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# EFFECT OF CYTOCHROME P450 1A1 (CYP1A1) POLYMORPHISM ON ASPHALT EXPOSURE - RELATED BIO-MARKERS

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## ABSTRACT

Asphalt fumes contain polycyclic aromatic compounds (PAC). There is a possibility of long-term health effects following chronic exposure by inhalation or skin contamination in asphalt road pavers. **Objective:** This study aimed at exploring the relationship between urinary 1-OH pyrene as a marker of internal dose of PAHs exposure and serum P53 protein as a response marker with the genetic polymorphism of CYP1A1 as a susceptibility marker among asphalt exposed workers. **Methods:** This work included 70 male individuals who were divided into an asphalt exposed group (n=43) and a matched control group (n=27). The exposed group was further subdivided into a group of paving and mixing (n=24) and a group of roller and vehicle drivers (n=19). Every participant was subjected to polymerase chain reaction (PCR) technique to detect the different genotypes of CYP1A1 gene and the expression of the p53 gene mutation. Besides, estimation of urinary 1-hydroxy pyrene (1-OHP) and serum level of mutant p53 protein was done as a PAHs exposure and response biomarkers, respectively. **Results:** CYP1A1*2A (Valine / Valine) was found to be the most prevalent CYP 1A1 genotype among both exposed and control groups. It was associated

with significantly higher percent (56.5%) of high expression of p53 gene mutation of high degree, in addition to higher levels of serum mutant p53 protein and urinary 1-OHP. Further analysis of results showed significantly higher urinary 1-OHP and serum mutant p53 protein ($P<0.005$) among the workers of mixing and paving than in the group of drivers indicating the impact of dermal absorption among the group of mixing and paving on the internal exposure level of PAHs. **Conclusion:** In the presence of genetic polymorphisms of cytochrome P4501A1 namely CYP1A1*2A (Valine / Valine) genotype, there is increased susceptibility to higher risk of cancer among people occupationally exposed to PAHs as in asphalt industry.

**Key Words:** Asphalt-PAHs-CYP1A1- CYP1A1*2A-CYP1A1*1- CYP1A1*2C-Polymorphism-P53 gene mutation- Urinary 1-OH Pyrene.

## Introduction

Asphalt pavement refers to any paved road surfaced with asphalt. Hot Mix Asphalt is a combination of approximately 95% stone, sand, or gravel bound together by asphalt cement, a product of crude oil [1]. Asphalt production is dictated by performance specifications rather than by a specific chemical composition. The precise chemical composition and physical properties of the resulting products are influenced by the composition of the original crude petroleum oil and the manufacturing processes. The basic chemical components of crude petroleum oil include paraffinic, naphthenic, and aromatic hydrocarbons as well as heterocyclic molecules containing sulfur, oxygen, and nitrogen. The proportions of these chemical components may vary significantly because sources of crude petroleum oil occur in various locations throughout the world involving different geologic formations Therefore, no two asphalts are chemically identical, and chemical analysis defining the precise structure and size of the individual molecules found in asphalt is almost impossible [2].

Asphalt fumes are defined as the cloud of small particles created by condensation from the gaseous state after volatilization of asphalt. The major route of occupational exposure to asphalt fumes (e.g., paving, roofing, and asphalt-based paints) is by inhalation and may also be through the skin absorption [3].

Exposure to asphalt fumes is a health concern due to the presence of polycyclic aromatic hydrocarbons (PAHs) in asphalt. PAHs are a group of over 100 different

chemicals that are formed during the incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco[4]. An important and very extensively studied prototype of this class of compounds is benzo (a) pyrene (BaP). PAHs such as BaP must be metabolically activated to exert their toxic effects. They are known to be potent substrates for cytochrome P4501A1 enzyme CYP1A1, CYP1B1, glutathione S-transferase M1 (GSTM1), glutathione S-transferase T1 (GSTT1) and microsomal epoxide hydrolase enzymes [5].

CYP1A1, is constitutively nil but ubiquitous after induction by PAHs. Hundreds of studies performed in vitro and in cell culture have demonstrated clearly that CYP1A1 is involved in the metabolic activation of BaP into reactive intermediates capable of binding to DNA and proteins which are the ultimate carcinogenic diol epoxides (B(a)PDE). Numerous reports have shown that these reactive intermediates, rather than the non metabolized parent compound, are responsible for BaP-mediated toxicity, mutations, cancer, and birth defects. Exploring the associations between genetic polymorphisms of metabolic enzymes and susceptibility to polycyclic aromatic hydrocarbon (PAH)-induced damage is of great significance for understanding PAH carcinogenesis [6].

As the presumed guardian of the genome, the p53 tumor suppressor gene (also known as TP53) coordinates a delicate balance between arrest of the cell cycle to allow repair of damage and apoptosis if the damage is irreparable. Unfortunately, the p53 gene is one of the most commonly mutated genes observed in human tumors. It is mutated in more than 50% of all human cancers and in about 60% of human lung cancers. Now, it is clear that, p53 is just one of the components of a network that culminate in tumor formation[7].

## Aim of the Work

This study aimed at evaluating the relationship between exposure markers such as urinary 1-OH pyrene as a marker of internal dose and serum P53 protein as a response marker with the genetic polymorphism of CYP1A1 as a susceptibility marker among asphalt exposed workers.

## Subjects & Methods

### I) Subjects:

This study was conducted at one of road paving stations located in Pyramids zone, Giza Governorate. Subjects of this study were composed of 43 male workers who constituted the exposed group. They were divided into 2 subgroups, a group of mixing raw materials and spreading the asphalt on the road (n=24) and a group of

drivers of vehicles and rollers (n= 19). Both exposed groups have been exposed to asphalt regularly for at least 2 years. The control subjects (n= 27) were randomly selected from the cleaning workers at Cairo University. The controls had no history of occupational exposure to (PAHs) . Both control and exposed groups were matched as regards age, sex, smoking habits, and socioeconomic standard.

**II) Methods :**

All participants in the study were subjected to the following:

a) A comprehensive personal and occupational history taking .

b) Laboratory procedures that included:

**i) Urinary 1-Hydroxypyrene (1-OHP) Analysis**

High performance liquid chromatography (HPLC) with electrochemical detector (waters) was used to measure the urinary 1-hydroxypyrene (1-OHP). Accuracy and precision were assessed using urine reference materials. The concentration of urinary 1-hydroxypyrene at mid-shift was used as an index of exposure. The results of urinary 1-OHP were then corrected for urinary creatinine. A cumulative index of exposure would have been a more reliable index of exposure, being based on the worker's mean 1-OHP for each quarter of the year. Unfortunately, no previous analysis was available. Chromatographic separation was performed using 3µm BAS phase II ODS analytical column (100# 302mm I-D) preceded by 7µm BAS phase II ODS pre column (15#302mm). The method involved extraction of 100µl urine sample with 500µl perchloric acid containing 0.01% cystine as antioxidant, 500pg/ 100µl internal standard. The mobile phase consisted of 0.1M monochloroacetic acid, 0.65mM sodium octylsulphate, 0.5mM EDTA per millililter water and pH was adjusted at 3.05 with 6m NaOH. All chemicals were HPLC graded (SIGMA, st Louis, MO, USA). Following filtration through GV 0.22µm filter (waters), acetonitrile was added to a final concentration of 2.9%. The mobile phase was degassed through vaccum degasser (BAS-LC26). The chromatographic separation was performed isocritically at flow rate of 0.1ml/ min The detector potential was 0.45volt versus silver electrode AG/AG column. Injection volume was 50µl.

**ii) Detection of cytochrome P450 and gene expression of mutant p 53 by PCR:**

**DNA extraction:**

Genomic DNA was extracted from 100 l of whole blood by a silica gel col-

umn method ( QIA amp DNA blood mini kit, Qiagen Gmbh, Hilden, Germany). Extracted DNA was quantitated at 260 nm by spectro-photometer. This extracted DNA was used for:

**a) Gene expression of mutant P 53.**

The PCR mixture contained 10 mmol/ L Tris-Hcl pH 8.3, 50 m M KCL, 1.5 m M KCL2, 250 µm of DNTPS mixure, 2.5 U of Taq polymerase and 100 uM of each of specific pnmer with the following sequence:

F : 5´ ATG GCA GAA GGA GGG CAG AT-3´

R : 5´GAT GTT GAG CGA GAA AGT-3´

This reaction mixture was then subjected to 40 cycles of 95ºc for 1 min and 72ºc for 2 mins and finally an extension cycle for 10 min at 72ºc was done. The PCR product were electrophoresed on 210 agarose gel and visualized by UV transilluminator.

**b) Genotyping of cytochrome $P_{450}$ (CYP1A1) gene :**

The sequence flanking Cyt. $P_{450}$ (T/V) polymorphism were PCR amplified from genomic DNA using a pair of oligonucleotide primers:

5´- CTG GAG ACC ACT CCC ATC CTT TCT-3´.

The PCR was carried out in 10 ul of 10 x buffer containing Tris-HCL, KCL, mg $cl_2$, 2-4 units of Taq, DNTPS and 100 umol of each primer. After an initial denaturation at 94 ºc for 3 mins, the DNA was amplified by 30 PCR cycles of denaturation at 94ºc for 30 secs, annealing at 58ºc for 45 secs, and extension at 68 ºc for 45 secs, and followed by final extension at 70ºc for 8 mins.

The PCR products were separated by electrophoresis and visualized by UV Transilluminator. The cytochrome $P_{450}$ (CYP1A1 2A) (valine / valine) was detected at 490 bp band, the genotype (CYP1A1*2C) (isoleucine / valine) was visualized at 190 bp band, the last type (CYP1A1* 1) (isoleucine / isoleucine) was detected at 153 bp band.

**iii) Estimation of $P_{53}$ level by ELISA:**

Mutant $P_{53}$ level was measured by ELISA kit supplied by ( Diagnostic Products Corp., Apeldoorn, The Neherlands) according to manufacture's instructions [8].

**III ) Statistical analysis:**

The distribution of different CYP1A1 genotypes and expression of p53 gene mutation among the study population, was expressed as frequency distribution using chi2 test. The serum level mutant p53 protein and the urinary 1-OHP results were

expressed as means and standard deviations (SD). Analysis of variance (ANOVA) was used for multiple comparisons between the groups. Post-hoc test was then used to study the inter-relation between the different groups. Pearson correlation coefficient was used to relate between the different parameters. A P value of <0.05 was considered to be the level of significance. Computer based statistical package for social sciences (SPSS) for windows 9.1 program was used.

## Results

The study population consisted of 70 male subjects divided into an asphalt exposed group (n=43) and a control group (n=27). Both groups were matched (P >0.05) as regards age, smoking index (S.I) (Pack / year) and duration of exposure as shown in Table (1). Concerning pre-shift Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine) and serum level of mutant p53 protein (pgmol/ ml), both parameters were remarkably higher (P<0.001) among the exposed versus the control group (1.575± 1.626, 2278.44 ± 1039.311) versus (0.327 ± 0.625, 973.220 ± 346.710), respectively, the differences being statistically significant (table2). Demonstration of the mean± SD of serum level of mutant p53 protein (pgmol/ ml) in association with different degrees of expression of mutant p53 gene was presented in table 3.

Frequency distribution of CYP1A1 genotypes (CYP1A1*2A, CYP1A1*2C, CYP1A1*1) was demonstrated in table (4). Workers carrying the genotype CYP1A1*2A, constituted 53.5% of the exposed workers and 55.5% of the controls while the other two genotypes constituted less percentages (21 % for CYP1A1*2C, and 25.5% for CYP1A1*1). The distribution of the different genotypes did not differ statistically between the control and exposed groups. For the associated degrees of expressed mutant p53 gene detected by PCR technique among the exposed subjects, high degree of p53 gene mutation (56.5%) was found among individuals carrying the genotype CYP1A1*2A , while 43.5% had moderate expression of p53 gene mutation and none had a low degree of mutation.. The other 2 genotypes, CYP1A1*2C and CYP1A1*1, showed more per cents of moderate and low degree of gene p53 mutation. As regards Table (5), it shows the mean± SD of Urinary (1-OHP), serum level of mutant p53 protein and smoking index (S.I) among different CYP1A1 genotypes. The exposed workers carrying the CYP1A1*2A allele showed higher levels of the two parameters (1.625 ±0.536, 2756.0 ±843.332) than those carrying CYP1A1*2C or

Table (1): Mean ± SD of age, duration of exposure (in years) and smoking index (S.I) (Pack / year) among the exposed workers and the control subjects.

| | exposed N=43 | Control N=27 | t | P |
|---|---|---|---|---|
| Age | 35.90 ± 11.052 | 33.533±9.164 | 0.677 | n.s* |
| S.I (pack/year) | 8.650 ± 8.290 | 7.966±7.122 | 0.256 | n.s* |
| Duration (in years) | 9.50 ± 7.330 | ----- | ------ | ----- |

Table (2): Mean ± SD of Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine) and serum level of mutant p53 protein (pgmol/ ml) among exposed and control groups.

| | Pre-shift exposed N=23 | Control N=27 | t | P |
|---|---|---|---|---|
| 1-OHP (µg/mg creatinine) | 1.575±1.626 | 0.327±0.625 | 2.811 | 0.008* |
| P53 protein (Pg mol/ml) | 2278.44±1039.311 | 973.220±346.710 | 4.658 | 0.000* |

*= highly significant P<0.005.

Table (3) : Mean ±SD of serum level of mutant p53 protein (pgmol/ ml) in relation to different degrees of expression of p53 gene mutation among exposed group.

| Expression of gene mutation | P53 mutant protein (Pg mol/ml) |
|---|---|
| High degree | 3018.76±327.95 |
| Moderate degree | 2251.80±632.32 |
| Low degree | 703.75±117.14 |

CYP1A1*1 genotypes but ANOVA test did not show statistically significant difference as regards the urinary (1-OHP) (P >0.05). Multiple comparison using Post Hoc test for the previous data showed no significant difference (P>0.05) between workers with the genotypes (CYP1A1*2A) and (CYP1A1*2C) regarding both the urinary 1-OHP and p53 mutant protein although they were higher in (CYP1A1*2A) genotype, while there was highly significant difference concerning them in both CYP1A1*2A and CYP1A1*1 genotype (table 6).

Table (4): Frequency distribution of CYP 1A1 genotypes (CYP1A1*2A, CYP1A1*2C, CYP1A1*1) and the associated degrees of expressed mutant p53 gene detected by polymerase chain reaction (PCR) technique among the exposed and the control subjects.

| CYP1A1 genotypes | Degree of P53 mutation | exposed N=43 | | Control N=27 | | Chi 2 | P |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | | |
| [1]CYP1A1*2A | | 23 | 53.5 | 15 | 55.5 | 0.029 | n.s |
| | High | 13 | 56.5 | 1 | 7 | 0.532 | <0.01 |
| | Moderate | 10 | 43.5 | 1 | 7 | 0.530 | <0.01 |
| | Low | 0 | 0 | 3 | 20 | -- | -- |
| | [4]None | 0 | 0 | 10 | 66 | -- | -- |
| [2]CYP1A1*2C | | 9 | 21 | 5 | 18.5 | 0.060 | n.s |
| | High | 0 | 0 | 0 | 0 | --- | ---- |
| | Moderate | 5 | 55.5 | 1 | 20 | 0.739 | n.s |
| | Low | 4 | 44.5 | 1 | 20 | 0.739 | n.s |
| | None | 0 | 0 | 3 | 60 | ---- | ---- |
| [3]CYP1A1*1 | | 11 | 25.5 | 7 | 26 | 0.001 | n.s |
| | High | 2 | 18 | 0 | 0 | ------ | ----- |
| | Moderate | 7 | 64 | 1 | 14 | 0.366 | n.s |
| | Low | 2 | 18 | 2 | 28 | 0.350 | n.s |
| | None | 0 | 0 | 4 | 58 | --- | ---- |

1= Valine/ Valine genotype

2=valine/ isoleucin genotype.

3=isoleucin/ isoleucin genotype.

4= no detection of p53 gene mutation.

Further, the exposed group was divided into mixing and paving group (n=24) and driver group (n=19). Both groups were matched in age , SI and duration of work. Table (7) reveals that the urinary (1-OHP) and serum level of mutant p53 protein are higher among the asphalt paving workers (1.819± 1.385 µg/mg creatinine,2661.44 ± 986.03 pg mol/ml, respectively) compared to drivers (0.680±0.515µg/mg creatinine, 156.143 ± 753,581 pg mol/ml, respectively) (P< 0.001). Multiple comparison using Post Hoc test for the previous data. Table (8) shows that there was no difference between urinary (1-OHP) of the group of drivers (0.680±0.515µg/mg creatinine) and

Table (5): Mean ± SD of Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine), serum level of mutant p53 protein (pgmol/ ml) and smoking index (S.I) among differtent CYP1A1 genotypes detected among the exposed group.

| | CYP1A1*2A N=23 | CYP1A1*2C N=9 | CYP1A1*1 N=11 | F | P |
|---|---|---|---|---|---|
| 1-OHP (µg / mg creatinine) | 1.625 ±0.536 | 1.244 ±0.776 | 0.736 ±0.444 | 5.012 | n.s |
| P53 protein (Pg mol/ml) | 2756.0 ±843.332 | 1828.980 ±589.411 | 1436.650 ±549.559 | 10.562 | <0.05 |
| S.I Pack/year | 9.00 ± 8.79 | 11.20 ±8.526 | 6.000 ± 8.000 | 0.523 | n.s |

Table (6): Multiple comparison using Post Hoc Test between mean ± SD of Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine) and serum level of mutant p53 protein (pgmol/ ml) among differtent CYP1A1 genotypes detected among the exposed group.

| | CYP1A1*2A N=23 | CYP1A1*2C N=9 | CYP1A1*1 N=11 | P |
|---|---|---|---|---|
| 1-OHP (µg / mg creatinine) | 1.625±0.536 | 1.244±0.776 | ------- | n.s |
| | ---------- | 1.244±0.776 | 0.736±0.444 | n.s |
| | 1.625±0.536 | -------- | 0.736±0.444 | <0.04* |
| P53 protein (Pg mol/ml) | 2756.0±843.332 | 1828.980±589.411 | ------ | n.s |
| | -------- | 1828.980±589.411 | 1436.650±549.559 | n.s |
| | 2756.0±843.332 | -------- | 1436.650±549.559 | <0.007* |

* statistically significant P<0.05

the control (0.327±0.625µg/mg creatinine). Also the serum p53 mutant protein in the asphalt paving workers (2661.44 pmol/ml) was statistically significantly higher than that of group of drivers (1567.143 pmol/ml) (P<0.05).

Inter-variable correlations (table 9) revealed highly significant positive correlation between the urinary (1-OHP) and serum level of mutant p53 protein (r=0.850 P<000). Insignificant correlation was found between the duration of exposure and SI on one hand and the urinary (1-OHP) on the other hand.

## Discussion

Asphalt is a mixture of mineral matter and bitumen. Road pavers are exposed to asphalt fumes through dermal contact, in-

Table (7): Mean ± SD of Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine) and serum level of mutant p53 protein (pgmol/ ml) among mixing and paving group, group of drivers and the control subjects.

| | Paving & Mixing group n=24 | Vehicles & roller drivers n=19 | Control group n=27 | F | P |
|---|---|---|---|---|---|
| Age | 39.0 ±11.68 | 33.00 ±9.66 | 33.533 ±9.164 | 0.615 | n.s |
| Duration (in years) | 10.750 ±7.472 | 8.571 ±7.184 | ------- | 0.165 | n.s |
| S.I (pack/year) | 8.416 ±7.864 | 10.285 ±9.411 | 7.966 ±7.122 | 0.264 | n.s |
| 1-OHP (µg / mg creatinine) | 1.819 ±1.385 | 0.680 ±0.515 | 0.327 ±0.625 | 21.55 | <0.001 |
| P53 protein (pg mol/ ml) | 2661.44 ±986.03 | 156.143 ±753.581 | 973.220 ±346.710 | 19.145 | <0.001 |

Table (8): Multiple comparison using post Hoc test between mean ± SD of Urinary hydroxy pyrene (1-OHP) (µg / mg creatinine) and serum level of mutant p53 protein (pg/ ml) among mixing and paving group, group of drivers and the control subjects.

| | Paving & Mixing group n=24 | Vehicles & roller drivers n=19 | Control group n=27 | P |
|---|---|---|---|---|
| 1-OHP (µg / mg creatinine) | 1.819±1.385 | 0.680±0.515 | ------- | <0.005 |
| | ------- | 0.680±0.515 | 0.327±0.625 | n.s |
| | 1.819±1.385 | ------ | 0.327±0.625 | <0.001 |
| P53 protein (pg/ ml) | 2661.44±986.03 | 156.143±753.581 | ----- | <0.001 |
| | -------- | 156.143±753.581 | 973.22±346.710 | n.s |
| | 2661.44±986.03 | -------- | 973.22±346.710 | <0.001 |

Table (9): Correlation coefficient® between duration of exposure, smoking index (SI), and urinary 1-OHP with serum level of mutant p53 protein among the exposed workers.

| | 1-OHP | P53 protein |
|---|---|---|
| Duration of exposure | R=0.104 P >0.05* | ----- |
| SI Pack/year | R=0.062 P >0.05* | ------ |
| 1-OHP | ------ | R= 0.850 P <000** |

* no statistical significance

** high statistical significance

halation, or ingestion. These fumes contain polycyclic aromatic compounds (PACs), a class of compounds including polycyclic aromatic hydrocarbons (PAHs). The PAHs include compounds such as pyrene and benzo (a) pyrene and a large number of other compounds of known or suspected carcinogenicity[9]. Prolonged, extensive exposure to asphalt fume has been associated with several adverse health effects. Emitted (PAHs) from asphalt fumes have been suspected of inducing such effects [10].

In vitro genotoxicity and mechanistic studies demonstrated a mutagenic effect of bitumen fume condensates (BFC). However, accumulation of mutations in genes responsible for the maintenance of growth control and genomic integrity results in loss of these essential functions and ulti-

mately leads to tumor formation. In 2001, the results of the IARC epidemiological study confirmed an excess of lung cancer despite a lower cancer mortality. Some studies provided a suggestive evidence of an excess risk of bladder cancer among asphalt workers [11].

It is widely held that humans differ in their susceptibilities to cancer. This may be due to a number of factors including health, nutritional status, and gender. From what is known about the mechanism of action of carcinogens, it is thought that genetic background could play a significant role. Variable levels of expression of genes that are encoding the xenobiotic-metabolizing enzymes (XMEs) could result in increased or decreased carcinogen activation [12]. The cytochrome P450 (CYP)-dependent monooxygenases represent the first line of defense against toxic lipophilic chemicals. Unfortunately, certain chemicals are activated to their ultimate carcinogenic form rather than being detoxified. The main CYPs in humans that metabolize carcinogens are CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1, CYP3A4, and CYP3A5 [13].

The CYP1A gene family has two members: CYP1A1, which is predominantly expressed in extrahepatic tissues such as the lung, and CYP1A2, which is concentrated in the liver [22]. CYP1A1 is involved, e.g., in the metabolic activation of polycyclic aromatic hydrocarbons (PAHs) to their carcinogenic metabolites in the lung[14]. As an example, CYP1A1-dependent aryl hydrocarbon hydroxylase (AHH) activities in human lung tissue (microsomes) correlate with activation of benzo(a)pyrene 7,8-diol to the ultimate carcinogen. Furthermore, the AHH activities correlate with the benzo(a)pyrene 7,8-diol-9,10-epoxide (BaPDE) DNA adduct levels in human lung tissue [15]. Moreover, some results demonstrate that asphalt fume condensate (AFC) exposure induced CYP1A1 activity and increased the enzyme levels of CYP1A1 in lung microsomes, suggesting that AFC exposure may alter metabolism of PAHs by the cytochrome P-450 system in the lung. Alteration of cytochrome P-450 metabolism of PAHs may contribute to the AFC-induced genotoxic effects demonstrated as micro nuclei (MN) formation [16].

In recent years the impact of inherited polymorphisms in the CYP1A1 gene on susceptibility to lung cancer has received particular interest since this enzyme plays a central role in the metabolic activation of (PAHs). Three common polymorphisms of the CYP1A1 have been identified and several systematic nomenclatures for

CYP1A1 have been proposed. The CYP1A1 m1 as CYP1A1*2, CYP1A1*2A or Valine / Valine genotype, CYP1A1 M2 as CYP1A1*2C or Isoluecin / Valine genotype, and wild type as CYP1A1*1 or Isoluecine / Isoluecine genotype[17].

In the current study, polymerase chain reaction (PCR) analysis of the CYP1A1 gene revealed that CYP1A1*2A (valine / valine ) genotype constitutes 53.5% (n=23 out of 43)of the exposed workers versus, 21% for the CYP1A1*2C (n=9 out of 43) and 25.5% (n=11 out of 43) for CYP1A1*1 indicating that it is the most prevalent genotype among the study subjects particularly if this percentages did not differ significantly from that of the controls ( 55.5%, 18.5 %and 26%), respectively (table 3). Genotype frequencies were known to vary by geography and race. However, many studies reported the rarity of CYP1A1*2C genotype among the Caucasian populations compared to Asians [18]. Besides, Alexandrie et al.,[5] reported the predominance of CYP1A1*2A among the Caucasians.

On the other hand, PCR analysis of the p53 gene revealed different degrees of expression of p53 gene mutation ranging from high, moderate to low degree among the whole exposed population. Workers carrying the genotype CYP1A1*2A (valine /valine) showed the highest percent of expressing high degree of p53 gene mutation (56.5%) while 43.5% had moderate expression of p53 gene mutation. None of the exposed individuals carrying this genotype had low degree of mutation. The other 2 genotypes showed statistically significantly higher percentage of moderate and low degree of gene p53 mutation compared to controls. This mutation can be explained by studies that have demonstrated that, B(a)PDE induces guanine adducts at mutational hotspots, including codons 157, 248, and 237 of p53 gene in normal human bronchial epithelial cells. Recently, Alexanerov et al.,[19] reported that, the persons with CYP1A1*2A -GSTM1 null genotype combination had higher level of BDPE-DNA adducts . Additionally, this is consistent with the prevalence of G:T mutations in the p53 gene at the hotspot codons in the lung tumors of smokers (being exposed to PAHs), a point mutation that is indicative of PAHs related mutational damage [20]. What complicates the situation, is that in the p53 gene the same codons are preferential targets for not only mutagenesis but also tumorigenic selection[7].

Therefore, It was not surprising to find out this remarkable increase in the serum level of mutant p53 gene protein among

the exposed workers (2278.44±1039.311 versus 973.220±346.710 in the controls) (P<0.000) (table 2). Our results correspond with Rossener et al., [21] who detected a significant increase in p53 proteins (the mutant and the wild types) in workers exposed to carcinogenic PAHs >1 microg/m3 as compared with the group exposed to carcinogenic PAHs <1 microg/m3. A similar trend was observed for p21(WAF1) protein, eventhough no correlation exists between the levels of both proteins.

Normally, p53 protein regulates the transcription of genes responsible for cell cycle arrest and apoptosis. P53 protein binds DNA, which in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein (cdk2). When p21 is complexed with cdk2 ; it induces cell cycle arrest either in the G(1), S, or G[2] phases[22].Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors [21] .

However, it has been shown that carcinogenic B(a) pyrene is able to induce the expression of both p53 and p21(WAF1) proteins in vitro .This induction occurs subsequent to the induction of DNA adducts which are correlated with both p53 and p21(WAF1) levels regardless of the PAH exposure and the phase of cell growth. This induction requires a minimal DNA adduct level of approximately 200-250 adducts/10 nucleotides for PAHs tested suggesting that the level of adducts rather than their structure triggers the p53 and p21(WAF1) responses [22] .

As pyrene is a major component of most PAH mixtures, it is often used as a indicator for total exposure. Accordingly, many studies have confirmed urinary 1-hydroxypyrene (1-OHP) as a biological measure for the internal exposure of (PAHs)[23]. Pyrene undergoes simple metabolism and is rapidly bio-transformed into various metabolites that undergo major enterohepatic recycling. Part of the initially formed and part of the recirculated 1-OHP eventually undergo urinary excretion such that close to 60% of pyrene is eliminated as metabolites in urine by 24 h after exposure while 20% is excreted in the feaces over the same period [24].

In the current work, there was highly significant statistical difference (P< 0.001) between the mean level of pre-shift urinary (1-OHP) among exposed workers (n=43) (1.575±1.626 µg/mg creatinine) compared to control subjects (n=27) (0.327± 0.625µg/mg creatinine) (table 2). These

levels are comparable to results obtained by Mc Clean et al.,[25] . The authors detected (1.4 μg/mg creatinine) of 1-OHP among paving workers in the pre-shift samples taken on the 4th day of the working week which was 3.5 times higher than the results of day 1. The level of urinary 1-hydroxypyrene (1-OHP) was significantly higher in the post-shift samples compared to the pre-shift samples and the controls in another study made by Vaananen et al.,[26] . indicating the reliability of using (1-OHP) as a biological indicator for PAHs exposure. Unfortunately , it was not feasible to perform post- shift sampling in our study.

When 1-OHP level were related with CYP1A1 genotypes, an association was observed for the CYP1A1*2A genotype, so that the asphalt exposed workers carrying the CYP1A1*2A allele showed significantly higher 1-OHP levels than those carrying CYP1A1*2C or CYP1A1*1 genotypes. This is in accordance with Adonis et al.,[27] who detected no significant correlation between urinary 1-OHP levels and GSTM1 null genotype, although higher levels of the urinary metabolite were found in individuals carrying the combined CYP1A1*2A and GSTM1 null genotype. This suggests an association between levels of the exposure biomarker 1-OHP and presence of the CYP1A1*2A genotype, as a potential genetic susceptibility biomarker which might be useful in identifying individuals at higher risk among people exposed to high PAH levels in paving occupation. In addition, CYP1A1*2A genotype exhibited a higher cytokinesis - block micronucleus ( CBMN ) frequency than in CYP1A1*1 (Ile/Ile) or (Ile/Val) CYP1A1*2C genotypes(29). Most reports from Japan point to the strong association between CYP1A1 *2A with the risk of lung cancer, especially in relation to tobacco smokers and in lung squamous cell carcinoma(15) .

Other studies reported that, the highest 1-OHP levels were observed in individuals carrying the CYP1A1 Ile/Val (CYP1A1*2C ) genotype who were also of the GSTM1 null Genotype[29]. In the current work, there was no significant difference (P>0.05) (table 5) between workers with the genotypes (CYP1A1*2A) and (CYP1A1*2C ) regarding both the urinary 1-OHP and p53 mutant protein although they were higher in (CYP1A1*2A) genotype (1.625±0.536μg/mg creatinine, 2756.0 ±843.332 pgmol/ml) versus (1.244 ±0.776μg/mg creatinine,,1828.98±589.411 pgmol/ml) in (CYP1A1*2C ).

This variation in enzyme inducibility could be due to genetic polymorphism in CYP1A1 or in genes involved in the con-

trol of its expression. Some studies showed effects of glutathione S- transferase (GST) M1 and aryl hydrocarbon polymorphisms on CYP1A1 activity, while others found no key role of these polymorphisms in CYP1A1 inducibility [30]. Other studies reported less association between the concentrations of 1-OHP and the GSTM1, polymorphism but these research pointed to a ratio between CYP1A1 and GST enzyme activities as a critical determinant of the target dose of carcinogenic BPDE [31] .

Further analysis of the results, revealed higher levels of urinary (1-OHP) among the asphalt paving workers (1.819± 1.385µg/mg creatinine) compared to workers involved in maintenance and drivers (0.680±0.515µg/mg creatinine) (P< 0.005) (table, 6). The variation in PAHs contamination on the skin may explain this significant difference as many studies have suggested dermal exposure to be a major determinant of the total PAH dose absorbed by road pavers from bitumen fumes [32,33]. Vanrooij et al., [34] concluded that an average of 75% of the total absorbed amount of pyrene enters the body through the skin. Furthermore, dermal exposure that occurred during the preceding 32 h had a statistically significant effect on urinary 1-OHP, while the effect of inhalation exposure was not significant[25]. However, among the asphalt exposed workers inhalation and dermal PAHs exposures varied significantly by task, crew, work rate (inhalation only). The inhalation exposures were consistent with the workers' proximity to the primary source of asphalt fume while the dermal exposures were consistent with the degree to which the workers have actual contact with asphalt-contaminated surfaces[35] . Usually, paving workers had inhalation (mean 0.3 micro g/$m^3$) and dermal (5.7 ng/$cm^2$) exposures to pyrene[36] .

On the other hand, there was no difference between urinary (1-OHP) of the group of drivers (0.680±0.515µg/mg creatinine) and the control (0.327±0.625µg/mg creatinine). As was discussed before, the exposure of the group of drivers is mainly through inhalation of PAHs which is far less than the dermal absorption making the drivers have higher levels but statistically not significantly different from the controls (P>0.05). Additionally, it should be taken into consideration that PAHs constitute about 1% of asphalt fumes while the remaining 99% is formed by aliphatic straight chain hydrocarbons[37] .

Similarly, the serum p53 mutant protein in the asphalt paving workers (2661.44 pmol/ml) was statistically significantly

higher than that of group of drivers (1567.143 pmol/ml) ($P<0.05$). This can be explained by the lower level of urinary 1-OHP detected in this group . In support of these results is the positive significant correlation detected between the urinary (1-OHP) and the serum level of mutant p53 protein ($r=0.580$, $P= 0.007$). This is consistent with the study of Pan et al. who demonstrated a significant correlation between serum p53 protein levels and the cumulated benzo(a)pyrene exposure dose [38].

Inter-variable correlations between the data revealed no significant correlation between the smoking index versus the urinary 1- OHP ($r=0.0262$ $P>0.05$), figure (2). Similarly, Lu et al . (39) reported no significant effect of smoking on the excretion of urinary(1-OHP) among coke oven workers but other studies reported that smoking caused a significant increase of urinary 1- OHP[40] .

Again no significant correlation was obtained between the level of 1-OHP and the exposure duration in spite of excessive exposure as evidenced by high 1-OHP levels in urine. In similar studies, no statistically significant correlation was observed between biomarker levels and the level of individual PAHs among exposed population[41]. Besides, in a longitudinal study on PAH biomarker levels, it was reported that urinary 1-OHP has a day-to-day and week-to-week individual variation, indicating the necessity for multiple sampling [42].

The highly significant positive correlation ($r = 0.850$ , $P<0.001$) detected between the urinary 1- OHP and serum mutant p53 protein suggest the necessity of using urinary 1- OHP in biological monitoring with the intention to prevent excessive occupational exposure to PAHs. Besides, genetically based metabolic polymorphisms must be taken into account in the future as detected from the higher level of urinary 1-OHP , high degree of p53 gene mutation and the high level of serum p53 mutant protein among individuals carrying CYP1A1 *2A genotype.

The current work recommends using control strategies that focus on reduction of dermal contamination by PAHs than on the reduction of inhaled dose to reduce occupational exposure to asphalt-related PAHs. An exposure assessment of PAHs that does not consider dermal exposure may considerably underestimate cumulative exposure.

However, the small size of the study limits the potential application of the results to the asphalt industry in general. More research is needed to develop a valid and inexpensive method of assessing total bitumen exposure and susceptibility to the encountered potential hazards .

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