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HEALTH EFFECTS OF AIRBORNE PARTICULATE MATTERS (PM_{10}) DURING DUST STORM AND NON-DUST STORM CONDITIONS IN TEHRAN

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ABSTRACT:

Introduction: Air pollution is a serious health concern worldwide, accounting for high premature morbidity and mortality annually. In this article, we explain the framework of a series of interrelated researches for studying particulate matter (PM_{10}, PM <10 μm) health impacts from dust storm and non-dust storm conditions in Tehran, Iran.

Materials and methods: PM_{10} samples will be collected daily from two representative stations simultaneously during normal and dusty days by high volume samplers. PM chemical properties including ions, polycyclic aromatic hydrocarbons, metal (loid)s and oxidative potential will be analyzed. For in vitro biological toxicity, cytokine release from peripheral blood mononuclear cells and A549 cell line will be assayed. Also, DNA damage on A549 cell line will be examined using comet assay. In addition, DNA methylation of PBMCs and hemolysis of red blood cells (RBCs) will be accomplished. In a parallel panel study, after completing demographic and the International Study of Asthma and Allergies in Childhood (ISSAC) questionnaires, two groups of students will be selected. The exhaled breath condensate will be conducted to measure TNF-α, 8-IP, IL-6.

Conclusions: This study aimed to evaluate the components, oxidative potential and biological effects induced by PM. Our research will be provided information on the toxicity potential of PM related to dust storm and traffic to help policy makers to establish strategies and prioritize resource allocation in comprehensive plan for PM control.

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INTRODUCTION

Air pollution is a serious public health concern globally. Numerous studies have demonstrated a strong link between exposure to ambient air pollution and morbidity and mortality [1]. A broad range of particulate matter (PM) health outcomes is associated with chronic and acute exposure, including school absence, increasing hospital admission, lung dysfunction, chronic obstructive pulmonary disease (COPD), respiratory symptoms, cancer and death [2-4]. Man-made airborne PM can be disseminated from cars and factories but natural PM originated from natural sources such as dust storm which has become a major challenge in Iran over recent years [5]. Dust storm is a meteorological phenomenon generally in arid and semi-arid areas [6]. Iran is located in Southwest Asia affected by dust storm each year. People who live in western and central parts of Iran, including the metropolitan capital city Tehran, are generally exposed to high levels of dust storm PM [7]. In Iran most of the dust storms that affect western and central parts are coming from the neighbor countries such as Iraq, desert lands in the north and northeast of Arabian Peninsula, and east and southeast of Syria [8]. Air pollution from multiple man-made sources is intensified by high mountain chain around the city and the dominant wind direction [7].

Physical and chemical characterizations of PM that cause toxicity and health adverse effects are still not conclusively recognized. However, potential health problems may be due to organic constituents, metals and other chemically active compounds. These are the characteristics that determine how the body will react and how much oxidative stress will induce when the particles are deposited in the lungs [9, 10].

Oxidative potential (OP) has been suggested as a more health relevant PM metric than mass alone that measure the capacity of ambient particles to generate reactive oxygen species (ROS) [11, 12]. ROS as one of the most important effects caused by PM can lead adverse health effects. Particles components such as organic carbon, PAHs and quinones are involved in the formation of ROS. High levels of ROS may change the cell redox potential and lead to acute effects such as lung inflammation [11,13,14]. It has been assumed that PM has a capacity to bring out oxidative stress in lung followed by acute and chronic health effects on people who exposed to high concentrations of PM [3]. Researchers have introduced several chemical compounds, including PAHs, transition metals, water-soluble organic carbon (WSOC) and quinones that may participate in this reaction. However, a lack of general agreement on the relative contribution of these compounds in overall activity of DTT currently does not exist [11, 13, 14].

Recent studies have emphasized that the mechanisms of PM-induced health effects include inflammation and oxidative stress [15]. Although the mechanisms of ROS production are vague, many studies showed its role in inflammation causing subsequent effects both in vitro or in vivo [16, 17]. The collection of free radicals are superoxide, hydroxyl and hydrogen peroxide that can be produced during metabolic process or exposure to ambient air PM [16].

PM_{10} can cause ROS inside the macrophage [18]. Instant ROS production accomplishes after exposure to PM, whereas inflammation progresses during time. Thus, PM can cause either oxidative stress (ROS-mediated) or inflammation or both of them resulting in DNA oxidation damage as a secondary phenomenon [19]. Macrophages and lung epithelial cells exposed to PM secrete pro-inflammatory and inflammatory chemokine and cytokines like interleukin IL-1β, IL-6, TNFα and IL-8 as biomarkers [20].

Based on latest researches, exposure to air pollution specially PM can modify host peripheral blood DNA [21] associate with genomic instability and cancer, higher systolic, diastolic and mean arterial blood pressure and increased risk of stroke [22].

Several studies have investigated the health effects of PM, but in this study the health effects of PM from dust storm and non-dust sources will be compared using chemical assay and biological toxicity tests. The main objective is to characterize PM and investigate their oxidative potential (OP) and biological effects during dust storm and urban air pollution. Additionally, the results of the dust storm-based PM will be compared with the PM originated from non-dust conditions. In the first phase, PM is sampled to measure OP, metals and metal (loid)s, water soluble ions and PAHs. Then, biological toxicity or health impacts of PM is assessed as in vitro and panel study by measuring biomarkers of oxidative stress and pro-inflammatory cytokines from Peripheral Blood Mononuclear Cells (PBMCs) and cell line, DNA damage in cell line, DNA methylation of PBMCs and RBCs hemolysis by individual sampling of blood and exhaled breath condensate (EBC). Finally, the association between PM components and observed biological effects will be differentiated during dust storm and non-dust air pollution. The results of this study will help the researchers to answer the question whether the difference between characteristics and the effects of particulate matter from dust storm and traffic are there. Also it will give some evidences about the different biological effects of them.

MATERIALS AND METHODS

Study area, sampling sites and schedule

In order to provide representative samples of dust and urban condition, two locations were chosen. Sampling sites were selected according to Environmental Protection Agency (EPA) criteria (i.e., more than 20 m away from trees and sources of pollution and 15 m distance from ground level) [23]. The samplers were placed on a roof at the height of 10 m above the ground level, without any obstacle to minimize the potential effects of natural and anthropogenic interferences on the air flow as well as PM concentration. PM_{10} samples will be collected from two sites simultaneously during different weather conditions on both normal and dusty days, using two PM_{10} high volume samplers (Grasebey Company) equipped with fiberglass filters (8×10 inch, grade G 653 Whatman, USA). Sampling was done every day during spring and autumn in 2016. The first sampler is placed on roof of the health school of TUMS located in the central part of Tehran metropolitan (urban area) with heavy traffic (35˚42′ 24. 63″ N, 51˚23′ 40.09″ E) and the second sampler is placed in a rural countryside (Raziabad), situated in the southwestern of Tehran metropolitan (35˚36′ 24, 35″ N, 51˚ 12′ 17.58″ E) (Fig.1). At the sampling time, local weather information will be received from the Meteorological Organization of Iran.

The sampler will be operated with a flow rate of 1.3-1.7 m^3/min during 24 h. However, the exact flow rate was calculated daily and will be corrected for variations in atmospheric pressure and actual differential pressure. The filters were pre- and post-conditioned in a dry and dark place for 48 h and weighed with an analytical balance (±0.1 mg) before and after sampling to calculate the PM mass trapped on the filter. For Quality Assurance and Quality Control (QA/QC), laboratory and field blank filters along with samples will be analyzed. Every sample will be cut into specific fractions and will be analyzed for determination of PAHs, metals, water soluble ions, OP and biological properties.

PM chemical analysis

In the present work, we investigated the characteristics of every fiberglass filter to determine water soluble ions, metal (loid)s, and polycyclic aromatic hydrocarbons (PAHs) in PM_{10} during dust storm and non-dust storm conditions at two sampling sites. Water soluble ions, metal (loid) s and PAHs will be analyzed by Ion Chromatograph (IC; model: Metrohm 8580), an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; model: Arcos), and a Gas Chromatography/Mass Spectrometry (GC/MS; model: 3800-GC Varian, USA), respectively.

OP of PM_{10} will be determined by Dithiothreitol (DTT) assay. In this method, 100 µm DTT and 1ml potassium phosphate buffer at pH 7.4 are added to a PM sample with 5-40 µg/L concentrations. Then sample will be incubated at 37˚C for 5-45 min at predetermined time (5, 15, 25, 35, 45 min). Finally 1ml of 10% thrichloro acetic acid will be added to 100µL of incubation mixture in order to stop reaction. Then 1ml of 0.4M Tris buffer, 20 m MEDTA, pH 8.9 and 50µL DTNB 10 mm will be added to incubation mixture. The concentration will be measured by spectrophotometer DR-5000 at 412 nm. The reaction rate that represents the consumption of DTT during the time is proportional to the concentration ROS of PM.

DNA damage evaluation

For DNA damage, we will use A549 cell line as a human epithelial lung cell model. Cells were plated in 24-well plates (80000 cells/well) and exposed to different concentrations of PM10 in a final volume of 1 ml. After passing 24 h, DNA degradation in A549 cell line will be analyzed by single cell electrophoresis or comet assay. After capturing the image, the length of each comet will be measured using an image analysis system [24].

Cytokine assay

For biological tests it will be necessary to pool similar daily filters to obtain enough PM_{10} mass. In Cytokine assay, we will use A549 cell line and

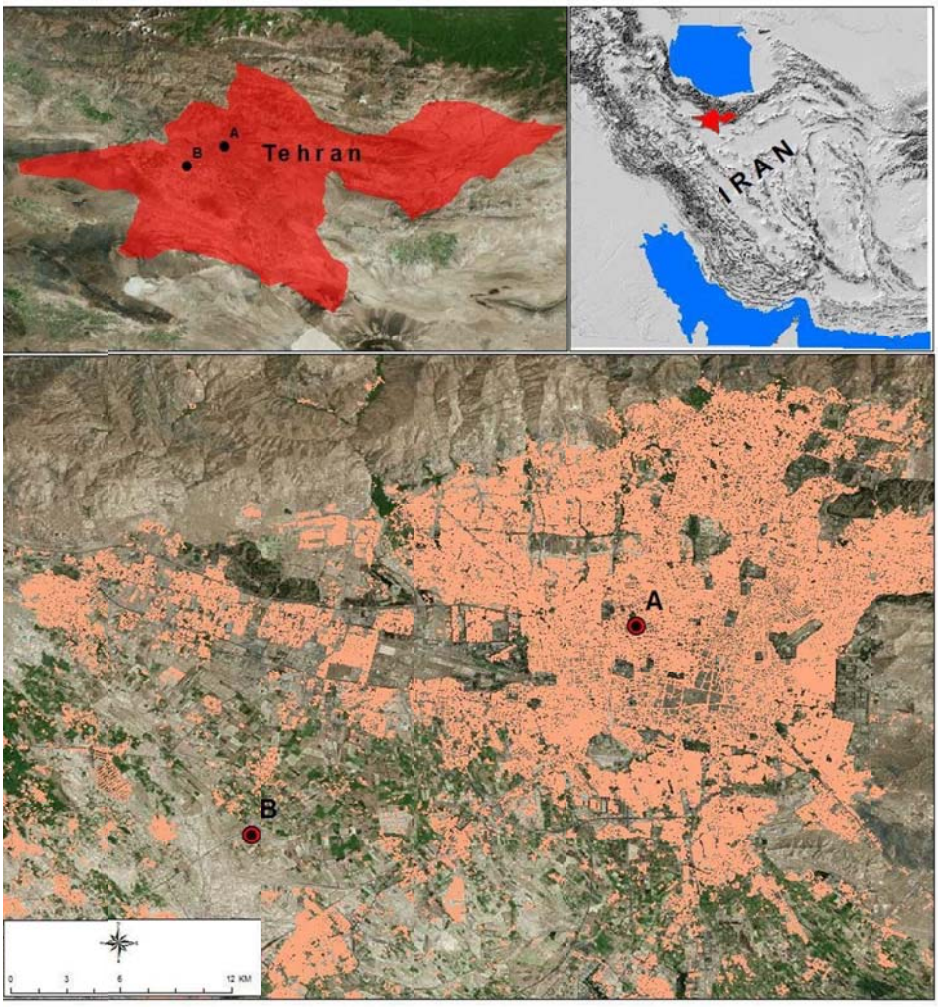

Fig.1. Location of sampling areas (A: urban sampling site, B: rural sampling site)

PBMCs. Peripheral blood will be obtained from 10 healthy donors and mononuclear cells isolated by standard protocol suspended in RPMI-1640. Three different concentrations of PM_{10} will be exposed to certain amount of PBMC suspension and A549 cell line at 37˚C in 96-well plates. Selected cells will be exposed to PM_{10} for specific time and incubated at 37°C, 5% CO_2 in humidified air, for 24 h. Then, supernatant aliquots will be removed for quantifying the Tumor Necrosis Factor-alpha (TNF-α), IL-1β and Interleukin-6 (IL-6) by cytokine ELISA assay. Therefore, cell supernatants were collected, centrifuged at 14,000 × g for 15 min, and frozen at –70˚C. The inflammatory mediators including, IL-6, IL-1β and TNF-α, will be measured with ELISA kit according to manufacturer's directions. Non-exposed cells were used as a negative control group and those cells exposed to 10 μg/mL LPS from Escherichia coli as positive control which stimulates pro-inflammatory cytokine production. All experiments will be repeated at least three times and results will be expressed as mean ± standard deviation. Also, association between biological effects and PM_{10} constituents (PAHs, ions and metal and metal (loide)s and OP will be analyzed.

DNA methylation and hemolysis

5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) percent will be measured by specific ELISA before and after exposure. Firstly, three different concentrations of PM_{10} will be exposed to certain amount of PBMCs suspension for proper exposure time based on set up experiments. Secondly, total DNA will be extracted using the DNA Purification Kit and will be stored at -80˚C. Then, one hundred nano grams of genomic DNA in each reaction will be used to measure global 5hmC and 5mC by specific ELISA kits according the manufacturer's protocols. All experiments will be run in duplicate. Results will be expressed as the percent of 5-methylated cytosine (%5mc) or the percent of 5-hydroxymethylated cytosine (%5hmc).

For hemolysis study, human red blood cells (RBCs) will be isolated from the healthy donor's whole blood by centrifugation. The cells will be washed three times with an isotonic solution of potassium chloride (KCl) and Tris buffer at pH 7.4. The released hemoglobin in the cell suspension supernatant will be quantified with a spectrophotometer at 540 nm before and after RBCs exposure to PM_{10}. The results will be expressed as hemolysis percent in relation to the hemoglobin released with the same amount of RBCs from each donor incubated with double-distilled water.

Exhaled breath condensate biomarkers

After completing demographic and ISSAC questionnaires (The International Study of Asthma and Allergies in Childhood), two groups of students (healthy and asthma cases) will be classified according to physical examination by a pulmonologist and the results of spirometers. A week before the EBC and blood sampling, concentrations of ambient PM_{10} will be measured in the study area using a high volume sampler and a portable GRIMM dust monitor. The EBC and blood sampling will be accomplished 4 times (2 times in normal concentration of air pollution in different seasons and 2 times during dust storm). EBC and blood samples will be immediately transported to the laboratory and stored at −80◦C until analysis. Tumor Necrosis Factor-alpha (TNF-α), 8-isoprostane (8-IP) and Interlukin-6 (IL-6) concentrations will be measured in EBC samples. According to the results, the relationship between biomarkers and concentration of ambient PM_{10} will be specified in normal and dust storms conditions. Also, the association between biomarkers and lung function indices (FVC, FEV_1, FEV_1 / FVC and FEF_{25-75}) will statistically be analyzed between two groups.

DISCUSSION

This is the first comprehensive study which will be done on the biological effects and chemical composition of PM originated from anthropogenic and natural sources in Tehran, Iran. Since the majority of national studies are limited to

measure the PM concentration, current study will be a new perspective on the potential effects of particles through OP and in-vitro cytotoxicity. We will develop in vitro models focusing on several biological effects of PM of dust storm and non-dust air pollution. Thus, we investigate the relationship between biological effects and PM with different compositions and origins. Study on PM temporal and spatial origin, components and composition, can help identify different biological effects. There are numerous studies investigated the health effects of air pollution throughout the world. Some researchers showed various health effects of particles including ROS production, inflammation biomarkers, DNA strand breaks and oxidatively damaged nucleobases in vitro and in vivo [19]. In a research, an increase was shown in TNF-α release of PBMCs [25]. Generally, there are a number of studies that have assessed diverse kinds of inflammatory cytokines in different blood cultured cells in Germany [26], Netherland [27], Switzerland [28] and Saudi Arabia [29]. It was investigated in few studies in Iran that air pollution biomarkers of DNA damage, vascular injury, cytokine profiles and lymphocyte immunophenotypes, respectively [30-32].

Moreover, several scientists have studied global and gene specific methylation induced by airborne PM, but as far as the authors know, there is no research in the world about PM originated from dust storm on methylation and its comparison with non-dust air pollution.

Searching multiple databases showed that methylation has not been studied neither during dust storm nor non-dust air pollution in Iran. Also, RBCs hemolysis that originates from PM during dust storm and its comparison with non-dust air pollution based on PM components have not been assessed in Asia. Some researchers measured DNA global 5mC and 5hmC by ELISA in the blood of 60 truck drivers and 60 office workers in Beijing, China in 2008. They evaluated the effects of ambient PM_{10} and personal $PM_{2.5}$ and its elemental components on blood global 5mC and 5hmC levels. An increase in PM_{10} was associated with an increase in 5hmC of 26.1% of office workers ($P = 0.004$), 20.2% in truck drivers ($P = 0.014$), and 21.9% in all participants combined ($P < 0.001$). The authors proposed that exposure to ambient PM_{10} affects 5hmC over time, but not 5mC [33]. In 2011, it was also assessed changes in DNA methylation associated with $PM_{2.5}$, black carbon (BC) and sulfates (SO_4) in 1,406 blood samples from 706 elderly participants in the USA [34]. In two separate studies (2011), the association between hemolysis, elemental compositions and OP of PM during dust storm in Mexico was evaluated. In one on the above mentioned researches it was concluded that hemolysis predominately induced by semi-urban PM_{10} ($P<0.05$) in comparison with urban-$PM_{2.5}$, urban-PM_{10} and semi-urban $PM_{2.5}$. Moreover, they reported stronger effects produced by PM_{10}. PM samples from semi-urban site presented lower concentrations of Cu and Zn, suggesting the influence of emissions from natural sources such as dust storm [35]. Additionally, in another study it was surveyed the effects of intermittent exercise in the polluted and clean air with air quality index (AQI) equal to 118 and 77, respectively on RBCs hemolysis in 10 endurance runners in Iran. Consequently, they found that an interval training session in high concentrations of air pollutants associated with more hemolysis of RBCs [36]. It was demonstrated in another study that local pollutants were important in the in vitro toxicity and OP [37]. There is some evidence that confirms the relationship between oxidative stress and biological effects [38]. Conversely, some studies demonstrated that oxidative stress has no important role in developing toxicity effects[39] and probably the result of multiple mechanisms caused by complex interaction between components such as metal synergism and organic participation can be a main reason for PM effects [40, 41].

Oxidative potential of PM correlated with DNA damage because of the presence of high level of metals such as Fe and Cu that are capable to generate a Fenton-type reaction [42-45]. Additionally, it was indicated in a research that three PM-

identified principal components could induce a defined cell response pattern. It was suggested that pro-inflammatory effects were the result of complex interactions, whereas cytotoxic effects were linked to S/K/Ca/Ti/Mn/Fe/Zn/Pb contents [39, 46].

But also a research carried out on the induction of IL-6 and inhibition of IL-8 secretion in the human air way cell line by the non-dust PM that indicated cytokine secretion induced by PM varies depending on the source through different mechanisms [47]. It has been proved that DNA breakage in epithelial cell line (A549) increased by PM due to seasonal variations both in summer and winter in Milan whereas it was found that winter PM mostly affected DNA breakage. Thus, the different seasons had different potency in DNA damage [48]. It has also been demonstrated that PM from different locations in a city or within different cities has different toxicity potential [43]. Some studies claimed that PM from combustion contain some compounds such as transition metals, PAHs and VOCs that generate ROS by various reactions that can release cytokine and DNA break and cellular damage [49].

As mentioned earlier, some studies showed significant relationship between the concentrations of air pollutants and EBC biomarkers; while some others did find a significant relationship between concentrations of ambient air pollutants and some of biomarkers. For example, in a study conducted in New Zealand, it was found that there is no significant correlation between effects of PM_{10}, $PM_{2.5}$ and PM_1 with oxidative stress in healthy or asthmatic students [50]. De Prins could not observe a link between 8-Isoprostane and various concentrations of $PM_{2.5}$ or PM_{10}; while BC exposure in the morning of sampling was associated with airway oxidative stress and 24-h and weekly exposures were linked with airway inflammation [51].

A study in Seoul compared health effects from dust storm and anthropogenic (local sources) components during the Asian dust storm (ADS) and measured peak expiratory flow rate (PEFR) in school children. It was showed that non-dust air pollution was affected by local pollution sources as well as by the ADS during the study period. Analysis of metals bound to the PM showed that natural metal concentrations were much higher than the anthropogenic metals. They found that ambient concentrations of $PM_{2.5}$ and PM_{10} were not significantly associated with PEFR in school children, except for asthmatics during the study period. Also, most of the metal concentrations bound to the particulates were significantly associated with decrease of the children's PEFR. They found that the effect of anthropogenic metals was not different from natural components of metals for reduction of PEFR. Exposure to the metals bound to particles during the ADS period reduces children's pulmonary function, but there was not a difference between the potency of anthropogenic and natural metal components for decreased pulmonary function [52].

According to a study in Japan, it was concluded that there was no association between the daily concentration of sand dust particles and air pollution aerosols, while both sand dust particles and air pollution aerosols had a significant association with suspended particulate matter (SPM) and $PM_{2.5}$. They showed that exposure to sand dust emission may intensify pulmonary disorders in children in East Asia [53].

Our study has some limitations. First, this study depends on the weather conditions which can affect sampling days and collection of adequate samples during dust storm compared with non-dust air pollution. This comprehensive project partly relies on importing standard solutions, culture media, kits and equipment from abroad that may be time-consuming.

CONCLUSIONS

Comparative evaluation of the components, OP and biological effects of PM during dust storm and non-dust air pollution, as one of the most important achievements of this study will help and inform policy makers in strategy establishment

and prioritizing resource allocation for PM control. This study will be a new perspective on the toxicity potential of particles. The result of this study provides valuable information on the toxicity potential of PM with different origins that can help to prevent biological adverse health effects in vulnerable groups of the society.

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COMPETING INTERESTS

All of the authors declare that they have no actual or potential personal or financial competing interests.

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ETHICAL CONSIDERATIONS

Informed consent will be obtained from research participants through a survey. Medical ethics committee of Tehran University of Medical Sciences approved this research protocol. Each participant will be asked to sign a written consent form. Also the results of the research will be published with the permission of the funders. Also All authors agree to submit their manuscript to JAPH. The authors confirm that the manuscript have not been submitted or published elsewhere in any language.

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