*Egyptian Journal of Occupational Medicine, 2019; 43 (1) : 145-159*

# 8-HYDROXY-2' -DEOXYGUANOSINE (8-OHDG) AND RUNX1-RUNX1T1 TRANSLOCATION: POTENTIAL RISK FACTORS FOR LEUKEMOGENESIS IN BENZENE EXPOSED WORKERS

By

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## Abstract

**Introduction:** Chronic benzene exposure has a detrimental effect on the hematopoietic system, disturbs its genetic material through the enhancement of oxidative DNA damage, increase the incidence of chromosomal aberrations that may result in direct hematotoxicity and an initiating step for leukemogenesis. **Aim of work:** To study the adverse impact of occupationally benzene exposure on peripheral blood cells, oxidative DNA damage and gene translocation of peripheral mononuclear blood cells through measurement of plasma 8-hydroxy-2'-deoxyguanosine (8-OHDG) levels and detection of RUNX1-RUNX1T1 translocation "t(8;21)". **Materials and methods:** The study was carried out on 91 subjects classified into two groups; group (I) occupationally benzene-exposed (57 subjects), which was subdivided into two subgroups: (A) " smokers" (No=41) and (B) "non-smokers" (No =16), Group (II) a control group and is also subdivided into two subgroups, (C) "smokers" (No =13) and (D) "non-smokers" (No =21) apparently healthy individuals not occupationally exposed to benzene. Data was collected using a specially designed questionnaire covering socio-demographic data, personnel and occupational histories. Investigations were done including: Blood cell count (CBC), examination of peripheral blood smears, Plasma 8-OHdG was measured by ELISA system, Translocation (8; 21) was detected. Working index and Smoking index were also calculated. **Results:** there was a significant increase in the plasma

levels of 8-OHdG in the occupationally benzene-exposed group both smokers and non-smokers and in control smokers in comparison to the control non-smoker individuals. The percentage of RUNX1-RUNX1T1 translocation was (3/41) 7.3% in occupationally benzene-exposed smokers, (1/13) 7.7% of control smokers, while it was negative among the non-smokers individuals either exposed to benzene or not.

**Conclusion:** There was an increase of plasma 8-OHDG levels and increased incidence of RUNX1-RUNX1T1 translocation t (8; 21) of mononuclear peripheral blood cells due to chronic benzene-exposure either occupationally or through tobacco smoking with obvious dysplastic features of peripheral blood cells.

**Keywords:** 8-hydroxy-2'-deoxyguanosine (8-OHdG), RUNX1-RUNX1T1 translocation t(8; 21), Benzene toxicity, Leukemogenesis and Smoking.

## Introduction

Chronic benzene exposure has been associated with a range of health hazards, including bone marrow depression (Yu et al., 2007), myelodysplastic syndrome, and acute myeloid leukemia through increase the incidence of chromosome aberrations and genetic damage of bone marrow cells (Schnatter et al 2012).

Cigarette smoking is one of the major sources of benzene exposure for the general population. Fustinoni et al (2005) reported that current smokers had higher levels of blood benzene (15-19 folds) than in non-smokers. In addition, cigarette smoke contains more than 4,000 chemicals capable of inducing free radical generation, resulting in oxidative DNA damage, creating 8-hydroxy-2'-deoxyguanosine (8-OHDG) (IARC, 2004 and El-Khawanky et al., 2016).

Several studies showed that low-level of benzene exposure for a long time was associated with an increased incidence of chromosome aberrations and translocations in mononuclear blood cells, particularly between chromosomes 8 and 21 "RUNX1-RUNX1T1 translocation", which is the most common translocation observed in acute myeloid leukemia (AML) (McHale et al., 2008). In addition, it was established that tobacco smoke is a risk factor for acute myeloid leukemia. The exact nature of this association is unclear, the smoking-associated risk for AML is restricted to the RUNX1-RUNX1T1 translocation "t(8;21) (q22;q22)" subgroup (Metayer et al., 2013). Remarkably, the prenatal parental smoking has been suggested as a possible risk factor for childhood leukemia (Milne et al., 2012).

The mechanism by which benzene and its metabolites exposure lead to increased levels of translocations between chromosomes 8 and 21 is not clear. One possible mechanism may be related to topoisomerase II inhibition as it was observed that t(8:21) exhibited a clonal abnormality associated with AML caused by topoisomerase-inhibiting drugs (Mondrala and Eastmond, 2010).

The activities of antioxidant enzymes in benzene-exposed workers were also significantly reduced which result in more oxidative DNA damage and chromosomal aberrations (Chang et al., 2008).

Several epidemiological studies show that benzene exposure is associated with an increased incidence of acute leukemia (Kirkeleit et al., 2008). For this, we concerned to detect RUNX1-RUNX1T1 translocation "t (8;21)" in benzene-exposed individuals as a risk factor for leukemogenesis, as it is mainly observed in acute myeloid leukemia (Paulsson and Johansson, 2007).

## Aim of work

To study the impact of occupationally benzene exposure on peripheral blood cells, oxidative DNA damage and gene translocation of peripheral mononuclear blood cells through measurement of plasma 8-OHDG and detection of RUNX1-RUNX1T1 translocation t(8; 21) as risk factors of leukemogenesis.

## Materials and methods

**Study design:** It is comparative cross sectional study.

**Place and duration of the study:** The study was conducted in the Clinical Pathology department, Faculty of Medicine, Al-Azhar University Hospitals, Cairo, Egypt; during the period from January to the end of September 2017.

**Study sample:** This study was carried out on 91 subjects who were available and accept to participate in the study. They were classified into 2 groups; group (I) occupationally benzene-exposed workers (No 57) working at motors fuel stations on the Belbies-Cairo desert highway, group (II) 34 apparently healthy individuals not occupationally exposed to benzene served as the control group working at Al-Azhar University Hospitals (administrative stuffs).

Group (I) was subdivided into 2 subgroups: (A) "smokers "; who were occupationally exposed to benzene and were cigarette smokers (No =41), (B)"

non- smokers "; who were occupationally exposed to benzene and non-smokers (No =16). Group II was subdivided also into 2 subgroups: (C) "smokers"; who were smokers and non- occupationally exposed to benzene (No =13) and (D) "non-smokers" (No =21); who were neither benzene-exposed nor smokers.

**Study methods**

**I-Questionnaire:** The studied groups were subjected to a full history taking that included personal, occupational and smoking histories, the history of any present disease, medications and the present history of any complaint.

**II- Laboratory investigations:**

**a- Blood cell count (CBC)** using automatic cell counter (Sysmex KX-21).

**b- Examination of peripheral blood smears** stained with Leishman's stain.

**c- Plasma 8-OHdG** was measured by ELISA system (TecanSpectra) using StressXpress® 8-OHdG enzyme immunoassay (EIA) kit (SKT120), which is a competitive assay. The EIA utilizes an anti-mouse IgG-coated plate and a tracer consisting of an 8-OHdG-enzyme conjugate. This format has the advantage of providing low variability and increased sensitivity.

We measured 8-OHDG in plasma (although many previous studies measured it in urine) (Valavanidis et al., 2009). Urinary 8-OHDG levels may be affected by renal impairment (Akagi et al., 2003), and need 24 hours urine sample collection and kidney function investigation (Hakim et al., 2008 and Sakano et al., 2009). Furthermore, 8-OHDG exists as either free nucleoside or incorporated in DNA circulating in plasma. Once the blood enters the kidney, free 8-OHDG is readily filtered into the urine, while larger DNA fragments remain in the bloodstream (Lin et al., 2004), so we relied on plasma sample as it is more reflective for oxidative DNA damage than the urine sample.

**d- Translocation (8; 21)** was detected by qualitative reverse transcription-polymerase chain reaction (RT-PCR) using the HemaVision-8; 21 kits (innuPREP Blood RNA Kit (Catalog# KS-2010050). The principle of this procedure consisted of four phases; 1- RNA extraction from whole blood providing the template for the synthesis of cDNA, 2- cDNA Synthesis by using the cDNA primer mix and Reverse Transcriptase. 3- PCR amplification of the resultant cDNA using the PCR primer mix. 4- Detection of PCR products by using

horizontal agarose gel electrophoresis that visualized by staining with ethidium bromide and detected under UV trans-illuminator.

**III- Working index** for benzene-exposed workers and the Smoking index for smokers (exposed and control) were calculated:

-**WI (Working Index):** is an indicator for measuring the worked hours over a long period of time. It was calculated by multiplying the number of worked hours per day by the number of years the person has worked.

**-SI (Smoking Index):** is an indicator for measuring the amount a person has smoked over a long period of time. It is calculated by multiplying the number of cigarettes smoked per day by the number of years the person has smoked.

## Consent

All research participants had given their informed consent to participate in this study.

## Ethical approval

The study was approved by the Ethical Committee of the department of Clinical Pathology, Faculty of Medicine, El Azhar University, Egypt. An approval from the motors fuel stations authority to perform this study was obtained.

## Data management

The collected data were revised, coded, tabulated, and analyzed statistically using the SPSS statistical software (IBM SPSS Inc., version 20, Chicago, Illinois, USA). Data were presented, and suitable analyses were carried out according to the type of data obtained for each parameter. Statistical difference was considered significant if the p value was less than 0.05 and highly significant if the p value was less than 0.001.

## Results

About the age of the subgroup exposed smokers the Mean ± SD was 37.8 ± 13.11 years. It was 36.4 ± 12.88 for exposed non-smokers, 43.4±8.88 years for control smokers and 33.4±9.66 years for control non-smokers. There were no significant differences among both studied groups (F = 1.968, p= .125) "ANOVA test" (The results are not tabulated).

About 85% of exposed smokers, 81.2% of exposed non-smokers and 46.2% of control smokers were complaining of fatigue symptoms (easy fatigability, bone ache, peripheral neuritis).

While none of control non-smokers had fatigue symptoms. (The results are not tabulated)

Fatigue symptoms were significantly higher among benzene exposed individuals in comparison to non-exposed (Chi-square value = 39.109, p= .000) and among smokers in comparison to non-smokers (Chi-square value = 10.021, p= .002). (The results are not tabulated)

**Table 1: Relation between 8-OHdG and smoking in the studied groups.**

| | **Exposed** | | **Control** | | **t-test and p value** |
|---|---|---|---|---|---|
| | **Exposed smokers (subgroup A)** | **Exposed non-smokers (subgroup B)** | **Control smokers (subgroup C)** | **Control non-smokers (subgroup D)** | |
| **No** | **41** | **16** | **13** | **21** | |
| **8-OhdG (pg/ml) Mean± SD** | 31.6 ± 7.6 | 28.2 ± 6.0 | 27.7 ± 7.8 | 12.6±3.0 | |
| **Sig. of each subgroup with subgroup D (t-test & p value)** | 11.046 **.000**** | 10.301 **.000**** | 6.422 **.016*** | --- | |
| **8-OhdG Sig. among subgroups A & B** | | | | | 4.186 **.046*** |
| **Sig. between subgroups A & C** | | | | | 5.306 **.032*** |

8-OHdG (8-hydroxy-deoxy-guanosine),

* Significant. ** Highly significant.

Table 1 showed that 8-OHdG plasma levels of exposed workers (smokers and non-smokers) and control smokers were significantly higher in comparison to control non-smokers, the p values were (.000, .000, .016) respectively. In addition, 8-OHdG plasma levels of smoking workers was significantly higher in comparison to non-smoking workers (p= .046), and control smokers (p= .032).

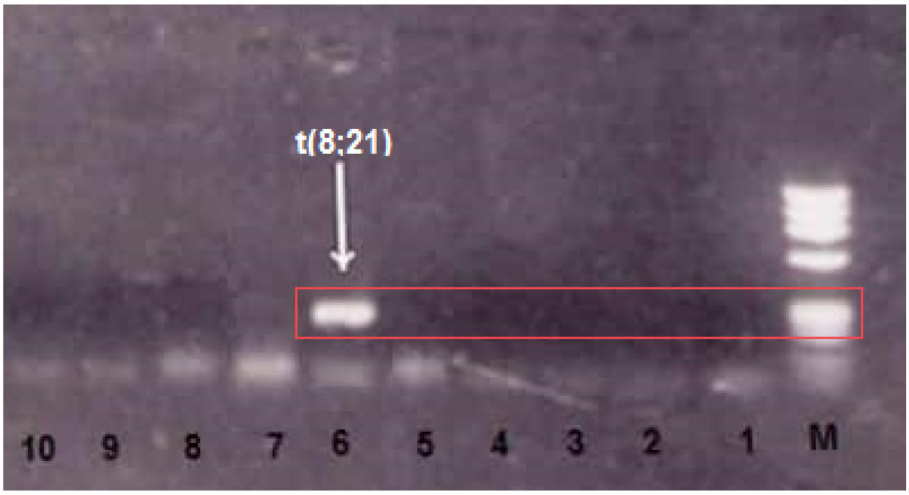


**Figure (1): Agarose gel electrophoresis plate showing a case carrier of the fusion gene t(8;21).**

Figure 1 showed a case carrier of the fusion gene t(8; 21) that was present in exposed smokers.

The incidence of translocation t(8;21) was (3/41) 7.3% among exposed smokers and only one case (1/13) 7.7% was positive among control smokers. While among non-smokers (exposed and control) all subjects were negative for t(8;21). (The results are not tabulated)

**Table 2: Correlation studies between Working Index (WI), Smoking Index (SI) and 8-OHdG with other parameters.**

| **WI correlations in smoking workers (subgroup A)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **8-OHdG** | **TLC** | **LYM** | **NEU** | **MON** | **RBC** | **HB** | **PLT** | **AGE** | **SI** |
| **WI** | **r** | .404 | .204 | .162 | .193 | .064 | .064 | .023 | -.016 | .333 | .312 |
| | **p** | **.009*** | .202 | .313 | .228 | .689 | .689 | .886 | .923 | **.033*** | **.047*** |
| **WI correlations in non-smoking workers (subgroup B)** | | | | | | | | | | |
| | | **8-OHdG** | **TLC** | **LYM** | **NEU** | **MON** | **EOS** | **BAS** | **RBC** | **HB** | **PLT** |
| **WI** | **r** | .213 | -.094 | -.208 | .087 | .079 | .222 | .186 | .135 | .192 | .038 |
| | **p** | .429 | .728 | .439 | .749 | .771 | .409 | .490 | .618 | .476 | .889 |
| **SI correlations in control smokers (subgroup C)** | | | | | | | | | | |
| | | **8-OHdG** | **TLC** | **LYM** | **NEU** | **MON** | **EOS** | **BAS** | **RBC** | **HB** | **PLT** |
| **SI** | **r** | .309 | .301 | .149 | .283 | -.089 | .003 | -.042 | .557 | .180 | .139 |
| | **p** | .303 | .317 | .625 | .348 | .773 | .993 | .892 | **.048*** | .556 | .651 |
| **8-OHdG correlations in control non-smokers (subgroup D)** | | | | | | | | | | |
| | | **AGE** | **TLC** | **LYM** | **NEU** | **MON** | **EOS** | **BAS** | **RBC** | **HB** | **PLT** |
| **8-OHdG** | **r** | -.096 | -.021 | -.058 | -.058 | -.008 | -.001 | .026 | .182 | .328 | .060 |
| | **p** | .678 | .928 | .804 | .804 | .972 | .996 | .911 | .430 | .147 | .795 |

8-OHdG (8-hydroxy-deoxy-guanosine), TLC (total leucocyte count), LYM (lymphocyte), NEU (neutrophil), MON (monocyte), EOS (eosinophil), BAS (basophil), RBC (red blood cells), HGB (hemoglobin), PLT (platelets). *: Significant

Table 2 showed that 8-OHdG levels did not show any significant correlation with any of blood parameters. Working index (WI) and smoking index (SI) showed a positive correlation with plasma 8-OHdG levels among only smoking exposed workers.

In control smokers, SI showed none significant correlation with any component of blood cells nor 8-OHdG, except RBCs count showed a positive correlation ($r = .557$, $p = .048$)

**Table 3: The difference of complete blood count (CBC) between exposed and control non-smokers.**

| CBC | Exposed Non smokers No = 16 (Mean ± SD) | Control Non-smokers No = 21 (Mean ± SD) | t | p |
|---|---|---|---|---|
| **TLC x $10^9$/L** | 7.2±2.6 | 7.3±2.2 | .046 | .963 |
| **LYMx $10^9$/L** | 3.0±1.8 | 2.8±1.12 | .377 | .708 |
| **NEU x $10^9$/L** | 3.4±1.0 | 3.7±1.4 | .596 | .555 |
| **MONx $10^9$/L** | 0.6±0.4 | 0.6±0.2 | .150 | .881 |
| **EOS x $10^9$/L** | 0.26±0.09 | 0.23±0.07 | .182 | .864 |
| **BAS x $10^9$/L** | .001±.0 | .001±.0 | .021 | 0.974 |
| **RBCs $10^{12}$/L** | 5.1±0.3 | 4.8±0.5 | 1.891 | .067 |
| **HGB g/dl** | 13.7±1.0 | 13.6±1.1 | .143 | .887 |
| **PLT $10^9$/L** | 289.3±71.9 | 273.4±62.6 | .718 | .478 |

CBC (complete blood count), TLC (total leucocyte count), LYM (lymphocyte), NEU (neutrophil), MON (monocyte), EOS (eosinophil), BAS (basophil), RBCs (red blood cells), HGB (hemoglobin), PLT (platelets).

Table 3 showed that there was no statistically significant difference detected on comparing the blood parameters of exposed and control non-smokers.

**Table 4: Data of subjects with or without fusion gene t (8;21) in benzene exposed workers.**

| Groups | t(8;21) | | t | p |
|---|---|---|---|---|
| | Non-carrier of the gene No =54 Mean± SD | Carrier of the gene No=3 Mean± SD | | |
| **8-OHdG (pg/ml)** | 30.7 ± 7.3 | 30.9 ± 9.3 | .054 | .957 |
| **Age** | 37.9±13.1 | 27.7±4.7 | 1.343 | .185 |
| **WI** | 162.7±104.4 | 108.0±90.6 | .888 | .375 |
| **SI(Subgroup A)** | 462.4±354.4 | 178.7±168.9 | 1.361 | .181 |

Group I (benzene-exposed) Subgroup A (smoking exposed workers)

8-OHdG : 8-hydroxy-deoxy-guanosine WI :Working index

SI : Smoking index.

Table 4 showed that there were no statistically significant difference (p= .957) in the levels of 8-OHdG between gene carriers and non-gene-carrier carriers of t(8;21) among the benzene-exposed group. In addition, age, working index and smoking index did not show any significant differences between both groups.

## Discussion

Each human cell metabolizes approximately $10^{12}$ molecules of oxygen per day, resulting in the generation of radical oxygen species, which represents an injurious endogenous oxidants (Beckman and Ames, 1997). Normally, there is a balance maintained between endogenous oxidants and antioxidant defenses. When an imbalance occurs, extensive oxidative damage to DNA occurs in the form of 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Valavanidis et al., 2009).

It has been established that 8-OHdG is an important biomarker to assess the risk to cancer after exposure to various carcinogenic substances and environmental pollutants as benzene, and tobacco smoking (Nilsson et al., 2004 and Valavanidis et al., 2009).

In the present study, high levels of 8-OHdG were observed in non-smoking benzene exposed workers with highly significant increased levels (p= .000) as compared to the control non-smokers subgroup. The Mean ± SD of plasma 8-OHdG concentrations were (28.2 ± 6.0) pg/ml and (12.6 ± 3.0) pg/ml, respectively (Table 1).

Several studies confirmed the present results and reported increased in 8-OHdG as single-strand DNA breaks circulating in blood among workers exposed to benzene (Pilger and Rüdiger, 2006).

In addition we reported that cigarette smoking (CS) increases the level of 8-OHdG significantly among smoking workers (P=.046) and control smokers (p= .016) more than the observed in control non-smokers subgroup. The mean of 8-OHDG concentration among smoking workers was (31.6±7.6) pg/ml, and was (27.7 ± 7.8) pg/ml among control smokers, while for control non-smokers was (12.6±3.0) pg/ml (Table 1). Cigarette smoking is one of the major sources of benzene exposure (Kirkeleit et al., 2008).

The increase of 8-OHdG concentration mostly returns to the benzene constituent in tobacco. Smokers reached a concentration of benzene in blood up to 13 nmol/l after smoking four or five cigarettes, which is estimated to be equivalent to benzene exposure in the breathing zone of as much as 0.3 ppm averaged over an 8-hour shift (Kirkeleit et al., 2008).

In control smokers subgroup; the smoking index (SI) showed a significant

positive correlation with red blood cells count and tended to be positively correlated with 8-OHdG levels (Table 2). Increased number of erythrocytes can be explained by the fact that tissue hypoxia caused by the increased creation of carboxyhemoglobin from cigarette smoking leads to an increased secretion of erythropoietin, thus increasing erythropoiesis (Nadia et al., 2015).

Several studies confirmed that plasma 8-OHDG levels were or tended to be positively correlated with the number of cigarettes smoked per day when adjusted for confounding factors (Chen et al., 2007). In contrast, other studies showed negative correlations between passive smoking and 8-OHDG levels (Smith et al., 2001). Although the reason for this discrepancy is unknown, the grade of the cigarette filter or differences in the quality of cigarettes among countries might be a factor (Sakano et al., 2009).

In smoking workers, working index (WI) showed a positive correlation with 8-OHDG and smoking index (SI) (Table 2) which indicates that the more exposure to benzene and/or smoking amount the more oxidative DNA damage the more incidences for gene translocation.

Our study showed one positive case (1/13) for t(8;21) among control smokers and three cases (3/41) among smoking workers while the non-smoking workers showed none positive cases . The common factor in all positive cases for RUNX1-RUNX1T1 translocation "t(8;21)" was cigarette smoking. This translocation is implicated in leukemogenesis. It was reported that environmental tobacco smoke is a possible cancer risk factor in offspring of experimental animals (Maciag et al., 2003 and Reikvam et al., 2011).

Several studies agreed with our results and showed that exposure to benzene was associated with increased levels of translocations t(8; 21) (Wong et al., 2010; McHale et al., 2011).

Contrary to expectations our results showed that carrier of the gene t(8;21) had lower means of age, smoking index and working index than negative cases (Table 4). Collectively, it is possible to predict that t(8;21) is formed in the early stages of benzene exposure either occupationally or through cigarette smoking and eliminated over time. It was postulated that some translocations may be eliminated by time and are not completely persistent, while others confirm persistence (Lloyd et al., 1998 and Fucic et al., 2007).

Lindholm and Edwards (2004) observed a decrease in translocation frequency in the highly renewable cells "unstable cells", while in stable cells, the results showed that the yield of translocations is constant with time.

Several studies have reported that long-term benzene exposure yields various abnormalities in peripheral blood cells (Qu et al., 2002 and Lan et al., 2004). In the present study, all blood components including red blood cells, hemoglobin levels, white blood cells, lymphocytes, neutrophils and platelet count had shown none significant variations between both groups although some cases in the benzene-exposed group showed leucopenia, neutropenia, and anemia (Table 3).

It was postulated that benzene metabolites rather than benzene itself can potentially damage bone marrow cells, genomic reprogramming that would result in aberrant gene expression inducing extensive apoptosis in the cells, and hence thrombocytopenia could occur (Zhao et al., 2009).

Several studies have reported that benzene-exposed workers showed decreased blood cell count and particular white blood cells subtypes (Zhang et al., 2010), including a decrease in circulating CD4 ± T cells, B cells, platelet count (Kirkeleit et al., 2008), and red blood cells (Qu et al., 2002). On the contrary, other studies reported no decrease in blood cell counts among benzene-exposed workers (Ray et al., 2007).

The differences in the findings of the studies could be related to the different exposure levels to benzene. Further, most of the positive studies done on Asian people have shown a higher risk of benzene toxicity than other ethnic groups due to a genetic polymorphism in some enzymes involved in metabolizing benzene (Nebert et al., 2002).

## Conclusion

Exposure to benzene occupationally or through cigarette smoking increases the detrimental effect on DNA integrity which results in increased 8-OHdG levels whilst cigarette smoking was the common factor for RUNX1-RUNX1T1 translocation occurrence.

## Conflicts of interest

There are no conflicts of interest.

## Funding

None

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