SYSTEMATIC REVIEW

Correlation Between Biomarkers of Benzene Exposure and 8-ohdg Metabolites as Biomarkers of DNA Damage: Systematic Review and Meta-analysis

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ABSTRACT

Introduction: Benzene exposure that occurs in the environment or occupational environment can cause malignancy, especially leukemia. Malignancy begins with DNA damage, and one of its biomarkers is 8-hydroxy-2-deoxy-Guanosine (8-OHdG). Objectives: This study aims to examine and summarize the relationship between biomarker of benzene exposure and 8-OHdG metabolites as biomarkers of DNA damage. **Materials and methods:** This study was guided by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 method. Studies were collected from 3 databases (PubMed, ScienceDirect, and ProQuest) on July 28th, 2024. Heterogeneity tests, summary effect calculations, and examination of study bias were carried out using JASP Statistics version 0.19.0. **Results:** The results of the meta-analysis showed that there was a significant positive correlation between benzene exposure and 8-OHdG metabolites with a moderate correlation strength. **Conclusion:** Despite heterogeneity in study design and effect size, the data suggest that individuals exposed to benzene have a higher risk of DNA damage as evidenced by the metabolite biomarker 8-OHdG.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP7): 199-209. doi:10.47836/mjmhs.21.s7.24

Keywords: Benzene exposure, 8-OHdG, Meta-analysis, Systematic review

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INTRODUCTION

Benzene exposure is an important issue in the realm of public health. This is because benzene is known as a toxicant that can cause both acute and chronic health impacts in humans. Benzene exposure can occur in both the environment and the work environment (1). In urban environments, the presence of benzene in the air can come from tobacco smoke (cigarettes), motor vehicle exhaust. Outside urban areas, benzene can come from forest fires, and in industry, benzene comes from industrial emissions (2).

The International Agency for Research on Cancer (IARC) classifies benzene as a carcinogenic agent in humans and can cause Acute Myeloid Leukemia (AML). IARC also notes that there are studies linking benzene exposure to chronic lymphocytic leukemia (CLL) and Acute lymphocytic leukemia (ALL), and multiple myeloma (3). This is because the potential target of benzene exposure is the hematopoietic stem cell (HSC) niche (4). Previous studies have reported that one of the mechanisms of benzene-induced genotoxicity in exposed hematopoietic stem cells (HSCs) is through DNA damage (5).

DNA damage can cause mutations in genes that control cell division so that cell division becomes uncontrolled which ultimately triggers the growth of cancer cells (6). There is no specific standard for markers of DNA damage due to benzene exposure. However, the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a popular marker of DNA damage in various studies, mainly to describe short-term DNA damage, including that caused by benzene exposure (7–9). This is because 8-OHdG is a very specific marker and its occurrence is abundant in DNA (10). The 8-OHdG metabolite is formed as a result of oxidation of one of the bases that make up DNA, namely Guanosine, by hydroxyl radicals produced from the benzene metabolism process (11). Oxidized Guanosine then becomes 8-hydroxy-2-deoxy-Guanosine or 8-OHdG (12). This DNA damage is somatic and not inherited.

In relation to benzene exposure, there have been several epidemiological studies examining the correlation between low-dose benzene exposure and DNA

damage (9,13–15). There has also been a meta-analysis discussing this. However, the DNA damage markers used do not include 8-OHdG (5). In addition, in several epidemiological studies conducted to determine the correlation between benzene exposure and 8-OHdG metabolites as biomarkers of DNA damage, there were inconsistent results (16,17). Therefore, the purpose of this literature review and meta-analysis was to examine the relationship between biomarker of benzene exposure and 8-OHdG metabolites as biomarkers of DNA damage. This knowledge is important for the development of research and strategies for preventing the adverse effects of benzene exposure, considering that 8-OHdG is an efficient biomarker and the samples used are less invasive (such as blood and urine).

MATERIALS AND METHODS

Study Design

This systematic review and meta-analysis study is a correlational study. We conducted the systematic review and meta-analysis based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 method (18). In this systematic review and meta-analysis, we examined benzene exposure in relation to DNA damage with the biomarker 8-OHdG.

Searching Methods

The reviewed studies were taken from 3 databases, namely Pubmed, ScienceDirect, and Proquest. The keywords used in searching for studies in all databases were "Benzene" AND "8-OHdG". Studies included in the review were studies that met the following inclusion criteria: (1) Studies published between January 1, 2014 and July 31, 2024; (2) Free full text studies; (3) Studies examining the correlation between benzene exposure and 8-OHdG; (4) Studies containing information on the number of analysis units/samples (n) and correlation coefficient (r). Studies will be excluded if they meet the following exclusion criteria: (1) Books, reviews, meta-analyses, experimental studies on experimental animals; (2) Studies that are irrelevant or out of scope; (3) Required data is incomplete. The number of studies identified, screened, and determined as studies included in this study is shown in Figure 1.

Screening of studies based on inclusion criteria points 1-2, and exclusion criteria point 1 was carried out automatically using the "advance search" option on the database website. Inclusion criteria points 3-4, and exclusion criteria point 3 were carried out manually by two researchers separately. If there was a difference of opinion between the two researchers, it would be discussed and decided by involving a third researcher.

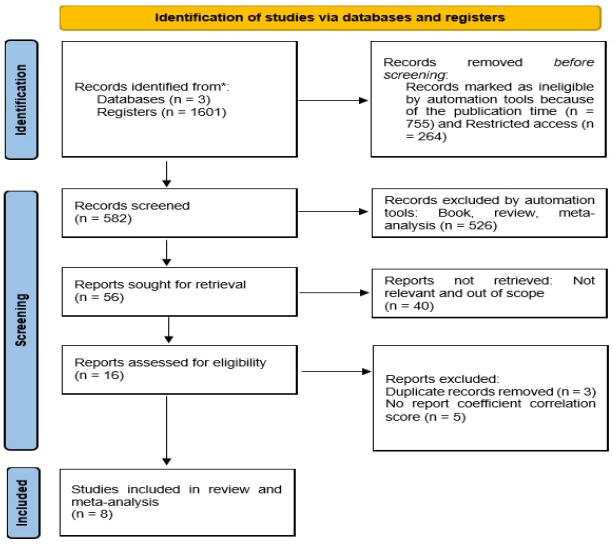

Figure 1: Preferred Reporting Items for Systematic Review and Meta Analyses (PRISMA) Flow Diagram of Study Selection

The identification process was carried out by searching for studies in 3 databases, namely Pubmed, ScienceDirect, and Proquest. The number of studies found was (n = 1601). There were studies that did not meet the requirements so they were automatically excluded by the search engine due to publication time (n = 755) and limited access (n = 264). Screening was carried out by excluding books, reviews, meta-analyses (and = 526); irrelevant and not in accordance with the scope (n = 40); Duplication (n-3); not reporting the coefficient correlation score (n = 5). In the end, there were 8 studies included in this literature review and meta-analysis.

Search outcome and audit trail

The studies collected for review and analysis in this study were managed using Zotero software. The researcher then checked the completeness of the study information in the software and completed the missing data manually if necessary. The next process, we read the entire study to re-ensure that all the studies that had been collected met the inclusion and exclusion criteria. The researcher also checked the references in the study bibliography to find relevant references to enrich the discussion in this study.

Quality appraisal

The types of studies reviewed and analyzed in this study were observational analytic with cross-sectional and case-control designs. All studies were quality checked using an instrument developed by National Institutes of Health (NIH) of The United States. Studies with cross-sectional designs that were collected were checked

using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies instrument, while case-control studies were checked using the Quality Assessment of Case-Control Studies instrument. The Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies contains 14 questions. The Quality Assessment of Case-Control Studies contains 12 questions. Both instruments provide 5 answer options, namely "Yes", "No", "CD (Cannot Determine)", "N/A (Not Applicable)" and "NR (Not Reported)". The final assessment score is obtained by adding up the "Yes" answers. Study quality is categorized into three: Poor <50%, Fair 50–75%, Good ≥75 (19). A summary of the results of the study quality check is presented in Table I. We used a funnel plot to check for possible bias in the reporting of pooled study results. This diagram presents the effect size plotted on the horizontal axis against its standard error on the vertical axis. If there is publication bias, the funnel plot is expected to be skewed or asymmetrical (20). Because this skewness can be subjective, researchers included Egger's test to confirm that the funnel plot is symmetrical/asymmetrical. If the p value in Egger's Test > 0.05, then the funnel plot is confirmed to be symmetrical. The funnel plot and Egger's test were performed using the online application JASP Statistics version 0.19.0 (21).

Table I: Study Quality Assessment Summary

Study Design	The United States National Institutes of Health (NIH) Quality Assessment Tool														Quality scoring	Quality rating*
	Cohort and Cross-Sectional Studies															
Questions	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**	**Q9**	**Q10**	**Q11**	**Q12**	**Q13**	**Q14**		
Goethel et al. (2014)	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	No	Yes	NR	CD	Yes	10/14	Fair
Li et al. (2015)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	NR	CD	Yes	10/14	Fair
Kun et al. (2020)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR	CD	Yes	12/14	Good
Pillia et al. (2021)	Yes	Yes	Yes	Yes	Yes	NR	Yes	Yes	Yes	Yes	Yes	NR	CD	Yes	11/14	Good
Kim *et al.* (2016)	Yes	Yes	Yes	No	Yes	Yes	NR	Yes	Yes	No	Yes	Yes	CD	Yes	10/14	Fair
Fenga *et al.* (2017)	Yes	Yes	Yes	Yes	Yes	NR	NR	Yes	Yes	No	Yes	Yes	CD	NR	9/14	Fair
Radu *et al.* (2024)	Yes	Yes	Yes	Yes	NR	Yes	NR	Yes	Yes	CD	Yes	Yes	CD	NR	9/14	Fair
Study Design	**Case-Control Studies**															
Questions	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**	**Q9**	**Q10**	**Q11**	**Q12**				
Kuang *et al.* (2021)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NR	Yes			11/12	Good

*Quality rating categories: Poor <50%, Fair 50–75%, Good ≥75

Data Abstraction

The studies that have been collected and agreed to be included in this study were reviewed and analyzed by two researchers separately to then be discussed and decided together. For review purposes, we use the help of a table to facilitate the process. The table contains the author's name, research title, method, results, and conclusions (Table II). These things are then stated in the results and discussion.

To conduct a meta-analysis, the data taken from the collected studies are the sample size (n) and the correlation coefficient (r). Both data are used to calculate the effect size (z) and standard error of the effect size (SEz) which will be used in the meta-analysis. The calculation of the z and SEz values is carried out using Excel to then be analyzed using JASP Statistics version 0.19.0. If there is more than one r value in one study, then each r value will be calculated for its effect size and included in the meta-analysis and considered as a separate study.

Synthesis

The narrative approach was used to summarize and discuss important issues related to benzene exposure, 8-OHdG metabolites and the correlation between the two. Furthermore, a statistical approach was used for heterogeneity testing, summary effect calculations, and examination of research bias. All statistical analyses were performed using JASP Statistics version 0.19.0. The heterogeneity test in this meta-analysis study aims to find out whether there are differences in each effect size. The heterogeneity test was evaluated using the Q value with a significance level of 0.05. The summary effect calculation was carried out to summarize the effect sizes of the studies studied. Thus, the strength of the correlation (rRE) between the two variables studied (benzene exposure and 8-OHdG metabolites) can be determined. Examination of research bias has been described in the Quality appraisal section. The completeness of the meta-analysis study was checked using the MOOSE Checklist for Meta-analysis of Observational Studies (22).

RESULTS

Systematic Review

The studies that were successfully collected as written in Figure 1, were 8 studies. Most of the studies came from countries in Asia (4 studies), the year of publication was almost evenly distributed in the last ten years, and most of the studies used a cohort design (6 studies). The results of the analysis of the selected studies were summarized and presented in several sub-sections as shown in Table II.

Based on the results of the study quality examination (Table I), it is known that the quality of the studies is quite varied. Most of the selected studies (62.5%) were of fair quality and the rest were of good quality (37.5%).

This could be due to the relatively strict inclusion criteria which limited the number of studies that could be analyzed. However, in order to minimize possible research bias, the studies included in this study had first undergone a research bias examination. Thus, the results of the review and meta-analysis of this study can still provide a valid picture of the relationship between benzene exposure and 8-OHdG metabolites as biomarkers of DNA damage.

Table II: Systematic review of Correlation between Biomarkers of Benzene Exposure and 8-OHdG Metabolites as Biomarkers of DNA Damage: Systematic Review and Meta-Analysis

No	Name (Year)	Title	Study design	Conclusion
1	Goethel *et al.* (2014)	Evaluation of genotoxicity in workers exposed to benzene and atmospheric pollutants	**Study Design:** Cross-Sectional **Population:** Gas station attendants and taxi drivers in Rio Grande do Sul/RS, Brazil **Sample:** Total 133 male workers: 43 gas station attendants (GSA group), 34 taxi drivers (TD group), and 22 subjects without known occupational exposures (NE group) **Ages of the respondents:** Older than 62 years **Biomarker of benzene exposure:** Urinary t,t-muconic acid (t,t-MA) **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman's rank. *p*-value = <0.001 and r-value = 0.439 (moderate correlation)	This study found that exposure to low concentrations of benzene (as indicated by t,t-MA concentrations) and other atmospheric pollutants in the workplace may be associated with genotoxicity and oxidative DNA damage (as indicated by 8-OHdG).
2	Li *et al.* (2015)	Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose–effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China	**Study Design:** Cross-Sectional **Population:** Kindergarten-aged children in Guangzhou, China **Sample:** 87 kindergarten-aged children **Ages of the respondents:** Children aged 3–6 years old **Biomarker of benzene exposure:** Urinary t,t-MA; Urinary 1,2-diethylbenzene (1,2-DB); Urinary S-Phenylmercapturic Acid (S-PMA) **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman's correlations (two tailed). *p*-value = <0.05 for t,t-MA; <0.01 for 1,2 DB; <0.01 for S-PMA and r-value = 0.349 for urinary t,t-MA (weak correlation); 0.363 for 1,2 DB (weak correlation); 0,415 for S-PMA (moderate correlation).	This study found that exposure to polycyclic aromatic hydrocarbons (PAHs) or benzene and toluene (BT) can cause oxidative DNA damage. The metabolite 8-OHdG is a good biomarker to describe the presence of DNA damage. This study found that there was a significant dose-effect relationship between benzene exposure, and urinary 8-OHdG concentration. Toddlers (aged 3–4 years) faced a higher burden of benzene exposure compared to older children
3	Kim *et al.* (2016)	Health Effect Assessment on Cleanup Workers of an Oil Spill in Yeosu	**Study Design:** Case Control **Population:** Cleanup Workers of an Oil Spill in Yeosu, South Korea **Sample:** 108 Worker (84 oil spill cleaner and 24 control) **Ages of the respondents:** Average age 63.8 ± 10.4 years old **Biomarker of benzene exposure:** Urinary t,t-muconic acid (t,t-MA) **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Multiple regression analysis. *p*-value = 0.614 and r-value = 0.081 (no correlation).	This study shows that oil spill cleanup activities affect VOC (including benzene) exposure and the health of cleanup workers. There is no correlation between benzene exposure (t,t-MA) and Urinary 8-OHdG. The results indicate the need for health screening for participants in oil spill cleanup work.

CONTINUE

Table II: Systematic review of Correlation between Biomarkers of Benzene Exposure and 8-OHdG Metabolites as Biomarkers of DNA Damage: Systematic Review and Meta-Analysis (CONT.)

No	Name (Year)	Title	Study design	Conclusion
4	Fenga (2017)	8-Hydroxydeoxyguanosine as a biomarker of oxidative DNA damage in workers exposed to low-dose benzene	**Study Design:** Cross-Sectional **Population:** Gasoline stations workers located in East Sicily **Sample:** 143 workers (80 men, employed in gasoline stations 63 men control group) **Ages of the respondents:** Average age 37.44 ± 9.13 years old **Biomarker of benzene exposure:** Urinary t,t-muconic acid (t,t-MA **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Pearson's test. *p*-value = <0.001 and r-value = 0.377 (weak correlation).	These results suggest that chronic low-level exposure to benzene among gas station attendants may induce oxidative damage to DNA, as indicated by changes in 8-OHdG which may be a non-invasive biomarker of early genotoxic damage in exposed subjects.
5	Kun (2020)	Distribution of S-phenylmercapturic acid and 8-hydroxy-2'-deoxyguanosine in urine of workers exposed to low-concentration benzene	**Study Design:** Cross-Sectional **Population:** Paint mixing and painting in the automobile factory in Yangzhou, China **Sample:** 206 employees **Ages of the respondents:** Average age 33 years old **Biomarker of benzene exposure:** Urinary S-PMA **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman correlation. *p*-value = <0.05 and r-value = 0.488 (moderate correlation).	Under low concentration benzene exposure in the workplace, the level of S-PMA in the urine of workers remained elevated, there was a positive correlation between S-PMA (a marker of benzene exposure) and 8-OHdG (a marker of DNA injury), and oxidative damage increased, indicating a relatively high health risk.
6	Kuang (2021)	Exposure to volatile organic compounds may be associated with oxidative DNA damage-mediated childhood asthma	**Study Design:** Case Control **Population:** Children were diagnosed as asthma by doctors from Guangzhou Women and Children's Medical Center, Guangzhou, China **Sample:** 321 (252 asthmatic children and 69 healthy children) **Ages of the respondents:** Children aged 6-11 years old **Biomarker of benzene exposure:** Urinary 1,2-DB **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman correlation. *p*-value = 0.1112 and r-value = 0.102 (no correlation).	Results showed that there is significant dose-response relationships between most VOC metabolites and 8-OHdG were observed but there is no correlation between benzene exposure (1,2-DB) and 8-OHdG.

CONTINUE

Table II: Systematic review of Correlation between Biomarkers of Benzene Exposure and 8-OHdG Metabolites as Biomarkers of DNA Damage: Systematic Review and Meta-Analysis (CONT.)

No	Name (Year)	Title	Study design	Conclusion
7	Pillia (2021)	Biomarkers of Low-Level Environmental Exposure to Benzene and Oxidative DNA Damage in Primary School Children in Sardinia, Italy	**Study Design:** Cross-Sectional **Population:** Children attending primary school in three different locations in two locations of southern Sardinia (Italy): the metropolitan area of Cagliari, the main city and the regional capital, and Sarroch, a small town immediately bordering one of the largest oil refineries in Europe **Sample:** 83 children (35 living in an urban area and 48 in area near a petrochemical plant) **Ages of the respondents:** Children between 3 and 13 years old **Biomarker of benzene exposure:** Urinary t,t-muconic acid (t,t-MA) **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman correlation. *p*-value = 0.043 for morning sample; 0.025 for evening sample and r-value = 0.222 for morning sample (weak correlation); 0.246 for evening sample (weak correlation).	This study found that there was a significant relationship between benzene exposure (urinary t,t-MA) and urinary 8-OHdG. The results suggest the importance of biological monitoring of low-level environmental exposure and its relation to risk of genotoxic effects among children.
8	Radu (2024)	Urinary Biomarkers of Benzene Exposure and Oxidative Damage in Residents of the Oil Extraction Area of Muanda, Dr Congo	**Study Design:** Case Control **Population:** Residents of the Muanda Region, on the Atlantic coast of the Democratic Republic of the Congo (an area of offshore and onshore oil extraction)**Sample:** 53 (34 adults and 19 children) **Ages of the respondents:** No information available **Biomarker of benzene exposure:** Urinary t,t-muconic acid (t,t-MA) **Biomarker of DNA Damage:** Urinary 8-OHdG **Type of correlation analysis:** Spearman correlation. *p*-value = <0,01 and r-value = 0.37 (moderate correlation).	This biomonitoring study revealed concentrations of S-PMA were increased among residents of the oil extraction areas and petroleum products area compared with controls. Although the levels of benzene metabolites were low, the positive correlation with 8-OHdG possibly indicates that pollution by hydrocarbons induced oxidative damage in this study population

Meta-Analysis

Research Bias Checking

The results of the study bias examination using the funnel plot are presented in Figure 2. Given that there were only 8 studies with 11 effect sizes analyzed, it is difficult to conclude whether the funnel plot results are symmetrical or not. However, through Egger's test it is known that $p > 0.05$ confirms that the funnel plot is symmetrical. Thus it can be concluded that there is no problem of publication bias in this meta-analysis study.

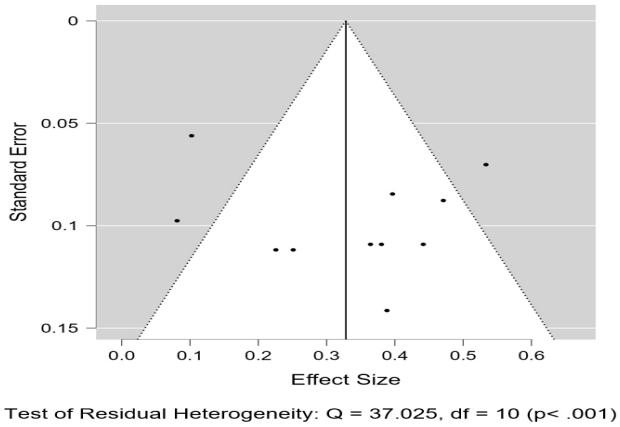

Figure 2: Funnel plot shows the examination of bias in reporting the results of the studies that have been collected and the result of heterogeneity test with Q value parameters

The potential bias of the studies analyzed in this study has been examined first and presented using a funnel plot. Given that there were only 8 studies with 11 effect sizes analyzed, it is difficult to conclude whether the funnel plot results are symmetrical or not. However, through the Egger test, it is known that $p > 0.05$ confirms that the funnel plot is symmetrical. Thus, it can be concluded that there is no problem of publication bias in this meta-analysis study.

Heterogeneity Test

Among the 8 studies that have been collected, there are 2 studies with r values of more than one so that the effect size is calculated separately as explained in the data abstraction section (14,15). In the study by Pilia et al., there were 2 r values, each representing the correlation coefficient between benzene exposure and 8-OHdG metabolites of study respondents in the morning and evening. In the study by Li et al., there were 3 r values representing the correlation coefficient between benzene exposure and 8-OHdG metabolites. In this study, there was a variation in the r value because benzene exposure was measured using 3 biomarkers and all three were tested for correlation with 8-OHdG metabolites. Thus, the number of effect sizes included in the meta-analysis was 11 scores.

The results of the heterogeneity test of 11 effect sizes from 8 studies are shown in Figure 2. The results of the analysis showed that the 11 effect sizes of the studies analyzed were heterogeneous ($Q = 37.025$; $p < 0.001$). Thus, the Random Effect model is more suitable for estimating the mean effect size of the 11 studies analyzed. The results of the analysis also indicate that there is potential to investigate moderator variables that affect the relationship between benzene exposure and 8-OHdG metabolites as markers of DNA damage.

Summary Effect Calculation

The results of the summary effect calculation using the Random Effect model showed that there was a significant positive correlation between benzene exposure and 8-OHdG metabolites as markers of DNA damage ($z=6.615$; $p<0.001$; 95%CI [0.231; 0.425]). The correlation between biomarker of benzene exposure and 8-OHdG metabolites as markers of DNA damage was included in the moderate category (rRE=0.33). The summary effect of the studies analyzed is shown in Figure 3.

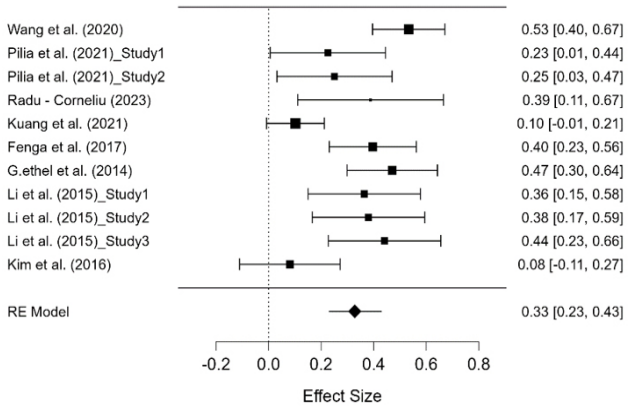

Figure 3: Forest plot showing correlation between biomarker of benzene exposure and 8-OHdG metabolites as biomarkers of DNA damage

The results of the summary effect calculation using the Random Effect model showed that there was a significant positive correlation between benzene exposure biomarkers and 8-OHdG metabolites as markers of DNA damage ($z=6.615$; $p<0.001$; 95%CI [0.231; 0.425]). The correlation between benzene exposure and 8-OHdG metabolites as markers of DNA damage was included in the moderate category (rRE=0.33).

DISCUSSION

Research Subjects and Benzene Exposure

The total research subjects in the reviewed studies were 1066 people. It was found that the research subjects in the benzene exposure studies were almost balanced between exposure that occurred due to work (with workers as research subjects) and non-work (with the general population as research subjects) with a proportion of 49%:51%. The research subjects from the benzene exposure studies that occurred due to work totaled 522 people (exposed group n=327, and control group n=195). Occupational benzene exposure in the reviewed studies mostly involved gas station attendants as research subjects (7,23). The rest, exposure occurred in workers in the car painting industry and crude oil spill cleanup workers (16,24).

The difference test in all the reviewed studies showed that the levels of internal benzene exposure in exposed respondents were significantly different compared to the control group (7,16,23,24). The highest occupational benzene exposure based on benzene exposure biomarker levels occurred in gas station attendants, where the average level of the metabolite t,t-muconic acid (t,t-MA) was 439.80 ± 97.30 µg/g creatinine, almost

four times that of the control group not exposed to benzene (23). These results are in line with the results of Rahimpoor's study which stated that the highest concentration of occupational benzene inhalation exposure was reported in two types of industries, namely solvent-related industries and oil and gas-related industries (25). However, in this study it was found that the levels of t,t-MA in gas station attendants were still below the Biological Exposure Index (BEI) of benzene set by the American Conference of Governmental Industrial Hygienists (ACGIH) which is 500 µg/g, so it can be considered as low-level exposure (26).

The subjects of the non-occupational benzene exposure study were 544 people (14,15,17,27). Of these, 93.75% were children (3-12 years old), while the rest were adults. Most of the subjects experienced exposure because they lived around industrial areas, especially the oil processing industry. The rest, significant benzene exposure was suspected to come from the combustion of motor vehicle fuel and cigarette smoke. The highest level of non-occupational benzene exposure measured by the biomarker t,t-MA reached 381.16 µg/g urine creatinine.

The most widely used benzene exposure biomarkers in the studies reviewed were t,t-MA and urine S-PMA. There was one study that used 1,2-Dihydroxybenzene (1,2-DB) or Catechol (17). A systematic review study also stated that t,t-MA and S-PMA are the two most commonly used metabolites by studies examining benzene exposure because they show better correlation with environmental exposure (28). ACGIH also uses these two metabolites in determining the Biological Exposure Index (BEI) (26).

The concentration of inhaled benzene exposure plays an important role in selecting the optimal biological benzene exposure biomarker (25). A study stated that t,t-MA appears to be a more specific biomarker for high levels of benzene exposure than S-PMA (29). The levels of t,t-MA can reflect the actual levels of benzene in the air with a concentration range of 1.10-86.91 mg/m3. The study also showed no effect of smoking habits or diet on t,t-MA (30). In relation to smoking, there is another study stating that t,t-MA is positively correlated with smoking (31). Not only cigarettes, other studies also mention that diet can significantly affect the concentration of t, t-MA in urine, especially for low-level exposure (≤0.5 ppm) (32).

Meanwhile, it is said that S-PMA is more reliable for low-level benzene exposure (33). Due to its specificity, S-PMA allows to determine benzene exposure up to 0.3 ppm. S-PMA is also a more reliable biomarker compared to t, t-MA for occupational benzene exposure during a 12-hour shift because it has a longer half-life (34). S-PMA is also said to be a more suitable biomarker for non-occupational benzene exposure compared to t, t-MA (30).

8-OHdG Metabolites as Biomarkers of DNA Damage

The formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) was first reported by Kasai and Nishimura in 1984. 8-OHdG is one of the most studied oxidized metabolites and is considered a biomarker for oxidative DNA damage (35). 8-OHdG can be measured in body fluids. The reliability of 8-OHdG makes it a standard marker of oxidative DNA modification. 8-OHdG is formed when guanine bases in DNA are oxidized by reactive oxygen species (ROS). Guanine is the main target because of its lower reduction potential compared to other nitrogen bases. As a result of the oxidation reaction, guanine experiences the addition of a hydroxyl group at the 8th position of its purine ring, forming 8-hydroxy-2-deoxyguanosine (36,37). So far there are no regulations or laws governing the use of 8-OHdG as a biomarker of DNA damage due to benzene exposure. The reason 8-OHdG was widely used as a marker of oxidative DNA damage due to benzene exposure is because 8-OHdG is very specific and occurs abundantly in DNA (10). In addition, changes in 8-OHdG levels may be a non-invasive biomarker of early genotoxic damage in subjects exposed to benzene and could therefore be used as an early screening to prevent more serious health impacts (7,14).

According to previous studies, the levels of 8-OHdG metabolites can be measured in urine, blood, and inhalation samples using certain procedures and instruments (38). However, the measurement of 8-OHdG metabolite biomarkers in this systematic review is known to have been carried out entirely on urine samples. Measurement of 8-OHdG in urine samples has several advantages, namely 8-OHdG is very stable in urine and less invasive than blood samples (39). Detection of 8-OHdG in urine can be done using several methods: Liquid chromatography-coupled tandem mass spectrometer (LC-MS/MS), High-performance liquid chromatography (HPLC), Enzyme-linked immunosorbent assay (ELISA) (40,41).

The analytical methods used to determine 8-OHdG levels in the reviewed studies were balanced between High-Performance Liquid Chromatography (HPLC) and ELISA with a proportion of 50:50. Both methods have their own advantages and disadvantages. The advantage of HPLC is that this assessment is very precise and sensitive, but on the other hand there is a requirement for DNA isolation which causes additional DNA oxidation during purification. In addition, quantification of 8-OHdG levels with this method requires a standard curve that covers both linearity dynamic ranges of more than 6 logarithms. This method also provides total quantification data of cellular oxidation damage and the data is not based on single cells. The advantage of the ELISA method is that currently ELISA kits have been developed commercially so that they are easy to obtain,

but the limitation of this method is in its accuracy and precision, for that specific antibodies are needed for proper testing. Another disadvantage is that there is also a requirement for DNA isolation in this method which results in additional DNA oxidation during purification (40).

Correlation between Benzene Exposure and 8-OHdG Metabolites

Although there are variations in the results of the studies reviewed, the calculation of the summary effect shows that there is a correlation between benzene exposure and the metabolite 8-OHdG as a marker of DNA damage (Figure 2). The strength of this correlation is included in the moderate category. DNA damage marked by 8-OHdG due to benzene exposure can occur through the mechanism of ROS formation and oxidative stress (23,42). This mechanism is explained in several studies although not in detail (7,24). In the body, benzene will be metabolized in the liver by the cytochrome P450 enzyme, especially CYP2E1. This metabolism converts benzene into more reactive metabolites such as benzene oxide, phenol, catechol, hydroquinone, and benzoquinone. Metabolites such as catechol and hydroquinone can undergo repeated redox reactions that can produce superoside anions (O2-) which are one form of ROS. Furthermore, O2- can react through various chemical pathways and produce hydrogen peroxide (H2O2) which under certain conditions can be converted into highly reactive and damaging hydroxyl radicals (OH) (43,44). The accumulation of ROS, if it exceeds the neutralization capacity of antioxidants in the body, can cause oxidative stress. Oxidative stress can damage various cellular components including DNA. One of the nitrogen bases in DNA that is very vulnerable is guanine. OH, one of the most reactive forms of ROS, can attack guanine, producing a chemical modification of 8-OHdG (36,37).

Metabolites of 1,4-benzoquinone (1,4-BQ) known as benzene metabolites that are genotoxic also have an indirect role in oxidative stress (45). Although 1,4-BQ does not directly produce ROS like catechol and hydroquinone, 1,4-BQ can trigger oxidative stress by participating in redox reactions. 1,4-BQ can deplete glutathione (GSH) which can weaken the antioxidant defense system so that cells are more susceptible to oxidative damage (46,47). This oxidative damage can be characterized by 8-OHdG. Various studies have shown that increased levels of 8-OHdG in human urine are closely related to the accumulation of mutations that trigger carcinogenesis (40,48).

Limitations of The Study

The limitations of this study include variations in the age of respondents and the type of urinary biomarker used to describe benzene exposure. This limitation was due to the limited number of studies that met the inclusion criteria, making it unfeasible to limit the analysis to only one age group or a single biomarker to describe benzene exposure. Nevertheless, this study can still provide valuable initial insights into the relationship between benzene exposure biomarkers and 8-OHdG metabolites as biomarkers of DNA damage more broadly. These findings can open up opportunities for further research that is specific to age and certain benzene exposure biomarkers to improve the precision of future research results.

CONCLUSION

In this study, it was found that the most widely used biomarkers of benzene exposure were t, t-MA and S-PMA. The 8-OHdG biomarker was detected by HPLC and ELISA. In addition, there were variations in the results of studies examining the relationship between benzene exposure and 8-OHdG metabolites. However, this study found that there was a moderate correlation between benzene exposure and 8-OHdG metabolites as markers of DNA damage. These results can be used as a basis for policy making by authorized agencies in strengthening regulations on benzene emissions and early detection programs for diseases related to genotoxicity, especially in high-risk groups.

REFERENCES

1. WHO. Preventing Disease Through Healthy Environments Exposure To Benzene: A Major Public Health Concern. In Switzerland; 2019.
2. CDC. Emergency Preparedness and Response CDC. Facts About Benzene. (2018). Accessed: 2024 Aug 5, 2024. https://emergency.cdc.gov/agent/benzene/basics/facts
3. ACS. American Cancer Society. Benzene and Cancer Risk. (2023). Accessed: Aug 5, 2024. https://www.cancer.org/cancer/risk-prevention/chemicals/benzene.
4. Chow PW, Rajab NF, Chua KH, Chan KM, Abd Hamid Z. Differential responses of lineages-committed hematopoietic progenitors and altered expression of self-renewal and differentiation-related genes in 1,4-benzoquinone (1,4-BQ) exposure. Toxicol In Vitro. 2018;46:122–8. doi: 10.1016/j.tiv.2017.10.001
5. Fracasso ME, Doria D, Bartolucci GB, Carrieri M, Lovreglio P, Ballini A, et al. Low air levels of benzene: Correlation between biomarkers of exposure and genotoxic effects. Toxicol Lett. 2010;192(1):22–8. doi: 10.1016/j.toxlet.2009.04.028
6. Morihito RVSA, Chungdinata SE, Nazareth TA, Pulukadang MI, Makalew RAM, Pinontoan B. Identifikasi Perubahan Struktur Dna Terhadap Pembentukan Sel Kanker Menggunakan Dekomposisi Graf. J Ilm Sains. 2017;17(2):153–60. doi: https://doi.org/10.35799/jis.17.2.2017.17368
7. Fenga C, Gangemi S, Teodoro M, Rapisarda V, Golokhvast K, Docea AO, et al. 8-Hydroxydeoxyguanosine as a biomarker of

oxidative DNA damage in workers exposed to low-dose benzene. Toxicol Rep. 2017;4:291–5. doi: 10.1016/j.toxrep.2017.05.008
8. Honda M, Yamada Y, Tomonaga M, Ichinose H, Kamihira S. Correlation of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, and clinical features of hematological disorders: a pilot study. Leuk Res. 2000;24(6):461–8. doi: 10.1016/S0145-2126(00)00006-0
9. Indraprasta S, Zulkarnain I, Ervianti E. Peningkatan Kadar 8-Hydroxydeoxyguanosine (8-OHdG) Urine pada Pasien Dermatitis Atopik Anak. Berk Ilmu Kesehat Kulit Dan Kelamin. 2016;28(3). doi: https://doi.org/10.53730/ijhs.v6nS6.9983
10. Awooda HA. Pathophysiology of Cerebral Ischemia: Role of Oxidative/Nitrosative Stress. J Biosci Med. 2019;07(03):20–8. doi: 10.4236/jbm.2019.73003
11. Niu BY, Li WK, Li JS, Hong QH, Khodahemmati S, Gao JF, et al. Effects of DNA Damage and Oxidative Stress in Human Bronchial Epithelial Cells Exposed to PM2.5 from Beijing, China, in Winter. Int J Environ Res Public Health. 2020;17(13):4874. doi: 10.3390/ijerph17134874
12. Abriyani E, Mulyawan I, Shakira NA, Haryadi R, Kholisoh T. Aktivitas Antioksidan Pada Bunga Telang (Clitoria ternatea L.) Secara Metode Spektrofotometeri Uv-Visible. J Compr Sci. 2022;1(5):1351–4. doi: https://doi.org/10.59188/jcs.v1i5.169
13. Mm E, Am S, Ma S, M A. 8-hydroxy-2′-deoxyguanosine (8-OHdG) and Runx1-Runx1t1 Translocation: Potential Risk Factors For Leukemogenesis In Benzene Exposed Workers. Egypt J Occup Med. 2019;43(1):145–59. doi: 10.21608/ejom.2019.25129
14. Pilia I, Campagna M, Marcias G, Fabbri D, Meloni F, Spatari G, et al. Biomarkers of Low-Level Environmental Exposure to Benzene and Oxidative DNA Damage in Primary School Children in Sardinia, Italy. Int J Environ Res Public Health. 2021;18(9):4644. doi: 10.3390/ijerph18094644
15. Li J, Lu S, Liu G, Zhou Y, Lv Y, She J, et al. Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose–effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China. Sci Total Environ. 2015;524–525:74–80. doi: 10.1016/j.scitotenv.2015.04.020
16. Kim G, Kang TS, Yoon M, Jo H, Joo Y, Yu SD, et al. Health Effect Assessment on Cleanup Workers of an Oil Spill in Yeosu. Korean J Environ Health Sci. 2016;42(6):385–95. doi: 10.5668/JEHS.2016.42.6.385
17. Kuang H, Li Z, Lv X, Wu P, Tan J, Wu Q, et al. Exposure to volatile organic compounds may be associated with oxidative DNA damage-mediated childhood asthma. Ecotoxicol Environ Saf. 2021;210:111864. doi: 10.1016/j.ecoenv.2020.111864
18. PRISMA. PRISMA 2020. (2024). Accessed: Aug 2, 2024. https://www.prisma-statement.org/prisma-2020
19. NIH. National Institutes of Health. Study Quality Assessment Tools. (2021). Accessed: Aug 1, 2024. https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools
20. Lin L, Chu H. Quantifying Publication Bias in Meta-Analysis. Biometrics. 2018;74(3):785–94. doi: 10.1111/biom.12817
21. JASP. JASP. Jeffreys's Amazing Statistics Program (JASP). (2024). Accessed: Jul 20, 2024. https://jasp-stats.org/download/
22. Elsevier [Internet]. MOOSE (Meta-analyses Of Observational Studies in Epidemiology) Checklist. Accessed: Aug 2, 2024. https://legacyfileshare.elsevier.com/promis_misc/ISSM_MOOSE_Checklist.pdf
23. Guethel G, Brucker N, M. Moro A, F. Charro M, Fracasso R, Barth A, et al. Evaluation of genotoxicity in workers exposed to benzene and atmospheric pollutants. Mutat Res Toxicol Environ Mutagen. 2014;770:61–5. doi: 10.1016/j.mrgentox.2014.05.008
24. Wang K, Wang T, Zhou Y hua, Pu Y, Zang J. Distribution of S-phenylmercapturic acid and 8-hydroxy-2′-deoxyguanosine in urine of workers exposed to low-concentration benzene. J Environ Occup Med. 2020;37(5):413–20. doi: 10.13213/j.cnki.jeom.2020.19802
25. Rahimpoor R, Jalilian H, Mohammadi H, Rahmani A. Biological exposure indices of occupational exposure to benzene: A systematic review. Heliyon. 2023;9(11):e21576. doi: 10.1016/j.heliyon.2023.e21576
26. NIH. NIH National Library of Medicine. Benzene. (2018). Accessed: Aug 11, 2024. https://www.ncbi.nlm.nih.gov/books/NBK550161/
27. Radu - Corneliu D. 68 Urinary Biomarkers of Benzene Exposure and Oxidative Damage in Residents of the Oil Extraction Area of Muanda, Dr Congo. Ann Work Expo Health. 2023;67(Supplement_1):i22–i22. doi: 10.1093/annweh/wxac087.057
28. Carrieri M, Bonfiglio E, Scapellato ML, Macca I, Tranfo G, Faranda P, et al. Comparison of exposure assessment methods in occupational exposure to benzene in gasoline filling-station attendants. Toxicol Lett. 2006;162(2–3):146–52. doi: 10.1016/j.toxlet.2005.09.036
29. Zhang L, Ye F li, Chen T, Mei Y, Song S zhen. Trans, Trans-Muconic Acid as a Biomarker of Occupational Exposure to High-Level Benzene in China. J Occup Environ Med. 2011;53(10):1194–8. doi: 10.1097/JOM.0b013e31822cfd36
30. Cui S, Pang B, Yan H, Wu B, Li M, Xing C, et al. Using Urinary Biomarkers to Estimate the

Benzene Exposure Levels in Individuals Exposed to Benzene. Toxics. 2022;10(11):636. doi: 10.3390/toxics10110636
31. Farmer PB, Kaur B, Roach J, Levy L, Consonni D, Bertazzi PA, et al. The use of S-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. Chem Biol Interact. 2005;153–154:97–102. doi: 10.1016/j.cbi.2005.03.013
32. Jalai A, Ramezani Z, Ebrahim K. Urinary Trans, Trans -Muconic Acid is Not a Reliable Biomarker for Low-level Environmental and Occupational Benzene Exposures. Saf Health Work. 2017;8(2):220–5. doi: 10.1016/j.shaw.2016.09.004
33. Protano C, Andreoli R, Manini P, Vitali M. Urinary trans, trans-muconic acid and S-phenylmercapturic acid are indicative of exposure to urban benzene pollution during childhood. Sci Total Environ. 2012;435–436:115–23. doi: 10.1016/j.scitotenv.2012.07.004
34. Boogaard PJ, Van Sittert NJ. Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans,trans-muconic acid, and phenol. Occup Environ Med. 1995;52(9):611–20. doi: 10.1136/oem.52.9.611
35. Kasai H, Hayami H, Yamaizumi Z, Saito H, Nishimura S. Detection and Identification of Mutagens and Carcinogens as Their Adduct with Guanosine Derivates. Nucleic Acids Res. 1984;12(4):2127–36. doi: 10.1093/nar/12.4.2127
36. Goriuc A, Cojocaru KA, Luchian I, Ursu RG, Butnaru O, Foia L. Using 8-Hydroxy-2′-Deoxiguanosine (8-OHdG) as a Reliable Biomarker for Assessing Periodontal Disease Associated with Diabetes. Int J Mol Sci. 2024;25(3):1425. doi: 10.3390/ijms25031425
37. Andrйs CMC, Lastra JMPDL, Juan CA, Plou FJ, Pйrez-Lebeca E. Chemical Insights into Oxidative and Nitrative Modifications of DNA. Int J Mol Sci. 2023;24(20):15240. doi: 10.3390/ijms242015240
38. Omari Shekaftik S, Nasirzadeh N. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker of oxidative DNA damage induced by occupational exposure to nanomaterials: a systematic review. Nanotoxicology. 2021;15(6):850–64. doi: 10.1080/17435390.2021.1936254
39. Graille M, Wild P, Sauvain JJ, Hemmendinger M, Guseva Canu I, Hopf NB. Urinary 8-OHdG as a Biomarker for Oxidative Stress: A Systematic Literature Review and Meta-Analysis. Int J Mol Sci. 2020;21(11):3743. doi: 10.3390/ijms21113743
40. Korkmaz KS, Debelec Butuner B, Roggenbuck D. Detection of 8-OHdG as a diagnostic biomarker. J Lab Precis Med. 2018;3:95–95. doi: 10.21037/jlpm.2018.11.01
41. Cooke MS, Olinski R, Loft S, members of the European Standards Committee on Urinary (DNA) Lesion Analysis (ESCULA). Measurement and Meaning of Oxidatively Modified DNA Lesions in Urine. Cancer Epidemiol Biomarkers Prev. 2008;17(1):3–14. doi: 10.1158/1055-9965.EPI-07-0751
42. Nishikawa T, Izumo K, Miyahara E, Horiuchi M, Okamoto Y, Kawano Y, et al. Benzene Induces Cytotoxicity without Metabolic Activation. J Occup Health. 2011;53(2):84–92. doi: 10.1539/joh.10-002-OA
43. Barreto G, Madureira D, Capani F, Aon-Bertolino L, Saraceno E, Alvarez-Giraldez LD. The role of catechols and free radicals in benzene toxicity: An oxidative DNA damage pathway. Environ Mol Mutagen. 2009;50(9):771–80. doi: 10.1002/em.20500
44. Peng D, Jiaxing W, Chunhui H, Weiyi P, Xiaomin W. Study on the cytogenetic changes induced by benzene and hydroquinone in human lymphocytes. Hum Exp Toxicol. 2012;31(4):322–35. doi: doi.org/10.1177/096032711143390
45. Yang J, Bai W lin, Chen Y jiao, Gao A. 1,4-benzoquinone-induced STAT-3 hypomethylation in AHH-1 cells: Role of oxidative stress. Toxicol Rep. 2015;2:864–9. doi: 10.1016/j.toxrep.2015.05.013
46. Mathialagan RD, Abd Hamid Z, Ng QM, Rajab NF, Shuib S, Binti Abdul Razak SR. Bone Marrow Oxidative Stress and Acquired Lineage-Specific Genotoxicity in Hematopoietic Stem/Progenitor Cells Exposed to 1,4-Benzoquinone. Int J Environ Res Public Health. 2020;17(16):5865. doi: 10.3390/ijerph17165865
47. Zhang J, Cao M, Yang W, Sun F, Xu C, Yin L, et al. Inhibition of Glucose-6-Phosphate Dehydrogenase Could Enhance 1,4-Benzoquinone-Induced Oxidative Damage in K562 Cells. Balbaa M, editor. Oxid Med Cell Longev. 2016;2016(1):3912515. doi: 10.1016/j.envpol.2023.121765
48. Al-Hashimi NN, Shahin RO, El-Sheikh AH, Jibreel MJ, Alsakhen NA, Alqudah AM, et al. A new approach for determination of urinary 8-hydroxy-2′-deoxyguanosine in cancer patients using reinforced solid/liquid phase microextraction combined with HPLC-DAD. Acta Chromatogr. (2023). Accessed: Jul 4, 2024. https://akjournals.com/view/journals/1326/aop/article-10.1556-1326.2023.01142/article-10.1556-1326.2023.01142.xml