ORIGINAL ARTICLE

ASSESSING BIOMARKERS ON EXPOSURE, EFFECTS AND SUSCEPTIBILITY FOR ENVIRONMENTAL AND OCCUPATIONAL EXPOSURE OF VARIOUS RANGE OF BENZENE

Noor Fatihah MF[1], Suhaili A[2], Juliana J[1]

[1]Department of Environmental and Occupational Health, Faculty of Medicine and Health Science, 43400 Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia

[2]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, 43400 Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia

ABSTRACT

Background: Benzene is primarily routed by inhalation which highly sensitive to blood parameters as bone marrow is their target organ. The ability of benzene even in low exposure levels may induce human bone marrow suppression resulting in blood diseases such as leukopenia, anemia, thrombocytopenia, aplastic anemia, and pancytopenia. In the occupational setting, the most common benzene-exposed workers are from the petrochemical industries and petrol distribution such as gasoline pumps. Benzene also generated primarily by mobile exhaust and some from various of anthropogenic sources at environmental atmosphere and occupationally exposed in the policemen traffic, taxi and bus drivers, and street vendors in long-length time with low concentration exposure. Methodology: This paper reviewed published articles on biomarkers exposure, effects and susceptibility as the useful tools for benzene exposure assessment in the occupational and environmental setting. Data from previous epidemiological studies relevant to benzene exposure in various occupational and environmental setting is also summarized. Results: Based on these analyses, the findings agreed that these biomarkers are could suggest in linking the benzene exposure with possible adverse health effects. The biological monitoring used in epidemiological studies is useful in providing an understanding of activation and detoxification of benzene in both the occupational and general population as they are exposed to wide range of benzene concentration. Conclusion. The biomarkers of exposure, effects, and susceptibility utilized for benzene exposure assessment are valid tools in determining the greatest potential risk as well as an early biological effect which then caused a related specific disease.

Keywords: *benzene exposure, biomarkers, genetic polymorphisms and environmental and occupational population*

INTRODUCTION

Benzene is a high-concern compound emitted in the industrial and urban ambient air from both, natural process and anthropogenic source[21]. The Class I carcinogens of benzene[60] which is highly sensitive to the blood parameters such as erythrocytes, leukocytes and platelets and causes leukemogenic risk is well-established. In an adult, the benzene toxicity has shown to disrupt the blood-forming (hematopoietic) cells. Thus, benzene leads to particular blood disease such as hematoxicity even in low levels of benzene concentrations[17] and also able to cause aplastic anemia and leukemia.

The toxicity of benzene on bone marrow as its target organ is well-established[42] which further causes risk of leukemia and other hematological cancers[40]. There are also numerous of epidemiological studies on the biomarker on exposure, effects, or susceptibility[46, 10, 28, 2, 3, 24, 25, 19, 51] including effects of hepatic, hematological, genotoxicity, cytotoxicity, reproductive, and neurological effects[56]. The biomarker assessment is a useful tools in understanding the markers of exposure, effects, and susceptibility related to benzene exposure for both either in the environmental or occupational setting. This paper reviewed the studies on influenced of genetic susceptibility involved in benzene metabolic pathway and DNA-repair capacity to makers of exposure and effects for benzene exposure in the environmental and occupational setting which further able to identifying the high-risk populations[18].

Environmental and occupational exposure to benzene Ambient air pollutants comprises of particulate matter 10 (PM_{10}), particulate matter 2.5 ($PM_{2.5}$), ultrafine particle (UFP) and volatile organic compounds (VOCs) such as benzene and 1,3-butadiene which posing the greatest risks to human health to the variety of exposed population group[33, 34, 22, 9]. Benzene in atmosphere primarily originated from vehicle emission and the wide range of benzene concentration are to be in high as that vicinity surrounded by heavy traffic, roadside, tunnel, industrial development and petrol stations[32]. There is no safe level and guidelines develop for the benzene ambient[62]. The general guidance for benzene in ambient refer to the lifelong exposure to ambient benzene concentration of 1 $\mu g/m^3$ is estimated to cause six cases per million of people to develop the risk of leukemia[63].

In the occupational setting, benzene is used as a primary aromatic solvent in the chemical industries, adhesive, shoe manufacturing, printing industry, synthetic rubbers, lubricants, dyes, pharmaceuticals and pesticides[56]. The most common benzene-exposed workers are from the petrochemical industries and petrol distribution such as gasoline pumps as well as factories with the usage of organic chemicals and paint production[50]. The permissible exposure limit for 8 hours' time-

weighted average (TWA) of benzene-exposed workers is up to 1 ppm in United States and European countries[57] and Malaysia has a limit of 0.5 ppm[61]. Even, a person who is exposed below than 1 ppm of benzene still can have an altered the blood count[26] or do potential having genotoxic effects in susceptible individuals even at the ambient benzene concentration[46].

Biomarkers monitoring in environmental and occupational exposure to benzene Biomarkers monitoring is valued tools in the practice of occupational safety and health (OSH)[27] as well as for the environmental exposure. The application of biomarkers in research allows to viewed and understanding the mechanism of chemicals pass through the body which further develop the potential disease. Commonly, there are three well-known categories of biomarkers named as exposure, effect, and susceptibility[25, 38].

METHODOLOGY

The search strategy databases are through Science direct & PubMed are been utilized in this analysis. The main articles focused are on the biomarker of susceptibility for benzene exposure. The keywords used for the search strategy are including benzene exposure, biomarkers, genetic polymorphism and environmental & occupational population. The periods of reviewed articles are from year of the 2003 until the year of 2014. The inclusion criteria for articles reviewed are comprises the original scientific papers and reviews types, the measurement the biomarkers are included either biomarkers of exposure & susceptibility, effects & susceptibility or/and three of them among environmental and occupational population and the language medium of articles is English. The articles which used non-English language and no study on biomarkers susceptibility are excluded.

RESULTS

Biomarkers of exposure

Table 1 showed the collected findings of the biomarkers of exposure, effects, and susceptibility from year 2003 to year 2014**.** Biomarkers of exposure for benzene can be detected through biological samples for example unmetabolized benzene in the blood, breath and urine, urinary benzene metabolites, benzene adducts in DNA, hemoglobin and albumin[49]. However, the authors only addressed on the biomarkers of exposure of blood, urinary benzene and urinary benzene metabolites such as S-phenylmercapturic acid (*S*-PMA) and trans, trans-muconic acid (*t,t*-MA). Five publications have evaluated the benzene metabolites of *S*-PMA and/or *t,t*-MA which three studies focused on combination both *S*-PMA and *t,t*-MA[46, 28, 19] and other two studies have determined only on either *S*-PMA[2] or *t,t*-MA[10].

In 2003, Sørensen and colleagues[46] found that men excreted more *t,t*-MA compared than women and revealed the significant association with personal exposure (median exposure is 2.5 μg/m^3) to benzene in living and working population in a central Copenhagen[46]. In the year 2010, Manini et al.[28] observed the *S*-PMA level significantly higher in both smoker and non-smoker of taxi drivers while *t,t*-MA excretion significantly higher among non-smoker of taxi drivers only. However, all subgroups (traffic policemen, gasoline pump attendants and taxi drivers) showed that the smokers excreted significantly higher both *S*-PMA and *t,t*-MA level than non-smokers with exception of *t,t*-MA in taxi driverswith personal exposure concentration is 38.3 μg/m^3 (median exposure)[28]. Garte*et al.*[19] reported that *S*-PMA and *t,t*-MA level have a significant relationship with 1.8 ppm benzene ambient exposure among the occupational group. Then in 2011, Angelini and co-workers[2] confirmed the higher *S-PMA* excretion observed in the traffic policemen compared than the indoor workers.

In urban schoolchildren population, the excretion of muconic acid or known as *t,t*-MA observed to has a statistically significant difference from rural areas at three times (day 0, morning, day 0, afternoon; and day 1, morning) and correlated well with the personal exposure level[10]. Only a paper focused on the biomarker of exposure by assessed the urinary benzene and blood benzene level[10]. Both markers of exposure have a significant correlation with the personal exposure levels of benzene in the Bangkok schoolchildren who those lived in areas with high traffic density which exposed to high level of benzene rather than schoolchildren in the rural areas.

Biomarkers of effects

Biomarker of effects refers to the early health effects which further can cause health impairment or cancer. In this reviewed, biomarkers of effects for benzene exposure generally been studied on micronuclei (MN) frequency[2, 3, 51, 25], comet assay for measuring DNA damage[46], chromosomal aberrations (CA)[24, 25], DNA Single Strands Breaks (SSB)[19] and oxidative stress such as 8-hydroxy-2'-deoxyguanosine (8-OHdG)[10] and 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG)[46] including nucleic acid oxidation[28] such as 8–oxodGuo, 8-oxoGuo, and 8-oxoGua that reflect the repair system within the human body.

Micronuclei (MN) frequency

Four publications have assessed MN frequency in determining the early of health effects. MN assay is useful in monitoring the human exposure to occupational and environmental genotoxin[14]. The Falck*et al.*[58] has defined the micronuclei frequency as a small, additional nuclei, are formed in mitosis from acentric chromosomal fragments or chromosomes that lag behind in anaphase and eventually fail to be included in either of the daughter nuclei. It is accepted that the high frequency of micronu-

clei formation is revealed the indicator for the high risk to develop the cancer[8]. All studies resulted that there was significantly higher of MN frequency was recorded among individual with the high level of exposure to benzene compared than those who exposed in the low level of benzene.

Comet assay

The comet assay was studied[46]by observing the strand break, fapyguanineglycosylase (FPG) and endonuclease III (ENDO) sensitive sites which useful for DNA damage detection[47, 48]. However, there are no significance differences of comet score reported with respect to the exposed and comparative group. No further results observed for the association between biomarkers of exposure and genetic polymorphism using this assay.

Chromosomal aberrations (CA)

The chromosomal aberrations (CA) are applicable for many years of exposure[15] and one of tools for future cancer predictor[7]. Kim *et al*.[24] reported that there was a significant association between benzene exposure and the frequencies of monosomy and trisomy of chromosomes 8 and 21. The benzene-exposed group has shown the significantly increased frequencies of monosomy and trisomy of chromosomes 8 and 21 by translocation for eightfold compared than the control group. Similarly, another study[25] revealed that the frequencies of CA were significantly higher in the benzene-exposed workers compared than unexposed workers.

DNA Single Strands Breaks (SSB)

Garte*et al*.[19] reported that the benzene-exposed workers having significantly higher SSB compared than the unexposed workers particularly in individual with variant *NQO1* T alleles.

Oxidative stress and nucleic acid oxidation

The individuals with *NQO*+/- genotype (variant-types) showed a significant correlation between *S*-PMA excretion and 8-oxodG compared than individuals carrying *NQO*+/+ genotypes (wild-types)[46]. An article by Buthbumrung*et al*.[10] by reported that 8-OHdG in leukocytes DNA and urine was higher in Bangkok schoolchildren compared with the rural schoolchildren. Similarly, the level of 8-OHdG in leukocytes DNA was statistically associated with benzene exposure level but not in urinary 8-OHdG. Sørensen*et al*.[46] observed a significant correlation between *S*-PMA excretion and 8-oxodG in lymphocytes. The urinary biomarkers of nucleic acid oxidation, namely 8-oxodGuo, 8-oxoGuo and 8-oxoGua were found to have a well correlation with each other and *S*-PMA and *t,t*-MA level[28]. All smokers group showed higher concentration of 8-oxoGua in traffic policemen, 8-oxodGuo in the gasoline pump attendants and 8-oxoGuo and 8-oxoGua in the taxi drivers, more than non-smoking in all subgroups.

Genetic polymorphisms as biomarkers of susceptibility Variation among individuals genome increases the sensitivity of target molecules or metabolism are able to increase the target dose20 which is known as biomarker of susceptibility. The pathways of benzene activation and detoxification involving phase I in liver and phase II in the bone marrow. The polymorphism of Cytochrome P450 2E1 (CYP2E1), NAD(P)H-quinoneoxidoreductase (NQOI), myeloperoxidase (MPO), Glutathione S-transferases T1 (GSTT1) and Glutathione S-transferases M1 (GSTM1) are involved in benzene activation and detoxification, may affect individual susceptibility to benzene toxicity51. In the phase I, CYP2E1 mainly oxidized benzene into intermediate metabolites such as phenol, hydroquinone, catechol and 1,2,4- benzenetriol39.

Meanwhile, the phase II of benzene metabolism occur in the bone marrow which MPO is responsible in converting these intermediate metabolites into toxic quinones, 1,4-benzoquinones. This quinones are may induces the protein and DNA adducts formation as well as reactive oxygen species (ROS)52. In fact, the production of the electrophilic metabolites such as benzene oxide (BO), benzene dihydrodiol epoxide (BDE), hydro-quinone (HQ), 1,2-, 1,4-, and 1,2,4-semiquinones (SQ) and benzoquinones (BQ), 1,2,4-benzenetriol (BT) and t,t-muconaldehyde as well as reactive oxygen species (ROS) from the biotransformation of benzene capable to form the covalent bonding with macromolecules cells for example RNA, DNA, protein and lipids which then impairing their functions12.

NQO1 was agreed to be responsible in detoxification of benzoquinones into less toxic via the reduction process36 which preventing oxidative damage35. The GSTs also considered involved in detoxification process by conjugated the benzene metabolites such as benzene oxide into less toxic compound, S-phenylmercapturic acid (S-PMA). In this articles reviewed, the genetic polymorphisms of the studied genes are involved in the phase 1 and II of benzene metabolism such as Cytochrome P450 2E1 (CYP2E1), NAD(P)H-quinoneoxidoreductase (NQOI), myeloperoxidase (MPO), Glutathione S-transferases T1 (GSTT1), Glutathione S-transferases M1 (GSTM1) and Glutathione S-transferases P1 (GSTP1). The genes responsible for DNA repair system are known as potential markers of genetic susceptibility to benzene toxicity. The other genes that claimed involved in the metabolism of benzene toxicity are Gutathione S-transferase alpha 1 (GSTAI), epoxide hydrolase (mEH) and N-acetyltransferase (NAT2).

Cytochrome P450 2E1 (CYP2E1)

CYP2E1 is responsible to metabolize benzene into epoxy benzene and spontaneously form phenol which further catalyzed into hydroquinone and catechol, more toxic than phenol itself53. Four studies have been evaluated the modulating effects of CYP2E1 genes towards marker of exposure; blood benzene level, urinary MA and 8-OHdG level10 and marker of effects; micronuclei frequency51 and aneuploidy and structural chromosomal

aberration24. Among the few "positive studies", Zhang et al.51 reported that the shoes-workers who carried CYP2E1 variant alleles genotype for promoter rs3813867 (CC+GC) and rs2031920 (CC+TT) showed to be associated with a significantly higher MN frequency compared than wild-type. Meanwhile there was no significant effect of CYP2E1 genotype on the blood benzene level, urinary MA and 8-OHdG level measured in leukocytes or urine10 are documented. However, the combination of variant genotype of CYP2E1*1/*5 and CYP2E1*5/*5 group revealed the slightly lower of urinary MA level in urine than in will type group, CYP2E1*1/*1.

Similarly, in 2004, Kim and colleagues24 observed there was no significant effect of CYP2E1 genetic polymorphisms on the increased frequencies of aneuploidy and structural chromosomal aberration. However, the combination of CYP2E1 DraI and RsaI variant-type revealed an association in increased frequency chromosomal aberrations compared than wild-type group. Angelini et al. 2 unable to analyze the CYP2E1 genotype in relation to MN frequencies and S-PMA excretion due an extremely low background frequency, genotype data of CY2PE1 genotype.

NAD(P)H-quinoneoxidoreductase (NQO1)
Seven publications have determined the influence of NQO1 polymorphism on variety marker of exposure and effects46, 10, 28, 2, 19, 24, 25. The five articles described that the variants of the NQO1 genotype significantly has modulating effects on t,t-MA and S-PMA excretion46, oxidation damage to nucleic acid28 particularly, RNA, single strands breaks (SSB)19, and increased frequency of translocation between chromosome 8 and 2124. After adjustment for age, smoking status, and alcohol intake, Kim et al.25 observed there were significantly affect frequencies of CA and MN among benzene-exposed workers with variant T/T genotype compared than controls with C/C and C/T genotypes.

Most of the articles revealed that NQO1 for variant genotype group (C/T and T/T) were found to significantly affect either the biomarker of exposure or/and effects compared than wild-type group (C/C). Meanwhile, only an article revealed that variant genotype of NQO1 had lower levels of urinary MA and 8-OHdG measured in leukocytes or urine10. However, Angelini et al. 2 did not describe between NQO1 and MN frequencies and S-PMA excretion as the data was not analyzed.

Glutathione S-transferases T1 (GSTT1), Glutathione S-transferases M1 (GSTM1) and Glutathione S-transferases P1 (GSTP1) Six publications evaluated the Glutathione S-transferases (GSTs) family for genes addressed are GSTT1, GSTM1, and/or GSTP12, 28, 10, 24, 46, 51. The previous study by Zhang et al.51 documented GSTM1-null, GSTT1-null, and GSTP1 rs1695, rs6413432, rs1051740 and rs2234922 polymorphisms showed no association with biomarker of effect, MN frequency. Angelini et al. 2 evaluated that only men who is carried the GSTM1-null genotype had a significantly higher median MN frequency compared with men with wild type of GSTM1 genotype. However no association of GSTM1 polymorphism has been found with S-PMA excretion. In 2010, Manini and colleagues28 quantified that the individuals with GSTM1-null and GSTT1-null genotypes excreted the lowest S-PMA levels than individuals with GSTM1-pos and/or GSTT1-pos type.

Buthbumrug and colleagues10 have found that schoolchildren who carried the GSTM1*1/ genotype (at least one undeleted allele) excreted more urinary MA than those with the GSTM1*2/*2 genotype (both alleles deleted). However, no significant effect was noted for GSTM1 genotypes on 8-OHdG level both in leukocytes and urine. Meanwhile, GSTT1 genotypes did not affect significantly urinary MA and 8-OHdG level measured in leukocytes or urine. In year 2004, Kim and co-workers24 found that the individual who exposed to benzene showed the higher of monosomy of frequency of chromosome 8 and 21 for those carried null-genotype of GSTM1 compared than GSTM1-positive genotype.

Similarly, individuals who bearing the GSTT1-null genotype revealed the higher in frequencies of chromosome 21 monosomy and chromosome 8 and 21 trisomy than for those bearing the GSTT1-positive genotype in the benzene-exposed group. However, the GSTP1 genetic polymorphism did not show any significant effects on the increased frequencies of aneuploidy and structural chromosomal aberration. In year 2003, Sørensen and co-workers46 observed the GSTP1, GSTM1 and GSTT1 genotypes did not affect the excretion of S-PMA or t,t,-MA significantly. Although not significant, there appeared to be an additive effects for those carrying GSTM1 and GSTT1-wild type as they exhibited to excrete more t,t-MA than individuals whose carrying the GSTM1 and GSTT1-variant type.

Gutathione S-transferase alpha 1 (GSTAl)
In 2010, Manini and co-workers28 were confirmed a significant modulating effect of GSTA1 ($p = 0.048$) polymorphisms on S-PMA excretion while Buthbumrunget al.10 found that individuals whose carried the variant genotype of GSTA1 had lower levels of urinary MA.

Myeloperoxidase (MPO)
Kim et al.25 documented the benzene-exposed workers with the MPO wild-type (G/G) genotype have a 2.3-fold rise in CA and MN frequency compared to controls with heterozygote (G/A) or variant-type (A/A) genotypes, after adjusting for age, smoking status, and alcohol intake. The other study by Angeliniet al.2 did not rigorously discussed on MPO genotype.

Epoxide hydrolase (mEH)
Angeliniet al.2 reported that high epoxide hydrolase (mEH) (predicted) enzyme activity was significantly correlated with a lower median MN frequency (P = 0.003). Zhang et al.51 evaluated no association the mEH polymorphisms rs1051740 and rs2234922 with the MN frequency which may due to a limited number of mEH mutant allele carrier.

N-acetyltransferase (NAT2)
Only an article evaluated the polymorphism of NAT2 which the finding revealed the frequency of chromosome 8 trisomy was significantly higher for the NAT2 slow acetylator genotype compared than for the fast acetylator genotype in the benzene-exposed group (P = 0.030)24.

DNA Repair genes
The DNA repair genes studied (hOGG1, NBS1, XPD, XRCC1, and XRCC3 exception APEX1) did not reveal any association with the median MN frequency3. However, a gender effect which is men subjects reported to have the relationship between median MN frequency and APEX1 genotype for homozygous variant allele type3. Kim et al.25 observed that the benzene-exposed workers with XRCC1 (Gln/Gln) variant-types showed significant 2.2-fold increases in the CA and MN frequencies, respectively, compared to the wild-type or heterozygous controls.

DISCUSSION

Most studies in this paper review addressed the biological markers of exposure urinary benzene metabolites such as S-PMA and t,t-MA as the biological indicator for the benzene exposure. Only an article found to include the markers of blood and urinary benzene as another biological marker of exposure to benzene. The production S-PMA and t,t-MA as well as other urinary benzene metabolites (phenol, hydroquinone, catechol, 1,2,4-trihydroxybenzene (trihydroxybenzene) yielded from metabolize of benzene oxide–oxepin in both enzymatic and non-enzymatic pathways which eliminated in urine either unchanged or as a sulphate and glucuronide43, 45. The use of S-PMA as a biomarker of exposure to benzene are most sensitive, specific and reliable30, 31, 46 as compared than t,t-MA which less specificity2 which easily influenced by sorbic acid in most of preservative food intake29. However, both markers of exposure are sensitive to benzene exposure level exceed 1 ppm18.

The potentiality of benzene in cause toxicity and cellular damage in the body is considered responsible by the benzene metabolites produced from activation and detoxification of benzene. Some of benzene metabolites such as phenol, catechol and hydroquinone able to form reactive oxygen species (ROS)46 which can cause cell damage. The production of phenolic metabolites as well as the most toxic polyphemolic metabolites such as hydroquinone (HQ), catechol and 1,2,4-benzene triol able to induce chromosomal damage and oxidative stress which further lead to DNA damage37. Thus there a number of assessment which established in determining the early health effects such as MN, CA, comet assay, SSB, oxidative DNA damage and nucleic acid oxidation2, 3, 51, 24, 25, 46, 19, 10, 28.

MN and CA assay are suitable for past exposure detection59 which CA is an indicator for increase cancer risk25. All the articles reviewed revealed the increased MN and CA frequencies for benzene exposure level at occupational and ambient level specifically among exposed population. The oxidative stress such as 8-OHdG in urine and 8-oxodG in lymphocytes are good indicators for the biological early health effects such as oxidative DNA damage10, 46. These findings strengthen by previous studies which observed the increased 8-oxodG levels in the bone marrow after benzene exposure47. However, both are not specifically for benzene exposure which is they are potentially influenced by other pollutants. Meanwhile, the nuclei acid oxidation such as urinary 8–oxodGuo, 8-oxoGuo, and 8-oxo-Gua has specificity and efficiency of the involved repair systems28. The most sensitive to oxidative damaged was 8-oxoGuo which arises from RNA turnover and/or repair system28.

The findings showed that among the studied genetic polymorphism for benzene exposure, NQO1, GSTM1 and GSST1 genotypes seem reported the consistencies in influencing the biomarkers of exposure and effects. NQO1 play important role in detoxification benzoquinones by reduction process to less toxic hydroxybenzenes55. NQO1 act to reduce hematoxic agent into less harmful hydroxybenzenes1 to prevent oxidative damaged35. It is to be thought that the shortage of NQOI to be a reason individual more susceptible to benzene toxicity which has been proven by a number of epidemiological studies41 and has the greatest risk to have related bone-marrow cancer43. Kim et al.23 suggested that GSTT1 influenced S-PMA and the metabolized genes (NQO1 and CYP2E1) which would affect the benzene metabolism in the liver.

The MPO and NAT2 genotypes less studied but showed the positive association with either biomarker of exposure and/or effects. The high levels of MPO in bone marrow5 able to causes polyphenolics oxidase into reactive quinones, semiquinones, and oxygen radicals which further inhibit topoisomerases and microtubule and lead to strand breaks occurrence. The other markers susceptibility closely related to benzene toxicity is NAT2 as this kind of genes responsible for the metabolism of carcinogenic agents and its polymorphism indicators for human cancer susceptibility24. Meanwhile, the others (CYP2E1, mEH, GSTA1 and XRCC1 genotypes) revealed the fluctuation result in their association with biomarkers of exposure and effects. The DNA repair genes (hOGG1, NBS1, XPD,

and XRCC3 exception for APEX1) and GSTP1 reported did not show any association with either biomarker of exposure and/or effects. Benzene toxicity also considered in influencing the DNA-repair genes such as APEX1 and XRCC154 as the benzene metabolites such as phenols and quinones potentially to form DNA lesions13, 11.

Others source suggested that XRCC1 responsible for repairing the DNA damages which may induce by benzene metabolites25. The genotypes CYP2E1, NQO1, and XRRC1 which documented have influenced both biomarkers of exposure and/or effects were contributed by variant-type exception for MPO with wild-type have increased in CA and MN frequency in the benzene-exposed group. Meanwhile, the null-genotype of GSTM1 and GSTT1 has modulating effects on either biomarker of exposure and/or effects. These studies suggesting that the variant-type of CYP2E1 and NQO1 genotypes that responsible metabolism of benzene exposure do not induce functional of this genotype meanwhile variant-type in XRCC1 genotype lead to less efficient in repairing the DNA damage25 compared than wild-type. The null-genotypes of GSTT1 and GSTM1 frequently linked with the increment of several types of cancer risk4. An individual with wild-type of MPO genotype claimed to have increased the risk of the chromosomal abnormality25 including lower white blood cells26.

CONCLUSION

The susceptibility genotype involved in the major metabolic pathway of benzene are CYP2E1, GSTs family, NQO1, and MPO. The polymorphisms of this genotype may affect every inter-individual susceptibility to benzene toxicity. The genes of CYP2E1 play as major role in oxidation and activation of benzene, while GSTT1 and NQOI involved in detoxification process6, 11. Similarly, DNA-repair genes also revealed the linking with biomarkers of exposure and effects. However the overall effect of polymorphic sites in several genes still arguable particularly CYP2E, mEH, GSTA1 and XRCC1 and other related genes due to the fluctuation result observed. In fact, human variable response played by these genes at the low exposure levels is not fully elucidated and should be highlighted in ambient benzene exposure group.

ACKNOWLEDGEMENTS

The authors would like to thank to the anonymous reviewers and editors for their helpful and constructive comments on this paper as these comments led to an improvement of the work.

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Appendix

Table 1.Summary of the results of the biomarkers of exposure, effects, and susceptibility

Author (s)	Benzene concentration	Biomarker			Exposure	Results
		Exposure	Effects	Susceptibility		
Sørensen*et al*.[46]	Personal exposure: All: 3.9 (1.86-3,61) µg/m^3	S-PMA and *t,t*-MA level	Benzene-induce oxidative damage: 8-oxodG	1.*GSTT1* 2.*GSTM1* 3.*GSTP1* 4.*NQO*	Environmental exposure	1. Men excreted significantly more *t,t*-MA level than women and significantly associated with the personal benzene exposure.
	Partly outdoor: 3.6 (2.5-7.0) µg/m^3 Office work: 2.5 (1.9-3.3) µg/m^3		DNA damage: Use of FPG and ENDO by comet assay			2. S-PMA excretion has a significant correlation with 8-oxodG in lymphocytes.
	(median exposure)					3. Individuals with variant-types of *NQO1* genotype was significantly excreted higher *t,t*-MA and S- PMA level compared than individuals with the wild-type of *NQO1* genotype.
continued						
Buthbumrung*et al*.[10]	Ambient air: Inside school: Urban area: 2.20 to 7.40 ppb Rural area: 2.71 ppb Roadside: Urban area: 17.75±2.23) ppb Rural area: 2.20-7.40) ppb	1.Blood benzene 2.urinary benzene 3.*t,t*-MA level	8-hydroxy-2'-deoxyguanosine (8-OHdG) in leukocytes and in urine	1.*CYP2E1* 2.*NQO1* 3.*GSTA1* 4.*GSTM1* 5.*GSTT1*	Environmental exposure	1. A significant correlation found between urinary MA and 8-OHdG in leukocytes. 2. *GSTM1* genotypes had a significant effect on urinary MA excretion.
Manini*et al*.[28]	Personal exposure: 38.3 µg/m^3	S-PMA and *t,t*-MA	Biomarker of nucleic acid oxi-	1. *NQO1* 2.*GSTM1* 3.*GSTT1*	Environmental exposure	1.Biomarker of nuclei acid oxidation was correlated with

	(median exposure)	level	dation: 1.8-oxodGuo 2.8-oxoGuo 3.8-oxoGua	4. *GSTA1*		each other and with *S*-PMA and *t,t*-MA level 2. Benzene exposure is associated with oxidation damage to nucleic acid particularly, RNA. 3. There is a significant modulating effect of *NQO1* polymorphism on oxidation damage to nucleic acid particularly, RNA. 4. There is a significant modulating effect of *GSTM1*, *GSTT1*, and *GSTA1* polymorphism on *S*-PMA excretion.
Angelini*et al*.[2]	Personal exposure: Outdoor workers: 19.33 (13.46-31.44) µg/m^3 Indoor workers: 2.95 (1.43-7.55) µg/m^3 (median exposure)	*S*-PMA level	MN frequency	1.*CYP2E1* 2.*NQO1* 3. *MPO* 4. *mEH* 5.*GSTM1* 6.*GSTT1* 7.*GSTP1*	Environmental exposure	1. Biomarkers of effects, MN frequency associated with*S*-PMA level. 2. *GSTM1*-null genotype associated with a significantly higher median MN frequency in men.
continued						
Angelini*et al*.[3]	Personal exposure : 1.43 to 31.41 µg/m^3	Not studied	MN frequency	DNA-repair genes: 1.*APEX1* 2.*hOGG1* 3. *NBS1* 4.*XPD* 5.*XRCC1* 6.*XRCC3*	Environmental exposure	1. A significantly higher median MN frequency was recorded in traffic wardens than in controls. 2. *APEX1* variant genotype was associated with significantly lower median MN frequency in men.

Kim *et al*.[24]	Ambient air: Geometric mean of benzene concentration 0.557 (0.014–0.743) ppm	Not studied	CA frequency	1. *GSTM1* 2.*GSTT1* 3.*GSTP1* 4.*NAT2* 5.*NQO1* 6.*CYP2E1*	Occupational exposure	1. Benzene exposure was significantly associated with the increased frequencies of monosomy and trisomy of chromosomes 8 and 21. 2. All studied genes polymorphisms showed the influence the susceptibility of individuals to chromosomal aberrations in relation to benzene exposure.
Garte*et al*.[19]	1.8 ppm	*S*-PMA and *t,t*-MA level	DNA Single Strands Breaks (SSB)	*NQO1*	Occupational exposure	1. *S*-PMA and *t,t*-MA showed dose-response relationship with benzene air exposure. 2. SSB detected was higher in benzene-exposed workers compared than the control group especially subjects with the variant *NQO1* T alleles
Kim *et al*.[25] *continued*	0.51 ppm for full-shift workers Time-weighted average (TWA) ranged from 0.004 to 4.25 ppm	Not studied	MNand CA frequency	1.*NQO1* (rs1800566) 2.*MPO* (rs2333227) 3.*XRCC1* (rs25487)	Occupational exposure	1. The frequencies of MN and CA were significantly higher in workers exposed to benzene than the unexposed group. 2. All studied genes have significant effect on MN and CA.
Zhang *et al*.[51]	Ambient exposure: 2.6 mg/m^3 - 57.0 mg/m^3 (median, 6.4 mg/m^3)	Not studied	MN frequency	1.*GSTM1* 2.*GSTT1* 3.*GSTP1* (rs1695) 4.*CYP2E1* (rs3813867) 5.*CYP2E1* (rs2031920) 6.*CYP2E1* (rs6413432) 7.*mEH* exon 3 8.(rs1051740) 9. *mEH*exon 4 (rs2234922)	Occupational exposure	1. Benzene-exposed workers had significantly increased MN frequency compared with the controls. 2. The results indicate that two promoter polymorphisms in the *CYP2E1* gene, especially the rs2031920 variant allele, were

	involved with the benzene-induction of MN.