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 NO₂, SO₂, O₃) 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]. 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, CeO₂ 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 outdoors or in doors. Samples were collected in heparin-coated tubes and stored at -80°C until being used. Blood pressure was recorded for each participant. Samples were collected from different locations in Greater Cairo as shown in Figure 1, where traffic stations were located.

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Received November 21, 2018; Accepted December 04, 2018; Published December 13, 2018

Citation: Shaat R, Abdel-Ghany SE, Nasr GM, Sabit H, El-Zawahry M (2019) Modulation of Epigenetic Profiles in Traffic Workers Exposed to Car Fumes in Egypt. Mol Biol 8: 225. doi: 10.4172/2168-9547.1000225

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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.

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

Table 2: Studied population characteristics according to their field of work (N=199).
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 μg/m³ 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 levels 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.

Acknowledgments

The authors thank the Minister of Interior and the Officers of Traffic department in the Ministry of Interior for cooperation with providing samples.

References

1. Cui JY, Li CY (2018) Epigenetics in toxicology in: McQueen C (ed.) Comprehensive Toxicology (Third Edition). Oxford: Elsevier.
2. Delport B, Mariet AS, Blanc C, Bejot Y, Riroud M, et al. (2018) Environmental Air Pollution: An Emerging Risk Factor for Stroke. In: Vasan RS and Sawyer DB (eds.) Encyclopedia of Cardiovascular Research and Medicine. Oxford: Elsevier.
3. Jeffries MA (2018) Epigenetic editing: How cutting-edge targeted epigenetic modification might provide novel avenues for autoimmune disease therapy. Clin Immunol 152:61-66: 30015-30041.
4. Finch CE (2018) The Three Smokes in Global Mortality. In: Finch CE (ed.) The Role of Global Air Pollution in Aging and Disease. Academic Press.
5. Ding R, Jin Y, Liu X, Zhu Z, Zhang Y, et al. (2016) Characteristics of DNA methylation changes induced by traffic-related air pollution. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 796: 46-53.
6. Krauskopf J, Calmet F, van Veldhoven K, Chadeau-Hyam M, Sinharay R, et al. (2018) The human circulating miRNome reflects multiple organ disease risks in association with short-term exposure to traffic-related air pollution. Environ Int 113: 26-34.
7. lei X, Muscat JE, Zhang B, Sha X, Xiu G (2018) Differentially DNA methylation changes induced in vitro by traffic-derived nanoparticulate matter. Toxicol 395: 54-62.
8. Shahadim MS, Ab Mutalib NS, Latif MT, Greene CM, Hassan T (2018) Challenges and future direction of molecular research in air pollution-related lung cancers. Lung Cancer 118: 69-75.
9. Leclercq B, Platel A, Antherieu S, Allemann LY, Hardy EM, et al. (2017) Genetic and epigenetic alterations in normal and sensitive COPD-diseased human bronchial epithelial cells repeatedly exposed to air pollution-derived PM2.5. Environ Pollut 230: 163-177.
10. Provenzi L, Guida E, Montiroso R (2018) Preterm behavioral epigenetics: A systematic review. Neurosci Biobehav Rev 84: 262-271.
11. Garcia-Gomez A, Rodriguez-Utrera J, Ballestar E (2018) Epigenetic interplay between immune, stromal and cancer cells in the tumor microenvironment. Clin Immunol S1521-6616: 30015-30019.
12. Moses C1, Garcia-Bloj B, Harvey AR, Bancafort P (2018) Hallmarks of cancer: The CRISPR generation. Eur J Cancer 93: 10-18.
13. Khan MI, Rath S, Adhami VM, Mukhtar H (2018) Targeting epigenome with dietary nutrients in cancer: Current advances and future challenges. Pharmacol Res 129: 375-387.
14. Aslani S, Sobhani S, Gharibdoost F, Jamshidi A, Mahmoudi M (2018) Epigenetics and pathogenesis of systemic sclerosis: the ins and outs. Hum Immunol 79: 178-187.
15. Supic G, Zeljic K, Magic Z (2018) Epigenetic nutraceuticals in cancer treatment. In: Hoibian AM, Grunzezescu AM (eds.) Therapeutic Foods. Academic Press: 449-494.
16. Esteller M (2018) The Human Epigenome—Implications for the Understanding of Human Disease. In: Coleman WB and Tsonisalis GJ (eds.) Molecular Pathology (Second Edition). Academic Press. 165-182.
17. Munir MT, Ponce C, Powell CA, Taradarkar K, Yanagita T (2018) The contribution of cholesterol and epigenetic changes to the pathophysiology of breast cancer. J Steroid Biochem Mol Biol 183: 1-9.
18. Porcellini E, Lapirovitera N, Riefolo M, Ravaoli M, Garavaja I, et al. (2018) Epigenetic and epithelial changes in colorectal cancer: Diagnostic, prognostic, and treatment implications. Cancer Lett 419: 84-95.
19. Barrow TM (2018) Epigenetic epidemiology for cancer risk. In: Epigenetic Mechanisms in Cancer. Boston: Academic Press.
20. Clifford RL, Jones MJ, Macksaas JL, McElwen LM, Goodman SJ, et al. (2017) Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation. J Allergy Clin Immunol 139: 112-121.
21. Higgins SP, Tang Y, Higgins CE, Mian B, Zhang W (2018) TGF-beta1/p35 signaling in renal fibrogenesis. Cell Signal 43: 1-10.
22. Zeng Y, Shen Z, Gu W, Wu M (2018) Inhibition of hepatocellular carcinoma tumorigenesis by curcumin may be associated with CDKN1A and CTGF. Gene 651: 183-193.
23. Dai M, Al-Odaini AA, Arakelian A, Rabbani SA, Ali S, et al. (2012) A novel function for p21cip1 and acetyltransferase pCAF as critical transcriptional regulators of TGFbeta-mediated breast cancer cell migration and invasion. Breast Cancer Res 14: R127.
24. Zhang P, Zhao G, Ji L, Yin J, Lu L (2018) Knockdown of survivin results in inhibition of epithelial to mesenchymal transition in retinal pigment epithelial cells by attenuating the TGFbeta pathway. Biochem Biophys Res Commun 498: 573-578.
25. Fang P, Xue Y, Zhang Y, Fan N, Ou L, et al. (2018) SIRT7 regulates the TGF-B1-induced proliferation and migration of mouse airway smooth muscle cells by modulating the expression of TGF-beta receptor I. Biomedicine & Pharmacotherapy 104: 781-787.
26. de Araujo Farias V, Carrillo-Gálvez AB, Martin F, Anderson P (2018) TGF-beta and mesenchymal stromal cells in regenerative medicine, autoimmunity and cancer. Cytokine Growth Factor Rev 43: 25-37.
27. Rodgers KM, Udesey JO, Rudel RA, Brody JG (2018) Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. Environ Res 160: 152-182.
28. Reis H, Reis C, Sharip A, Reis W, Zhao YS, et al. (2018) Diesel exhaust exposure, its multi-system effects, and the effect of new technology diesel exhaust. Environ Int 114: 252-265.
29. Bowe B, Xie Y, Li T, Yan Y, Xian H , et al. (2018) The 2016 global and national burden of diabetes mellitus attributable to PM2.5 air pollution. The Lancet Planetary Health 2: e301-e312.
30. Olova Alvarez HA, Kubzansky LD, Campen MJ, Slavich GM (2018). Early life stress, air pollution, inflammation, and disease: An integrative review and immunologic model of social-environmental adversity and lifespan health. Neurosci Biobehav Rev 92: 226-242.
31. Thiering E, Heinrich J (2015) Epidemiology of air pollution and diabetes. Trends Immunol S1521-6616: 30015-30041.
32. Clifford RL, Jones MJ, Macksaas JL, McElwen LM, Goodman SJ, et al. (2017) Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation. J Allergy Clin Immunol 139: 112-121.
33. Higgins SP, Tang Y, Higgins CE, Mian B, Zhang W (2018) TGF-beta1/p35 signaling in renal fibrogenesis. Cell Signal 43: 1-10.
34. de Araujo Farias V, Carrillo-Gálvez AB, Martin F, Anderson P (2018) TGF-beta and mesenchymal stromal cells in regenerative medicine, autoimmunity and cancer. Cytokine Growth Factor Rev 43: 25-37.
35. Rodgers KM, Udesey JO, Rudel RA, Brody JG (2018) Environmental chemicals and breast cancer: An updated review of epidemiological literature informed by biological mechanisms. Environ Res 160: 152-182.
36. Reis H, Reis C, Sharip A, Reis W, Zhao YS, et al. (2018) Diesel exhaust exposure, its multi-system effects, and the effect of new technology diesel exhaust. Environ Int 114: 252-265.
37. Bowe B, Xie Y, Li T, Yan Y, Xian H , et al. (2018) The 2016 global and national burden of diabetes mellitus attributable to PM2.5 air pollution. The Lancet Planetary Health 2: e301-e312.
38. Olova Alvarez HA, Kubzansky LD, Campen MJ, Slavich GM (2018). Early life stress, air pollution, inflammation, and disease: An integrative review and immunologic model of social-environmental adversity and lifespan health. Neurosci Biobehav Rev 92: 226-242.
39. Thiering E, Heinrich J (2015) Epidemiology of air pollution and diabetes. Trends Immunol S1521-6616: 30015-30041.
37. Billet S, Landkocz Y, Martin PJ, Verdin A, ledoux F, et al. (2018) Chemical characterization of fine and ultrafine PM, direct and indirect genotoxicity of PM and their organic extracts on pulmonary cells. J Environ Sci.

38. Ros XR, Vermeulen L (2018) Turning Cold Tumors Hot by Blocking TGF-β. Trends in Cancer 4: 335-337.

39. Apparicio P, Gelb J, Carrier M, Mathieu ME, Kingham S (2018) Exposure to noise and air pollution by mode of transportation during rush hours in Montreal. J Transp Geog 70: 182-192.

40. Mccullough SD, Dhingra R, Fortin MC, Diaz-Sanchez D (2017) Air pollution and the epigenome: A model relationship for the exploration of toxicoepigenetics. Curr Opin Toxicol 6: 18-25.

41. Clifford RL, Jones MJ, MacIsaac JL, McEwen LM, Goodman SJ, et al. (2017) Inhalation of diesel exhaust and allergen alters human bronchial epithelium DNA methylation. J Allergy Clin Immunol 139: 112-121.

42. Ding R, Jin Y, Liu X, Zhu Z, Zhang Y, et al. (2016) Characteristics of DNA methylation changes induced by traffic-related air pollution. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 796: 46-53.