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Detection of Benzo[a]Pyrene Diol Epoxide-DNA Adducts in White Blood Cells of Asphalt Plant Workers in Syria

Razan Zohairee

Abstract

Benzo[a]pyrene (B[a]P) is a major polycyclic aromatic hydrocarbon (PAH), it can bind the DNA to produce DNA-adducts, which has major carcinogenic potential. Enzyme-linked immunosorbent assay (ELISA) is the method used to detect these DNA adducts of B[a]P diol epoxide (BPDE) within the living cells. The aim of this study is to evaluate exposure to bitumen fumes, and to B[a]P in asphalt plant workers by measuring the BPDE-DNA adducts in their peripheral white blood cells (WBC), which are considered biological markers for exposure risk assessment. In this study, Hemostatic blood (CBC, AST) were measured, and the levels of BPDE-DNA adducts were measured in DNA samples of WBC obtained from asphalt plant workers in Syria and compared to those measured from a control group. The measurement was performed using BPDE-DNA Adducts ELISA kit. The sample size was determined to be 50 with 25 asphalt plant workers and 25 healthy volunteers with no occupational exposure to PAHs. The results showed some diseases associated with exposure to asphalt fumes among the workers in the study group and a statistically significant difference in the values of (CBC; WBC, leukocytes, HCT, MCHC and AST) between the study group and the control group. BPDE-DNA adducts were detected in WBC of 11 asphalt plant workers with concentrations ranging between 0 and 2.75 ng/ml and only one individual in the control group with concentration of 0.75 ng/ml. These results indicate significant positive relationship between exposure to the bitumen fumes and formation of BPDE-DNA adducts. BPDE-DNA adducts is potential biomarker for PAHs exposure and likely helpful indicator of PAH-induced DNA damage and possibly carcinogenesis.

Keywords: benzo[a]pyrene, CBC, AST, BPDE-DNA adducts, asphalt workers

1. Introduction

Workers are exposed during the asphalt industry to bitumen fumes that contain poly aromatic hydrocarbons (PAHs) [1], organic vapors, silica dust, diesel exhaust, asbestos fibers and coal tar [2].

Exposure to bitumen fumes causes headache, fatigue, lack of appetite, eye, skin and bronchitis irritation, coughing, bronchitis, asthma [3], genotoxic effects [4], damaging DNA [5], cancer lung [6], increases the risk of developing bladder cancer [7].

PAHs are known environmental pollutants with harmful effect on human health. Benzo [*a*] pyrene (B[a]P) is a lead compound in this group and one of the most studied carcinogenic PAHs [8]. B[a]P is formed during incomplete combustion of organic materials and pyrolysis of inorganic compounds [9] B[a]P is also found in cigarette smoke [10], cooked food [11], and various combustion gases such as vehicle exhaust [12]. It is also generated from some industrial operations such as those of cooking ovens, heavy oil plants [13] and asphalt plants [14]. Given its harmful effects on health, B[a]P was classified by the International Agency for Research on Cancer (2012) among the highly genotoxic compounds and categorized to “group 1 carcinogenic to humans” [15].

Asphalt workers are exposed to B[a]P through two major ways: though inhalation of emanating fumes from the chimney for those working in the mixing plants, or through inhalation of the vaporous gas while paving asphalt at roadside workshops [16]. Notably, when asphalt is being prepared for road paving, the mixture reaches very high temperature ranging between 130° and 145°, which results emission of large of amount of vaporous fumes, that workers inadvertently inhale. To a lesser extent, some workers are exposed to B[a]P via touching B[a]P-containing compounds [16]. This particularly occurs among mechanics that are often exposed to petroleum compounds. According to the occupational safety and health administration, the allowed concentration of B[a]P in environment when workers perform their duty should not exceed 0.2 mg/ and exposure should not exceed more than 8 hours a day [17].

Although B[a]P is metabolized in all human tissues, the hepatic catabolism of B[a]P through cytochrome P450 (specifically cytochrome P450 1A1) results in production of several metabolites such as epoxides, dihydrodiols, phenols and quinones. First, B[a]P undergo phase I metabolism into epoxides. Subsequently, these epoxides undergo further metabolism though one of three ways: through spontaneous rearrangement to phenols, through hydration to transdihydrodiols by epoxide hydrolase or though phase II detoxification. Phase II dextoxification occurs via binding to glutathione, which can occur spontaneously or though catabolism by glutathione-S-transferases [18]. Given this extensive hepatic metabolism, the amount of B[a]P that reaches the systemic circulation is minimal. Therefore, gastro-intestinal route appears to be a less important route of exposure to B[a]P.

B[a]P appears a carcinogenic compound which is metabolized by cytochrome 450 1A1 to form DNA-adducts, that play key roles in its carcinogenesis [9].

The mechanism of carcinogenesis of B[a]P is dependent on a 3-step enzymatic metabolism discussed above (**Figure 1**) [18]. These steps eventually form the final mutagen BPDE [19], which is a very reactive and binds covalently to lipids, protein and DNA to form BPDE adducts [20, 21]. These BPDE-DNA adducts can result in permanent mutations if not promptly repaired, which can lead to development of cancer.

In this study, we examine the relationship between being an asphalt worker and detection of BPDE-DNA adducts in the white blood cells (WBC), which can be a useful surrogate exposure risk.

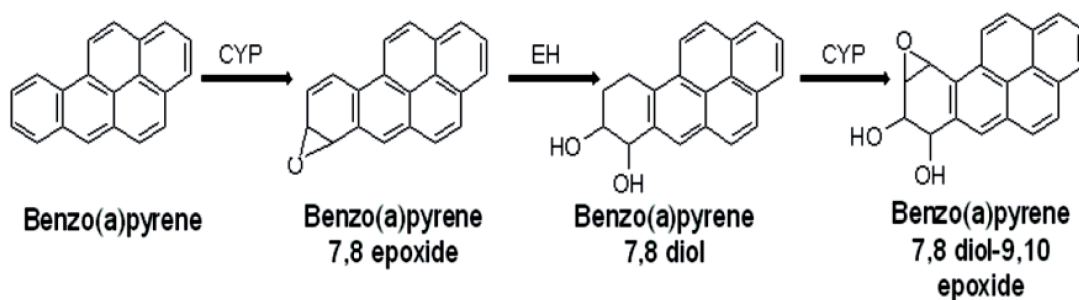


Figure 1.
B[a]P converts to the final carcinogen, BPDE.

2. Subjects and methods

This was a cross-sectional study that aimed to evaluate the exposure to asphalt fumes (i.e., by measure the hemostatic blood CBC, and Aspartate Amino Transeferase AST), and to evaluate the exposure to B[a]P through asphalt fumes among asphalt workers by determining the levels of BPDE-DNA adducts in their WBC. It was conducted on 25 male workers exposed to asphalt fumes and 25 healthy male individuals without such exposure.

2.1 Comparison groups

Group 1 (study group): This group consisted of 25 male individuals who worked at an asphalt plant in Syria. Among those, 12 subjects worked in the mixing plant (9 were employed to monitor the equipment operation and 3 mechanics to maintain the equipment). The remainder 13 subjects were paving workers at road-side workshops (5 subjects to operate the equipment, 4 mechanics and 4 manual construction workers). These subjects were exposed to B[a]P vapors and aerosols of bitumen for many years ranging from 3 to 31 years. On average, all subjects worked 8 hours a day, 4 days a week. Their mean age was 46 years (range: 33–58 years).

The subjects within the study group were carefully selected to avoid external bias. All subjects did not drink alcohol. They all had somewhat comparable dietary habits, that consisted predominantly of vegetables. All subjects did not work outside their primary site of employment. Subjects with significant non-occupational exposure to PAHs (e.g. use burning woods for heat) or those who work a second job were excluded.

Group 2 (control group): The control group consisted of 25 healthy volunteers. These individuals were predominantly university administrators and hospital workers. They did not work outside their primary site of employment. There all had no significant occupational or personal exposure to PAHs (e.g. did not use burning woods for heat). They all took no prescription medications and lived remotely from bitumen fumes. The subjects in the control group were selected to match the subjects in exposure group in gender, age, smoking status and dietary habits.

Subgroups: Subjects in the study group were categorized into subgroups based on age, and smoking status. Based on age, they were categorized by age: <45 years (8 workers, ranging from 33–44 years) and ≥ 45 years (17 workers, ranging from 46–58 years). Based on smoking status, they were categorized to smokers (22 subjects) and non-smokers (3 subjects).

Study protocol: All subjects signed written consent prior to enrollment in the study. The study protocol was approved by the Ethical Committee of Damascus University prior to the start of the study.

The study was begun with a questionnaire, that was administered to all subjects to collect general personal information such as age, weight, health status, smoking status, dietary and lifestyle habits and other demographic data.

After enrollment of qualified subjects, blood samples were collected via cubital venipuncture and 5 mL of blood were collected from each subject and placed in sterile tubes. For each subject, one of these tubes was placed immediately in ice bags at 4° and transported immediately to the hospital to be stored in the freezer at –80° until the staff were ready to analyze it. The remainder of the specimen was used immediately to perform various laboratory biomarkers such as complete blood count CBC; WBC, red blood cells RBC, hematocrit (HCT), hemoglobin (HGB) and mean corpuscular hemoglobin concentration (MCHC) using (Sysmex Cooperation, Japan) and (AST) (reference range 0–35 unit/L) using (Biosystem S.A. Spain).

All blood samples were collected in April, 2016.

2.2 DNA isolation

Genomic DNA was isolated from peripheral WBC by using DNA Kit (Thermo Scientific Gene JET Genomic DNA Purification Kit) (Qiagen, USA). DNA concentrations were measured using Gene Nano Drop (Biochrom, England), which occurred in the biology laboratory at Alassad University Hospital in Damascus, Syria. Repeated thawing and freezing of the samples were avoided.

2.3 Determination of BPDE-DNA adduct levels

After DNA was isolated from WBC, the samples were subsequently diluted to a concentration of 2 μ g of DNA in 1 mL. Phosphate-buffered Saline with PH 7.2 (1X) (Gibco by life technologies) was used for dilution and washing. BPDE-DNA adduct levels (ng/ml) were measured according to the standard method provided by OxiSelect BPDE-DNA Adduct ELISA Kit (Cell Biolabs, Inc., San Diego, USA). This ELISA kit is an immunoassay enzyme developed for rapid detection of BPDE-DNA adducts. The quantity of BPDE adduct in DNA samples is determined by relative comparison of a known BPDE-DNA standard curve. The kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown DNA samples. The results were expressed as nanograms of BPDE-DNA adducts per microgram of DNA. The analyses were applied twice.

Apparatus: ELISA (Tecan, Switzerland) in the Blood Bank of Damascus University.

Statistical analysis: The statistical analysis of this study was performed using SPSS software version 13.0. P value of <0.05 was considered to be statistically significant. Mann–Whitney U test was used to compare continuous data with non-normal distribution, χ^2 test was used to compare categorical variables between the two groups, and spearman coefficient and independent T- student tests were used.

3. Results

3.1 Medical conditions and basic laboratory data

Review of the subject's basic data revealed that some study group suffers from a health condition, particularly respiratory diseases (chest pains, shortness of breath, asthma). This was particularly true among mechanics, one of the workers has olfactory deficits. In total, 44% of asphalt plant workers had at least one medical condition.

3.1.1 Hemostatic assays

Laboratory studies showed significantly higher the number of WBC with T-student test among the exposed group compared to the control group (8.72 ± 1.92 and $P = 0.015$), particularly lymphocytes (32.09 ± 6.99 and $P = 0.003$). (HCT) and (MCHC) were significantly higher among the study group compared to the control group ($P = 0.034$ and $P < 0.0001$, respectively). The asphalt workers have significantly higher levels of (AST) (40.59 ± 7.56 unit/l, $p < 0.0001$).

3.1.2 BPDE-DNA adducts levels in the study group

In the study group, BPDE-DNA adducts were detected in the WBC of 11 out of 25 individuals (44%), 2 non-smokers and 9 smokers. The concentrations of

BPDE-DNA adducts among the exposed group ranges between(0 to 2.75 ng/mL) except for one individual who had exceptionally high concentration at 16.5 ng/mL. He was a smoker.

In the control group, BPDE-DNA adducts were detected in only one individual with concentration of 0.75 ng/ml (**Table 1**). He was a smoker.

Using statistical analysis, the number of individuals with detected BPDE-DNA adducts in their WBC was higher among the study group compared to the control group (χ^2 test, $p = 0.001$).

3.1.3 BPDE-DNA adducts levels according to smoking status

In the study group, there was no significant difference in the number of individuals with detected BPDE-DNA adducts between smokers and non-smokers (Mann–Whitney U test, $P = 0.43$), the results of Levels of BPDE-DNA adducts for Group Study below (**Table 1**).

3.1.4 BPDE-DNA adducts levels according to age

Among asphalt workers, the concentrations of BPDE-DNA adducts ranges from 0 to 2.75 ng/mL for subjects <45 year-old and ranges from 0 to 2.25 ng/mL for those ≥ 45 year-old except for one subject with unusually high concentration of 16.5 ng/mL. He was from the older age category.

3.1.5 BPDE-DNA adduct levels according to disease status

As discussed above, 44% of asphalt workers had health conditions and/or symptoms such as chest pain, dyspnea, and asthma. Among the asphalt workers, there was a statistically non-significant trend toward positive relationship between the concentrations of BPDE-DNA adducts and disease status (χ^2 test, $p = 0.08$). Nonetheless, one asphalt worker suffered from olfactory deficits (his BPDE-DNA adducts concentration was 2.25 ng/mL) and another suffered from spinal disc disease (his BPDE-DNA adducts concentration was 16.5 ng/mL).

3.1.6 BPDE-DNA adducts levels according various laboratory biomarkers

The relationship between BPDE-DNA adducts levels and various laboratory biomarkers (CBC variables and AST) among asphalt workers was assessed using spearman coefficient. There was no significant correlation identified between BPDE-DNA adducts levels and any of these variables.

Group Study	Sample Number (N)			Percentage (%)		
	Sum	BPDE-DNA adducts	non- BPDE-DNA adducts	Sum	BPDE-DNA adducts	non- BPDE-DNA adducts
Study group	25	11	14	100	44	56
Control group	25	1	24	100	4	96

Table 1.
Levels of BPDE-DNA adducts.

4. Discussion

When the responses to the administered questionnaires were reviewed, a significantly higher number of health illnesses were identified among asphalt workers, particularly respiratory diseases. They were identified in both smokers and non-smokers and therefore, they are likely attributed to exposure to bitumen fumes, where inhalation is the primary modality through which asphalt workers were exposed to it. Prior studies such as conducted by (Gamble et al.,1999) [22] align with our observation and indicate significant positive relationship between exposure to bitumen fumes and respiratory diseases.

It was also noted that mechanics that work in asphalt plants are at higher risk to develop respiratory disease [22], which could be attributed to the impact of PAHs they were exposed.

In our study, we noted changes in some of the hematological parameters such as increase in the number of lymphocytes, which aligns with the study published by (Tompa et al) [23] where may attributed this increase to the harmful effects of PAHs.

In addition, the subjects in our study group had significantly higher HCT and MCHC levels compared to the control group, which is likely due to exposure to PAHs. These findings are in alignment with the study conducted by (Wang et al) [24] which showed significantly higher hemoglobin levels with some atypical appearance of the red blood cells, which were attributed to exposure to PAHs.

We also noted higher hemoglobin levels among smokers in both groups, which is a compensatory mechanism that occurs due to decrease in the oxygen level and increase carbon monoxide levels due to smoking [25–27].

We also noted significantly higher AST level between the study and control groups, in contrast to the findings of (Atasoy et al) [28]. The contradictory observations between these two studies could be explained by difference in the type of asphalt used, or the method of use and the extend and level of exposure to PAHs.

In our study, we noted relationship between exposure to PAHs by inhaling bitumen fumes from working closely with asphalt and detection of BPDE-DNA adducts ($P = 0.001$). On the other hand, (Pavanello et al). demonstrated significant relationship between chronic inhalation of high levels of PAHs and detection of BPDE-DNA adducts [29].

Interestingly, although smoking has somewhat similar effect on DNA as exposure to B[a]P, this study showed no correlation between smoking and the levels of BPDE-DNA adducts ($p = 0.43$). These findings are in agreement with those of (Pavanello et al) [29] and (van Schooten et al) [30]. However, other studies found significant correlation between the levels of BPDE-DNA adducts and smoking in subjects with occupational exposure PAHs where exposure to tobacco products and PAHs acted synergistically to form BPDE-DNA adducts as reported by (Rojas et al) [31]. These discrepancy between these findings could be explained by inter-individual factor variability and route of exposure to PAHs which can play a major role in formation of BPDE-DNA adducts.

Statistically, there was no significant difference in the adducts concentrations between the younger and older age groups (χ^2 test, $p = 0.18$). On the other hand, age did not significantly correlate with the presence of BPDE-DNA adducts among subjects in the study group ($p = 0.18$). These results are in agreement of those reported by (McClean et al) [32]. and is likely due to the similar metabolic and excretion ability in both age groups.

Given the known carcinogenic effect of B[a]P [8], the presence of BPDE-DNA adducts when measured in the WBC using ELISA technique correlates of the risk of development of B[a]P-induced diseases such as lung cancer [33]. Therefore,

BPDE-DNA adducts can serve as a useful biomarker to assess prior exposure to PAHs and could potentially determine cancer risk [34].

Our results suggests that importance of measurement of BPDE-DNA adducts and its role as potential biomarker for exposure to PAHs. As it involves the DNA, it could be a surrogate marker to assess the risk for development of cancer [35, 36].

5. Conclusions

AST levels and some vital blood parameters can be considered warning indicators for rapid diagnosis of exposure for the asphalt workers. Our results highlight that importance of measurement of BPDE-DNA adducts and its role as potential biomarker for exposure to PAHs and being considered a biomarker for unrepaired DNA damage.

As it involves the DNA, it could be a surrogate marker to assess the risk for development of cancer.

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Conflict of interest

The authors declare no conflict of interest.

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