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ORIGINAL ARTICLE

Toxicological Effects of Benzene on Blood and Biochemical Profiles Among Fuel Station Workers in Southern Saudi Arabia

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ABSTRACT

Background: **Benzene is a widely-used industrial solvent recognized for its toxic effects on multiple organ systems. Occupational exposure, particularly among gasoline station attendants, has been associated with hematological disturbances and alterations in lipid metabolism. This study aimed to evaluate the impact of benzene exposure on hematological and biochemical markers among gasoline station workers in the Asir region of southern Saudi Arabia.**

Methods: **A total of 206 participants were enrolled, comprising 106 benzene-exposed workers that were categorized into three subgroups based on duration of exposure: less than 2 years, 2 to 4 years, and more than 4 years. An additional 100 unexposed individuals, matched for age and gender, served as the control group. Laboratory assessments included CBC, electrolytes, GLU, lipid profile, PO_4^{3-}, ALP, Ca^{2+}, and Mg^{2+}.**

Results: **Benzene exposure was significantly associated with a reduction in RBC count and related indices ($p < 0.05$), while HGB and HCT levels were elevated ($p < 0.005$). Lipid profile analysis revealed significant increases in LDL, VLDL, and TG, along with a marked decrease in HDL ($p \leq 0.0001$). Electrolyte analysis showed disturbances, particularly in potassium levels ($p \leq 0.0001$). Furthermore, significantly elevated levels of PO_4^{3-}, ALP, and Mg^{2+} ($p \leq 0.0001$) in the exposed group suggested potential negative effects on bone metabolism.**

Conclusions: **This study demonstrates that chronic occupational exposure to benzene is associated with significant hematological abnormalities, dyslipidemia, electrolyte imbalances, and alterations in bone-related biochemical markers. These findings highlight the need for regular health monitoring and the implementation of protective measures for gasoline station workers and others in similar occupational environments.**

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KEYWORDS

benzene, complete blood count, lipid profile, electrolytes, alkaline phosphatase, occupational exposure

LIST OF ABBREVIATIONS

ALP - Alkaline phosphatase
AUC - Area under curve
GLU - Blood glucose
BMI - Body mass index
Ca^2 - Calcium
CBC - Complete blood count

DNA - Deoxyribonucleic acid
EDITA - Ethylenediaminetetraacetic acid
HCT - Hematocrit
HBG - Hemoglobin
HDL - High-density lipoprotein
LDL - Low-density lipoprotein
MG^{2+} - Magnesium
MCH - Mean corpuscular hemoglobin
MCHC - Mean corpuscular hemoglobin concentration
MCV - Mean corpuscular volume
OR - Odds ratio
ANOVA - One-way analysis of variance
R^2 - Pearson's correlation coefficient
PO_4^{3-} - Phosphate
K^+ - Potassium
PR - Prevalence ratios
PPAR - Proliferator-activated receptor
ROS - Reactive oxygen species
ROC - Receiver operating characteristic
RBC - Red blood cell
rpm - Revolutions per minute
Na^+ - Sodium
SD - Standard deviation
TC - Total cholesterol
TG - Triglycerides
VLDL - Very low-density lipoprotein
VOCs - Volatile organic compounds
WBCs - White blood cells

INTRODUCTION

Benzene is a volatile, aromatic hydrocarbon derived from crude oil and serves as a key industrial chemical and a major constituent of gasoline. It is extensively used as fuel for automobiles, motorcycles, diesel engines, and power generators and also functions as a solvent for oils, lipids, and marine fuels. Benzene is a colorless, highly flammable liquid with a characteristic sweet odor and is one of more than 500 hydrocarbons found in motor gasoline, which is predominantly composed of volatile organic compounds (VOCs) [1].

Occupational exposure to benzene and related BTEX compounds (benzene, toluene, ethylbenzene, and xylene) poses significant health risks, particularly affecting the reproductive, developmental, and immune systems and contributing to systemic toxicity [2]. Globally, benzene exposure is estimated to account for approximately 0.07% of occupational fatalities, with 5.3% of the global workforce exposed to associated hazards [3]. Workers in the petroleum sector, particularly gasoline station attendants, are at increased risk due to continuous contact with fuel vapors and vehicular exhaust. Environmental factors such as ambient temperature, fuel composition, altitude-related hypoxia, and the use of VOC-emitting equipment can further influence exposure levels and the severity of toxic effects [4].

Benzene exerts a wide range of toxic effects, including hematological disorders such as leukemia, lymphoma, aplastic anemia, and chromosomal abnormalities [5]. In addition, benzene exposure has been shown to impair the function of the respiratory, hepatic, renal, and nervous systems [6]. The primary routes of exposure are inhalation and dermal absorption, through which benzene is metabolized into reactive intermediates such as phenol, benzene oxide, and hydroquinone. These metabolites contribute to cellular toxicity by inducing oxidative stress, DNA damage, and apoptosis [7].

Due to its high volatility, benzene is rapidly absorbed through the lungs, whereas dermal absorption occurs at a slower and less efficient rate [8]. Benzene metabolism primarily takes place in the liver and bone marrow via cytochrome P450 2E1 (CYP2E1), resulting in the formation of reactive intermediates implicated in its hepatotoxic and carcinogenic effects [9]. However, the metabolic pathways active within the bone marrow remain inadequately characterized, highlighting the need for further research into benzene-induced hepatotoxicity and its systemic impact [10]. Excretion occurs predominantly via exhalation and urinary elimination, depending on the level of exposure and the degree of metabolic saturation [11].

Despite extensive research on benzene's systemic toxicity, there remains a lack of region-specific data examining its subclinical hematological and biochemical effects under environmental conditions unique to high-altitude regions, such as the Asir area of Saudi Arabia. Chronic hypoxia at high altitudes may exacerbate benzene's oxidative stress-mediated damage, alter hematological responses, and influence organ function [12]. In Saudi Arabia, where gasoline station workers are frequently exposed to benzene and other VOCs through routine handling of fuel and proximity to vehicular emissions, such investigations are especially warranted [13].

This study therefore aimed to evaluate the impact of chronic benzene exposure on hematological indices and biochemical markers among gasoline station workers in the Asir region, a high-altitude environment characterized by chronic hypoxia. The findings are intended to provide novel insights into how altitude-related hypoxia interacts with occupational benzene exposure, thereby enhancing understanding of health risks in real-world occupational environments and informing preventive strategies and evidence-based monitoring protocols.

MATERIALS AND METHODS

Study design and ethical approval

This cross-sectional study was conducted in the Asir region, located in the southern part of the Kingdom of Saudi Arabia. Ethical approval was obtained from the Research Ethics Committee at King Khalid University (approval no. ECM#2023-608). The study adhered to the ethical principles outlined in the Declaration of Helsinki (1975), as revised in 2013. All participants were informed of the study objectives and provided written

informed consent prior to enrollment. Participant data were anonymized to ensure confidentiality.

Study population

This study was conducted from January through April 2024, and it included all available gasoline station workers from the three largest cities of the Asir region (Abha, Khamis Mushait, and Ahad Rufaidah). A total of 106 male gasoline station workers aged between 18 and 56 years were recruited from various fuel stations across the region. All participants were occupationally exposed to benzene through routine work activities, typically performing either 8-hour or 12-hour shifts, six days per week. To minimize potential confounding variables, individuals with a history of renal or hepatic failure, thyroid disorders, diabetes mellitus, cardiomyopathy, acute or chronic inflammatory conditions, or those currently receiving corticosteroids or lipid-lowering medications were excluded from the study. A control group of 100 healthy, unexposed individuals was recruited to provide a comparison for the benzene-exposed workers. Controls were carefully matched to the exposed group by age and gender to minimize confounding effects from demographic factors. All control participants reported no occupational or environmental exposure to benzene or other volatile organic compounds and were screened using the same exclusion criteria applied to the exposed group. This rigorous matching and screening ensured that observed differences between the exposed and control groups could be primarily attributed to occupational benzene exposure.

Demographic and anthropometric characteristics, including age, marital status, weight, height, and body mass index (BMI), were recorded. Based on the duration of occupational exposure, the gasoline station workers were stratified into three subgroups: less than 2 years (n = 23), 2 to 4 years (n = 42), and more than 4 years (n = 41). A control group of 100 healthy, unexposed individuals, matched for age and gender, was recruited for comparative analysis.

Sample collection

Venous blood samples were collected under aseptic conditions using standard phlebotomy techniques. Blood was drawn from the antecubital vein using a sterile, single-use needle and vacuum collection system after participants had fasted for at least 8 hours. Each sample was transferred into two separate vacutainer tubes: one containing ethylenediaminetetraacetic acid (EDTA) for hematological analysis and one plain tube for serum separation.

EDTA tubes were gently inverted several times to prevent coagulation and were analyzed within four hours of collection. Plain tubes were allowed to clot at room temperature for 20 - 30 minutes and were then centrifuged at 3,000 rpm for 10 minutes to obtain serum. The resulting serum was liquated into labeled cry vials and stored at -20°C until further biochemical analysis.

Laboratory analysis

Hematological parameters, including red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), and red cell indices, were analyzed using an automated hematology analyzer (Sysmex XN-2000™, Sysmex Corporation, Kobe, Japan). Serum biochemical markers were assessed using a fully automated clinical chemistry system (Cobas 6000, Roche Diagnostics, Mannheim, Germany). The parameters measured included fasting blood glucose (GLU), lipid profile [total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL)], electrolytes (sodium ($Na+$), potassium ($K+$), calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphate (PO_4^{3-}), and alkaline phosphatase (ALP). All analyses were performed according to the manufacturer's instructions and under standard laboratory quality control protocols to ensure accuracy and reproducibility.

Statistical analysis

All statistical analyses were conducted using Graph Pad Prism version 10.4.2 (Graph Pad Software, Inc., San Diego, CA, USA). Data were assessed for normality prior to analysis. As most variables were non-normally distributed, non-parametric methods were employed.

Group comparisons between two independent samples were performed using the unpaired *t*-test, while comparisons across more than two groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to identify significant intergroup differences. Pearson's correlation coefficient **(R^2)** was used to evaluate the strength and significance of associations between continuous variables. Results are presented as mean ± standard deviation (SD) and visualized using box plots. A p-value < 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of study participants

A total of 206 individuals were included in this study. Out of these, 106 participants (51%) were occupationally exposed to benzene vapors, while 100 participants (49%) served as unexposed controls. Demographic data are presented as mean ± SD. There was no statistically significant difference in age between the exposed and control groups ($p = 0.82$), as shown in Table 1 and Figure 1A. In contrast, the BMI was significantly lower in the benzene-exposed group compared to controls ($p = 0.018$), as illustrated in Figure 1C. When participants were stratified by exposure duration, those with more than four years of benzene exposure had a significantly higher mean age than the control group ($p < 0.0001$), as presented in Table 2 and Figure 1B. However, no significant differences in BMI were observed among the exposure subgroups based on duration ($p > 0.05$), as shown in Table 2 and Figure 1D.

Table 1. The hematological and biochemical parameters among the control group and exposure group.

Categories	Control group n = 100	Exposures sample n = 106	p-value
Age	30.43 ± 8.9	30.16 ± 8.4	0.822
BMI	24.53 ± 4.6	25.47 ± 3.5	0.099
RBC	5.47 ± .547	5.28 ± .518	0.046
HGB	15.61 ± 1.40	16.47 ± 1.06	< 0.0001
HCT	45.91 ± 3.49	47.38 ± 3.21	0.0016
MCV	85.49 ± 4.97	80.62 ± 7.48	< 0.0001
MCH	29.48 ± 2.3	28.38 ± 2.54	0.051
MCHC	34.43 ± 1.48	33.76 ± 2.27	0.26
PLT	286.2 ± 70.08	281.6 ± 52.07	0.998
WBC	7.45 ± 2.6	8.35 ± 1.85	0.006
Na^+	138.4 ± 2.55	138.9 ± 1.86	0.149
K^+	4.29 ± 0.43	4.63 ± 0.49	< 0.0001
Cl^-	103.7 ± 2.87	103.9 ± 2.22	0.77
Mg2+	1.99 ± 0.21	2.19 ± 0.14	< 0.0001
PO43-	3.76 ± 0.65	4.31 ± 0.85	< 0.0001
Ca2+	9.55 ± 0.53	9.7 ± 0.55	0.051
ALP	75.06 ± 20.8	93.92 ± 24.0	< 0.0001
LDL	139.4 ± 59.08	115.5 ± 27.08	0.023
VLDL	116.9 ± 42.02	43.18 ± 25.36	< 0.0001
HDL	45.35 ± 11.99	39.3 ± 8.19	< 0.0001
TC	188.5 ± 38.81	188.7 ± 38.10	0.980
TG	28.98 ± 19.07	190.3 ± 82.82	< 0.0001
GLU	88.46 ± 7.65	91.13 ± 25.93	0.19

Impact of benzene exposure on hematological and biochemical parameters

Assessment of alterations in hematological indices between the benzene-exposed and control groups is presented in Table 1 and Figure 2. RBCs were significantly lower in the exposed group compared to controls ($p = 0.046$), while HGB and HCT levels were significantly elevated ($p < 0.0001$ and $p = 0.0016$, respectively; Figures 2B and 2C). These findings may reflect compensatory erythropoietic activity or hem concentration. MCV was also significantly reduced in the exposed group ($p < 0.0001$), suggesting potential alterations in red cell morphology. PLT did not differ significantly between groups ($p = 0.998$). However, WBCs count was significantly elevated in the exposed group ($p = 0.006$; Figure 2A), potentially suggesting an underlying inflammatory or immune response.

ALP levels were significantly elevated in the exposed group ($p < 0.0001$). Serum K+, Mg^{2+}, and PO_4^{3-} levels were also significantly higher in the exposed group ($p < 0.0001$ for all; Figures 2D). No significant differences were observed in Cl^- or Ca^{2+} concentrations ($p = 0.77$ and $p = 0.051$; Figures 3C and 3F, respectively).

TG were significantly elevated in the exposed group ($p < 0.0001$; Figures 3D), while LDL, VLDL, and HDL levels were significantly decreased ($p = 0.023$, $p < 0.0001$; Figure 3A, B, and C). However, no significant difference was observed in TC ($p = 0.980$) or GLU ($p = 0.19$).

Hematological and biochemical alterations by duration of exposure

Hematological parameters were assessed across benzene exposure subgroups categorized by duration (< 2 years, 2 - 4 years, and > 4 years), along with the control group, as presented in Figure 4 and Table 2.

RBC counts decreased progressively with longer exposure duration, with the lowest values observed in the > 4-year group compared to controls ($p < 0.025$). HGB levels were significantly elevated in the 2 - 4-year subgroup ($p < 0.0001$) and remained higher in the > 4-year group ($p < 0.0001$). HCT was significantly increased in the 2 - 4-year subgroup relative to the control group ($p < 0.004$). MCV values were significantly lower across all exposed groups when compared to the control ($p < 0.0001$; Figure 4A). WBC counts were significant-

Table 2. The hematological and biochemical parameters among the control group and exposure group based on occupational duration.

Variable	Control group (n = 100)	Exposure group of less than two years (n = 23)	Exposure group of 2 - 4 years (n = 42)	Exposure group of more than 4 years (n = 41)	p-value
Age	30.16 ± 8.4	27.13 ± 6.24	26.67 ± 5.95	39.198.87	< 0.0001
BMI	25.47 ± 3.5	25.28 ± 3.9	23.55 ± 4.16	26.22 ± 4.75	0.0088
RBC	5.48 ± 0.53	5.36 ± 0.51	5.3 ± 0.55	5.21 ± 0.52	0.025
HGB	15.61 ± 1.41	16.24 ± 0.93	16.68 ± 0.92	16.27 ± 1.43	< 0.0001
HCT	45.91 ± 3.49	47.29 ± 2.40	48.07 ± 2.86	46.66 ± 3.82	0.0047
MCV	85.49 ± 4.97	81.0 ± 6.75	81.76 ± 4.91	80.12 ± 8.38	< 0.0001
MCH	29.48 ± 2.29	28.38 ± 3.0	28.53 ± 2.22	28 + 23 ± 2.63	0.015
MCHC	34.43 ± 1.48	33.97 ± 1.96	33.45 ± 2.47	33.95 ± 2.23	0.048
PLT	282.2 ± 70.08	286.8 ± 49.11	285.3 ± 53.43	275.1 ± 55.46	0.823
WBC	7.45 ± 2.06	8.22 ± 1.9	8.12 ± 1.83	8.53 ± 1.79	0.016
Na^+	138.4 ± 2.55	138.8 ± 1.97	138.8 ± 1.74	139.2 ± 1.78	0.34
K^+	4.29 ± 0.43	4.57 ± 0.54	4.56 ± 0.52	4.74 ± 0.41	< 0.0001
Cl^-	103.72 ± 2.87	103.5 ± 2.08	103.4 ± 1.90	104.9 ± 2.39	0.06
Mg^{2+}	1.99 ± .21	2.16 ± 0.14	2.22 ± 0.14	2.16 ± 0.14	< 0.0001
$PO4^{3-}$	3.76 ± 0.65	4.09 ± 0.66	4.3 ± 0.71	4.31 ± 0.88	< 0.0001
Ca^{2+}	9.55 ± 0.53	9.81 ± 0.49	9.54 ± 1.46	9.46 ± 0.49	0.11
ALP	75.06 ± 20.8	90.65 ± 20.12	91.67 ± 17.47	96.07 ± 20.1	< 0.0001
LDL	80.92 ± 19.36	116.9 ± 21.91	116.7 ± 31.15	118.0 ± 25.46	< 0.0001
VLDL	113.7 ± 35.49	43.90 ± 27.61	38.56 ± 16.92	45.91 ± 27.66	< 0.0001
HDL	45.75 ± 11.36	37.41 ± 6.51	40.39 ± 8.01	39.24 ± 9.14	< 0.0001
TC	188.52 ± 38.81	190.2 ± 37.2	189.0 ± 32.04	186.0 ± 44.73	0.79
TG	28.98 ± 19.07	189.0 ± 83.91	176.8 ± 73.53	203.6 ± 88.86	< 0.0001
GLU	88.46 ± 7.65	89.29 ± 20.79	85.77 ± 18.29	98.55 ± 35.99	0.045

ly elevated in the > 4-year subgroup relative to other groups ($p < 0.016$). No significant differences were observed in PLT counts across the groups ($p = 0.823$).

Serum ALP, K^+, Mg^{2+}, PO_4^{3-} levels were significantly elevated in all exposed groups relative to the control ($p < 0.001$; Figures 4B, C and). While Ca^{2+} was significantly elevated in the < 2-year group only but not significantly between all groups ($p < 0,11$). However, no significant differences were observed in Cl^- levels among the groups ($p = 0.06$).

LDL and TG levels were significantly increased in all exposure subgroups compared to controls ($p < 0.001$; Figures 5A and 5D). In contrast, VLDL and HDL levels were significantly decreased in all exposure subgroups ($p < 0.0001$; Figures 5B and 5C). TC levels showed no significant differences across groups ($p = 0.79$), while GLU levels were significantly higher in the > 4-year exposure group compared to control ($p < 0.05$).

Association between hematological and biochemical parameters with duration of occupational exposure

As presented in Table 3 and Figure 6, Pearson´s correlation analysis demonstrated significant positive associations between exposure duration and serum levels of K^+ ($R^2 = 0.081$, $p < 0.0001$), Mg^{2+} ($R^2 = 0.053$, $p = 0.0009$), TG ($R^2 = 0.22$, $p < 0.0001$), VLDL ($R^2 = 0.21$, $p < 0.0001$), and GLU ($R^2 = 0.044$, $p = 0.0025$). An inverse correlation was observed for HDL ($R^2 = 0.027$, $p = 0.0173$). Additionally, WBC count showed a weak but significant positive correlation ($R^2 = 0.023$, $p = 0.0283$). Other parameters, including RBC, HGB, HCT, MCV, MCH, MCHC, PLT, Na^+, Cl^-, Ca^{2+}, PO_4^{3-}, TC, and LDL, did not exhibit statistically significant correlations with duration of exposure.

Diagnostic performance of hematological and biochemical parameters

Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic utility of hematological and biochemical parameters that demon-

Table 3. Pearson correlation analysis between hematological and biochemical parameters with duration of occupational exposure.

Parameter	95% confidence interval	R squared	p-value	p-value summary
WBCs	0.02 to 0.28	0.023	0.0283	*
K^+	0.15 to 0.41	0.081	< 0.0001	****
Mg^{+2}	0.1 to 0.36	0.053	0.0009	***
HDL	-0.30 to -0.03	0.027	0.0173	*
VLDL	-0.56 to -0.35	0.21	< 0.0001	****
TG	0.36 to 0.57	0.22	< 0.0001	****
GLU	0.08 to 0.34	0.044	0.0025	**
RBCs	-0.08 to 0.20	0.0037	0.3836	ns
HBG	-0.05 to 0.22	0.0083	0.193	ns
HCT	-0.16 to 0.11	0.00052	0.7453	ns
MCV	-0.22 to 0.05	0.008	0.2016	ns
MCH	-0.16 to 0.12	0.00036	0.7873	ns
MCHC	-0.03 to 0.24	0.012	0.1142	ns
PLT	-0.20 to 0.071	0.0045	0.3398	ns
Na^+	-0.04 to 0.23	0.009	0.1739	ns
Cl^-	-0.03 to 0.23	0.01	0.1506	ns
Ca^{+2}	-0.21 to 0.07	0.0049	0.3154	ns
PO_4^{-3}	-0.07 to 0.22	0.0065	0.2476	ns
TC	-0.15 to 0.12	0.00034	0.7934	ns
LDL	-0.24 to 0.03	0.011	0.1413	ns

Table 4. Diagnostic performance of hematological and biochemical parameters based on ROC curve analysis.

Parameter	AUC	95% CI	p-value
Hematological parameters			
HGB	0.980	0.961 - 1.000	< 0.0001
HCT	0.887	0.841 - 0.932	< 0.0001
WBC	0.837	0.780 - 0.894	< 0.0001
RBC	0.763	0.698 - 0.827	< 0.0001
MCV	0.717	0.649 - 0.784	< 0.0001
MCH	0.689	0.618 - 0.760	< 0.0001
MCHC	0.667	0.595 - 0.740	< 0.0001
Biochemical parameters			
TG	0.994	0.985 - 1.000	< 0.0001
Mg^{2+}	0.779	0.718 - 0.841	< 0.0001
ALP	0.743	0.677 - 0.809	< 0.0001
K^+	0.704	0.634 - 0.774	< 0.0001
PO_4^{3-}	0.686	0.614 - 0.759	< 0.0001
Ca^{2+}	0.582	0.505 - 0.659	0.0365
HDL	0.521	0.443 - 0.598	0.6423
LDL	0.544	0.468 - 0.621	0.2932
VLDL	0.539	0.462 - 0.616	0.3424

Table 5. Association of hematological and biochemical parameters with benzene exposure.

Parameter	PR	95% CI	Z statistic	p	OR	95% CI	Z statistic	p
RBCs	14.524	6.17 to 34.13	6.14	p < 0.0001	82.82	29.79 to 230.22	8.46	p < 0.0001
HGB	0.6827	0.38 to 1.20	1.31	p = 0.1887	0.5015	0.20 to 1.25	1.47	0.1396
MCV	3.2143	1.53 to 6.73	3.09	p = 0.0020	3.9808	1.72 to 9.26	3.20	p = 0.0013
MCH	1.6745	1.31 to 2.14	4.11	p < 0.0001	3.3607	1.77 to 6.37	3.71	p = 0.0002
K^+	0.8386	0.34 to 2.09	0.38	p = 0.7053	0.8254	0.31 to 2.23	0.37	p = 0.7052
LDL	1.3273	1.07 to 1.64	2.59	p = 0.0094	0.2466	0.14 to 0.45	4.60	p < 0.0001
HDL	1.6771	1.24 to 2.27	3.34	p = 0.0008	2.709	1.54 to 4.76	3.46	p = 0.0005
TG	32.547	8.19 to 129.25	4.95	p < 0.0001	91.378	21.31 to 391.84	6.07	p < 0.0001
Mg^{2+}	1.9766	1.27 to 3.08	3.02	p = 0.0025	2.6697	1.44 to 4.95	3.11	p = 0.0018
Na^+	1.0684	0.45 to 2.52	0.15	p = 0.8799	1.0757	0.42 to 2.77	0.15	p = 0.7053
Ca^{2+}	3.869	2.31 to 6.46	5.17	p < 0.0001	7.2597	3.72 to 14.18	5.81	p < 0.0001

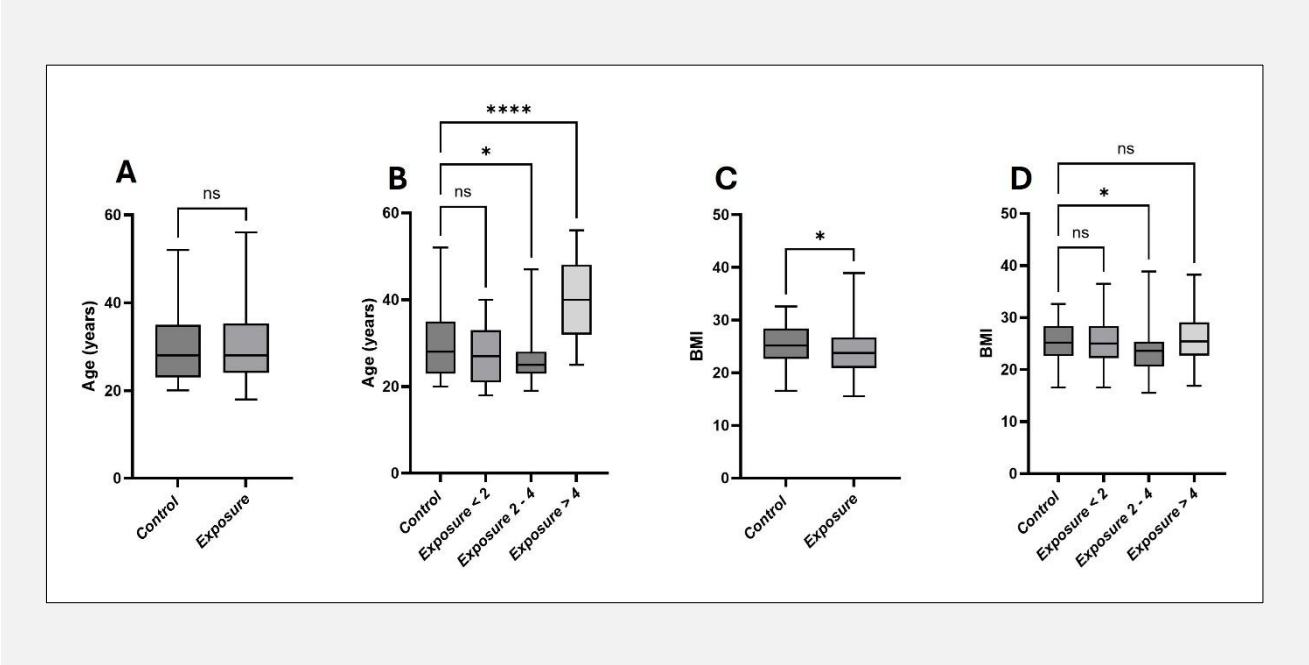

Figure 1. Comparison of age and BMI between control and benzene-exposed groups.

A: Age, C: BMI shows the distribution between control and exposed participants, while distributions across exposure subgroups stratified by duration (< 2 years, 2 - 4 years, and > 4 years) of age (B) and BMI (D). Each box represents the median and interquartile range (IQR), the horizontal line indicates the median, and whiskers represent the minimum and maximum values. ns: indicates non-significant comparisons, while * p < 0.05, ** p < 0.01, ** p < 0.001, and **** p < 0.0001.

strated statistically significant differences between the exposed and control groups. As presented in Figures 7 and summarized in Table 4, HGB, HCT, and WBC showed the highest classification performance, with AUC values of 0.980, 0.887, and 0.837, respectively ($p < 0.0001$ for all). Additional hematological indices, including RBC count, MCV, MCH, and MCHC), yielded AUCs between 0.667 and 0.763 ($p < 0.0001$). Among the biochemical parameters, TG displayed the highest AUC (0.994; 95% CI: 0.985 - 1.000; $p < 0.0001$), followed by Mg^{2+} (AUC = 0.779), ALP (AUC = 0.743), K^+ (AUC = 0.704), and PO_4^{3-} (AUC = 0.686),

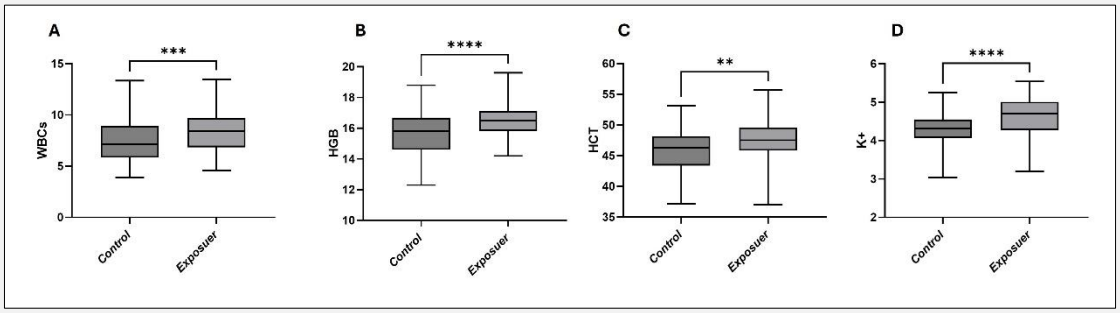

Figure 2. Box plots illustrating hematological and biochemical parameters in benzene-exposed and control groups.

Median and IQR of A: WBC count, B: HGB, C: HCT, and D: K+. The horizontal line indicates the median, and whiskers represent the minimum and maximum values. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

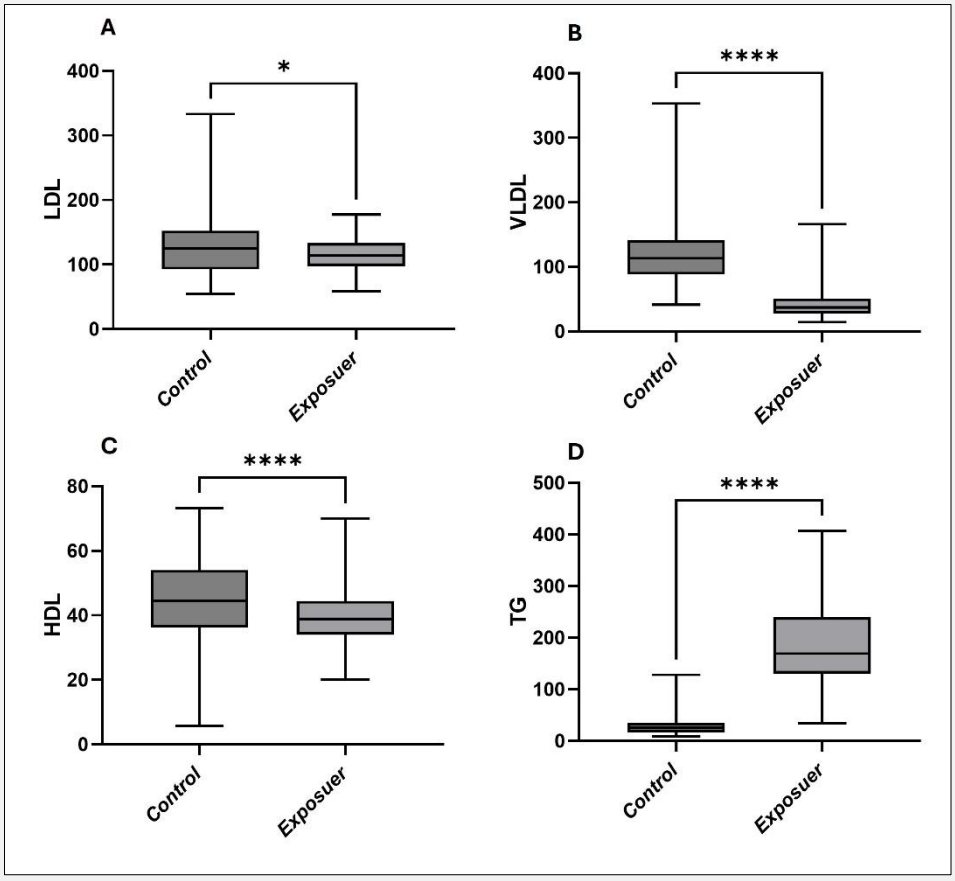

Figure 3. Box plots illustrating lipid profile levels in benzene-exposed and control groups.

Median and IQR of A: LDL, B: VLDL, C: HDL, and D: TG. The horizontal line indicates the median, and whiskers represent the minimum and maximum values. * $p < 0.05$, and **** $p < 0.0001$.

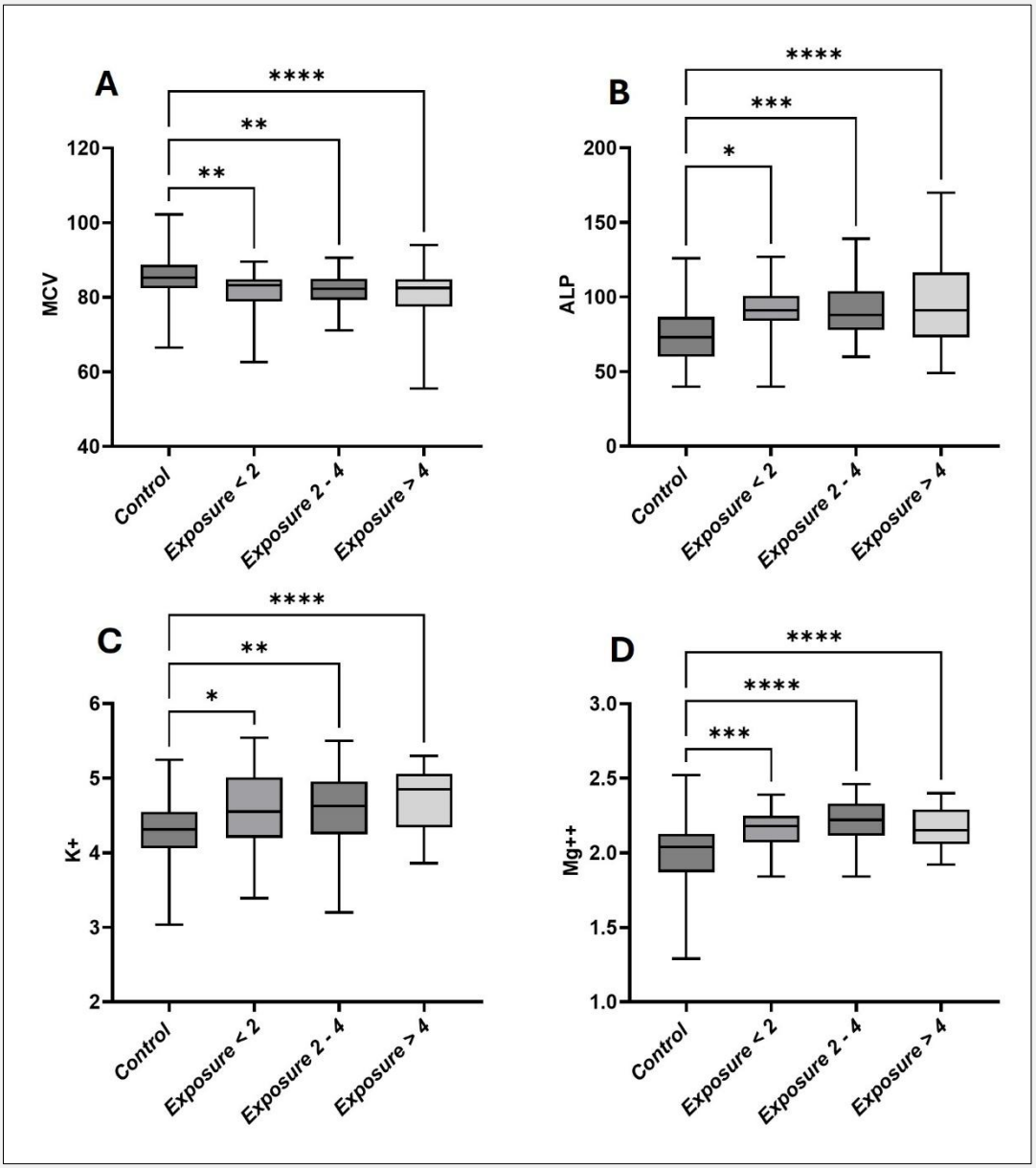

Figure 4. Box plots illustrating hematological and biochemical parameters across benzene exposure subgroups stratified by duration (< 2 years, 2 – 4 years, and > 4 years), with the control group included for comparison.

Median and IQR of A: MCV, B: ALP, C: K+, and D: Mg++. The horizontal line indicates the median, and whiskers show the minimum and maximum values. Statistical comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$, and **** $p < 0.0001$.

all with $p < 0.0001$. In contrast, Ca^2, HDL, LDL, and VLDL exhibited lower AUC values (< 0.60), indicating limited differentiating performance.

Prevalence ratio and odds ratio analysis of biochemical markers in relation to clinical outcomes

Table 5 explores the association between various blood and biochemical markers and clinical condition, utilizing prevalence ratios (PR) and odds ratios (OR) for statistical analysis. The results highlight significant associ-

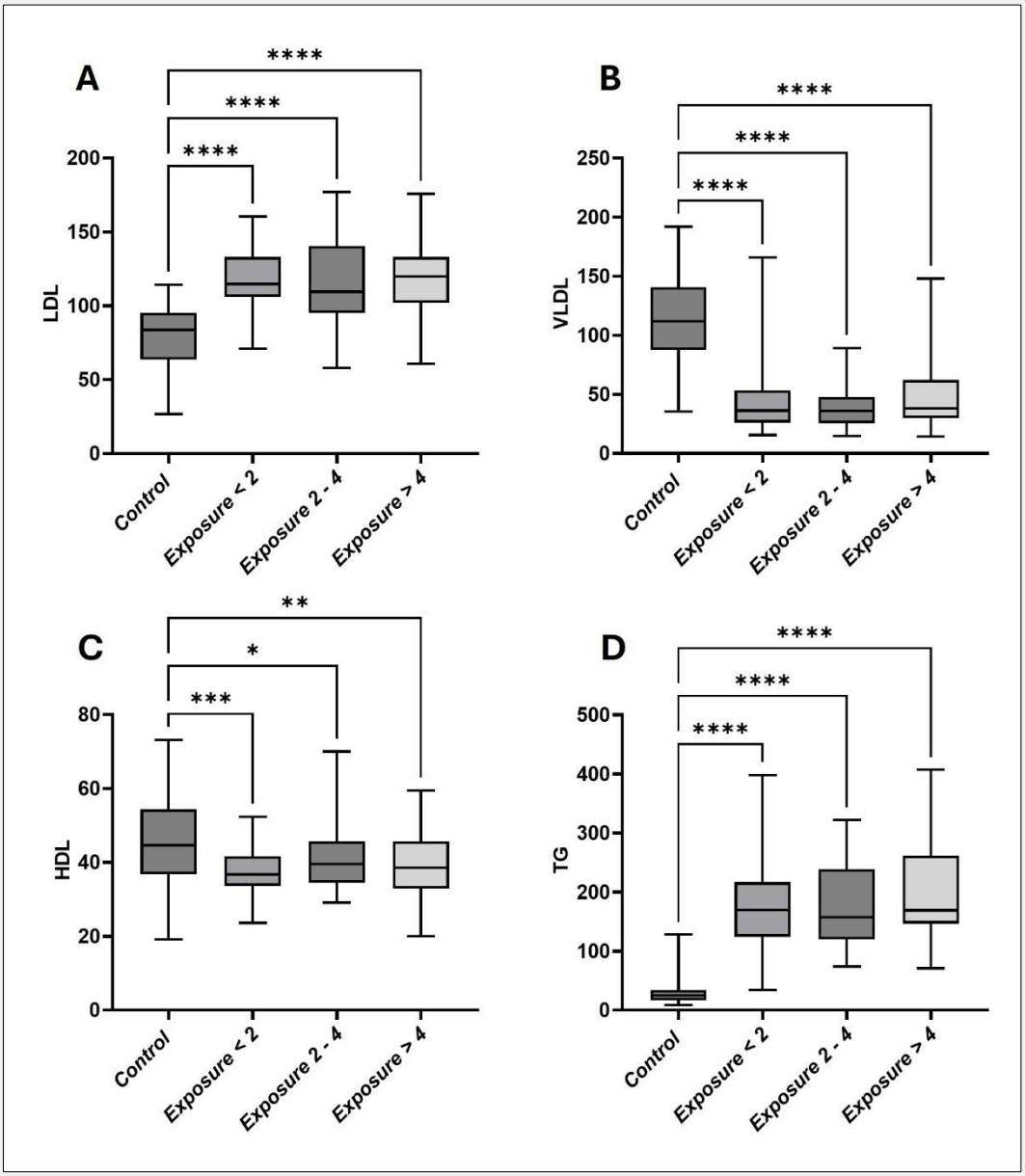

Figure 5. Box plots illustrating lipid profile levels across benzene exposure subgroups stratified by duration (< 2 years, 2 - 4 years, and > 4 years), with controls included for comparison.

Median and IQR of A: LDL, B: VLDL, C: HDL, and D: TG. The line within the box indicates the median, and whiskers show the full data range. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. ns: indicates non-significant comparisons, while * $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$, and **** $p < 0.0001$.

ations for several markers, including RBCs, MCV, MCH, LDL, HDL, TG, Mg^{2+}, and Ca^{2+}. These markers demonstrated substantial statistical significance, both in terms of prevalence and odds, suggesting their potential relevance in the clinical manifestation of the condition. RBC count was associated with a significantly higher prevalence (PR = 14.524) and odds ratio (OR = 82.82) in the exposed group, indicating a strong relationship with the condition. Similarly, MCV, MCH, HDL, TG, Mg^{2+}, and Ca^{2+} exhibited high PR and OR values, further supporting their potential role as biomarkers in the condition. Notably, TG displayed an exceptionally high OR of 91.38, reinforcing the strength of this association. The significant findings for these markers imply their

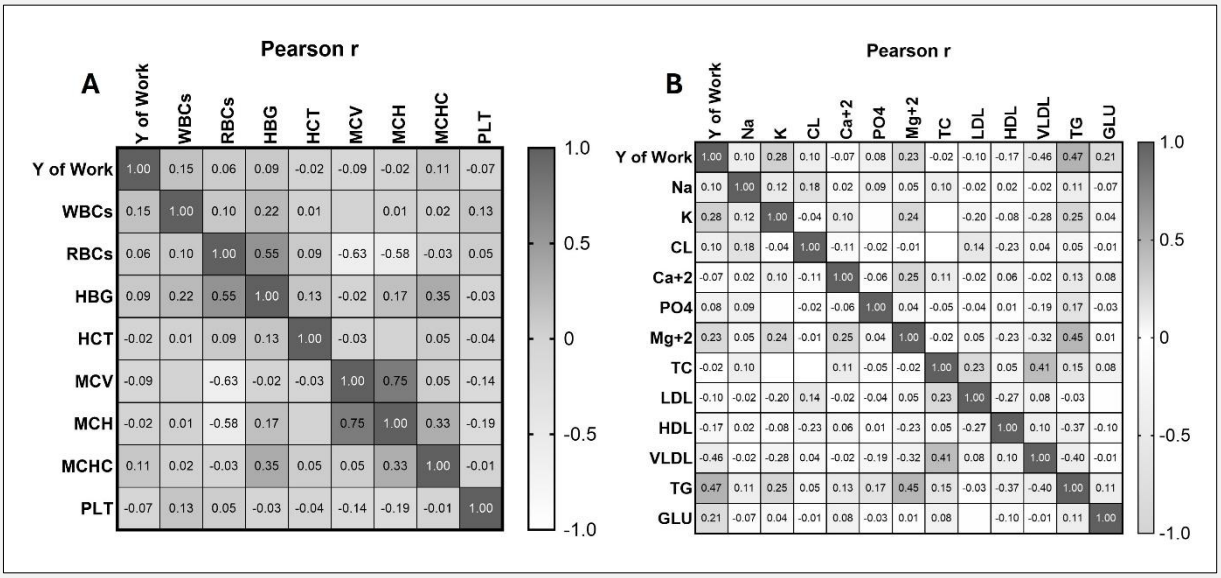

Figure 6. Heat map of Pearson´s correlations between work duration and hematological/biochemical parameters.

Heat maps display Pearson´s correlation coefficients (r) between years of work exposure and hematological (A) and biochemical (B) parameters. Blue indicates positive and red indicates negative correlations. Notable findings include strong negative correlations between RBC count and MCV/MCH, and inverse associations of years of exposure with VLDL and TG levels.

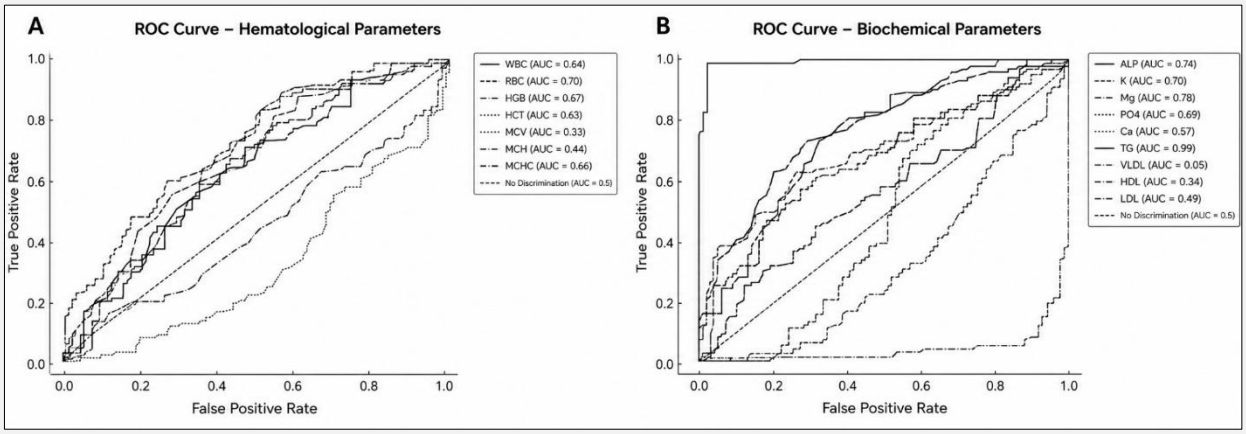

Figure 7. ROC curve analysis of hematological and biochemical parameters in benzene-exposed individuals.

Hematological parameters A: include RBC, HGB, HCT, WBC, MCV, MCH, and MCHC. Biochemical parameters B: include ALP, K^+, Mg^{2+}, PO_4^{3-}, Ca^{2+}, TG, HDL, LDL, and VLDL. The x-axis represents the false positive rate (1 - specificity), and the y-axis represents the true positive rate (sensitivity). The diagonal gray line indicates no discrimination performance (AUC = 0.5).

potential utility in diagnosing or monitoring the clinical condition, given their robust statistical associations.

In contrast, HGB and K+ did not exhibit a statistically significant association with the condition. Hemoglobin demonstrated a PR of 0.6827 and an OR of 0.5015, both suggesting a negligible difference between the affected and control groups, with a p-value of 0.1887 and 0.1396, respectively, indicating non-significance. Likewise, Na+ levels showed no meaningful association, with both PR and OR values indicating no significant difference between the groups, and corresponding p-values (0.7053 and 0.8799) further reinforcing the lack of statistical significance.

DISCUSSION

This study provides compelling evidence that chronic occupational exposure to benzene among gasoline station workers in southern Saudi Arabia is associated with significant perturbations in both hematological and biochemical profiles. Demographic analysis revealed no significant difference in age between exposed and control groups; however, BMI was significantly lower in exposed individuals. Furthermore, age was significantly elevated among those with more than four years of exposure, indicating a correlation between exposure duration and demographic variation.

The observed hematological alterations, including elevated HGB, HCT, and WBC counts, along with reductions in RBC indices such as MCV, MCH, and MCHC, suggest subclinical hematotoxic effects potentially induced by chronic benzene exposure. These findings may reflect early compensatory mechanisms, such as increased erythropoietic activity or alterations in erythrocyte morphology and lifespan, possibly due to oxidative damage or bone marrow stress. Mechanistically, benzene undergoes hepatic metabolism primarily via cytochrome P450 enzymes - particularly CYP2E1 - producing reactive intermediates such as hydroquinone, catechol, phenol, and benzoquinone [14,15]. These metabolites exert toxicity by generating reactive oxygen species (ROS), inducing oxidative stress, impairing mitochondrial membrane integrity, and inhibiting oxidative phosphorylation, ultimately disrupting hematopoietic progenitor cell function [11,16,17]. Several studies have shown that benzene exposure leads to suppression of hematopoietic stem cells, chromosomal aberrations, DNA strand breaks, and apoptosis, which contribute to both early hematologic changes and long-term carcinogenic outcomes [11,18,19]. Elevated HGB and HCT levels may reflect stress erythropoiesis due to transient hypoxia or shortened red blood cell survival caused by oxidative injury, whereas decreased MCV and MCH may indicate microcytosis or impaired hemoglobin synthesis [16,20].

Furthermore, ROC curve analysis in this study demonstrated that HGB, HCT, and WBC exhibited strong differentiating power, supporting their potential utility as early biomarkers of benzene-induced hematotoxicity. These findings are consistent with previous research documenting benzene's hematopoietic and leukemogenic toxicity and underscore the importance of early hematological monitoring in exposed populations [11,19, 21].

Biochemical assessment revealed elevated levels of TG, ALP, Mg^{2+}, K^{+}, and PO_4^{3-}, suggesting systemic biochemical disruptions extending beyond hematopoietic toxicity. Among these markers, TG exhibited the highest differentiating power for benzene exposure with an AUC of 0.994, highlighting its potential utility as a biomarker of systemic metabolic perturbation. This is consistent with findings by Qu et al. (2002), who reported dyslipidemia in benzene-exposed populations [22]. Elevated triglyceride levels may reflect hepatic dysfunction, altered lipid metabolism, or inflammatory responses, as benzene and its metabolites can disrupt peroxisome proliferator-activated receptor (PPAR) signaling and impair lipid homeostasis 6. The observed increases in ALP and PO_4^{3-} may indicate induction of hepatic enzymes or increased bone turnover, possibly linked to oxidative stress-mediated tissue remodeling or secondary effects on calcium-phosphate homeostasis. Benzene exposure has been shown to increase ALP activity due to hepatic microsomal enzyme induction and hepatocellular injury [17,23]. Furthermore, elevated PO_4^{3-} can result from impaired renal clearance or enhanced bone resorption due to systemic inflammation or oxidative damage [19,23].

Elevations in Mg^{2+} (PR = 3.46, $p < 0.0001$) and K^{+} (PR = 8.49, $p = 0.041$) further point to renal and cellular membrane involvement, as benzene metabolites can impair ion channel function and mitochondrial energy metabolism. Magnesium homeostasis is tightly regulated by the kidneys, and its elevation may reflect impaired renal excretion, potentially due to subclinical nephrotoxicity. Potassium elevation could result from intracellular leakage due to membrane instability, impaired Na^{+}/K^{+} ATPase activity, or renal tubular dysfunction all of which are plausible under the oxidative burden imposed by benzene metabolites [11,24]. These electrolyte disturbances align with reports of benzene-induced oxidative damage to renal and hepatic tissues, supported by increased lipid peroxidation and reduced antioxidant defenses [25]. Overall, the constellation of biochemical abnormalities underscores the multi-system toxicological impact of benzene exposure. In addition to its hematopoietic and leukemogenic effects, benzene appears to exert significant metabolic, hepatic, renal, and oxidative stress-mediated damage, consistent with its known capacity to disrupt cellular redox homeostasis, ion transport, and mitochondrial integrity [6,11,17,24,25].

The analysis of PR and OR provided robust quantitative support for the observed biochemical alterations associated with benzene exposure. Among the evaluated markers, PO_4^{3-} exhibited a statistically significant association with exposure (PR = 2.06, $p = 0.0068$), suggesting an increased likelihood of disrupted phosphate ho-

meostasis in affected individuals. This could reflect alterations in renal phosphate handling or bone remodeling activity, possibly secondary to benzene-induced oxidative stress and mitochondrial dysfunction [11,25]. Benzene and its metabolites are known to impair renal tubular function, which may contribute to phosphate retention and altered electrolyte excretion [26]. Remarkably, TG showed a dramatically elevated association (PR = 129.32, p = 0.0006), indicating a strong relationship between benzene exposure and dysregulation of lipid metabolism. This aligns with previous studies demonstrating benzene-induced hepatic steatosis, lipid peroxidation, and altered expression of lipid-regulatory enzymes such as lipoprotein lipase and acetyl-CoA carboxylase [27,28]. Benzene exposure has also been shown to influence the activity of nuclear receptors such as PPAR-α and SREBP-1, which play central roles in triglyceride synthesis and fatty acid oxidation [29]. Interestingly, VLDL showed an inverse association (PR = 0.70, p < 0.0001), which may indicate dysfunctional lipid transport or altered hepatic VLDL secretion. VLDL is the primary carrier of endogenously synthesized triglycerides, and its reduction may suggest impaired assembly or secretion of lipoproteins due to benzene-induced hepatocellular injury [17,30]. This finding supports the hypothesis that benzene disrupts the lipoprotein profile in a nonlinear and complex manner, possibly depending on the dose and duration of exposure. Additionally, HDL levels were modestly elevated (PR = 1.14, p = 0.0011), which stands in contrast to previous findings where HDL levels were often reduced in toxicant-exposed or metabolically-stressed populations.

This unexpected elevation may be indicative of population-specific metabolic responses or variability in benzene biotransformation pathways, which can differ based on genetic polymorphisms in detoxifying enzymes, nutritional status, or co-exposures [31,32]. Notably, interindividual variability in CYP2E1, NQO1, and GST polymorphisms can modulate the extent of benzene metabolism and its systemic toxicity [6,32]. Our findings show that chronic benzene exposure among fuel station workers is associated with significant hematological and biochemical changes, suggesting oxidative stress-mediated organ damage. This study is unique in focusing on workers in the high-altitude Asir region, where hypoxia may exacerbate benzene's effects.

Recent studies in Thailand and Brazil report fatigue, headache, dizziness, and hematological alterations among fuel station workers exposed to benzene, highlighting occupational risks and the importance of regular health monitoring. Although direct regional comparisons are limited, our study provides novel insights into benzene exposure under hypoxic conditions and emphasizes the need for region-specific preventive strategies [33,34].

Limitations

This study has several limitations. Its cross-sectional design limits the ability to establish causal relationships, and the findings may not be fully generalizable due to the specific population of fuel station workers. The lack of detailed exposure assessment (e.g. benzene concentration) prevents establishing dose-response relationships. Potential confounders, such as smoking and pre-existing health conditions, were not fully accounted for. Additionally, the study did not assess long-term clinical outcomes or explore the underlying toxicological mechanisms of the observed alterations. A more comprehensive evaluation, including a larger and more diverse sample, is needed for a clearer understanding of benzene's health impact.

CONCLUSION

In conclusion, this study provides valuable insights into the hematological and biochemical alterations associated with occupational benzene exposure. The results indicate that prolonged exposure to benzene vapors is linked to significant changes in hematological indices, electrolyte balance, lipid metabolism, and glucose regulation, all of which may contribute to the increased risk of developing chronic diseases. Biochemical markers, particularly those related to lipid metabolism and glucose control, show promise as potential early biomarkers for monitoring the health of individuals exposed to benzene. Further research is needed to better understand the long-term health implications of benzene exposure and to validate these biomarkers for clinical use.

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Consent to Participate and Publish:
The authors accept responsibility for the entire content of this submitted manuscript and approved submission.

Data Availability Statement:
All data used in this manuscript is available from the corresponding author and will be provided upon reasonable request for non-commercial purposes.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

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