

Alteration of Hematological and Cardio-Vascular Parameters Among Petrol Filling Workers of West Bengal, India

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ABSTRACT

This study aimed to investigate the effects of volatile organic compounds exposure on blood and cardiovascular parameters of Petrol station workers of West Bengal. The study included 153 male Petrol station workers and 50 male control group individuals of similar socio economic status having age range 20-60 years. Different hematological parameters and cardiovascular parameters (blood pressure and heart rate) has been evaluated. Blood samples were collected and analyzed by fully automated hematology analyzer. The analysis of the results showed significant hematological changes in exposed workers and 21% workers were anaemic. There is a significant decline in hemoglobin and RBC count but significantly higher neutrophil, ESR and eosinophil count were observed compared to the control group. Besides, significantly higher heart rate, systolic and diastolic blood pressure was found in exposed group indicating cardiovascular risk, reflected as hypertension (41.17%). Again, hemoglobin, Red Blood Cell, White Blood Cell and platelet count declined insignificantly and eosinophil count increased significantly with increase in exposure duration. ESR and Neutrophil-Lymphocyte ratio (NLR) values increased maximum above 20 years and between 11-20 years of exposure respectively. This high NLR value is associated with anaemia and hypertension among petrol filling workers. Thus, NLR value can be used in routine clinical assessment of anaemia and hypertension. Thus, health of workers of fuel station should be protected from exposure to benzene and other toxic substances by introduction of petrol vapour recovery system and minimizing leakage and spillage, by wearing protective gadgets and by giving health education and awareness to these workers.

Keywords: Petrol filling workers, hematological parameters, Cardiovascular parameters, BTEX, Neutrophil –Lymphocyte ratio (NLR).

INTRODUCTION

Benzene is an aromatic hydrocarbon and a natural component of crude oil and natural gas. It is toxic to the blood and blood-forming organs. Chronic hematotoxic effects of benzene exposure including reduced lymphocyte, neutrophil and platelet count in peripheral blood have been detected [1]. The previous studies were carried out on composite fumes evaporating from

kerosene, petrol and diesel mainly on experimental animals. Benzene, lead and volatile nitrates have been shown to produce harmful effects on the bone marrow, spleen and lymph nodes [2]. Chronic and long period of exposure to benzene was found to be associated with progressive degeneration of bone marrow [3]. Elderderly et al [4] showed a significant decrease in hemoglobin and RBC levels among Petrol Filling workers (PFW). There is a significant harmful health effect on human exposure to benzene and might be associated with a risk of blood abnormalities including aplastic anemia, leukemia, lymphoma, pancytopenia and chromosomal aberration. But exact pathophysiology of hematological effects following exposure to benzene is still unclear.

Clinical laboratory investigation on effects of benzene exposure on blood level might help in diagnosis of hematological disorders like anaemia. Complete blood count (CBC) is known to be an easy, quick method of screening hematotoxicity of benzene [4]. Benzene is metabolized in liver and bone marrow, hence these are major site of benzene toxicity.

A major effect of long-term benzene exposure is on the blood. Besides causing anaemia, it can also lead to excessive bleeding and increase the chance of infection. Benzene has a role in causing human leukemia [5]. Benzene exposure is also linked to the development of multiple hematological malignancies [6]. Along with benzene exposure, petrol filling workers are also exposed to air pollutant due to vehicular exhausts. Air pollutants exposure also induces cardiovascular changes and elevated blood parameters is an established risk factor for coronary heart disease.

Besides, neutrophil to lymphocyte ratio (NLR) has now adays considered as a novel inflammatory and oxidative stress marker. The NLR comprises two immune pathways that are related to the oxidative response and angiotensin II mediated hypertensive response [7,8]. NLR has been applied in predicting the prognosis of malignancies, mortality and chronic diseases.

Therefore, the aim of the present study is to

1. Assess the effects of occupational exposure to benzene and air pollutants on hematopoietic system of petrol filling workers.
2. Assess the effects of work place environmental effluents on Pulse rate and Blood Pressure among PFW.
3. Investigate the correlation between Neutrophil -Lymphocyte ratio (NLR) and prevalence of hypertension and anaemia among petrol filling workers of West Bengal.

METHODS AND MATERIAL

This cross-sectional study was carried on petrol filling workers of petrol stations located in various regions of the Howrah and Hooghly districts of West Bengal. The study population consisted of 153 male petrol filling workers and 50 male control individuals of same age and socioeconomic background. The study participants were selected through a simple random sampling method, which ensures a representative and unbiased sample.

Ethical Considerations

The study has been approved by the Institutional Ethical Committee of Raja Peary Mohan College under University of Calcutta. Prior to the commencement of the study, written consent was taken from both the petrol station owners and the workers. The data were only used for

research purposes and subject to Helsinki Declaration. Individual workers willing to participate in health checkup was assured confidentiality and anonymity of the data.

Inclusion Criteria

- Participants had to be male.
- Age ranges from 20-60 years.
- Participants must have worked for a minimum 8 hours per day over a period of one year.
- Participants must not have any prior history of cardiorespiratory and metabolic disorders.

Sample Size Determination and Sampling Technique

One hundred fifty-three petrol filling workers of 15 petrol stations selected at random mainly from Howrah and Hooghly district of West Bengal and 50 control group individuals were included from staff members of college, laboratory workers, shopkeepers and sedentary office workers. The representative sample size was calculated using PS Power and Sample Size Calculator Software (Version 3.1.6).

Study of Physical Parameters

Physical characteristics like age, height, weight and Body Mass Index were documented as standard health criteria. To keep accuracy on demographic data, Aadhar card, anthropometric rod and weighing machine were used to note age, height and weight respectively. BMI was calculated using the formula: body weight (in kg) divided by the square of body height (in meters)².

Data Collection and Laboratory Methods

Socio-demographic Data:

Socio-demographic related data were collected using a pretested questionnaire by interview method comprising of duration of service, smoking habit, education, monthly income, family member, no. of working days per week, use of protective equipment, any type of cardiac problem like chest pain, respiratory discomfort during walking and stair climbing etc.

Collection of Blood Samples:

A volume of 5 ml of venous blood was collected from median cubital vein of which 3.5 ml and 1.5 ml were immediately transferred to heparinized and EDTA vial respectively. The anticoagulant tube blood samples were gently but thoroughly mixed by inversion to ensure the blood did not clot and then stored in the refrigerator (at 4⁰ C) for a maximum period of 3 days, within which the samples were analyzed for the study of hematological parameters.

Study of Complete Hemogram:

Blood samples collected in heparinized vials were used to analyze complete hemogram by the help of hematology auto analyzer. The complete hemogram includes

- Hemoglobin concentration
- Total Count of RBC
- Total Count of WBC
- Total Count of Platelets

- Differential Count of Neutrophils
- Differential Count of Lymphocytes
- Differential Count of Monocytes
- Differential Count of Eosinophils
- Differential Count of Basophils
- Packed Cell Volume (PCV)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)
- Erythrocyte Sedimentation Rate (ESR).

The hemiglobincyanide method by Van Kampen and Zijlstra [9] was used to quantify hemoglobin concentration whereas method provided by Dacie & Lewis [10] was used for Total Count, Differential Count, cell volumes of blood cell and ESR. In addition, neutrophil to lymphocyte ratio (NLR) was calculated.

Statistical Analysis

Data were entered and exported into Statistical Package for Social Science (SPSS Version 25.0) for analysis. The results were summarized as mean \pm standard deviation (SD), P value < 0.05 was set as statistically significant for all analysis. Student t-test for normally distributed data and one way ANOVA was used for comparison between groups.

RESULTS

Table 1 represents demographic pattern of both the experimental group and the control group. 37.2% of petrol filling workers were in the lower age group (20-35 years) and 62.7% of workers were from a higher age group (36-60 years) in contrast to 56% of control group individuals were in a lower age group and 44% were in a higher age group. 54.9% PFW and 14% control group individuals were overweight but 5.8% PFW and 4% control group individuals were underweight. 55.5% of PFW had 1-10 years of working exposure, 18.3% had 11-20 years and 26.1% had >20 years of exposure. 58.8% of PFW and 72% of control group workers were smoker but 41.2% PFW and 28% of control group workers were nonsmokers.

Table 1: Demographic pattern of both study group and control group

Socio-demographic Characteristics		Petrol Filling Workers (n=153)		Control (n=50)	
		No. of participant	Percentage	No. of participants	Percentage
Age in Years	20- 35Years	57	37.2%	28	56 %
	36- 60Years	96	62.7%	22	44 %
BMI (kg/m ²)	Underweight	9	5.8%	2	4 %
	Normal	60	39.2%	41	82 %
	Overweight	84	54.9%	7	14 %
Type of work	Petrol fillers	153	100%	-	-
	Student	-	-	8	16 %
	Driver	-	-	3	6 %
	Lab Technician	-	-	7	14 %

	Office workers	-	-	23	46 %
	Shop keepers	-	-	9	18 %
Year of Exposure	1-10 Years	85	55.5%	-	-
	11-20 Years	28	18.3%	-	-
	>20 Years	40	26.1%	-	-
Smoking Habit	Smokers	90	58.8%	36	72 %
	Nonsmokers	63	41.2%	14	28 %

Table 2: Comparison of hematological parameters between PFW and control group

Parameters	PFW (n=153)	Control (n=50)	P - value
Hemoglobin (g/dl)	13.90 ± 1.37	15.50 ± 0.52	0.0001****
RBC (millions/cu.mm)	4.70 ± 0.43	5.07 ± 0.47	0.0001****
WBC (cu.mm)	8116.34 ± 1349.92	8462 ± 1138.18	0.1
Platelets (Lakhs/cu.mm)	2.56 ± 0.20	2.54 ± 0.26	0.5
Neutrophil (%)	60.63 ± 6.17	57.02 ± 4.71	0.0001****
Lymphocyte (%)	31.89 ± 6.27	32.32 ± 5.93	0.6
Monocyte (%)	2.52 ± 0.52	2.62 ± 0.49	0.2
Eosinophil (%)	5.14 ± 2.37	3.4 ± 0.85	0.0001****
PCV (%)	41.92 ± 4.08	41.86 ± 3.54	0.9
MCV (fl)	89.07 ± 1.04	89.47 ± 0.65	0.01*
MCH (pg)	29.48 ± 0.78	29.83 ± 0.55	0.004**
MCHC (gm/dl)	33.17 ± 0.25	33.24 ± 0.32	0.08
ESR (mm/hr)	14.65 ± 8.75	6.82 ± 2.36	0.0001****
NLR	2.01 ± 0.63	1.82 ± 0.38	0.04*

P<0.05-*, P<0.01-**, P<0.001-***, P<0.0001****

Table 2 represents a comparison of hematological parameters between the PFW and the control group. The percentage of hemoglobin and total RBC count are lower in PFW than control group. Neutrophil, ESR and eosinophil count is significantly (P<0.0001) higher in PFW than the control group. Neutrophil to lymphocyte ratio (NLR) which is considered as an inflammatory marker, can be utilized to examine various pathological conditions such as chronic kidney disease, metabolic syndrome, anaemia, cancer and infections. NLR value was significantly higher in exposed group workers compared to the unexposed control group.

Table 3: Comparison of cardiovascular parameters between PFW and control group

Parameters	PFW (n=153)	Control (n=50)	P - value
Heart Rate (bpm)	78.12 ± 10.03	74.64 ± 4.24	0.01*
SBP (Hg mm)	132.29 ± 17.46	125.44 ± 6.04	0.006**
DBP (Hg mm)	85.92 ± 12.07	81.08 ± 6.77	0.007**
Pulse Pressure (Hg mm)	46.50 ± 9.95	44.36 ± 5.85	0.1
Mean Arterial Pressure (Hg mm)	101.42 ± 13.18	95.88 ± 5.93	0.004**

P<0.05-*, P<0.01-**, P<0.001-***, P<0.0001****

Table 3 represents a comparison of cardiovascular parameters between the PFW and the control group. Heart rate, systolic and diastolic blood pressure and MAP (mean arterial pressure) were significantly (P< 0.01-0.007) higher in PFW than control group indicating cardiovascular stress among PFW.

Table 4: Comparison between hematological and cardiovascular parameters based on different duration of exposure

Parameters	Year of Exposure			F Value	P Value
	1-10 year (n=85)	11-20 years (n=28)	>20 years (n=40)		
Hemoglobin (g/dl)	14.09 ± 1.07	13.95 ± 1.35	13.47 ± 1.50	1.09	0.3
RBC (millions/cu.mm)	4.76 ± 0.41	4.72 ± 0.42	4.56 ± 0.47	0.69	0.8
WBC (cu.mm)	8043.52 ± 1468.51	8096.42 ± 1103.69	8285 ± 1254.23	1.071	.381
Platelets (Lakhs/cu.mm)	2.54 ± 0.21	2.55 ± 0.18	2.61 ± 0.18	0.74	0.8
Neutrophil (%)	60.54 ± 6.58	61.14 ± 5.91	60.47 ± 5.53	1.330	.127
Lymphocyte (%)	31.81 ± 5.92	32.5 ± 8.41	31.62 ± 5.34	1.057	.399
Monocyte (%)	2.54 ± 0.54	2.42 ± 0.50	2.55 ± 0.50	.945	.566
Eosinophil (%)	5.09 ± 2.60	5 ± 1.98	5.35 ± 2.14	2.062	.002**
PCV (%)	42.5 ± 3.83	42.3 ± 3.91	40.6 ± 4.48	1.1	0.3
MCV (fl)	89.15 ± 1.05	89.08 ± 0.93	88.88 ± 1.11	1.07	0.3
MCH (pg)	29.46 ± 1.01	29.55 ± 0.27	29.48 ± 0.35	0.3	1
MCHC (gm/dl)	33.16 ± 0.25	33.17 ± 0.27	33.17 ± 0.25	1.11	0.3
ESR (mm/hr)	13.22 ± 7.61	13.78 ± 9.32	18.27 ± 9.74	1.25	0.1
NLR	1.98 ± 0.63	2 ± 0.58	1.94 ± 0.55	1.1	0.3
HR (bpm)	78.61 ± 9.65	77.85 ± 12.43	77.17 ± 9.13	0.88	0.65
SBP (Hg mm)	128.54 ± 16.50	131.14 ± 15.02	140.8 ± 18.12	1.27	0.1
DBP (Hg mm)	84.02 ± 12.09	86 ± 10.95	89.55 ± 11.99	1.03	0.4
PP (Hg mm)	44.18 ± 8.22	45.57 ± 7.18	51.8 ± 12.7	1.44	0.07
MAP (Hg mm)	98.74 ± 12.97	101.18 ± 12.16	106.81 ± 12.63	1.18	0.2

P<0.05-*, P<0.01-**, P<0.001-***, P<0.0001****

Table 4 reflects a comparison between haematological parameters with different levels of year of exposure. Haemoglobin and RBC count decrease insignificantly with the duration of exposure to petrol vapour. WBC count and platelet count elevate insignificantly with an increase in year of exposure. No significant change was observed in neutrophil, lymphocyte and monocyte count. Percentage of eosinophil count increases with the duration of exposure. ESR value increases insignificantly with an increase in year of exposure with the maximum increase in >20 years of exposure. Heart rate and blood pressure values increase with duration of exposure, but the F values are not significant.

Table 5: Correlation between year of exposure, age, height, weight and BMI with hematological and cardiovascular parameters

Parameters	Year of Exposure	Age	Body Height	Body Weight	BMI
Hemoglobin (g/dl)	-0.25**	-0.27**	0.11	0.04	-0.01
RBC (millions/cu.mm)	-0.11	-0.08	0.06	0.02	0.001
WBC (cu.mm)	0.05	0.05	-0.05	0.13	0.18*
Platelets (Lakhs/ cu.mm)	0.14	0.13	-0.01	0.05	0.07
Neutrophil (%)	0.04	0.19*	-0.08	0.08	0.16*
Lymphocyte (%)	-0.01	-0.12	0.05	-0.12	-0.18*
Monocyte (%)	-0.02	-0.12	-0.08	-0.12	-0.10
Eosinophil (%)	-0.01	0.02	0.13	0.09	0.03

PCV (%)	-0.26**	-0.27**	0.11	0.04	-0.003
MCV (fl)	-0.11	-0.09	-0.02	0.01	0.02
MCH (pg)	-0.02	-0.09	0.05	-0.16*	-0.24**
MCHC (gm/dl)	-0.03	-0.07	0.06	-0.08	-0.14
ESR (mm/hr)	0.29**	0.29**	-0.11	-0.001	0.07
NLR	0.02	0.14	-0.10	0.10	0.18*
Heart Rate (bpm)	-0.03	-0.02	-0.008	0.01	-0.007
SBP (Hg mm)	0.34**	0.53**	0.10	0.25**	0.24**
DBP (Hg mm)	0.21**	0.35**	0.09	0.29**	0.29**
PP (Hg mm)	0.37**	0.48**	0.10	0.09	0.06
MAP (Hg mm)	0.29**	0.44**	0.11	0.29**	0.28**

P<0.05-*, P<0.01-**, P<0.001-***, P <0.0001****

Table 5 represents the correlation between year of exposure, age, body height, body weight and BMI with different haematological and cardiovascular parameters. A significant negative correlation has been found between haemoglobin and PCV with age and year of exposure, but a significant positive correlation exists between ESR, SBP, DBP, PP and MAP with age and duration of exposure. Neutrophil count value has a significant positive relation with age. A significant negative correlation has been found between body weight and BMI with MCH but a significant positive correlation has been present between body weight and BMI with SBP, DBP and MAP.

Table 6: Effects of smoking habit, high NLR (H-NLR) and high ESR (H-ESR) on the prevalence of hypertension and anaemia with ODDs Ratio and 95% CI among Petrol Filling Workers

Group	No. examined	Hypertension				Anaemia			
		No.	%	OR	95%CI	No.	%	OR	95%CI
Smokers	90	39	43.3%	1.24	0.64 -2.39	17	18.8%	0.74	0.34- 1.63
Nonsmokers	63	24	38.09%	1	-	15	23.8%	1	-
H-NLR	11	3	27.2%	0.51	0.13 - 2.01	2	18.1%	0.82	0.17 - 4.04
N-NLR	142	60	42.2%	1	-	30	21.1%	1	-
H-ESR	42	23	54.7%	2.14*	1.04 - 4.41	28	66.6%	53.5 ****	16.33 - 175.25
N-ESR	111	40	36.03%	1		4	3.60%	1	-
Total	153								

P<0.05-*, P<0.01-**, P<0.001-***, P <0.0001****

Table 6 represents the effects of smoking habit, high NLR and high ESR value on the prevalence of hypertension and anaemia with odds ratio and 95% CI. Odds ratio values indicate smoker PFW are more prone to hypertension. Odds ratio of high ESR are more prone to hypertension and anaemia. NLR and ESR both are prognostic markers for disease progression, including diabetes, cardiovascular disease, chronic kidney disease and hypertension. So these values have predictive abilities, severity and mortality of cardiovascular diseases and others.

Table 7: Multiple logistic regression equation of hypertension and anaemia among Petrol Filling Workers

Parameters	Coefficient	SE	P value	Adjusted OR	95% Confidence Interval	
					Lower	Upper

Hypertension						
Age	0.12	0.02	0.0001****	1.13	1.08	1.19
BMI	0.05	0.05	0.3	1.05	0.95	1.16
Smoking habit	-0.80	0.45	0.07	0.44	0.18	1.07
Year of Exposure	-0.005	0.02	0.8	0.99	0.95	1.04
NLR	0.11	0.33	0.7	1.12	0.58	2.13
ESR	0.008	0.02	0.7	1.00	0.96	1.05
Anaemia						
Age	0.01	0.04	0.8	1.01	0.93	1.09
BMI	-0.09	0.07	0.2	0.91	0.78	1.05
Smoking habit	0.79	0.77	0.3	2.20	0.48	9.99
Year of Exposure	-0.02	0.04	0.6	0.97	0.89	1.06
NLR	-0.83	0.61	0.1	0.43	0.12	1.44
ESR	0.39	0.07	0.0001****	1.48	1.28	1.70

P<0.05-*, P<0.01-**, P<0.001-***, P <0.0001****

Table 7 shows Multiple Logistic Regression equations of the prevalence of hypertension and anaemia. From the analysis of the logistic regression equation and the association of age, BMI, smoking habit and year of exposure, NLR and ESR with the prevalence of hypertension and anaemia at the multivariate level, it was observed that only age (P<0.0001) was significantly associated with hypertension. ESR value is significantly associated with anaemia.

Table 8: Comparison of haematological and cardiovascular parameters between anaemic and on-anaemic PFW

Parameters	Anaemic (n=32)	Non-anaemic (n=121)	P - value
Hemoglobin (g/dl)	11.84 ± 1.08	14.45 ± 0.79	0.0001****
RBC (millions/cu.mm)	4.04 ± 0.33	4.87 ± 0.26	0.0001****
WBC (cu.mm)	8087.50 ± 1209.30	8123.97 ± 1389.30	0.8
Platelets (Lakhs/cu.mm)	2.55 ± 0.23	2.56 ± 0.20	0.6
Neutrophil (%)	61 ± 5.46	60.54 ± 6.36	0.5
Lymphocyte (%)	31.34 ± 5.32	32.03 ± 6.52	0.6
Monocyte (%)	2.72 ± 0.52	2.47 ± 0.51	0.02*
Eosinophil (%)	4.94 ± 1.60	5.20 ± 2.54	0.4
PCV (%)	35.66 ± 3.05	43.58 ± 2.33	0.0001****
MCV (fl)	88.10 ± 1.19	89.32 ± 0.84	0.0001****
MCH (pg)	29.24 ± 0.45	29.54 ± 0.84	0.007**
MCHC (gm/dl)	33.18 ± 0.32	33.16 ± 0.23	0.7
ESR (mm/hr)	27.53 ± 7.70	11.24 ± 5.07	0.0001****
NLR	2.02 ± 0.51	2.00 ± 0.66	0.8
HR (bpm)	78.78 ± 9.94	77.95 ± 10.09	0.6
SBP (Hg mm)	132.31 ± 13.06	132.41 ± 18.50	0.9
DBP (Hg mm)	85.31 ± 8.82	86.08 ± 12.82	0.6
PP (Hg mm)	47.25 ± 8.79	46.31 ± 10.24	0.6
MAP (Hg mm)	101.05 ± 8.90	101.51 ± 14.12	0.8

P<0.05-*, P<0.01-**, P<0.001-***, P <0.0001****

Table 8 represents a comparison of hematological and cardiovascular parameters between anaemic and nonanaemic PFW. Hemoglobin, RBC count, PCV, MCV, MCH values are significantly lower in the anaemic group in comparison to the nonanaemic. Monocyte percentage and ESR

values increase significantly in anaemic group PFW than non anaemic. WBC and eosinophil count decrease insignificantly in the anaemic PFW group than the nonanaemic. No such changes have been found in cardiovascular parameters between the anaemic and the nonanaemic PFW groups.

Table 9: Comparison of hematological parameters between Hypertensive and Normotensive PFW

Parameters	Hypertensive (n=63)	Normotensive (n=90)	P – value
Hemoglobin (g/dl)	13.6 ± 1.52	14.1 ± 1.22	0.03*
RBC (millions/cu.mm)	4.61 ± 0.48	4.76 ± 0.39	0.04*
WBC (cu.mm)	8144.44 ± 1347.85	8096.67 ± 1358.57	0.8
Platelets (Lakhs/cu.mm)	2.59 ± 0.19	2.54 ± 0.21	0.1
Neutrophil (%)	61.75 ± 5.70	59.86 ± 6.40	0.05*
Lymphocyte (%)	31.14 ± 6.81	32.41 ± 5.85	0.2
Monocyte (%)	2.43 ± 0.53	2.59 ± 0.51	0.06
Eosinophil (%)	5.14 ± 1.86	5.14 ± 2.68	0.9
PCV (%)	41.10 ± 4.51	42.50 ± 3.66	0.04*
MCV (fl)	88.98 ± 1.15	89.13 ± 0.97	0.3
MCH (pg)	29.34 ± 1.18	29.58 ± 0.22	0.1
MCHC (gm/dl)	33.13 ± 0.26	33.19 ± 0.25	0.2
ESR (mm/hr)	16.73 ± 7.47	13.19 ± 7.01	0.02*
NLR	2.09 ± 0.63	1.94 ± 0.63	0.1

P<0.05-*, P<0.01-**, P<0.001-***, P<0.0001****

Table 9 represents a comparison of hematological parameters between the hypertensive and non-hypertensive group of PFW. Hemoglobin, RBC count, PCV values decrease significantly but neutrophil count and ESR value increase significantly (P<0.05) in the hypertensive PFW group compared to the non-hypertensive. An insignificant increase in WBC count and NLR has been observed in the hypertensive PFW group in comparison to the non-hypertensive group. Increase in NLR value, ESR and WBC count has high prognostic importance. ESR and NLR values are also inflammatory markers and indicators of cardiorespiratory and renal function impairment.

DISCUSSION

The study aimed to investigate the changes in hematological and some biochemical parameters among petrol station workers exposed to VOCs compared to control group. Environmental exposure to benzene has long been known as a carcinogen to human blood components. In addition, occupational exposure to benzene and other volatile organic compounds may cause non-carcinogenic effects including hematologic, hepatic, neurologic, renal and immunologic dysfunctions [11]. But the precise mechanism of benzene, xylene, toluene, dust particles (PM₁₀, PM_{2.5}), SO₂, and NO₂ present in the workplace air is not fully understood. Several studies indicates that bone marrow failure was found to be one of the serious health problems of benzene exposure which can impair blood cell formation and can increase the risk of developing cancer [12]. The principal screening tool for clinical assessment of benzene toxicity is complete blood count (CBC) including hemoglobin and red blood indices [13].

From the result of this study hemoglobin and total RBC count decreased significantly ($p < 0.0001$) in petrol filling workers but WBC count decreased insignificantly compared to control group. A consistent result had been reported from Sudan, Korea and Nigeria [4, 14, 15, 2]. It was suggested that a reduction in the cell size could be due to the membrane alterations. Benzene may have an impact on flexibility and permeability of the cell although its mechanism is not clearly known [16]. Again, metabolism of petrol chemicals leads to the formation of free radicals that can damage the cell membrane. Further, free radicals produced from benzene and other VOCs can affect erythrocyte membranes and heme synthesis, resulting in shortened life span as well as impairment of heme synthesis and as a result lowering of hemoglobin content and RBC count [17]. This result was in agreement with Ray et al [18]; Lan et al [19]; Mohammed [20] and D'Andrea and Reddy [21]. But RBC indices – MCV and MCH decreased significantly in PFW than control group in our study which was inconsistent with the study of Binsaleh et al [22] where MCH and MCHC values elevated significantly, and no significant difference was found in MCV between studied group. The difference in these findings might be due to individual susceptibility to petroleum products, such as benzene [23].

Our study revealed that neutrophil and eosinophil count increased significantly in PFW compared to control group but total WBC count, lymphocyte and monocyte count decreased insignificantly in studied groups. The result of our study corroborates with the findings of D'Andrea and Reddy [21] and Abdel Azia et al [24]. Using animal models Orisakwe et al [25] reported significantly decreased in PCV and total WBC in a group of rat for 7 days treatment with 200mg/kg Bonny light crude oil compared to the respective control and before treatment groups. Chu et al [26] in a 14 days study reported that light gas oil caused a decrease in Hb, PCV and RBC with bone marrow myeloid hyperplasia and dyserythropoiesis. Except PCV that showed insignificant variation in our study with human subject, all other blood parameters mentioned above showed similar results. The variation might be due to variation in nature of exposure of petroleum product. In animal experiment petroleum products directly or invasively introduced into the animal body but in our study petroleum product exposure was made through inhalation and skin contact. In contrast, Salem et al [27] reported significantly higher Hb level, WBCs and Platelet count in PFW of Libya than those of comparison group. Again, Salem et al [11] found significantly lower hematocrit value, MCV, MCH and MCHC in Petrol station attendants of Libya, Tripoli than comparison group. Sirdah et al [28] reported similar result among workers exposed to petroleum vapour of Gaza except WBCs count where significantly lower values were recorded. In another study conducted in India Ray et al [18] also showed a significant increase in WBC count, neutrophils, eosinophils and monocytes in benzene exposed workers. D'Andrea and Reddy [21] reported that platelet count was significantly elevated in benzene exposed group compared with the control group. In contrast, Teklu et al [17] and Ray et al [18] observed lower platelet count in benzene exposed group than non-exposed group. In present study no significant difference has been found in platelet count between exposed group and control group.

These conflicting findings could be attributed to many factors like adaptation to petroleum products such as benzene in the bone marrow with little response from leucocytes [22] which was supported by the findings of Schlebusch et al [29] where adaptation to toxic chemical has been reported and ability to tolerate an environmental stressor by Andean population [22]. Another possible explanation could be that exposure to other types of pollutants could influence the susceptibility to hematotoxicity. Besides, nutritional status, genetic

polymorphism and individual's natural ability to detoxify chemicals like benzene might be the reason for variation in results of different studies. Nourozi et al [30] concluded that certain specific genotypes increase the susceptibility to benzene induced hematological disorder. Ye et al [31] and Wan et al [32] indicated that individual with high levels of metabolic enzymes that oxidize benzene into more toxic metabolites (such as CYP2E1) and low level of metabolic enzyme that detoxify pathways. In our study ESR value is significantly high in PFW compared to control group. This finding is the result of the increased inflammatory process in exposed workers. ESR is very useful nonspecific test for certain chronic infection with or without fever, inflammatory conditions, anaemia and neoplastic conditions particularly if there is tissue degeneration or extensive metastasis [33].

In the present study hemoglobin, total RBC, WBC and platelet count decrease insignificantly with increase in year of exposure. Similar observation of reduction of hemoglobin concentration was found in studies by Bharmota et al [34]; Mahapatra et al [35]; Joshi et al [1] and Jabbar et al [36] between 1-5 years and > 5 years of exposure. But RBC count significantly increase within 1-5 years of exposure and decrease with >5 years of exposure in comparison to control was observed by Bharmota et al [34]; Firouzkouhi et al [37] and Getu et al [23]. No significant difference has been found in RBC count in studies by Shilpi et al [38] and Jothery et al, 2017 [39].

In our study total leucocyte count increase insignificantly with increase in duration of exposure and maximum increase was observed in >20 years of exposure. No statistically significant difference in leucocyte count was found in studies by Bharmota et al [34]; Qafishesh et al [40] and Mohammad [20]. In contrast study of Lavanya et al [41] showed significant decrease while study of Awodele et al [42] showed significant increase in total WBC count compared to controls. Besides in present study, neutrophil and lymphocyte count increased maximum in between 11-20 years of exposure. Eosinophil count increased significantly with increase in year of exposure. Bharmota et al [34] reported significant neutropenia and lymphocytosis with increasing duration of exposure in comparison to controls. This finding corroborates with the study of Elderderly et al [4]. No significant difference in basophil, eosinophil and monocyte count was observed in a study by Bharmota et al [34] which was not in accordance with our study. Mahapatra et al [35] observed significant decrease in eosinophil count with increase in duration of exposure. ESR value increased maximum above 20 years of exposure although this value was not significant. Neutrophil Lymphocyte ratio increased maximum between 11-20 year of exposure, monocyte count increased maximum between >20 years of exposure.

In the present study insignificant decrease in MCV value was obtained with increase in duration of exposure. This finding is not in accordance with the studies by Teklu et al [17]; Nair et al [43] and Bharmota et al [34].

No significant difference has been found in MCH and MCHC values in our study with increase in duration of exposure. In contrast Bharmota et al [34] and Ajugwo et al [44] found significant decrease in these parameters with increase in duration of exposure. But values observed by Mohammed et al [45] corroborate with our study. Hematological parameters when compared between anaemic and nonanaemic PFW, significant decrease was found in Hb concentration, RBC count, WBC count, PCV, MCV, MCH and ESR values might be due to long term exposure to gasoline that can create blood toxicity and decrease in Hb levels. According to Kponee et al [46]

and Owusu et al [47] polycyclic aromatic hydrocarbons (PHAs) detected in the residue after gasoline combustion are persistent organic pollutants which can reduce Hb content after long term exposure. Thus, occupational gasoline exposure may be a potential risk factor for anaemia, suggested by Getu et al [23] and Garcia-Casal et al [48]. A positive correlation exists between gasoline exposure concentration and severity of anaemia, consistent with the studies by Ezejiogor [33]; Ramirez et al [49] and Cakmak et al [50].

The toxic components in gasoline such as benzene may also trigger autoimmune reactions, causing the immune system to attack its own RBC and resulting in immune mediated hemolytic anaemia [51]. Besides, benzene has genotoxic effects, causing damage of DNA which can affect gene expression related to RBC production, resulting anaemia [52].

Previous studies recognize that alterations in hematologic indices have strong link with hypertension [53, 54, 55]. Sun et al [56] revealed that WBC can independently predict hypertension in chinese adults. Nakamishi et al [57] demonstrated that WBC count might be an important risk factor for hypertension in Japanese adult male office workers. Significant association between WBC count and development of hypertension after adjustment for the effects of confounding factors such as age, gender, smoking habit, alcohol intake, regular exercise habits, obesity, diabetes and dyslipidemia was established by Ishida et al [58]. In our study significant increase in heart rate, systolic and diastolic blood pressure and mean pressure was observed in PFW than control group. Hypertensive and normotensive PFW in our study when compared, significant reduction in hemoglobin, total RBC count and PCV was observed but total WBC count, neutrophil and ESR value increased significantly in hypertensive compared to normotensive person. Siedlinski et al [59] concluded that high blood lymphocyte count may play a causal role in the development of hypertension. It was also observed that blood lymphocyte count might influence albuminuria which might be the underlying mechanism of hypertension. It is hypothesized that WBC count might reflect chronic inflammation which could lead to endothelial dysfunction [60]. Other possible mechanism of hypertension might be arteriosclerotic changes, vasoconstriction, greater sympathetic tone and chronic kidney disease [61-66].

In the present study neutrophil lymphocyte ratio (NLR) increases in hypertensive PFW compared to control group which is associated with increased risk of hypertension suggested by Sarejloo et al [67]. Wang et al [68] indicated that the NLR might be a predictor of mortality and cardiovascular events in patients undergoing angiography. Balta et al [69] proposed that NLR might have correlation with vascular disease associated risk factor.

In the present study it is focused that NLR value is insignificantly higher in anaemic which is in agreement with the study by Singh [70]. Sharma et al [71] indicated that elevated NLR combined with decrease Hb predicted the onset of major adverse cardiovascular events in patients with ST- elevation myocardial infraction. NLR is also an emerging marker in patients with heart failure, acute coronary syndrome, hypertension and diabetes [71-73, 7]. In our study NLR value is insignificantly high in hypertensive PFW in comparison to non-hypertensive PFW, indication association of NLR value with hypertension as well as anaemia [71].

Neutrophil and lymphocyte count are valuable blood parameters provide insights into systemic inflammatory status and balance between natural immunity (neutrophil) and acquired immunity (lymphocyte). The ratio of this count is called NLR, a more predictive indicator than either parameter alone [74]. NLR has demonstrated a predictive ability towards severity and mortality of cardiovascular diseases [75, 76]. Zhang et al [77] have underscored the pivotal role of inflammation and immune responses in the onset and persistence of hypertension, contributing to elevated blood pressure by triggering vascular inflammation and micro vascular remodeling. Thus, our study demonstrated elevated NLR in anemic subjects as well as hypertensive PFW. So NLR can be used in routine chemical assessment of anemia and hypertension among PFW.

CONCLUSION

This study concluded that there are variations in hematological parameters among PFW mainly hemoglobin, total RBC count, neutrophil and eosinophil count, RBC indices compared to control although variation in result are not always consistent with other studies. These mixed results highlight the multifaceted nature of workplace environment in petrol stations that can influence hematological health. However, long term exposure to volatile organic compounds such as benzene, xylene, toluene might have effects on human hematopoietic system and suppression of bone marrow to cause abnormal hematological profile and other related health complications like hypertension and anaemia.

There is statistically significant correlation between age, year of exposure and BMI with most of the hematological parameters indicating that age, year of exposure and nutritional status have strong impact on variation of hematological parameters of PFW. Crude OR values indicate smoking habit as important risk factor for hypertension and high ESR value is the risk factor for prevalence of hypertension and anaemia. Multiple logistic regression equations indicate age is the risk factor for prevalence of hypertension and ESR value is the significant risk factor for prevalence of anaemia among PFW. The results indicate increase exposure to VOC of petrol station is associated with higher incidence of anaemia and decrease in Hb concentration. Besides higher NLR value indicates the incidence of hypertension among PFW.

Therefore, it is necessary to improve the workspace air quality of petrol station by application of sophisticated technologies and equipments like Petrol vapour recovery system, use of protective gadgets like gloves, masks and protective clothing, periodic health assessment, screening and health education. Thus, health of the workers will remain protected that can reduce the absenteeism as well as early mortality among these petrol station workers and society will be benefitted and productivity will be increased.

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