Short-term effects of particle size fractions on circulating biomarkers of inflammation in a panel of elderly subjects and healthy young adults*

Mohammad Sadegh Hassanvand a,b, Kazem Naddafi a,b, Homa Kashani c, Sasan Faridi b, Nino Kunzli d,e, Ramin Nabizadeh a,b, Fatemeh Momeniha f, Akbar Gholampour g, Mohammad Arhami h, Ahad Zare i, Zahra Pourpak i, Mohammad Hoseini j, Masud Yunesian k,l,*

a Center for Air Pollution Research (CAPR), Institute for Environmental Research (IER), Tehran University of Medical Sciences, Tehran, Iran
b Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
c Department of Research Methodology and Data Analysis, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran
d Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland
e University of Basel, Basel, Switzerland
f Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
g Department of Environmental Health Engineering, School of Public Health, Tabriz University of Medical Sciences, Tabriz, Iran
h Department of Civil Engineering, Sharif University of Technology, Tehran, Iran
i Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran
j Department of Environmental Health Engineering, School of Public Health, Shiraz University of Medical Sciences, Fars, Iran

ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form 27 January 2017
Accepted 1 February 2017
Available online 10 February 2017

Keywords:
Particulate matter
Circulating biomarkers
Elderly panel
Healthy young panel

ABSTRACT

Systemic inflammation biomarkers have been associated with risk of cardiovascular morbidity and mortality. We aimed to clarify associations of acute exposure to particulate matter (PM10 (PM < 10 μm), PM2.5-10 (PM 2.5–10 μm), PM2.5 (PM < 2.5 μm), PM1-2.5 (PM 1–2.5 μm), and PM1 (PM < 1 μm)) with systemic inflammation using panels of elderly subjects and healthy young adults.

We followed a panel of 44 nonsmoking elderly subjects living in a retirement home and a panel of 40 healthy young adults living in a school dormitory in Tehran city, Iran from May 2012 to May 2013. Blood biomarkers were measured every 7–8 weeks and included white blood cells (WBC), high sensitive C-reactive protein (hsCRP), tumor necrosis factor-soluble receptor-II (sTNF-RII), interleukin-6 (IL-6), and von Willebrand factor (vWF). We measured hourly indoor and outdoor exposure to PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1 mass concentration to derive weighted averages of personal exposure based on simultaneously collected time-activity data. The random intercept linear mixed effects model was used for data analysis.

We observed significant positive associations for WBC and IL-6 with exposure to PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1; sTNF-RII with PM2.5, PM1-2.5, and PM1; hsCRP with PM2.5 and PM1; and vWF with PM10 and PM2.5-10. PM2.5, PM1-2.5, and PM1 mass concentration to derive weighted averages of personal exposure based on simultaneously collected time-activity data. The random intercept linear mixed effects model was used for data analysis.

We observed significant positive associations for WBC and IL-6 with exposure to PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1; sTNF-RII with PM2.5, PM1-2.5, and PM1; hsCRP with PM2.5 and PM1; and vWF with PM10 and PM2.5-10. PM2.5, and PM1-2.5 mass concentration in elderly subjects from the current-day and multiday averages. For healthy young adults, we found significant positive associations for WBC and IL-6 with exposure to PM10, PM2.5-10, PM2.5, and PM1-2.5, but not with PM1. The results showed that increase of hsCRP, sTNF-RII, and vWF were not significantly associated with any of the PM sizes investigated in the healthy young subjects.

Our results provided some evidence that short-term exposure to PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1 was associated with inflammation and coagulation blood markers, but associations were depended on PM size and also differed across the various time lag.

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1. Introduction

Air pollution is a major issue for the global community and has a wide range of adverse effects on human health (Rückerl et al., 2011). Numerous studies have shown that environmental exposure to particulate matter (PM) is associated with increased cardiovascular hospitalization and mortality (Cosselman et al., 2015; Pope III and Dockery, 2006). Although biological mechanisms related to the PM exposure and cardiovascular diseases have not been fully illustrated, toxicological studies have demonstrated that PM may induce inflammatory and oxidative stress and prothrombotic responses mediated through vascular endothelial cells, leukocytes, and platelets, with expression of inflammatory cytokines, cellular adhesion molecules, and coagulation factors (Brook et al., 2010).

PM10 (PM < 10 μm) deposited in lungs activate inflammation with 1) generation of pro-inflammatory cytokines by macrophages that have phagocytized coarse particles, and 2) induction of chemokine production by the pulmonary epithelium (Fujii et al., 2001; Valavanidis et al., 2008; Wang et al., 2015). The mediators released by alveolar macrophages can influence both local and systemic inflammatory responses (Brook et al., 2010; Cosselman et al., 2015; Newby et al., 2008). Some toxicological studies indicate that PM2.5, PM1-2.5, and PM1 would be associated with increased biomarkers of systemic inflammatory responses in the healthy young adults and the elderly subjects. To investigate these acute responses, we carried out a study involving repeated measurements of PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1 exposure and circulating biomarkers (WBC, hsCRP, TNF-RII, IL-6, and vWF) in a panel of elderly subjects living in a retirement home and of healthy young adults living in a school dormitory in Tehran, the capital of Iran. Tehran is the largest metropolitan area in western Asia. About 9 million residents of this city are regularly exposed to severe air pollution including smog episodes where schools were closed due to air pollution. Despite the significance of PM pollution in Tehran, there is little information on effects of PM on human health in this area.

2. Materials and methods

2.1. Study participants and design

The study design consisted in two parallel panel studies, one among healthy young adults and one among elderly subjects. The panel approach with repeated measurements comes with the advantage of each participant acting as his or her own control, thus, individual characteristics that do not vary over time will not confound the acute effect associations of interest. We recruited 44 non-smoking elderly volunteer subjects included men and women (>65 years of age) living in a retirement home and 40 healthy, non-smoking male high school students between 15 and 18 years of age living in a school dormitory in the city of Tehran who had consent to take part in the study.

Detailed information about the study sites can be found in our previous publications (Hassanvand et al., 2014, 2015). Briefly, the retirement home and school dormitory were located in central urban area of Tehran. The retirement home is located about 650 m away from a major freeway and the school dormitory was approximately 200 m away from a major freeway and 1.1 km away from the retirement home. Of 60 elderly volunteers, 10 were not eligible, 3 died, and 3 had insufficient biomarker data due to exclusions for frequent infections, leaving 44 subjects. Of 45 healthy young volunteers, 3 were not eligible, and 2 had insufficient biomarker data due to exclusions for frequent infections, leaving 40 subjects.

Between May 2012 to May 2013, the healthy young and the elderly participants were recruited to take part in six blood draws scheduled every seven to eight weeks on the same day of the week and the same time of the day (Wednesday afternoons between 13:00–15:00) to control day-of-week effects and circadian rhythm. Each participant contributed six blood draws (n = 240 (40 × 6) and 264 (44 × 6) total samples, respectively, for the healthy young and the elderly subjects). We chose longer time periods between the measurements to possibly increase the variability in PM concentrations which tend to show seasonal patterns. At each step of blood sampling, participants were visited by a physician and data on health status, medication use and disease was collected and subsequently venous blood samples were drawn. We did not take blood during times with acute infectious illnesses. Finally, we used participants with six complete blood samples.

All participants provided written informed consent prior to participating in the study. The Research Ethics Boards of Tehran University of Medical Sciences approved the study protocol.
2.2. Measurement of blood biomarkers

Venous blood samples were centrifuged at 4 °C for 15 min and plasma aliquots were immediately stored at −70 °C until tested. Five biomarkers were considered: high sensitive C-reactive protein (hsCRP), white blood cells (WBC), Interleukin-6 (IL-6), tumor necrosis factor-soluble receptor-II (sTNF-RII), and von Willebrand factor (vWF). IL-6, sTNF-RII, and vWF markers were analyzed with enzyme linked immunosorbent assay (Quantikine, R&D Systems) at Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences (Tehran, Iran). hsCRP and WBC were analyzed in an immunoturbidimetric method (Sentinel CRP Vario List No. 6K26-02) and WBC of whole blood was counted using an automatic hematological analyzer (CellDyn 4000, Abbott). All samples were analyzed in duplicate to ensure reproducibility.

2.3. PM measurement

Details of the PM measurement were described elsewhere (Hassanvand et al., 2014, 2015). Twenty-four-hour PM sampling was conducted in each sampling site. Simultaneous indoor and outdoor air sampling was measured in the school dormitory and retirement home. Measurements started always six days before each blood draw and ended after the health assessment. Real-time data collected by the GRIMM dust monitors were logged at 1-min intervals. The 24-h averages were calculated from the 6-day average exposure (0–95 h), the 5-day average (0–119 h), the 3-day average (0–72 h), the 4-day average (0–95 h), and the 2-day average (0–67 h), the 3-day average (0–143 h), and the 2-day average (0–47 h), the 3-day average (0–71 h), the 4-day average (0–95 h), the 5-day average (0–120 h), the 6-day average exposure (0–143 h).

2.4. Time spent indoors and outdoors

To ultimately estimate the individual exposure to PM as well as relative humidity and temperature, we added a time-budget survey (TBS) as a tool to estimate time spent by each individual in different microenvironments (Almeida-Silva et al., 2015). A data collection form was designed, which included information about environments (indoor/outdoor) where participants spend their time (hourly) in each environment. The data collection form was based on 5-min intervals and then this data converted to hourly data. We collected information about the exact amount of time (e.g. 5 min) spent in indoor or outdoor environments. The data collection forms were distributed among participants six days before blood sampling and collected at the end of each day. In the retirement home the collection data forms were completed with the help of the elderly’s nurses, and in the school dormitory the forms were completed by the participants. Finally, the time-budget data were used to derive for each participant the hourly pattern of time spent indoors and outdoors.

2.5. Exposure assessment

Daily average personal exposure was calculated for each participant by integrating the results obtained from the time-budget survey with the PM concentrations measured in indoor and outdoor environments.

We considered the first 24 h immediately before the blood draw up to 5 days before the blood sampling (lag 0: 0–23 h, lag 1: 24–47 h, lag 2: 48–71 h, lag 3: 72–95 h, lag 4: 96–119 h, lag 5: 120–143 h), and the 2-day average (0–47 h), the 3-day average (0–71 h), the 4-day average (0–95 h), the 5-day average (0–120 h), the 6-day average exposure (0–143 h).

2.6. Statistical analysis

Demographic and clinical characteristics of the study participants as well as the concentration of PM10, PM2.5, PM1-2.5, PM1, and PM2 were described through mean ± standard deviation (SD) or frequency (%), as appropriate. The single-pollutant linear mixed-effects regression model was used to assess the relationship between the PM exposure and blood biomarkers. Since the outcomes were measured repeatedly within-individuals every seven to eight weeks, and therefore each subject acted as his/her own control over time, we applied the random intercept term for each subject and fixed effect for each exposure variable in our models using PROC MIXED in SAS 9.2 software (SAS Institute, Cary, NC, USA). Also, only the effect of time-dependent covariates including temperature and relative humidity was adjusted as fixed effects because by this study design time-independent potential confounders between subjects were controlled and needed no adjustment. The analyses were performed separately for healthy young adults and elderly subjects.

To evaluate the delayed and cumulative effect of exposure, the concentration of particulate matters from 1 to 6 days before blood sampling as well as the multiday averages were considered. Residuals were examined for the deviations from linear mixed-model assumptions. Residuals for IL-6 both in the healthy young adults and the elderly showed skewed distribution. Moreover, sTNF-RII had a skewed distribution in the elderly. Hence, the logarithm of these variables was considered. Percent change in biomarker levels associated with interquartile range (IQR) change in exposure concentration (with 95% confidence interval [CI]) were reported and computed as $\frac{10^{(\beta*\text{IQR})}-1}{\beta}$*100% for log-transformed biomarkers and $\frac{10^{(\beta*\text{IQR})}-1}{\beta}$*100% for other biomarkers where $\beta$ represents the estimated regression coefficient.

The temporal correlation between all PM size fractions were computed with the Pearson correlation coefficients. Accordingly, PM1 and PM1-2.5 were almost uncorrelated. On the other hand, the results of the single-pollutant models revealed that PM2.5 was mainly associated with the studied biomarkers. Therefore, we ran two-pollutant linear mixed effects regression model for the components of PM2.5, including PM1 and PM1-2.5, to determine what size fraction has greater association with biomarkers adjusting for each other and also temperature and relative humidity. Because of a bit stronger associations for blood marker for the lag 0 exposure were seen for PM1 and PM1-2.5 in single pollutant models, the
results for lag 0 were presented. In all analyses, p-values less than 0.05 were considered as statistically significant.

3. Results

The mean age of the study participants for the elderly and healthy young adults were 75.4 and 16.2 years, respectively. Table 1 presents the summary information on blood markers and participants’ demographic and clinical characteristics. A total of 264 and 240 samples, respectively, for the elderly and the healthy young subjects for each blood markers were available for analyses.

3.1. PM mass concentrations

Table 2 displays the indoor and outdoor PM10, PM2.5-10, PM2.5, PM1.2.5, and PM1 mass concentrations in both the retirement home and the school dormitory. The highest PM10 concentration occurred in the retirement home during a dusty day in May, which was 360.0 \(\mu g/\text{m}^3\). The highest PM2.5 and PM1 levels occurred in December, which were 118.0 and 108.3 \(\mu g/\text{m}^3\), respectively, in the school dormitory. The results show that in the retirement home, the average indoor/outdoor ratio was 0.52, 0.50, 0.59, 0.71, and 0.54 for PM10, PM2.5-10, PM2.5, PM1.2.5, and PM1, respectively. Therefor in the retirement home, the ratio was lower than the unity for all PM size fractions. This indicates the significant mass of indoor PM in the retirement home originated from outdoor environment. In the school dormitory, the average indoor/outdoor ratio was 0.76, 1.21, 0.59, 1.07, and 0.45 for PM10, PM2.5-10, PM2.5, PM1.2.5, and PM1, respectively. The I/O ratios of PM2.5-10 and PM1.2.5 were higher than 1 in the school dormitory, suggesting an indoor sources for these PM. This could be attributed to pupils’ activity leading to the resuspension of PM2.5-10 and PM1.2.5 in the school dormitory. The results show that the elderly and healthy young adults spend 97.0 ± 0.4% and 90.1 ± 4.2%, respectively, of their time in indoor.

The descriptive statistics for distribution of the range of exposure within each subject, across the 6 days are shown in Table S1 (from Supplementary material). The distribution of the exposure range was calculated for each subject as the difference between the highest level of each PM size fraction experienced during the six periods (regardless of lag times) and the lowest value of the six periods. It is the within-subject range of exposure that determines the statistical power in panel studies with repeated measurements. Indeed, if a subject experienced the same level of pollution on all six blood withdrawal days it would not contribute informative data.

3.2. Associations with biomarkers

The relationships of PM with blood biomarkers are shown in Figs. 1–5. Fig. 1 presents the associations between IL-6 and PM mass concentrations. In the healthy young adults exposure to PM10, PM2.5-10, and PM1.2.5 was associated with increased IL-6 at nearly all lag times and with PM2.5 only at lag 1. IL-6 was not associated with exposure to PM1 nor PM2.5, while the association was negative for PM1 at lag 3-day-average. Except for all PM size fractions at lag 5 and PM1-2.5 at lag 4, strong positive associations were found between PM exposure and IL-6 in the elderly population. In addition the highest level of IL-6 were observed with PM2.5 86.9% (95% CI: 42.1, 144.0) and with PM1 190.3% (95% CI: 133.7, 260.5) for the healthy young adults and the elderly subjects, respectively.

Fig. 2 and Fig S1 (from Supplementary material) present the percent changes in blood biomarker of hsCRP per IQR increase in PM10, PM2.5-10, PM2.5, PM1.2.5, and PM1 mass concentrations for elderly subjects and healthy young adults (see IQR in Table S2 in Supplementary material). As shown in Fig. 2, exposure to PM was not associated with increased hsCRP in healthy young adults, while in the elderly subjects hsCRP increased with PM2.5

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Elderly (n = 44)</th>
<th>Healthy young adults (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%) or mean ± SD</td>
<td>N (%) or mean ± SD</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19 (43.2)</td>
<td>40 (100)</td>
</tr>
<tr>
<td>Female</td>
<td>25 (56.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.4 ± 5.8</td>
<td>16.2 ± 0.5</td>
</tr>
<tr>
<td>History of MI</td>
<td>14 (31.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Positive angiogram or stress test</td>
<td>14 (31.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hyper tension</td>
<td>13 (29.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>8 (18.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>8 (18.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>9 (20.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hay fever</td>
<td>1 (2.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>2 (4.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stroke</td>
<td>5 (11.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>12 (27.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>HMG CoA reductase inhibitors (statins)</td>
<td>10 (22.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>26 (59.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antihyperlipidemic medication</td>
<td>10 (22.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antihypertensive and cardiac medication</td>
<td>9 (20.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>4 (9.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Blood Samples</td>
<td>264</td>
<td>240</td>
</tr>
<tr>
<td>hsCRP (ng/ml)</td>
<td>16405 ± 956715</td>
<td>6842.73 ± 3106.86</td>
</tr>
<tr>
<td>WBC (k/\mu l)</td>
<td>6.43 ± 1.61</td>
<td>6.38 ± 1.45</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>18.59 ± 30.28</td>
<td>15.61 ± 26.32</td>
</tr>
<tr>
<td>sTNF-RII (pg/ml)</td>
<td>4828 ± 2664</td>
<td>1979.33 ± 846.51</td>
</tr>
<tr>
<td>vWF (ng/ml)</td>
<td>833 ± 351</td>
<td>1220.01 ± 451.21</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; n, number; MI, myocardial infarction; hsCRP, high sensitivity C-reactive protein; WBC, white blood cells; IL-6, interleukin-6; sTNF-RII, tumor necrosis factor-soluble receptor-II; vWF, von Willebrand factor.
19.4% (95% CI: 5.9, 32.8) at lag 0, and with PM1 17.3% (95% CI: 4.6, 30.1) at lag 0, 12.2% (95% CI: 1.6, 22.8) at lag 3, 5.6% (95% CI: 1.2, 10.0) at 2-day-ave., and nearly with 3-6 day average. The results show that exposure to PM10, PM2.5-10, and PM1-2.5 was not consistently associated with hsCRP in both elderly and healthy young adults, and finer particles were the most important PM with hsCRP. There was an unexpected inverse association of hsCRP with lag 5 PM10 in the elderly panel.

Fig. 3 shows the results for percent changes in WBC per an IQR increase in PM concentrations in single-pollutant models. In the healthy young panel exposure to PM10, PM2.5-10, and PM1-2.5 (coarse fractions) were associated with increased WBC at different lag times whereas PM2.5 and PM1 were not associated with WBC (lag 1). In healthy young adults the strongest associations of WBC were observed with PM2.5 17.6% (95% CI: 10.5, 25.2) at lag 1. In the elderly population, exposure to PM10, PM2.5-10, PM2.5, PM1-2.5, and PM1 was

### Table 2

Descriptive statistics for daily indoor and outdoor measurements of PM mass concentrations (in μg m⁻³), temperature (°C) and relative humidity (RH) (%) for the study period (May 2012 to May 2013).

<table>
<thead>
<tr>
<th>School dormitory</th>
<th>Outdoor (n = 132)</th>
<th>Indoor (n = 132)</th>
<th>Retirement home</th>
<th>Outdoor (n = 144)</th>
<th>Indoor (n = 144)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Min-Max</td>
<td>Mean ± SD</td>
<td>Min-Max</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>PM10</td>
<td>77.9 ± 28.7</td>
<td>25.9–162.0</td>
<td>56.2 ± 34.3</td>
<td>8.5–145.0</td>
<td>76.3 ± 27.3</td>
</tr>
<tr>
<td>PM2.5-10</td>
<td>45.9 ± 23.3</td>
<td>2.9–140.2</td>
<td>37.6 ± 25.3</td>
<td>3.5–117.2</td>
<td>47.6 ± 27.4</td>
</tr>
<tr>
<td>PM2.5</td>
<td>32.1 ± 18.8</td>
<td>10.0–118.0</td>
<td>18.6 ± 11.1</td>
<td>5.0–52.7</td>
<td>27.4 ± 14.1</td>
</tr>
<tr>
<td>PM1.2.5</td>
<td>7.9 ± 2.6</td>
<td>3.7–19.9</td>
<td>8.3 ± 5.4</td>
<td>1.5–26.7</td>
<td>8.1 ± 4.0</td>
</tr>
<tr>
<td>Temperature</td>
<td>20.5 ± 9.2</td>
<td>−0.3–38.2</td>
<td>26.5 ± 5.4</td>
<td>22.2–33.0</td>
<td>21.3 ± 9.6</td>
</tr>
<tr>
<td>RH</td>
<td>32.8 ± 16.4</td>
<td>9.0–91.6</td>
<td>29.9 ± 8.5</td>
<td>13.8–42.7</td>
<td>33.2 ± 17.7</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; Max, maximum; Min, minimum.

Fig. 1. Associations between Interleukin 6 (IL-6) and PM in single-pollutant model in the panel of healthy young adults (n = 40) (a) and the elderly panel (n = 44) (b). Estimated percent changes in the blood marker (adjusted coefficient and 95% CI) corresponds to an IQR increase in PM concentrations (see IQR in Table S2 in Supplementary material), adjusted for temperature and relative humidity.
Fig. 2. Associations between high sensitivity C-reactive protein (hsCRP) and PM in single-pollutant model in the panel of the elderly panel (n = 44). Estimated percent changes in the blood marker (adjusted coefficient and 95% CI) corresponds to an IQR increase in PM concentrations (see IQR in Table S2 in Supplementary material), adjusted for temperature and relative humidity.

Fig. 3. Associations between White blood cells (WBC) and PM in single-pollutant model in the panel of healthy young adults (n = 40) (a) and the elderly panel (n = 44) (b). Estimated percent changes in the blood marker (adjusted coefficient and 95% CI) corresponds to an IQR increase in PM concentrations (see IQR in Table S2 in Supplementary material), adjusted for temperature and relative humidity.
strongly associated with increase WBC, while that association was not observed at lag 5 for all of PM size fractions. In addition, PM10 and PM2.5-10 were not associated with WBC at lag 4. We found an unexpected inverse association of WBC with PM1 at lag 0, 2- and 3-day-average among the young.

Associations between exposure to PM mass concentrations and sTNF-RII are presented in Fig. 4 and Fig S2 (from Supplementary material). In health young adults, PM was not associated with sTNF-RII. Instead in the elderly, sTNF-RII was significantly associated with PM2.5, PM1, and a few lags of PM1-2.5 while findings were null for PM10, PM2.5-10, and most lags of PM1-2.5.

Fig. 5 and Fig S3 (from Supplementary material) show the associations between vWF and exposure to PM in the panel of the elderly and healthy young adults. In young adults vWF was not associated with any PM fraction. Among elderly subjects, vWF was weakly associated with PM1-2.5, PM2.5, PM2.5-10, and PM10 but not with PM1. We found an unexpected inverse association of vWF with PM1 and PM2.5 at lag 0 in the elderly panel.

3.3. Two pollutant models

Since our study panels are exposed to PM size fractions simultaneously during the study, their exposures to size fractions of PM can be considered as co-pollutants. Base on the correlation coefficients across all PM size fractions at lag 0, only PM1 and PM1-2.5 can be considered to be uncorrelated ($r = 0.028$). Moderate to high correlation is observed among other PM size fractions. For instance, PM2.5-10, PM2.5 and PM1-2.5 were highly correlated to PM10 ($r > 0.7$). PM10 showed only weak correlation with PM1 ($r = 0.177$). In addition, PM2.5-10 and PM1-2.5 had a high correlation coefficient of 0.802. Hence, we ran two-pollutant models for the components of PM2.5 (including PM1 and PM1-2.5) in the elderly panel.

Fig. S4 (from Supplementary material) presents an example of two-pollutant models, which shows the percent changes in hsCRP, WBC, IL-6, sTNF-RII, and vWF per interquartile increase in PM1 and PM1-2.5 adjusted for each other at lag 0 for the elderly subjects. With the exception of PM1 versus IL-6, results of the two-pollutant models remained mostly the same as in single pollutant analyses. In particular, the very strong associations of PM1-2.5 with IL-6 remained in both models. On the contrary, PM1 was strongly and significantly associated with IL-6 in two-pollutant models only. In the elderly subjects, our results in two-pollutant models showed that PM1 was more strongly associated with hsCRP and sTNF-RII
than PM_{1-2.5}. By contrast, PM_{1-2.5} had greater effects on WBC and IL-6 than PM_{1}.

4. Discussion

We investigated the association of blood biomarkers reflecting inflammation and coagulation with PM in the elderly subjects and healthy young adults. Associations were stronger in the elderly subjects with clear increases in hsCRP, WBC, IL-6, sTNF-RII, and weak for vWF with PM mass concentration. For the panel of healthy young adults blood biomarkers of WBC and IL-6 were increased in association with PM mass concentrations.

4.1. PM mass concentration

The Iranian national ambient PM standards are the same as WHO guidelines, which are 10 and 20 \(\mu g/m^3\) for annual average PM_{2.5} and PM_{10} concentrations, respectively. Currently, no guidelines or standard levels have been proposed for PM_{2.5-10}, PM_{1-2.5}, and PM_{1} levels. Our results demonstrate that in both the school dormitory and the retirement home, the annual indoor and outdoor PM_{10} and PM_{2.5} concentrations exceeded the ambient air quality guidelines issued by the WHO: Indoor and outdoor PM_{10} and PM_{2.5} mass concentrations were higher than those known from many studies to have adverse effects in humans (Karotki et al., 2015; Nazaroff and Goldstein, 2015). In Tehran, high levels of PM originate in particular from the more than three million gasoline- and diesel-fueled vehicles but regional dust is of relevance as well (Hassanvand et al., 2015). Our results showed that the maximum daily outdoor PM_{10} concentration in the retirement home reached 360.0 \(\mu g/m^3\). This high level of PM_{10} was measured during a dusty day which occurred on May 25, 2012 that had most likely originated from the Middle East. The results showed that in the retirement home the I/O ratio was below 1 for all PM size fractions. In the school dormitory, although this ratio was always below 1 for PM_{10}, PM_{2.5} and PM_{1}, in some occasions it surpassed 1 for PM_{2.5-10} and PM_{1-2.5} which indicates the existence of an important indoor PM_{2.5-10} and PM_{1-2.5} emitting source such as pupils’ activity. Across both study sites and indoors as well as outdoors, the average PM_{2.5-10}/PM_{10}, PM_{2.5}/PM_{10}, and PM_{1-2.5}/PM_{10} ratios ranged from 0.59 to 0.65, 0.33 to 0.42, and 0.25 to 0.42, respectively. This indicates that about 60% of PM_{10} studied in the present study were made up of coarse PM (PM_{2.5-10}). In addition, about 58–75% of fine particles studied in the present study were made up of submicron particles.

4.2. Biomarkers

In general, several studies support the hypothesis that exposure to PM can affect the cardiovascular system by 3 biological pathways (Brook et al., 2010). One of the pathways includes pulmonary oxidative stress and inflammation resulting in systemic oxidative stress and inflammation, indicated by increases in levels of cytokines, activated immune cells and platelets. A second pathway involves activation of lung autonomic nervous system resulting in autonomic nervous system imbalance (e.g., heart rate variability). A third pathway – conjectured in particular for the finest size fractions – includes translocation of PM or particle compositions directly into the systemic circulation. In this study, we assessed inflammation and coagulation blood markers.

Prior to discuss findings for each biomarker, let us emphasize a few general patterns. Our study confirms that claims of “particular health relevance” being related to any specific size fraction are not supported. Instead, the study provides further evidence that all size fractions result in acute changes, but that effects of various PM may have different time patterns and depend on personal characteristics. For example, effects were in general stronger and more consistent in the elderly than in the young adults. Or whereas in the young, larger particles appear to be more relevant for WBC than small ones, the opposite pattern is seen in the elderly. Whilst effects on hsCRP may be very immediate — with mostly lag 0 being of relevance, IL6 or WBC show a pattern of more sustained effects across several days.

IL-6 is an important stimulant of immune cells, and could represent important risk factors for coronary disease (Kritchevsky et al., 2005). Also, increased IL-6 levels have been associated with total mortality (Salvi et al., 1999). We found increase in IL-6 in association with PM_{10}, PM_{2.5-10}, PM_{2.5}, PM_{1-2.5}, and PM_{1} in the elderly subjects. In healthy young panel exposure to PM_{10}, PM_{2.5-10}, PM_{2.5}, and PM_{1-2.5} was associated with increase of IL-6 but not with PM_{1}. There are inconsistencies among the studies for the impact of PM on IL-6. Elevated IL-6 was found in healthy subjects (Räckerl et al., 2007) and in elderly panel (Delfino et al., 2009). Other previous studies reported no association between exposure to PM and IL-6 (Bräuner et al., 2008; Liu et al., 2015; Zuurber et al., 2011). This inconsistency might be due to different characteristics of the study participants or different PM components. Our study adds to the conclusion that PM may increase IL-6 not only in the elderly subjects but also in healthy young subjects.

Associations between elevated levels of hsCRP and cardiovascular diseases have been shown in numerous studies. We found increase in hsCRP in association with PM_{2.5} and PM_{1} in the elderly panel which indicates that inflammation and oxidative stress are the main biological pathways linking PM exposure with cardiovascular disease. Our analyses show the strongest effects with lag 0 (0–23-h) exposure to PM_{1} and PM_{2.5}. Regarding to the elderly subjects, our study results are compatible with the observed results regards to PM and hsCRP in several previous studies (Brook et al., 2010; Hampel et al., 2015). In contrast to results in the elderly panel, no associations were found between PM and hsCRP in the healthy young adults panel that is consistent with other studies (Zuurber et al., 2011), although some studies have found associations (Chuang et al., 2007; Kelshadi et al., 2014; Yang et al., 2015). A systematic review on the effects of PM on hsCRP reported that the correlations are not consistent in healthy young adults (Li et al., 2012). In the elderly panel, our data show more and stronger associations for PM_{2.5} than PM_{1}. The obtained results showed that exposure to larger particles, PM_{10} and PM_{2.5-10}, was not associated with hsCRP. Although PM_{2.5} and PM_{1} were associated with hsCRP effects were not significant for PM_{1-2.5}. Whether this indicates stronger effects of smaller particles or larger random errors in the PM_{1-2.5} exposure terms – which combines measurement errors of both size fractions – cannot be determined. The percentage of particle deposition in different parts of the respiratory tract and mechanisms that affect human health depend on PM size (Araujo and Nel, 2009). There is conflicting evidence in the studies about the effect of PM on hsCRP. These inconsistencies could reflect differences of study participants, or differences in the composition of PM. The indication of very immediate effects of the smaller PM’s on hsCRP with significant results restricted to lag 0 is of interest as well. Further analyses based on hourly exposure data may further elucidate the immediacy of these reactions.

WBC is a stable marker of vascular inflammation and is a predictor of cardiovascular diseases (Chen and Schwartz, 2008; Huttunen et al., 2012). Short-term exposures to ambient PM were associated with high WBC counts (Huttunen et al., 2012; Schwartz, 2001). In the present study, we found that elevations of PM_{10}, PM_{2.5-10}, PM_{2.5}, PM_{1-2.5} and PM_{1} mass concentrations were associated with increases in WBC count in the elderly subjects. In addition, in the healthy young adults increase of PM_{10}, PM_{2.5-10}, and
PM$_{1-2.5}$ were associated with WBC but PM$_{2.5}$ and PM$_{1}$ were not associated with WBC. This indicates that in healthy young subjects larger particles were more effective than smaller ones for WBC. The observed results demonstrated the importance of inflammation pathways linking PM exposure with cardiovascular diseases.

To our knowledge, studies are more scarce for sTNF-RII (Delfino et al., 2008; Rohr and Wyzga, 2012). sTNF-RII has higher values and the longer life time than TNF-α in plasma (Delfino et al., 2008). Some studies showed that soluble receptors have major roles in adverse health effects (Heaney and Golde, 1998). We observed strong associations of sTNF-RII with PM$_{2.5}$ and PM$_{1}$ and weak association with PM$_{10}$ and PM$_{1-2.5}$ in the elderly subjects, whereas no associations were found for sTNF-RII and PM size fractions in the healthy young panel. We found higher increase of sTNF-RII in smaller size fractions of PM for the elderly subjects. Our findings for sTNF-RII are consistent with other studies that have reported increased levels of sTNF-RII are associated with elevated PM concentrations (Delfino et al., 2008).

vWF is a marker of the coagulation process (Monroe and Hoffman, 2006). It may also serve as an indicator of endothelial dysfunction (Rückerl et al., 2011). In general, vWF is known as a predictor of coronary events (Andrews et al., 2004). We found no associations between PM$_{10}$ exposure and the coagulation marker vWF, but positive associations with PM$_{10}$, PM$_{2.5-10}$, PM$_{2.5}$, and PM$_{1-2.5}$ in the elderly panel. Our results for healthy young subjects were different from the elderly people and we observed no associations between PM size fractions and vWF. This inconsistency between the elderly and healthy young adults panel is difficult to explain. There are conflicting evidences in the studies about the effect of PM on vWF. Several previous studies reported associations between PM exposure and vWF (Liao et al., 2005; Mills et al., 2008; Riediker et al., 2004), although other studies could not confirm such associations (Bräuner et al., 2008; Samet et al., 2009; Zuurbier et al., 2011).

4.3. Strengths and limitations

We conducted a panel study, thus each subject represents his or her own control and individual characteristics do not confound hypothesized associations. To eliminate the probability that the detected associations are unintentionally resulted from acute infections in the participants, we excluded blood samples of participants with acute infection during the seven days before the blood sampling. As we run several tests to find out which lags were associated with the biomarkers, multiple testing might be one of the limitations in this study. The other limitation is that the concentrations on the rooftop for outdoor measurements may differ substantially from the individual’s inhalation zone. Strengths of this study are the large number of (six) repeated measurements within a time period of a year, covering all seasons. The very large within-subject contrasts in exposure are a very powerful feature of our study. Also, we investigated both the elderly and healthy young subjects, confirming that age related factors may amplify the acute effects of air pollution. Another clear strength is the parallel subjects, controlling for confounding factors. Also, we investigated both the elderly and healthy young panels. We found higher increase of sTNF-RII in the elderly subjects and vWF with PM$_{10}$ and PM$_{2.5-10}$, PM$_{2.5}$, and PM$_{1-2.5}$ mass concentration. Therefore, our results show that various size fractions of PM increase biomarkers of inflammation and coagulation in the elderly subjects and – to a lesser extent – in healthy young adults. This confirms our hypothesis of PM induced pulmonary inflammation and oxidative stress. Whereas the elderly population may be particularly susceptible to air pollutants, the role of other characteristics needs to be elucidated in future studies.

Competing interests

There is no actual or potential conflict of interest among authors.

Acknowledgments

The authors wish to thank the Institute for Environmental Research (IER) of Tehran University of Medical Sciences for financially and technically supporting this research (grant number 90-03-46-15705). The authors also grateful to the administrations of Hejari School and Tohid retirement home for their cooperation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.02.005.

References


