Determinants of carbon load in airway macrophages in pregnant women☆

Mohammad Miri [a], Hossein Rezaei [b], Seyed Mojtaba Momtaz [c], Moslem Lari Najafi [d], Abolfazl Adli [e], Nasim sadat Pajohanfar [e], Mina Abroudi [e], Malihe Sadat Bazghandi [e], Zahra Razavi [e], Lucia Alonso [f,g,h], Cathryn Tonne [f,g,h], Xavier Basagaña [f,g,h], Mark J. Nieuwenhuijsen [f,g,h], Jordi Sunyer [f,g,h], Tim S. Nawrot [i,j], Payam Dadvand [f,g,h,*]

[a] Non-Communicable Diseases Research Center, Department of Environmental Health, School of Health, Sabzevar University of Medical Sciences, Sabzevar, Iran
[b] Student Research Committee, Department of Environmental Health, School of Health, Sabzevar University of Medical Sciences, Sabzevar, Iran
[c] Department of Environmental Health Engineering, Zarand School of Nursing, Kerman University of Medical Sciences, Kerman, Iran
[d] Pharmaceutical Sciences and Cosmetic Products Research Center, Kerman University of Medical Sciences, Kerman, Iran
[e] Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
[f] ISGlobal, Barcelona, Spain
[g] Universitat Pompeu Fabra (UPF), Barcelona, Spain
[h] CIBER Epidemiología y Salud Pública (CIBERESP), Spain
[i] Centre for Environmental Sciences, Hasselt University, Hasselt, Belgium
[j] Department of Public Health & Primary Care, Leuven University, Leuven, Belgium

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ABSTRACT

The airway macrophages carbon loading (AMCL) has been suggested to be a biomarker of the long-term exposure to air pollution; however, to date no study has characterized AMCL for the pregnancy period. Therefore, this study aimed to assess the determinants of AMCL during pregnancy in Iran, a middle-income country. This study was based on a sample of 234 pregnant women with term and normal vaginal delivery who were residing in Sabzevar, Iran (2019). We characterized 35 potential determinants of personal exposure to air pollution for each participant, including six personal, nine indoor, and 20 home-outdoor factors. We applied Deletion/Substitution/Addition algorithm to identify the most relevant determinants that could predict AMCL levels. The median (IQR) of AMCL level was 0.12 (0.30) μm^2 with a successful sputum induction in 82.9% (194) of participants. Ambient residential $PM_{2.5}$ levels were positively associated with higher AMCL levels. On the other hand, increased residential distance to the traffic lights, squares and ring-roads, the duration of opening window per day, and opening window during cooking were inversely associated with AMCL levels. Our findings provide novel insights on the different personal, indoor, and outdoor determinants of personal exposure to air pollution during pregnancy in a middle-income country.

1. Introduction

Air pollution is a major environmental hazard worldwide. More than 98% of the global population lives in areas that do not meet World Health Organization (WHO) air quality guidelines and, at the same time, there is a notable difference between high-, middle-, and low-income countries in this regard (WHO, 2021). While, in high-income countries, about half of the cities with a population of more than 100 000 inhabitants do not meet WHO air quality recommendations, this proportion reaches 97% in low- and middle-income countries (LMICs)

cities. This difference in air pollution levels is accompanied by a considerably greater health burden of air pollution in LMICs. More than 90% of the annual 4.2 million premature deaths that could be attributed to air pollution worldwide, occur in LMICs (WHO, 2021). At the same time, whilst studies on determinants of the personal exposure to air pollution could provide an evidence base necessary to fine-tune targeted interventions to tackle the burden of air pollution, a vast majority of these studies have been conducted in high-income countries whose finding are not necessarily generalizable to LMICs, for which such evidence is still very scarce (Milà et al., 2018; Curto et al., 2019; Sanchez

et al., 2020; Shupler et al., 2020).

Pregnant women and developing foetus are particularly vulnerable to air pollution exposure. This exposure has been associated with a higher risk of pregnancy complications (e.g., preeclampsia, gestational diabetes) as well as adverse birth outcomes (e.g., low birth weight and preterm birth) (Melody et al., 2019; Bai et al., 2020). In this context, identifying the determinants of personal exposure to air pollution during pregnancy is of importance for both researchers and policymakers. Given the logistical and perhaps ethical constraints of conducting long-term personal monitoring of exposure to air pollution in pregnant women using personal samplers (Valero et al., 2009; Schembari et al., 2013), studies for identifying the determinants of personal air pollution exposure during pregnancy could benefit from novel biomarkers of this exposure (e.g. (Øvrevik, 2019; Li et al., 2020; Zhao et al., 2020), notably carbon load in airway macrophages (Bai et al., 2015a). Airway macrophages phagocyte the inhaled particles that are presented on the epithelial surfaces in the lumen of the bronchi and alveoli (Lundborg et al., 2006). Therefore, the carbon core of particles emitted from different sources, e.g., traffic, smoking and indoor sources, can be visualized inside the airway macrophages using light microscopy (Lundborg et al., 2006; Bai et al., 2015a). As such, airway macrophages carbon load (AMCL) has been used as a marker of personal exposure to ambient and indoor air pollution (Kulkarni et al., 2005; Kulkarni et al., 2006; McCreanor et al., 2007; Fullerton et al., 2009; Bai et al., 2015b; Bai et al., 2018a; Bai et al., 2018b; Momtaz et al., 2019). In this context, the AMCL has been suggested as a marker of long-term exposure to air pollution for up to six months (Bai et al., 2015a); thus, the AMCL based on the samples taken at the end of pregnancy could surrogate exposure to air pollution during the most of pregnancy period. However, to date, no study has applied AMCL in pregnant women. Accordingly, we aimed to investigate the level and determinants of AMCL levels in a sample of pregnant women in Iran, a middle-income country.
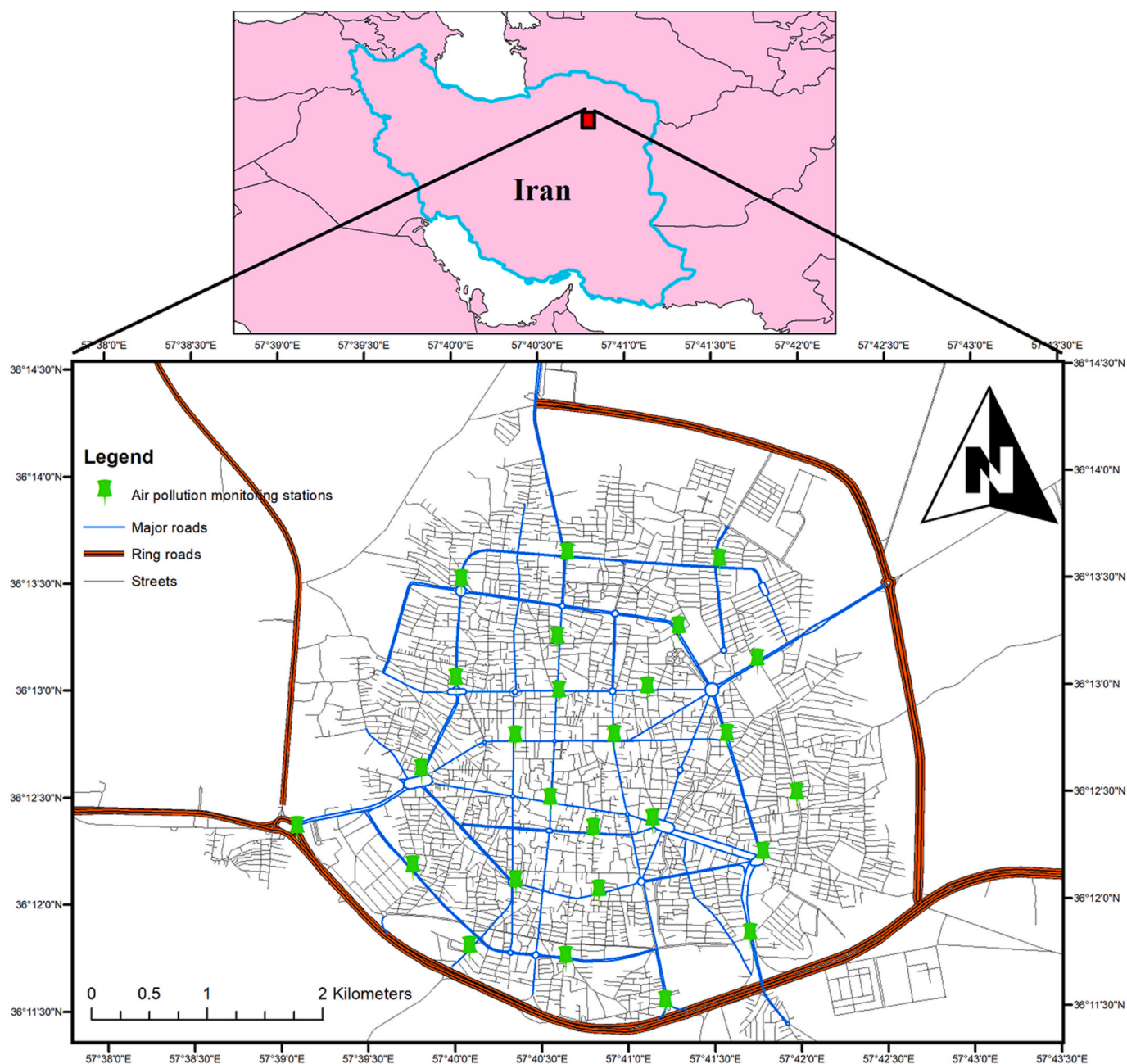

Fig. 1. Location of study area, monitoring stations of air pollution, roads and other traffic indicators.

2. Material and methods

2.1. Study area and population

This study was conducted in Sabzevar, a town with a 25 km^2 area and a population of about 240 000 inhabitants in the northeast of Iran (Fig. 1). Being located at the verge of the central desert in the Iran plateau, Sabzevar has an arid climate with an annual average precipitation and relative humidity of 170 mm and 43%, respectively (Office, 2020). The annual mean, average minimum and average maximum temperature in Sabzevar is respectively 18, -2 and 45$^°$C. One of the main highways in Iran passes through Sabzevar, contributing to the traffic-related air pollution in the city.

This study was based on a pregnancy cohort of 234 pregnant women enrolled at their delivery time during summer months (June to August) of 2018 in the Shahidan Mobini Hospital, the only maternity hospital of the Sabzevar. We included pregnant women with (i) normal vaginal delivery, (ii) gestational age of 37–43 weeks at delivery (i.e. term delivery), (iii) no chronic diseases such as diabetes or hypertension or pregnancy complications such as preeclampsia, (iv) who lived in Sabzevar during the entire pregnancy, (v) did not work outside the home, (vi) were not active smoker, and (vii) did not change their home during pregnancy. First, inclusion/exclusion criteria, aims and steps of the study were fully described for mothers who attended the hospital for delivery (about 1200 mothers) and 234 eligible mothers accepted our invitation to participate in the study. We carried out face-to-face interviews to obtain questionnaire data on socioeconomic and demographic characteristics as well as lifestyle habits. The Clinical Research Ethical Committee of Sabzevar University of Medical Sciences approved our study (IR.MEDSAB.REC.1395.82) and all participants signed an informed consent form before their enrolment into the cohort.

2.2. Sputum induction and AMCL measurements

The sputum samples were optioned one day after delivery at the hospital. The sputum induction was based on the method described by Scheicher et al. (2003), with slight modifications. An ultrasonic nebulizer device (1.7 MHz, NE-U17, Omron Healthcare Co., Ltd., Kyoto, Japan) was used to assist the inhalation of a 4.5% saline solution three consecutive times, each lasting 5-min (cumulative duration of 15 min) with a 5 min interval between each inhalation. We used salbutamol spray for participants who had coughs during inducing sputum. The sputum samples were collected in a sterilized plate and were transported to the laboratory within less than an hour near dry ice at four$^°$C. After the initial preparation, the slides of sputum samples were dried for 2 h at room temperature and then were stained by Diff Quick (Pavord et al., 1997). Light microscopy under oil immersion at 100× magnification was used to visualize the AMCL. Those participants whose sputum samples had less than 50 macrophages (Jacobs et al., 2010; Brugha et al., 2014; Bai et al., 2018b) were excluded from our analyses (40 participants). Finally, the images of 194 participants were analysed using ImageJ software (National Institutes of Health, USA) (ImageJ, 2020) to calculate the AMCL level in 50 randomly selected macrophages per participant, defined as the median area (μm^2) occupied by carbon (Jacobs et al., 2010; Brugha et al., 2014; Bai et al., 2018b). To provide more accuracy in our measurements, we checked some pictures with high level of spurious colors manually in the software. Moreover, the images with very high level of spurious colors/bacteria (where it was difficult to separate and detect carbon particles) were excluded from our analyses and replaced with new images from the slide. The image analyses for all participants were performed by two researchers independently (they were blinded to the exposure status of participants or any baseline information as well as their each other's results) and their results were compared by a third researcher (Brugha et al., 2012; Bai et al., 2015a). If the results of carbon loading measurements for each participant were exactly similar between the two raters (defined as the difference of lower

than ± 0.001 μm^2 between the median AMCL levels of 50 images reported by the two raters), we reported this value as the final AMCL level for that sample. For the samples for which the difference in the reported median levels of carbon loading was more than ±0.001 μm^2, we reanalysed it again by three researchers at the same computer through an open discussion about each image. Finally, an agreement was reached between the three researchers on the level of AMCL in the discussed samples.

2.3. Determinants of exposure

We obtained data on six, nine, and 20 personal, home-indoor, and home-outdoor factors, respectively, that we hypothesized could influence personal exposure to air pollution.

2.3.1. Personal determinants

The participant's information including age (year, continuous), pre-pregnancy body mass index (kg/m^2, continuous), pre-pregnancy weight (kg, continuous), height (meter, continuous), education level (high school and/or lower, university degree) and family income (<15 million, ≥15 million Rials) were collected using a questionnaire through a face-to-face interview.

2.3.2. Indoor determinates

Indoor determinants encompassed second-hand tobacco smoke at home during pregnancy (yes/no), duration of second-hand tobacco smoke at home during pregnancy (min/day), second-hand tobacco smoke in other places than home (e.g. public places, cafes, friend's homes, etc.) (yes/no), type of kitchen (open-plan kitchen vs. walled kitchen), use of kitchen hood during cooking (yes/no), opening window during cooking (yes/no), duration of opening window per day (min/day), the average daily cooking time during pregnancy (min/day), and type of home (Single-family house/apartment). It should be noted that in Sabzevar, like most cities in Iran, residents have access to natural gas through the national gas pipeline and as such, all participants of the study used natural gas for cooking and heating at their homes; therefore, we did not use this information in our analyses. The data on these indoor determinates were collected through face-to-face interview.

2.3.3. Home-outdoor determinants

The annual mean concentration of PM (PM$_1$, PM$_{2.5}$, PM$_{10}$) at residential address of participants was estimated based on land use regression (LUR) models, developed for Sabzevar. These LUR models were generated based on data obtained from 26 air pollution monitoring sites in Sabzevar (Fig. 1). These models could predict between 68% and 75% of the variation in the annual mean of these PMs. The details of PM measurement campaigns and LUR model development and performance were fully described elsewhere (Miri et al., 2019).

The data on total street length in 100, 300 and 500 m buffers around the residential address of participants and the residential distance to the nearest ring roads and major roads were calculated based on the street map of Sabzevar, provided by the Municipality of Sabzevar (2019). The data on the residential distance to the bus terminal, transportation land use (parking, cargo terminals, etc.), industrial land use, residential-industry land use, squares, mosque/religious places, and government offices were calculated based on the aforementioned land use map of Sabzevar. Moreover, distance from home to the nearest public parking, bus station, heavy traffic-light (based on the classification of Open Street Map (OSM)), gas stations and ATM banks were calculated based on data of OSM of Sabzevar.

2.4. Statistical analysis

We used different correlation coefficients (Spearman's correlation for two continuous variables, polychoric correlation for two categorical variables, or polyserial correlation for one categorical and one

continuous variable) to generate the correlation matrix of the determinants of AMCL.

The Deletion/Substitution/Addition (DSA) algorithm was applied to identify the most relevant determinants that could predict AMCL levels. Barrera-Gomez et al. and Agier et al. have suggested the DSA algorithm as a method with better performance in selecting variables compared other methods on the basis of its sensitivity, low false detection proportion, and ability to correct for correlated exposures (Agier et al., 2016; Barrera-Gómez et al., 2017). In the DSA algorithm, an empty model is started and the final model is created based on iteratively applying the following steps: i) removing a variable from the model (deletion) if this improves model fit, ii) replacing a variable in the model by another one not in the model (substitution) if this improves model fit or iii) adding a variable in the model (addition) if this improves model fit. An improvement of model fit is considered if the new model considered at a given step reduces the residual mean squared error (RMSE) calculated using 5-fold cross-validation. To stabilize the results for the random splitting of cross-validation, we ran DSA 50 times and we selected the variables that were selected by the algorithm at least once. For comparability between the estimates, all continuous determinants were divided by its interquartile range (IQR) before applying the DSA, so the reported associations were corresponding to one IQR increase in each exposure value. All analyses were performed with the R statistical software (version 3.6.2), using the DSA package.

3. Results

3.1. Determinants

Descriptive statistics of personal, indoor and home-outdoor determinants, as well as AMCL level in pregnant women are presented in Table 1. The median (IQR) age and pre-pregnancy BMI of the participants were 27.5 (8.0) years and 23.9 (5.7) kg/m^2, respectively. Most of the mothers had primary or high school education levels (71.7%). Moreover, 42 (21.7%) and 77 (39.7%) of mothers had second-hand tobacco smoke exposure at respectively home and other places than home during pregnancy. About 47% of mothers used kitchen hood during cooking and 60% of mothers used natural ventilation (opening the window) during cooking. The median (IQR) of estimated annual ambient levels of PM$_{10}$, PM$_{2.5}$, PM$_1$ at participants' homes were 47.7 (12.2), 43.8 (10.3), and 38.9 (9.4) μg/m^3, respectively.

The success rate of sputum acquisition for measuring AMCL in participants was 82.9% (194 participants). Fig. 2 shows the airway macrophages of sputum samples with a high carbon load level (Fig. 2 –A) and without any carbon load in the airway macrophages (Fig. 2 –B). The median (IQR) of AMCL in pregnant women was 0.12 (0.30) μm^2.

3.2. Correlation between determinants

The correlation between personal, indoor and outdoor determinates of personal exposure to air pollution during pregnancy are presented in Fig. 3. There was a positive but weak correlation between using hood and the amount time of cooking during pregnancy (r = 0.11). We also observed a negative correlation between second-hand tobacco smoke as well as duration of exposure to tobacco smoke at home during pregnancy and maternal education. Moreover, there was a weak correlation between personal and indoor determinants with estimated PMs as well as traffic indicators. There was a negative correlation between PMs and traffic indicators, e.g., distance to major roads, ring roads, the bus terminal and heavy-traffic lights. Moreover, a strong correlation was observed between the estimated PM$_1$ and PM$_{2.5}$, while PM$_{10}$ was moderately correlated with PM$_{2.5}$ and PM$_1$.

3.3. Main analyses

The estimated associations of determinates of personal exposure to

Table 1
Descriptive statistics of personal, indoor and outdoor determinants of exposure to air pollution during pregnancy.

	Description[a]
Personal determinants	
Maternal age (yr)	27.5 (8.0)
Mothers weight (kg)	61.5 (15.0)
Mothers hight (m)	1.6 (0.1)
Pre-pregnancy BMI (kg/m2)	23.9 (5.7)
Matenal education,	
Elementry and high-school	139 (71.7)
University	55 (28.3)
Income	
<1.5 milion Rials	139 (71.7)
≥1.5 milion Rials	55 (28.3)
Indoor determinants	
The average daily cooking time during pregnancy (min/day)	120.0 (120.0)
Duration of second-hand tobacco smoke at home during pregnancy [b]	30.0 (78.0)
Duration of opening window per day during pregnancy (min/day)	180.0 (60.0)
Second-hand tobacco smoke exposure at home during pregnancy (Yes)	42 (21.7)
Using kitchen hood during cooking (Yes)	102 (52.6)
Second-hand tobacco smoke exposure in other places than home (Yes)	77 (39.7)
Type of home	
Single-family house (vila)	103 (53.1)
Apartement	91 (46.9)
Type of kitchen	
Open-plan kitchen	28 (14.4)
Walled kitchen	166 (85.6)
Opening window during cooking	
Rarely/No	117 (60.3)
Some time/Always	77 (39.7)
Outdoor determinants	
PM$_{10}$ (μg/m^3)	47.7 (12.2)
PM$_{2.5}$ (μg/m^3)	43.8 (10.3)
PM$_1$ (μg/m^3)	38.9 (9.4)
Total street length around residential address (m)	
100 m buffer	886.4 (206.1)
300 m buffer	7417.6 (2200.2)
500 m buffer	19834.2 (6971.4)
Distance to major roads (m)	232.1 (298.7)
Distance to the bus terminal (m)	2983.6 (1916.7)
Distance to transportation land use (m)	168.4 (212.1)
Distance to industrial land use (m)	197.4 (268.4)
Distance to residential-industry land use (m)	122.5 (212.7)
Distance to ATM banks (m)	577.3 (608.7)
Distance to gas stations (m)	996.1 (413.5)
Distance to heavy trrafic_lights (m)	471.5 (561.2)
Distance to squares (m)	289.4 (256.0)
Distance to the bus station (m)	817.5 (788.8)
Distance to musque/religious places (m)	599.1 (629.8)
Distance to ring roads (m)	1022.7 (721.8)
Distance to public parking (m)	814.7 (559.1)
Distance to government offices (m)	200.3 (238.7)
Airway macrophages carbon loading (μm^2)	0.12 (0.30)

[a] Continuous variables are presented as median (interquartile range) and categorical variables as count (%).
[b] Reported as mean (standard deviation).

air pollution based on AMCL are presented in Table 2. The results of the DSA algorithm of 20 outdoor, nine indoor and six personal determinants indicated that home-outdoor levels of PM$_{2.5}$, residential distance to heavy-traffic lights, squares and ring-roads, existence and duration of the daily opening window at home, duration of the second-hand tobacco smoke exposure at home, type of kitchen, and participant's age were the most relevant indicators in association with AMCL.

An IQR increase in residential ambient PM$_{2.5}$ concentration was related with an increase of 0.351 μm^2 (95% confidence interval (CI): 0.186,0.517, P-value <0.01) in AMCL. Moreover, longer exposure to the second-hand tobacco smoke at home during pregnancy was related with higher AMCL levels. An IQR increase in the residential distance to
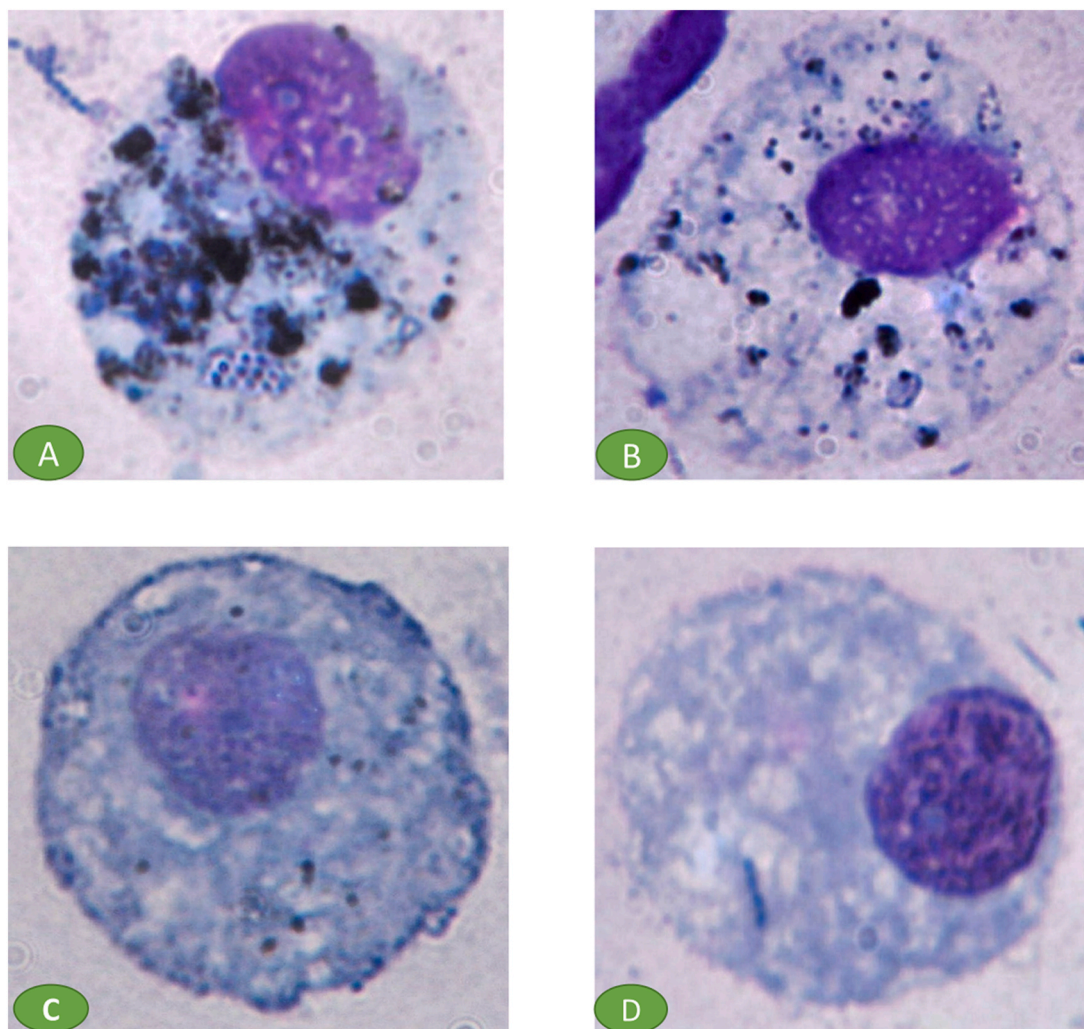
Fig. 2. Fig. 2: Airway macrophages of sputum samples with a high carbon load level (Fig. 2 –A and 2 –B), with low carbon load level (Fig. 2 –C) and without any carbon load (Fig. 2 –D).

heavy-traffic lights, squares, and ring-roads was related with a decrease of respectively −0.486 μm^2 (95%CI: 0.833, −0.139, P-value <0.01), −0.266 μm^2 (95%CI: 0.505, −0.026, P-value = 0.03) and −0.511 μm^2 (−0.835, −0.187, P-value <0.01) in AMCL levels. Furthermore, longer duration of daily opening window of home (β = −0.223, 95% CI: 0.392, −0.053, P-value = 0.03), opening window during cooking (β = −0.576, 95% CI: 0.971, −0.181, P-value <0.01), and older age (β = −0.028, 95% CI: 0.063, 0.007, P-value = 0.11) were inversely associated with AMCL levels. Additionally, maternal age, kitchen type, and the duration of exposure to the second-hand tobacco smoke at home during pregnancy stayed in the final model; however, their association with AMCL did not attain statistical significance (Table 2).

4. Discussion

To the best of our knowledge, this study is the first to characterize AMCL during pregnancy. Furthermore, this is the first study to explore the personal, indoor and outdoor determinates of personal exposure to air pollution in pregnant women in an LMIC. Moreover, this is one of the largest reported studies on characterization of AMCL levels. Furthermore, measuring the AMCL was performed by two independent investigators and checked by third investigator to reduce the AMCL measurement bias. We observed that the estimated ambient PM$_{2.5}$ levels at residential address was positively associated with AMCL. On the other hand, residential distance to heavy-traffic lights, squares and ring-roads,

the duration of opening window per day, and opening window during cooking were inversely associated with AMCL levels.

4.1. AMCL level and sputum induction

The median (IQR) of AMCL level in our study was 0.12 (0.30) μm^2, with a sputum acquisition success rate of 82.9%, which are compatible with those previous studies. A study by Bai et al. (2018), in 43 adult subjects reported a median (IQR) AMCL level of 0.346 (0.314) μm^2 with a success rate of 76.0% in sputum induction (Bai et al., 2018a). Another study by Kulkarni et al. (2006), reported a median (range) AMCL of 0.41 (0.03–1.14) and 0.00 (0.00–0.12) μm^2 for 64 healthy children and nine children with asthma with a successful ratio of sputum induction of 56% and 100%, respectively (Kulkarni et al., 2006). A study by Jacobs et al. (2010), on 137 adults with diabetes reported a median (range) of 0.19 (0.09–0.34) μm^2 for AMCL levels with a success rate of 87% (Jacobs et al., 2010). Moreover, a review study by Bai et al. (2015) on AMCL reported a success ratio between 56 and 100% based on six different studies (Bai et al., 2015a). Obtaining the sufficient number of macrophages in the samples could be influenced by personal factors (e.g., the squamous epithelial contamination), subject-specific physiological features, and technical factors such as time course of inhalation (cumulative duration of 15–21 min), pre-treatment with salbutamol, incremental changes in saline concentration, and the use of an ultrasonic nebulizer (Bai et al., 2015a).
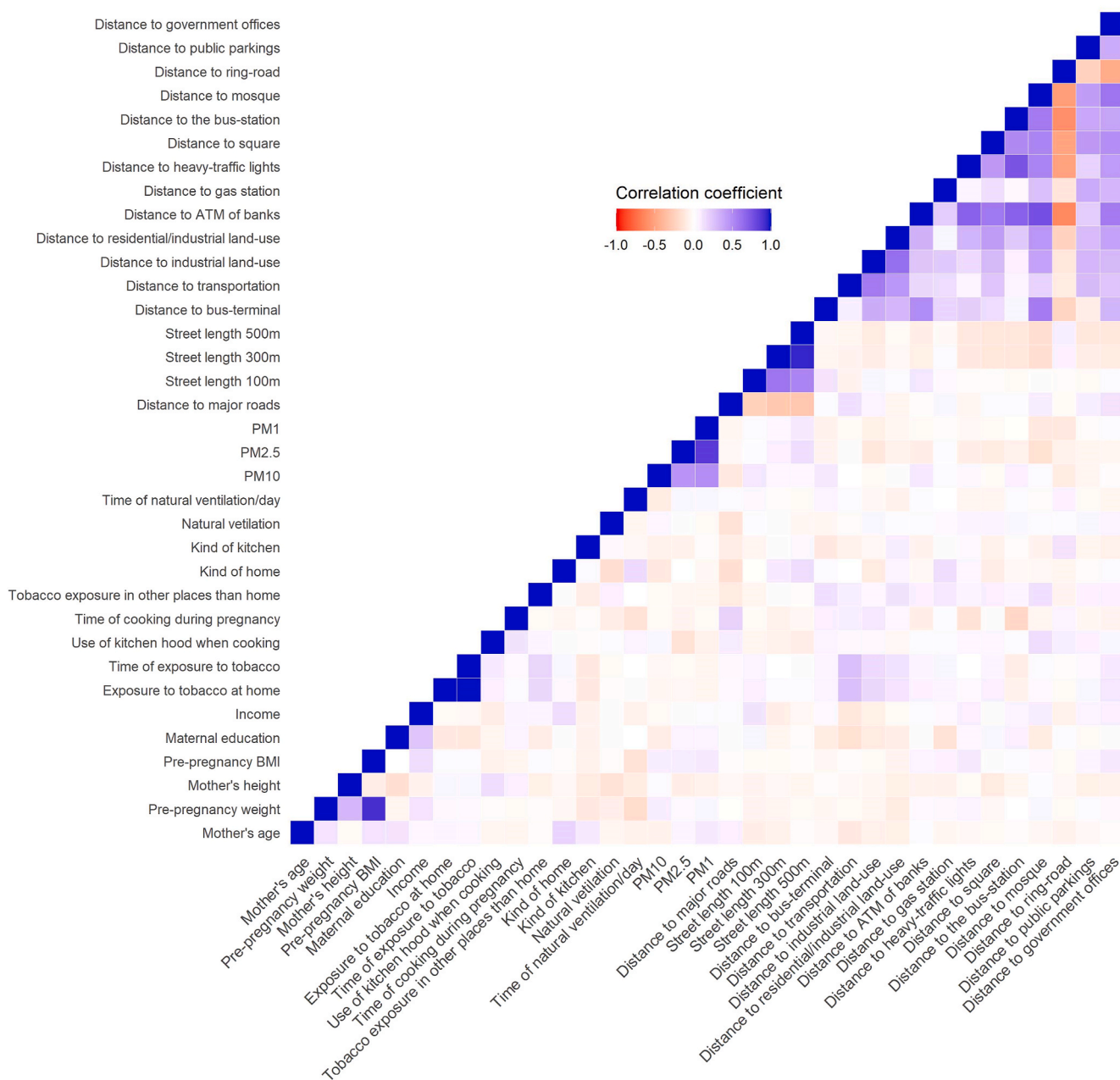
Fig. 3. Corrlation of personal, indoor and outdoor determinants of expsoure to air pollution.

4.2. Determinants of AMCL

We are not aware of any study on the determinates of AMCL levels in pregnant women; therefore, we are not able to compare our observations with those of previous research. However, the available evidence in other populations suggested that traffic-related air pollution, indoor air pollution (e.g., exposure to tobacco smoke and biomass burning) and personal characteristics could be associated with AMCL level (Bai et al., 2015a; Belli et al., 2016; Whitehouse et al., 2018; Momtaz et al., 2019; Rezaei Hachesu et al., 2019). Regarding personal determinants, we observed that the participant's age was inversely, although statistically non-significant, associated with AMCL levels. Majority of the previous studies did not find any relationship between personal characteristics such as age, sex, weight, height and BMI with AMCL level (Jacobs et al., 2010; Bai et al., 2015a). Similarly, in our study, weight, height, and BMI did not appear to influence the AMCL levels. However, experimental research showed that particle deposition in the respiratory tract could be

affected by different mechanisms and physical characteristics of participants (e.g., BMI and age) could have various effects on it (Bennett et al., 1996; Bennett and Zeman, 2004; Bai et al., 2015a). Furthermore, human and animal model studies suggested that with aging, alveolar macrophages phagocytosis and their number could reduce significantly (Linehan and Fitzgerald, 2015; Wong et al., 2017), which, in turn could result in reducing AMCL levels.

Regarding indoor determinants, we observed an inverse association between having and the duration of opening window per day with AMCL levels. Moreover, open-plan kitchen and the duration of exposure to the second-hand tobacco smoke at home during pregnancy stayed in the final model and showed positive, though statistically non-significant, association with AMCL. Lack or less time of opening windows during cooking and/or the amount time of opening window per day could lead to the accumulation of indoor air pollutants and higher exposure levels. Previous studies suggested that natural ventilation (e.g., opening window) could significantly decrease indoor air pollution (Chang et al.,

Table 2
The determinates of airways macrophage carbon load based on Deletion/Substitution/Addition (DSA) algorithm.

	Estimate (95% CI)	P value
Ambient residential PM$_{2.5}$ [a]	0.351 (0.186, 0.517)	<0.01
Distance to heavy traffic-lights [a]	−0.486 (−0.833, −0.139)	<0.01
Distance to squares [a]	−0.266 (−0.505, −0.026)	0.03
Distance to ring roads [a]	−0.511 (−0.835, −0.187)	<0.01
Duration of opening window per day during pregnancy [a]	−0.223 (−0.392, −0.053)	0.01
Duration of second-hand tobacco smoke at home during pregnancy	0.002 (0.000, 0.004)	0.12
Walled kitchen	−0.412 (−0.975, 0.151)	0.15
Opening window during cooking	−0.576 (−0.971, −0.181)	<0.01
Maternal age [a]	−0.028 (−0.063, 0.007)	0.11

[a] Effect estimates are reported as change in macrophages carbon loading (μm^2) with their 95% confidence interval (95% CI) per one-interquartile range increase in each determinant and estimates are mutually adjusted.

2017; Spiru and Simona, 2017). A study by Schembari et al. (2013), for example, showed that natural ventilation of the home and the time spent cooking were statistically significant determinants of personal and indoor air pollution exposure in Spanish pregnant women (Schembari et al., 2013).

Previous studies suggested that tobacco smoke is a major source of indoor air pollution (Tarnoki et al., 2011; Masjedi et al., 2019). A study by Momtaz et al. on 160 healthy adults in Yazd, Iran, reported that the level of AMCL in passive smokers was significantly higher than those not exposed to tobacco smoke at home (Momtaz et al., 2019). Moreover, a study by White et al. (2019) on nine non-smoker adults reported that environmental tobacco smoke could be one of the major sources of AMCL level (White et al.). Our observed higher AMCL levels in participants living at homes with open-plan kitchen were in line with findings of the aforementioned study by Momtaz et al. in Iranian adults that reported that walled kitchen was significantly associated with a lower AMCL level (Momtaz et al., 2019).

In our study, the outdoor determinants, including PM$_{2.5}$ levels (directly) and the residential distance to heavy-traffic lights, squares and ring-roads (inversely), were associated with AMCL levels. Fuel combustion is one of the main sources of PM$_{2.5}$ in Iranian urban areas (Arhami et al., 2018; Soleimani et al., 2018); therefore, PM$_{2.5}$ contains different carbonaceous species such as organic and black carbon (BC) (Hand et al., 2013; Kim et al., 2015; Pongpiachan et al., 2015; Arfaeinia et al., 2016; Al Hanai et al., 2019). Moreover, PM$_{2.5}$-bounded carbonaceous species can easily be deposited deep into the lungs due to their small size. As such alveolar macrophages can directly encounter PM$_{2.5}$ after inhaling polluted air and phagocyte them (Wei and Tang, 2018). A study by Belli et al. (2016) on 20 former smokers, showed that there was a significant direct relationship between short-term (5–7 days) and long-term (three to six months) exposure to indoor PM$_{2.5}$ and AMCL; however, short and long-term exposure to outdoor PM$_{2.5}$ were not statistically significantly associated with AMCL levels (Belli et al., 2016). Another study by Whitehouse et al. (2018) on 57 non-pregnant Malawian women reported that there was not any association between short-term exposure (48 h personal monitoring) to PM$_{2.5}$ and AMCL level (Whitehouse et al., 2018).

Regarding distance variables, we observed that longer distance to traffic sources (i.e., heavy traffic lights, square and ring roads) was associated with lower AMCL level, which could be due to lower exposure to ambient PM-bounded carbonaceous species. It has been reported that heavier vehicle categories (e.g., tracks) emit higher PM to the

atmosphere (Timmers and Achten, 2016), and many of them cross the ring roads every day in Sabzevar. Moreover, in the heavy traffic lights and/or squares, the vehicles work in idle mode or move with heavy gear for a long time, leading to emitting BC (Rahman et al., 2013; Turri et al., 2016). Previous studies reported an inverse relationship between distance to traffic indicators and AMCL levels (Mason et al.; Bunn et al., 2001; Jacobs et al., 2010; Brugha et al., 2014; Momtaz et al., 2019). Momtaz et al. (2019) reported that there was a significant inverse relationship between distance to major roads and AMCL level (Momtaz et al., 2019). Moreover, a study by Jacobs et al. (2010) on 137 non-smoking adults with diabetes showed that the AMCL level was significantly higher in participants living near to major roads (>100 m) compared to those living away from major roads (Jacobs et al., 2010). Another study by Bunn et al. (2001) on 22 healthy children reported that the percentage of AMCL was significantly lower in children who lived on a quiet residential road compared with those living near major busy roads (Bunn et al., 2001). These results are in line with our findings. However, other studies on 49 children with asthma (Brugha et al., 2014) and on 14 COPD patients (Mason et al.) reported a non-significant relationship between distance to major roads and AMCL level, which are inconsistent with our findings.

4.3. Study limitations

We estimated the outdoor PM based on LUR models and we did not have any measurement of indoor PMs levels, while the pregnant women spent much of their time at home-indoor. We estimated PMs exposure based on the annual mean, which might not have perfectly matched with the pregnancy period of the participant. However, given that all pregnant women were recruited at their time of delivery and that our recruitment only lasted three months, the pregnancy periods of the study participants covered similar months of the year. Hence, we expect that the exposure misclassification due to our use of annual levels could be comparable among the participants. Moreover, we did not have data on personal monitoring of PM2.5 or black carbon for our participants. Similarly, we did not have access to data on traffic count and also the types of vehicles, which could be relevant determinants of the AMCL. The composition of PMs (e.g. elemental and organic carbon) was not measured, which might have been related to AMCL levels. Furthermore, we relied on self-reported weight and height of mothers before pregnancy that could have resulted in measurement errors. We also selected the healthy pregnant women with no pregnancy complications and they were housewives (i.e. they did not have occupational exposure), non-active smokers, did not have any exposure to biomass smoke and did not have any background diseases. These inclusion and exclusion criteria were applied to increase the internal validity of our study; however, they could have limited the external validity of our findings. In our study setting about 83% of married women are housewives (based on last census in 2016) and only 8.7% of Iranian women are active smokers (Halimi et al., 2013). Finally, since AMCL gives an integrated 6-month exposure window, it might be difficult to infer the critical exposure period for specific pregnancy outcomes.

5. Conclusions

In this study, among 34 personal, indoor and home-outdoor determinates, we found that estimated PM$_{2.5}$ concentration at the residential address was positively associated with AMCL level in pregnant women in a middle-income country. Moreover, distance to heavy-traffic lights, squares and ring-roads and having and duration of natural ventilation were inversely associated with AMCL. Furthermore, we observed some suggestions for a potential direct association of the duration of second-hand tobacco smoke at home and open-plan kitchen type and an indirect association of age with AMCL. If confirmed by other studies, these findings inform future studies of the health effects of the maternal exposure to air pollution during pregnancy about the

important determinants of such an exposure to be considered in their studies. Moreover, they can provide the necessary evidence base for policy- and decision-makers to fine-tune and implement interventions to reduce maternal exposure to air pollution during pregnancy, particularly in LMICs. We recommended further research in other population settings with different potential determinates including home-indoor measures of air pollution to shed further light on the relationship between these determinants and AMCL levels in pregnant women.

Author statements

MM, conceptualizing and designing the study, collecting the data and drafting the manuscript. HR, SMM, MLN, AA, NP, MA, MSB and ZR, collecting the samples and conducting the laboratory analyses. LA, carrying out the statistical analyses and contributing to model development. CT, XB, MN, JS and TN, conceptualizing the study and revising the manuscript. PD, conceptualizing and designing the study and revising the manuscript. All authors reviewed the manuscript, provided feedback, and concurred with its submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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