

ORIGINAL ARTICLE

Impact of Chronic Benzene Exposure on Biochemical Profiles

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ABSTRACT

Background: Occupational exposure to benzene constitutes a considerable health risk, particularly for fuel station workers who experience chronic exposure to benzene vapors. This study aims to investigate biochemical markers indicative of vital organ functions in fuel station workers within the Asir region, Saudi Arabia.

Methods: A comparative cross-sectional analysis was conducted on 106 benzene-exposed fuel station workers and 100 matched healthy controls. Participants were categorized according to duration of benzene exposure (< 2, 2 - 4, and > 4 years). Blood samples were collected to evaluate hepatic, renal, cardiac, and pancreatic biochemical markers.

Results: Workers exposed to benzene showed significantly elevated serum levels of uric acid ($p = 0.002$), liver enzymes (ALT, AST, ALP; all $p < 0.001$), GGT ($p = 0.017$), total protein, albumin, and bilirubin (all $p < 0.001$). Notably, amylase levels were also significantly increased, indicating possible pancreatic involvement. Principal Component Analysis (PCA) revealed that the first two components explained 58% of the variance, with distinct clustering of hepatic and tissue injury markers, suggesting coordinated biochemical disruptions.

Conclusions: Chronic occupational exposure to benzene adversely affects biochemical parameters indicative of vital organ functions, potentially through benzene-induced oxidative stress mechanisms. Enhanced occupational health awareness and adherence to protective measures are strongly recommended to mitigate these risks among fuel station workers.

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KEYWORDS

benzene, Saudi Arabia, fuel workers, biochemical parameters, hematological parameters, Asir region

INTRODUCTION

Benzene is a clear, volatile solvent characterized by a sweet, gasoline-like odor, readily evaporating into vapors in the atmosphere. It is a significant organic constituent of petroleum and refined gasoline, produced primarily from the incomplete combustion of fossil fuels such as crude oil and coal [1]. Due to its chemical properties, benzene serves as an essential intermediate in the manufacturing of numerous industrial products. Common applications include use in vehicles, motorcycles, commercial transport, boating fuels, power generators, and as a solvent for oils and lipids [1].

Certain occupations, particularly fuel station workers, experience heightened exposure to benzene and its derivatives, as the volatile nature of petroleum products facilitates continuous environmental presence at fuel stations and storage facilities [2]. The adverse health effects associated with benzene exposure often result from its specific chemical constituents, including lead and oxygenates. Acute exposure to benzene vapors can cause respiratory issues, nausea, vomiting, headaches, dizziness, and irritation of the throat and nasal passages. Skin exposure may lead to dermatitis, redness, swelling, and, although rare, hypersensitivity reactions [1].

Benzene, toluene, ethylbenzene, and xylene isomers [collectively known as BTEX] represent harmful volatile organic compounds commonly found in gasoline. Numerous studies have reported that BTEX exposure adversely affects neurological development and contributes to chronic health conditions [3]. Specifically, benzene exposure is linked to severe hematological disorders such as leukemia, lymphoma, aplastic anemia, pancytopenia, and chromosomal abnormalities, alongside detrimental effects on pulmonary, hepatic, renal, and nervous system functions [4].

Benzene enters the human body primarily via inhalation and dermal absorption. Upon inhalation, benzene is rapidly absorbed, metabolized, and excreted in the urine as intermediate metabolites such as phenol, benzene oxide, benzoquinone, monoaldehydes, hydroquinone, and catechol [5]. The liver and kidneys are vital organs responsible for detoxification, metabolism, and homeostasis, including the elimination of harmful substances. Hepatic cytochrome P450 2E1 metabolizes benzene to reactive metabolites, generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress, lipid peroxidation, and damage to nucleic acids, enzymes, and structural proteins [6].

Oxidative stress caused by benzene and its metabolites significantly impacts organ function, notably hepatic and pancreatic cells. Reduced antioxidant enzyme levels in pancreatic cells make them particularly vulnerable to ROS-induced oxidative damage, potentially impairing glucose metabolism and pancreatic function [7-9]. Chronic benzene exposure severity depends on factors such as dose, route, duration, individual age, and health status, frequently resulting in hepatic and renal toxicity [10].

Despite extensive research on benzene's systemic toxicity, region-specific data on its subclinical biochemical effects remain limited, particularly in occupational settings involving sustained low-to-moderate exposure levels. In Saudi Arabia, gasoline station workers frequently experience benzene and other volatile organic compounds (VOCs) exposure through routine fuel handling. This study investigates benzene exposure effects on vital organ function by assessing biochemical parameters related to liver, kidney, cardiac, and pancreatic health among fuel station workers in the Asir region of southern Saudi Arabia.

MATERIALS AND METHODS

Study design and ethical approval

This cross-sectional research was carried out in Saudi Arabia's southern Asir region. Approval from the Research Ethics Committee at King Khalid University was secured (Approval No. ECM#2023-608), ensuring compliance with ethical standards. The study followed the ethical guidelines of the Declaration of Helsinki (1975), including its 2013 revisions. Before participating, all individuals were briefed on the study's purpose and submitted written informed consent. To maintain privacy, participant information was anonymized.

Study population

The study included 106 fuel station workers (aged 18 - 56 years) with regular occupational exposure to benzene, working 8 - 12 hours per day for six days weekly. Participants were excluded if they had pre-existing liver, kidney, heart, or pancreatic conditions or were taking medications that could alter relevant biochemical parameters. A control group of 100 age-matched volunteers (20 - 52 years) without benzene exposure was also recruited. All participants provided written informed consent before enrollment.

Demographic and anthropometric data - including age, marital status, weight, height, and BMI - were documented. Based on exposure duration, workers were categorized into three subgroups: Group 1 (< 2 years exposure, n = 23), Group 2 (2 - 4 years, n = 42), and Group 3 (> 4 years, n = 41). This stratification allowed for analysis of potential dose-dependent effects of benzene exposure.

Sample collection and analysis

Certified laboratory technicians collected venous blood samples from all participants under sterile conditions, following standard phlebotomy procedures. Using disposable needles and vacuum tubes, 5 mL of blood was drawn from the antecubital vein and placed in serum separator tubes (SST). The samples were then centrifuged at 4,000 rpm for 5 minutes to isolate serum. For fuel station workers, blood draws were performed at their workplace administrative offices during normal shifts.

Biochemical analysis was conducted at Asser Central Hospital's laboratory using a Cobas 6000 analyzer (Roche Diagnostics, Germany). The automated system measured liver function tests (LFT), kidney function tests (KFT), cardiac markers (CFT), and serum amylase levels, adhering to strict quality control protocols to ensure precise and consistent results.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 9.5.1 (GraphPad Software, Inc., San Diego, CA, USA). Data were assessed for normality prior to analysis. As most variables were not 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. Results are presented as mean $\pm$ standard deviation (SD) and visualized using box plots. Pearson's correlation coefficient (*r*) was used to evaluate the strength and significance of associations between continuous variables.

Package R (R 4.3.2) were used to assess Receiver Operating Characteristic (ROC). A *p*-value < 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of study participants

A total of 206 individuals participated in this study, with data presented as the median and interquartile range (IQR). Participants were divided into two groups: the benzene-exposed group, consisting of 106 fuel station workers (51%), and the unexposed control group, comprising 100 individuals (49%). Among the exposed group, 50 participants were single and 56 were married, while the control group included 54 single and 46 married individuals.

The mean age of participants in the exposed and unexposed groups was 30.43 ± 8.9 and 30.16 ± 8.4 years, respectively, with no statistically significant difference observed ($p = 0.82$). Similarly, the mean BMI did not differ significantly between the two groups (24.5 ± 4.6 vs. 25.4 ± 3.5 kg/m²; $p = 0.09$), as shown in Figure 1 (A & C) and Table 1. However, when analyzed by duration of occupational exposure, participants with more than four years of benzene exposure had a significantly higher average age compared to controls ($p < 0.0001$), as shown in Figure 1 (B). Additionally, a significant difference in BMI was found among exposed subjects when stratified by exposure duration ($p = 0.008$), as illustrated in Figure 1 (B & D) and Table 2.

Comparison of renal, hepatic, cardiac, and pancreatic parameters between exposed and control groups

Assessment of alterations in biochemical markers between the benzene-exposed and control groups is presented in Table 1 and Figure 2. Renal function assessment revealed that serum creatinine levels were slightly but significantly elevated in the exposed group compared to controls (0.94 ± 0.01 vs. 0.90 ± 0.11 mg/dL; $p = 0.026$). In contrast, urea levels did not differ significantly between the two groups ($p = 0.99$). Uric acid levels were significantly lower in the exposed group than in controls (5.55 ± 0.99 vs. 6.03 ± 1.18 mg/dL; $p = 0.0014$), suggesting possible alterations in purine metabolism associated with benzene exposure.

Liver function enzymes showed significant reductions in the exposed group, with serum ALT, AST, and ALP levels markedly decreased (all $p < 0.0001$), while GGT

levels were modestly reduced ($p = 0.039$). Additionally, total protein and albumin concentrations were significantly lower among exposed individuals (TP: 7.57 ± 0.56 vs. 7.92 ± 0.46 g/dL; Albumin: 4.65 ± 0.45 vs. 5.02 ± 0.34 g/dL; $p < 0.0001$ for both), indicating a potential compromise in hepatic protein synthesis. Total bilirubin levels were also significantly lower in the exposed group (0.48 ± 0.23 vs. 0.67 ± 0.39 mg/dL; $p < 0.0001$), whereas direct bilirubin levels showed no statistically significant difference ($p = 0.54$).

Cardiac and pancreatic biomarkers, including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and amylase, were significantly reduced in the exposed group compared to controls, with *p*-values < 0.0001 for all three parameters, suggesting potential subclinical alterations in myocardial and pancreatic function.

Stratified analysis of renal, hepatic, cardiac, and pancreatic parameters based on duration of benzene exposure

A stratified comparison of biochemical parameters was conducted between the control group and three benzene-exposed subgroups, categorized by duration of occupational exposure: less than 2 years, 2 to 4 years, and more than 4 years as presented in Figure 3 and Table 2. The analysis revealed several significant differences across groups.

In the subgroup with less than two years of exposure, serum creatinine levels were significantly lower than those of the control group (0.85 ± 0.09 vs. 0.94 ± 0.11 mg/dL), whereas creatinine values in the 2 - 4 year and > 4 year groups were similar to those in controls (0.93 ± 0.11 and 0.90 ± 0.12 mg/dL, respectively; $p = 0.0036$). In contrast, uric acid concentrations were significantly elevated in all exposed subgroups compared to controls (5.42 ± 0.84 mg/dL), with the greatest increase observed in the < 2 year group (6.26 ± 1.24 mg/dL), followed by the > 4 year (6.14 ± 0.75 mg/dL) and 2 - 4 year (5.90 ± 0.97 mg/dL) groups ($p < 0.0001$). No significant differences in urea levels were observed across any of the exposure groups.

Liver function enzymes (ALT, AST, ALP, and GGT) demonstrated a progressive increase with longer durations of exposure. ALT levels, for example, ALT rose from 17.65 ± 7.46 U/L in the control group to 27.23 ± 10.67 , 29.26 ± 15.12 , and 31.51 ± 17.66 U/L in the < 2 , 2 - 4, and > 4 year exposure groups, respectively ($p < 0.0001$). Similar trends were observed for AST, ALP, and GGT, indicating potential cumulative hepatic stress or enzyme induction associated with prolonged benzene exposure (all $p < 0.0001$).

Total protein and albumin levels were significantly higher in all exposed subgroups compared to controls. Albumin levels increased from 4.65 ± 0.45 g/dL in the control group to 5.01 ± 0.28 , 5.01 ± 0.25 , and 5.07 ± 0.40 g/dL in the < 2 , 2 - 4, and > 4 year exposure groups, respectively ($p < 0.0001$). Total bilirubin levels also showed a marked increase, especially in the < 2 year group (0.75 ± 0.48 mg/dL vs. 0.47 ± 0.19 mg/dL in

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

Categories	Non-Exposures (control sample)	Exposures sample	p-value
Number of samples	n = 100	n = 106	-
Age	30.16 ± 8.4	30.43 ± 8.9	0.822
BMI	25.4 ± 3.5	24.5 ± 4.6	0.099
Creatinine	0.90 ± 0.11	0.94 ± 0.01	0.026*
Urea	26.7 ± 5.1	26.8 ± 6.2	0.99
Uric acid	6.03 ± 1.18	5.55 ± 0.99	0.0014 **
ALT	27.82 ± 16.11	17.65 ± 7.5	< 0.0001 ****
AST	25.0 ± 9.2	18.77 ± 5.4	< 0.0001 ****
ALP	93.9 ± 24.0	75.06 ± 20.8	< 0.0001 ****
GGT	25.97 ± 11.27	22.47 ± 9.23	0.039 *
TP	7.92 ± 0.46	7.57 ± 0.56	< 0.0001 ****
Albumin	5.02 ± 0.34	4.65 ± 0.45	< 0.0001 ****
T. BIL	0.67 ± 0.39	0.48 ± 0.23	< 0.0001 ****
D. BIL	0.19 ± 0.08	0.19 ± 0.07	0.54
CK-MB	22.02 ± 10.12	15.44 ± 4.22	< 0.0001 ****
LDH	211.9 ± 48.11	157.5 ± 29.39	< 0.0001 ****
Amylase	85.51 ± 29.86	68.23 ± 21.75	< 0.0001 ****

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

Variable	Control group	Exposures group < 2 y	Exposures group from 2 - 4 y	Exposures of > 4 y	p-value
	(n = 100)	(n = 23)	(n = 42)	(n = 41)	
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 **
Creatinine	0.94 ± 0.11	0.85 ± 0.09	0.93 ± 0.11	0.90 ± 0.12	0.0036 **
Urea	26.8 ± 6.19	25.70 ± 5.33	27.58 ± 6.37	27.03 ± 4.73	0.67
Uric acid	5.42 ± 0.84	6.26 ± 1.24	5.9 ± 0.97	6.14 ± 0.75	< 0.0001 ****
ALT	17.65 ± 7.46	27.23 ± 10.67	29.26 ± 15.12	31.51 ± 17.66	< 0.0001 ****
AST	18.77 ± 5.41	23.35 ± 5.73	24.80 ± 7.77	26.50 ± 11.31	< 0.0001 ****
ALP	75.06 ± 20.80	90.65 ± 20.12	91.76 ± 17.47	96.07 ± 29.10	< 0.0001 ****
GGT	21.33 ± 7.46	26.26 ± 7.50	27.0 ± 7.20	29.29 ± 12.10	< 0.0001 ****
TP	7.11 ± 0.70	7.85 ± 0.44	7.95 ± 0.51	7.92 ± 0.44	< 0.0001 ****
Albumin	4.65 ± 0.45	5.01 ± 0.28	5.01 ± 0.25	5.07 ± 0.40	< 0.0001 ****
T. BIL	0.47 ± 0.19	0.75 ± 0.48	0.62 ± 0.30	0.63 ± 0.37	< 0.0001 ****
D. BIL	0.19 ± 0.07	0.22 ± 0.10	0.19 ± 0.07	0.19 ± 0.09	0.53
CK-MB	15.44 ± 4.2	18.99 ± 8.10	21.99 ± 8.40	23.79 ± 12.39	< 0.0001 ****
LDH	157.5 ± 29.39	208.8 ± 47.26	208.5 ± 47.09	216.6 ± 50.38	< 0.0001 ****
Amylase	67.73 ± 20.44	75.13 ± 17.97	82.24 ± 27.73	94.61 ± 34.84	< 0.0001 ****

Table 3. Pearson's correlation coefficient between parameters and duration of work in fuel stations.

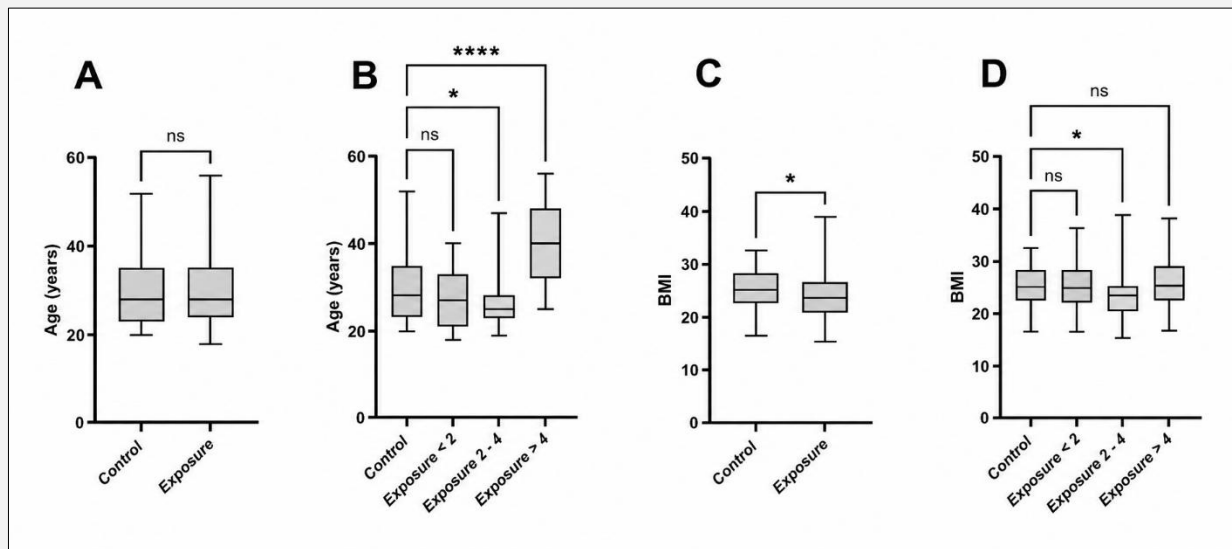
	r	95% confidence interval	p-value
BMI	-0.09221	-0.23 to 0.05	0.18
Age	0.1543	0.014 to 0.29	0.026 *
Creatinine	-0.1004	-0.24 to 0.04	0.15
Urea	0.02435	-0.12 to 0.16	0.72
Uric acid	0.2902	0.16 to 0.41	< 0.0001 ****
AST	0.394	0.27 to 0.51	< 0.0001 ****
ALT	0.393	0.27 to 0.51	< 0.0001 ****
GGT	0.1847	0.045 to 0.32	0.007 ***
T. BIL	0.2581	0.12 to 0.38	0.0002 ***
D. BIL	-0.02487	-0.1650 to 0.1162	0.72
TP	0.2854	0.15 to 0.41	< 0.0001 ****
Albumin	0.3619	0.23 to 0.48	< 0.0001 ****
CK-MB	0.3713	0.24 to 0.49	< 0.0001 ****
LDH	0.5325	0.42 to 0.63	< 0.0001 ****
Amylase	0.2736	0.14 to 0.39	< 0.0001 ****

Table 4. Association of biochemical parameters with benzene exposure.

		Score	95% CI	z Statistic	p-value
Creatinine	PR	0.943	0.06 to 14.89	0.041	p = 0.97
	OR	0.943	0.06 to 15.28	0.041	p = 0.97
Urea	PR	0.972	0.79 to 1.18	0.289	p = 0.77
	OR	0.919	0.52 to 1.64	0.289	p = 0.77
Uric Acid	PR	14.15	1.90 to 105.17	2.589	p = 0.009 ***
	OR	16.32	2.11 to 126.03	2.677	p = 0.007 ***
AST	PR	9.91	2.38 to 41.16	3.155	p = 0.001 ***
	OR	12.11	2.76 to 53.14	3.304	p = 0.001 ***
ALT	PR	31.15	1.89 to 512.43	2.407	p = 0.016 *
	OR	36.65	2.17 to 619.65	2.496	p = 0.012 *
GGT	PR	21.710	1.30 to 363.64	2.14	p = 0.032 *
	OR	24.20	1.41 to 416.47	2.195	p = 0.028 *
ALP	PR	14.16	0.82 to 244.73	1.823	p = 0.068
	OR	15.15	0.85 to 268.86	1.852	p = 0.064
T. BIL	PR	3.53	1.01 to 12.27	1.981	p = 0.048 *
	OR	3.82	1.03 to 14.14	2.01	p = 0.044 *
D. BIL	PR	0.94	0.66 to 1.33	0.372	p = 0.709
	OR	0.89	0.49 to 1.61	0.373	p = 0.709
TP	PR	4.25	1.83 to 9.85	3.368	p = 0.0008 ***
	OR	5.35	2.10 to 13.62	3.522	p = 0.0004 ***
Albumin	PR	1.89	0.35 to 10.08	0.743	p = 0.457
	OR	1.92	0.34 to 10.73	0.744	p = 0.456
CK-MB	PR	29.25	4.07 to 210.24	3.354	p = 0.0008 ***
	OR	40.92	5.46 to 306.57	3.612	p = 0.0003 ***
LDH	PR	0.43	0.26 to 0.74	3.142	p = 0.0017 **
	OR	0.33	0.17 to 0.65	3.232	p = 0.0012 **
Amylase	PR	4.48	1.58 to 12.72	2.818	p = 0.0048 **
	OR	5.24	1.72 to 16.01	2.908	p = 0.0036 **

Table 5. Diagnostic sensitivity and specificity of biochemical markers distribution width in all study subjects.

Variable	AUC (95% CI)	Cutoff value	Sensitivity	Specificity	p-value
Creatinine mg/dL	0.572 (0.494 - 0.651)	0.855	34.9	81	0.073
Urea mg/dL	0.504 (0.425 - 0.584)	18.9	98.1	11	0.912
Uric acid mg/dL	0.687 (0.615 - 0.759)	5.45	78.3	53	< 0.0001 ****
AST U/L	0.754 (0.689 - 0.820)	19.65	75.5	64	< 0.0001 ****
ALT U/L	0.751 (0.683 - 0.818)	19.75	71.7	73	< 0.0001 ****
GGT U/L	0.618 (0.542 - 0.694)	21.5	68.9	52	0.003 ***
TP g/dL	0.707 (0.637 - 0.777)	7.685	74.5	58	< 0.0001 ****
Albumin g/dL	0.750 (0.682 - 0.818)	4.785	86.8	58	< 0.0001 ****
ALP U/L	0.743 (0.676 - 0.811)	79.5	73.6	68	< 0.0001 ****
T. BIL mg/dL	0.665 (0.591 - 0.739)	0.4	84.9	45	< 0.0001 ****
D. BIL mg/dL	0.499 (0.419 - 0.580)	0.298	51.7	95	0.988
CKMB U/L	0.719 (0.649 - 0.788)	21.1	46.2	91	< 0.0001 ****
LDH U/L	0.824 (0.769 - 0.879)	178.5	72.6	78	< 0.0001 ****
Amylase U/L	0.674 (0.601 - 0.746)	85.5	81.4	83	< 0.0001 ****


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

Age A, 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 IQR, the horizontal line indicates the median, and whiskers represent the minimum and maximum values. “ns” indicates non-significant comparisons while (*) for $p < 0.05$, and (****) for $p < 0.0001$.

controls; $p < 0.0001$), whereas direct bilirubin levels did not significantly differ across groups ($p = 0.53$). Cardiac biomarkers exhibited significant, exposure-dependent increases. CK-MB levels rose from $15.44 \pm$

4.20 U/L in controls to 18.99 ± 8.10 , 21.99 ± 8.40 , and 23.79 ± 12.39 U/L in the < 2, 2 - 4, and > 4 year groups, respectively ($p < 0.0001$). LDH levels similarly increased, peaking at 216.6 ± 50.38 U/L in the > 4 year

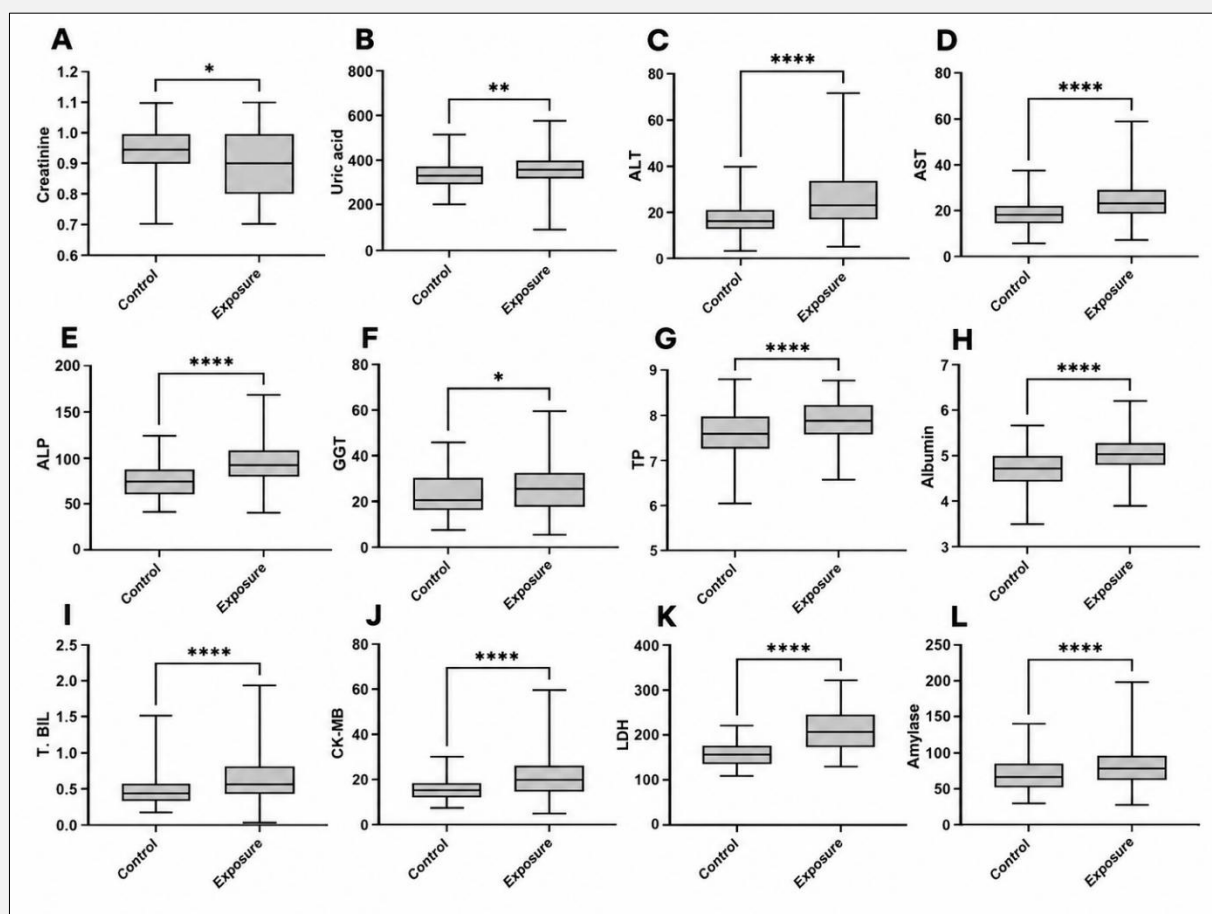


Figure 2. Box plots illustrating biochemical parameters in benzene-exposed and control groups.

Median and IQR of A: Creatinine, B: Uric acid, C: ALT, D: AST, E: ALP, F: GGT, G: TP, H: Albumin, I: T. Bil, J: CK-MB, K: LDH and L: Amylase. The horizontal line indicates the median, and whiskers represent the minimum and maximum values. (*) indicates significant comparisons for $p < 0.05$, (**) for $p < 0.01$, and (****) for $p < 0.0001$.

group compared to 157.5 ± 29.39 U/L in controls ($p < 0.0001$). Amylase levels followed a comparable pattern, increasing significantly with exposure duration and reaching 94.61 ± 34.84 U/L in the > 4 year group versus 67.73 ± 20.44 U/L in controls ($p < 0.0001$).

Correlation between duration of occupational exposure and biochemical parameters

Pearson's correlation analysis was performed to investigate the relationship between the duration of employment at fuel stations and selected anthropometric and biochemical parameters. The results demonstrated several statistically significant correlations, as illustrated in Table 3. A positive and statistically significant correlation was observed between duration of exposure and age ($r = 0.1543$; $p = 0.026$), indicating that older partici-

pants tended to have longer employment histories. While BMI showed a weak inverse correlation with exposure duration ($r = -0.0922$), this was not statistically significant ($p = 0.18$).

Among renal markers, uric acid showed a significant positive correlation with exposure duration ($r = 0.2902$; $p < 0.0001$). Creatinine and urea were not significantly correlated with exposure duration ($p = 0.15$ and 0.72 , respectively). A strong positive correlation was identified between exposure duration and liver enzymes, including AST ($r = 0.394$; $p < 0.0001$), ALT ($r = 0.393$; $p < 0.0001$), and GGT ($r = 0.1847$; $p = 0.007$).

Additionally, significant positive correlations were found for TP ($r = 0.2854$; $p < 0.0001$), albumin ($r = 0.3619$; $p < 0.0001$), and T. Bil ($r = 0.2581$; $p = 0.0002$), while D. BIL was not significantly associated

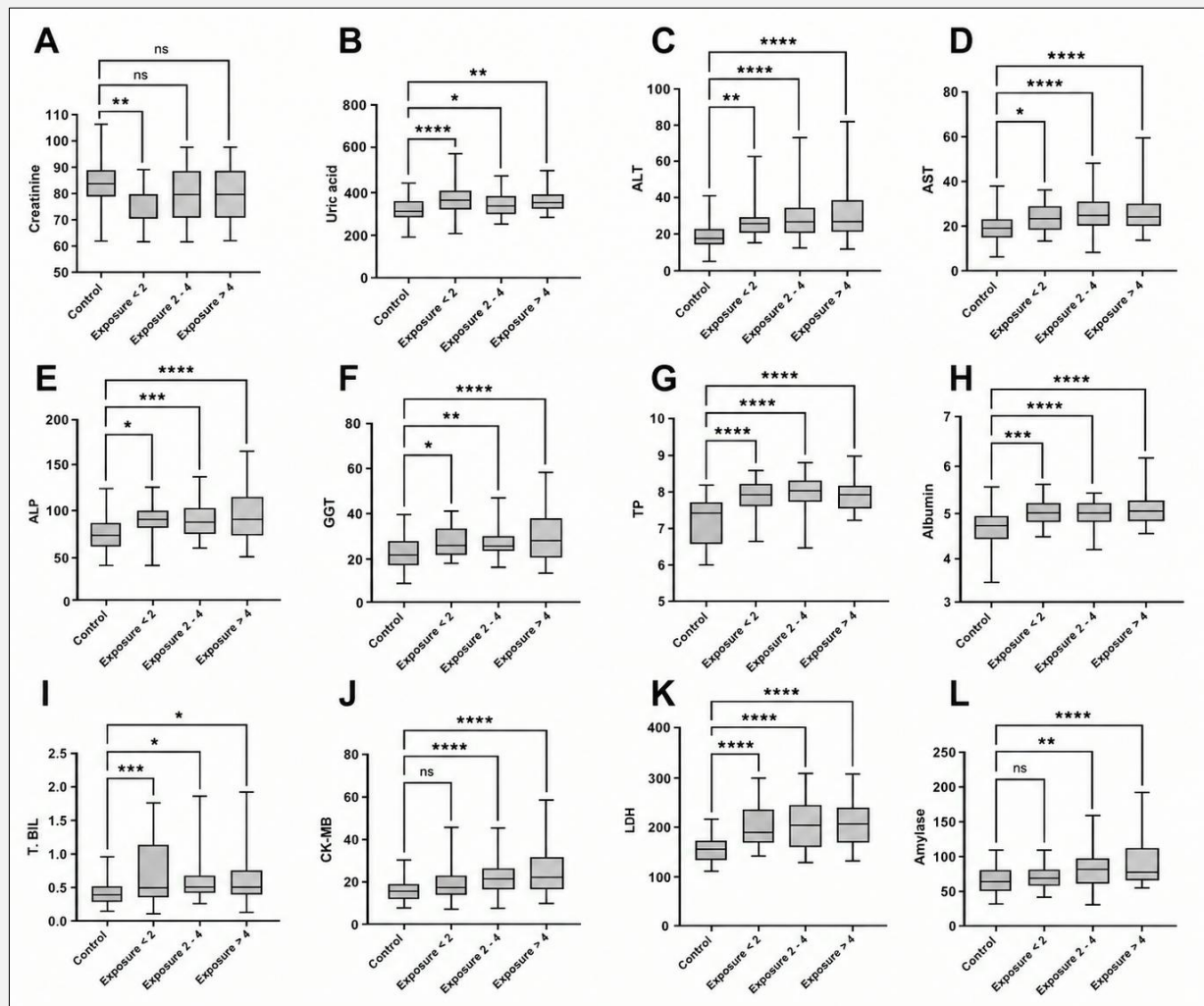


Figure 3. Box plots illustrating biochemical parameters across benzene exposure subgroups stratified by duration (< 2 years, 2 - 4 years, and > 4 years), with the control group included for comparison.

Median and IQR of A: Creatinine, B: Urea, C: Uric acid, D: ALT, E: AST, F: ALP, G: GGT, H: TP, I: Albumin, J: T. BIL, K: D. BIL, L: CK-MB, M: LDH and N: Amylase. The horizontal line indicates the median, and whiskers represent the minimum and maximum values. “ns” indicates non-significant comparisons while (*) for $p < 0.05$, (**) for $p < 0.01$, (***) for $p < 0.001$, and (****) for $p < 0.0001$.

with duration of exposure ($p = 0.72$). Cardiac markers also demonstrated strong associations. Both CK-MB ($r = 0.3713$; $p < 0.0001$) and LDH ($r = 0.5325$; $p < 0.0001$) exhibited significant positive correlations. Similarly, amylase, a pancreatic marker, showed a significant positive correlation ($r = 0.2736$; $p < 0.0001$).

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

Table 4 explores the association between various biochemical markers and clinical condition, utilizing prevalence ratios (PR) and odds ratios (OR) for statistical

analysis. The results highlight significant associations for several markers, including kidney, liver, cardiac and pancreas function tests. These markers demonstrated substantial statistical significance, both in terms of prevalence and odds, suggesting their potential relevance in the clinical manifestation of the condition.

ALT count was associated with a significantly higher prevalence (PR = 31.15) and odds (OR = 36.65) in the exposed group, indicating a strong relationship with the condition. Similarly, uric acid, AST, GGT, ALP, CK-MB exhibited high PR and OR values, further supporting their potential role as biomarkers in the condition.

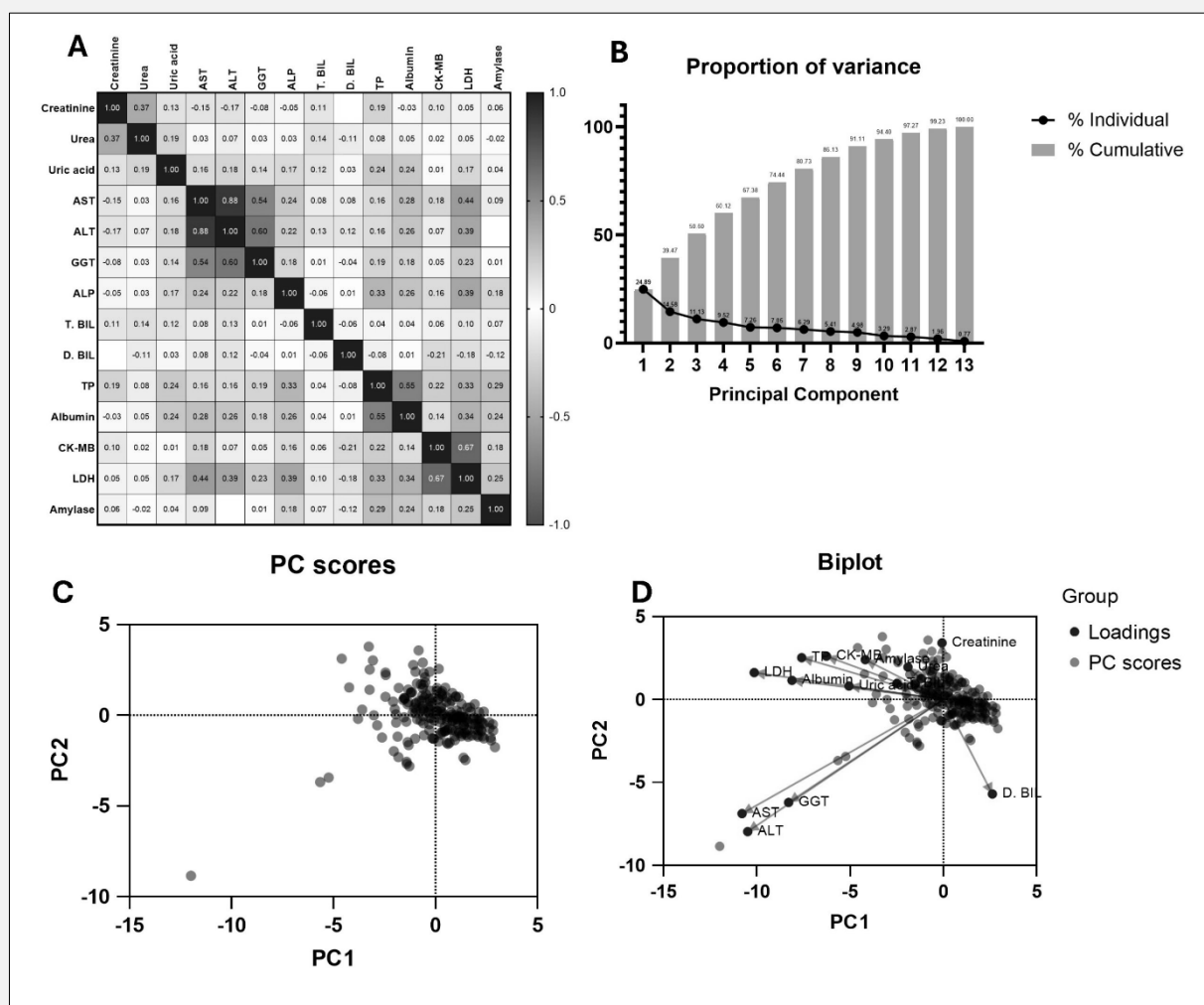


Figure 4. Principal component analysis (PCA) of interrelationships between clinical biomarkers and their association with benzene exposure.

A: Correlation matrix heatmap showing pairwise Pearson's correlation coefficients among biomarkers, with blue indicating positive correlations and red indicating negative correlations (p-values in parentheses). **B:** Scree plot displaying the proportion of variance explained by each principal component, with cumulative variance shown in grey bars and individual variance in black dots. **C:** PCA score plot showing the distribution of individual cases along the first two principal components (PC1 and PC2). **D:** PCA biplot combining variable loadings (blue vectors) and individual scores (grey points), indicating the contribution of each biomarker to group separation. Biomarkers such as creatinine, amylase, uric acid, LDH, and CK-MB showed stronger positive loadings along PC1, suggesting a close association with benzene exposure, while AST, ALT, and GGT contributed predominantly along PC2, indicating potential liver-related alterations in exposed individuals.

Notably, CK-MB displayed an exceptionally high OR of 40.92, reinforcing the strength of this association. The significant findings for these markers imply their potential utility in diagnosing or monitoring the clinical condition, given their robust statistical associations as well as amylase.

In contrast, creatinine, urea and direct bilirubin did not exhibit a statistically significant association with the condition. Creatinine demonstrated both PR and OR of

0.943, both suggesting a negligible difference between the affected and control groups, with a p-value of 0.97, indicating non-significance.

Principal component analysis (PCA) of biochemical markers in benzene-exposed workers

PCA was conducted to identify patterns and correlations among biochemical markers in benzene-exposed fuel station workers. The first two principal components

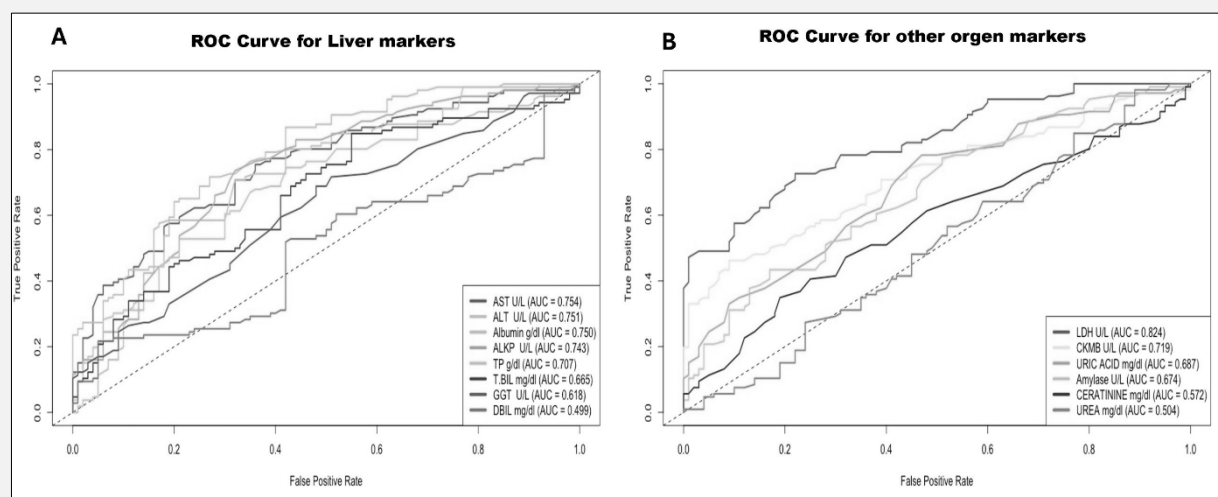


Figure 5. ROC curve analysis of biochemical parameters in benzene-exposed individuals.

Liver parameters (A) include AST, ALT, ALP, GGT, TP, Albumin, T&D, bilirubin, kidney, heart, and pancreatic parameters (B) include creatinine, uric acid, urea, CK-MB, LDH and amylase. 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. ROC.

(PC1 and PC2) accounted for 53% of the total variance, with PC1 alone explaining 39.4%, as shown in Figure 4. The PCA biplot demonstrated distinct clustering of parameters. ALT, AST, and GGT showed strong negative loadings on PC1, indicating their significant contribution to hepatic variability. In contrast, LDH, CK-MB, albumin, and TP exhibited positive loadings on PC1, reflecting associations with tissue injury and protein metabolism. Direct bilirubin contributed mainly to PC2, indicating a separate dimension of variability with minimal influence on PC1. These findings highlight coordinated shifts in liver and cardiac biomarkers and suggest distinct biochemical patterns associated with the duration and extent of occupational benzene exposure.

Diagnostic utility of biochemical markers in fuel station workers

Receiver Operating Characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of selected biochemical markers in distinguishing fuel station workers from unexposed controls. As illustrated in Figure 5 and Table 5, ROC analysis revealed LDH as the most discriminative marker (AUC = 0.824, 95% CI: 0.769 - 0.879, sensitivity 72.6%, specificity 78%, $p < 0.0001$). AST (AUC = 0.754), ALT (AUC = 0.751), albumin (AUC = 0.750), ALP (AUC = 0.743), and CKMB (AUC = 0.719) also demonstrated good diagnostic performance, with balanced sensitivity and specificity values ranging between 64 - 91%. Total protein,

uric acid, amylase, and T. BIL showed fair accuracy (AUC = 0.665 - 0.707), whereas GGT had lower accuracy (AUC = 0.618) but remained statistically significant ($p = 0.003$). In contrast, creatinine (AUC = 0.572), urea (AUC = 0.504), and D. BIL (AUC = 0.499) exhibited poor and non-significant discrimination.

DISCUSSION

Benzene is a well-established hematotoxic and carcinogenic agent, with additional toxic effects on hepatic, renal, neurologic, and immune systems [1]. Following inhalation, benzene undergoes hepatic metabolism into reactive phenolic intermediates that can accumulate in bone marrow and induce oxidative stress, genetic damage, and immunosuppression [11,12]. Petrol station workers are consistently exposed to benzene vapor, placing them at increased risk of systemic biochemical disturbances [13]. Therefore, the present study aimed to evaluate the biochemical alterations in renal, hepatic, cardiac, and pancreatic markers among fuel station workers in the Asir region of Saudi Arabia, and to examine the association of these changes with duration of occupational benzene exposure.

In this study, the demographic profile showed no statistically significant differences between the exposed and control groups in terms of age or BMI. However, workers with longer durations of occupational exposure (> 4

years) had significantly higher mean age and BMI values, suggesting possible age-related vulnerability and exposure-related physiological shifts.

Renal function parameters provided further insights into early systemic effects. No statistically significant differences were observed in serum creatinine and urea levels between exposed and control groups, aligning with findings from previous studies in Saudi Arabia and Iraq [14,15]. These metabolites reflect glomerular filtration, and their stability may suggest early or subclinical renal involvement, or compensatory mechanisms preventing marked dysfunction [16]. However, the significant elevation in uric acid levels among exposed workers, consistent with prior reports from Iraq and Palestine [16, 17], may reflect altered purine metabolism or oxidative stress, as uric acid functions as a major plasma antioxidant [18].

Similarly, hepatic enzymes - ALT, AST, ALP, and GGT - were significantly elevated in exposed individuals, consistent with prior studies in Sudan and Iraq [19]. This suggests hepatocellular injury and possible cholestasis induced by benzene metabolism. Benzene exposure is known to trigger free radical formation, lipid peroxidation, and covalent binding to liver macromolecules, resulting in hepatocyte membrane disruption and enzyme leakage into circulation [20-22]. Elevated GGT levels also support oxidative stress induction, as GGT is implicated in glutathione homeostasis and antioxidant defense [23].

In addition to enzymatic indicators, protein-related markers provided further evidence of hepatic and systemic response. Increased total protein, albumin, and total bilirubin levels observed in this study are consistent with previous research [24]. Albumin, a major antioxidant and transport protein, may be upregulated in response to oxidative stress [25-27]. Elevated bilirubin levels - particularly unconjugated forms - could reflect increased hemolysis or altered hepatic clearance, though direct bilirubin levels remained unchanged, suggesting preserved conjugation capacity [28].

Findings related to cardiac function were also notable. Cardiac enzymes CK-MB and LDH were significantly elevated among exposed workers, aligning with findings from Uganda and other regions [29,30]. These enzymes are markers of myocardial stress or injury, potentially resulting from oxidative damage, lipid peroxidation, or altered membrane permeability [31]. Benzene inhalation may also impact baroreflex sensitivity, affecting cardiovascular regulation [32,33].

Amylase levels, assessed for the first time in this population, were significantly higher in exposed individuals. As a marker of pancreatic function, elevated amylase may indicate subclinical pancreatic stress or inflammation, possibly driven by ROS and low antioxidant defenses in acinar cells [34].

To further explore these relationships, correlation analysis revealed that duration of benzene exposure was significantly associated with elevations in hepatic enzymes (ALT, AST, ALP, GGT), uric acid, total protein, albu-

min, total bilirubin, CK-MB, LDH, and amylase. These findings suggest a time-dependent association and provide evidence of progressive biochemical alterations with increasing duration of occupational exposure. This pattern is consistent with previous findings that prolonged exposure to volatile organic compounds among petroleum workers is significantly associated with elevated hepatic and cardiac enzyme levels, aligning with the observations of D'Andrea and Reddy (2014), who reported significant elevations in liver enzymes following a large-scale benzene exposure, suggesting the potential for cumulative hepatotoxic effects [35]. However, other studies suggest that population-level responses to benzene exposure may vary based on factors such as exposure levels, biomarker specificity, and methodological limitations, potentially explaining inconsistencies in observed biochemical correlations. [36].

Complementing these findings, PCA provided a multivariate perspective on biochemical marker interactions, highlighting distinct clustering of liver enzymes (ALT, AST, GGT) separate from protein synthesis and cellular injury markers (albumin, TP, CK-MB, LDH). These patterns suggest coordinated physiological alterations linked to occupational exposure, offering insight into underlying systemic responses. Similar biomarker clustering patterns have been reported by Wahlang et al. (2021), who demonstrated associations between exposure to volatile organic compounds and liver injury biomarkers, supporting the use of PCA for identifying systemic biochemical shifts [37]. Conversely, overlapping biochemical groupings in PCA analyses have been observed by Li and Ralph (2016), supporting how population structure and exposure variability can influence multivariate interpretations [38].

The high AUC values observed in ROC analysis further support the diagnostic value of several markers - particularly ALT, AST, uric acid, albumin and CK-MB - in distinguishing exposed individuals. These markers may serve as early indicators of biochemical alterations resulting from benzene exposure. This aligns with findings by Hegazy and Kamel (2014), who demonstrated significant elevations in liver enzymes among fuel station workers, highlighting their diagnostic potential in early detection of occupational benzene toxicity [39]. Conversely, another study reported that while individual biomarkers were informative, combining multiple biochemical indicators, including oxidative stress markers, enhanced diagnostic sensitivity for detecting low-level benzene exposure effects [40].

CONCLUSION

This study demonstrates that prolonged occupational exposure to benzene is associated with significant alterations in renal, hepatic, cardiac, and pancreatic biochemical parameters among fuel station workers. These changes, particularly in hepatic and cardiac enzymes, show time-dependent patterns and may serve as early

indicators of subclinical organ dysfunction. The findings highlight the need for regular biomonitoring and implementation of occupational health strategies, including the use of personal protective equipment and exposure reduction measures. Future research should focus on longitudinal studies and include oxidative stress and inflammatory biomarkers to better characterize the health risks associated with chronic benzene exposure.

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This study was approved by the Research Ethics Committee at King Khalid University with an approval number (ECM#2023-608).

Consent to Participate and Publish:

The author accepts responsibility for the entire content of this submitted manuscript and approved submission.

Data Availability:

All data used in this manuscript is available from the author and will be provided upon reasonable request for noncommercial purposes.

Declaration of Generative AI in Scientific Writing:

Generative AI tools (ChatGPT, GPT-5, OpenAI) were used only to refine the language of the manuscript. All scientific content, study design, data analysis, and interpretations were created and verified entirely by the authors to ensure accuracy and ethical compliance.

Declaration of Interest:

The authors declare that they have no conflicts of interest.

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