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Modulation of Epigenetic Profiles in Traffic Workers Exposed to Car Fumes in Egypt

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Abstract

Air pollution is one of the most crucial public health concerns over the globe. Car fumes are major component of the pollution burden, and thought to modulate the methylation landscape of the exposed people. In the present investigation, 199 blood samples were collected from male traffic workers belonging to the Traffic Department, Ministry of Interior in Egypt. Global DNA methylation was quantified using real time PCR, and the obtained results indicated a hypermethylation in the outdoor group compared to the indoor group. Transforming growth factor (*TGF*-β) gene expression was assessed using real time PCR as an indicator for the transformation of cells from the normal to the malignant state. Data indicated that the outdoor group had an elevated level of *TGF*-β expression as normalized by β actin compared to the indoor group. Blood glucose was also reported an increased level in the outdoor compared to the indoor group. These data could be considered as a preliminary study to a larger one to underlie the risk of exposure to car fumes on traffic workers, especially the outdoor workers.

Keywords: Air pollution; Epigenetics; Methylation; *TGF-β;* Car Fumes; Egypt

Introduction

Environmental air pollution is one of the most serious public health problems throughout the world [1]. About 7 million people die prematurely every year as a result of air pollution [2]. A vast array of studies have indicated a strong link between exposure to air pollution and human morbidity and mortality [3]. Ambient air pollution caused 6% of total mortality, or more than 40,000 attributable cases, per year worldwide [4]. Increased number of cars raises a new challenge; Traffic-related Air Pollution (TRAP), which has been considered as the most important source of air pollution in urban areas [5]. TRAP is a complex mixture of gases (including NO_2, SO_2, O_3) and Particulate Matters (PMs) with different aerodynamic equivalent diameters, which could access the respiratory system easily *via* inhalation, and affect human health directly and indirectly [6]. Exhaust fumes are extremely dangerous and can seriously injure and even kill people who are exposed [7].

Recent epidemiological studies have shown that ambient air pollution exposure is associated with increased mortality and higher incidence of several diseases such as asthma, chronic obstructive pulmonary disease, neurologic disease, and cancer [8,9].

The ever-changing environment has great effects on human health both at genetic and epigenetic levels [10,11]. The epigenome represents the interface between the environment and the genome [12] , and it is frequently changed in response to the environmental conditions such as diet, exercise, disease and even aging [13,14]. Changes in epigenome might be of transient nature, where it has minor effects on the individual phenotype [15]. When it is inheritable, epigenetic changes might lead to increased disease susceptibility and progression [16,17]. Therefore, understanding the epigenetic changes induced by exposure to PM may provide an important tool for dissecting the association between PM exposure and cancer because of their carcinogenicity, cytotoxicity, embryotoxicity, genotoxicity, and reproductive toxicity [18-20].

Several studies have linked changes in DNA methylation with traffic-related air pollution, although the underlying mechanisms have not been explored [21-23].

Transforming Growth Factor beta (*TGF*-β) is a member of the transforming growth factor superfamily of cytokines [24]. It performs many cellular functions, including the control of cell growth, proliferation, differentiation, and apoptosis [25,26]. Furthermore, they do not always induce cellular transformation, and are not the only growth factors that induce cellular transformation [27]. Exposure to particle matter (as a main component in polluted air) increases the expression of *TGF*-β along with other genes such as fibronectin [28]. In addition, CeO_2 exposure resulted in increased level of expression of *TGF*-β [29].

Here in the present study, the changes in methylation profile of *TGF*-β gene has been profiled in cohort population of traffic workers in Egypt.

Materials and Methods

Sample collection

About 199 peripheral blood samples were collected from traffic workers belonging to the Ministry of Interior, Egypt upon written approval obtained from the Minister of Interior. Clear, comprehensive informed consent was obtained from every participant. Participants were classified according to the duration they spent out door or in door. Samples were collected in heparin-coated tubes and stored at -80°C until being used. Blood pressure was recorded for every participant. Samples were collected from different locations in Greater Cairo as shown in Figure 1, where traffic stations were located.

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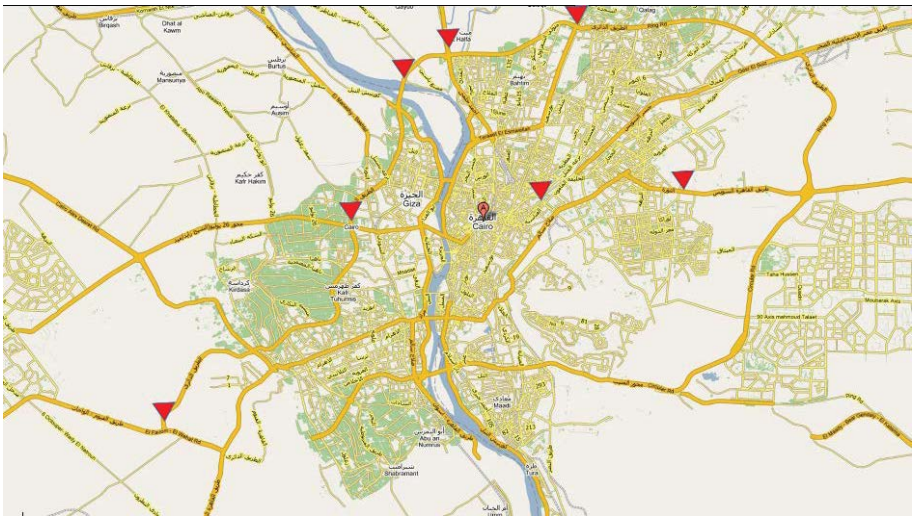

Figure 1: Greater Cairo map with the locations from which samples were collected

RNA extraction

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Germany).

cDNA synthesis

The extracted RNA was converted into cDNA using First Strand cDNA Synthesis Kit (Qiagen, Germany).

DNA extraction

Genomic DNA was extracted from all samples using QIAamp DNA Mini Kit (Qiagen, Germany). The procedures were performed according to the manufacturer protocol.

Target gene

Tumor growth factor was chosen for its function as preliminary warning system for the transformation of normal cells to malignant state. The primer sequences used in the present study are presented in Table 1.

Real time amplification

Normalize the primer concentrations and mix gene-specific forward and reverse primer pair. Each primer (forward or reverse) concentration in the mixture is 5 pmol/μL. The experiments were performed according to the following PCR program on ABI Step One Plus: 50°C for 2 min, 1 cycle as a pre-PCR step, followed by 95°C for 10 min, 1 cycle at 95°C for 15 s, 60°C for 30 s, 72°C for 30 s, 40 cycles, and 72°C 10 min, 1 cycle as a final extension step. All samples were performed in triplicates. Beta actin gene was used as internal control for normalization. Normalization was performed using the equation: $2^{-\Delta\Delta CT}$.

Diabetes measurements

In all blood samples obtained, the concentrations of glucoses were measured using OneTouch Verio Flex meter (USA) according to the manufacturer protocol.

Global methylation quantification

Real time PCR-based global 5-mC was performed using MethylQuant (Epigentek, USA). We followed the kit's instructions. All samples were performed in triplicates as recommended by the manufacturer.

Results and discussion

Sample categorization

Samples were categorized based on the duration spent out door or in door. A total of 199 samples were collected from outdoor and indoor male traffic worker participants as shown in Table 2. Average age was 34.9 ± 9.14 years as shown in Table 3, and average duration was 10.2 ± 6.6 years as shown in Table 4. Participants with history of any malignancies were excluded from the study. In addition, participants with any chronic illnesses that require long-term medications were also excluded. Blood glucose level were recorded for each participant as shown in Figure 2.

	Forward (5-3)	Reverse (5-3)
TGF- β	CATCCATGACATGAACCGACCCTT	ACGAAGTTGGCATGGTAGCCCTT
B-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG

Table 1: The primer sequences used in the present study.

In or outdoor	Frequency	Percent	Mean	Std. Deviation
Indoor	63	31.70%	1.78	0.604
Outdoor	117	58.80%		
Indoor and outdoor	19	9.50%		
Total	199	100.00%		

Table 2: Studied population characteristics according to their field of work (N=199).

Age	Frequency	Percent	Mean	Std. Deviation
from 20 to 30 years old	55	27.60%	34.93	9.146
from 31 to 35 years old	32	16.10%		
from 36 to 40 years old	56	28.10%		
from 41 to 45 years old	38	19.10%		
more than 45 years	18	9.00%		
Total	199	100.00%		

Table 3: Age Characteristics of the studies population (N=199).

Duration of exposure	Frequency	Percent	Mean	Std. Deviation
Less than 5 years	62	31.20%	10.2	6.604
from 5 to 10 years	22	11.10%		
from 11 to 15 years	71	35.70%		
more than 15 years	33	16.60%		
Total	188	94.50%		
Missing	11	5.50%		
Total	199	100.00%		

Table 4: Duration of exposure to car fumes outdoor (N=188).

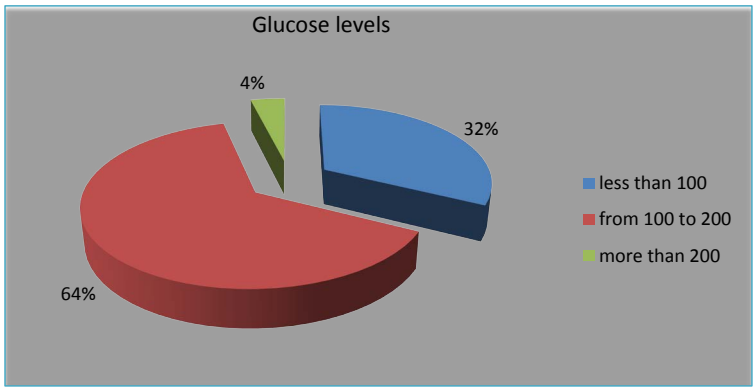

Figure 2: Blood glucose levels for participants (mg/dL).

Diabetes as an indicator

Air pollution, the most pervasive environmental concern, is estimated to cause around 800,000 deaths every year worldwide [30,31]. Our results indicated that the outdoor group have suffered from higher levels of blood glucose compared to indoor group as shown in Figures 2 and 3. Cases with blood glucose level higher than 200 mg/dL were detected in the outdoor group but not in the indoor group. It has been indicted earlier that air pollution could be responsible for 3.2 million new cases of type 2 diabetes every year globally [30,32,33]. Several studies have indicated that a 10 $\mu g/m^3$ increase in PM2·5 was associated with increased risk of diabetes [22,23,34].

Gene expression analysis

Transforming growth factor beta (*TGF*-β) gene is a multifunctional cytokine that plays crucial roles from modulation of immune-surveillance to angiogenesis [35-37]. Several literature indicated the correlation between tumor suppressor gene expression and car fumes [35,38]. Data obtained indicated that *TGF*-β gene was upregulated in outdoor group compared to indoor group as shown in Figure 4. This might be attributed to the chronic exposure to car fumes as indicated elsewhere [35,39]. Some cases reported an increase in the fold change as normalized against beta actin in indoor group, and this might refer to an early transformation of their cells from the normal to malignant state, taken in consideration that this indoor group had served outdoor for some time during the past 10 years. *TGF*-β elevated expression was detected in several types of cancers.

Methylation quantification

Global methylation was quantified in the genomes of selected participants (23 participants) as shown in Figures 5a-5d using real time PCR. Participants were originally categorized into outdoor group, who spent more than 10 years of working in the field (outdoor) and indoor group, who spent more than 10 years working indoor. Results obtained indicated that indoor group has lower concentration of global 5-methylCytosine (5-mC). However, a lower concentration of 5-mC was also obtained in outdoor cases, which might indicated an individual variation rather that being a trend in this cohort group [1,36,40]. The lower concentrations of 5-mC in indoor group could be attributed to the lower levels of car fumes they exposed to, and this might resemble, in turn, the normal level of genome methylation in the total population [20,41,42]. The relatively elevated level of 5-mC in the genomes of outdoor group might be due to the prolonged periods of exposure to car exhaust, as several researches indicated that chronic exposure to car fumes results in changes in the methylation landscape either globally or on loci-specific fashion [5,41].

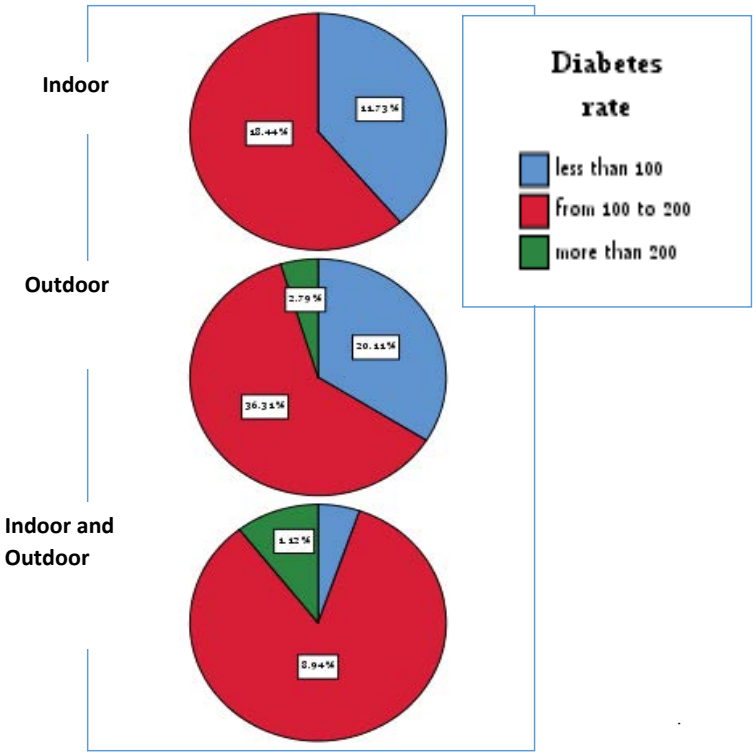

Figure 3: Prevalence of blood glucose levels in the Outdoor and Indoor groups

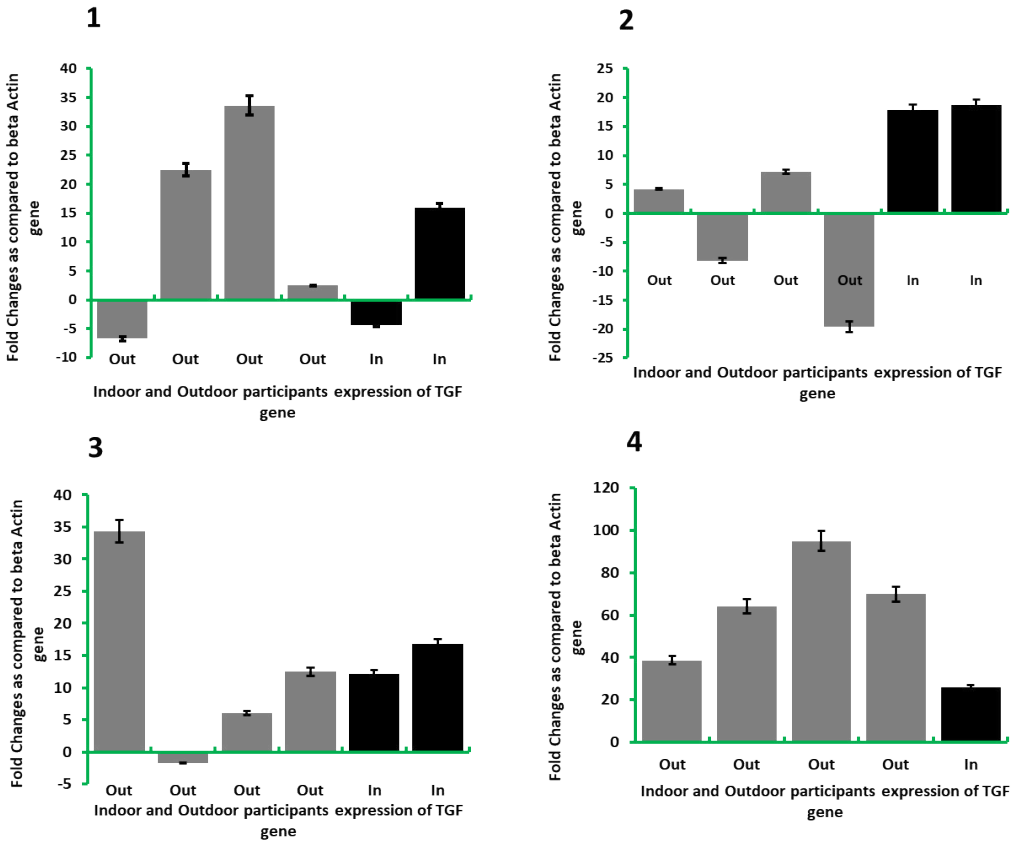

Figure 4: Real time PCR-based gene expression analysis of *TGF*-β for indoor and outdoor participants.

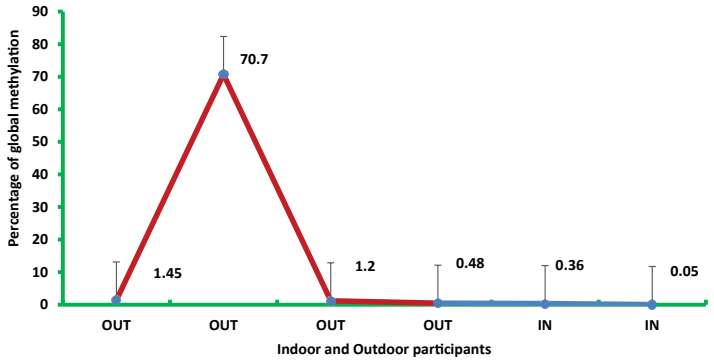

Figure 5a: Real time PCR-based global DNA methylation quantification in indoor and outdoor participants (OUT: Outdoor participants, and IN: Indoor participants).

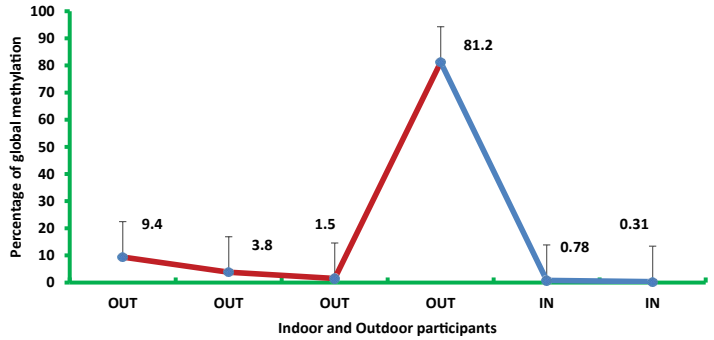

Figure 5b: Real time PCR-based global DNA methylation quantification in indoor and outdoor participants (OUT: Outdoor participants, and IN: Indoor participants).

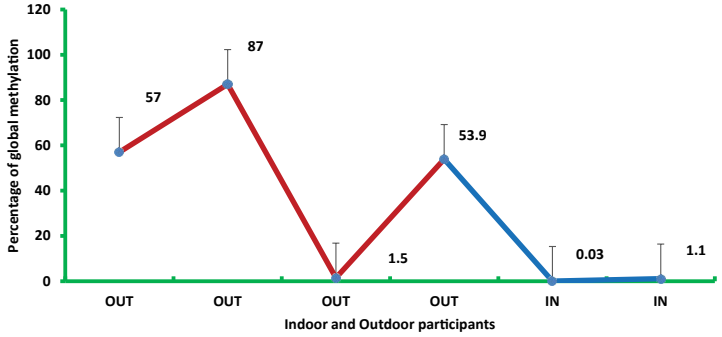

Figure 5c: Real time PCR-based global DNA methylation quantification in indoor and outdoor participants (OUT: Outdoor participants, and IN: Indoor participants).

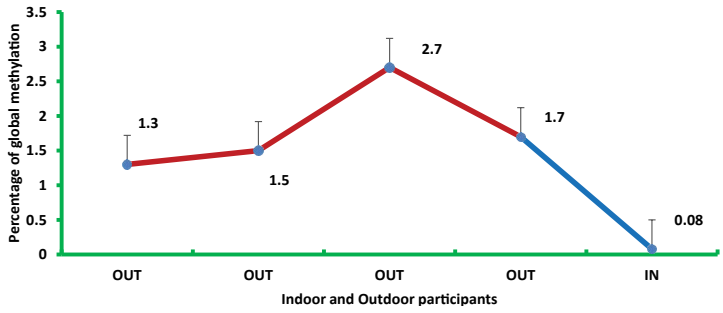

Figure 5d: Real time PCR-based global DNA methylation quantification in indoor and outdoor participants (OUT: Outdoor participants, and IN: Indoor participants).

Conclusion

In the present study, blood samples were collected from male traffic workers in Egypt with clear informed consent being obtained. Blood glucose level were recorded for each participant. RNA was extracted and converted to cDNA and subjected real time PCR to assess the level of *TGF*-β gene expression as normalized with beta actin. Results indicated that *TGF*-β was up regulated generally in the outdoor group compared to the indoor group. Meanwhile, global DNA methylation was quantified using real time PCR-based technique. Data obtained showed elevated level of 5-mC in the outdoor group compared to the indoor group. These data could be consisted as preliminary to conduct a more intensive, large-scaled study to highlight the risks of developing tumors in traffic workers in Egypt, especially who spend more than 10 years working outdoors.

Conflict of interest

The authors declare no conflict of interests.

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