developing acute myeloid leukemia and morphological changes in blood cells.[7] Benzene is an aromatic hydrocarbon and a natural component of crude oil and natural gas. It is a colorless, highly flammable liquid with a sweet odor. Systemic exposure to benzene may cause acute and chronic clinical disorders associated with a risk of hematologic abnormalities, including leukemia, lymphoma, and chromosomal aberrations.[8] Several mechanisms are involved during benzene-induced toxicity including oxidative stress, DNA damage, cell cycle disturbance, and apoptosis.[9]

The production of benzene metabolites takes place mainly in the liver, where they are then transported to the bone marrow. Benzene is metabolized to phenol, its main metabolite, by the liver enzyme, cytochrome P4502E1 (CYP2E1), via benzene oxide. It is then metabolized by CYP2E1 to hydroquinone (HQ). Hydroquinone is transported to the bone marrow and oxidized to a number of metabolites that can accumulate in the bone marrow, which are then bioactivated by myeloperoxidase and other heme protein peroxidases to form semiquinone, quinine, and benzochinone. This causes formation of reactive oxygen species (ROS). These reactive oxygen species can affect signaling cascades by altering the activity of certain protein kinases and transcription factors.[10] Finally, adverse effect of benzene is associated with bone marrow failure and hematological malignancies.[11]

As reported by the World Health Organization (WHO) in 2010, exposure to benzene has been related with a wide range of short-term and long-term health disorders.[12] Long-term benzene exposure is associated with suppression of bone marrow function, leading to a reduction in the number of red blood cells, white blood cells and its harmful impacts depends on the amount, route and duration of exposure, as well as the age and pre-existing medical condition of the exposed person.[13]

Evidence has shown that exposure to gasoline results in several health effects in humans, including decline in all blood cell formation, and increased risk of developing malignancies.[14] Studies on the blood cell parameters of gasoline station workers in countries have yielded contradictory results. Some studies have shown that there are no statistically significant differences between hematological parameters of people exposed to gasoline and the unexposed group in terms of red blood cell count, hemoglobin (Hgb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell count (WBC) and differential white blood cell count, and platelet count (PLT).[15] However, another study showed that RBC counts and Hct values were significantly higher in people exposed to gasoline than in the unexposed group.[16,17] Other studies have reported that the WBC count, RBC count, PLT counts, Hgb, Hct, and MCH values were significantly lower in the exposed group than in the unexposed groups.[18–20] Moreover, information on Ethiopia, particularly in the study area is limited. Results obtained from hematologic analyzers can provide change in hematological parameters such as red blood cell indices, WBC count and its differential, Hgb, Hct, PLT, mean platelet volume (MPV), and other parameters.[15,21] Studying these parameters helps study participants to understand the risk of occupational exposure on their hematological parameters and to take appropriate safety measures. Therefore, this study aimed to assess the hematological parameters of gasoline-exposed individuals in comparison with unexposed controls in Hossana Town, Southwest Ethiopia.

Methods and Materials

Study Design, Period and Area

A comparative cross-sectional study was conducted from May 01 to June 15, 2020, in Hossana Town, Southwest Ethiopia. Hossana town is the administrative center of the Hadiya zone in the south Ethiopia region, located 232 km away from the capital city of Ethiopia, Addis Ababa, and 157 km from the regional city of Hawassa, with a total population of 75,963. It has a latitude and longitude of 7°33′N 37°51′E with an elevation of 2177 meters above sea level. Five gas stations were located in the town of Hosanna, and 65 gasoline station workers were employed during the study period.

Study Participants

All adult workers at the five gas stations in Hossana town were recruited in the study. In addition, study participants working at Wachemo University Nigist Eleni Mohammed Memorial Teaching and Comprehensive Specialized Hospital (WUNEMMCSH) were included as controls. Study participants who had chronic diseases (cardiovascular, hematological malignancies, liver, and renal disease), participants who were on medication affecting blood cell count (Erythropoietin

therapy, hematin factors), participants with a history of blood transfusion in the last 3 months, pregnant women, and participants working in gasoline station for less than six months were not recruited in this study.

Sample Size Determination and Sampling Technique

One hundred and eighty study participants, comprising 60 gas station workers in the exposed group and 120 WUNEMMRTH staff members in the control group, were included. A total of 65 gasoline station workers worked in five substations in Hosanna town. Of the 65 gasoline station workers, 60 provided blood samples for analysis. Two gas station workers were not volunteers to participate, two were left out due to cardiovascular disease, and one pregnant mother. A convenience sampling technique was used to recruit staff members to the control groups.

Data Collection and Laboratory Methods

Socio-demographic and related data were collected using a pretested structured questionnaire via face-to-face interviews. After the interview, 4mL of venous blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes by laboratory professional. Complete blood counts (CBC) were performed using UniCel DxH 800 (BECKMAN COULTER, UniCel® DxH 800, USA) automated hematology analyzer. A peripheral blood film was prepared to investigate abnormalities in red blood cell, white blood cell, and platelet morphology of gasoline station workers. All laboratory tests were conducted at hematology laboratory of WUNEMMRH located in Hosanna Town.

Data Quality Assurance and Management

All quality assurance phases were considered to ensure data quality. The English version of the questionnaire was translated into the local language (Hadiyisa), translated back into English for accuracy and consistency. The questionnaire was pretested and data collectors were trained before actual data collection began. All blood samples were analyzed within two hours of collection. Manufacturer's instructions and standard operating procedures were strictly followed during sampling and experimental procedures. For automated hematology analyzer, background checks, repeated analysis of randomly selected samples to see reproducibility; randomly selected samples were verified using other similar hematology analyzer, and as part of the testing process, hospital laboratories evaluated the performance of the devices using the whole blood quality control.

Statistical Analysis and Interpretation

Data were cleaned and checked for its consistency and completeness. Then, the data entered into Epi-data version 3.1 (Epi-Data, Odense, Denmark) and exported into Statistical Package for Social Science (SPSS) version 25 (SPSS, Chicago, USA) statistical software for Windows. All continuous data were tested for normality by histogram and Shapiro–Wilk test. Hematological parameters were compared between gasoline-exposed workers and unexposed group using an independent-samples *t*-test for normally distributed data and the Mann–Whitney *U*-test for non-normally distributed data. One-way ANOVA was used for the comparison of hematological parameters according to duration of exposure. The results were summarized as mean ± standard deviation (SD), median, and interquartile range (IQR). P-value <0.05 was set as statistically significant for all analysis. The Hgb concentration was adjusted for altitude according to WHO standard guidelines to define anemia.

Ethical Consideration

This study was carried out according to the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) of Jimma University, Institute of Health with protocol number of IRB000130/2020. Written permission was obtained from the director of WUNEMMRTH and Gasoline Station Owners. Each participant gave their written informed consent after they are fully informed about the study's objectives, methods, and purpose of the study. Participants in the study were identified by codes rather than by individual identifiers to ensure confidentiality. Any abnormal results were communicated to healthcare provider to manage them appropriately.

Results

Socio-Demographic Characteristics of Study Participants

One hundred eighty 180 (60 gasoline station workers and 120 control group) study participants were included in this study. Gasoline station workers and control groups showed significant differences in terms of sex and educational status ($p<0.05$). The mean age was 30±5.23 for gasoline station workers and 31±5.49 years for control group. Out of the total participants, 4 (6.70%) and 116 (96.70%) had a higher educational level for gasoline station workers and controls, respectively. Thirty-eight (63.30%) and 54 (45.00%) participants were single for gasoline station workers and controls, respectively (Table 1).

Comparison of Hematological Parameters Among the Study Participants

Statistically significant differences were observed in the median (IQR) of Hgb (P=0.001) and mean ± SD of RBC count (P=0.007) between gasoline station workers and control group. The median absolute eosinophil count (IQR) was significantly higher in gas station employees than in control groups (P=0.01). Levels of other hematological parameters, such as MCHC, RDW, and absolute monocyte count, were not significantly different between groups (Table 2).

Comparison of Hematological Parameters Based on Duration of Exposure

The mean of MCV was significantly decreased among participants with longer duration of work experience as compared to their counter parts (P=0.04). Other parameters varied non-significantly with years of working experience in gasoline station workers (Table 3).

Magnitude of Hematological Abnormality Among Gasoline Station Workers

The magnitudes of leukopenia, anemia, and thrombocytopenia among the gasoline-exposed workers were 4 (6.60%), 7 (11.70%), and 6(10.00%), respectively. On the other hand, among those gasoline station workers, 5 (8.30%) had leukocytosis. Thrombocytosis was not observed in gasoline station workers. In addition, all gasoline station workers were examined for peripheral morphology. Of the examined peripheral blood films, 49 (81.70%) of the result were normocytic normochromic RBCs, 7 (11.70%) had macrocytic oval shaped RBCs with increasing corresponding MCV values, and 4 (6.6%) study participant had microcytic RBCs with reduced MCV values (66 fL).

Table 1 Socio-Demographic Characteristics of the Study Group at Hossana Town, Southwest Ethiopia, from May 01 to June 15, 2020 (n=180)

Variables	Categories	Gasoline Station Workers (n=60)	Control Group (n=120)	X^2-test
Gender	Male n (%)	56 (93.30%)	92 (76.7%)	P=0.006*
	Female n (%)	4 (6.70%)	28 (23.3%)	
Age in years	20–24	15 (25.00%)	30 (25%)	P=0.700
	25–29	33 (55.00%)	58 (48.3%)	
	30–39	11 (18.30%)	30 (25%)	
	≥40	1 (1.70%)	2 (1.7%)	
Marital status	Single	38 (63.30%)	54 (45%)	P=0.500
	Married	20 (23.30%)	62 (51.7%)	
	Divorced	1 (1.70%)	1 (0.8%)	
	Widowed	1 (1.70%)	3 (2.5%)	
Educational status	No formal education	13 (21.70%)	–	P=0.01*
	Read and write	13 (21.70%)	–	
	Primary	22 (36.70%)	3 (2.5%)	
	Secondary	8 (13.30%)	1 (0.8%)	
	College/University	4 (6.70%)	116 (96.7%)	

Note: *P-value <0.05 is considered as statistically significant.

Table 2 Hematological Parameters of the Study Participants at Hossana Town, Southwest Ethiopia, from May 01 to June 15, 2020 (n=180)

Parameters	Gasoline Station Workers (n=60)	Control Group (n=120)	P-value
WBC ($\times10^3$/μL)$^{\alpha}$	7.31 ±2.39	7.00 ±1.98	0.39
RBC ($\times10^6$/μL)$^{\alpha}$	4.87 ±0.62	5.08 ±0.41	0.007*
Hgb (g/dl)	14.38 (1.70)	15.40 (1.30)	0.001*
HCT (%)	45.70 (5.80)	47.70 (6.50)	0.04*
MCV (fl)$^{\alpha}$	89.08 ±4.23	89.30 ±3.59	0.74
MCH (pg)	29.20 (2.07)	29.30 (1.60)	0.192
MCHC (g/dl)	32.90 (1.17)	32.90 (1.00)	0.82
RDW (%)$^{\alpha}$	13.80 ±0.65	13.80 ±0.70	0.62
PLT ($\times10^3$/μL)$^{\alpha}$	264.67 ±73.19	272.61 ±62.00	0.44
MPV (fl)$^{\alpha}$	7.903 ±0.75	7.83 ±0.60	0.78
Neutrophil absolute	4.20 (3.85)	4.30 (2.50)	0.58
Lymphocyte absolute	1.85 (1.17)	1.70 (1.00)	0.57
Monocyte absolute	0.50 (0.20)	0.50 (0.20)	0.16
Eosinophil absolute	0.57 (0.30)	0.30 (0.20)	0.01*
Basophil absolute	0.00 (0.10)	0.00 (0.10)	0.79
Neutrophil relative	58.40 (23.60)	63.60 (19.00)	0.66
Lymphocyte relative$^{\alpha}$	24.36 ±9.38	27.00 ±7.70	0.04*
Monocyte relative	6.60 (2.95)	6.65 (3.00)	0.61
Eosinophil relative	6.92 (3.40)	3.70 (2.45)	0.09
Basophil relative	0.57 (0.50)	0.60 ±0.50	0.17

Notes: $^{\alpha}$P-value derived from independent sample *T*-test, *p-value <0.05 is considered as statistically significant.
Abbreviations: WBC, White Blood Cell; RBC, Red Blood Cell; Hgb, Hemoglobin; HCT, Hematocrit; MCV, Mean Cell Volume; MCH, Mean Cell Hemoglobin; MCHC, Mean Cell Hemoglobin Concentration; RDW, Red Cell Distribution Width; MPV, Mean Platelet Volume.

Table 3 Comparison of Hematological Parameters Based on Duration of Exposure Among Gasoline Station Workers at Hosanna Town, Southwest Ethiopia, from May 01 to June 15, 2020

Parameters	Duration of Exposure (in Year)			P-value
	1	2–5	>5	
WBC ($\times10^3$/μL) (Mean ±SD)	7.16±2.32	7.5±2.31	7.2±2.60	0.80
RBC ($\times10^6$/μL) (Mean ±SD)	5.09±0.65	4.8±0.50	4.75±0.66	0.20
MCV (fl) (Mean ±SD)	90±2.94	89.4±4.60	87.9±3.00	0.04*
RDW (%) (Mean ±SD)	13.67±0.65	13.83±0.50	13.88±0.78	0.58
LYM (%) (Mean ±SD)	26.23±9.90	27.65±8.20	21.46±9.60	0.31
PLT ($\times10^3$/μL) (Mean ±SD)	266.5±51	279.6±79	253±88.00	0.50
MPV (fL) (Mean ±SD)	8.1±0.78	7.79±0.69	7.8±0.79	0.37

Notes: *P-value <0.05 is considered as statistically significant.
Abbreviations: WBC, White Blood Cell; LYM%, Relative Lymphocyte; RBC, Red Blood Cell; MCV, Mean Cell Volume; RDW, Red Cell Distribution Width; MPV, Mean Platelet Volume; SD, standard deviation.

Discussion

Human exposure to benzene is associated with many adverse health effects, primarily related to the impairment of the hematopoietic system and bone marrow suppression. The present study showed that the levels of red blood cells, Hgb and Hct in gas station workers were significantly reduced compared to those in the control group. These are consistent with the results of other studies.[18,22,23] The decrease in Hgb and red blood cell count may be explained by the effect of metabolic end products and free radicals which shortened red blood cell lifespan. These free radicals can also alter

erythrocyte membrane and heme protein synthesis in bone marrow.[18,22,24] Moreover, the decline in these parameters might be due to the hematotoxic effect of benzene, which causes impairment of the hemopoietic system with bone marrow suppression leading to pancytopenia.[25]

The findings of the current study are in contrast to those of a study done in Iraq on 38 subjects who were exposed to benzene and control group. Between the benzene-exposed workers (filling workers) and the control group, there were no significant differences in the mean values of red blood cell count, Hgb, Hct, MCV, MCH, MCHC, RDW, white blood cell count, differential white blood cell count, or PLT count.[15] This might be due to differences in the duration of exposure and daily duty hours of the participants, as well as differences in the sample size of the study. The red cell indices of the present study are in line with those of a study conducted in Surat City on 30 gasoline station worker with ≥1 year of in duration of exposure, and 30 controls showed that there were no significant differences in red blood cell indices such as MCV and MCH between the exposed workers and control groups, which is similar to the present study.[26] Contrary to the study done by Pune, India, MCV and MCH were significantly higher in the exposed workers than in the non-exposed control group.[22] This might be due to differences in the length of exposure and the small size of previous studies.

Similar to our findings, the study in Bhubaneswar found no statistically significant difference in platelet count between the gasoline exposed and control group.[27] In contrast to current findings, UK study from Texas City found that participants exposed to benzene had a non-significantly higher platelet count.[16,20] According to the current study, gasoline station worker had a higher WBC count than the control groups, but it is not statistically significant. This outcome was in line with one from a study done in Nigeria.[28] Various studies could provide an explanation for this: high infection rates in groups that have been exposed because of the toxic gasoline products' immune-suppressing effects, which then cause WBC counts to rise.[29] On the other hand, a study conducted in India showed a decrease in WBC count between gasoline station workers and control groups.[30] This might be due to differences in the length of exposure time of the study participants.

In this study, MCV was significantly decreased with respect to the duration of exposure, whereas other parameters such as platelet count, relative lymphocyte count, and RDW varied insignificantly with respect to the duration of working experience in gasoline station workers. This finding is in agreement with a study conducted in the Dehradun region, where petrol pump workers with longer exposure times experienced more adverse effects on hematological parameters.[17]

The results of present study displayed the prevalence of hematological abnormalities, such as leukopenia (6.6%), anemia (11.7%), and thrombocytopenia (10%), among gasoline station workers. The hematopoietic system is extremely sensitive to the most volatile organic solvents that reach the bloodstream. These solvents may interfere with the process of RBC proliferation, causing adverse effects on heme synthesis and life spans of RBCs.[31] Another reason is that the toxic effects of prolonged exposure to benzene metabolites might cause suppression of the bone marrow, resulting in changes in blood parameters, which could cause anemia, leukopenia, or thrombocytopenia.[22] The prevalence of anemia in this study among those gasoline station workers was 11.7%, which is in line with EDHS report, of which 11% of men aged between 15–49 were anemic,[32] and lower than the EDHS report of 2016, 15% of men aged between 15 and 49 were anemic.[32]

Regarding the peripheral blood film, 6.6% microcytic red cells with reduced MCV values gasoline station workers, which is inconsistent with study conducted in Sudan[8] from 2014 to 2015 reported regarding red blood cell morphology 50% of the gasoline station workers showed a microcytic picture. This inconsistency might be differences in the duration of exposure and sample size of the study. The current study revealed that macrocytosis of red cell morphology occurred in 11.7% of cases, which is consistent with research from Nigeria and Iran.[33,34] This macrocytosis might be due to the toxic component of gasoline, which can affect erythropoiesis, alter the RBC membrane, make it more susceptible to hemolysis, obstruct replication, and cause macrocytic anemia.[22]

Conclusion and Recommendation

This study showed that some hematological parameters are changed in gasoline station workers as compared to the control groups. These hematological alterations include decreased RBC, Hgb, and Hct counts and increased absolute eosinophil counts in gasoline station workers than control group. This study therefore came to the conclusion that there might be link between exposure to gasoline and hematological changes. Medical observations, including prior to

employment and periodic medical check-ups of the hematological profile before the development of chronic impairment should be considered. Further long-term prospective study with an increased sample size of gasoline workers is needed to obtain a more complete picture of the long-term effects and define effects of a single chemical to which gasoline station workers are exposed.

Data Sharing Statement

This paper is based on the thesis of Tamirat Ersino. It has been published on Jimma university website, which is available https://repository.ju.edu.et/bitstream/handle/123456789/4204/TAMIRAT-ERSINO%20final%20thesis.pdf?isAllowed=y&sequence=1.[2] All relevant data related to this work are included within the manuscript.

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Author Contributions

All authors made a significant contribution to the work reported in the conception, study design, execution, acquisition, analysis, and interpretation of data; took part in drafting, revising, and critically reviewing the manuscript. All authors have agreed on approval of the final manuscript to be published in the current journal and to be accountable for all aspects of the work.

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Disclosure

The authors declare that there is no conflict of interest for this work.

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