Novel Serum Proteomes Expressed from Benzene Exposure Among Gasoline Station Attendants

Biomarker Insights
Volume 19: 1–10

DOI: 10.1177/11772719241259604

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ABSTRACT

BACKGROUND: Research on the proteomes impact of benzene exposure in fuel station employees remains sparse, underscoring the need for detailed health impact assessments focusing on biomarker evaluation.

OBJECTIVES: This investigation aimed to analyze the differences in blood parameters and serum proteomes resulting from benzene exposure between gasoline station attendants (B-GSA) and a control group.

DESIGN AND METHODS: A cross-sectional analytical study was conducted with 96 participants, comprising 54 in the B-GSA group and 42 in the control group. The methodology employed included an interview questionnaire alongside urine and blood sample collections. The urine samples were analyzed for *trans,trans*-muconic acid (*t,t*-MA) levels, while the blood samples underwent complete blood count analysis and proteome profiling.

RESULTS: Post-shift analysis indicated that the B-GSA group exhibited significantly higher levels of *t,t*-MA and monocytes compared to the control group ($P < .05$). Proteome quantification identified 1448 proteins differentially expressed between the B-GSA and control groups. Among these, 20 proteins correlated with the levels of *t,t*-MA in urine. Notably, 4 proteins demonstrated more than a 2-fold down-regulation in the B-GSA group: HBS1-like, non-structural maintenance of chromosomes element 1 homolog, proprotein convertase subtilisin/kexin type 4, and zinc finger protein 658. The KEGG pathway analysis revealed associations with apoptosis, cancer pathways, p53 signaling, and the TNF signaling pathway.

CONCLUSION: The changes in these 4 significant proteins may elucidate the molecular mechanisms underlying benzene toxicity and suggest their potential as biomarkers for benzene poisoning in future assessments.

KEYWORDS: Proteomes, benzene, complete blood count, biomarker, gasoline station attendants

RECEIVED: March 8, 2023. **ACCEPTED:** May 17, 2024.

TYPE: Original Research Article

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Introduction

Fuel service workers face significant exposure risks to organic solvents, among which benzene is of particular concern due to its substantial health hazard and classification as a human carcinogen.[1] This occupation sees a significantly elevated likelihood of benzene absorption through inhalation,[2] which is identified as the predominant exposure pathway among these workers.[3,4] It is estimated that approximately 110 million individuals worldwide are exposed to fuel vapors at service stations.[5] It has been estimated that for every 30 L of gasoline containing 5% benzene pumped during the refueling process, approximately 700 mg of benzene is vaporized.[6]

The toxicological mechanism of benzene on human health initiates when the substance is inhaled, rapidly absorbed, and distributed into the alveoli of the lungs. Benzene then penetrates the alveolar walls, diffusing into the circulatory system, where it engages the aryl hydrocarbon receptor signaling pathway and undergoes metabolism through cytochrome P450 2E1 (CYP2E1),[4] producing reactive metabolites. These metabolites undergo further transformation into substances such as *trans,trans*-muconic acid (*t,t*-MA), catechol, and hydroquinone, involving the action of specific enzymes. Several biotransformations, facilitated by enzymes like myeloperoxidase, quinone oxidoreductase 1, and glutathione-*S*-transferases,[7] is instrumental in modifying benzene's structure for its excretion from the body. However, biomarkers such as *t,t*-MA and *S*-phenylmercapturic acid (S-PMA) have been identified for assessing occupational exposure to benzene.[8] Studies conducted in Italy revealed that fuel station workers and petrochemical industry operators exhibited significantly elevated levels of *t,t*-MA and S-PMA in their urine.[4] Benzene's toxicity is notably high, with no established safe exposure thresholds,[3]

and its effects on health can be both acute and chronic.[9] The precise toxicological mechanisms of benzene remain elusive.[10] The hematopoietic system is particularly vulnerable to benzene toxicity,[11] manifesting in reductions in red blood cell (RBC), white blood cell (WBC), and platelet (PLT).[1] More critically, benzene can influence genetic material through its biotransformation mechanism, which involves the generation of reactive oxygen species. This process can lead to DNA damage, covalent binding to DNA, interference in DNA repair mechanisms, and the initiation of apoptosis. Prolonged exposure to benzene has been associated with severe hematological disorders, including aplastic anemia and leukemia.[1,7] The International Agency for Research on Cancer (IARC) is an agency specializing in cancer research, reports benzene has been classified as a group 1 carcinogen, which means it has been confirmed to clearly cause cancer in both humans and laboratory animals, leading to leukemia.[1] In Thailand, reports indicate that 78 individuals suffered from poisoning due to exposure to organic solvents, with benzene accounting for 12 of these cases. This statistic may not fully capture the extent of the issue, as the accuracy of medical diagnoses and the efficacy of the reporting system could influence the reported figures.[12]

Protein level measurement serves as an exceptionally sensitive biomarker for health assessments.[13–15] Additionally, the assessment of protein expression is gaining recognition as a pivotal indicator of health outcomes,[16] with proteomics providing significant analytical insights. This methodology facilitates the examination of protein effects in both quantitative and qualitative aspects.[17] Historically, research efforts have been directed toward understanding the impact of benzene exposure on protein expression in specific occupational groups, including printer[18] and shoe factory workers.[19] The investigation conducted by Zhang et al[15] identified 3 significant proteins—apolipoprotein A1, alpha-1 antitrypsin, and complement C3. Notably, variations in protein expression were observed between individuals diagnosed with benzene poisoning and those merely exposed to benzene.[13] Nonetheless, prior studies have not thoroughly evaluated the relationship between benzene exposure and the proteomic profiles of workers,[15,20] leading to an inability to definitively categorize protein changes resulting from benzene exposure.

Given the potent effects and carcinogenic properties of volatile substances and solvents, it is critical to focus on specific populations at increased risk. For instance, industrial workers in Rayong province, Thailand, have been distinctly identified as being highly susceptible to benzene exposure.[12] A study was conducted among workers in Rayong province, where employees were found to have levels of *t,t-MA* that exceeded the exposure criteria for workers by as much as 29.5%.[21] Furthermore, a review of the literature indicates conflicting evidence regarding the effects of low-concentration benzene exposure on the hematopoietic system.[13,22] Consequently, a comprehensive preventive health impact assessment emphasizing biomarker and proteomic analysis is essential for elucidating the impact of benzene.[15,23] The current study aims to elucidate the differences in blood parameters and serum proteomes resulting from benzene exposure between gasoline station attendants (B-GSA) and a control group. By doing so, we seek to enhance the understanding of benzene's toxicological mechanisms and potentially identify novel biomarkers for future health screenings.

Methods

Determination of the sample size

The calculation of the sample size was based on estimating the mean *t,t*-MA levels across 2 distinct population groups, utilizing a formula derived from a previous study aimed at evaluating *t,t*-MA concentrations in employees' urine.[24] A 95% confidence level was established with a *z*-score of 1.96 and a margin of error set at 0.05. The sample encompassed 96 workers, divided into 54 participants in the B-GSA group and 42 in the control group, all of whom met specific inclusion and exclusion criteria.

For the B-GSA group, inclusion criteria were designated for employees engaged primarily in refueling or other fuel service activities, with a minimum employment duration of 3 months, and without any pre-existing hematopoietic system-related diseases. Conversely, the control group encompassed individuals employed within enclosed building environments, unaffected by any hematopoietic diseases, and exhibiting *t,t*-MA levels compliant with the American Conference of Governmental Industrial Hygienists (ACGIH) standard.[8] The exclusion criteria for both groups included any illness or absence from work on the day designated for sample collection. Cluster sampling was conducted in the Mueang Rayong District within the Eastern Economic Corridor of Thailand (Figure 1). Data were collected from February to November 2020, with subsequent laboratory analyses spanning from December 2020 to February 2021.

Research instruments

This study employed 3 primary instruments for data collection: an interview questionnaire, urine collection equipment, and blood collection tools. Further details on these instruments and the data collection process are provided below:

The interview questionnaire was divided into 2 sections: personal information and employment history, encompassing variables such as age, weight, height, smoking status, years of work experience, and weekly work duration. The questionnaire's validity was confirmed through a quality assessment conducted by an occupational medicine specialist and 2 occupational health experts, achieving an index of item-objective congruence score ranging from 0.67 to 1.00. Completing the questionnaire required approximately 10 minutes per participant.

Urine collection equipment facilitated the analysis of benzene exposure using *t,t*-MA as a biomarker. The collection

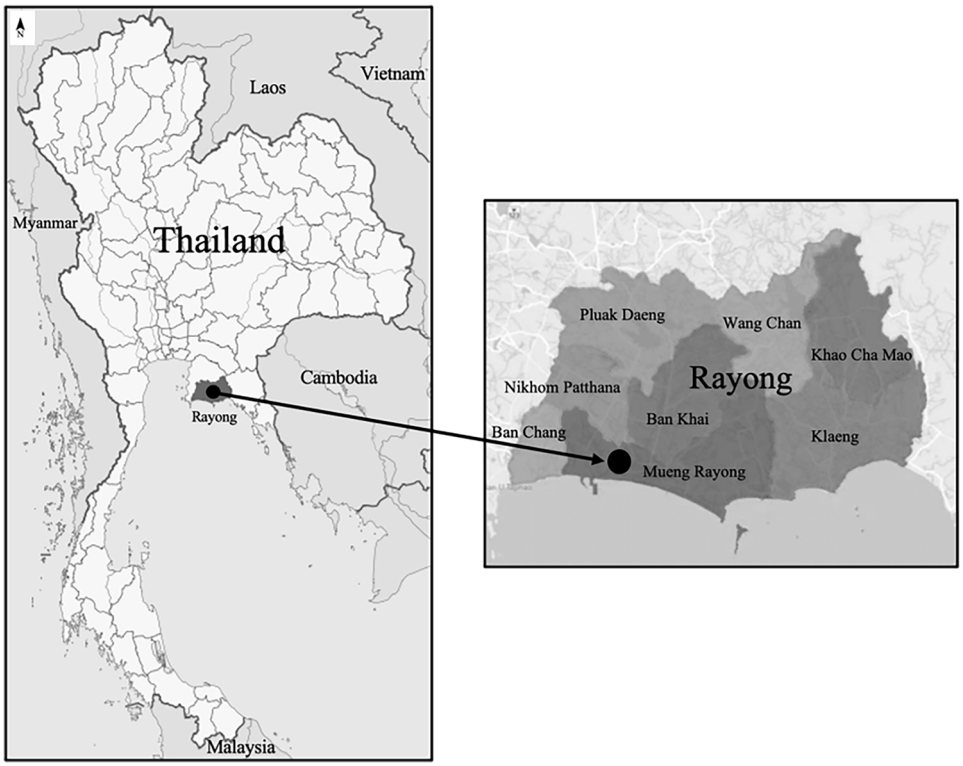

Figure 1. Location of research area, Mueang Rayong District, Rayong Province of Thailand.

setup included plastic cups, 50-mL polyethylene urine containers, foam boxes, and ice packs for sample preservation. The collection was performed post-shift, with instructions for participants to capture the midstream urine in plastic cups before transferring it to the polyethylene containers, ensuring minimal contact with the container's interior. A crucial aspect of the collection process was ensuring that the urine volume exceeded half of the container's capacity. Subsequently, the samples were stored in foam boxes at a temperature below 4°C to preserve the quality of the samples until laboratory analysis.

This study addressed important confounding variables. It has been noted previously that foods containing sorbic acid (eg, shrimp paste, sausage, stuffed bread, canned food, and fermented fruits) and smoking[25,26] can affect the metabolism of *t,t*-MA, respectively. The initial researcher collected data on these confounding variables for analysis. During this analysis, it was found that diet and smoking had no effect on *t,t*-MA. Therefore, it is believed that the *t,t*-MA detected was due to occupational exposure to benzene.

Similarly, blood collection tools played a crucial role in further analyzing the biochemical and proteomic markers for a comprehensive assessment of benzene exposure. The array of equipment included tourniquets, sterile cotton swabs, isopropyl alcohol, syringes, needles, 10 mL ethylenediaminetetraacetic acid (EDTA) tubes, coagulation tubes, adhesive bandages, and biohazard waste bags. Blood samples were collected concurrently with urine samples post-shift to ensure consistency in exposure assessment. Medical technicians and professional nurses collected 6 mL of blood, distributing it evenly between EDTA tubes for plasma separation and coagulation tubes for serum analysis. Subsequently, these samples were refrigerated at 4°C and reserved for further analysis.

Laboratory analysis

The determination of *t,t*-MA levels employed a method consistent with Onchoi's protocol.[27] This involved high-performance liquid chromatography using a C18 column and a mobile phase of water and acetonitrile in a 50:50 volume ratio, with a flow rate of 1.0 mL/minute at a temperature of 37.0°C. Methanol served as the sample solvent, and detection was conducted at an ultraviolet wavelength of 254 nm.[28] The quantification of *t,t*-MA was expressed in micrograms per gram of creatinine (μg/g Cr).

Biochemical marker analysis adhered to protocols established by the Thailand Department of Medical Sciences.[29] The complete blood count (CBC) included measurements of white blood cell (WBC), neutrophil (NE), lymphocyte (LY), monocyte (MO), eosinophil (EO), and basophil (BA) utilizing the fluorescent stain flow cell method. Hemoglobin (HB), hematocrit (HCT) values were obtained through direct measurement, while RBC and PLT counts were assessed using a dynamic single-flow cell approach. All CBC analyses were performed using the Sysmex XN 550 analyzer. The laboratory adhered to the International Organization for Standardization 15189:2012 standards, certification No. 4247/63, and complied with the standards set by the Thailand Ministry of Public Health.

Serum proteome analysis was conducted using a methodology established by Kerdsaeng et al,[30] focusing on protein quantitation and identification through liquid chromatography-mass spectrometry/mass spectrometry at the National Center for Genetic Engineering and Biotechnology of Thailand. The analysis included accession numbers of proteins and their influencing factors, utilizing the Multiple Array Viewer (MeV) program for analysis.[31] Subsequently, proteins were categorized into functional groups—biological processes, cellular components, and molecular functions—utilizing the Protein Analysis Through Evolutionary Relationships (PANTHER) tool, which interfaces with the gene ontology database (https://pantherdb.org).[32] Protein identification was conducted using the UniProt database (https://www.uniprot.org).[33] The study adhered to the STROBE guidelines.

Statistical analyses

Data adhering to a normal distribution, such as age, body mass index (BMI), and working time, were summarized and analyzed using the mean and standard deviation. Non-normally distributed variables, such as *t,t*-MA levels, were evaluated using the median, minimum, and maximum values. Categorical variables, including CBC parameters, were described with frequency and percentage. Inferential statistical analyses compared urinary *t,t*-MA levels between the B-GSA and the control group using an independent sample *t*-test. A Chi-square test was utilized to compare CBC proportions between the B-GSA and control groups. The relative fold change in protein levels between the B-GSA group and the control group was determined using the formula (protein levels in the B-GSA group − protein levels in the control group),[2] where a fold change exceeding 2-fold was indicative of significance.

Results

General information and work history

The demographic profile of the B-GSA group indicated an average age of 28.70 ± 9.74 years, with a mean BMI of 24.55 ± 5.75 kg/m^2. Regarding employment history, the average duration of work experience was 2.59 ± 2.73 years, with a typical workweek spanning 6.25 ± 4.34 days. An evaluation of health behaviors revealed that 21.9% of the workers were smokers, who, on average, had been smoking for 4.86 ± 3.19 years, consumed 8.14 ± 5.67 cigarettes daily, and smoked on an average of 6.24 ± 1.94 days per week. A comparison of general demographics and work history demonstrated no significant differences between the B-GSA group and the control group.

Benzene exposure

The B-GSA group had a median value of 459.11 µg/g Cr (with a range of 127.85–1202.56 µg/g Cr), and exposure to *t,t*-MA exceeded the specified threshold by as much as 37.0% (The ACGIH agency determines that urinary *t,t*-MA values in workers should not exceed 500 µg/g Cr). Conversely, the control group's median *t,t*-MA level stood at 116.31 µg/g Cr (min–max was 29.23–195.13 µg/g Cr), with no instances exceeding the exposure standard. Comparative analysis confirmed that *t,t*-MA levels were significantly higher in the B-GSA group compared to the control group ($P < .05$).

Blood biochemical parameters

In comparing the CBC parameters between the B-GSA group and the control group, it was observed that the MO counts were significantly higher in the B-GSA group compared to the control group ($P < .05$). No significant differences were noted in other CBC parameters (Table 1).

Protein expression in relation to urinary t,t*-MA levels*

The investigation identified a total of 1448 proteins with expressed variations. Utilizing the MeV software for analysis, 20 proteins were found to correlate with *t,t*-MA levels in urine. Subsequent comparative analysis between the B-GSA group and the control group revealed 4 proteins exhibiting a relative fold change greater than 2-fold: HBS1-like (HBS1L) protein, non-structural maintenance of chromosomes element 1 homolog (NSMCE1), proprotein convertase subtilisin/kexin type 4 (PCSK4), and zinc finger protein 658 (ZNF658) protein, all of which demonstrated down-regulation in the B-GSA group (Table 2).

Functional classifications of 20 proteins associated with urinary t,t*-MA levels*

Analysis through the PANTHER Classification System of the 20 proteins correlating with *t,t*-MA levels categorized them into 3 functional groups: (a) biological processes, including cellular processes and response to stimulus, each constituting 29.0%, (b) cellular components, including intracellular elements, protein-containing complexes, and cellular anatomical structures, each at 33.3%, and (c) molecular functions, with catalytic activity and binding activities representing 50% each (Figure 2).

Functional groups of 4 proteins exhibiting more than a 2-fold relative change

The UniProt database analysis provided insights into the functional categories of 4 proteins associated with *t,t*-MA levels, each showing a relative fold decrease of more than 2-fold (Table 3). For instance, NSMCE1 is involved in molecular functions such as double-strand break repair through homologous recombination, along with biological processes like protein dimerization activity and ubiquitin-protein ligase activity.

Table 1. Comparison of blood biochemical parameters between B-GSA and control groups.

BLOOD BIOCHEMICAL PARAMETERS	B-GSA (N=54), N(%)	CONTROL (N=42), N(%)	χ^2	P-VALUE
White blood cell (WBC)				
Normal	47(87.0)	37(88.1)	0.024	.876
Abnormal	7(13.0)	5(11.9)		
Neutrophil (NE)				
Normal	54(100.0)	40(95.2)	2.626	.105
Abnormal	0(0.0)	2(4.8)		
Lymphocyte (LY)				
Normal	38(70.1)	30(71.4)	0.013	.910
Abnormal	16(29.9)	12(28.6)		
Monocyte (MO)				
Normal	48(88.9)	42(100.0)	4.978	.026*
Abnormal	6(11.1)	0(0.0)		
Eosinophil (EO)				
Normal	54(100.0)	38(90.5)	1.536	.215
Abnormal	0(0.0)	4(9.5)		
Basophil (BA)				
Normal	54(100.0)	42(100.0)	–	–
Abnormal	0(0.0)	0(0.0)		
Red blood cell (RBC)				
Normal	46(85.2)	32(76.2)	1.255	.263
Abnormal	8(14.8)	10(23.8)		
Hemoglobin (HB)				
Normal	37(68.5)	29(69.0)	0.003	.956
Abnormal	17(31.5)	13(31.0)		
Hematocrit (HCT)				
Normal	44(81.5)	34(81.0)	0.004	.947
Abnormal	10(18.5)	8(9.0)		
Platelet (PLT)				
Normal	45(83.3)	40(95.2)	3.300	.069
Abnormal	9(16.7)	2(4.8)		

*$p<0.05$.

Discussion

The study revealed important findings: in the B-GSA group, urinary *t,t*-MA levels exceeded the standard by 37%, with the highest exposure recorded at 1202.56 μg/g Cr. The study's findings align with those of Tongsatia,[34] which reported elevated *t,t*-MA levels among refuelers and cashiers at fuel stations by 27.2% and 17.7%, respectively, underscoring the persistent risk of airborne benzene for individuals in this occupational setting.

The CBC analysis revealed a significantly higher proportion of abnormal MO counts in the B-GSA group compared to the control group ($P<.05$). MOs, a type of WBC, play a crucial role in capturing foreign substances in the body and migrating from the bloodstream into tissues. An elevation in MO counts may indicate inflammation or collagen vascular disease.[35] Furthermore, previous research has identified an inverse relationship between *t,t*-MA levels and circulating angiogenic cells, potentially heightening the risk of cardiovascular diseases.[36]

Table 2. Protein names and relative fold change of proteins expressed down-regulation between B-GSA and control groups.

PROTEIN NAMES	RELATIVE FOLD CHANGED B-GSA AND CONTROL
BK channel (BKCA alpha) (Calcium-activated potassium channel subunit alpha-1)	0.325
Bromodomain-containing protein 8	0.029
cDNA FLJ10755	1.285
cDNA FLJ45654	0.969
Dystonin	0.368
ENTH domain-containing protein 1	0.110
HBS1-like protein	4.175[a]
KIAA0323, isoform CRA_a	0.235
Laminin	0.273
NOGO-interacting mitochondrial protein	0.001
Non-structural maintenance of chromosomes element 1 homolog	9.277[a]
Olfactory receptor	0.211
Osteopontin	0.047
Proprotein convertase subtilisin/kexin type 4	6.869[a]
RNA helicase	1.417
Zinc finger protein 559, isoform CRA_a	0.092
ZNF658 protein	2.260[a]
Glycogen synthase kinase-3 alpha	0.830
KICSTOR complex protein SZT2	0.249
NACHT, LRR, and PYD domains-containing protein 6	0.246

[a]Relative fold change more than 2-fold.

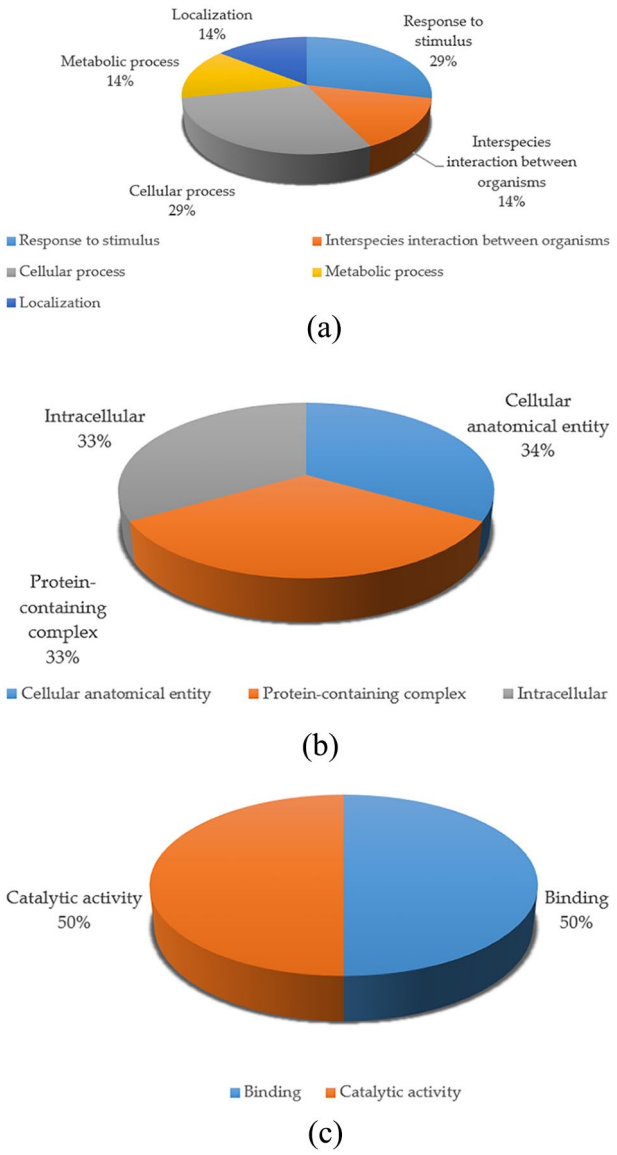

Figure 2. Function classifications of differentially expressed proteins: (a) biological process, (b) cellular component, and (c) molecular function.

Despite these findings, no significant differences were observed between the 2 groups in terms of RBC and PLT counts. Moro et al[37]'s study on refueling workers suggested that CBC parameters may not serve as a reliable screening tool for fuel exposure-related poisoning. Nevertheless, the literature has documented instances of chronic benzene toxicity affecting the blood system,[1,38] highlighting the importance of ongoing monitoring for individuals at risk.

In terms of proteomic analysis, this study explored the correlation between protein expression and urinary *t,t*-MA levels among employees, employing a distinct analytical approach.[13,15,19] Protein level measurement emerged as a sensitive indicator of chemical exposure-related changes.[39] Notably, 4 proteins exhibited reduced expression in the B-GSA group compared to the control group, with a relative fold change exceeding 2-fold, illustrating the impact of benzene exposure on protein expression.

Among these proteins, HBS1L (*Saccharomyces cerevisiae*) emerges as a protein of interest due to scholarly reports highlighting its linkage to the hematological system.[40] Recognized for its implications in benzene toxicity's impact on hematology,[1] HBS1L expression was found to decrease by a factor of 4.175 in the B-GSA group compared to the control group. In conjunction with DOM34, HBS1L is thought to assist in identifying stalled ribosomes and initiating the endonucleolytic cleavage of mRNA. This process is crucial for releasing non-functional ribosomes and degrading damaged mRNAs, thereby maintaining cellular integrity.[41]

The expression of HBS1L has been the focus of research in individuals with myeloproliferative neoplasms, revealing its significant roles beyond mere protein synthesis.[40,42] A notable

Table 3. Functional groups of proteins expressed related with *t,t*-MA and relative more than 2-fold changed.

PROTEIN	FUNCTIONAL GROUPS	
	MOLECULAR FUNCTION	BIOLOGICAL PROCESS
Non-structural maintenance of chromosomes element 1 homolog	Double-strand break repair via homologous recombination; intracellular signal transduction; positive regulation of response to DNA damage stimulus; post-replication repair	Metal ion binding; protein dimerization activity; ubiquitin-protein ligase activity; ubiquitin-protein transferase activity
Proprotein convertase subtilisin/kexin type 4	N/A	Serine-type endopeptidase activity
HBS1-like protein	Regulation of translation; translation	GTPhase activity; GTP binding
ZNF658 protein	N/A	N/A

Abbreviation: N/A, not available, does not appear in the Uniport program.

study conducted in Taiwan discovered a negative correlation between HBS1L and polycythemia vera, a condition characterized by the excessive production of RBCs. This abnormality underscores a failure in the body's regulation of blood production mechanisms.[42] Furthermore, the adverse effects of benzene poisoning on the hematopoietic system, such as anemia and acute myeloid leukemia, have been well documented,[1,43] reinforcing the need to investigate HBS1L's potential as a biomarker for benzene's toxic impact on blood health. Future research on human subjects is necessary to validate these findings.

The current study expanded its focus to include additional proteins influenced by benzene exposure. Notably, the analysis revealed that the expression of the NSMCE1 protein in the B-GSA group was markedly reduced, exhibiting a 9.277-fold decrease compared to the control group. NSMCE1 plays a critical role in mediating oxidative stress responses.[44] This protein functions as a RING-type, zinc finger-containing E3 ubiquitin ligase that collaborates with melanoma antigen gene proteins to facilitate the direct transfer of ubiquitin from E2 ubiquitin-conjugating enzymes to specific substrates. This process is pivotal for maintaining genomic integrity and responding to DNA damage.[45]

Research on patients with chronic lymphocytic leukemia has identified associations between NSMCE1 levels and DNA methylation patterns,[46] suggesting a potential link to the molecular mechanisms of benzene toxicity. However, these studies did not specifically target workers exposed to benzene under conditions similar to those in the present investigation or employ a comparable research design. Consequently, further studies involving human subjects are warranted to corroborate these findings and elucidate the role of NSMCE1 in the context of benzene exposure.

In addition, the current study revealed a significant reduction in PCSK4 levels within the B-GSA group, with a 6.869-fold decrease compared to the control group. Existing research highlights the reproductive relevance of PCSK4, which is found on the plasma membrane of sperm and ovary acrosomes. Variations in its levels have resulted in disruptions in protease activity, underscoring its biological significance.[47] Previous reports have documented sperm abnormalities in workers exposed to benzene.[48] Furthermore, a cohort study conducted by Katukam et al[49] found a decrease in the number of spermatozoa, with comet tail length significantly shorter than that observed in the control group. Regarding carcinogenic potential, PCSK4 has been associated with human breast cancer[50] and implicated in the activation of proteins that may promote tumor development into cancer.[51]

Given the observed reductions in PCSK4 levels and their potential implications, it becomes crucial to contextualize these findings within the broader epidemiological landscape of benzene exposure risks. A systematic review of 32 studies from reputable research databases highlighted the incremental lifetime cancer risk (ILCR) associated with elevated benzene concentrations in various countries, including Iran, Brazil, South Africa, and Thailand. Specifically, employees at Bangkok gas stations demonstrated ILCR values ranging between 1.82×10^{-4} and 2.5×10^{-4}. This comprehensive review underscores the pronounced genotoxic effects prevalent in gas station environments, potentially elevating cancer risk.[52]

Regarding ZNF658, the analysis revealed a notable decrease in expression levels within the B-GSA group, showing a 2.260-fold reduction compared to the control group. ZNF658 plays a crucial role in regulating gene transcription pertinent to zinc homeostasis and influences ribosome biogenesis via the zinc transcriptional regulatory element. Moreover, it is instrumental in governing the orchestrated cellular response to zinc availability.[53] Previous studies on proteomes comparing 11 patients with rheumatoid arthritis (RA) to a control group of 15 individuals without RA highlighted the significance of serum albumin and ZNF658 in active RA,[54] indicating an association of ZNF658 with inflammatory mechanisms within the body. Additionally, benzene exposure has been implicated in reducing the activity of natural killer cells and elevating pro-inflammatory biomarkers even at low exposure levels.[43]

This research identified 4 significant proteins, which were further analyzed for their involvement in KEGG pathways. The findings underscored critical pathways linked to benzene

exposure toxicity, including apoptosis, p53 signaling, TNF signaling pathways, and cancer pathways. Consequently, this study contributes to the academic discourse by elucidating the association of specific protein expressions with the mechanisms underlying benzene toxicity and offering insights into the biological impact of benzene exposure.

While this study has provided valuable insights into the biological impact of benzene exposure, it is essential to recognize certain constraints that influenced our findings, notably the sample collection period coinciding with the COVID-19 pandemic in Thailand. This unprecedented circumstance might have led to reduced benzene exposure among workers due to altered working conditions. A distinctive strength of the study is its novel assessment of urinary *t,t*-MA to verify benzene exposure due to refueling, differentiating it from previous studies. Additionally, the demographic and general information factors between the B-GSA and the control group showed no significant differences, enhancing the comparability of the 2 groups. A novel proteome analysis technique was employed, differing from those used in previous studies. This approach involved analyzing protein relationships with urinary *t,t*-MA using the MeV program prior to protein identification, thereby enhancing the reliability of the protein analysis results. However, it is important to note that this represents a preliminary pilot study within the B-GSA cohort. Future research should aim to examine these 4 proteins in a broader population and across varying levels of benzene exposure to validate the findings before practical application.

Conclusion

The current study demonstrated that the primary biochemical components in blood, including RBCs, select WBCs, and PLTs, exhibited no significant differences between the B-GSA group and the control group. However, a notable exception was observed in the levels of MOs, where the B-GSA group presented a significantly higher incidence of abnormal MO counts compared to the control group ($P < .05$). In the domain of proteome analysis, a comparative examination revealed that 20 proteins exhibited a correlation with urine *t,t*-MA levels between the B-GSA and control groups. Notably, within this subset, 4 proteins—HBS1L, NSMCE1, PCSK4, and ZNF658—demonstrated a more than 2-fold decrease in expression levels in the B-GSA group relative to the control group. This nuanced approach to examining the biochemical and proteomic impacts of benzene exposure provides a comprehensive overview of its physiological effects, underscoring the significance of abnormal MO levels and specific protein expressions related to occupational benzene exposure.

Abbreviations

ACGIH: American Conference of Governmental Industrial Hygienists; BA: Basophil; B-GSA: Benzene Gasoline Station Attendants; BMI: Body Mass Index; CBC: Complete Blood Count; CVD: Cardiovascular Disease; CYP-2E1: Cytochrome P450 2E1; DNA: Deoxyribonucleic Acid; EDTA: Ethylene Diamine Tetra Acetate; EEC: Eastern Economic Corridor of Thailand; EO: Eosinophil; HB: Hemoglobin; HBS1L: HBS1-like S. cerevisiae; HCT: Hematrocrit; HPLC: High-Performance Liquid Chromatography; IOC: Item Objective Congruence; KEGG: Kyoto Encyclopedia of Genes and Genomes; LY: Lymphocyte; MEV: Multiple Array Viewer; MO: Monocyte; NE: Neutrophil; NSMCE1: Non-Structural Maintenance of Chromosomes Element 1 homolog; PANTHER: Protein Analysis Through Evolutionary Relationships; PCSK4: Proprotein Convertase Subtilisin/Kexin type 4; PLT: Platelet; RA: Rheumatoid Arthritis; RBC: Red Blood Cell; ROS: Reactive Oxygen Species; S-PMA: S-Phenylmercapturic Acid; TNF: Tumor Necrosis Factor; *t,t*-MA: *trans,trans*-Muconic Acid; WBC: White Blood Counts; ZNF 658: Zinc Finger Protein 658

Declarations

Ethics Approval and Consent to Participate

This study was conducted with the approval of the Burapha University Institutional Review Board for Protection of Human Subjects in Research (BUU-IRB) (certificate no. 016/2562). Before beginning the survey, written informed consent was obtained from all the study participants.

Consent for Publication

Written informed consent for publication was obtained from all participants, following is the approved by the BUU-IRB.

Author Contributions

CPP: conceptualization, data curation, formal analysis, methodology, writing—original draft.
SR: investigation, resources, software, writing—review & editing.
JS: conceptualization, formal analysis, investigation, methodology, supervision, validation, writing—review & editing.
TY: methodology, validation, writing—review & editing.
AT: conceptualization, funding acquisition, methodology, project administration, supervision, validation, writing—original draft, writing—review & editing.

Acknowledgments

We would like to express our gratitude to the Health System Research Institute (HSRI) for funding this research. This work was supported by Burapha University. We thank the staff of Rayong Hospital in honor of Her Royal Highness Princess Maha Chakri Sirindhorn for allowing us to collect samples as well as for their participation. And we thank the BIOTEC proteome analysis laboratory.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Health System Research Institute (HSRI) grants No. 63-070.

Competing Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Supplemental Material

Supplemental material for this article is available online.

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