A Pilot Biomarker Study to Assess the Subclinical Health Impacts due to Exposures to Air Pollution

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Executive Summary

The epidemiological evidence of adverse effects on health is widely regarded as an important driver to support clean air policies. Effects of air pollution on the major registered causes of death include chronic degenerative, cardiovascular, cerebrovascular and pulmonary diseases in older people and respiratory diseases such as asthma in children. These health outcomes account for a major burden in the society especially in terms of quality adjusted life years lost and pain suffering from both the patients and the carers, health care costs and productivity loss, as measured by the sum of tangible and intangible costs. These costs could be substantial when long-term effects of air pollution are taken into account because of their effects being at least ten times larger than the estimated short-term effects of air pollution.

However, both the longer- and shorter-term exposure to air pollution could have directly contributed to the development of subclinical conditions but the associated community burden such as restricted activities and loss in well being could be substantial. According to a recent survey conducted by The University of Hong Kong (*http://hkupop.hku.hk/english/report/subhealth/content/resources/pr.pdf*), 97% of Hong Kong citizens have sub-optimal health problems. The measurement of biomarkers, which can be detected before the onset of diseases, could give an account of the subtle and subclinical pathophysiological changes associated with health impacts in the population due to air pollution. The core objective of this pilot study was to develop methodological approaches in measuring the effects of air pollution on health at molecular level in Hong Kong, which could be utilized to improve public awareness on implementing government's clean air policies for prevention of diseases and subtle signs of health problems.

Systematic Review

We conducted a systematic review with search strategies based on the PubMed database for selection of biomarkers to be used in the project. We focused on the health effects of the four criteria air pollutants documented by the World Health Organization (WHO) and routinely monitored by the Hong Kong Environmental Protection Department (EPD), including particulate matters (PM_{10} and $PM_{2.5}$), nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and ozone (O_3).

We justified the screening criteria for selection of literatures as well as for selection of biomarkers to be used in the project, considering the study design, level of evidence and sample size of the selected studies. When published data for a biomarker was sufficient, we conducted the meta-analysis and assessed whether there were publication biases.

Study Design for Biomarker Measurement

We identified residential buildings in suitable locations which were 0.1 to 1.5 km away from the air monitoring station in the Central/Western and Mongkok regions and sent out 2,810 invitation letters to the addresses there. We received 151 calls (4.3% response rate) from the invited residents. One hundred and twenty eligible Chinese participants aged 50 to 65 years, who were non-smokers and had no known chronic diseases were recruited.

We scheduled the appointments with the participants for four times of clinical visits. Each visit included questionnaire interviewing, blood collection, blood pressure and anthropometry measurement.

Data Analyses

Each person was measured at four times in different months of the study year (i.e. from July 2014 to April 2015) to maximize the temporal variation in $PM_{2.5}$ exposure. Blood samples collected from the participants were used to examine the oxidative stress biomarkers. We assessed the relationships between temporal changes in $PM_{2.5}$ exposure and temporal changes in levels of physiological parameters and biomarkers. We also assessed the relationship between spatial difference in $PM_{2.5}$ concentrations measured in the two EPD monitoring stations and the difference in bio-physiological levels with control for individual characteristics (for example, dietary habit and passive smoking exposure) and environmental conditions.

Key findings and discussion

1. We performed a systematic literature review on the use of biomarkers for air pollution and health studies, focusing on the oxidative stress effects of $PM_{2.5}$. Oxidative stress triggers a number of redox sensitive signaling pathways in the human body and it is one of the mechanisms of air pollution effects on the human health. The pulmonary inflammatory response and cardiovascular actions arising from exposure to air pollutants, were mediated via these oxidant signaling pathways. We selected three oxidative biomarkers in our study: 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and glutathione [GSH] and glutathione disulfide [GSSG] ratio.

2. The pilot biomarker study originally recruited 120 participants, with 62 of them living nearby the Central/Western ambient $PM_{2.5}$ monitor in a residential-only urban area, and with 58 living nearby the Mongkok roadside monitor in a commercial-residential area. For the 120 participants, 109 came to our study centre at the Laboratory Block, Li Ka Shing Faculty of Medicine,

The University of Hong Kong in July 2014, and 99 completed all the four times of visits. The successful follow-up rate was 90.8% which is regarded as satisfactory.

3. In this pilot study, the measured biomarkers levels were comparable to the values reported in other studies. For the three oxidative stress biomarkers, 8-OHdG, MDA and GSH/GSSG ratio, were negatively but statistically non-significantly ($p>0.05$) correlated with $PM_{2.5}$, adjusted for covariates including demographic, lifestyles and dietary factors. For blood lipid profile, the HDL-C level measured in the participants was negatively correlated with $PM_{2.5,}$ which was statistically significant ($p<0.05$). For triglyceride, the correlation was positive but not significant ($p>0.05$). All the autonomic measurements in the study were positively correlated with $PM_{2.5}$, in which only the correlation with diastolic blood pressure (DBP) was statistically significant ($p<0.05$). In the current day exposure window, we found a reduction of 0.35 ng/ml in 8-OHdG, and 0.06 in GSH/GSSG ratio; and an increase of 0.001 mmol/L in MDA were associated with every 10 $\mu g/m^3$ increase of $PM_{2.5}$. The associations for MDA and GSH/GSSG ratio with $PM_{2.5}$ were comparable to other studies. This pilot study showed that $PM_{2.5}$ could contribute to the development of subclinical diseases by changing the level of certain molecular biomarkers in the human body.

4. The measurements of the biomarkers using bioassay methods in this pilot study were subject to the limitation that they are not specific for oxidative stress. The non-significant association with $PM_{2.5}$ could be due to the non-specific nature of the measurement methods and may also be due to insufficient statistical power for the assessments. In the future study, analytical chemistry based methods (for example, high performance liquid chromatography-mass spectrometry) with a larger sample size (estimated to be at least 250 to achieve a statistical power of 80%) will be applied to further investigate the subtle and subclinical pathophysiological changes associated with health impacts in the population due to air pollution.

Conclusion

This pilot study demonstrated the feasibility of using molecular biomarkers to investigate the subclinical health impacts due to ambient air pollution in Hong Kong. The preliminary data showed that $PM_{2.5}$ could contribute to the development of subclinical diseases by changing the level of certain molecular biomarkers in the human body. The change in oxidative biomarkers can be detected if there were government interventions for alleviation of $PM_{2.5}$. Certain limitations in the pilot study, including the non-specific analytical methods, insufficient statistical power, and the lack of personal exposure assessment, will be addressed in the future biomarker study in Hong Kong.

Way Forward

In the future panel study, we recommend improving the study power by: (1) targeting a more specific and sensitive population such as the elderly, who are less mobile, with repeated biomarker measurements in shorter time scales, within cool and warm seasons, respectively; (2) increasing the sample size; and (3) measuring the personal exposure to air pollution besides the usage of ambient air pollution data.

1. Introduction

1.1 Background

To study the adverse effects of air pollution on health, the Environmental Protection Department (EPD) of the Hong Kong Special Administrative Region Government commissioned Dr. C.M. Wong of the School of Public Health, The University of Hong Kong to conduct a series of studies on the subclinical effects of exposure to fine particulate matter ($PM_{2.5}$) in residential-commercial and residential areas.

The study comprised four visits for two panels of participants, one residing in Mongkok (residential-commercial) region near EPD's Mongkok roadside air quality monitoring station and the other in Central/Western (residential) region near EPD's Central/Western ambient air quality monitoring station. The four visits were carried out in July 2014, October 2014, January 2015, and April 2015, respectively. Blood samples were taken from all the recruited participants for assessing the relationship between measures of biomarkers and air pollutant concentrations.

An inception report was submitted to the EPD in April, 2014 to provide the background information of the study and an interim report submitted in December, 2014 to present a literature review on health effects of air pollution in terms of changes in biomarker measurement. This final report incorporated the literature review from the Interim Report and summarized all the results from the four visits and the relationship between biomarkers and air pollutants with concentrations measured at the EPD monitoring stations in the two representative regions.

1.2 Objectives

The objectives of the study are as follows:

i) to conduct a literature review on the use of biomarkers for air pollution health studies in Hong Kong and overseas and recommend appropriate biomarkers for this pilot study;
ii) to collect samples from the participants residing in two representative regions in Hong Kong and carry out biomarkers analysis;
iii) to conduct questionnaire survey to assess the individual characteristics of the participants for biomarkers analysis;
iv) to assess the relationship between measures of biomarkers and air pollutant concentrations measured at the EPD monitoring stations in the two representative regions; and
v) to develop methodological approaches in measuring the effects of air pollution on health at molecular level in Hong Kong.

2. Literature Review

2.1 Methods

We conducted a systematic review with search strategies based on a well-known medical literature database. We justified the screening criteria for selection of literatures as well as for selection of biomarkers to be used in the project, considering the study design, level of evidence and sample size of the selected studies, and the total number of references that support potential health effects of the biomarkers. When published data for a biomarker was sufficient, we conducted the meta-analysis and assessed whether there were publication biases.

2.2 Literature Selection

We searched the PubMed database (last entry on 7th August, 2014) using the following terms from the title and abstract of published articles: ("particulate matter" OR "PM_{10}" OR "PM(10)" OR "$PM_{2.5}$" OR "PM(2.5)" OR "fine particle" OR "fine particles" OR "fine particulate" OR "fine particulates" OR "nitrogen dioxide" OR "NO_2" OR "NO(2)" OR "sulfur dioxide" OR "sulphur dioxide" OR "SO_2" OR "SO(2)" OR "ozone" OR "O_3" OR "O(3)" OR "air pollution") AND health AND ("biomarker" OR "biomarkers")

We focused on the health effects of four criteria air pollutants documented by the World Health Organization (WHO): particulate matter (PM_{10} and $PM_{2.5}$), nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and ozone (O_3) (WHO 2006). These are the air pollutants routinely monitored by the EPD.

There were 381 abstracts retrieved from PubMed on 7th August, 2014, from which 53 articles were selected for this review by a researcher using the following inclusion criteria:

(i) studies should be focused on the effects of the criteria air pollutants (PM, NO_2, SO_2 or O_3) on the biomarkers relevant to the research question;

(ii) subjects should be human subjects not including cell lines, tissues or animal models; and

(iii) the report must be written in English or Chinese.

In case there were more than one publication reporting the same results only one of them was selected. The selected study was adhered to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (Moher et al. 2010). Figure 1 below shows the flow chart in screening the articles.

The data extracted were: the first author's surname, year of publication, number of subjects in total and broken down into gender and age groups, biomarkers and pollutants examined (regardless

of statistical significance), and exposure time (e.g. lag days of the exposure). The percentage (%) change or absolute change in the biomarkers associated with unit increase of each pollutant and the standard error (SE) or 95% confidence interval (CI) were presented.

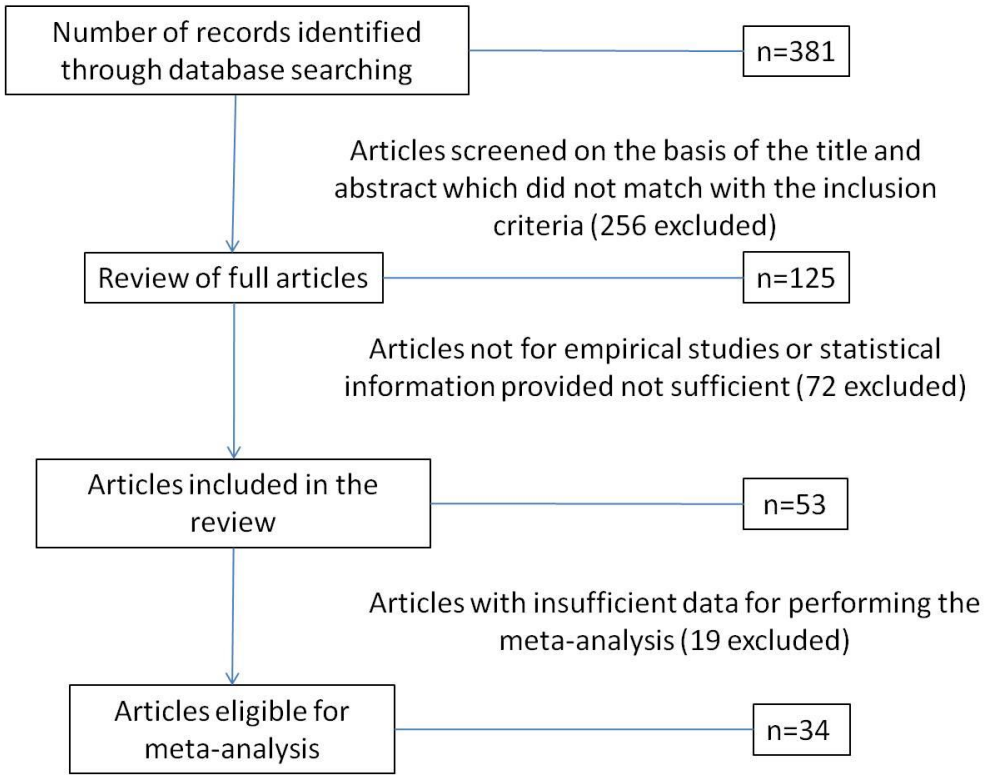


Figure 1: Screening Flow Chart for the Literature Review

2.3 Meta-analysis

For each biomarker, if there were two or more studies identified, a meta-analysis was conducted to estimate the pooled up effect of each pollutant, PM_{10}, $PM_{2.5}$, NO_2, SO_2 or O_3, in terms of percentage change of the biomarker per unit increase of the pollutant.

Risk estimates from the selected studies were pooled up by the overall average of these estimates weighted by the inverse of the variance. All risk estimates were expressed as or converted to percentage change or absolute unit change in the concentration of the biomarker associated with every 10 μg/m^3 increase in concentration of each pollutant.

For short-term effects of air pollutants, we selected the lag days based on the following criteria: (1) the lag-day presented by the author in the abstract or main text; (2) the smallest lag days which showed significant associations, and (3) lag 0 or 1 day, or the smallest lag day provided, if all the lag days presented were statistically non-significant. When pooling up the estimates, we used a random

effects model if I^2 statistics for heterogeneity was >25% or otherwise a fixed effect model (Woodward 2005). A fixed-effect meta-analysis estimates an effect which is assumed to be homogeneous in a population, while a random-effects meta-analysis estimates the mean of a distribution of effects, which is heterogeneous in a population (Borenstein et al. 2009). Here we use I^2 statistics >25% to detect existence of heterogeneity in our population.

2.4 Findings

A total of 53 articles were included in this review. Table 1 and Appendix Table 1a – Table 1c summarized the associations between $PM_{2.5}$ and biomarkers in all the studies reviewed. Although our main hypothesis in this project was focused on $PM_{2.5}$ and the constituents of $PM_{2.5}$ that may induce oxidative damages, we also presented the effects on systemic inflammation, and hemostasis, and effects associated with other air pollutants, such as O_3 and NO_2 (Appendix Table 2).

Among all the selected articles, 34 of them were considered to have sufficient data for performing the meta-analysis, for which the sample size, concentrations of the pollutants, the effect estimates and the corresponding standard error (SE) or 95% CI were presented. The number of studies for the association between long-term exposure to air pollutants and the biomarkers was limited. We therefore did not review studies for long-term effects.

We identified biological mechanisms through which air pollution may be related to changes in biomarkers as preclinical indicators for adverse effects on human health:

(1) Oxidative stress: 8-hydroxydeoxyguanosine [8-OHdG], 8-isoprostane, malondialdehyde [MDA], glutathione [GSH] and glutathione disulfide [GSSG] ratio, superoxide dismutase [SOD] and exhaled breath condensate [EBC] pH, Fe, nitrate, and nitric oxide [FeNO];

(2) Hemostasis or endothelial function: sP-selectin [sCD62p], soluble CD40 ligand [sCD40L], von Willebrand factor [vWF], Factor VII, and plasminogen activator inhibitor-1 [PAI-1];

(3) Systemic inflammation: fibrinogen, C-reactive protein [CRP], club cell secretory protein [CC16], interleukin-6 [IL-6], interleukin-8 [IL-8], myeloperoxidase, tumor necrosis factor alpha [TNF-α], and intercellular adhesion molecule-1 [ICAM-1]; and

(4) Metabolic function: blood profile such as white blood cells counts [WBC], blood pressure and heart rate.

We performed meta-analysis for 16 different biomarkers, namely 8-OHdG, CC16, CRP, EBC pH, FeNO, fibrinogen, Factor VII, IL-6, IL-8, lymphocytes, myeloperoxidase, neutrophils, PAI-1, sCD40L, TNF-alpha, and vWF (Table 1 & Appendix Table 1a – Table 1c). We found that each 10 $\mu g/m^3$ increase of $PM_{2.5}$ was significantly (p-value < 0.05) associated with 0.14% increase of EBC pH (in contrary to the expectation that increase in PM is associated with increase in airway acidity

and hence decrease in the biomarker), 4.42% increase of myeloperoxidase, and 1.08% increase of TNF-alpha.

For other biomarkers, we found that $PM_{2.5}$ had positive but statistically non-significant associations with 8-OHdG, FeNO, CC16, CRP, fibrinogen, IL-8, vWF, and lymphocytes; while negative but statistically non-significant associations were observed in IL-6, factor VII, PAI-1, sCD40L, and neutrophils level (Appendix Table 3). There were insufficient studies to assess variation of age, diseases or health status, and occupation of the participants, which might make a big difference in biomarker response to changes in PM level.

For the rest of the biomarkers, there were limited numbers of studies available. Meta-analyses were not performed in case there was only one single study identified or the measurements of the effects were different between studies (e.g. either present in percentage change or differences in absolute change). Health effects of the biomarkers were well-known for some toxic pollutants such as polycyclic aromatic hydrocarbon (PAH) or black carbon, but their associations with the WHO criteria air pollutants were largely unknown. More future researches are needed to assess the evidence for the association of these biomarkers with the WHO criteria pollutants.

In this project, we were interested in the oxidative stress effects of $PM_{2.5}$. The oxidative stress related biomarkers, including 8-OHdG, 8-isoprostane, Cu/Zn-SOD, MDA, EBC pH, Fe, nitrate and FeNO, which were found to have significant associations, were described in details.

For 8-OHdG, significant associations were found in four studies, one in China and three in the US. The one in China was a panel study for residents in Beijing, which reported the largest percent changes in 8-OHdG of 57.6% (95% CI: 26.1, 97.0) at lag 1 per inter-quartile range (IQR) increase in $PM_{2.5}$ exposure (Gong et al. 2014). For the US studies, one of them was a panel study for workers in trucking industry, for which 21% (95% CI: 2.0, 42.0) increase in 8-OHdG was associated with IQR increase in the exposure (Neophytou et al. 2013). The second US study in Boston focused on elderly, for which the corresponding increase in 8-OHdG was 30.8% (95% CI: 9.3, 52.2) (Ren et al. 2011). The third US study focused on workers in a power plant, for each 1 mg/m^3 increase in $PM_{2.5}$ exposure was associated with 1.67 μg/g increase (95% CI: 0.21, 3.14) in 8-OHdG levels (Kim et al. 2004).

For exhaled breath condensate [EBC] biomarkers, it was assessed in the above-mentioned Beijing study, showing the largest percentage increase in FeNO of 40.7% (95% CI: 26.1, 57.0) at lag 0; in EBC pH of 1.21% (95% CI: 0.39, 2.03) at lag 1; and in EBC nitrite of 21.9% (95% CI: 12.0, 32.6) at lag 0, for an IQR increase in $PM_{2.5}$ exposure (Gong et al. 2014). In another Beijing study focusing on children, an increase in FeNO of 18.7% (95% CI: 15.0, 22.5) was associated with an IQR increase in $PM_{2.5}$ exposure (Lin et al. 2011). The other study on FeNO was conducted for schoolchildren in California, in which 24 $\mu g/m^3$ increase of personal $PM_{2.5}$ was associated with 1.1

ppb increase (95% CI: 0.1, 1.9) in FeNO (Delfino et al. 2006). In a study of adolescents in New York City, a change (decrease for negative change) in EBC pH of -0.15 unit (95% CI: -0.28, -0.02) was associated with an IQR increase in $PM_{2.5}$ exposure at lag 1 (Patel et al. 2013). In a study of adults in London, a change (decrease for negative change) in EBC Fe of -116 units (95% CI: -223, -7.78) was associated with 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure (Zhang et al. 2009).

For 8-isoprostane, a study for adolescents in New York showed that a 9.9 $\mu g/m^3$ increase in 3-day average $PM_{2.5}$ was associated with a 0.38 unit increase (95% CI: 0.11, 0.56) in natural log-transformed measurement of the biomarker (Patel et al. 2013). For MDA, the same Beijing study showed the largest percentage increase of the biomarker measured in urinary of 15.3 (95% CI: 3.4, 28.4) was associated with an IQR increase of $PM_{2.5}$ exposure at lag 0 (Gong et al. 2014). In study of schoolchildren in four cities (two in China and two in Korea), 0.0275 mmol/g creatinine increase of MDA ($p<0.0001$) was associated with 10 $\mu g/m^3$ increase in $PM_{2.5}$ exposure measured from the current day to the two previous day (Bae et al. 2010). For Cu/Zn SOD, a study for elderly in Mexico, the biomarker was inversely related to $PM_{2.5}$ exposure ($\beta = -0.05$, $p = 0.001$) (Romieu et al. 2008).

For the other pollutants (PM_{10}, NO_2, SO_2 and O_3), we had performed the respective meta-analyses as shown in the Appendix Tables. There were significant associations between these pollutants and several biomarkers. Briefly, each 10 $\mu g/m^3$ increase of a pollutant, for PM_{10} was associated with 0.14 mg/dl increase of fibrinogen, and 0.58% increase in TNF-α; for NO_2 with 0.25% increase of fibrinogen, and 4.97% increase of myeloperoxidase; for SO_2 with 0.49% increase of fibrinogen; and for O_3 with 0.66% increase of CRP, and 1.42 mg/dl increase of fibrinogen.

Table 1 Percentage (%) change in oxidative stress biomarkers concentrations per 10 μg/m^3 increase in $PM_{2.5}$

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change$^+$	SE for % change	Reference
8-OHdG	2008	125 adults, age 22-27	Beijing, China	Panel	2.22 (3.7) mg/mol[a]	**4.977**	**2.022**	Gong et al 2014
	2009-2010	67 men in trucking industry	North-eastern US	Panel	55.9 (31.5) μg/g creatinine	**44.88**	**20.23**	Neophytou et al 2013
	2006-2008	320 elderly men	Boston, US	Longitudinal	20.8 (12.3) ng/ml	**30.58**	**13.09**	Ren et al 2010
	1999	20 power plant workers	^Boston, US	Repeated measures	13.26 (1.04) μg/g creatinine	**#0.0167**	**0.00852**	Kim et al 2004
					Random effect	*21.65*	*12.70*	
EBC pH	2008	125 adults age 22-27	Beijing, China	Panel study	7.43-7.61[a]	**0.154**	**0.0638**	Gong et al 2014
	2003-2005	60 adults age 18-55	London, UK	Crossover	7.9-8.1[a]	-0.17	0.29	Zhang et al 2009
	2005	36 adolescents age 14-19	^New York, US	Panel study	8 (5.8-8.2) [b]	**#-0.152**	**0.067**	Patel et al 2013
					Fixed effect	***0.138****	***0.0622***	
FeNO	2008	125 adults age 22-27	Beijing, China	Panel study	5.8-12.51[a] ppb	**5.301**	**0.971**	Gong et al 2014
	2007-2008	36 students age 9-12	Beijing, China	Panel study	13.7 (7.9) ppb	**1.255**	**0.127**	Lin et al 2011

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
	2003-2005	60 adults age 18-55	London, UK	Crossover	39.4-50.3[a] ppb	1.89	2.74	Zhang et al 2009
	2003-2004	45 school children age 9-18	California, US	Panel	25.6 (25.1) ppb	**#0.421**	**0.185**	Delfino et al 2006
					Random effect	*2.895*	*1.662*	
8-isoprostane	2005	36 adolescents age 14-19	New York, US	Panel	48.6 pg/ml (22.1-72.9)[b]	**#0.384**	**0.139**	Patel et al 2013
Cu/Zn-SOD	2001-2002	52 elderly	Mexico	Randomized controlled trial	0.68-0.76 (0.04-0.05) IU/mL	**#-0.05**	**0.02**	Romieu et al 2008
EBC Fe	2003-2005	60 adults age 18-55	London, UK	Crossover	68-323[a] nmol/L	**-116**	**54.6**	Zhang et al 2009
EBC nitrate	2008	125 adults age 22-27	Beijing, China	Panel	2.61-4.23[a] µM	**2.852**	**0.655**	Gong et al 2014
GSH	2001-2002	52 elderly	Mexico	Randomized controlled trial	3.66-4.38 (1.39-1.7) µM	#0.06	0.05	Romieu et al 2008
MDA	2008	125 adults age 22-27	Beijing, China	Panel	311-483 (1.1-12.8) nM	**1.988**	**0.786**	Gong et al 2014
MDA	2007	120 school children mean age 9.46-11.9	4 cities, Korea & China	Panel	1.2[c] mmol/g creatinine	**#0.0275**	**0.0049**	Bae et al 2010

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
TBARS	2003-2005	60 adults age 18-55	London, UK	Crossover	2.14-2.5[a] µmol/L	-3.37	6.44	Zhang et al 2009

Note: ^Studies were excluded from meta-analysis due to different in units or estimation.

+Text for statistical significant changes were **Bold**.

#The changes were in absolute unit change.

*$p < 0.05$

[a] Range of mean, SD is not provided.

[b] Only median (range) is provided in the study.

[c] Least square mean of 4 cities.

2.5 Discussion

Among the different mechanisms of air pollution effects on the human health, oxidative stress is one of them, for which the effects of traffic-related air pollution have been assessed (Miller 2014). Oxidative stress triggers a number of redox sensitive signaling pathways. There is a large body of evidence showing that the pulmonary inflammatory response and cardiovascular actions arising from exposure to air pollutants, are mediated via oxidant signaling pathways (Anderson et al. 2012; Auerbach and Hernandez, 2012; Mills et al. 2009). In this study we focus on the oxidative stress effects of $PM_{2.5}$ on 8-OHdG, MDA, and GSH/GSSG ratio.

2.5.1 8-OHdG as Biomarker for Exposure to $PM_{2.5}$

Our review clearly showed that 8-OHdG was associated with $PM_{2.5}$ exposure. Besides the effects of $PM_{2.5}$, a cross-sectional study on non-smoking bus drivers and healthy adults who stayed mostly indoors as controls in Prague, the effects of other air pollutants including PAH, benzopyrene, benzene, toluene, ethylbenzene, m-p-xylene, o-xylene and ozone were also assessed. The results consistently indicated that 8-OHdG levels were associated with both $PM_{2.5}$ and PM_{10}, in that when compared with the controls, bus drivers who were exposed to 10 and 15 $\mu g/m^3$ higher in the respective pollutants were associated with higher levels of the biomarker ($p<0.001$) (Rossner et al. 2007; Rossner et al. 2008). Thus 8-OHdG is clearly a biomarker for assessing exposure to traffic-related particulate pollutants.

2.5.2 MDA as Biomarker for Exposure to $PM_{2.5}$

The cross-sectional study of 120 schoolchildren in four cities (two in China and two in Korea) has shown that increase in urinary MDA was associated with the ambient daily PM concentrations (Bae et al. 2010). The associations may be due to the sub-species of $PM_{2.5}$, including magnesium, iron, strontium, arsenic, cadmium, zinc, aluminium, mercury, barium and copper, which also had significant associations with MDA level. These findings support the use of MDA as a biomarker for exposure to PM in epidemiology study.

2.5.3 GSH/GSSG Ratio as Biomarker for Exposure to $PM_{2.5}$

The ratio between Glutathione (GSH) and its oxidized form (glutathione disulfide, GSSG) would be a measure for oxidant-antioxidant balance in the body. In an animal study, for mice, decreased GSH/GSSG ratio was associated with exposure to residual oil fly ash, which contained high level of PM (Marchini et al. 2013). In a study of human aortic endothelial cells exposure to ultrafine particles (UFP, diameter <200 nm) was associated with exposure to increase in GSSG/GSH ratio (Du et al. 2013). A study in Indian children living in household using biomass as fuel, similar association

was shown (Padhy and Padhi, 2009). We believe that the role of $PM_{2.5}$ is a potential mediator in the oxidation pathway for reduction of GSH by reactive oxygen species and hypothesize it to be a metabolic disruptor.

3. Blood Sample Collection and Biomarker Measurement

3.1 Recruitment

In planning for recruitment of participants for blood sample taking and biomarker measurement, we had performed two site visits, one in Central/Western on 22 January, 2014 and the other in Mongkok on 28 January, 2014. We identified residential buildings in some suitable locations and sent out 2,810 invitation letters (Appendix 1) to the addresses there. The buildings were 0.1 to 1.5 km away from the air monitoring station in each region.

We received 151 calls from the invited residents from February to April, 2014. They were then screened for recruitment into the study by the following criteria:

1. Often live in the invited residential buildings
2. Chinese
3. Age 50 to 65 years
4. Non-smoker
5. No known chronic diseases

We successfully screened and recruited 120 eligible participants into the study. The overall response rate was 4.3% which is regarded as satisfactory.

3.2 Clinical Visit

We scheduled an appointment for taking blood samples, health measurement and administration of questionnaire interview with the participants at our study centre located in the Teaching Laboratory of the Li Ka Shing Faculty of Medicine, The University of Hong Kong, at 21 Sassoon Road, Pokfulam, Hong Kong for clinical visit. For the 120 eligible participants, 109 of them came to our study centre at Sassoon Road in July 2014 (Table 2).

Two eligible participants had changed their residential address to other region and were therefore excluded from the study. Nine of the eligible participants withdrew from the study, with the following reasons:

a) objection of family members;
b) not able to arrange the time for the study;
c) not willing to participate in the study; and
d) the research site too far away (living in Mongkok region).

When the participants came to the study center, they were given the information sheet (Appendix 1), which contained the background information of the study. For any enquires about the study, the investigator answered their questions on the site. After that, the participants signed the consent form (Appendix 2) for taking part in the study.

The visit included questionnaire interviewing, blood collection, blood pressure and anthropometry measurement. We obtained the smoking history and used a smokerlyzer (piCO$^+$ smokerlyzer, Bedfont Scientific Ltd., UK) for validation of smoking status and assessment of second-hand-smoke exposure of the participants (Table 2). The smoking-related data will be used mainly in sensitivity analyses.

Table 2. Description of the Smokerlyzer Readings

Description	Reading (ppm) [a]	%COHb [b]
Non smoker	0–6	0.79–1.59
Danger zone	7–10	1.75–2.23
Smoker	11–15	2.39–3.03
Frequent smoker	16–25	3.19–4.63
Addicted smoker	26+	4.79+

[a] ppm stands for parts per million, i.e. one part of carbon monoxide (CO) in one million parts of air (breath).
[b] %COHb stands for the percentage of CO combined with haemoglobin in the blood.

After validating the smoking status of the participants, we measured the blood pressures for the participants twice, with at least 5 minutes interval between two measurements. When the first two readings differed by more than 5 mmHg, additional readings (one or two) were obtained before taking the average.

Height and weight were measured by a calibrated medical scale and a stadiometer. The participants also completed the questionnaire (Appendix 3), which asked for the individual characteristics including socio-demographic, lifestyles (such as indoor and outdoor air pollution exposure, daily activities and recent diets), and medical history of the participants. We collected blood samples as described in Section 3.3 below.

We scheduled the second, third and fourth appointments with the participants for the follow-up clinical visits. The contents of the three follow-up visits were similar to those of the first visit, with slightly modified questionnaire questions. For the 109 participants in the July 2014 fieldwork, 103 of them came to the study centre again in October 2014, 100 of them came in January 2015, and 99 of them came in April 2015. In total, nine participants withdrew from the study after the first visit,

with the same reasons mentioned above in this section. One female participant was lost to follow-up due to change of her contact number and residential address. The successful follow-up rate was 90.8% which is regarded as satisfactory (Table 3).

Table 3. Number of Participants and Follow-up Rate in the Study

Visits	**Jul 2014**		**Oct 2014**		**Jan 2015**		**Apr 2015**	
Date of visit	**Date**	**No.**	**Date**	**No.**	**Date**	**No.**	**Date**	**No.**
	2nd	17	6th	13	7th	18	15th	18
	4th	17	8th	16	9th	16	17th	19
	7th	22	10th	15	12th	14	22nd	18
	8th	9	13th	10	14th	10	24th	17
	9th	33	17th	14	15th	9	25th	9
	11th	3	21st	6	16th	10	29th	11
	21st	5	22nd	9	21st	10	30th	7
	31st	3	24th	10	24th	6		
			25th	3	28th	7		
			29th	5				
			31st	2				
Participant (n)								
CW		58		57		56		56
MK		51		46		44		43
Total		**109**		**103**		**100**		**99**
Follow-up rate (%)								
CW		-		98.3		96.6		96.6
MK		-		90.2		86.3		84.3
Total		-		94.5		91.7		90.8

3.3 Blood Sample Taking and Processing

3.3.1 Justification for Collecting Blood Samples

Blood and urine are the two most feasible fluid from human body for the measurement of biomarkers. For taking other tissue samples, it would encounter sampling difficulty, analytic enrichment, and sample preparation complexity arising in the bio-monitoring (Shen et al. 2014). In a recent meta-analysis, the mean differences between the exposed and unexposed subjects for oxidized DNA including 8-OHdG was 0.53 (95% CI: 0.29, 0.76) in blood compared with 0.52 (95%

CI: 0.22, 0.82) in urine (Møller and Loft, 2010), indicating both blood and urine samples were comparable.

Given that there is no considerable difference between the levels of oxidative biomarkers in both blood and urine types of the human samples, and only blood samples which contain the blood profile information, we decided to collect blood samples only to avoid the complexity in the process of the multiple samples.

3.3.2 Blood Sample Taking

The research nurse took blood samples (fasting for at least 6 hours, total blood volume: 22 ml) from the participants using a vacutainer apparatus and obtained the blood with the plasma and serum blood collection tubes according to the following procedures:

1. Assess the participant for any recent surgery, history of difficult blood draws, fainting, and medications that may delay clotting, such as aspirin or Coumadin.
2. The participant sits in a comfortable position when the tourniquet is to be applied.
3. The vein is palpated to look for the best site for blood drawing.
4. The site is cleaned with alcohol swabs in a circular motion starting from the inside going outwards and alcohol is applied to dry.
5. The vein is stabilized with the non-dominant hand, while the dominant hand punctures the skin at a 30-degree angle using the vacutainer apparatus.
6. Blood samples are obtained using serum and plasma blood collection tubes.
7. If blood flowed freely, the tourniquet may be loosened; the tourniquet is removed just before the last blood sample has been obtained.
8. A clean gauze pad is applied over the puncture site and the needle is then withdrawn.
9. Pressure is applied to the site for approximately two to three minutes; a band-aid is then applied to the site.
10. The labeled blood collection tubes are inverted with additives for proper mixture.

3.3.3 Blood Sample Processing

The research assistant processed the blood samples within 1 hour after blood taking according to the following procedure:

1. For plasma in Heparin tubes, they are centrifuged at 3,000 rpm for 10 minutes.
2. The supernatant is then pipetted and aliquoted (1.0 ~ 1.5 ml) into 5 × 1.8 ml plastic conical vials labeled with the subject ID and date.
3. The plasma samples are then stored at -80^{o}C freezer for laboratory analysis.
4. For serum in Plain tubes, they are allowed to clot for 30 to 60 minutes at room temperature.
5. The tubes are then centrifuged for 1,800 g for 15 minutes.
6. The supernatant is then pipetted and aliquoted (1.0 ~ 1.5 ml) into 5 × 1.8 ml plastic conical vials

labeled with the subject ID and date.

7. The serum samples are then stored at -80^{o}C freezer for laboratory analysis.

3.3.4 Assay for Biomarkers

PM-induced reactive oxygen species (ROS) can cause oxidative damage to DNA and lipids of the cell membrane, leading to the formation of stable compounds such as the well-known 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Wu et al. 2004; Valavanidis et al. 2009) and malondialdehyde (MDA) (Tagesson et al. 2005; Inaba et al. 2011), respectively, which were analyzed in this study. The GSH/GSSG ratio, which reflects oxidant-antioxidant balance were also measured. In addition, we study the blood profile, including high density lipoprotein cholesterol (HDL-C) and triglycerides for understanding of the cardio-metabolic health status for covariate adjustments of the PM-induced oxidative stress. All the biomarkers were analyzed using commercially available assays kits (Table 4) according to manufacturer's protocols with appropriate amendment if necessary (Appendix 4).

We used colorimetric and fluorometric assay kits for the determination of MDA, GSSG/GSH ratio, HDL-C and Triglyceride. For 8-OHdG, we used the enzyme immunoassay and enzyme-linked immunosorbent assay (ELISA) developed by JaICA Ltd., which has been widely used in many studies (Miyaoka et al., 2015; Morillas-Ruiz et al., 2005; Saito et al., 2000). The advantage of using ELISA to determine the biomarkers is on their being highly sensitive to detect substances in the body (Gan and Patel, 2012), with high throughput, no requirement for pretreatment of the samples (Cooke et al., 2008), and thus minimum the loss in the valuable human samples. The use of commercially available kits reduces the time needed for assay standardization and optimization of the regents, as well as containing less health-hazardous chemicals.

Table 4: Assay Kits for Measurement of Biomarkers

Biomarkers	Company (Cat No.)
8-OHdG	JaICA (KOG-200S/E)
MDA	BioVision (K739-100)
GSSG/GSH ratio	Abcam (ab156681)
HDL-C	BioVision (K613-100)
Triglyceride	BioVision (K622-100)

3.4 Statistical Method

3.4.1 Descriptive Statistics

We performed descriptive statistics for each criteria air pollutant and for each health endpoint (biomarker). We calculated means, standard deviations, median and inter quartile range (IQR) for each visit and each region by evaluating all biomarkers as continuous responses using linear modelling techniques.

3.4.2 Relationships Between Pollutants and Biomarkers

Due to the repeated measures design for the subjects in the study, we applied the linear mixed model with random effect to assess the intraclass correlation coefficient (ICC) of biomarkers within the subjects (two-level), or within districts and subjects (three-level). We assumed equicorrelation between all observations within subjects in the model to account for correlation within subjects (Zhang et al. 2013). We evaluated the relationship between a biomarker and $PM_{2.5}$ across the entire study period. $PM_{2.5}$ concentrations were measured by the EPD air monitoring stations from the current day up to 7 days before the blood samples were taken. We examined the associations by adding the $PM_{2.5}$ concentrations to the mixed linear models (with period indicators) to adjust for the period effect (by a variable indicating whether the measurement was taken in July 2014, October 2014, January 2015, or April 2015). We conducted the analyses using the R software nlme and mixlm packages (R version 3.2.0).

3.5 Ethics and Institutional Review Board Approval

The study involved human subjects and the study protocol was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (IRB reference number UW 14-277). Written informed consent was obtained from all participants. Upon the completion of each clinical visit, an honorarium was offered to each participant to compensate them for their time and for transportation allowance. The questionnaire responses were with password protection. By securing the data and ensuring that only the investigators and designated study staff members had access to records, participants' identities were completely protected in compliance with human subject guidelines.

4. Results and Discussion

4.1 Descriptive Summary of the Participants

We had successfully invited 109 participants to complete the research in the July clinical visit, in which 58 (53.2%) were living in the Central/Western (CW) region, while the rest of 51 (46.8%) were living in the MongKok (MK) region. The characteristics of the participants were summarized in Table 5 below.

Table 5. Baseline Descriptive Summary of the Participants (n=109)

a) Socio-demographic factors

Region	Characteristics	CW n (%)	MK n (%)	Total n	P-value for Chi-square
Gender	Male	20 (34.5)	15 (29.4)	35	0.572
	Female	38 (65.5)	36 (70.6)	74	
Age	(mean ± SD)	58.4±4.9	58.9±4.6	58.6±4.7	0.609^
Floor level	(mean ± SD)	13.3±8.4	5.4±3.2	9.6±7.6	<0.001^
Year of living	(mean ± SD)	15.9±8.5	15.9±10.2	15.9±9.3	0.988^
Marital	Single	2 (3.5)	3 (5.9)	5	0.108
	Married	48 (82.8)	41 (80.4)	89	
	Divorced/widowed	8 (13.8)	7 (13.7)	15	
Education	Primary	2 (3.5)	6 (11.8)	8	0.299
	Form 1-5	17 (29.3)	16 (31.4)	33	
	Form 6-7	7 (12.1)	3 (5.9)	10	
	Diploma	7 (12.1)	3 (5.9)	10	
	Tertiary or above	25 (43.1)	23 (45.1)	48	
Monthly Expenditure	$2000-2999	8 (13.8)	2 (3.9)	10	0.272
	$3000-4499	11 (19.0)	8 (15.7)	19	
	$4500-6699	11 (19.0)	12 (23.5)	23	
	$6700-9999	17 (29.3)	13 (25.5)	30	
	≥$10000	11 (19.0)	16 (31.4)	27	
Housing	Self-owned flat	54 (93.1)	44 (86.3)	98	0.238
	Rented	4 (6.9)	7 (13.7)	11	
Occupation	Full-Time	21 (36.2)	16 (31.4)	37	0.823
	Part-Time	6 (10.3)	4 (7.8)	10	
	Housewife/retired	31 (53.5)	31 (60.8)	62	
Body Mass Index (BMI)	Underweight (<18.5)	5 (8.6)	2 (3.9)	7	0.510
	Normal (18.5-22.9)	26 (44.8)	20 (39.2)	46	
	Overweight (23-24.9)	13 (22.4)	17 (33.3)	30	
	Obesity (≥25)	14 (24.1)	12 (23.5)	26	

^ p-value for T-test.

b) Lifestyle factors

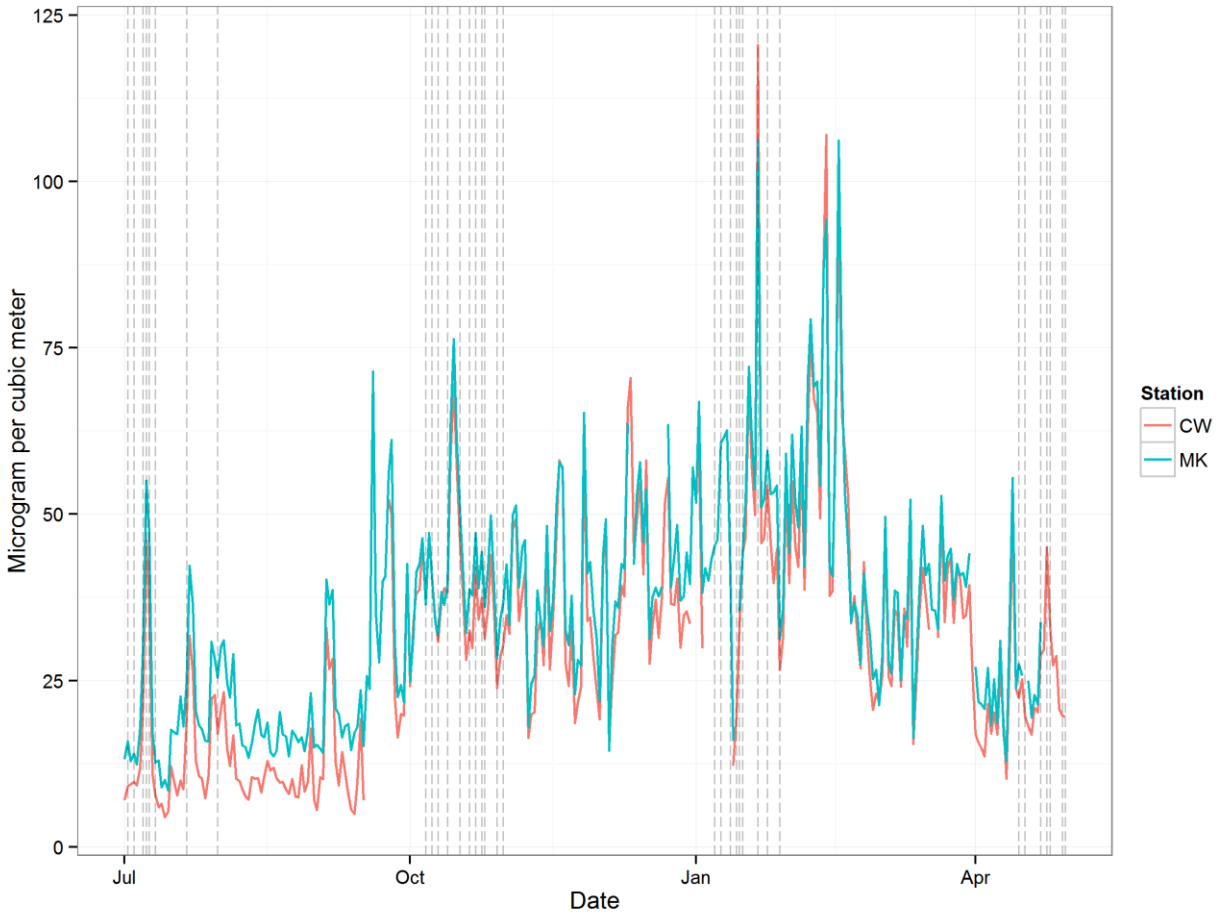
Region	Characteristics	CW n (%)	MK n (%)	Total n	P-value for Chi-square
Smoking history	Never smoker	55 (94.8)	49 (96.1)	104	0.891
	Ex-smoker	3 (5.2)	2 (3.9)	5	
Passive smoking	No	46 (79.3)	37 (72.5)	83	0.409
	Yes	12 (20.7)	14 (27.5)	26	
Exposure (Hour/week)	(mean ± SD)	2.7±3.0	1.2±0.8	1.9±2.2	0.395^
Alcohol Drinking Frequency	Never OR 1-2/year	37 (63.8)	28 (54.9)	65	0.247
	<1/month	7 (12.1)	5 (9.8)	12	
	<1/week	9 (15.5)	6 (11.8)	15	
	≥1/week	5 (8.6)	12 (23.5)	17	
Exercise Frequency	Every day	3 (5.2)	4 (7.8)	7	0.567
	4-6/week	6 (10.3)	7 (13.7)	13	
	1-3/week	15 (25.9)	17 (33.3)	32	
	<1/week	34 (58.6)	23 (45.1)	57	

^ p-value for T-test.

4.2 Air Pollutant Concentrations

The concentrations of $PM_{2.5}$ in the study period were retrieved from the EPD air monitoring stations. The levels of CW station and MK station were comparable to each other. The time-series pattern of the air pollutants over the study period was shown in Figure 2.

Figure 2. Time-series Pattern of $PM_{2.5}$ in the Study Period



--- The vertical dashed lines represent the sampling date.

Source: Past Air Quality Monitoring Data from the EPD Air Quality Monitoring Stations: http://epic.epd.gov.hk/EPICDI/air/station/?lang=en (Assessed 10 September 2015)

4.3 Biomarkers and Physiologic Endpoints

4.3.1 Reliability of Laboratory Measured Biomarkers

The concentration of oxidative stress biomarkers (8-OHdG, MDA, and GSH/GSSG ratio) and blood lipids (HDL-C and triglyceride) were estimated with the laboratory assay kits.

We used the coefficient of variation (CV) to assess the precision and reliability of quantitative assay (Reed, Lynn and Meade, 2002). The CV is calculated by the ratio of the *standard deviation* to the *mean* (Everitt, 1998) in the triplicate analysis of each individual sample. The value of CV less than 20% is set as the criteria to interpret the inter-sample precision and reliability of the biomarkers level as acceptable (DeSilva et al., 2003). Proportions of our samples with CV greater than 20% range from 0 to 4.6% (Table 7). The results showed that the current measurement of the biomarkers with medians CV less than 10% for all the measurements were reliable and highly precise.

In addition, the measured levels of the biomarkers were comparable to the values reported in other studies. The mean HDL-C and triglyceride levels for the two regions of participants were also within the normal level for generally healthy adults (Table 8).

Table 7. Coefficient of Variation (median, range); Percentage of Coefficient of Variation Greater than 20%

	July 2014		**October 2014**		**January 2015**		**April 2015**	
Biomarker	Median (range)	%*	Median (range)	%	Median (range)	%	Median (range)	%
8-OHdG	4.7 (0 - 28)	1.8	5.9 (0 - 17)	0	3.4 (0 - 21)	3.0	6.1 (0 - 14)	0
MDA	2.6 (0 - 27)	1.8	3.3 (0 - 21)	1.0	2.1 (0 - 29)	3.0	3.5 (0 - 28)	3.0
GSH/GSSG	3.0 (0 - 25)	4.6	2.6 (0 - 22)	1.0	0.8 (0 - 21)	1.0	0.7 (0 - 8)	0
HDL-C	2.9 (0 - 34)	1.9	2.6 (0 - 29)	1.0	3.2 (0 - 27)	1.0	5.3 (0 - 18)	0
Triglyceride	3.4 (0 - 50)	0.9	5.3 (0 - 24)	1.9	4.7 (0 - 26)	1.0	5.0 (0 - 26)	1.0

*%: the percentage of CV greater than 20%

Table 8. Comparability of the Measured Biomarker Level to Reported Level

Biomarker	Range of Mean Level (four visits, two regions)	Reported mean range value*/ Normal level
8-OHdG	7.3 – 10.5 ng/ml	0.2 – 12.3 ng/ml
MDA	1.32 – 1.95 nmol/ml	0.8 – 5.5 nmol/ml
GSH/GSSG	3.23 – 5.07	1.5 – 160
HDL-C	0.98 – 1.37 mmol/L	Normal level: Above 0.9 mmol/L
Triglyceride	0.42 – 0.65 mmol/L	Normal level: Below 1.5 mmol/L

*Please refer to Appendix Table 6 for the review on the reported levels of the oxidative biomarkers.

4.3.2 Descriptive Results for the Biomarkers

The levels of the oxidative stress and blood lipids biomarkers, and the heart rate and blood pressure level measured by the blood pressure monitor were summarized in Table 9. There was no statistically significant difference of the levels of biomarkers between the two regions in the four visits, respectively. Figure 3A-3F showed the concentrations of the oxidative biomarkers for the four visits. The patterns among the four visits were complex for both CW and MK.

Table 9. Summary Statistics of Biomarkers

A) Central/Western

Biomarker / Visit	Mean (SD)	Median (1st to 3rd quartile)
Oxidative Stress		
8-OHdG (ng/ml)		
Jul 2014	10.5 (7.88)	7.64 (5.98, 11.92)
Oct 2014	9.30 (7.49)	7.06 (4.61, 10.42)
Jan 2015	9.90 (9.13)	6.88 (5.15, 10.25)
Apr 2015	7.31 (6.92)	5.81 (3.92, 7.70)
MDA (nmol/ml)		
Jul 2014	1.40 (0.95)	1.02 (0.68, 1.85)
Oct 2014	1.47 (1.06)	1.35 (0.50, 2.17)
Jan 2015	1.72 (0.87)	1.83 (0.87, 2.33)
Apr 2015	1.94 (0.69)	1.48 (1.10, 1.69)
GSH/GSSG		
Jul 2014	3.38 (2.48)	2.55 (2.09, 3.86)
Oct 2014	3.23 (1.45)	2.9 (2.34, 3.87)
Jan 2015	3.28 (1.23)	2.99 (2.56, 3.76)
Apr 2015	3.33 (0.90)	3.09 (2.76, 3.63)
Lipid profile		
HDL-C (mmol/L)		
Jul 2014	1.28 (0.69)	1.03 (0.72, 1.78)
Oct 2014	0.99 (0.39)	0.93 (0.70, 1.23)
Jan 2015	0.98 (0.36)	1.03 (0.67, 1.29)
Apr 2015	1.15 (0.51)	1.05 (0.72, 1.39)
Triglyceride (mmol/L)		
Jul 2014	0.46 (0.29)	0.43 (0.29, 0.58)
Oct 2014	0.61 (0.33)	0.52 (0.36, 0.86)
Jan 2015	0.44 (0.30)	0.36 (0.23, 0.62)
Apr 2015	0.45 (0.24)	0.41 (0.24, 0.63)
Autonomic measurement		
Heart rate (bpm)		
Jul 2014	66.9 (8.48)	67.0 (61.5, 73.6)
Oct 2014	67.8 (8.77)	66.5 (61.5, 75.8)
Jan 2015	71.6 (9.12)	72.0 (65.4, 77.0)
Apr 2015	69.3 (8.45)	69.5 (63.0, 74.0)
DBP (mmHg)		
Jul 2014	78.2 (9.44)	77.0 (72.1, 86.1)
Oct 2014	80.6 (10.5)	80.0 (73.5, 87.8)
Jan 2015	82.3 (9.53)	80.3 (75.1, 88.9)
Apr 2015	78.4 (8.97)	76.5 (70.0, 82.5)
SBP (mmHg)		
Jul 2014	124.3 (15.3)	125 (113.3, 133.4)
Oct 2014	129.0 (17.0)	129 (117.8, 136.0)
Jan 2015	129.5 (16.3)	126 (117.6, 140.8)
Apr 2015	126.4 (15.2)	120 (117.5, 138.0)

B) Mongkok

Biomarker / Visit	Mean (SD)	Median (1st to 3rd quartile)
Oxidative Stress		
8-OHdG (ng/ml)		
Jul 2014	9.45 (6.88)	7.31 (4.90, 12.88)
Oct 2014	8.78 (6.67)	6.72 (4.39, 12.09)
Jan 2015	9.13 (6.90)	6.83 (4.42, 10.52)
Apr 2015	7.53 (5.25)	5.64 (3.71, 9.49)
MDA (nmol/ml)		
Jul 2014	1.32 (0.77)	1.13 (0.71, 1.61)
Oct 2014	1.68 (1.27)	1.53 (0.57, 2.97)
Jan 2015	1.60 (0.89)	1.33 (0.78, 2.31)
Apr 2015	1.95 (0.72)	1.46 (0.90, 1.77)
GSH/GSSG		
Jul 2014	4.87 (6.35)	2.66 (1.94, 5.20)
Oct 2014	4.03 (3.36)	2.68 (2.36, 4.93)
Jan 2015	3.41 (1.85)	2.69 (2.43, 3.75)
Apr 2015	3.40 (0.94)	3.21 (2.86, 3.63)
Lipid profile		
HDL-C (mmol/L)		
Jul 2014	1.37 (0.76)	1.06 (0.81, 1.80)
Oct 2014	1.02 (0.46)	0.92 (0.69, 1.43)
Jan 2015	1.12 (0.36)	1.16 (0.80, 1.42)
Apr 2015	1.05 (0.46)	0.98 (0.72, 1.32)
Triglyceride (mmol/L)		
Jul 2014	0.49 (0.39)	0.39 (0.24, 0.53)
Oct 2014	0.65 (0.34)	0.57 (0.34, 0.83)
Jan 2015	0.42 (0.27)	0.36 (0.22, 0.67)
Apr 2015	0.49 (0.32)	0.43 (0.25, 0.66)
Autonomic measurement		
Heart rate (bpm)		
Jul 2014	68.0 (6.15)	68.0 (64.0, 72.0)
Oct 2014	70.0 (8.94)	68.8 (63.8, 76.1)
Jan 2015	71.4 (9.56)	72.5 (64.0, 78.0)
Apr 2015	69.1 (8.29)	67.5 (60.5, 74.8)
DBP (mmHg)		
Jul 2014	77.8 (10.1)	77.5 (69.5, 84.5)
Oct 2014	78.1 (8.47)	79.0 (72.3, 82.6)
Jan 2015	81.8 (14.1)	81.0 (72.5, 87.5)
Apr 2015	77.6 (9.38)	75.5 (71.3, 86.8)
SBP (mmHg)		
Jul 2014	127.4 (18.3)	125.0 (113.5, 139.5)
Oct 2014	127.6 (14.4)	127.8 (117.3, 139.6)
Jan 2015	127.7 (18.8)	131.5 (114.0, 140.5)
Apr 2015	128.5 (17.5)	128.5 (113.8, 144.8)

Figure 3A. Level of 8-OHdG of Each Subject in the Four Visits in Central/Western

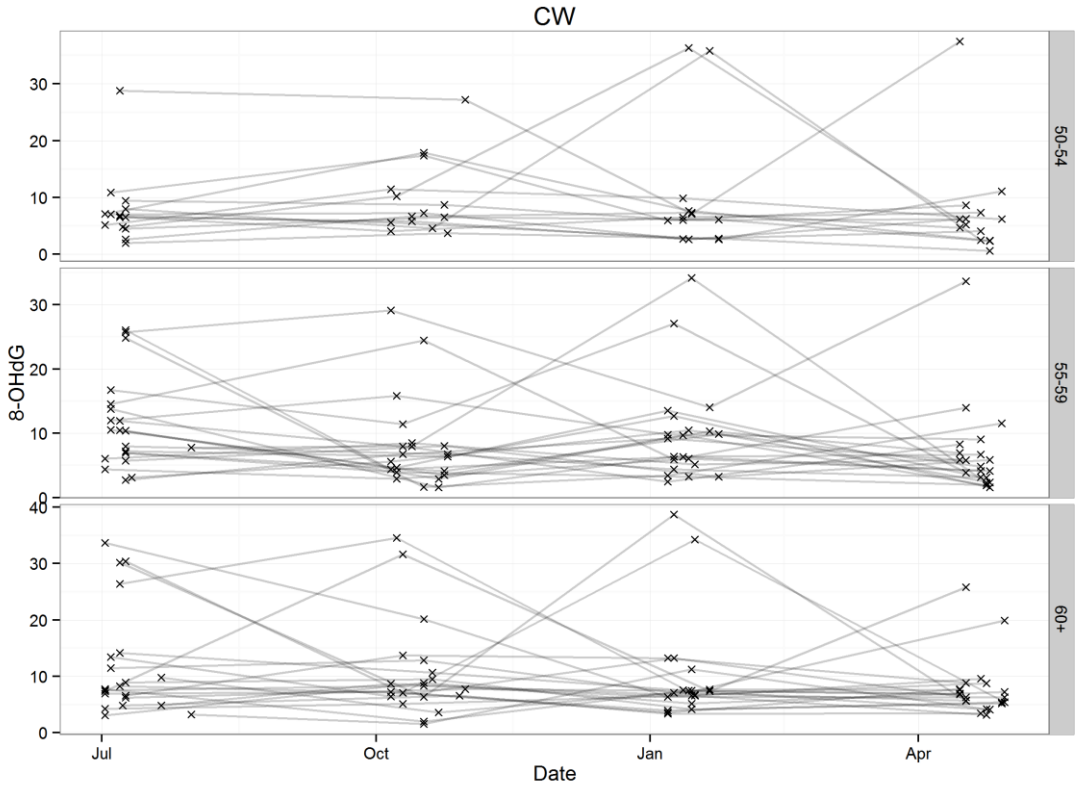


Figure 3B. Level of 8-OHdG of Each Subject in the Four Visits in Mongkok

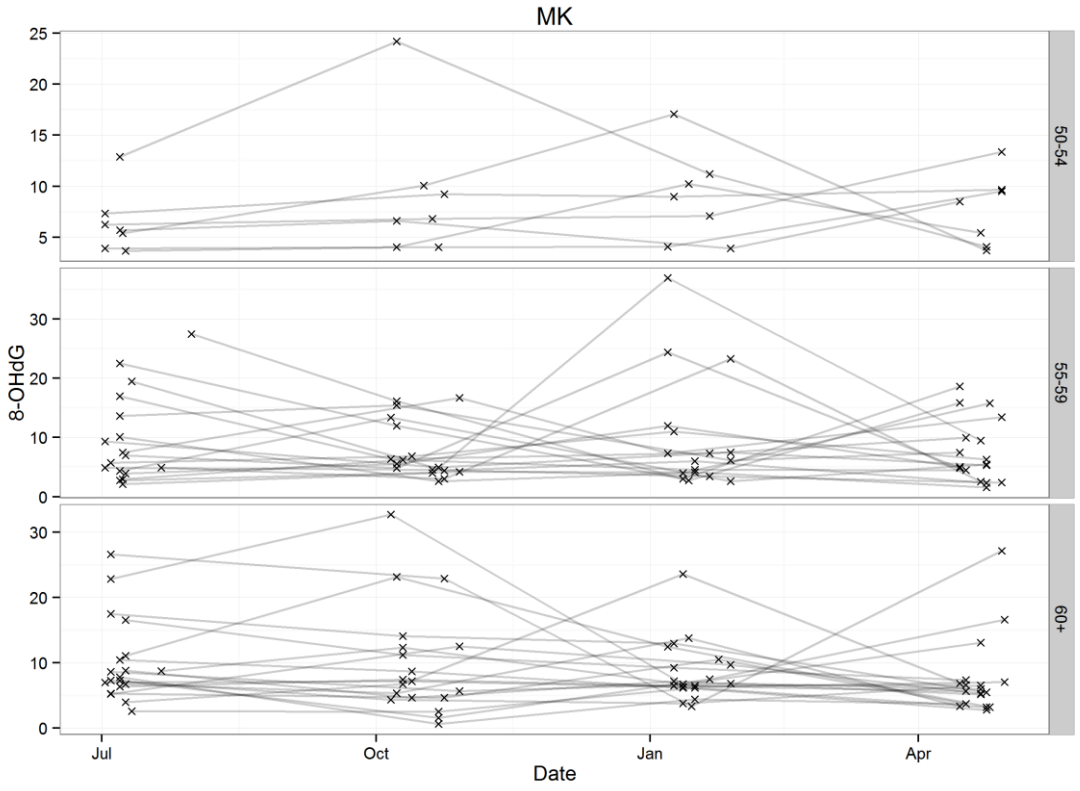


Figure 3C. Level of MDA of Each Subject in the Four Visits in Central/Western

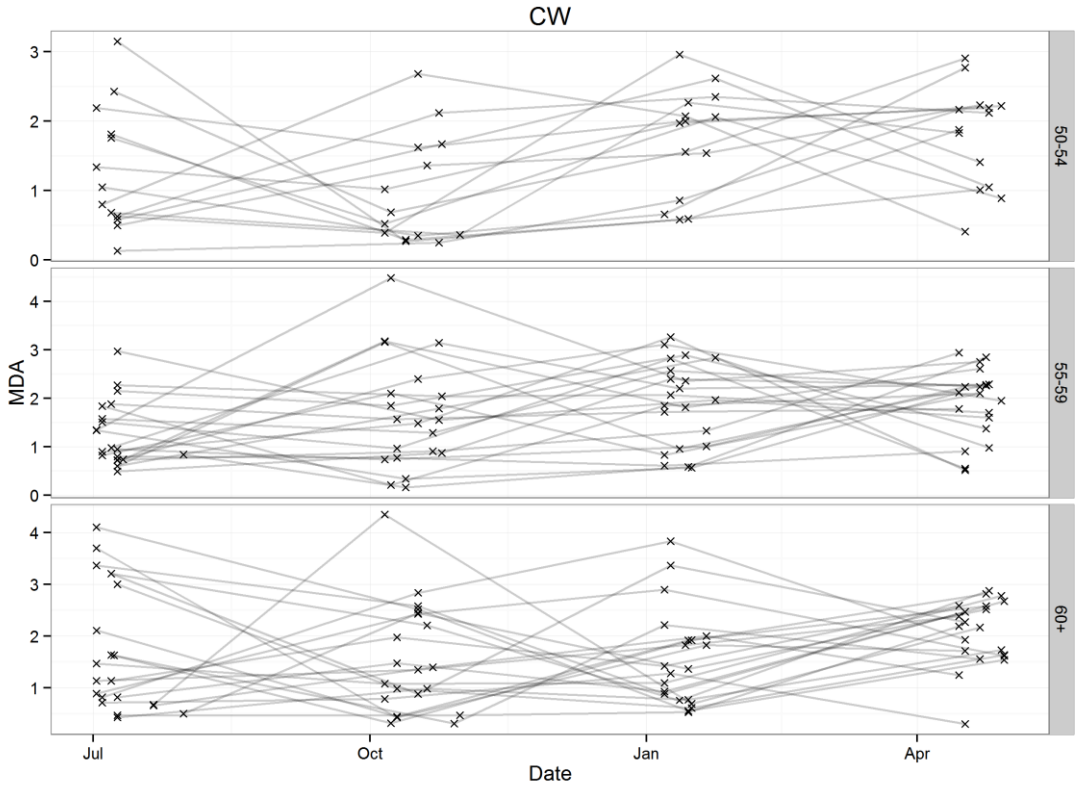


Figure 3D. Level of MDA of Each Subject in the Four Visits in Mongkok

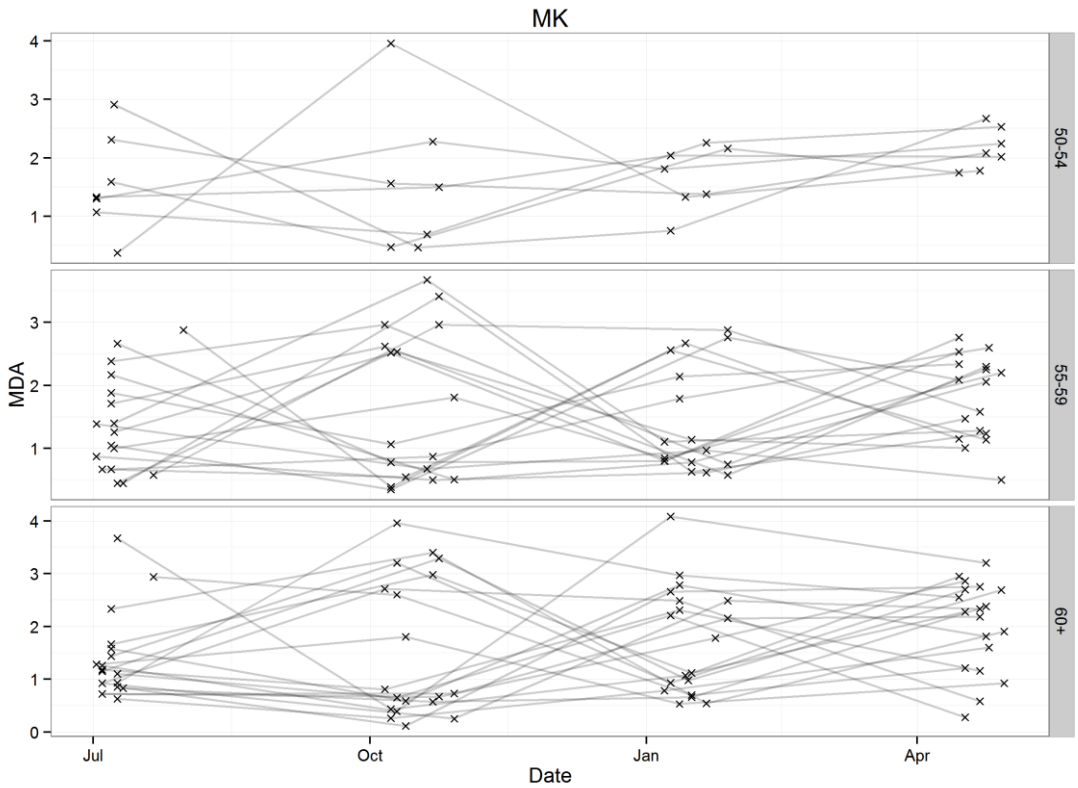


Figure 3E. Level of GSH/GSSG Ratio of Each Subject in the Four Visits in Central/Western

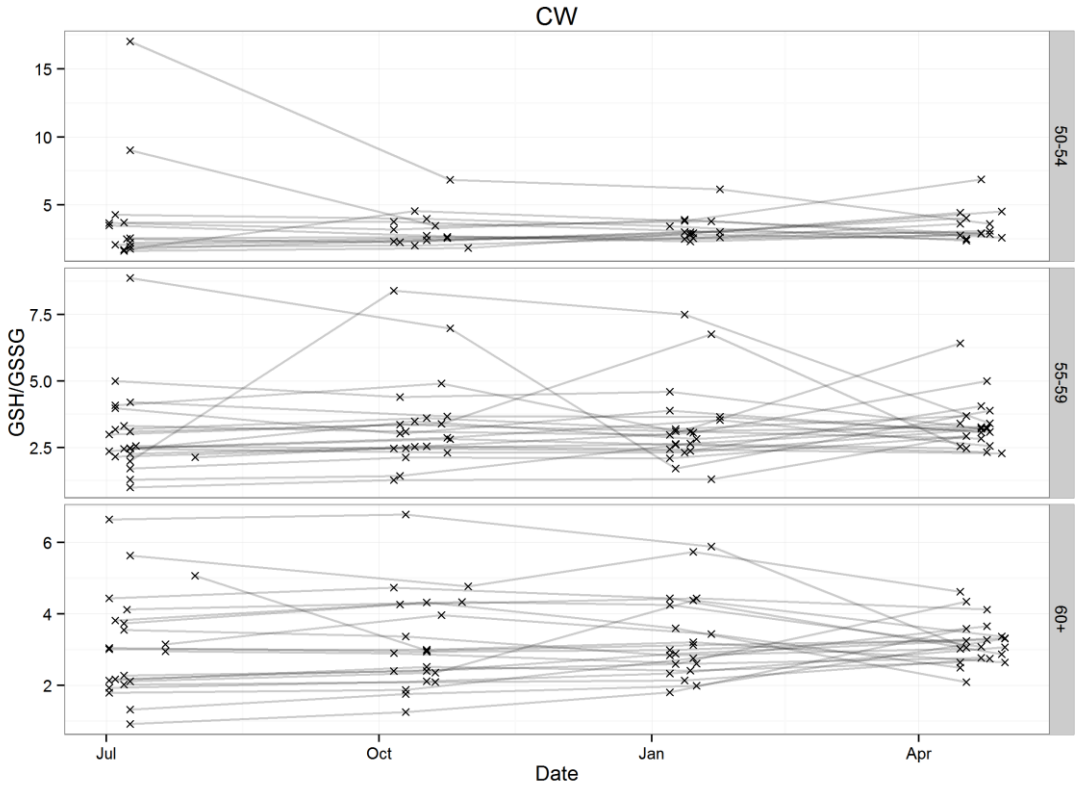
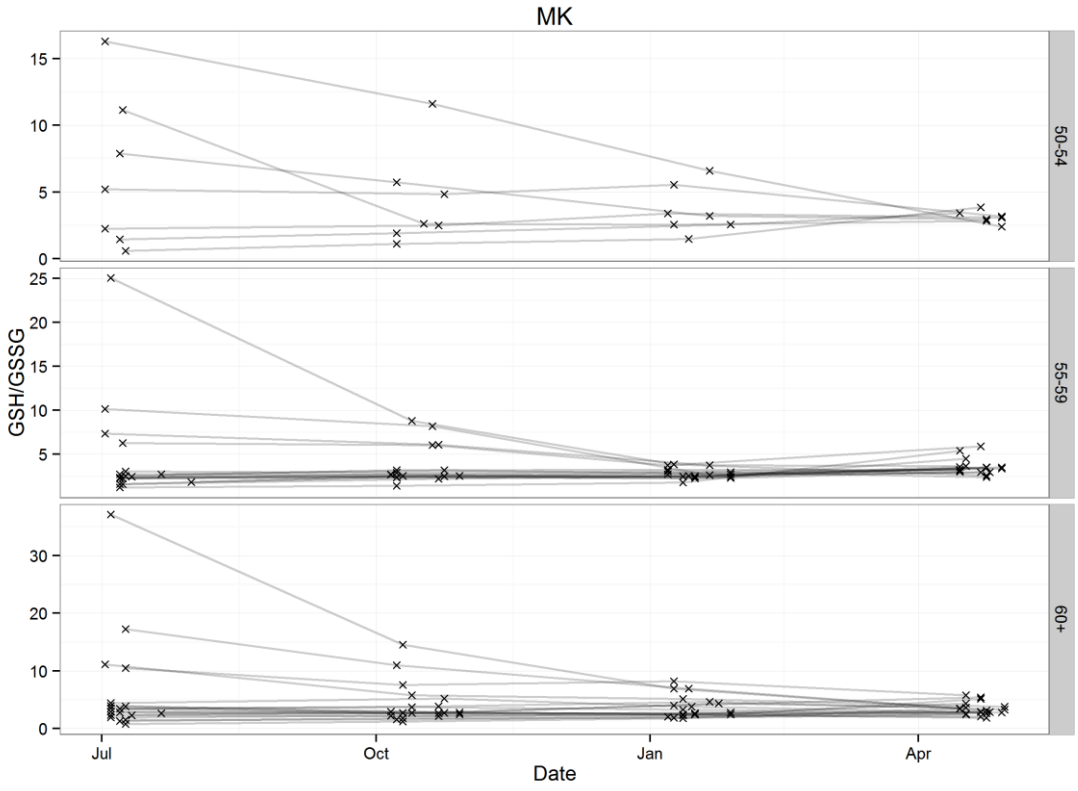


Figure 3F. Level of GSH/GSSG Ratio of Each Subject in the Four Visits in Mongkok



4.3.3 Associations of Biomarkers with Socio-demographic and Lifestyles of Participants

We compared the level of the biomarkers by the participants' demographic covariates including age, gender and BMI; lifestyles covariates including alcohol drinking frequency, exercise frequency, and passive smoking exposure; and dietary covariates including frequency of fresh fruits and vegetables intake, and drinking of tea (Table 10).

The correlations between these covariates and the level of biomarkers were summarized in Appendix Table 4. For demographic covariates, we found that gender was associated with 8-OHdG, HDL-C and blood pressure. BMI was associated with HDL-C, and age was associated with systolic blood pressure (SBP). For lifestyles covariates, heart rate was correlated with passive smoking. Interestingly, some significant associations were found between GSH/GSSG ratio and the intake of alcohol.

For dietary covariates, fresh fruits, vegetables, and tea are natural antioxidants (Gülçin, 2012). We found that tea and fresh vegetables were associated with oxidative stress biomarkers (8-OHdG and GSH/GSSG), in the direction as expected. However, we could not find any significant correlations between MDA and these anti-oxidant food and drinks. Heart rate had a strong association with green tea, which may reflect its protective action on cardiovascular diseases (Bhardwaj and Khanna, 2013).

We had also examined the correlations between biomarkers and other information obtained from the questionnaires, such as education, marital status, housing, etc. The results were not statistically significant (data not shown).

Table 10. Level of Biomarkers (Mean±SD), by Demographic and Lifestyles of Participants, July 2014

A) Demographic

	n	8-OHdG	MDA	GSH/ GSSG	HDL-C	Trigly-ceride	Heart rate	DBP	SBP
Demographic									
Age									
Age 50-59	63	9.41±7.15	1.30±0.75	4.03±4.22	1.37±0.76	0.46±0.37	66.5±7.45	77.0±10.0	122.4±17.9
Age ≥ 60	46	10.9±7.77	1.45±1.01	4.37±5.74	1.25±0.68	0.49±0.28	68.8±7.36	79.4±9.19	130.4±13.9
Gender									
Male	35	8.01±5.31	1.25±0.89	4.12±3.9	1.17±0.69	0.54±0.36	67.4±8.14	83.1±8.95	130.3±14.5
Female	74	11.0±8.09	1.42±0.86	4.2±5.33	1.39±0.73	0.44±0.32	67.5±7.19	75.6±9.17	123.6±17.4
BMI									
Underweight (<18.5)	7	12.5±5.01	1.57±1.15	4.06±2.42	1.42±0.70	0.35±0.16	71.6±10.8	76.9±6.20	121.0±8.78
Normal (18.5-22.9)	46	10.6±7.64	1.24±0.77	3.95±4.04	1.53±0.75	0.49±0.35	66.2±7.92	76.1±10.5	120.2±15.4
Overweight (23-24.9)	30	8.72±6.75	1.42±1.03	5.46±7.58	1.27±0.73	0.52±0.42	68.3±5.07	79.9±9.32	132.3±18.0
Obesity (≥25)	26	9.88±8.33	1.47±0.78	3.12±1.70	0.97±0.56	0.42±0.22	67.5±7.85	79.5±9.28	129.3±16.2

B) **Lifestyles**

	n	8-OHdG	MDA	GSH/ GSSG	HDL-C	Trigly-ceride	Heart rate	DBP	SBP
Lifestyles									
Exercise frequency									
4-7/week	20	9.76±7.29	1.29±0.74	4.11±3.82	1.64±0.88	0.56±0.51	66.6±5.78	79.7±9.13	126.5±16.1
1-3/week	32	11.7±8.03	1.29±0.88	3.95±3.32	1.31±0.75	0.43±0.27	66.3±7.51	78.2±11.1	124.3±19.7
<1/week	57	9.20±7.08	1.43±0.91	4.32±5.93	1.22±0.63	0.46±0.30	68.4±7.94	77.2±9.13	126.3±15.4
Passive smoking									
Yes	26	9.23±7.78	1.38±0.71	3.91±3.62	1.32±0.79	0.44±0.19	69.9±6.55	81.2±7.90	128.2±15.0
No	83	10.3±7.33	1.36±0.92	4.26±5.25	1.32±0.71	0.48±0.37	66.6±7.60	77.0±10.1	125.0±17.3
Alcohol drinking									
Never OR 1-2/year	65	9.68±6.83	1.40±0.89	4.05±5.32	1.18±0.65	0.41±0.30	67.3±7.80	77.7±9.33	127.0±14.1
<1/week	27	12.1±9.22	1.30±0.85	4.06±3.69	1.47±0.78	0.59±0.40	66.1±6.66	75.1±9.68	118.7±20.6
≥1/week	17	8.08±5.89	1.32±0.87	4.84±5.09	1.61±0.83	0.52±0.33	70.1±7.09	83.7±9.35	132.3±16.5
Dietary									
Fruits									
≥4-7/week	89	10.0±7.49	1.33±0.85	4.24±5.07	1.35±0.76	0.44±0.28	66.9±7.50	77.2±9.51	125.2±16.7
<3/week	20	9.96±7.25	1.51±0.95	3.86±4.12	1.17±0.55	0.59±0.52	69.6±7.10	81.3±10.2	128.2±17.1
Vegetables									
≥4-7/week	103	10.3±7.50	1.38±0.88	4.22±4.98	1.35±0.73	0.46±0.30	67.2±7.39	77.7±9.63	125.3±16.7
<3/week	6	4.99±3.22	1.16±0.59	3.47±3.28	0.89±0.46	0.71±0.73	71.6±8.30	82.5±10.9	133.4±16.7
Tea									
≥4-7/week	48	9.97±7.05	1.38±0.93	5.17±6.65	1.24±0.70	0.47±0.33	68.8±7.31	78.0±9.45	125.0±14.4
<3/week	61	10.1±7.75	1.35±0.83	3.39±2.65	1.38±0.75	0.47±0.34	66.3±7.47	78.0±9.99	126.4±18.5

4.4 Relationships between Biomarkers and Air Pollutants

4.4.1 Partial correlation of Biomarkers with $PM_{2.5}$

The partial correlation between measures of biomarkers and air pollutants, were controlled for individual demographic characteristics, lifestyles, and dietary. The results of the partial correlations for $PM_{2.5}$ in the baseline were summarized in Table 11.

For all the three oxidative stress biomarkers, there were no significant correlations with $PM_{2.5}$. For blood lipid profile, the HDL-C level measured in the participants was negatively correlated with $PM_{2.5}$ ($p<0.05$). For triglyceride, the correlations were positive and significant. Most of the autonomic measurements in the study were positively correlated with $PM_{2.5}$.

Table 11. Crude and Partial Correlation of Biomarker and $PM_{2.5}$ in Central/Western and Mongkok in Baseline

Biomarkers	Crude	Demographic [a]	Demographic & Lifestyles[b]	Demographic & Dietary[c]	Demographic, Lifestyles, & Dietary
CW					
8-OHdG	-0.0182	-0.0156	0.0421	0.0176	0.0922
MDA	-0.1598	-0.2197	-0.2050	-0.2649	-0.2587
GSH/GSSG	0.0560	0.0391	0.0745	0.0210	0.0553
HDL-C	**-0.3441****	**-0.2763***	-0.2512	**-0.3279***	-0.2905
Triglyceride	0.1025	0.1620	0.2618	0.1609	0.2790
Heart rate	0.0970	0.0857	0.0481	0.0667	0.0130
DBP	0.1588	0.1952	0.1604	0.2445	0.2161
SBP	0.1642	0.1936	0.1571	0.1970	0.1756
MK					
8-OHdG	-0.2175	-0.1787	-0.1248	-0.1800	-0.0892
MDA	0.0773	0.0702	0.1330	0.0466	0.1290
GSH/GSSG	-0.2282	-0.2745	**-0.3429***	-0.1579	-0.2390
HDL-C	-0.1681	-0.2205	-0.1742	-0.2246	-0.2076
Triglyceride	**0.3258***	**0.2941***	0.2194	0.2852	0.1961
Heart rate	-0.0458	-0.0702	-0.2221	-0.0373	-0.1504
DBP	0.0876	0.0389	-0.0840	0.0094	-0.1290
SBP	0.1784	0.1269	0.0482	0.1382	0.0252
All (CW and MK)					
8-OHdG	-0.1297	-0.1031	-0.0422	-0.1204	-0.0608
MDA	-0.0624	-0.0870	-0.0648	-0.0977	-0.0793
GSH/GSSG	-0.0817	-0.1197	-0.1155	-0.0921	-0.0999
HDL-C	**-0.2169***	**-0.2083***	**-0.2159***	**-0.2003***	**-0.2209***
Triglyceride	**0.2372***	**0.2394***	**0.2615****	**0.2398***	**0.2530***
Heart rate	0.0544	0.0529	0.0025	0.0448	-0.0052
DBP	0.1090	0.1157	0.0508	0.1059	0.0383
SBP	**0.1924***	0.1682	0.0978	0.1574	0.0858

[a] Partial correlation adjusted for demographic factors (age, gender and BMI)

[b] Partial correlation adjusted for demographic factors[a] and lifestyles (alcohol drinking frequency, exercise frequency, and passive smoking exposure)

[c] Partial correlation adjusted for demographic factors[a] and dietary (frequency of fresh fruits and vegetables intake, and drinking of tea)

*p-value < 0.05, **p-value < 0.01

4.4.2. Intraclass correlation coefficient of biomarkers

The intraclass correlation coefficient (ICC) was defined as the variance of the specified random effect component divided by the sum of the total variance of the random effect components and the error variance. It explained the proportion of the variance of the specified random effect component relative to the total variance in the model. All of the biomarkers showed very small ICC in the districts (<0.0001) except for the GSH/GSSG ratio (Table 12), which implied there should not have much variation in the district level. Therefore, we applied the two level model for all the outcomes except that the three level model was applied on GSH/GSSG ratio for the further analyses.

Table 12. Intraclass Correlation Coefficient (ICC) by Models

	Two level	Three level	
Biomarker	**Subject**	**District**	**Subject**
8-OHdG	0.2106	<0.0001	0.2717
MDA	0.0241	<0.0001	0.0289
GSH/GSSG	0.3089	0.0244	0.2930
HDL-C	0.0744	<0.0001	0.1020
Triglyceride	0.0657	<0.0001	0.1003
Heart rate	0.5683	<0.0001	0.6145
DBP	0.6086	<0.0001	0.6505
SBP	0.6676	<0.0001	0.7062

4.4.3.1 Relationships between Changes in Biomarkers and $PM_{2.5}$

The relationships between biomarkers level and $PM_{2.5}$ concentration were expressed as changes in the biomarkers level per 10 $\mu g/m^3$ increase of $PM_{2.5}$ concentration. Three exposure windows were examined, including current day exposure (lag 0), previous two-day average exposure (lag 0-1 days), and previous one-week average exposure (lag 0-7 days).

In the current day exposure window, we found a reduction of 0.35 ng/ml 8-OHdG, 0.06 GSH/GSSG ratio, 0.02 mmol/L of HDL-C, 0.002 mmol/L of triglyceride for every 10 $\mu g/m^3$ increase of $PM_{2.5}$. An increase of 0.001 mmol/L of MDA, 0.13 bpm of heart rate, 0.3 mmHg of DBP and 0.1 mmHg of SBP was associated with every 10 $\mu g/m^3$ increase of $PM_{2.5}$ (Table 13A). Similar associations were found in the lag 0-1 days exposure, except with triglyceride for which the association was positive instead of negative (Table 13B). However, for the lag 0-7 days exposure, pattern of associations was different (Table 13C). All the associations were statistically not significant.

Table 13. Estimated unit changes (95% CI) in biomarkers per 10 μg/m^3 increase of $PM_{2.5}$

A) Current day

Biomarker	Change per 10 μg/m^3	95% CI	p-value
8-OHdG	-0.3477	(-0.0825, 0.0129)	0.1544
MDA	0.0010	(-0.0062, 0.0064)	0.9755
GSH/GSSG	-0.0565	(-0.0238, 0.0125)	0.5413
HDL-C	-0.0157	(-0.0053, 0.0021)	0.4036
Triglyceride	-0.0018	(-0.0022, 0.0019)	0.8595
Heart rate	0.1251	(-0.0288, 0.0538)	0.5531
DBP	0.3047	(-0.0185, 0.0795)	0.2242
SBP	0.1225	(-0.0617, 0.0862)	0.7457

B) Lag0-1 days

Biomarker	Change per 10 μg/m^3	95% CI	p-value
8-OHdG	-0.3934	(-0.0965, 0.0178)	0.1784
MDA	0.0048	(-0.0072, 0.0081)	0.9030
GSH/GSSG	-0.0889	(-0.0303, 0.0125)	0.4163
HDL-C	-0.0226	(-0.0067, 0.0022)	0.3191
Triglyceride	0.0064	(-0.0018, 0.0031)	0.6157
Heart rate	0.0752	(-0.0417, 0.0567)	0.7649
DBP	0.4982	(-0.0078, 0.1074)	0.0913
SBP	0.2562	(-0.0629, 0.1142)	0.5712

C) Lag0-7 days

Biomarker	Change per 10 μg/m^3	95% CI	p-value
8-OHdG	-0.7797	(-0.1726, 0.0167)	0.1075
MDA	0.0496	(-0.0070, 0.0170)	0.4188
GSH/GSSG	-0.0510	(-0.0420, 0.0317)	0.7862
HDL-C	0.0499	(-0.0020, 0.0120)	0.1629
Triglyceride	-0.0229	(-0.0063, 0.0017)	0.2616
Heart rate	-0.7227	(-0.1561, 0.0115)	0.0921
DBP	0.6290	(-0.0330, 0.1588)	0.1995
SBP	0.6839	(-0.0814, 0.2182)	0.3716

Sensitivity Analysis

The distributions of some of the biomarkers were highly skewed (specifically, for 8-OHdG and GSH/GSSG ratio). Hence, we performed sensitivity analyses by dichotomized the biomarker levels based on data distribution into high and low categories. For 8-OHdG, MDA, GSH/GSSG, and heart rate, top quantile (median, tertile, quartile and quintile) values of the entire data set was used for the cut off. We tested visits and current day exposure for assessing $PM_{2.5}$ effects for these dichotomized biomarkers using logistic regression and found that the effects sign of $PM_{2.5}$ changed positively for 8-OHdG for top median, GSH/GSSG ratio for all top quantiles. Yet, most of the changes with increase in $PM_{2.5}$ were statistically non-significant (Table 14).

Table 14. Estimated Excess Risks (95% CI) in High Biomarkers per 10 μg/m^3 Increase of $PM_{2.5}$

Biomarker	Top median	Top tertile	Top quartile	Top quintile
8-OHdG	2.2(-12.6, 19.6)	-2.4(-17.9, 16.0)	-7.4(-23.9, 12.7)	-11.9(-30.0, 10.9)
MDA	-1.9(-14.8, 13.0)	3.7(-10.8, 20.6)	2.8(-12.9, 21.3)	3.0(-14.9, 24.7)
GSH/GSSG	13.3(-7.7, 39.0)	24.7(2.2, 52.1)*	25.2(1.3, 54.7)*	17.1(-6.0, 45.9)
HDL-C	7.3(-8.6, 25.9)	-1.7(-15.3, 14.0)	-16.5(-30.6, 0.6)	-24.7(-41.1, -3.7)*
Triglyceride	-4.8(-17.6, 10.0)	-9.7(-23.5, 6.6)	-12.6(-28.1, 6.3)	-7.1(-24.7, 14.7)
Heart rate	-10.9(-27.6, 9.8)	-7.4(-25.3, 14.9)	0.5(-19.5, 25.5)	8.9(-12.0, 34.7)
DBP	---	13.2(-9.7, 41.9)	---	---
SBP	1.00(-16.9, 22.6)	-1.10(-21.4, 24.5)	12.2(-12.0, 43.0)	18.3(-11.9, 58.9)

*$p<0.05$

--- : Models in DBP did not converge in top median, quartile and quintile.

4.4.4 Comparisons with Studies in Other Countries

Across the entire study period, we hypothesized that biomarker levels would be positively correlated with concentrations of $PM_{2.5}$ according to the literatures (Table 15).

Table 15. Change in Biomarkers for increase in $PM_{2.5}$ / Traffic-Related Pollutants in the Current Study and in the Literatures

Biomarkers	Current study	Literatures	References
8-OHdG	Decrease?	Increase	Refer to "Literature Review" section
MDA	Increase	Increase	Refer to "Literature Review" section
GSH/GSSG	Decrease	Decrease	Refer to "Literature Review" section
HDL-C	Decrease	Decrease	Tomao et al. 2002
Triglyceride	Increase	Increase	Tomao et al. 2002
DBP	Increase	Increase	Rich et al. 2012
SBP	Increase	Increase	Rich et al. 2012
Heart rate	Increase	Increase	Rich et al. 2012

Regarding oxidative markers, we found a decrease in 8-OHdG in the participants, pointing towards decreased oxidative stress in association with an increase of $PM_{2.5}$. Although reverse effects of $PM_{2.5}$ on 8-OHdG was found in the dichotomized logistic regression analysis for top median, the effects remained negative for the other cut off in the dichotomized model. The negative associations were unexpected. However, a Czech study showed that oxidative biomarkers did not differ between locations or between seasons (Rossner et al. 2013). The oxidative markers level may be affected by lifestyle factors. A study among 361 healthy male subjects indicated that oxidative biomarker level was inversely correlated with fruits consumption, physical activity, and total energy consumed per day (Tamae et al. 2009). Adjustment for dietary and exercise frequency, did not account for the lack of between-group differences in the levels of 8-OHdG observed in our study. However, we did not obtain any information on total energy consumption of the participants for assessing the correlation with these biomarker levels. On the other hand, we observed a different pattern of O_3 pollutants in the study period, in which the peak was found in October instead of January (the peak of PM). O_3 was also found to be associated with oxidative biomarkers, including 8-OHdG (Appendix Table 3). The level of 8-OHdG was affected not only by PM but other pollutants as well.

For the rest of the biomarkers, most of their associations with $PM_{2.5}$ were in the direction as expected, but were mostly not statistically significant and the $PM_{2.5}$-associated changes in these biomarkers did not always agree with each other in terms of the direction, magnitude, and timing of

health outcome response. This inconsistency may be partially due to the uncontrolled day-to-day variations in ambient concentrations of air pollutants in PM composition and pollutant mixture.

4.5 Methodological Approaches for Biomarker Study in Hong Kong

We have developed methodological approaches in measuring the effects of air pollution on health at molecular level in Hong Kong. A research protocol is summarized below:

(A) Subject Recruitment

1. Select the potential study area (e.g. by site visits) which is near the air quality monitoring stations
2. Send out invitation letters to the potential study address
3. Define the characteristic of the study population (e.g. age group, smoking conditions, health conditions, etc.)
4. Screen and recruit the eligible participants.

(B) Preparation for the Clinical Visit

1. Schedule appointment for clinical visit. Remind the participants to fast overnight.
2. Introduce the study objectives and answer any enquires from the participants as detailed as possible.
3. Ask the participants to sign the consent form for taking part in the study.
4. Obtain the smoking history and use a smokerlyzer for validation of smoking status.

(C) Clinical Visit

1. The visit includes questionnaire interviewing, blood collection, blood pressure and anthropometry measurement.
2. Blood pressures measurement is performed twice, with at least 5 minutes interval in between. When the first two readings differed by more than 5 mmHg, additional readings should be obtained before taking the average.
3. By means of questionnaire ask for individual characteristics including socio-demographic, lifestyles (such as indoor and outdoor air pollution exposure, daily activities and recent diets), and medical history of the participants.
4. Collect blood samples in the well-labelled tubes by a research nurse.
5. Schedule the next appointments for follow-up clinical visits.

(D) Storage of samples after the Clinical Visit

1. The blood samples should be processed within one hour after blood taking.
2. For plasma samples, they should be centrifuged. For serum samples, they should be allowed to clot for at least 30 minutes at room temperature, and then be centrifuged.
3. All the samples should be well labelled and stored at -80^{o}C freezer for laboratory analysis.

(E) Laboratory Analysis

1. The use of high-performance liquid chromatography–mass spectrometry [HPLC-MS] is more specific and thus is recommended to quantity the level of the biomarkers.
2. Before the biomarkers analysis, assay standardization and optimization of the regents is required.
3. Perform biomarkers analysis with at least triplicate samples.

(E) Statistical Analysis

1. Check for reliability of the measured biomarkers using the coefficient of variation.
2. Data cleaning by means of descriptive summary of the participants; checking of air pollutant levels for different exposure windows; examining quantified biomarkers levels.
3. Analysis for the relationship between air pollutants, subjects' characteristic and biomarkers, including partial correlations, intraclass correlation coefficient, association quantified in excess risks (per 10 $\mu g/m^3$ increase of pollutants), and sensitivity analysis.

4.6 Pilot Study Achievements and Limitations

This pilot study successfully accomplished the objectives of: (1) literature review on the use of biomarkers for air pollution health studies in Hong Kong and overseas; (2) biomarker sample collection and laboratory analysis for two representative regions in Hong Kong; (3) questionnaire survey of the individual characteristics of the participants that may relate to air pollution exposure and biomarker levels; (4) a preliminary analysis of the relationship between measures of biomarkers and air pollutant concentrations measured at the EPD monitoring stations; and (5) developing methodological approaches in measuring the effects of air pollution on health at molecular level in Hong Kong.

A few limitations were observed in the pilot study and will be addressed in the future biomarker study in Hong Kong.

No Repeated Measurements for the Same Individuals within Season

In the study, we measured the biomarkers once in each of the four seasons. A recent study showed that seasonal expression profiles were found in the immune system (Dopico et al. 2015). The level of biomarkers inside the human body may also be subjected to this kind of seasonal variations. We recommend several repeated measurements should be taken within a short study period (for example, in terms of weeks or days), to eliminate any variations due to seasonality or other time dependent covariates.

Insufficient Statistical Power of the Study

In our study, we did not find statistically significant associations between biomarkers and $PM_{2.5}$ levels in Hong Kong over the study period. This may be due to the insufficient statistical power for the assessments. Based on the data from the Beijing Olympic study (Zhang et al. 2013), we calculated that a sample size of 108 in which 80% of the participants (N = 86) had completed all four repeated measurements, would have sufficient statistical power to detect the main effects of the biomarkers. Therefore, we expected our final sample size of 109 participants in which 91% of participants committed all the four visits should have enough power to detect meaningful effects sizes of these biomarkers.

However the variations of $PM_{2.5}$, expressed as the inter-quartile range (IQR), in Beijing was much higher than that of Hong Kong. Taking daily exposure of $PM_{2.5}$ in year 2014 as an example, the IQR was 82.3 $\mu g/m^3$ for Beijing (http://www.stateair.net/web/historical/1/1.html) (data from the U.S. Embassy Beijing Air Quality Monitor), which was more than 3 times that of Hong Kong in the same period (26.0 $\mu g/m^3$ and 22.4 $\mu g/m^3$ for CW and MK, respectively).

We estimated a sample size (Liu and Liang, 1997) of 250 based on the data we obtained in this pilot study (225 after accounting for 10% loss to follow up) would achieve an adequate statistical power of 80%.

Mobility of the Participants

About 43% of the participants had a full-time or part-time job. Most of their working locations (77%) were not in the same regions as their residential area. By comparing the mean difference of the $PM_{2.5}$ concentration, the day time $PM_{2.5}$ was significantly higher than that in night time in both regions (means 28.0 - 34.1 $\mu g/m^3$ versus 30.3 - 36.5 $\mu g/m^3$) (p-value < 0.001, paired sample T-test). The use of PM data from the monitoring stations near their residential area may not reflect their actual ambient PM exposure during the daytime. However, the $PM_{2.5}$ exposure in their working area is hard to estimate as the air monitoring stations may not be located near their working area.

In future studies, it may be feasible to request the participants stay most of their time near the residential region at least for 24 hours before the clinical visits. This could be done by: (1) recruit only the housewife, retired participants or the elderly; (2) recruit only the participants whose residential and working area are near; and (3) arrange the clinical visits early in the morning on Monday mornings for participants who work in week days and rest in weekends.

Low specificity of ELISA methods

The use of ELISA method for the determination of 8-OHdG level, although sensitive in most cases, may be less specific for detecting a particular biomarker (Shimoi et al., 2002). This is because the method does not directly measure a compound, rather it measures immuno-responses of antibodies relating to the compound and to all other compounds which have similar immuno-functions. The ELISA methods could be influenced largely by other constituents in samples. In addition, it was also found to have higher detection limits above the normal ranges for healthy people compared to the use of analytical chemistry based methods (for example, the high-performance liquid chromatography–mass spectrometry [HPLC-MS]) (Koide et al., 2010). For a future full scale study, we recommend the use of HPLC-MS instead of ELISA to quantity the level of the biomarkers.

5. Conclusion

This pilot study demonstrated the feasibility of using molecular biomarkers to investigate the subclinical health impacts due to ambient air pollution in Hong Kong. The preliminary data showed that $PM_{2.5}$ could contribute to the development of subclinical diseases by changing the level of certain molecular biomarkers in the human body. The change in oxidative biomarkers can be detected if there were government interventions for alleviation of $PM_{2.5}$.

The pilot study successfully accomplished the objectives of: (1) literature review on the use of biomarkers for air pollution health studies in Hong Kong and overseas; (2) biomarker sample collection and laboratory analysis for two representative regions in Hong Kong; (3) questionnaire survey of the individual characteristics of the participants that may relate to air pollution exposure and biomarker levels; (4) a preliminary analysis of the relationship between measures of biomarkers and air pollutant concentrations measured at the EPD monitoring stations; and (5) developing methodological approaches in measuring the effects of air pollution on health at molecular level in Hong Kong. A few limitations were observed in the pilot study and will be addressed in the future biomarker study in Hong Kong.

In the future panel study, we recommend improving the study power by: (1) targeting a more specific and sensitive population such as the elderly, who are less mobile, with repeated biomarker measurements in shorter time scales, within cool and warm seasons, respectively; (2) increasing the sample size; and (3) measuring the personal exposure to air pollution besides the usage of ambient air pollution data.

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Abbreviations

8-OHdG	8-hydroxy-2-deoxyguanosine
BMI	body mass index
bpm	beats per minute
CC16	club cell secretory protein (Clara)
CI	confidence interval
CRP	C-reactive protein
Cu/Zn-SOD	copper/zinc superoxide dismutase
CV	coefficient of variation
CW	Central/Western
DBP	diastolic blood pressure
EBC	exhaled breath condensate
EPD	Environmental Protection Department
ELIZA	enzyme immunoassay and enzyme-linked immunosorbent assay
FeNO	exhaled nitric oxide
GSH	glutathione
GSH/GSSG	ratio of glutathione and glutathione disulfide
GSSG	glutathione disulfide
HDL-C	high density lipoprotein cholesterol
ICAM-1	intercellular cell adhesion molecule-1
ICC	intra-class correlation within-participant
IL-10	Interleukin-10
IL-6	Interleukin-6
IL-8	Interleukin-8
IQR	interquartile range
MDA	malondialdehyde
MK	Mongkok
mmHg	millimeters of mercury
NO_2	nitrogen dioxide
O_3	ozone
PAH	polycyclic aromatic hydrocarbon
PAI-1	plasminogen activator inhibitor-1
PM_{10}	particular matter with aerodynamic diameter < 10 μm
$PM_{2.5}$	particular matter with aerodynamic diameter < 2.5 μm
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RBC	red blood cells
SBP	systolic blood pressure
sCD40L	soluble CD40 ligand

sCD62P	sP-selectin
SD	standard derivations
SE	standard error
SO_2	sulphur dioxide
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
TNF-α	tumor necrosis factor alpha
UFP	ultrafine particles
vWF	von Willebrand factor
WBC	white blood cells
WHO	World Health Organization

Appendices

Appendix Table 1a: Percentage (%) change in systemic inflammation biomarkers concentrations per 10 µg/m^3 increase in $PM_{2.5}$

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change[+]	SE for % change	Reference
CC16	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	37.4 (0.83) µg/l	-0.291	0.223	Zuurbier et al 2011
	2000	1,004 elderly men	Oslo, Norway	Cross-sectional	9.5 (1.7) µg/l	**20**	**5.1**	Madsen et al 2008
			Random effect			*9.214*	*10.13*	
CRP	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	3.7 (6.5) mg/l	**9.820**	**4.091**	Rückerl et al 2014
	2009	31 students age 19-26	Netherlands	Semi-experimental	1 (0.1-14.5)[b] mg/l	**1.508**	**0.769**	Strak et al 2013
	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	1.2 (0.1) mg/l	-0.259	0.182	Zuurbier et al 2011
	1997-2001	1,696 pregnant women	Allegheny, US	Longitudinal	Not provided	**2.981**	**0.393**	Lee et al 2011
	2009-2010	67 men in trucking industry	North-eastern US	Panel	1.66 (1.8) mg/l	-13.73	20.90	Neophytou et al 2013
	2000-2002	5,634 adult age 45-84	6 US community	Longitudinal	1.84[c] mg/l	3	2.55	Diez Roux et al 2006
	1999-2001	88 elderly	^Utah, US	Panel	0.5 (0.6) mg/dl	**#0.081**	**0.018**	Pope et al 2004
	2000-2001	57 male age 51-76	Erfurt, Germany	Prospective panel	3.7 (6.5) mg/l	**0.915**[a]	**0.187**	Ruckerl et al 2006

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
					Random effect	*2.039*	*1.124*	
Fibrinogen	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	3.3 (0.5) g/l	-0.270	0.414	Rückerl et al 2014
	2008	125 adults age 22-27	Beijing, China	Panel study	250-261[a] mg/dl	**0.178**	**0.0824**	Gong et al 2014
	2008-2012	40 male college students	Beijing, China	Prospective panel	1.98-2.13 (0.7-0.79) g/l	0.470	0.427	Wu et al 2012
	2009	31 students age 19-26	Netherlands	Semi-experimental	3.02 (1.43-5.19)[b] g/l	0.234	0.146	Strak et al 2013
	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	3 (0.035) g/l	-0.0162	0.0743	Zuurbier et al 2011
	1989-1994	~20,000 mean age 49	^US	Longitudinal	318 (89) mg/dl	**#5.14**	**1.72**	Schwartz 2001
					Random effect	*0.105*	*0.0713*	
IL-6	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	1 (0.9) pg/ml	**-4.054**	**2.068**	Rückerl et al 2014
	2009-2010	67 men in trucking industry	North-eastern US	Panel	1.38 (1.2) pg/ml	7.190	13.23	Neophytou et al 2013
	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	0.41 (0.023) ng/l	-0.210	1.131	Zuurbier et al 2011
					Random effect	*-1.492*	*1.604*	

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change[+]	SE for % change	Reference
IL-8	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	3 (0.33) ng/l	-0.0134	0.0130	Zuurbier et al 2011
	2003-2005	60 adults age 18-55	London, UK	Crossover	89-91.5[a] ng/ml	28.3	22.91	Zhang et al 2009
					Random effect	*4.876*	*10.70*	
Myeloperoxidase	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	14.5 (12.3) ng/ml	**4.414**	**1.931**	Rückerl et al 2014
	2003-2005	60 adults age 18-55	London, UK	Crossover	5.69-6.53[a] ng/ml	235	336	Zhang et al 2009
					Fixed effect	***4.422****	***1.931***	
TNF-α	2008-2012	40 male college students	Beijing, China	Prospective panel	3.13-3.22 (1.32-1.43) pg/ml	**1.125**	**0.332**	Wu et al 2012
	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	2 (0.11) ng/l	-0.0971	1.602	Zuurbier et al 2011
					Fixed effect	***1.075****	***0.325***	
ECP	2003-2005	60 adults age 18-55	London, UK	Crossover	13.7-38.9[a] ng/ml	43.6	64.8	Zhang et al 2009
ICAM-1	2009-2010	67 men in trucking industry	North-eastern US	Panel	202 (40) ng/ml	7.625	5.113	Neophytou et al 2013
ICAM-1	2000-2001	57 male age 51-76	Erfurt, Germany	Prospective panel	272 (75.7) ng/ml	**0.427**[d]	**0.0933**	Ruckerl et al 2006

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
IL-10	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	0.37 (0.017) ng/l	-0.874	0.462	Zuurbier et al 2011

Note: ^Studies were excluded from meta-analysis due to different in units or estimation.

+Text for statistical significant changes were **Bold**.

#The changes were in absolute unit change.

[a] Range of mean, SD is not provided.

[b] Range, SD is not provided.

[c] SD is not provided

[d] The estimation was in odd ratio.

Appendix Table 1b: Percentage (%) change in hemostasis or endothelial function biomarkers concentrations per 10 µg/m^3 increase in $PM_{2.5}$

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change$^+$	SE for % change	Reference
Factor VII	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	90.5 (0.94) %	0.0647	0.0743	Zuurbier et al 2011
	2000-2001	57 male age 51-76	Erfurt, Germany	Prospective panel	124 (61) %	**-2.134**	**0.902**	Ruckerl et al 2006
					Random effect	*-0.851*	*1.084*	
PAI-1	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	3.6 (3.1) ng/ml	**-5.586**	**2.114**	Rückerl et al 2014
	2008-2012	40 male college students	Beijing, China	Prospective panel	27.3-29.5 (7.2-8.4)	0.131	0.280	Wu et al 2012
					Random effect	*-2.343*	*2.832*	
sCD40L	2007-2008	87 adult mean age 55.9	Augsburg, Germany	Prospective panel	1001 (773) pg/ml	**-4.685**	**2.022**	Rückerl et al 2014
	2008	125 adults age 22-27	Beijing, China	Panel	1.76-1.92[a] ng/ml	**0.382**	**0.170**	Gong et al 2014
					Random effect	*-1.751*	*2.501*	

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
VWF	2008	125 adults age 22-27	Beijing, China	Panel	79.5-106.4[a] %	**0.646**	**0.252**	Gong et al 2014
	2008-2012	40 male college students	Beijing, China	Prospective panel	304-334 (66.8-86.2) ng/ml	**-0.660**	**0.230**	Wu et al 2012
	2009	31 students age 19-26	Netherlands	Semi-experimental	89.4 (37.7-200)[b] %	**0.503**	**0.203**	Strak et al 2013
	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	107 (1.9) %	0	0.0826	Zuurbier et al 2011
	2000-2001	57 male age 51-76	Erfurt, Germany	Prospective panel	135 (59) %	2.195	1.462	Ruckerl et al 2006
					Random effect	*0.165*	*0.239*	
sCD62p	2008	125 adults age 22-27	Beijing, China	Panel	4.16-6.29[a] ng/ml	**1.076**	**0.257**	Gong et al 2014
sCD62p	2008-2012	40 male college students	Beijing, China	Prospective panel	45.2-52.7 (19.6-22.9) ng/ml	0.527	0.440	Wu et al 2012
activated partial thromboplastin	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	28.2 (0.12) sec	-0.0485	0.0330	Zuurbier et al 2011
time tissue-type plasminogen activator	2008-2012	40 male college students	Beijing, China	Prospective panel	9.5-10.2 (3.7-4.6) ng/ml	0.400	0.522	Wu et al 2012

Note: +Text for statistical significant changes were **Bold**.

The changes were in percentage changes (%).

[a] Range of mean, SD is not provided.

[b] Range, SD is not provided.

Appendix Table 1c: Percentage (%) change in metabolic function and blood profile concentrations per 10 µg/m^3 increase in $PM_{2.5}$

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change$^+$	SE for % change	Reference
lymphocytes	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	2.4 (0.042) 10^9/l	0.210	0.182	Zuurbier et al 2011
	2003-2005	60 adults age 18-55	London, UK	Crossover	0.62-1.03[a] %	-9.42	30.19	Zhang et al 2009
					Fixed effect	*0.210*	*0.182*	
neutrophils	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	3.3 (0.058) 10^9/l	-0.388	0.272	Zuurbier et al 2011
	2003-2005	60 adults age 18-55	London, UK	Crossover	36.3-39.9[a] %	-7	14	Zhang et al 2009
					Fixed effect	*-0.391*	*0.272*	
eosinophils	2003-2005	60 adults age 18-55	London, UK	Crossover	1.53-1.92[a] %	-6.59	30.06	Zhang et al 2009
epithelial cells	2003-2005	60 adults age 18-55	London, UK	Crossover	2.83-3.14[a] %	-1.17	15.93	Zhang et al 2009
erythrocytes	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	4.9 (0.021) 10^{12}/l	-0.0485	0.0330	Zuurbier et al 2011
leukocytes	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	6.5 (0.082) 10^9/l	-0.113	0.157	Zuurbier et al 2011
macrophages	2003-2005	60 adults age 18-55	London, UK	Crossover	54.8-57.8[a] %	12.6	7.403	Zhang et al 2009
platelet counts	2009	31 students age 19-26	Netherlands	Semi-experimental	268 (130-416)[b] 10^9/l	0.0607	0.106	Strak et al 2013
platelet counts	2007-2008	34 adults age 23-55	Netherlands	Longitudinal	Not provided	-0.0162	0.0578	Zuurbier et al 2011
WBC	2008	125 adults age 22-27	Beijing, China	Panel	5210-5400[a] µl	-0.151	0.375	Gong et al 2014
WBC (number)	1989-1994	~20,000 mean age 49	^US	Longitudinal	7.2 (2.3)	**0.062**	**0.026**	Schwartz 2001
SBP	2008	125 adults age 22-27	Beijing, China	Panel	101-111[a] mm hg	**0.134**	**0.0445**	Gong et al 2014
DBP	2008	125 adults age 22-27	Beijing, China	Panel	60.1-60.2[a] mm hg	-0.0586	0.0664	Gong et al 2014

Biomarker	Year	Subjects	Region	Study design	Mean (SD)	% Change+	SE for % change	Reference
heart rate	2008	125 adults age 22-27	Beijing, China	Panel	65.4-66.5[a] /min	**0.194**	**0.083**	Gong et al 2014

Note: +Text for statistical significant changes were **Bold**.

The changes were in percentage changes (%) otherwise specified.

[a] Range of mean, SD is not provided.

[b] Range, SD is not provided.

Appendix Table 2: Effects of air pollutants (per each 10 μg/m^3 increase) on biomarkers in reviewed papers

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes$^+$	SE for % change
Bae et al 2010	Panel study of 120 schoolchildren in Ala Shan & Beijing, China; Jeju & Seoul, Korea in 2007	MDA	0.9-1.74 [a] mmol/g creatinine	PM_{10}	**#0.0156**	**0.0030**
Delfino et al 2006	Panel study of 45 school-children age 9-18 in California, US in 2004	FeNO	25.6 (25.1) ppb	NO_2	**#0.510**	**0.192**
Delfino et al 2008	Panel study of 29 elderly in Los Angeles, US in 2005-2006	Cu/Zn-SOD CRP IL-6 TNF-α soluble receptor-II sCD62p	5260 (1671) U/g Hb 3134 (3796) ng/ml 2.95 (2.32) pg/ml 3933 (1555) pg/ml 37.6 (15.1) ng/ml	NO_2	**#-95.99** #199.6 **#0.234** #51.60 #0.909	**43.19** 132.5 **0.0755** 31.54 0.581
Gong et al 2014	Panel study of 125 adults age 22-27 in Beijing, China in 2008	MDA DBP heart rate	311-483 (1.1-13) nM 60.1-60.2[a] mm hg 65.4-66.5[a] /min	SO_2 NO_2 SO_2 SO_2	6.362 **3.698** -0.3538 **0.721**	3.246 **1.306** 0.4280 **0.368**

Author	**Study**	**Biomarker**	**Mean (SD)**	**Pollutant**	**Changes[+]**	**SE for % change**
Huang et al 2012	Panel study of 125 adults age 22-27 in Beijing, China in 2008	8-OHdG	2.22-3.7 [a] mg/mol	NO_2 O_3	**14.76** **-5.845**	**4.102** **1.674**
Kim et al 2012	Panel study of 560 elderly in Seoul, Korea in 2008-2010	MDA	2 (1) µmol/l	PM_{10}	**7.332**	**3.594**
Lee et al 2011	Longitudinal study of 1696 pregnant women in Allegheny, US in 1997-2001	CRP	Not provided	PM_{10}	**1.485**	**0.211**
Liao et al 2005	Cohort study of 10208 adults mean age 54 in US in 1987-1989	Fibrinogen VWF	302 (65) mg/dl 118 (48.4) %	PM_{10} NO_2 SO_2 PM_{10} NO_2 SO_2	#0.127 #-0.964 #-0.382 -0.422 -0.140 0.620	0.590 0.519 0.897 0.453 0.372 0.639

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes+	SE for % change
		Factor VIII-C	131 (39.9) %	NO_2	-0.1463	0.3059
				O_3	-0.0870	0.1621
		Albumin	3.86 (0.28) g/dl	PM_{10}	#0.0227	0.0133
				NO_2	#-0.0027	0.0020
				O_3	#-0.0006	0.0012
		WBC	6 (1.9) x $10^3/mm^3$	PM_{10}	#0.0164	0.0148
				NO_2	#-0.0113	0.0140
				O_3	#-0.0117	0.0069
Liu et al 2007	Longitudinal study of 25 adults age 18-65 in Windsor, Canada in 2005	TBARS	1.7 [a] nmol/ml	PM_{10}	**#16.12**	**4**
		CRP	1.7 µg/ml		#0.11	0.07
		IL-6	1.7 pg/ml		#0	0.05
		TNF-α	1.8 pg/ml		#0.03	0.05
		SBP	124 mm Hg		#0.17	0.19
		DBP	77 mm Hg		#0.19	0.16
Madsen et al 2008	Cross-sectional study of 1,004 elderly men in Oslo, Norway in 2000	CC16	9.5 (1.7) µg/l	PM_{10}	1	1.53

Author	**Study**	**Biomarker**	**Mean (SD)**	**Pollutant**	**Changes[+]**	**SE for % change**
NorbaÈck et al 2000	central Sweden	Eosinophil Cationic Protein	1.6 [c] µg/l	NO_2	**#2.7**	**0.61**
Patel et al 2013	Panel study of 36 adolescents age 14-19 New York, US in 2005	EBC pH	7.9-8.1 (5.8-8.2) [b]	NO_2 O_3	#-0.014 #-0.0364	0.100 0.0255
		8-isoprostane	42.3-54.8 (22.1-72.9) pg/ml	NO_2 O_3	**#0.448** **#-0.109**	**0.143** **0.0417**
Pekkanen 2000	Cross-sectional study of 10,308 office workers in London, UK in 1991-1993	Fibrinogen	2.42 [a] g/l	PM_{10} NO_2 SO_2 O_3	0.0725 **0.201** 0.358 0.0448	0.251 **0.0778** 0.263 0.213
Provost et al 2014	Cross-sectional study of 825 adolescents in Belgium in 2006	CC16	9.2 (3.7) µg/l	PM_{10}	**#1.04**	**0.214**
Ren et al 2010	Longitudinal study of 320 elderly men in Boston, US in 2006-2008	8-OHdG	20.8 (12.3) ng/ml	NO_2 O_3	**43.78** **15.92**	**18.49** **7.510**

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes[+]	SE for % change
Rich et al 2012	Panel study of 125 adults age 22-27 in Beijing, China in 2008	Fibrinogen	250-261 mg/dl [a]	NO_2	**0.495**	**0.239**
				SO_2	**0.782**	**0.385**
				O_3	**-0.568**	**0.0871**
		sCD40L	1.76-1.92 ng/ml	NO_2	0.0952	0.384
				SO_2	**2.105**	**0.890**
				O_3	0.1558	0.397
		VWF	79.5-106.4 %	NO_2	**1.654**	**0.577**
				SO_2	**3.966**	**0.895**
				O_3	**-1.117**	**0.255**
		sCD62p	4.16-6.29 ng/ml	NO_2	1.365	0.477
				SO_2	**6.179**	**1.244**
				O_3	**-2.467**	**0.438**
		WBC	5210-5400 µl	SO_2	**-1.840**	**0.753**
				NO_2	**-0.741**	**0.319**
				O_3	-0.277	0.332
		DBP	60.1-60.2 mm hg	NO_2	-0.0208	0.2439
				O_3	-0.2864	0.2432
		heart rate	65.4-66.5/min	NO_2	0.151	0.135
				O_3	-0.0802	0.180

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes+	SE for % change
Ruckerl et al 2006	Prospective panel study of 57 male age 51-76 in Erfurt, Germany in 2000-2001	CRP	3.7 (6.5) mg/l	PM_{10}	OR:**1.316**	**0.302**
				NO_2	OR: **1.163**	**0.237**
		ICAM-1	272 (75.7) ng/ml	PM_{10}	OR**: 2.039**	**0.369**
				NO_2	OR: **0.349**	**0.0593**
		Factor VII	124 (61) %	PM_{10}	**-4.342**	**1.276**
				NO_2	-1.163	1.038
		VWF	135 (59) %	PM_{10}	**3.947**	**1.813**
				NO_2	**3.314**	**1.483**
Rückerl et al 2014	Prospective panel study of 87 adult mean age 55.9 in Augsburg, Germany in 2007-2008	CRP	3.7 (6.5) mg/l	PM_{10}	**9.424**	**3.157**
				NO_2	9.016	3.806
		Fibrinogen	3.3 (0.5) g/l	PM_{10}	-0.144	0.294
				NO_2	**1.066**	**0.418**
		IL-6	1 (0.9) pg/ml	PM_{10}	**-3.525**	**1.652**
				NO_2	**-4.590**	**1.924**
		Myeloperoxidase	14.5 (12.3) ng/ml	PM_{10}	3.165	1.432
				NO_2	4.918	1.840
		PAI-1	3.6 (3.1) ng/ml	PM_{10}	-4.892	1.651
				NO_2	-4.344	1.924
		sCD40L	1001 (773) pg/ml	NO_2	-1.148	1.966

Author	**Study**	**Biomarker**	**Mean (SD)**	**Pollutant**	**Changes⁺**	**SE for % change**
				PM_{10}	**-3.669**	**1.615**
Schwartz 2001	Longitudinal study of ~20,000 US adults mean age 49 in 1989-1994	Fibrinogen	318 (89) mg/dl	NO_2	#2.170	0.782
				SO_2	#0.504	0.603
				O_3	#0.811	0.776
		WBC	7.2 (2.7) count	NO_2	#-0.0117	0.0144
				SO_2	**#0.0252**	**0.0103**
				O_3	#-0.0189	0.0138
Seaton et al 1999	Panel study of 112 elderly in Belfast & Edinburgh, UK in 1996-1998	CRP	Mean level not provided	PM_{10}	**0.147**	**0.0648**
		Fibrinogen			-0.009	0.005
		IL-6			0.01	0.024
		Haemoglobin	g/dl		**#-0.073**	**0.019**
		WBC			-0.002	0.0046
		RBC	$x10^{12}$/l		**#-0.018**	**0.0056**
Steinvil et al 2007	Longitudinal study of 3659 adults mean age 46 in Israel in 2003-2006 (changes of all the	CRP	1.5 (2.8) mg/l	PM_{10}	-0.290	0.165
				NO_2	-0.0704	1.126
				SO_2	-2.022	5.547
				O_3	1.184	1.349

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes+	SE for % change
	biomarkers was estimated from the pooled effects of male and female)	Fibrinogen	280 (60) mg/dl	PM_{10}	**#0.139**	**0.0676**
				NO_2	**#-2.886**	**0.611**
				SO_2	**#-11.50**	**3.393**
				O_3	**#1.736**	**0.555**
		WBC	6.8 (1.7) x 10^3 cell/µl	PM_{10}	#0.316	8.333
				NO_2	**#-40.65**	**19.53**
				SO_2	#-191.9	101.5
				O_3	#12.17	31.06
Strak et al 2013	Semi-experimental study of 31 students age 19-26 in Netherlands in 2009	CRP	1 (0.1-14.5) [a] mg/l	PM_{10}	0.548	0.283
				NO_2	10.26	6.346
		Fibrinogen	3.02 (1.43-5.19) g/l	PM_{10}	0.0741	0.0529
				NO_2	0.275	1.152
		VWF	89.4 (37.7-200) %	PM_{10}	**0.163**	**0.0756**
				NO_2	1.129	1.544
		platelet counts	268 (130-416) 10^9/l	PM_{10}	0.0296	0.0416
				NO_2	-1.603	0.828
Wang et al 2011	Case-control study of 110 male workers in	8-OHdG	11.5-15.8 [c] µg/g	PM_{10}	#0.00071	0.0033
		1-hydroxypyrene	0.075-0.13 µmol/mol		**#0.0035**	**0.0017**

Author	**Study**	**Biomarker**	**Mean (SD)**	**Pollutant**	**Changes⁺**	**SE for % change**
	Guangzhou, China	SOD	91.7-97.7 µM		#-0.0058	0.0089
		MDA	3.26-4.11 µM		**#0.0045**	**0.0017**
Wu et al 2012	Panel study of 40 university students in Beijing, China in 2010-2011	Fibrinogen	1.98-2.13 (0.7-0.79) g/l	PM_{10}	0.192	0.286
				NO_2	0.121	0.856
		TNF-α	3.13-3.22 (1.32-1.43) pg/ml	PM_{10}	**0.581**	**0.222**
				NO_2	**1.345**	**0.651**
		PAI-1	27.3-29.5 (7.2-8.4)	PM_{10}	0.0794	0.198
				NO_2	0.695	0.758
		VWF	304-334 (66.8-86.2) ng/ml	PM_{10}	-0.302	0.175
				NO_2	-0.503	0.540
		sCD62p	45.2-52.7 (19.6-22.9) ng/ml	PM_{10}	0.352	0.314
				NO_2	0.684	0.930
		tissue-type plasminogen activator	9.5-10.2 (3.7-4.6) ng/ml	PM_{10}	0.0912	0.436
				NO_2	0.708	1.918
Zhang et al 2009	Crossover study of 60 London, UK adults age 18-55 in 2003-2005	EBC pH	7.9-8.1[a]	NO_2	**-0.15**	**0.051**
		FeNO	39.4-50.3 ppb		-0.07	0.56
		EBC Fe	68-323 nmol/L		-1.92	15.14
		TBARS	2.14-2.5 µmol/L		-0.84	0.82

Author	Study	Biomarker	Mean (SD)	Pollutant	Changes+	SE for % change
		Myeloperoxidase	5.69-6.53 ng/ml		69.9	64.6
		Eosinophil Cationic Protein	13.7-38.9 ng/ml		-1.72	12.23
		IL-8	89-91.5 ng/ml		**10.9**	**4.22**
		lymphocytes	0.62-1.03 %		-7.58	8.378
		neutrophils	36.3-39.9 %		1.14	3.64
		eosinophils	13.7-38.9 ng/ml		-3.62	7.74
		epithelial cells	2.83-3.14 %		-1.46	4.04
		macrophages	54.8-57.8 %		0.78	2.25
Zuurbier et al 2011	Longitudinal study of 34 adults age 23-55 in Netherlands in 2007-2008	CC16		PM_{10}	-0.673	0.540
		CRP			-0.337	0.442
		Fibrinogen			-0.0481	0.172
		IL-6			-1.490	2.918
		TNF-alpha			-0.914	3.704
		IL-8			-0.192	0.614
		IL-10			-0.192	1.055
		Factor VII			0	0.172
		VWF			0.0481	0.221
		activated partial thromboplastin time			**-0.1442**	**0.0491**
		lymphocytes			-0.433	0.417

Author	**Study**	**Biomarker**	**Mean (SD)**	**Pollutant**	**Changes+**	**SE for % change**
		Neutrophils			-0.337	0.662
		erythrocytes			-0.0962	0.0736
		leukocytes			-0.337	0.368
		platelet counts			-0.289	0.147

Note: +Text for statistical significant changes were **Bold**.

#The change was in unit change.

[a] Range of mean, SD is not provided.

[b] Only median (range) is provided in the study.

[c] Only median is provided in the study.

Appendix Table 3: Meta-analysis for percentage changes (%) of biomarkers per 10 µg/m^3 increase in pollutants

Biomarker	Pollutants	Region	Changes$^+$	SE for % change	References
8-OHdG	NO_2	Beijing, China	**14.76**	**4.102**	Huang et al 2012
		Boston, US	**43.78**	**18.49**	Ren et al 2010
		Random effect	*23.67*	*13.38*	
	O_3	Beijing, China	-5.845	1.674	Huang et al 2012
		Boston, US	**15.92**	**7.510**	Ren et al 2010
		Random effect	*3.807*	*10.81*	
CC16	PM_{10}	Netherlands	-0.673	0.540	Zuurbier et al 2011
		Oslo, Norway	1	1.53	Madsen et al 2008
		Fixed effect	*-0.488*	*0.509*	
CRP	PM_{10}	Augsburg, Germany	**9.424**	**3.157**	Rückerl et al 2014
		Belfast & Edinburgh, UK	**0.147**	**0.0648**	Seaton et al 1999
		Netherlands	0.548	0.283	Strak et al 2013
		Netherlands	-0.337	0.442	Zuurbier et al 2011
		Allegheny, US	**1.485**	**0.211**	Lee et al 2011
		Israel^{++}	-0.290	0.165	Steinvil et al 2007
		Random effect	*0.415*	*0.307*	
	NO_2	Augsburg, Germany	9.016	3.806	Rückerl et al 2014
		Netherlands	10.26	6.346	Strak et al 2013

Biomarker	Pollutants	Region	Changes[+]	SE for % change	References
		Israel[++]	-0.0704	1.126	Steinvil et al 2007
		Random effect	*5.098*	*3.902*	
	O_3	Allegheny, US	0.652	0.133	Lee et al 2011
		Israel[++]	1.184	1.349	Steinvil et al 2007
		Random effect	***0.657****	***0.132***	
Fibrinogen (%)	PM_{10}	Augsburg, Germany	-0.144	0.294	Rückerl et al 2014
		Beijing, China	0.192	0.286	Wu et al 2012
		Belfast & Edinburgh, UK	-0.009	0.005	Seaton et al 1999
		London, UK	0.0725	0.251	Pekkanen 2000
		Netherlands	0.0741	0.0529	Strak et al 2013
		Netherlands	-0.0481	0.172	Zuurbier et al 2011
		Fixed effect	*-0.0082*	*0.0051*	
	NO_2	Augsburg, Germany	**1.066**	**0.418**	Rückerl et al 2014
		Beijing, China	0.121	0.856	Wu et al 2012
		Beijing, China	**0.495**	**0.239**	Rich et al 2012
		London, UK	**0.201**	**0.0778**	Pekkanen 2000
		Netherlands	0.275	1.152	Strak et al 2013
		Fixed effect	***0.254****	***0.0724***	
	SO_2	Beijing, China	**0.782**	**0.385**	Rich et al 2012
		London, UK	0.358	0.263	Pekkanen 2000
		Fixed effect	***0.493****	***0.217***	

Biomarker	Pollutants	Region	Changes[+]	SE for % change	References
	O_3	Beijing, China	**-0.568**	**0.0871**	Rich et al 2012
		London, UK	0.0448	0.213	Pekkanen 2000
		Random effect	*-0.293*	*0.305*	
Fibrinogen (mg/dl)	PM_{10}	Israel[++]	**0.139**	**0.0676**	Steinvil et al 2007
		US	0.127	0.590	Liao et al 2005
		Fixed effect	***0.139****	***0.0671***	
	NO_2	Israel[++]	**-2.886**	**0.611**	Steinvil et al 2007
		US	2.170	0.782	Schwartz 2001
		US	-0.964	0.519	Liao et al 2005
		Random effect	*-0.6039*	*1.307*	
	SO_2	Israel[++]	**-11.50**	**3.393**	Steinvil et al 2007
		US	0.504	0.603	Schwartz 2001
		US	-0.382	0.897	Liao et al 2005
		Random effect	*-1.696*	*1.619*	
	O_3	Israel[++]	**1.736**	**0.555**	Steinvil et al 2007
		US	0.811	0.776	Schwartz 2001
		Fixed effect	***1.423****	***0.451***	
IL-6	PM_{10}	Augsburg, Germany	**-3.525**	**1.652**	Rückerl et al 2014
		Belfast & Edinburgh, UK	0.01	0.024	Seaton et al 1999

Biomarker	Pollutants	Region	Changes+	SE for % change	References
		Netherlands	-1.490	2.918	Zuurbier et al 2011
		Random effect	*-1.240*	*1.287*	
Myeloperoxidase	NO_2	Augsburg, Germany	4.918	1.840	Rückerl et al 2014
		London, UK	69.9	64.6	Zhang et al 2009
		Fixed effect	***4.971****	***1.839***	
TNF-α	PM_{10}	Beijing, China	**0.581**	**0.222**	Wu et al 2012
		Netherlands	-0.914	3.704	Zuurbier et al 2011
		Fixed effect	***0.576****	***0.222***	
Factor VII	PM_{10}	Netherlands	0	0.172	Zuurbier et al 2011
		Erfurt, Germany	**-4.342**	**1.276**	Ruckerl et al 2006
		Random effect	*-1.987*	*2.163*	
PAI-1	PM_{10}	Augsburg, Germany	-4.892	1.651	Rückerl et al 2014
		Beijing, China	0.0794	0.198	Wu et al 2012
		Random effect	*-2.136*	*2.471*	
	NO_2	Augsburg, Germany	-4.344	1.924	Rückerl et al 2014
		Beijing, China	0.695	0.758	Wu et al 2012
		Random effect	*-1.515*	*2.500*	

Biomarker	Pollutants	Region	Changes+	SE for % change	References
sCD40L	NO_2	Augsburg, Germany	-1.148	1.966	Rückerl et al 2014
	NO_2	Beijing, China	0.0952	0.384	Rich et al 2012
		Fixed effect	*0.0496*	*0.377*	
VWF	PM_{10}	Beijing, China	-0.302	0.175	Wu et al 2012
		Erfurt, Germany	**3.947**	**1.813**	Ruckerl et al 2006
		Netherlands	**0.163**	**0.0756**	Strak et al 2013
		Netherlands	0.0481	0.221	Zuurbier et al 2011
		US	-0.422	0.453	Liao et al 2005
		Random effect	*-0.0236*	*0.177*	
	NO_2	Netherlands	1.129	1.544	Strak et al 2013
		Beijing, China	-0.503	0.540	Wu et al 2012
		Beijing, China	**1.654**	**0.577**	Rich et al 2012
		Erfurt, Germany	**3.314**	**1.483**	Ruckerl et al 2006
		US	-0.140	0.372	Liao et al 2005
		Random effect	*0.698*	*0.577*	
	SO_2	Beijing, China	**3.966**	**0.895**	Rich et al 2012
		US	0.620	0.639	Liao et al 2005
		Random effect	*2.234*	*1.672*	

Note: Text for statistical significant changes were **Bold**.

+ The changes were in percentage changes (%) otherwise specified.

++For the Israel study, changes of all the biomarkers was estimated from the pooled effects of male and female.

*$p<0.05$ for fixed or random effects

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THE UNIVERSITY OF HONG KONG
香 港 大 學 公 共 衛 生 學 院

邀請參與研究信

Appendix 1

致　貴住戶：

香港環境保護署 委託 **香港大學** 進行一項研究，我們誠邀閣下參加，以助制訂改善空氣質素政策。以下是有關研究的資料：

題目：【空氣污染與生物標記】

背景： 空氣污染氧化物可破壞人體細胞內的基因、脂肪和蛋白質組織，並產生一些獨特的分子。這些分子可以作為生物標記，以了解空氣污染所致的健康影響。

目的： 我們的目標是評估居民暴露在城市中商業及住宅區的微細懸浮粒子($PM_{2.5}$)所引致的健康影響。

對象： 50 至 65 歲，無長期病患，必須居住在受邀請的單位之內，並為非吸煙人仕（呼氣測試將會在到訪時提供）

地點： 香港沙宣道 21 號 香港大學醫學院 實驗室大樓

日期： 2014 年 7 月和 10 月; 2015 年 1 月和 4 月(共 4 次來訪)

時間： 早上 8 時至 11 時 30 分

過程： 參加者在研究時期內 4 次到訪上述研究地點，進行抽血(~25ml)及手指血糖測試。抽血過程由有20年在香港紅十字會工作經驗的護士負責。參加者亦需要填寫問卷及量度身高、體重、血壓和肺功能。

資助： 每次到訪，參加者將會得到港幣 400 元的交通資助。當完成全部 4 次到訪後，將會有額外港幣 400 元的獎勵，合共 2000 元。

報名及查詢： 2014 年 4 月 30 日前聯絡 曾小姐 (Hilda)
電話: **2831 5057** (辦公時間為上午 10 時至下午 6 時)
電郵地址：**tsangh@hku.hk**

我們會回覆合適這研究的 120 位人仕。你的踴躍參與將會協助香港政府有效地改善空氣質素，令香港的居住環境更美好！

祝你　馬年身心健康，福杯滿溢！

香港大學 公共衛生學院
環境健康研究組 研究助理教授
黎克勤博士 謹啟

二零一四年三月二十八日

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Appendix 2

研 究 資 料 書

研究標題 : 空 氣 污 染 與 生 物 標 記

您現正被邀請參加一項研究, 在您決定參加與否前, 您必須清楚明白為何會有此研究, 以及其所涉及的內容。請仔細和小心閱讀以下的資料, 如有需要, 請與您的家人親友和家庭醫生討論。如有任何疑問, 請詢問我們。請仔細考慮您是否願意參加是項研究。

****

這項研究的目的是什麼?

研究背景: 分子水平上的氧化壓力評估, 是解釋空氣污染所引致的肺部和其他健康影響的生物機制的核心假設之一。這個可以利用生物標記來分析, 即是空氣污染物氧化破壞人體的脫氧核糖核酸(DNA), 脂肪組織和蛋白質後所產生的分子。

研究目的: 我們的目標是評估在研究時期內, 暴露在香港城市中商業及住宅區的微細懸浮粒子（$PM_{2.5}$）所引致的亞臨床影響。

研究時期: 12個月 (2014年7月 至 2015年6月)

為何我會受到邀請?

我們挑選的受試者為華裔人仕, 50~65歲, 無已知的慢性疾病，住在空氣質素監測站附近。這項研究中共有120名受試者。

我是否要參加?

這是由您來決定是否參加的。如果您決定參加, 您將需要簽署一份同意書。在您決定參加後, 您仍然可以隨時自由地退出研究,而毋須任何原因。

如果我參加, 會發生什麼事?

您將參與一項為期12個月的研究, 即從 2014年7月 至 2015年6月。您需要在12個月當中到訪我們的研究所4次(2014年7月和10月; 2015年1月和4月), 每次持續2小時。每次到訪，我們將會提供港幣400元的交通資助, 當完成4次到訪後, 將會有額外港幣400元的獎勵。

我們將收集您的血液樣本(30毫升)進行分析，抽血過程會由曾經在香港紅十字會有20年工作經驗的護士負責。您亦需要填寫問卷, 量度身高、體重、血壓和肺功能。

我需要做什麼準備嗎?

您需要空腹進行血液測試, 即在測試前6~8小時內, 除了喝水外不能進食任何東西。您不應該是吸煙者(曾在過去12個月吸煙),亦不能在12個月的研究期間吸煙。您將被要求進行呼氣測試，以確認您的非吸煙狀況。

如果出現錯誤呢？

如果您在參與研究過程中受到傷害，將不會有特殊的補償安排。如果您受到損害是由於別人的疏忽，你可向香港大學的有關部門投訴。

請問我參與這項研究會被保密嗎?

在研究過程中所收集的個人資料將被嚴格保密, 任何輸出大學的資料將會移去您的姓名和地址等個人信息，您將不會從中被識別。

研究的結果會怎樣?

研究的結果將會發佈於科學期刊上, 您可以索取已發佈的論文, 而您將不會在任何報告/刊物中被識別。

資料保密

有需要的話，每個研究參與者都有權利獲得公開報告的研究結果。
根據香港法律（特別是「個人資料（私隱）條例」，第486章），您有權對您個人資料進行保密，如在本項研究中或與本項研究有關的個人資料的收集、保管、保留、管理、控制、使用（分析或比較）、在香港內外轉讓、不披露、消除和/或任何方式處理。如有任何問題，您可以諮詢隱私資料私隱專員或致電到其辦公室（電話號碼：2827 2827），以適當監管或監督您個人資料保護，以便您能完全認識和瞭解確保遵守法律保護隱私資料的意義。

同意參與該項研究，您明確作出以下授權:

* 為了監督該項研究，授權主要研究者及其研究團隊和倫理委員根據本項研究和本知情同意書規定的方式獲得、使用並保留您的個人資料，並且
* 為了檢查和核實研究資料的完整性、評估研究協定與相關要求的一致性，授權相關的政府機構（如香港環境保護署）可獲得您個人資料。

誰在籌備和資助這項研究？

香港特別行政區環境保護署是此項的資助者。環保署將支付香港大學公共衛生學院以包括您在這項研究中。

誰人已審閱本研究？

香港大學及醫管局港島西醫院聯網研究倫理委員會。

****

感 謝 您 參 加 這 項 研 究 ！

SCHOOL OF PUBLIC HEALTH
THE UNIVERSITY OF HONG KONG
香 港 大 學 公 共 衞 生 學 院

研 究 同 意 書

參加者編號：

研究名稱：空 氣 污 染 與 生 物 標 記

請剔選

1. 本人確定已細閱及明白上述研究的資料書 (版本1.00) ，亦確定本人提出的所有與是次研究有關的問題已得到解答。 ☐
2. 本人明白是次的參與研究是自願性的，本人可於任何時候退出研究而毋需任何理由。 ☐
3. 本人明白本人的個人資料或會被香港大學相關的人仕或有關團體查閱，本人在此允許有關人仕查閱本人的個人資料。 ☐
4. 本人同意參加以上的研究。 ☐

參加者姓名	日期	簽署

Sassoon Road Campus:
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Appendix 3

2014年6月17日

Version 1.0

空氣污染與生物標記

問 卷 調 查

香港大學

公共衛生學院

（由研究員填 寫）研究編號：

問卷日期：

第一部分：背境資訊

1.1 性別 ☐ 男 ☐ 女

1.2 婚姻狀況

☐ 未婚 ☐ 已婚 ☐ 同居
☐ 分居 ☐ 離婚 ☐ 喪偶

1.3 家中同住的成員(可多選)

☐ 獨身 ☐ 異性配偶 ☐ 父母 ☐ 其他長輩
☐ 兄弟姊妹 ☐ 同輩親戚 ☐ 朋友 ☐ 子女
☐ 孫兒女 ☐ 其他晚輩 ☐ 其他，請說明：_____

1.4 包括您本人，現時居住的單位共有幾人? _____人

1.5 出生日期 _____年_____月_____日

1.6a 您居住在現時的地址多少年? _____年

1.6b 你居住在哪一區? ☐ 中西區 ☐ 旺角區 ☐ 其他，請說明：_____

1.7 您的教育程度是?

☐ 沒正式教育/幼稚園 ☐ 小學 ☐ 中一至中五
☐ 預科 ☐ 專業文憑 ☐ 大學或以上

1.8 過去12個月，您**個人**的**每月平均開支**是多少? （只需包括衣，食，行）

☐ <2000元 ☐ 2000～3000元 ☐ 3000～4500元
☐ 4500～6700元 ☐ 6700～10000元 ☐ ≥10000元，請說明：_____元

1.9 您現時居住的樓宇是什麼類型?

☐ 私人房屋(自置) ☐ 租住整個單位 ☐ 租住一個房
☐ 租住床位 ☐ 其他，請說明：_____

1.10 您現在的**工作狀況**是?

☐ 全職 ☐ 兼職 ☐ 家庭主婦→*第二部分*

☐ 已退休→*第二部分* ☐ 其他，請說明：_____

1.11 這份**工作**是在**哪一區**?

港島： ☐ 灣仔區 ☐ 東區 ☐ 中西區 ☐ 南區

九龍： ☐ 觀塘區 ☐ 黃大仙區 ☐ 九龍城區 ☐ 深水埗區

☐ 油尖區 ☐ 旺角區

新界： ☐ 葵青區 ☐ 荃灣區 ☐ 屯門區 ☐ 元朗區

☐ 沙田區 ☐ 大埔區 ☐ 北區 ☐ 西貢區 ☐ 離島

1.12 **過去12個月**，您主要的**工作環境**是?

☐ 室內→*到1. 12a* ☐ 戶外→*到1. 12b*

1.12a 在室內工作時，會否曝露於**燃燒氣體／揮發性氣體**等的空氣污染中?

☐ 沒有 ☐ 有，每天1~2小時 ☐ 有，3~4小時 ☐ 有，5小時或以上

1.12b 在戶外工作時(包括上班和下班的時間)，會否長時間**接觸路面的交通**?

☐ 沒有 ☐ 有，每天1~2小時 ☐ 有，3~4小時 ☐ 有，5小時或以上

第二部分：吸煙與被動吸煙

2.1 **過去**您的吸煙習慣?

☐ 從不吸煙 →*到2. 5* ☐ 偶然吸煙（不是每天） ☐ 每天吸煙

2.2 您戒煙已有幾年? _____年

2.3 您在戒煙前平均每天吸煙多少支? _____支/天（少於一支填“0”）

2.4 您在戒煙前已吸煙多少年? _____年（少於一年填“0”）

2.5 過去12個月，您每週在室內接觸**二手煙**的時間是多長? _____小時/週

2.6 與你同住的人中有多少人吸煙? ☐ 沒有 ☐ 有，多少人? _____人

第三部分： 飲食習慣

3.1 下列的哪一種情況最能反映您過去12個月的飲酒習慣？

☐ 完全沒飲／僅在特別場合飲(一年1-2次)
☐ 每月少於1次
☐ 每週少於1次
☐ 每週1-3次
☐ 每週4-6次
☐ 每天或幾乎每天

3.2 每週有多少餐(正餐)在家中煮食／進食? _____餐

3.3 您進食以下各類食物平均有多頻密?			每月		每星期		
		不食	少於1次	1-3次	1-3次	4-6次	每天
a	新鮮水果						
b	新鮮蔬菜						
c	豆腐、豆漿、腐皮、豆腐花						
d	牛奶/奶粉、芝士						
e	中國茶 – 綠茶 (如:龍井、碧螺春)						
f	中國茶 - 半發酵/全發酵 (如:普洱、鐵觀音)						
g	魚類 – 淡水魚						
h	魚類 – 海鮮						
i	肉類 – 紅肉(如:豬、牛)						
j	肉類 – 白肉(如:雞)						
k	蛋類						

3.4 您平均每天進食以下各組食物的比例是多少?

	第一組	第二組	第三組
食物種類	**穀類、麵包、飯、粉、麵**	**水果、蔬菜、瓜類**	**瘦肉、家禽、魚類、豆類、蛋類、牛奶、奶類產品、芝士類**
進食的比例 (請填上適當數字，進食愈多數值愈大)	*例如:* *3* : *2* : *1*		
		:	:

第四部分：體力活動狀況

4.1 過去7天您平均每天做**家務或體力勞動**的時間是? _____小時_____分鐘

4.2 過去7天您平均每天的**步行**時間是? _____小時_____分鐘

4.3a 除了做**家務或體力勞動**外您做**比步行較劇烈的運動**平均有多**頻密**?

☐ 每天 ☐ 每星期4-6天 ☐ 每星期1-3天 ☐ 每星期少於1天

4.3b 過去7天您平均每天做**比步行較劇烈的運動**時間是? _____小時_____分鐘

4.3c 您通常在哪裏做這些**較劇烈的運動**?

室內：☐ 家中 ☐ 體育館/社區中心 ☐ 其他，請說明：_____

戶外：☐ 公園 (哪個公園? _____) ☐ 其他，請說明：_____

第五部分：室內空氣污染

5.1 您在家中煮食是否用**明火煮食爐**? ☐ 是 ☐ 否

5.2 過去7天每天在家中的時間(包括睡覺)? _____小時/日

5.3 過去7天每天**睡眠**時間? _____小時/日

5.4 過去7天使用**冷氣**的時間? _____小時/日

5.5 過去7天使用**暖氣**的時間? _____小時/日

5.6 過去7天使用**電風扇**的時間? _____小時/日

5.7 過去7天使用**空氣清新機**的時間? _____小時/日

5.8a 過去7天當您在家中時，平均每天有多少時間**打開窗戶**? _____小時

5.8b 過去7天，您有曾經因為**防止蚊蟲**而關窗嗎? ☐ 是 ☐ 否

5.8c 過去7天，您有曾經因為**噪音**而關窗嗎? ☐ 是 ☐ 否

第六部分：個人健康狀況

6.1 與其他同齡的人比較，您認為您的健康狀況是?

☐ 非常好 ☐ 好 ☐ 普通 ☐ 差 ☐ 非常差

6.2 過去7天您有否服用藥物?

☐ 沒有 ☐ 有，請說明：_____

個人病歷

6.3a 您有否心臟、循環系統的疾病？ ☐ 否→*到6. 4a* ☐ 有

6.3b 是哪種疾病？（可多選）

☐ 高血壓 ☐ 冠心病

☐ 中風 ☐ 其他，請說明：_____

6.4a 您有否呼吸系統的疾病？ ☐ 否→*到6. 5a* ☐ 有

6.4b 是哪種疾病？（可多選）

☐ 慢性支氣管炎　☐ 哮喘　☐ 肺結核
☐ 肺氣腫　☐ 其他，請說明：_____

6.5a 您有否神經系統、精神科的疾病?　☐ 否→*到6. 6a*　☐ 有

6.5b 是哪種疾病？（可多選）

☐ 帕金森遜症　☐ 腦退化症 (老年癡呆)
☐ 抑鬱　☐ 其他，請說明：_____

6.6a 您有否內分泌、新陳代謝的疾病?　☐ 否→*到6. 7a*　☐ 有

6.6b 是哪種疾病？（可多選）

☐ 甲狀腺疾病　☐ 痛風症　☐ 糖尿病
☐ 高膽固醇　☐ 其他，請說明：_____

6.7a 您有否消化系統的疾病？　☐ 否→*到6. 8a*　☐ 有

6.7b 是哪種疾病？（可多選）

☐ 胃潰瘍　☐ 其他，請說明：_____

6.8a 您有否生殖泌尿的疾病？　☐ 否→*到6. 9a*　☐ 有

6.8b 是哪種疾病？（可多選）

☐ 小便失禁　☐ 前列腺病　☐ 其他，請說明：_____

6.9a 您有否肌肉骨骼的疾病？　☐ 否→*到6. 10a*　☐ 有

6.9b 是哪種疾病？（可多選）

☐ 退化性關節炎　☐ 肩周炎　☐ 痛風
☐ 骨質疏鬆症　☐ 其他，請說明：_____

6.10a 您有否眼耳鼻喉的疾病？　☐ 否→*到6. 11a*　☐ 有

6.10b 是哪種疾病？（可多選）

☐ 失聰　☐ 白內障　☐ 青光眼
☐ 其他，請說明：_____

6.11a 您有否癌症？　☐ 否→*到6. 12*　☐ 有

6.11b 是哪種癌症？（可多選）

☐ 乳癌　☐ 子宮頸癌　☐ 腸癌
☐ 肺癌　☐ 其他，請說明：_____

6.12 您有否其他疾病？

☐ 否
☐ 有，請列明：_____ _____ _____
_____ _____ _____
_____ _____ _____

**********問卷調查完畢**********

<空氣污染與生物標記>

（由研究員填 寫）研究編號：

日期：

物理體檢 (由研究員填寫)

1 抽血時間 _____

2 呼氣測試(一氧化碳濃度) _____ ppm

3 身高(脫鞋) _____ 釐米

4 體重(脫鞋、單衣) _____ 公斤

5 血壓和心率 (靜坐5分鐘後測量)

	收縮壓	舒張壓		心率	
1.	☐☐☐	☐☐☐	mmHg	☐☐☐	次/分鐘
2.	☐☐☐	☐☐☐	mmHg.	☐☐☐	次/分鐘
平均值	☐☐☐	☐☐☐	mmHg.	☐☐☐	次/分鐘

6 肺功能(用力肺活量FVC，第一秒吐出量FEV_1)

☐ 完成2次 ☐ 完成1次 ☐ 未能完成

Appendix 4 Assay Protocol for the biomarkers

Note: Modifications of the assay from the manufacturer protocols was Underlined.

i) Assay Protocol for 8-OHdG level

1. Bring all reagents and samples to room temperature (20-25ºC) before use.
2. Reconstitute the Primary Antibody with the Primary Antibody Solution.
3. Add 50 µl of sample or Standard per well.
4. Add 50 µl of reconstituted primary antibody per well. Shake the plate from side to side and mix fully. Cover the plate with an adhesive strip, making sure it is sealed tightly. Incubate at 37°C for 1 hour.
5. Mix 1 volume of Washing solution (5X) with 4 volumes of distilled water.
6. Pour off contents of wells into sink. Pipette 250 µl washing solution into each well. After washing thoroughly by shaking the plate from side to side, dispose of washing solution. Invert plate and blot against clean paper towel to remove any remaining washing buffer. Repeat wash two times more.
7. Reconstitute the Secondary Antibody with the Secondary Antibody Solution.
8. Add 100 µl of constituted secondary antibody per well. Shake the plate from side to side and mix fully. Cover the plate with an adhesive strip. Incubate 37°C for 1 hour.
9. At the end of the incubation period, repeat washing as in Step 6.
10. Prepare substrate solution. Add 1 volume of the Chromatic Solution to 100 volumes of the Diluting Solution just before use. Add 100 µl of substrate solution per well. Shake the plate from side to side and mix fully. Incubate at room temperature for 15 minutes in the dark.
11. Add 100 µl of the Reaction Terminating Solution. Shake the plate from side to side and mix fully.
12. Measure the absorbance at 450 nm using micro-titer plate reader.
13. Use a standard curve to determine the amount of 8-OHdG present in test samples. Generate the standard curve by plotting absorbance vs log (concentration of standards). Then use the absorbance values obtained for the test samples to determine the concentrations.

ii) Assay Protocol for MDA level

A) *Reagent Reconstitution:*

1. Take one vial of TBA and add of 7.5 ml Acetic Acid and mix.
2. Transfer the slurry to another tube and add ddH_2O to a final volume of 25 ml. Mix well to dissolve.

B) *Sample Preparation:*

3. Mix 10 µl with 500 µl of 42 mM H_2SO_4 in a microcentrifuge tube.
4. Add 125 µl of Phosphotungstic Acid Solution and vortex.
5. Incubate at room temperature for 5 minutes, then centrifuge for 3 minutes at 13,000 x g.
6. Collect the pellet and re-suspend on ice with 100 µl ddH_2O (with 2 µl BHT).
7. Adjust the final volume to 200 µl with ddH_2O.

C) *MDA Standard Curve:*

8. Dilute 10 µl of the MDA standard with 407 µl of ddH_2O to prepare a 0.1 M MDA solution, then dilute 20 µl of the 0.1 M MDA solution with 980 µl of ddH_2O to prepare a 2 mM MDA Standard.
9. For colorimetric analysis, add 0, 0.5, 1, 1.5, 2, 2.5 µl of the 2 mM MDA Standard into separate microcentrifuge tubes and adjust the volume to 200 µl with ddH_2O to generate 0, 1, 2, 3, 4, and 5 nmol Standard.

D) *Develop:*

10. Add 600 µl of TBA reagent into each vial containing standard and sample. Incubate at 95°C for 60 minute. Cool to room temperature in an ice bath for 10 minutes. Pipette 200 µl (from each 800 µl reaction mixture) into a 96-well microplate for analysis.

E) *Measure:*

11. For colorimetric analysis, read the absorbance at 532 nm.

F) *Calculation:*

12. Plot the MDA Standard Curve and determine the MDA amount in the test sample in nmol by interpolation from the standard curve.

iii) Assay Protocol for GSH/GSSG ratio

A) Preparation of stock solution:

1. Add 200 µl of Assay Buffer into the vial of GSH Standard to make 1 mM GSH standard stock solution.
2. Add 200 µl of ddH2O into the vial of GSSG Standard to make 1 mM GSSG standard stock solution.
3. Add 100 µl of DMSO into the vial of Thiol Green Indicator to make 100X Thiol Green stock solution.

B) Preparation of assay mixtures:

4. Add 100 µl of 100X Thiol Green stock solution into 10 ml of Assay Buffer and mix well to make the GSH Assay Mixture (GAM)

5. Add 5 ml of GAM into the bottle of GSSG probe and mix well to make the total GSH Assay Mixture (TGAM).

C) Preparation of GSH Standards:

6. Add 10 µl of GSH standard stock solution into 990 µl of Assay Buffer to generate 10 µM GSH standard solution.
7. Add 200 µl of 10 µM GSH standard solution to perform 1/2 serial dilutions to get 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1563 and 0 µM serially diluted GSH standards.
8. Add GSH standards and test samples into a solid black 96-well microplate.

D) Preparation of GSSG Standards:

9. Add 10 µl of GSSG standard stock solution into 990 µl of Assay Buffer to generate 10 µM GSSG standard solution.
10. Take 200 µl of 10 µM GSSG standard solution to perform 1/2 serial dilutions to get 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, and 0 µM serially diluted GSSG standards. The concentrations of Total GSH standard solutions should be twice the concentrations of GSSG standard solutions as 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, and 0 µM.
11. Add GSSG standards and test samples into a solid black 96-well microplates.

E) Run GSH and Total GSH Assay:

12. Add 50 µl of GSH Assay Mixture into the wells of GSH standard, blank control, and test samples to make the total assay volume of 100 µl/well.
13. Add 50 µl of Total GSH Assay Mixture into the wells of GSSG standard, a new blank control, and the other set of test sample to make the total assay volume of 100 µl/well.
14. Incubate the reaction at room temperature for 30 minutes, protected from light.
15. Monitor the fluorescence increase at Ex/Em = 490/520 nm with a fluorescence plate reader.

F) Data analysis:

16. The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the GSH reactions.

iv) Assay Protocol for Triglyceride level

A) Sample preparation:

1. Add 12 µl test samples to a 96-well plate. Adjust the volume was adjusted to 50 µl/well with Triglyceride Assay Buffer.
2. A background control is performed by replacing 2 µl Lipase with 2 µl Triglyceride Assay Buffer. The background is subtracted from all readings.

B) Standard curve preparation:

3. For the colorimetric assay, dilute 40 µl of the 1 mM Triglyceride into 160 µl Triglyceride Assay Buffer, mix to generate 0.2 mM Triglyceride Standard.
4. Add 0, 10, 20, 30, 40, 50 µl of the 0.2 mM Triglyceride Standard into a series of wells.
5. Adjust volume to 50 µl/well with Triglyceride Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard.

C) Lipase:

6. Add 2 µl of Lipase to each Standard and sample well.
7. Mix and incubate for 20 minutes at room temperature to convert triglyceride to glycerol and fatty acid.

D) Triglyceride reaction mix:

8. Mix enough reagent for the number of assays to be performed: For each well, a total 50 µl Reaction Mix is prepared with 46 µl Reaction Mix Triglyceride Assay Buffer, 2 µl Triglyceride Probe, and 2 µl Triglyceride Enzyme Mix.
9. Add 50 µl of the Reaction Mix to each well containing the Triglyceride Standard, samples and background control. Mix well. Incubate at room temperature for 60 minutes. Protect from light.

E) Measurement and calculations:

10. Measure absorbance at 570 nm in a microtiter plate reader for colorimetric assay.
11. Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the TG Standard Curve.

v) Assay Protocol for HDL-C level

A) Separation of HDL-C and LDL-C:

1. Mix 100 µl of 2X Precipitation Buffer with 100 µl of serum sample in microcentrifuge tubes. Incubate 10 minutes at room temperature, centrifuge at 5000 rpm on bench-top microcentrifuge for 10 minutes.
2. Transfer the supernatant into new labelled tubes. This is the HDL-C fraction.

B) Standard curve and sample preparation:

3. Dilute the Cholesterol Standard to 0.25 µg/µl by adding 20 µl of the Cholesterol Standard to 140 µl of Cholesterol Assay Buffer, mix well.
4. Add 0, 4, 8, 12, 16, 20 µl into a series of wells in a 96-well plate.
5. Adjust volume to 50 µl/well with Cholesterol Assay Buffer to generate 0, 1, 2, 3, 4, 5 µg/well

of the Cholesterol Standard.

6. For sample testing, using 1 to 20 µl of the HDL-C fraction, adjust the total volume to 50 µl/well with the Cholesterol Assay Buffer.

C) Reaction mix preparation:

7. Mix enough reagent for the number of assays performed. For each assay, prepare a total 50 µl Reaction Mix containing: 44 µl Cholesterol Assay Buffer, 2 µl Cholesterol Probe, 2 µl Enzyme Mix, and 2 µl Cholesterol Esterase.
8. Add 50 µl of the Reaction Mix to each well containing the Cholesterol Standard or test samples, mix well.
9. Incubate the reaction for 60 minutes at 37°C, protect from light.

D) Measurement and calculations

10. Measure O.D. at 570 nm in a micro-titer plate reader.
11. Subtract 0 standard reading from readings. Plot the standard curve. Apply the sample readings to the standard curve to determine sample cholesterol amount in the reaction well.

Appendix Table 4. ANOVA F-Ratio for Differences Between Groups Adjusted for Time of Visit

	df 1[a]	df 2	8-OHdG	MDA	GSH/ GSSG	HDL-C	Trigly-ceride	Heart rate	DBP	SBP
Demographic										
Age	1	107	0.60	0.64	0.19	0.17	0.55	2.17	0.02	6.45*
Gender	1	107	4.27*	0.12	0.15	12.5***	0.66	0.07	13.8***	6.32*
BMI	4	294	0.66	0.83	1.07	4.56**	0.41	0.41	1.64	1.32
Lifestyles										
Exercise frequency	3	105	0.55	0.14	0.27	1.17	0.30	0.84	0.67	0.42
Passive smoking	1	107	0.31	0.27	0.09	0.01	0.28	6.55*	1.52	0.49
Alcohol drinking	4	104	0.31	0.86	3.19*	0.82	1.62	0.85	1.83	2.04
Dietary										
Fruit	1	107	0.69	1.06	0.70	2.70	0.01	0.44	1.92	0.12
Vegetables	1	107	5.47*	0.45	0.34	4.76*	0.22	0.28	1.71	1.95
Green tea	1	107	0.67	0.02	5.47*	1.02	0.00	10.2**	0.02	0.00
Red tea	1	107	1.51	0.04	5.58*	0.20	0.17	0.40	0.40	0.20

[a] df stands for degrees of freedom

*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001

Appendix Table 5. Crude and Partial Correlation of Biomarkers and O_3 in Central/Western and Mongkok in Baseline

Biomarkers	Crude	Demographic[a]	Demographic & Lifestyles[b]	Demographic & Dietary[c]	Demographic, Lifestyles, & Dietary
CW					
8-OHdG	-0.2767	-0.1904	-0.1676	-0.2336	-0.2185
MDA	**-0.3394***	-0.2332	-0.2475	-0.2739	-0.2629
GSH/GSSG	0.0569	-0.1413	-0.1020	-0.1084	-0.0668
MK					
8-OHdG	0.0340	0.0449	-0.2960	0.0193	**-0.5295***
MDA	-0.3311	-0.3070	-0.3198	**-0.4031***	-0.3608
GSH/GSSG	0.0419	-0.0157	-0.1356	-0.0346	-0.1217
All (CW and MK)					
8-OHdG	-0.0099	0.0155	-0.0511	0.0356	-0.0189
MDA	**-0.2538***	-0.2420	-0.2505	**-0.2683***	**-0.2739***
GSH/GSSG	0.0252	0.0000	-0.0710	0.0075	-0.0788

[a] Partial correlation adjusted for demographic factors (age, gender and BMI)

[b] Partial correlation adjusted for demographic factors[a] and lifestyles (alcohol drinking frequency, exercise frequency, and passive smoking exposure)

[c] Partial correlation adjusted for demographic factors[a] and dietary (frequency of fresh fruits and vegetables intake, and drinking of tea)

*p-value < 0.05

Appendix Table 6. Literature Review on the Level of the Oxidative Stress Biomarkers

Biomarkers	Region	Mean	References (by publication year)
8-OHdG	Turkey	7.1	Sertan CU, et al. Psychiatry Res 2015;229:200-5
(ng/ml)	Austria	9.4	Matzi V, et al. Clin Lab 2015;61:587-93
	Taiwan	0.2	Lee HT, et al. Int J Mol Sci 2015;16:3757-68
	Taiwan	4.4	Tsai MC, et al. J Affect Disord 2015;173:22-6
	Turkey	3.7	Tabur S, et al. Tumour Biol 2015;36:2667-74
	Turkey	1.9	Kocael A, et al. Can J Surg 2014;57:183-7
	Turkey	12.1	Hendek MK, et al. J Periodontol 2015;86:273-82
	India	0.2	Basu S, et al. J Perinatol 2014;34:519-23.
	China	12.3	Ma Y, et al. Oxid Med Cell Longev 2013;543760
	Turkey	0.7	Bayram F, et al. Growth Horm IGF Res 2014;24:29-34
	Iran	34.9	Ghorbanihaghjo A, et al. J Health Popul Nutr 2013; 31:343-9
	China	1.4	Gao H, et al. PLoS One 2013;8:e67727
	China	0.8	Lin LY, et al. Sci Total Environ 2013;463-4:176-81
	China	6.3	Chang D, et al. Oxid Med Cell Longev 2013;587826
	Netherlands	0.2	Fischer SG, et al. Int J Mol Sci 2013;14:7784-94
	Poland	0.003	Płonka PE, et al. Pharmacol Rep 2013;65:99-106
	Austria	0.3	Müllner E, et al. Mol Nutr Food Res 2013;57:328-38
	Turkey	9.5	Gönenç A, et al. Eur J Intern Med 2013;24:39-44
	Slovenia	1.1	Letonja MS, et al. Mol Biol Rep 2012;39:10121-30
	Sweden	0.5	Harms-Ringdahl M, et al. Nutr J 2012;11:29
Mean range*			**0.2-12.3 ng/ml**
MDA	India	1.1	Mukhopadhyay B, et al. J Clin Diagn Res 2016;10:BC08-10
(nmol/ml)	Spain	1.1	Lorente L, et al. PLoS One 2015;10:e0125893
	Turkey	0.4	Erem C, et al. Endocr J 2015;62:493-501
	Brazil	9.6	De Souza GF, et al. BMJ Open 2015;5:e006048
	Austria	0.9	Matzi V, et al. Clin Lab 2015;61:587-93
	Turkey	3.2	Ari E, et al. Int Urol Nephrol. 2014;46:1843-9.
	Turkey	1.1	Erden ES, et al. Eur Rev Med Pharmacol Sci 2014;18:3477-83
	India	2.0	Basu S, et al. J Perinatol 2014;34:519-23.
	Italy	1.2	Alvarez SJM, et al. J Nutr Biochem 2014;25:289-94
	India	1.0	Nagamma T, et al. Asian Pac J Cancer Prev 2014;15:9467-70
	Poland	1.3	Wojciechowska C, et al. Mediators Inflamm 2014;147040
	Poland	1.0	Adamczyk-Sowa M, et al. J Physiol Pharmacol 2014;65:543-50
	Czech Republic	0.8	Kanďár R, et al. Physiol Res 2014;63:753-62

	Austria	3.9	Walker J, et al. Hum Psychopharmacol 2014;29:537-43
	China	6.2	Ma Y, et al. Oxid Med Cell Longev 2013;543760
	China	2.6	Gao H, et al. PLoS One 2013;8:e67727
	China	3.3	Chang D, et al. Oxid Med Cell Longev 2013;587826
	Netherlands	5.4	Fischer SG, et al. Int J Mol Sci 2013;14:7784-94
	Turkey	5.5	Gönenç A, et al. Eur J Intern Med 2013;24:39-44
	Turkey	4.9	Kaya Y,et al. Int J Med Sci 2012;9:621-6
Mean range*			**0.8-5.5 nmol/ml**
GSH/GSSG ratio	UK	17.5	Seshadri S, et al. Acta Ophthalmol 2015;93:e266-74
	Italy	160	De Felice C, et al. Mediators Inflamm 2014;560120.
	Italy	6.3	Ceci R, et al. Redox Biol 2013;2C:65-72
	Italy	9.5	Bellanti F, et al. Redox Biol 2013;1:340-6
	France	0.35	Turki A, et al. Free Radic Biol Med 2012;53:1068-79
	Spain	4.0	Tasset I, et al. Clin Biochem. 2012;45:440-4
	Mexico	7.4	Calderón-Salinas JV, et al. Mol Cell Biochem. 2011;357:171-9
	Mexico	11.5	Guevara-Arauza JC, et al. Chem Cent J 2011;5:10
	Panama	6.7	Rusanova I, et al. Eur J Haematol 2010;85:529-37
	Italy	96	Calabrese V, et al. Neurochem Res. 2010;35:2208-17
	USA	200	Elokda A, et al. J Neurol 2010;257:1648-53
	Turkey	27.8	Sahin E, et al. Ann Clin Biochem 2008;45:369-74
	Portugal	1.5	Machado MV, et al. Scand J Gastroenterol. 2008;43:95-102
	Italy	33.6	De Mattia G, et al. Diabetes Res Clin Pract. 2008;79:337-42
	Chile	7.2	Rodrigo R, et al. Hypertens Res 2007;30:1159-67
	Chile	7.5	Rodrigo R, et al. Mol Cell Biochem. 2007;303:73-81
	Cuba	5.7	Pardo-Andreu GL, et al. Arch Med Res 2006;37:158-64
	Italy	15.2	Veglia F, et al. Biomarkers. 2006;11:562-73
Mean range*			**1.5-160**

*Mean range is 2.5% to 97.5% range of the reported mean.

Note: We searched in PubMed with the following string in either title or abstract: [biomarkers] AND (serum OR plasma) AND healthy AND human, where "[biomarkers]" could be either 8-OHdG or MDA or GSH/GSSG.

The most recent 5-year publications for 8-OHdG and MDA, and 10-year publications for GSH/GSSG ratio (last search date 12 May 2016) with full text and the following criteria for each biomarker will be selected to calculate the reported mean level: (1) reported mean level for the biomarker, (2) human blood samples, (3) healthy adult, and (4) non-smoking.