

Polycyclic Aromatic Hydrocarbons: Role of Apoptosis in Dermatotoxic and Carcinogenic Effects in Some Asphalt Road Paving Egyptian Workers

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Abstract This study aimed firstly to assess the dermatotoxic and carcinogenic risk associated with chronic asphalt fumes exposure, and secondly to investigate the cancer causal relationship by examining the expression pattern of P53, Bax and Bcl-2 apoptotic proteins in skin specimens from road paving workers. The study was conducted on one hundred and fifty two male subjects classified into 122 asphalt fumes exposed workers and 30 non exposed workers; skin examination and skin biopsies were obtained from all participants after written consent. Biopsies were examined histopathologically and immunohistochemically. Results showed that 58.19 % of exposed workers had erythema, itching, excoriations, chronic dermatitis, chemical keratosis, keratoacanthoma (K.A) and 7.38% of exposed workers had squamous cell carcinoma (SCC). Immunohistochemically, wild type P53 was significantly higher in all asphalt exposed workers and mutant type P53 was significantly higher in SCC lesions when compared with control. A significant increase in Bax expression was observed in all exposed workers. A significant decrease in Bcl-2 expression was noted in all workers while none of SCC cases were positive for Bcl-2. It could be concluded that chronic exposure to asphalt fumes may increase the risk for developing dermatotoxic and/or cancer through disturbing P53, Bax and Bcl-2 apoptotic proteins.

Keywords Polycyclic aromatic hydrocarbons (PAHs), dermatotoxicity, apoptosis, skin cancer, asphalt workers

Introduction

Asphalts are complex hydrocarbon mixtures with molecular weights ranging from 500 to 2000, high boiling temperature (above 500°C) and carbon numbers predominantly higher than C25. Asphalts produced from the atmospheric and vacuum distillation of crude oil are used primarily in paving applications (Ceinwen, 2011). Asphalt typically contains about 80% by weight of carbon; around 10% hydrogen; up to 6% sulfur; small amounts of oxygen and nitrogen; and trace amounts of metals such as iron, nickel, and vanadium (Michael, 1999).

Asphalt fumes produced during heating of asphalts to facilitate their use commonly are encountered by workers in road paving operations. They are complex mixtures of aerosols and vapors that contain various organic compounds including polycyclic aromatic hydrocarbons (King et al., 1984).

Polycyclic aromatic hydrocarbons "PAHs" are vapors resulting from incomplete combustion or

pyrolysis of coal tar, pitch, coke and asphalt. They precipitate as particles or condense onto soot (Harrison, 2004). These fumes may be inhaled or deposited on skin or clothing (Ceinwen, 2011).

The adverse effects of PAHs on humans are diverse (Yvonne, 2007). PAHs have been reported to cause many skin disorders (Bull, 2008); they were reported to be phototoxic (Wang et al., 2007) and verrucae inducer (ATSDR, 1995). Numerous studies have described an excess risk of cancer among asphalt-exposed workers (Boffetta et al., 1997; Sivak et al., 1997; Boffetta et al., 2003; Okona-Mensah et al., 2005). PAHs have been shown to be mutagenic in rat lung (Zhaoa et al., 2004), reproductive and developmental toxicant (Parker et al., 2011) as well as genotoxic in humans (Monika et al., 2011).

Apoptosis is a natural, genetically controlled series of steps that occurs when a cell is old, unhealthy, or severely damaged. The end result is cell destruction.

Apoptosis in the skin kills off harmed cells that they do not turn cancerous. In some cases, genetic mutations or other factors derail apoptosis. If this occurs; the cells can become "immortal" and continue to proliferate, resulting in skin cancer (Timothy, 2009).

The process of apoptosis is controlled by important genes as P53 gene (tumour suppressor gene) and proteins such as Bcl-2 family i.e. several pro-apoptotic (Bax, Bak, Bad) and anti-apoptotic (Bcl-2, Bcl-xl) proteins and Caspases (Erb et al., 2005).

PAHs compounds have been shown to induce apoptosis in hepatoma cells (Lei et al., 1998), in human B cells (Salas and Burchiel, 1998), in macrophages (Chin et al., 1998) and in rat lungs (Gosset et al., 2003). Though numerous studies have described an excess cancer risk among asphalt workers, a causal relationship has not been established. Accordingly, the aim of this work firstly was to search for the dermatotoxic and carcinogenic risk associated with chronic PAHs exposure in asphalt workers and secondly to evaluate this cancer causal relationship through studying the PAHs effect on apoptosis by examining the expression of P53, Bax and Bcl-2 apoptotic proteins in skin specimens from road paving workers using immunohistochemical techniques.

Subjects and methods

The study was conducted on 152 male subjects from Minia Governorate classified into two groups: 122 road paving workers who were exposed nearly daily to asphalt fumes "exposed workers" and 30 age-matched non-paving outdoor construction workers with no history of occupational asphalt fumes exposure "control group".

Written informed consent was obtained from each participant after discussing the purpose and the protocol of the study. All procedures were conducted in accordance with a standardized human subject's protocol that was approved by the ethics committee of Faculty of medicine, Minia University.

Over 10-month period, for all subjects, a detailed history of medical status, occupational, and drug administration was taken. Smokers were excluded. Full general, local examination and photographing for any observed skin lesions were undertaken.

Histopathology and immunohistochemistry study

Four mm punch biopsies were taken from squamous cell carcinoma (SCC) lesions "if found" and from normal uninvolved forearm skin if no lesion were detected (non-lesional skin). Patients with evident skin lesions were then referred to be properly managed. Biopsies were taken according to Alguire and Mathes (1998) who said that the skin biopsy is relative simple, but essential procedure in the diagnosis and management of skin disorders. Properly performed, it may confirm a diagnosis, remove cosmetically unacceptable lesions, and provide definitive treatment for a number of skin conditions. Skin biopsies are unique because the lesion can be visualized, allowing

for proper selection biopsy site and technique skin biopsy can be performed with minimal risk even in critically ill patients, and may a timely skin biopsy may avoid other more invasive procedures.

Biopsy specimens underwent routine haematoxylin and eosin light microscopic histopathological examination then evaluated immunohistochemically.

Immunohistochemistry was performed on paraffin wax embedded skin tissue according to Petrusz and Ordronneau (1983). Four μm tissue sections were immunostained for P53, Bax and Bcl-2 proteins using a standard avidin-biotin-peroxidase method. Sections were dewaxed in two changes of xylene, hydrated in graded alcohol. Endogenous peroxidase was blocked with 0.3 % H_2O_2 (volume/volume) in methanol for 30 minutes at room temperature. Samples were then incubated overnight with rabbit monoclonal antibody to human P53 antibody (code no.: RMPD 016, ready-to-use 1ry antibody, DBS, CA, USA) which reacts with both the wild and the mutant types of human P53 protein, Bax epitope specific rabbit antibody (E3381, Spring Bioscience, CA, USA) and mouse monoclonal antibody to human Bcl-2 oncoprotein (code no.: PDM 016, ready-to-use 1ry antibody, DBS, CA, USA) at 4°C. Sections were then immersed in a biotinylated secondary antibody diluted at 1:100 for 30 minutes and in avidin biotinylated peroxidase complex for 60 minutes (code no.: # Kp-50L, DBS, CA 94566, USA). Slides were then treated with 100 μl of freshly prepared DAB (3' 3' diaminobenzidine) solution for 10 min. Finally, slides were rinsed in running water, dehydrated in graded methanol, cleared in three changes of xylene and mounted in DPX then examined. Negative control sections were obtained by omitting the specific primary antibody from the staining process.

The level of expression was evaluated quantitatively according to scoring system made by Liang et al., (1999). Application of this system gives a score for both the degree of positivity (the number of positively stained keratinocytes/ 100 examined cells) and the degree of intensity of staining (Table, 1).

Table (1): Scoring system for quantifying the level of P53, Bax or Bcl-2 expression in epidermal keratinocytes (Liang et al., 1999).

Score	Positivity	Score	Intensity
0	< 1.0%	0	No staining (negative)
1	1 – 10%	1	Faint brown (mild-weak)
2	10 – 50%	2	Brown (moderate)
3	> 50%	3	Deep brown (strong)

Statistical analysis

Statistical analysis of data was performed by using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) for windows. Results were expressed as mean values (M) and standard deviation ($\pm\text{SD}$). Unpaired two-tailed Students't-test was performed to compare results. P Values of less than <0.05 was considered statistically significant.

Results

The age of the road paving workers ranged from 30–55 years with a mean of 42 years and the exposure period to asphalt fumes ranged from 9 – 25 years with a mean of 11.5 years.

General examination revealed that all participants were free of any medical diseases except ten complaining of hypertension. By local examination, many dermatologic toxic symptoms and signs were observed in 71 asphalt fumes exposed workers out of 122 presented (58.19 %) as erythema (8 cases), itching (19 cases), excoriations (13 cases), chronic dermatitis (16 cases), chemical keratosis (9 cases) and keratoacanthoma (K.A) (6 cases). For the last three disorders; the diagnosis was clinically and confirmed histopathologically.

Nine out of 122 (7.38%) workers examined were found to have skin lesions in the form of three skin ulcers, two hyperpigmented lesions and four fungating masses. They gave history of working in asphalt paving for more than 15 years before noting skin lesions. They were exposed to asphalt fumes on a nearly daily basis. Lesions were limited to lower lip (Fig.1), ear pinna (Fig.2), back of scalp, knee and the nostrils entrance. Clinical as well as histopathological examination revealed squamous cell carcinoma (SCC).

Histopathological examination of squamous cell carcinoma lesions revealed seven moderately differentiated SCC cases and two poorly differentiated SCC cases. Squamous cell carcinoma was characterized by typical keratinocytes presented in groups and nests of malignant squamous cells breaking through the basal membrane into the dermis. The keratinocytes lie in complete disorder. Cellular atypia, including pleomorphism, hyperchromatic nuclei, and mitoses are prominent. The moderately differentiated SCC showed an increased number of atypical keratinocytes and fewer horn-pearls, while the poorly differentiated SCC exhibited numerous atypical keratinocytes and rare horn pearl within atypical keratinocytes “with increasing malignancy; the atypical cells increased and the horn pearl become scanty” (Fig.3, 4).

The level of P53 expression in the epidermal keratinocytes of the control group ranged from 0.7–1.1 with a mean of 0.84 ± 0.16 . Immunoreactive

keratinocytes exhibited nuclear staining and were limited to the basal cell layer of the epidermis indicating wild type (diffuse pattern) P53 expression (Fig.5). While the level of wild P53 expression in the epidermal keratinocyte of the asphalt fumes exposed uninvolved skin ranged from 0.62– 1.9 with a mean of (1.3 ± 0.46) (Fig.6). Mutant type P53 (compact pattern) was over-expressed in SCC cases with a level ranged from 16.64–63.48 with a mean of 49.60 ± 32.7 (Fig.7). A significant increase in P53 expression level in epidermal keratinocytes of asphalt fumes exposed normal uninvolved skin “non lesional” ($t= 2.51$, $P= 0.04$) and in SCC cases ($t= 4.79$, $p= 0.003$) was observed when compared with the control group (Histogram 1).

Bax was overexpressed in all asphalt fumes exposed workers “either within PAHs exposed normal uninvolved skin or SCC lesions”. The expression was cytoplasmic and its level in epidermal keratinocytes was 2.2–2.46 with a mean of 2.3 ± 0.099 , 1.57–3.75 with a mean of 2.81 ± 0.59 and 2.3 – 3.91 with a mean of (2.9 ± 0.02) in the control group, asphalt fumes exposed normal uninvolved skin and SCC cases respectively (Fig. 8, 9, 10). A significant increase in Bax expression in epidermal keratinocytes of the asphalt fumes exposed workers and in SCC cases was observed when compared with the control group ($t= 2.73$, $P= 0.03$ & $t = 2.98$, $P= 0.01$, respectively [Histogram 1]).

Bcl-2 expression of control group was confined to basal keratinocytes. They exhibited cytoplasmic staining with perinuclear enhancement (Fig. 11) with mean expression level of 2.69 ± 1.7 . In asphalt fumes exposed normal uninvolved skin; Bcl- 2 was down regulated with a mean level of 1.3 ± 1.2 (Fig. 12). On other hand; none of the 9 tested SCC cases were positive for Bcl-2 (Fig. 13). A significant decrease in Bcl-2 expression in epidermal keratinocytes of PAHs exposed subjects was noted ($t= 2.49$, $P= 0.047$) (Histogram 1).

Number of positively immunostained cases, immunostaining intensity and the expression score for P53, Bax and Bcl-2 in both asphalt fumes exposed normal uninvolved skin and SCC cases in asphalt fumes exposed group are illustrated in tables 2, 3 & Fig. 14A & 14B.



Fig.1: Photograph showing SCC of lower lip.



Fig.2: Photograph showing SCC of ear.

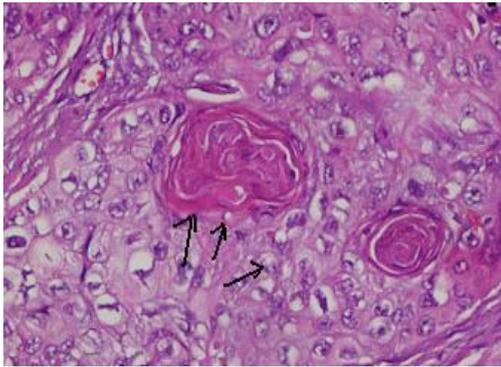


Fig.3: Photomicrograph showing moderately differentiated SCC, an increased of number atypical keratinocytes and fewer horn pearls (H&E, x400).

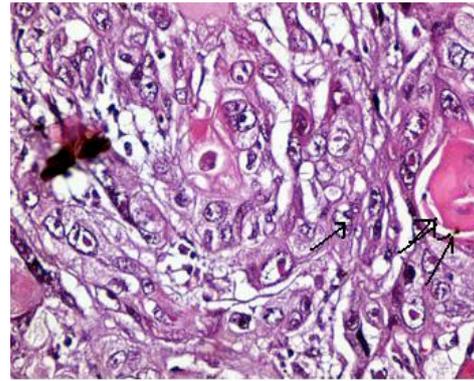


Fig.4: Photomicrograph showing poorly differentiated SCC with numerous atypical keratinocytes. Occasional horn pearl can be seen within atypical keratinocytes (H&E, x400).

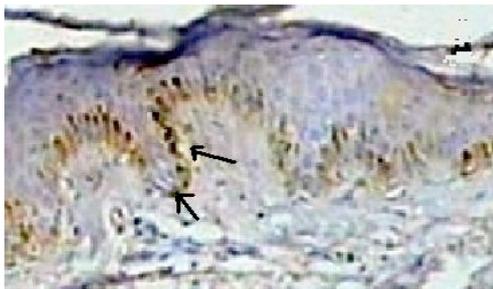


Fig.5: Photomicrograph showing positive nuclear P53 expression in basal layer of epidermal keratinocytes of control group (Immunoperoxidase; x200).

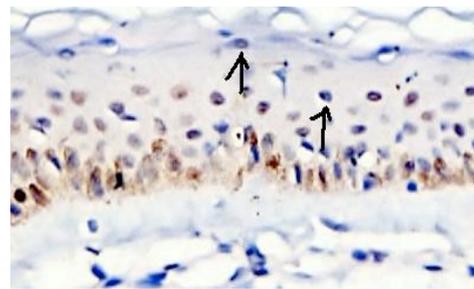


Fig.6: Photomicrograph showing increased nuclear P53 expression in epidermal keratinocytes of normal uninjured skin of asphalt fumes exposed group. The expression extended from basal to granular cell layer (Immunoperoxidase; x400).

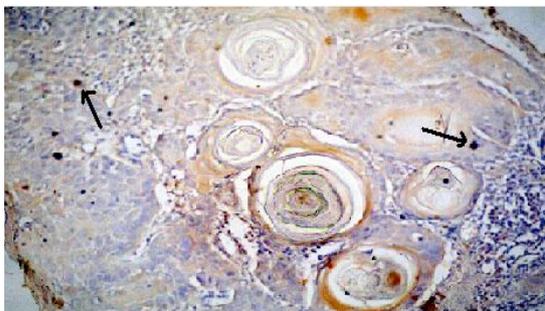


Fig.7: Photomicrograph showing positive nuclear P53 expression in SCC among asphalt fumes exposed group. (Immunoperoxidase; x200).

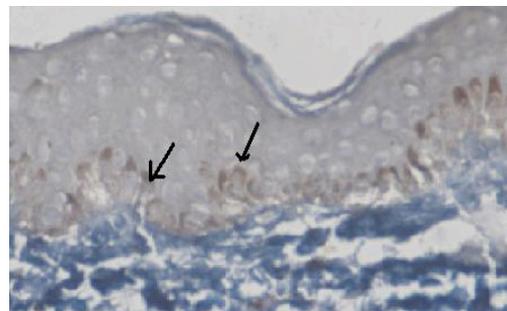


Fig.8: Photomicrograph showing cytoplasmic expression pattern of Bax in basal cell layer of the epidermal keratinocytes of control group (Immunoperoxidase x400).

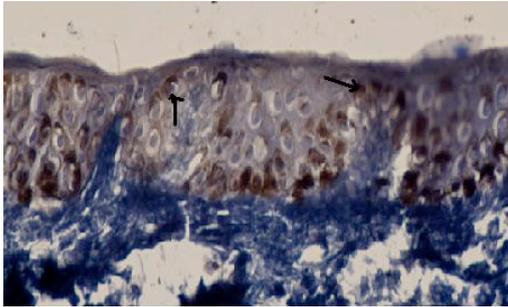


Fig.9: Photomicrograph showing Bax expression in all layers of epidermal keratinocytes of asphalt fumes exposed normal uninvolved group (Immunoperoxidase; x400).

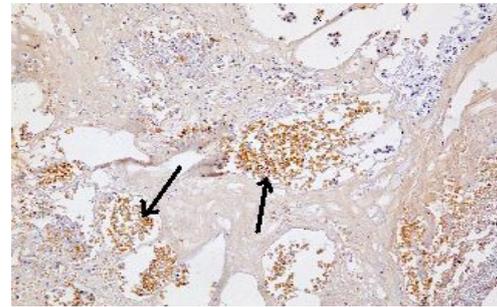


Fig.10: Photomicrograph showing higher Bax expression within SCC (Immunoperoxidase; x200).

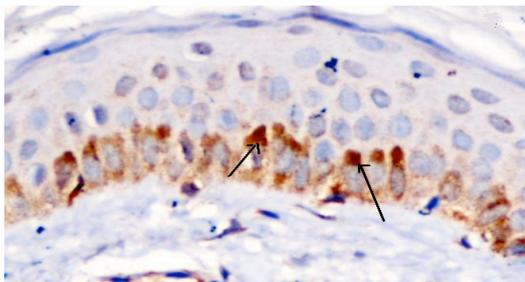


Fig.11: Photomicrograph showing cytoplasmic expression of Bcl-2 of basal cell layer in control skin (Immunoperoxidase; x400).

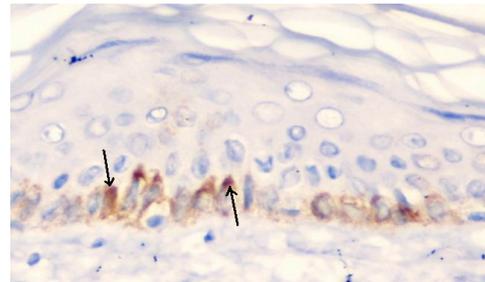


Fig.12: Photomicrograph showing low Bcl-2 expression in asphalt fumes exposed workers (Immunoperoxidase; x400).

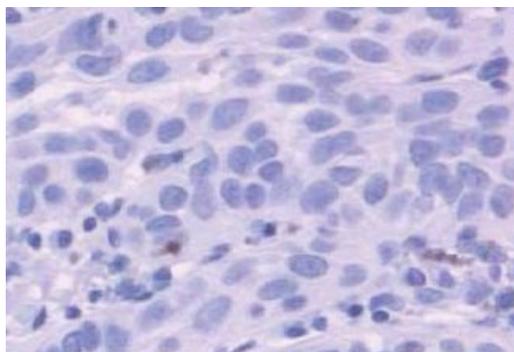
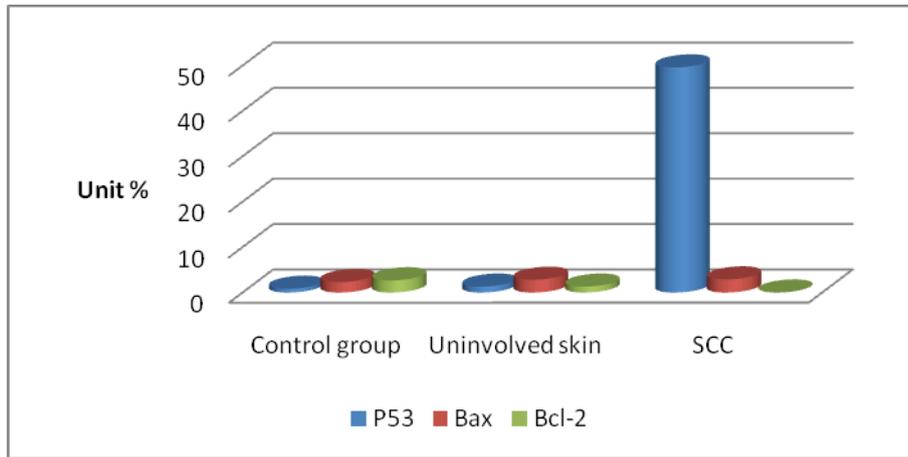


Fig. 13: Photomicrograph showing negative Bcl-2 expression in SCC (Immunoperoxidase; x400).



Histogram 1: The mean levels of P53, Bax and Bcl-2 among control and asphalt exposed groups (Control group= 30, exposed group=122 {uninvolved skin= 113, SCC cases= 9}).

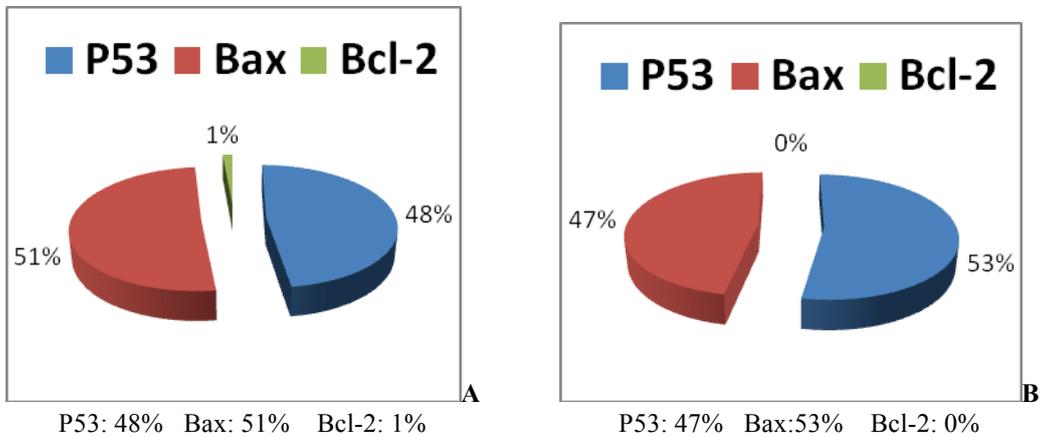


Figure 14: Pie chart shows immunostaining intensity of P53, Bax and Bcl-2 in uninvolved skin (A) and SCC (B) cases of asphalt-exposed group.

Table (2): Immunostaining intensity of P53, Bax and Bcl-2 in uninvolved skin and SCC cases of PAHs exposed group.

Immunostaining intensity		No. of positive cases	No. of cases with mild (weak) staining intensity	No. of cases with moderate staining intensity	No. of cases with strong staining intensity
Marker					
PAHS exposed uninvolved skin (n=113)	P53	101	7	88	6
	Bax	107	6	38	63
	Bcl-2	3	3	0	0
SCC cases (n=9)	P53	9	0	7	2
	Bax	8	1	6	1
	Bcl-2	0	0	0	0

Table (3): Apoptotic markers expression score in uninvolved skin and SCC cases of PAHs exposed group

Score		No. of positive samples	< 1.0% (score 0)	1-10% (score 1)	10 – 50% (score 2)	> 50% (score 3)
Markers						
PAHS exposed uninvolved skin (n=113)	P53	101	12	15	80	6
	Bax	107	6	6	23	78
	Bcl-2	3	110	3	0	0
SCC cases (n=9)	P53	9	0	1	4	4
	Bax	8	1	0	2	6
	Bcl-2	0	9	0	0	0

Discussion

Occupational exposure to hot asphalt may increase the risk of cancer (lung, stomach, bladder, leukemia and non-melanoma skin cancer) among asphalt workers (McClellan et al., 2007). Changed expression of apoptosis related molecules could be detected during the process of malignant transformation and in malignant cells (Teo et al., 2007). As dermatotoxic and carcinogenic potentials of asphalt fumes are not fully clarified and mechanisms involved in PAHs-induced apoptosis are less characterized; so this work aimed to study chronic toxic effects of PAHs fumes on skin and to search for cellular pathways of apoptosis underlying malignant transformation through studying P53, Bax and Bcl-2 apoptotic protein expression in skin of a population of road paving workers to assess the toxic role of asphalt fumes on apoptosis using immunohistochemical techniques.

The observed increased toxic skin symptoms in asphalt fumes exposed workers agree with what reported by (ATSDR, 1995; Wang et al., 2007; Bull, 2008). This may be explained on the basis that asphalt fumes exposure alters cutaneous barrier integrity and induce skin disease as the Egyptian workers don't follow safety protective measures. Results also proved an association between cancer risk and exposure to asphalt fumes where 9 chemical keratosis cases (with pre-cancerous potential) and 9 SCC cases were diagnosed which in line with (Partanen and Boffetta, 1994; Voelter et al., 2007).

Apoptosis which is equated to programmed cell death is an evolutionary conserved cell death process that plays a major role during normal development, homeostasis and elimination of potentially dangerous cells. The ordered execution of this internal death program leads to typical morphological and biochemical changes (Sinha Hikim and Swerdloff, 1999). Dysregulation of the apoptotic process may result in a wide range of pathological conditions as cancers, autoimmune diseases, and viral infections (Cecilia et al., 2007).

In this study; asphalt fumes were found to induce activation of apoptosis as defense mechanism from the body, where both wild type P53 (tumour suppressor gene) and Bax protein (pro-apoptotic protein) were over-expressed and Bcl-2 (the major anti-apoptotic protein) was downregulated which is in the same line with (Ponten and Lundeberg, 2003; Solhaug et al., 2004; Moll et al., 2005). The activated apoptosis act as a defense mechanism to prevent PAHs toxic effect as it helps exposed workers to get rid of harmed cells and to prevent progression to carcinoma. Increased P53 expression level suggests that PAHs induced apoptosis is triggered by the induced DNA damage in P53-dependent pathway. P53 in its natural form (wild-type) upregulates P21 causing cell cycle arrest to allow repair of damaged DNA (Barakat and Abd-Elghany, 2007). Road paving asphalt fume was found by De Meo et al., (1996) to be mutagenic and induced DNA damage and adduct formation. DNA damage initiates a number of cell signaling pathways that may result in cell cycle delays, senescence or cell

death. The key point of these changes is the tumour suppressor P53 (Niida and Nakanishi, 2006). Following DNA damage P53 is phosphorylated thereafter translocated to the nucleus where it may result in cell cycle arrest, allowing time for DNA repair or triggering apoptosis (Efeyan and Serrano, 2007). If damage is extensive, P53 induces apoptotic cell death by downregulating Bcl-2 and upregulating Bax expression (Barakat and Abd-Elghany, 2007).

Comparatively, we were able to further substantiate this hypothesis as we found an increased level of Bax which is involved in triggering the apoptotic process as Bax is known to be a downstream target of P53 and is often induced and/or translocated to mitochondria as a part of the apoptotic process (Landvika et al., 2010). The enhanced apoptosis was also confirmed from the observed lowered expression level of Bcl-2. Bcl-2 is a proto-oncogene that encodes the largest known anti-apoptotic protein. Bcl-2 regulates apoptosis through the formation of homodimer proteins with the other proteins belonging to the Bcl-2 group, especially the pro-apoptotic protein Bax (Verhaegh et al., 1995).

Asphalt fumes, induced apoptosis may also have a role in cancer initiation and promotion. Results showed significantly increased expression levels of mutant P53 in SCC cases. The observed higher level of mutant P53 accompanied with high Bax expression may explain the aggressive behavior of SCC as the mutant type P53 protein was unable either to induce apoptosis or to suppress SCC development and progression (Chan et al., 1995). Mutation in P53 gene leads to the loss of its repair function and thus apoptosis resistance of the DNA-damaged cell and hence cancer development (Peter et al., 2005). Hussain and Harris (2007) found that mutations in P53 gene are the most frequently observed genetic alteration in cancer. P53 mutation may occur due to chromosomal instability, reactive nitrogen and oxygen species and formation of reactive metabolites which induce mutations (Vom et al., 2009).

The lack of Bcl-2 expression in SCC confirm the hypothesis that most of tumor cells originate from the suprabasal keratinocytes which normally have negative Bcl-2 expression (Lu et al., 1993). The negative Bcl-2 expression in SCC was unexpected as Bcl-2 is known to protect tumor cells from apoptosis (Xie et al., 1999). This may be possibly compensated by over expression of other anti-apoptotic proteins (not included in the study). So, it is suggested that disturbed apoptosis and SCC formation in PAHs exposed workers is not Bcl-2 dependent. Although the relatively high expression level of Bax; it appears to be unable to suppress the tumor progression. This may be due to expression of functionally inactive Bax (Kurabayashi et al., 2001). Another explanation to tumor formation was reported by Holme and Solhaug (2005) where they stated that although apoptosis of cells exposed to PAHs is considered to function anti-carcinogenic since cells with extensive DNA damage will be removed, it is possible on other hand that removal of these cells may

give survival and proliferating signals to surrounding cells with less DNA-damage, increasing their probability of having mutations and give promotion signals to cell proliferation and hence cancer formation.

Conclusion and Recommendation

- This work makes it clear that asphalt workers who exposed continuously to asphalt fumes are at great risk to develop serious skin disorders including cancers so, it is recommended that they follow protective safety measures including wearing protective gloves, boots, aprons, and gauntlets as needed to prevent prolonged or repeated skin exposure. Goggles and face shields should be used in areas where splashing may occur.
- Workers who developed skin disorders must seek professional medical help and are advised to move to another job away from continuous asphalt exposure.
- Asphalt fumes exposure may increase the risk for developing dermatotoxic and/or cancer through disturbing apoptosis. Although PAHs exposure can disturb P53, Bax and Bcl-2 apoptotic proteins, more extensive researches on other factors involved in controlling apoptosis as Bcl-xl, Caspase family and Fas are to be undertaken.

Abbreviation: STDs: Sexually Transmitted Disease

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الملخص العربي

الهيدروكربونات العطرية المتعددة الحلقات: تأثيرات الموت المبرمج للخلية في سمية الجلد و التأثير المسرطن في بعض عمال رصف الطرق الإسفلتية المصريين

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تهدف هذه الدراسة أولاً لتقييم سمية الجلد والتأثير المسرطن المرتبط بالتعرض المزمن لأبخرة الإسفلت و ثانياً للتحقيق في العلاقة السببية للسرطان عن طريق فحص نمط التعبير لكل من بروتين بي 53 و باكس و بي سي ال 2 في عينات الجلد من عمال رصف الطرق الإسفلتية أجريت هذه الدراسة على 152 ذكر قسموا إلى 122 شخصاً يمثلون المجموعة المتعرضة لأبخرة الإسفلت و 30 شخصاً لم يتعرضوا لهذه الأبخرة. تم فحص الجلد و أخذ عينات جلدية من المشاركين بعد أخذ موافقتهم الكتابية. ثم فحصت هذه العينات هستوباثولوجياً و خلويًا مناعياً. وقد أوضحت النتائج أن 58.19% من العمال المعرضين للأبخرة الإسفلتية يعانون من أعراض تسمم جلدية مثل الحمامي والحكة وتسحجات والتهاب مزمن وتقران كيميائي و ورم شائكي متقرن، كما تبين إصابة 7.38% من العمال المعرضين للأبخرة بسرطان الخلايا الحرشفية. بالفحص الخلوي المناعي وجد زيادة ذات دلالة إحصائية للنوع البري لبروتين بي 53 بالمجموعة المتعرضة لأبخرة الإسفلت مقارنة بالمجموعة الضابطة. أما حالات سرطان الجلد وجد بها ارتفاع ذو دلالة إحصائية للنوع الطفري لبروتين بي 53 مقارنة بالمجموعة الضابطة. كما وجدت زيادة ذات دلالة إحصائية في نسبة بروتين باكس في العمال المعرضين لأبخرة الإسفلت مقارنة بالمجموعة الضابطة. كما وجد انخفاض ذو دلالة إحصائية في نمط التعبير لبروتين بي سي ال 2 في كل العمال المعرضين مقارنة بالمجموعة الضابطة. في حين لم يتواجد أي نمط للتعبير عن بروتين بي سي ال 2 بأي من حالات سرطان الخلايا الحرشفية. لذا فقد خلصت الدراسة إلى أن التعرض المزمن لأبخرة الإسفلت قد يؤدي إلى زيادة مخاطر حدوث التسمم الجلدي و/أو نمو الأورام السرطانية عن طريق اختلال بروتين بي 53 و باكس و بي سي ال 2.

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