Introduction

Hairdressers are exposed to chemical substances during their work due to the usage of cosmetic hair products such as hair dyes, permanent wave solutions and bleaches [1–3]. Several constituents of cosmetic hair products are airway irritants and may impair pulmonary function or induce chronic bronchitis [4]. Exposure to ammonia during hair bleaching can lead to mucosal irritation [5], and the persulphates present in hair bleaching agents can act as allergens and airway irritants [6], placing hairdressers at an increased risk for developing asthma [7]. Also, volatile organic compounds (VOCs) present in sprays may cause adverse reproductive effects [8]. Recently, an epidemiological study reported that exposure to ambient VOCs is associated with an increased risk of cardiovascular death in heavily polluted cities [9]. The American Heart Association (AHA) has published a statement on the important role that ambient particles, gases and chemical substances play in the development of cardiovascular disease [10]. However, the relationship between occupational exposure to VOCs and resultant cardiovascular effects among young healthy hairdressing assistants has not been well characterized.

The biological mechanisms linking air pollution to cardiovascular diseases in the AHA statement involve the direct effects of air pollution on the cardiovascular system through a neural mechanism that alters central nervous system function as well as indirect effects mediated by pulmonary inflammation and oxidative stress that eventually lead to a systemic inflammatory response [10]. Several studies have provided support for these mechanisms in human subjects. Some of these studies have documented...
an association between air pollution and autonomic dysfunction by measuring heart rate variability (HRV) indices [11,12]. Some have also documented an association between air pollution and activation of inflammatory oxidative stress pathways by measuring serum C-reactive protein (CRP) or oxidative DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels [13,14]. Accordingly, we designed this cross-sectional study to investigate whether or not exposure to VOCs was associated with autonomic dysfunction, inflammation and oxidative stress among young healthy assistants in hair salons in Taipei, Taiwan.

Methods

Our study was designed to investigate exposure to various levels of VOCs and markers of autonomic function, inflammation and oxidative stress among hair salon assistants. The selection criteria for participants were as follows: participants could not have been taking any medication that had the potential to affect cardiac rhythm, and the participant must have had no known cardiovascular disease or history of cardiovascular disease, such as coronary artery disease, arrhythmias, hypertension, diabetes mellitus or dyslipidemia. We recruited young, healthy, non-smoking hairdressing assistants working in different hair salons in Taipei via a mailed invitation.

We collected blood samples from each participant and also conducted electrocardiographic (ECG) monitoring during the four times between August 2008 and June 2009. Following a first visit inclusion examination at home on a non-working day (9:00 a.m. to 9:00 p.m.), three follow-up visits in hair salons were scheduled every 3 months on working days (9:00 a.m. to 9:00 p.m.). During the first visit (a non-working day), a baseline screening questionnaire was administered in which age, sex, body mass index (BMI), medications, home characteristics (building structure, number of windows, presence of pets, usage of stove and air conditioning, etc.) and time-activity patterns (timetable for rising, dining, walking, showering, sleeping, etc.) were noted, followed by air pollution monitoring, blood sample collection and ECG monitoring. On subsequent visits (on working days), participants were requested to stay at home and keep gas stoves off during the first visit.

The study protocol was reviewed and approved by the Human Subject Committee of the St. Mary’s Medicine Nursing and Management College.

We took a 10 ml blood sample on each occasion from each participant at 9:00 p.m. on the sampling day and kept the sample on ice before centrifugation. These blood samples were first centrifuged by a refrigerated centrifuge at 3000 r.p.m. for 15 min within 90 min of collection and then stored at −80°C before the assay was run. The serum level of the inflammatory marker serum CRP was determined using a two-site chemiluminescent enzyme immunoassay (IMMULITE hs-CRP; Diagnostic Products Corporation, Los Angeles, CA). The oxidative stress marker, oxidative DNA adduct 8-OHdG, was measured by enzyme-linked immunosorbent assay using the monoclonal antibody N45.1 [15] (Japan Institute for the Control of Aging, Fukuori City, Japan). Each participant underwent approximately four serum CRP and four 8-OHdG measurements during the four visits.

A detailed description of the continuous ambulatory ECG monitoring we used as well as the details regarding our data analysis is detailed in our previous study [12]. In brief, we performed 12 h continuous ambulatory ECG monitoring for each participant during the daytime (9:00 a.m. to 9:00 p.m.) using a PacerCorder three-channel device (model 461A; Del Mar Medical Systems LLC, Irvine, CA) with a sampling rate of 250 Hz (4 ms). ECG tapes were analysed using a Delmar Avionics model Strata Scan 563 (Del Mar Medical Systems LLC, Irvine, CA). A complete 5 min segment of normal-to-normal (NN) intervals was taken for HRV analysis, including the standard deviation of the NN intervals (SDNN) and the square root of the mean of the sum of the squares of the differences between adjacent NN intervals (r-MSSD). Each participant obtained ~576 successful 5 min SDNN and 5 min r-MSSD measurements during the four visits (144 measurements for each visit) that were used for data analysis.

We performed 12 h continuous VOCs monitoring for each participant during the daytime (9:00 a.m. to 9:00 p.m.). Personal exposure to VOCs was measured continuously in 62 hair salons using a total VOC monitor (ppbRAE Plus, model PGM-7240; RAE Systems Inc., San Jose, CA), which is a compact instrument with a microprocessor control that uses a photo-ionization detector to detect a wide range of organic vapours. Organic vapours pass through a ultraviolet lamp and are photoionized. The ejected electrons are then detected as a current. The calibration gas used is isobutylene.

We performed 12 h continuous indoor particles monitoring for each participant during the daytime (9:00 a.m. to 9:00 p.m.). Indoor particles, temperature and humidity were measured continuously using a personal dust monitor (DUST-check portable dust monitor, model 1.108; Grimm Labortecnik Ltd, Airing, Germany), which measured and recorded 1 min mass concentrations of particulate matter <2.5 μm in diameter (PM₂.₅) as well as temperature and humidity. The DUST-check
portable dust monitor measured PM$_{2.5}$ concentrations by measuring a particle’s light-scattering properties and using a correction factor to derive mass concentrations from reference aerosols that had a density of 0.92 and reflective index of 1.45. Collocated Rupprecht and Patashnick 1400a tapered element oscillating microbalance samplers (Thermo Electron Corporation, East Greenbush, NY) were used to calibrate the mass concentrations of PM$_{2.5}$ measured by our DUST-check monitor before and after each session.

After sampling, the raw data for 1 min total VOC and PM$_{2.5}$ measurements were matched with the sampling time of blood samples and HRV monitoring and then computed to hour-long moving averages if 75% of the data were present.

We created linear mixed-effects regression models in S-PLUS 2000 (MathSoft Inc., Cambridge, MA) to examine the association between indoor air pollution exposure, blood markers and log$_{10}$-transformed HRV. The exposure variables were 1–8 h averages of total VOC and PM$_{2.5}$ exposure and the outcome variables were serum CRP, 8-OHdG and log$_{10}$-transformed 5 min HRV indices (5 min SDNN and 5 min r-MSSD). We treated participants’ sex, age and BMI, as well as the hour of day, temperature, relative humidity and level of indoor air pollution as fixed effects and fitted each participant as a random effect in our mixed-effects models. The models were also adjusted for several smooth function terms as fit by a natural spline. These terms included hour of day, temperature and humidity. Model selections were made based on the minimization of Akaike’s Information Criterion [16]. Sensitivity analyses were also performed by exploring all models with the influential outliers or participants excluded. Pollution effects were expressed as percent changes by interquartile range (IQR) changes as $[10^{(\beta \times IQR)} - 1] \times 100\%$ for log$_{10}$-transformed HRV indices and $[\beta \times IQR/M] \times 100\%$ for blood markers, where $\beta$ and $M$ are the estimated regression coefficient and the mean of each blood marker, respectively.

**Results**

A total of 62 hairdressing assistants were willing to participate in our study after we explained our monitoring protocols to them (response rate = 31%). Two hundred and forty-eight visits were performed in which 245 serum CRP, 231 8-OHdG and 30 351 successful 5 min HRV measurements were obtained and included in data analyses. The mean age of our participants was 25.2 years (SD = 2.1 years), the mean BMI was 22.7 kg/m$^2$ (SD = 3.1 kg/m$^2$) and the male:female ratio was ~1:1. The mean (SD) values of blood markers for our study participants over their four visits were 0.23 mg/dl (0.1) for serum CRP and 1.2 ng/ml (0.4) for 8-OHdG. The mean value (SD) of the log$_{10}$-HRV indices were 1.6 ms$^2$ (0.3) for SDNN and 1.3 ms$^2$ (0.2) for r-MSSD.

Table 1 summarises the indoor air pollution, indoor temperature and relative humidity data averaged over 1 h for the 62 study participants. During the study period, the total levels of VOCs and PM$_{2.5}$ measured on working days were relatively high compared with those on non-working days. The Pearson correlation analysis showed that the level of total VOC was moderately correlated with the level of total PM$_{2.5}$ ($r = 0.34$). The indoor condition was pleasant with a temperature range of 20.4–25.9°C and a relative humidity range of 58.4–69.3% during the study period.

The effect of pollution on inflammatory and oxidative stress markers as estimated by mixed-effects models are listed in Table 2. After adjusting for sex, age, BMI, smooth functions of hour of day, temperature and humidity in the mixed-effects models, total VOC exposure was found to be significantly associated with increases in serum CRP and 8-OHdG. For interquartile increases in total VOC exposure, the mixed-effects models showed 6.2–10.7% increases in serum CRP at 3–5 h averages and 1.6–5.8% increases in 8-OHdG at 1–5 h averages. For interquartile increases in PM$_{2.5}$, the mixed-effects models showed 6.8 and 7.7% increases in serum CRP at 4 and 5 h averages and 1.0–1.8% increases in 8-OHdG at 2 and 3 h averages. The association between total and inflammatory and oxidative stress factors remained after adjusting for PM$_{2.5}$ in the mixed-effects models.

Table 3 lists the associations between indoor air pollution and 5 min HRV indices estimated by the mixed-effects models. We found that both 5 min SDNN and 5 min r-MSSD were significantly associated with total

Table 1. Summary statistics for the indoor air pollution exposure, indoor temperature and humidity levels experienced by assistants in hair salons during the study period

<table>
<thead>
<tr>
<th></th>
<th>All data</th>
<th>Working days</th>
<th>Non-working day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VOC, ppb</td>
<td>Mean ± SD</td>
<td>56.1 ± 37.5</td>
<td>75.3 ± 47.2</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>68.6</td>
<td>84.7</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>2940</td>
<td>2200</td>
</tr>
<tr>
<td>PM$_{2.5}$, μg/m$^3$</td>
<td>Mean ± SD</td>
<td>31.7 ± 10.4</td>
<td>42.6 ± 22.7</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>21.0</td>
<td>29.0</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>2960</td>
<td>2220</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>Mean ± SD</td>
<td>22.7 ± 2.1</td>
<td>21.1 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>2960</td>
<td>2220</td>
</tr>
<tr>
<td>Relative humidity, %</td>
<td>Mean ± SD</td>
<td>63.1 ± 4.0</td>
<td>60.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>IQR</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>2960</td>
<td>2220</td>
</tr>
</tbody>
</table>

*Values reported are 1 h means.*
VOC and PM$_{2.5}$ exposure. For interquartile increases in total VOC exposure, the mixed-effects models showed 2.3–4.0% decreases in 5 min SDNN and 2.8–6.0% decreases in 5 min r-MSSD at 1–4 h averages, respectively. For an interquartile increase in PM$_{2.5}$, the mixed-effects models showed 3.0% decrease in 5 min SDNN at 4 h average and 4.1 and 5.7% decreases in 5 min r-MSSD at 3 and 4 h averages, respectively. The associations between total VOC exposure and 5 min HRV indices remained after adjusting for PM$_{2.5}$ exposure in the mixed-effects models.

We further performed effect modification of the association between total VOC exposure and health data by adjusting for working status (working day versus non-working day) (Table 4) and found a consistent effect modification of total VOC exposure effect on working
days. During visits on working days, participants’ serum CRP and 8-OHdG increased by 10.9 and 4.5% per IQR of total VOC, respectively, at the 4 h average. During visits on non-working days, participants showed a smaller change or no change in 8-OHdG and serum CRP levels. Similar effect modification was suggested in models evaluating the effects of total VOC exposure on 5 min HRV indices.

**Discussion**

Our study results suggest that both total VOC and PM2.5 exposure could lead to elevated serum CRP and 8-OHdG levels as well as depressed HRV indices. VOCs are regarded as hazardous air pollutants and can cause pulmonary and cardiovascular symptoms [17]. A previous study showed that indoor VOCs concentrations are usually greater than outdoor VOCs concentrations [18]. It has also been proposed that VOCs were mostly associated with PM2.5 [19] and better represent air pollution exposure than a measure of exposure to particulate matter [20]. Such findings might explain the consistent association observed between total VOC exposure and inflammation, oxidative stress and autonomic dysfunction as compared to the lack of association between these parameters and exposure to PM2.5 that we found in our study. Our data regarding total VOC exposure and the associated changes in clinical parameters also support the results of previous epidemiological studies regarding the association between VOCs exposure and cardiovascular effects [9,21].

Our findings regarding PM2.5 exposure and HRV indices are compatible with the autonomic responses to particulate air pollution observed in several other studies. The effects of PM2.5 exposure on HRV indices among our subjects in hair salons were greater than those observed in previous studies. The magnitude of the effect of indoor PM2.5 exposure on SDNN [3.0% decrease in SDNN per IQR of indoor PM2.5 using a 4 h moving average (19.8 µg/m³)] in our study was greater than the effect of ambient PM2.5 exposure on boilermakers reported by Magari et al. [22] (a 2.66% decrease in SDNN per 1.0 mg/m³ of ambient PM2.5 at 4 h moving average) and elderly subjects reported by Park et al. [23] (0.1% decrease in SDNN per SD of ambient PM2.5 at 4 h moving average (8.0 µg/m³)). However, the effects of ambient PM2.5 exposure on elderly subjects with known cardiovascular conditions reported by Gold et al. [11] (0.24/0.25 ms decrease in SDNN/r-MSSD per 1.0 µg/m³ of ambient PM2.5 at 4 h moving average) were greater than the effects of PM2.5 exposure on HRV indices presented in our study (0.06/0.05 ms decrease in SDNN/r-MSSD per 1.0 µg/m³ of PM2.5 at 4 h moving average). Although the comparisons can be only approximate, these findings imply that the effect of occupational PM2.5 exposure on SDNN among assistants working in hair salons were more dramatic than the ambient PM2.5 effects observed in previous studies. Elderly patients with cardiovascular disease appear to be more susceptible to PM exposure than young adults. Moreover, our findings regarding PM2.5, serum CRP and 8-OHdG also support previous studies of particle-induced inflammation and oxidative stress. For instance, previous studies have reported that increases in serum CRP levels were associated with PM10 exposure in patients with coronary heart disease [24] and with PM2.5 exposure in young healthy adults [14]. Additionally, increases in 8-OHdG levels were reported to be induced by PM2.5 exposure in an in vivo study [25] as well as in a human study [14].

Another interesting finding of this study was that working status (working day versus non-working day) seemed to modify the effect of total VOC exposure on the health parameters we measured: greater effects on serum CRP, 8-OHdG and HRV were observed during visits that occurred on working days as compared to those during the visits that occurred on non-working days. These findings suggested that occupational exposure to VOCs in hair salons was associated with adverse cardiovascular effects. Time spent away from the workplace could modify the effects of VOCs exposure on serum CRP, 8-OHdG and HRV in young healthy adults in Taipei, Taiwan.
It has been proposed that inhalation of particulate matter can induce pulmonary oxidative stress and inflammation, which can develop into a systemic inflammatory response and lead to alterations in central nervous system function and an associated increased risk of cardiovascular events [10]. Such biological causality may also be applied to the association between VOCs exposure and cardiovascular health. Ozone (O₃) is a major secondary pollutant formed by the photochemical oxidation of VOCs derived from solvents or automobile emissions. Studies have shown that exposure to concentrated ambient O₃ produces an inflammatory response in humans [26] and in in vitro cellular models [27] as well as autonomic dysfunction in humans [11]. Such effects are possibly induced by VOCs exposure, although at this time, there is no proof available for this hypothesis. Because VOCs and PM₂.₅ are two types of primary pollutants and represent similar groups or sources of pollutants in most outdoor and indoor air pollution, our findings imply that risk-based air pollution control policies should focus not only on ambient pollutants but also on indoor aerosol precursors in order to maximize health risk reduction.

Our study design has several limitations. The association of VOCs with an increase in serum CRP and 8-OHdG and decrease in HRV indices found in the study could be confounded by unavailability of indoor exposure data for other air pollutants [O₃, nitrogen dioxide (NO₂), formaldehyde and ammonia]. As these unmeasured air pollutants are usually correlated with VOCs, the outcomes of this study may be biased [28].

We did not measure outcome variables or collect blood samples from each subject continuously over several hours within 1 day. Therefore, we cannot investigate the time sequence of inflammation and oxidative stress that occur when assistants are exposed to VOCs in hair salons.

Although we have adjusted for a set of individual-level confounders, we could not rule out the possibility of unmeasured confounders. Also, we did not have a control group in this study. Therefore, we cannot make a comparison of VOCs-induced cardiovascular effects between people working in hair salons and normal population.

The limited number of intra-individual measurements we conducted in this study (i.e. measurement of variables at four time points per participant) may not be sufficient to fully control for inter-individual differences in health outcomes in our mixed-effects models.

Our data generally supports the hypothesis that occupational exposures to VOCs and PM₂.₅ in hair salons can lead to increases in serum CRP and 8-OHdG levels and decreases in HRV indices. Time spent away from workplace could modify the effects of VOCs on serum CRP, 8-OHdG and HRV indices in young healthy hairdressing assistants.

Key points
- Occupational exposure to volatile organic compounds in hair salons can lead to increases in inflammatory blood markers and decreases in heart rate variability indices.
- Time spent away from hair salons could modify the effects of volatile organic compounds on autonomic dysfunction, inflammation and oxidative stress.

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Conflicts of interest
None declared.

References


