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Original Article

Alteration in Leukocyte Subsets and Expressions of FcγR and Complement Receptors among Female Ragpickers in Eastern India

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ABSTRACT

Background: There are a million ragpickers in India who gather and trade recyclable municipal solid wastes materials for a living. The objective of this study was to examine whether their occupation adversely affects their immunity.

Methods: Seventy-four women ragpickers (median age, 30 years) and 65 age-matched control housemaids were enrolled. Flow cytometry was used to measure leukocyte subsets, and leukocyte expressions of Fcγ receptor I (CD64), FcγRIII (CD16), complement receptor 1 (CD35) and CR3 (CD11b/CD18), and CD14. Serum total immunoglobulin-E was estimated with enzyme-linked immunosorbent assay.

Results: Compared with the controls, ragpickers had significantly ($p < 0.0001$) higher levels of CD8+T-cytotoxic, CD16+CD56+natural killer, and CD4+CD45RO+memory T-cells, but depleted levels of CD19+B-cells. The percentage of CD4+T-helper-cells was lower than the control group ($p < 0.0001$), but their absolute number was relatively unchanged ($p = 0.42$) due to 11% higher lymphocyte counts in ragpickers. In ragpickers, the percentages of CD14+CD16+intermediate and CD14dim CD16+nonclassical monocyte subsets were elevated with a decline in CD14+CD16-classical monocytes. The expressions of CD64, CD16, CD35, and CD11b/CD18 on both monocytes and neutrophils, and CD14 on monocytes were significantly higher in ragpickers. In addition, ragpickers had 2.7-times more serum immunoglobulin-E than the controls ($p < 0.0001$). After controlling potential confounders, the profession of ragpicking was positively associated with the changes.

Conclusion: Ragpicking is associated with alterations in both innate (neutrophils, monocytes, and natural killer cell numbers and expression of complement and Fcγ receptors) and adaptive immunity (numbers of circulating B cells, helper, cytotoxic, and memory T cells).

1. Introduction

In the past two decades, India has been undergoing rapid urbanization and industrialization. More people are moving from rural to urban localities, thus the populations of the cities are increasing day by day. The population in urban areas is consuming additional resources and generating more waste with a bigger purchasing power. As a consequence, in the past few decades, there has been a significant increase in municipal solid waste (MSW) generation in this country [1]. It has been reported that about 25% and 51%, respectively, of household waste and waste produced by paper industries are recyclable. A large number of poor, downtrodden, marginalized urban people are thriving on these recyclable wastes. In recent years, there are about 1,000,000 ragpickers in India who search the city's trash cans and solid waste disposal sites to retrieve recyclable materials like plastic, glass, and paper for a living. About 100,000 ragpickers are found in Kolkata alone and another 300,000 operate in Delhi.

Ragpickers who are at the bottom of the hierarchy in the informal solid waste management sector after sorting the waste,

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sell to middlemen from where the waste undergoes further processing and recycling through wholesale dealers [2]. Ragpickers contribute significantly to the environment; however, most often they do not have social recognition or occupational recognition in this country. A considerable number of ragpickers in India are children and women [3]. They live in urban slums or pavements of the city in unhygienic conditions. While collecting recyclable waste, the ragpickers rarely use any protective gear such as masks, gloves, etc. In the majority of cases, they wear old sandals retrieved from the waste that exposes a considerable part of their foot to the trash. MSW in the city holds extra moisture due to the hot and humid climate, and it facilitates breeding of different pathogens. Moreover, medical wastes such as blood and body fluid-stained cotton, sanitary napkins, diapers, and used needles are often mixed with MSW despite government rules forbidding it [4]. Therefore, the ragpickers expose themselves to a host of pathogens while waste picking. This may lead to the spread of various communicable diseases [3]. Although ragpickers constitute the poorest of the poor in Kolkata, little is known about the health hazard associated with this profession. Earlier we reported the problems of the general respiratory health of ragpickers in India [5,6]. We found a significant reduction in lung function in the majority of ragpickers and the majority of them had airway obstruction [5].

Although the ragpickers are highly exposed to microorganism present in the solid waste there is hardly any study on the functioning of their immune systems with respect to lymphocyte and monocyte subtypes, adhesion molecule expression on circulating leukocytes, and the cytokine–chemokine network that plays a serious role in host defense against attacking pathogens [7]. The phagocytic cells of the immune system recognize the invading pathogens by opsonin-dependent as well as opsonin-independent mechanisms. The opsonin-dependent mechanism involves the opsonins like immunoglobulin (Ig)G and the complement system. IgG arbitrates its effector functions by binding its Fc domain with Fcγ receptors (FcγRs)—FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16)—present on the surface of phagocytic cells such as the neutrophils and the monocytes [8]. The complement system is the other component of the opsonin-dependent mechanism of phagocytosis and consists of more than 30 soluble and cell-surface proteins. The complement system has numerous effector functions in host defense, comprising opsonization of microbes to facilitate phagocytosis, release of anaphylatoxins to endorse inflammation, and killing of microbes via the membrane attack complex [9,10]. Complement opsonization is mainly vital for the recognition, binding, and internalization of particles including encapsulated bacteria by neutrophils. Phagocytes recognize complement-opsonized particles using complement receptor 1 (CR1: CD35) that binds C3b, C4b, and C1q, and the integrin complement receptor 3 (CR3: CD11b/CD18) and receptor 4 (CR4: CD11c/CD18) that specifically binds iC3b. Human neutrophils use both CR1 and CR3 for phagocytosis [11]. Infections, mostly due to bacterial and viral origin, induce different expression patterns of complement regulators in the human leukocyte membrane. For example, an increase in membrane-bound CD35 on neutrophils and monocytes is a strong marker of bacterial infection [12].

Like the lymphocytes, human monocytes are a heterogeneous cell population that plays subset-specific functions and phenotypes. They can be segregated into three functionally distinct populations based on CD14 and CD16 expression [13,14]. Classical or “traditional” monocytes express high levels of CD14 but lack CD16 (CD14+CD16−). They produce proinflammatory cytokines in response to microbial components, however, to a lesser degree than intermediate or “inflammatory” monocytes (CD14+CD16+) do. Nonclassical or “patrolling” monocytes (CD14dim CD16+) produce interleukin-6 and -8 in response to viral rudiments, and patrol vascular endothelium.

During any host–pathogen interaction, Toll-like receptor (TLR) family members are first activated. TLRs are responsible for identifying microbial products and tempting innate and adaptive immunity [15]. For this, TLRs require the cooperation of CD14, a pattern recognition receptor expressed on the plasma membrane of the phagocytic cells such as the monocytes and the macrophages. Expression of CD14 reflects functional properties of the monocytes as CD14 contributes to TLR4-mediated immune responses to lipopolysaccharide (LPS), a gram-negative bacteria endotoxin [16]. The LPS-binding protein acts as the opsonin, while CD14 represents the opsonin receptor to mediate phagocytosis of LPS-coated microorganisms [17].

In view of these reports, in this study we investigated lymphocyte and monocyte subsets and the expression of cell-surface receptors engaged in phagocytosis such as CD14, CR1 (CD35), CR3 (CD11b/CD18), FCgRI (CD64), and FcγRIII (CD16) on circulating leukocytes in a group of premenopausal female ragpickers of Kolkata in Eastern India and compared the findings with that of an age- and sex-matched control group.

2. Materials and methods

2.1. Study design, population, and working conditions

Seventy-four premenopausal female ragpickers (age, 21–39 years; median age, 30 years) and 65 control women (age, 22–41 years; median age, 31 years) of Kolkata (former Calcutta) in Eastern India were signed up for this cross-sectional study with controls matched for age, sex, and socio-economic conditions. They were randomly selected from the eastern part of the city including the area surrounding the landfill site at Dhapa. Informed consent was obtained from all individual participants included in the study. In West Bengal, the majority of the ragpickers were migrants from rural regions who lived in slums or on city’s footpaths. They do their daily job in groups of two to five from early morning until late afternoon, usually 8–12 h/d, 6–7 d/wk. They do not use gloves, masks, or shoes. They search through the garbage while holding a short-curved iron stick in their bare hands for segregating the wastes and picking up anything valuable. After a hard day’s work, they usually get 5–10 kg of recyclable materials such as glass, paper, plastic, and burned-out batteries from the community garbage bins of different city localities and landfill areas at Dhapa in East Kolkata that usually fetches a price of approximately 50–70 rupees (approximately US$1.0). The present study considered housemaids as the control group. Housemaids work 8–12 h/d, 6–7 d/wk within the city. For this cross-sectional study, sample size and power calculation with a total sample size of 139 participants (65 controls and 74 ragpickers) were carried out following the procedure of previously published literatures [18,19]. We estimated that we could identify statistically significant mean differences between different measured parameters of these two groups to achieve more than 90% power with a significance level (α) of 0.050 using a two-sided two-sample t test.

The Ethics Committee members of the Chittaranjan National Cancer Institute, Kolkata, and West Bengal, India approved the study protocol and the research was conducted according to the principles of the most recent version of the Declaration of Helsinki [20].

2.2. Inclusion and exclusion criteria

A premenopausal married woman who had engaged actively for the previous 5 years or more in waste handling and selling for a

livelihood was considered in this study as a ragpicker. Those who were currently on medication, pregnant, or lactating were excluded. Background demographic and socioeconomic characteristics such as age, family, marital status, tobacco smoking, betel quid chewing habit, education, monthly income, and working conditions were collected through individual interview with a questionnaire by female researchers of the study team.

2.3. Hematology

Venous blood was collected in K3EDTA-anticoagulated vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) from antecubital plexus by 5-mL sterile plastic disposable syringe fitted with a 21-gauge needle. Total hemoglobin, red blood cells (RBC), white blood cells, and platelets counts were evaluated using previously published procedures [21]. Morphological variations of leukocytes were examined in Leishman-stained blood slides under the microscope (Leitz, Leitz, Wetzlar, Germany).

2.4. Analysis of lymphocyte subtypes

Flow cytometric analysis of lymphocyte subsets were done within 3 hours of blood sample collection. Firstly, 25 μL of anticoagulated whole blood was mixed with 75 μL of phosphate-buffered saline (PBS; pH 7.3). The mixture was then incubated with 10 μL each of fluorescence isothiocyanate (FITC) and phycoerythrin (PE) conjugated monoclonal antibodies (Becton Dickinson) specific for human lymphocyte surface markers viz CD4-PE (T-helper), CD8-FITC (T-cytotoxic/suppressive), CD19-FITC (B cell), CD 16-FITC and CD56-PE [natural killer (NK) cell], CD4-PE and CD45RO-FITC (memory T-cells), CD4-PE/CD25-FITC (T-regulatory), and isotype controls for 30 minutes in the dark at room temperature. Then, 2 mL of RBC lysing buffer (Becton Dickinson) was added for 5 minutes at room temperature for the lysis of red cells in the mixture. The cells were fixed with paraformaldehyde (PFA) solution (0.5%), and 10,000 events were acquired and analyzed in a flow cytometer (FACS Calibur with sorter, Becton Dickinson, San Jose, CA, USA). Dot plot lymphocyte gating was done from their characteristic forward and side scatters (forward-scattered light and side-scattered light, respectively) profile. Acquisition of data and analysis of FITC and channel-2 (PE) were done using Cell Quest software (Becton Dickinson). The relative proportion of each subset of lymphocyte (CD4+ or CD8+) was obtained from quadrant gate settings for CD4, CD8, CD19, CD16, and CD56 and isotype negative controls. Individual lymphocyte subset data was expressed as the percentage of total lymphocytes.

2.5. Assessment of monocyte subtypes

Monocyte subsets were defined based on surface expression of CD14 and CD16 [12,13]. Briefly, whole blood samples (25 μL) anticoagulated with K3EDTA were incubated with the saturating concentration of PE-conjugated mouse antihuman CD16 and FITC-conjugated antihuman CD14 (Becton Dickinson) for 30 minutes in the darkness. The samples were lysed with RBC lysing solution (Becton Dickinson), centrifuged for 5 minutes at 350 $\times g$ and resuspended in PBS. Approximately 10,000 events were acquired in a flow cytometer (Becton Dickinson) and monocyte cell populations were selectively gated based on their forward-scattered light and side-scattered light. Cell isotype control antibodies were used to define background levels. Percentages of CD14+CD16− (classical), CD14+16+ (intermediate), and CD14dimCD16+ (nonclassical) were calculated from the dot plots using statistical package of the Cell Quest software (Becton Dickinson). Isotype matched PE- and FITC-conjugated mouse IgG served as controls for nonspecific staining.

2.6. Flow cytometric assessment of surface molecules

Twenty-five microliters of blood sample were added to a polypropylene tube, incubated for 20 minutes in the dark at normal temperature with FITC-conjugated antihuman CD35; CD11b-PE and CD18-FITC; CD16-PE and CD64-FITC; and CD14-FITC monoclonal antibodies, (Becton Dickinson), and isotype controls. Then the RBCs were lysed with lysing solution (Becton Dickinson), and the samples were centrifuged at 500*g* for 5 minutes. Ice-cold PBS with 0.1% sodium azide was used to wash cell pellets, resuspended in 500 μL of PFA in PBS (1% solution) and analyzed in a flow cytometer. Measurements were made on the FL1 and FL2 channel, and the gates were adjusted to the negative control quadrant. A total of 15,000 events were recorded. Results were expressed as mean fluorescence intensity (MFI) in an arbitrary unit.

2.7. Serum IgE determination

Serum separation by centrifugation were done within 2 hours of blood collection and stored at −20°C for further use. The serum IgE level was measured using a commercially available enzyme-linked immunosorbent assay kit for the total IgE (IBL Immunobiological Laboratories, Hamburg, Germany; analytical sensitivity 0.8 IU/mL) following the manufacturer's protocol. Ten-microliters (10 μL) of a sample was poured in duplicate into wells of microtitration plates percolated with monoclonal antibody (mouse antihuman IgE) along with peroxidase-conjugated antihuman IgE. After 30 minutes of incubation at normal temperature, the plate was rinsed with wash buffer for the removal of unbound material. A substrate solution (tetramethyl benzedrine) was mixed and incubated for 15 minutes to develop color. Stop solution was added to end the reaction. The final intensity of the color was measured at 450-nm wavelength against the blank in a microplate reader (model 680; Bio-Rad, Osaka, Japan). The IgE concentration was read from the standard curve. The level of total IgE was calculated from mean values of two separate determinations from each sample. Data were expressed as IU/mL. Serum IgE levels were highly skewed, so log-transformed (Log10) was performed to obtain a Gaussian shape.

2.8. Data analysis

The results were analyzed by SPSS statistical software package (Statistical Package for Social Sciences for windows, release 10.0; SPSS Inc., Chicago, IL, USA). Statistical differences were calculated by Student *t* test, Mann–Whitney *U* test, and Chi-square test as applicable. Logistic regression analysis was done to detect the impact of all variables on measurable health parameters for the identification of potential confounders. Step-wise regression (multivariate logistic) analysis was carried out to evaluate the collective impact of these factors on health factors. Multivariate statistical analysis was conducted by variable selection, such as step-wise regression, deviance comparisons of various candidate models, and assessments of goodness of fit. Any measured parameter was considered as either continuous variable (when computing univariately for correlation) or dichotomous variable (when examining association). A p value < 0.05 was considered significant.

3. Results

3.1. Demography and socioeconomic status of study population

The two groups of the study population were similar to each other with regards to age, body mass index, family members, income, and food habit. Use of tobacco products and excess alcohol

consumption habits were much higher among ragpickers. They also were less educated than the control population (Table 1). Moreover, the use of traditional biomass (dung, crop residues, and wood) and kerosene were found to be the main cooking fuel among ragpickers.

3.2. Hematological changes

Table 2 shows significantly lower levels of hemoglobin and erythrocyte, but higher values of total leukocyte and platelet counts in ragpickers compared with the control group. White blood cell differential count of the ragpickers showed a marked increase in all cell types, especially eosinophils and monocytes. The leukocytes of the ragpickers illustrated toxic granulation in neutrophils (in 21.6% ragpickers vs. 6.1% in the controls, $p < 0.001$ in Student t test) and an abundance (>5% total neutrophils) of immature neutrophils like myelocytes, metamyelocytes, and band cells (36.5% vs. 12.3% in the controls, $p < 0.001$).

3.3. Change in lymphocyte subtypes

Significantly reduced % of CD4+ T-helper cells, elevated percentage, and absolute number of CD8+ T-cytotoxic cells were observed in the blood samples of the ragpickers in comparison to the controls (Table 3). Thus, the CD4:CD8 ratio was reduced from 1.52:1 to 1.09:1. However, there was no appreciable change in the absolute numbers (number/μL of blood) of CD4+ cells between those two groups of the study population. The absolute number of CD19+ B-lymphocytes was 53% below the control level, while CD16+CD56+ NK-cells were more than 2-fold than that of the control levels (Table 3). We did not find any significant changes both in the % and absolute count of CD4+CD25+ T-regulatory cells between the ragpickers and controls. It was also evident that the ragpickers had a significantly greater percentage as well as increased absolute numbers of CD4+CD45RO+ memory T-cells in circulation when compared with the controls (Table 3).

Table 1
Demographic and socio-economic features of the study groups*

Characteristics	Control women ($n = 65$)	Ragpicker ($n = 74$)
Age (y), median (IQR)	31 (22–41)	30 (21–39)
BMI (kg/m^2), median (IQR)	21.0 (19.4–22.8)	20.6 (19.2–22.6)
Y of schooling, n (%)		
Y 0	2 (3.1)	14 (18.9)‡
Y 1–5	63 (96.9)	53 (71.6)‡
Food habit, n (%)		
Vegetarian,	2 (3.1)	2 (2.7)
Mixed	63 (96.9)	73 (98.6)
History of smoking, n (%)	0 (0)	0 (0)
History of tobacco/betel quid use, n (%)	24 (36.9)	52 (70.3)‡
Substance abuse history, n (%)		
Abuse of ethyl alcohol	1 (1.5)	8 (10.8)‡
Abuse of drugs	0 (0)	0 (0)
Family size, median (IQR)		
Total members in family	4 (2–6)	4 (2–7)
Number of children	2 (1–3)	2 (1–4)
Smoking history of spouse		
Present smoker, n (%)	36 (55.4)	39 (52.7)
No. of beedi†/cigarettes smoked/d, median (IQR)	7 (5–15)	8 (6–12)
Mosquito repellent used at home, n (%)	48 (73.8)	53 (71.6)
Cooking fuel used at home, n (%)		
LPG (liquefied petroleum gas)	7 (10.8)	1 (1.4)‡
Biomass fuel and kerosene	58 (89.2)	73 (98.7)‡
Cooking time per d (h), median (IQR)	3 (2–5)	3 (2–4)
Monthly earnings in US$, mean ± SD	48 ± 12	44 ± 10

* Demography and socio-economic features of control women versus ragpickers were compared statistically by Mann–Whitney U test (for median with IQR), χ^2-test (for results in % values), and Student t test (for results with mean ± SD) as appropriate.
† Hand-made local cigarettes.
‡ $p < 0.05$ considered significant.
BMI, body mass index; IQR, interquartile range; LPG, liquefied petroleum gas; SD, standard deviation of mean; Y, year.

Table 2
Hematological parameters

Parameters	Control ($n = 65$)	Ragpicker ($n = 74$)	p
Hemoglobin (g/dL)	14.3 ± 0.4	12.2 ± 0.3	<0.0001*
RBC (× 10^6/μL)	6.4 ± 0.2	4.3 ± 0.2	<0.0001*
WBC (× 10^3/μL)	6.8 ± 0.4	8.7 ± 0.4	<0.0001*
Platelet (× 10^5/μL)	2.3 ± 0.2	2.8 ± 0.3	<0.0001*
Neutrophil/μL	3968 ± 170	5240 ± 257	<0.0001*
Eosinophil/μL	284 ± 27	428 ± 21	<0.0001*
Lymphocyte/μL	2352 ± 89	2640 ± 105	<0.0001*
Monocyte/μL	182 ± 11	332 ± 22	<0.0001*

Results are presented as mean ± standard deviation.
*$p < 0.05$ considered significant in unpaired Student t test when compared to controls.
RBC, red blood cells; WBC, white blood cells.

Table 3
Comparison of change in lymphocyte subset in peripheral blood of control women and ragpickers

Lymphocyte subset	Control women ($n = 65$)	Ragpickers ($n = 74$)	p
T-helper cells (CD 4+)			
% positive cells	41.5 ± 2.3	37.2 ± 2.6	<0.0001*
Cell no./μL	976 ± 56	983 ± 47	0.4245
T-cytotoxic cells (CD8+)			
% positive cells	27.2 ± 1.2	34.0 ± 1.6*	<0.0001*
Cell no./μL	641 ± 30	899 ± 29	<0.0001*
CD4+:CD8+	1.52:1	1.09:1	0.9342
B-lymphocytes (CD19+)			
% positive cells	21.0 ± 1.2	8.6 ± 1.2*	<0.0001*
Cell no./μL	489 ± 48	231 ± 28*	<0.0001*
Natural killer cells (CD 16+/CD56+)			
% positive cells	10.2 ± 1.1	20.1 ± 1.2*	<0.0001*
Cell no./μL	243 ± 21	532 ± 53*	<0.0001*
T-regulatory cells (CD4+/CD25+)			
% positive cells	4.9 ± 1.8	4.6 ± 1.7	0.3143
Cell no./μL	48.2 ± 16.5	45.2 ± 15.8	0.2759
T-memory cells (CD4+/CD45RO+)			
% positive cells	39.3 ± 4.8	64.3 ± 8.5	<0.0001*
Cell no./μL	385 ± 42	631 ± 76	<0.0001*

Results are presented as mean ± standard deviation.
*$p < 0.05$ considered significant in unpaired Student t test when compared with controls.

3.4. Change in monocytes subsets

Considering heterogeneity in phenotype, morphology, and function, circulation monocytes in humans have been classified into three groups depending on their expression of CD14 and CD16 receptors. In ragpickers, the percentage of CD14+CD16+ intermediate and CD14dim CD16+ nonclassical monocyte subsets were elevated with a decline in CD14+CD16− classical monocytes (Figs. 1A–C). Since the total number of monocytes was 1.8-tmes higher than the controls in ragpickers (Table 2), the absolute number of classical monocytes was 225 ± 19/μL in ragpicker versus 147 ± 7/μL in the controls ($p < 0.001$ in the Student t test), intermediate monocytes was 76 ± 8 versus 25 ± 5/μL ($p < 0.001$), and nonclassical monocytes was 32 ± 4/μL versus 9 ± 2/μL in the

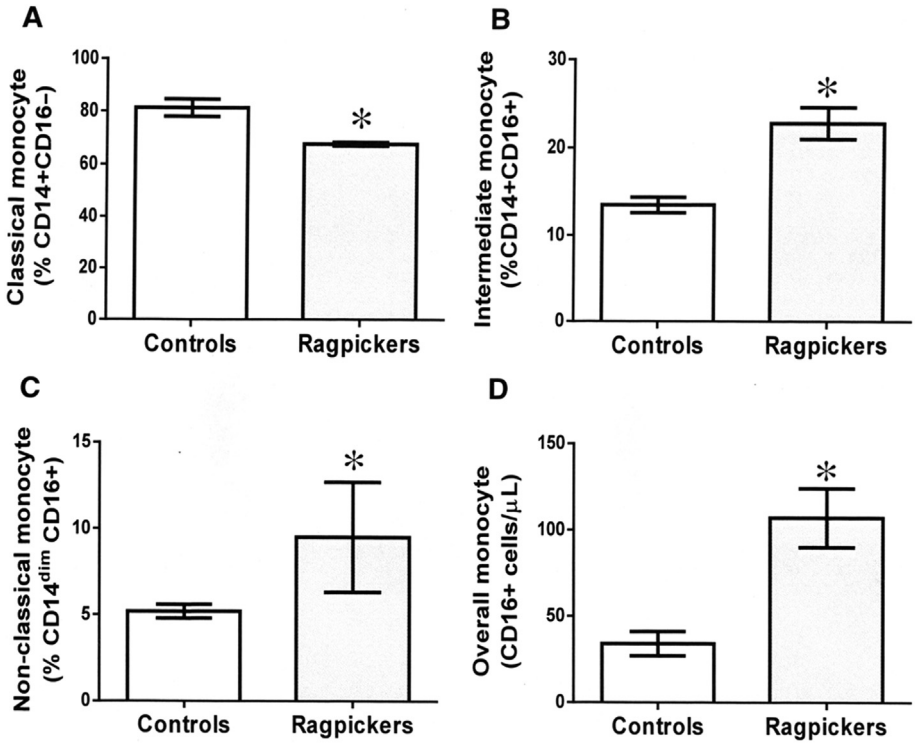

Fig. 1. Histograms showing relative distribution (%) of (A) classical, (B) intermediate, and (C) nonclassical monocyte subsets, and (D) absolute numbers of overall monocytes in peripheral blood among control and ragpickers. Bars indicate standard deviation of the mean. * $p < 0.05$ considered significant compared with controls in unpaired Student *t* test.

controls ($p < 0.0001$). Overall, the number of CD16+ monocytes was 3.1-tmes more than the controls (Fig. 1D).

3.5. Changes in expression of Fcγ receptors on monocytes and neutrophils

The expression of FcγRI (CD64) and FcγRIII (CD16) on the surface of both monocytes and neutrophils were significantly higher in ragpickers in comparison to the housemaid control women (Fig. 2). The monocytes of ragpickers exhibited 51% higher MFI for FcγRI with respect to the controls (Fig. 2A). Likewise, a 42% increase in the MFI of FcγRI was recorded on the surface of neutrophils of the ragpickers (Fig. 2B). The MFI of FcγRIII was 53% and 40% higher respectively in monocytes and neutrophils of the ragpickers compared with controls (Figs. 2C, 2D).

3.6. Changes in expression of complement and pattern recognition receptors

The MFI of CR1 (CD35) on circulating monocytes and neutrophils of the ragpickers was 56% and 64% higher, respectively, than the control. Likewise, the MFI of both the CD18 and CD11b in monocytes and neutrophils of ragpickers were significantly higher than that of the controls, indicating elevated expression of CR3. Also, the expression of pattern recognition receptor (CD14) in monocytes was 2.6-fold higher in the ragpickers when compared with the housemaid controls (Table 4). Collectively, the ragpickers showed higher expressions of CR1, CR3, and CD14.

3.7. Changes in serum IgE level

The total IgE level in serum of ragpickers was 2.7-times more than that of the controls (362.4 ± 56.2 IU/mL vs. 132.5 ± 18.4 IU/mL, $p < 0.0001$ in the Student *t* test). In multivariate logistic regression analysis, we found that the profession of rag picking was positively associated with the total serum IgE level (odds ratio = 1.42, 95% confidence interval: 1.22–2.04) even after controlling for potential confounders.

3.8. Association of ragpicking with immune cell population

After controlling for potential confounders (age, education, tobacco/betel quid chewing, alcohol drinking habits, and use of highly polluting biomass and kerosene for cooking) in multivariate logistic regression analysis, the profession of ragpicking was found to be positively associated with the numbers of NK-cells, T-regulatory, memory T-cells, Fc-γ, and CRs in monocytes and neutrophils (Table 5).

4. Discussion

Our study showed that the ragpickers had altered lymphocyte and monocyte subsets and altered expressions of complement receptors and Fc-γ receptors on circulating leukocytes when compared with that of the control women. The change in lymphocyte subsets included higher levels of CD8+ T-cells, CD4+CD45RO+ memory T-cells, and CD 16+CD56+ NK cells with depleted CD19+ B-cell numbers and an altered CD4+:CD8+ cell ratio. A rise in NK-cell numbers is a risk factor for deterioration of renal function [22] and human papillomavirus infection and progression to cervical cancer in women [23]. Although we did not find any significant change in the number of T-regulatory cells that play a crucial role in the maintenance of immunological self-tolerance against self and foreign antigens [24], significant increase of CD4+CD45RO+ memory T-cells was recorded in ragpickers. The generation of the immunological memory is the

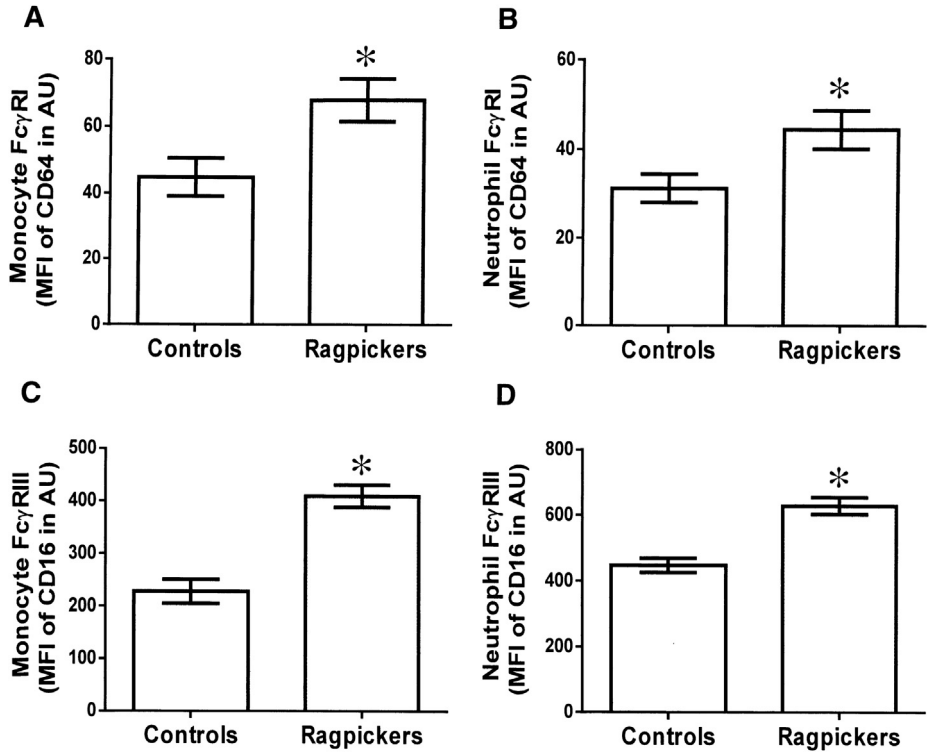

Fig. 2. Histograms showing mean fluorescence of (A) FcγRI in monocytes, (B) FcγRI in neutrophils, (C) FcγRIII in monocytes, and (D) FcγRIII in neutrophils in peripheral blood among control and ragpickers. Bars indicate standard deviation of the mean. * $p < 0.05$ considered significant compared with control in unpaired Student *t* test. AU, arbitrary unit, FcγR, Fcγ receptor; MFI, mean fluorescence intensity.

Table 4
Comparison of changes in expression of the surface receptors on monocyte and neutrophils in control women and ragpickers

Surface molecule & cell type	Control women ($n = 65$)	Ragpicker ($n = 74$)	*p*
Complement receptor type 1 expression (MFI of CD35 in AU)			
Circulating monocyte	52.1 ± 6.2	81.3 ± 7.4	<0.0001*
Circulating neutrophil	64.4 ± 8.1	105.6 ± 1.6	<0.0001*
Expression of complement receptor type 3 (MFI of CD18 & CD11b in AU)			
Expression of CD18			
Circulating monocyte	232.5 ± 17.8	322.7 ± 31.5	<0.0001*
Circulating neutrophil	204.9 ± 21.7	289.8 ± 19.4	<0.0001*
Expression of CD11b			
Circulating monocyte	465.4 ± 26.2	702.7 ± 42.8	<0.0001*
Circulating neutrophil	417.8 ± 23.7	678.6 ± 34.3	<0.0001*
Expression of pattern recognition receptor (MFI of CD14 in AU)			
Circulating monocyte	164.9 ± 10.8	433.7 ± 48.9	<0.0001*

Results are presented as mean ± standard deviation.
*$p < 0.05$ considered significant in unpaired Student *t* test when compared with controls.
AU, arbitrary unit; MFI, mean fluorescence intensity.

Table 5
Analysis of multivariate logistic regression for the association between immune cell population and ragpicking after controlling potential confounders

Immune cell parameters	With ragpicking job		*p*
	Odds ratio	95% confidence intervals	
Natural killer cells (no./μL)	1.53	1.22–2.15	<0.001
T-cytotoxic cells (no./μL)	1.22	1.06–1.65	<0.001
T-regulatory cells (no./μL)	1.23	0.98–1.66	<0.001
T-memory cells (no./μL)	1.38	1.16–1.81	<0.001
FcγRI in monocyte (MFI)	1.20	1.07–1.47	<0.001
FcγRI in neutrophil (MFI)	1.18	1.05–1.37	<0.001
FcγRIII in monocyte (MFI)	2.68	1.72–4.33	<0.001
FcγRIII in neutrophil (MFI)	1.33	1.12–1.78	<0.001
CR1 in neutrophil (MFI)	1.19	1.08–1.42	<0.001
CR3 in monocyte (MFI)	1.58	1.25–2.03	<0.001
CR3 in neutrophil (MFI)	1.37	1.14–1.62	<0.001

CR, complement receptor; FcγR, Fcγ receptor; MFI, mean fluorescence intensity.

hallmark of the adaptive immune response [25]. During the development of T-lymphocytes in the thymus, a shift from CD45RO to CD45RA happens, which results in the end of negative selection and aids to eliminate autoreactive T-cells and the prevention of autoimmune disease [26]. Memory T-cells have encountered their antigen previously and recirculate to be restimulated and differentiate into effector cells. The higher level of memory T-cells in ragpickers could in part be due to the conversion of CD4+CD45RA+ cells into CD4+CD45RO+ T-cells following stimulation by antigens, as observed in the case of infections [27] and other health conditions [26].

The ragpickers showed an alteration in monocyte subsets too. There was a significant rise in the intermediate and nonclassical monocyte subsets along with an overall 3-fold increase in CD16 expressing monocytes among the ragpickers. CD16+ monocytes are major producers of inducible tumor-necrosis factor in human blood, and the number of CD16+ monocytes is increased during infections [28] and various inflammatory conditions including rheumatoid arthritis [29]. Current studies indicate that blood platelets can contribute to inflammation in various ways [30,31]. Activated platelets make physical contact with the monocytes and form monocyte-platelet aggregates via P-selectin–P-selectin ligand-1 interaction. These aggregates induce CD16 upregulation on CD14+CD16− monocytes that give rise to a phenotypic change in circulating monocytes from CD14+CD16− subpopulation to CD14+CD16+ cells subgroup with higher proinflammatory activity

[32]. The key components of defense against invading microorganisms are inflammation and repair of tissue injury. In view of these reports, the increase in CD16+ monocytes in ragpickers can be attributed in part to higher platelet P-selectin expression as we reported earlier in female ragpickers of Kolkata [6].

The ragpickers showed significantly higher expression levels of CR1 and CR3 on circulating neutrophils and monocytes along with higher expression of pattern recognition receptor CD14 on monocytes. Phagocytosis is an integral part of the body's innate immunity. Neutrophils are the foremost leukocytes to be delivered to the site of inflammation. Active neutrophils express cell surface CR1 (CD35) and CR3 (CD11b/CD18) that are essential for their transmigration to protect the host against invading microorganisms [33]. Increase in membrane-bound CD35 [12] and CD11b [34,35] in both neutrophils and monocytes has been reported following bacterial and viral infections, especially the latter [35]. In fact, CD35 expression on neutrophils and monocytes is considered effective markers of bacterial infection [12]. Neutrophil influx from blood to the tissues is driven in large part by interleukin-8, a strong neutrophil chemoattractant. Therefore, the upregulation of CR1 and CR3 expressions on circulating leukocytes along with higher interleukin-8 levels, as we have reported earlier among female ragpickers [6], may suggest increased neutrophilic inflammation in ragpickers. However, the complement molecules may induce undesirable inflammation if activated improperly or uncontrolled; thus acting as a double-edged sword in that sense.

In addition to CRs, the expressions of FcγRI (CD64) and FcγRII (CD16) were higher on the surface of monocytes and neutrophils of the ragpickers. These receptors bind to the infected cells or pathogens to stimulate cytotoxic cells and/or phagocytosis for the clearance of the invading microbes. These phenomenon are commonly known as antibody-dependent cell-mediated cytotoxicity and/or antibody-mediated phagocytosis. These molecules are important for containing bacterial and viral infections [35]. Therefore, activation of the expressions of CD16 and CD64 in phagocytes of the ragpickers implies that both neutrophils and monocytes of the ragpickers are stimulated presumably to combat the onslaught of infectious agents present in the solid waste.

Compared with controls, significantly higher serum total IgE level was observed in ragpickers, suggesting hypersensitivity reaction. Higher serum total IgE has been shown associated with gastrointestinal complaints [36] and dermatitis [37], which are common complications among the ragpickers [5].

LPSs, the major membrane constituent of gram-negative bacteria like *Escherichia coli*, may be released from the bacteria during multiplication or lysis. CD14 is the receptor for the LPS of gram-negative bacteria and may bind with LPS either in soluble condition or on membrane [38]. *E. coli*-induced activation of granulocytes was more dependent on complement, and activation of the monocytes is more dependent on CD14 [16]. In the present study, there was 2.6-fold increase in the expression (MFI) of CD14 on the monocytes of the ragpickers and the total number of monocytes expressing CD14 was remarkably higher in the circulation of the ragpickers when compared with that of the control, suggesting activation of the immune response against gram-negative bacterial infection.

However, our study has certain limitations. Firstly, the immune parameters have not been correlated with the health status of the ragpickers due to the nature of the study design. Secondly, the study was conducted in a small population without any environmental monitoring data on allergens and endotoxin concentrations in the areas surrounding the ragpickers' work zones. The measurement of endotoxin and β-glucan concentrations would be very useful as these seem to play important roles through a synergic action in promoting inflammation in airways. Thirdly, collection of waste along with other working activities can expose ragpickers to urban outdoor air pollutants such as particulate matter, oxides of nitrogen, oxides of carbon (CO_2, CO), and ozone. All these may be the causative agents for the onset of inflammatory and allergic diseases [39].

Despite these limitations, the present study, the first of its kind in India or elsewhere, has documented alterations in innate (blood neutrophils, monocytes, and NK-cell numbers and expression level of CRs and Fcγ receptors) and adaptive immunity (numbers of circulating B cells, helper, cytotoxic, and memory T cells) in female ragpickers in their reproductive age. A study in Mumbai, the financial capital of India, has shown that female ragpickers marry at a young age, have multiple pregnancies, high addiction, and high morbidity, especially in those who operate at the MSW dumping site [2]. The situation deserves immediate awareness of all concerned to this otherwise overlooked field of occupational exposure that affects millions of deprived people. In this investigation, we advocate compulsory use of protective gear such as shoes, nose masks, and gloves for the ragpickers, and regular health checkups. In an effort to long-term measure, we put emphasis on the finding of substitute professions for these ragpickers who are an important part of the ecology and economy of the society.

Conflicts of interest

All authors have no conflicts of interest to declare.

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References

[1] Mor S, Ravindra K, De Visscher A, Dahiya RP, Chandra A. Municipal solid waste characterization and its assessment for potential methane generation: a case study. Sci Total Environ 2006;371:1–10.
[2] Uplap PA, Bhate K. Health profile of women ragpicker members of a nongovernmental organization in Mumbai, India. Indian J Occup Environ Med 2014;18:140–4.
[3] Hunt C. Child waste pickers in India: the occupation and its health risks. Environ Urbanization 1996;8:111–8.
[4] Patil AD, Shekdar AV. Health-care waste management in India. J Environ Manage 2001;63:211–20.
[5] Ray MR, Mukherjee G, Roychoudhury S, Lahiri T. Respiratory and general health impairments of ragpickers in India: a study in Delhi. Int Arch Occup Environ Health 2004;77:595–8.
[6] Ray MR, Roychoudhury S, Mukherjee S, Siddique S, Banerjee M, Akolkar AB, Sengupta B, Lahiri T. Airway inflammation and upregulation of b2 Mac-1 integrin expression on circulating leukocytes of female ragpickers in India. J Occup Health 2009;51:232–8.
[7] Yan SR, Sapru K, Issekutz AC. The CD11/CD18 (beta 2) integrins modulate neutrophil caspase activation and survival following TNF-alpha or endotoxin-induced transendothelial migration. Immunol Cell Biol 2004;82:435–46.
[8] Lilius EM, Nuutila J. Bacterial infections, DNA virus infections, and RNA virus infections manifest differently in neutrophil receptor expression. Sci World J 2012;2012:527347.
[9] Walport MJ. Complement: first of two parts. N Engl J Med 2001;344:1058–66.
[10] Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010;11:785–97.
[11] Fällman M, Andersson R, Andersson T. Signaling properties of CR3 (CD11b/CD18) and CR1 (CD35) in relation to phagocytosis of complement-opsonized particles. J Immunol 1993;151:330–8.
[12] Nuutila J, Jalava-Karvinen P, Hohenthal U, Kotilainen P, Pelliniemi TT, Nikoskelainen J, Lilius EM. Use of complement regulators, CD35, CD46, CD55, and CD59, on leukocytes as markers for diagnosis of viral and bacterial infections. Human Immunol 2013;74:522–30.
[13] Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;116:e74–80.
[14] Zawada AM, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, Heine GH. Super SAGE evidence for CD14++CD16+ monocytes as a third monocyte subset. Blood 2011;118:e50–61.
[15] Akira S, Takeda K. Toll-like receptor signaling. Nat Rev Immunol 2004;4:499–511.

[16] Brekke OL, Christiansen D, Fure H, Pharo A, Fung M, Riesenfeld J, Mollnes TE. Combined inhibition of complement and CD14 abolish E. coli-induced cytokine-, chemokine- and growth factor-synthesis in human whole blood. Mol Immunol 2008;45:3804–13.
[17] Wright SD, Tobias PS, Ulevitch RJ, Ramos RA. Lipopolysaccharide (LPS) binding protein opsonizes LPAS-bearing particles for recognition by a novel receptor on macrophages. J Exp Med 1989;170:1231–41.
[18] Campbell MJ, Julious SA, Altman DG. Estimating sample sizes for binary, ordered categorical and continuous outcomes in two group comparisons. BMJ 1995;311:1145–8.
[19] Chow S, Shao J, Wang H. Sample Size Calculations in Clinical Research. 2nd ed. Chapman & Hall/CRC Biostatistics Series. 2008 http://www.crcnetbase.com/doi/pdfplusdirect/10.1201/9781584889830.fmatt.
[20] Williams JR. Revising the Declaration of Helsinki. World Med J 2008;54:120–2.
[21] Dacie JV, Lewis SM. Practical Haematology. 8th ed. London (UK): Churchill-Livingstone; 1996.
[22] Iwatani H, Nagasawa Y, Yamamoto R, Iio K, Mizui M, Horii A, Kitahara T, Inohara H, Kumanogoh A, Imai E, Rakugi H, Isaka Y. CD16+CD56+ cells are a potential culprit for hematuria in IgA nephropathy. Clin ExpNephrol 2015;19:216–24.
[23] Pita-Lopez ML, Ortiz-Lazareno PC, Navarro-Meza M, Santoyo-Telles F, Peralta-Zaragoza O. CD28-, CD45RA(null/dim) and natural killer-like CD8+ T cells are increased in peripheral blood of women with low-grade cervical lesions. Cancer Cell Int 2014;14:97.
[24] Mills KHG. Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 2004;4:841–55.
[25] Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004;22:745–63.
[26] McNeill L, Cassady RL, Sarkardei S, Cooper JC, Morgan G, Alexander DR. CD45 isoforms in T cell signaling and development. Immunol Lett 2004;92:125–34.
[27] Bozdogan G, Erdem E, Demirel GY, Yildirmak Y. The role of Treg cells and FoxP3 expression in immunity of β-thalassemia major and β-thalassemia trait patients. Pediatr Hematol Oncol 2010;27:534–45.
[28] Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007;81:584–92.
[29] Rossol M, Kraus S, Pierer M, Baerwald C, Wagner U. The CD14 (bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. Arthritis Rheum 2012;64:671–7.
[30] Beaulieu LM, Clancy L, Tanriverdi K, Benjamin EJ, Kramer CD, Weinberg EO, He X, Mekasha S, Mick E, Ingalls RR, Genco CA, Freedman JE. Specific inflammatory stimuli lead to distinct platelet responses in mice and humans. PLoS One 2015;10:e0131688.
[31] Stokes KY, Granger DN. Platelets: critical link between inflammation and microvascular dysfunction. J Physiol 2012;590:1023–34.
[32] Passacquale G, Vamadevan P, Pereira L, Hamid C, Corrigal V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. PLoS One 2011;6:e25595.
[33] Paulsson JM, Jacobson SH, Lundahl J. Neutrophil activation during transmigration *in vivo* and *in vitro*: a translational study using the skin chamber model. J Immunol Methods 2010;361:82–8.
[34] Pauksens K, Fjaertoft G, Douhan-Håkansson L, Venge P. Neutrophil and monocyte receptor expression in uncomplicated and complicated influenza A infection with pneumonia. Scand J Infect Dis 2008;40:326–37.
[35] Nuutila J, Hohenthal U, Laitinen, Kotilainen P, Rajamäki A, Nikoskelainen J, Lilius EM. Simultaneous quantitative analysis of FcγRI (CD64) expression on neutrophils and monocytes: a new, improved way to detect infections. J Immunol Methods 2007;328:189–200.
[36] Vara EJ, Svanes C, Skorge TD, Berstad A, Florvaag E, Jarvis D, Omenaas E, Waatevik M, Johannessen A, Lied GA. Functional gastrointestinal symptoms are associated with higher serum total IgE levels, but less atopic sensitization. Dig Dis Sci 2016;61:189–97.
[37] Kiiski V, Karlsson O, Remitz A, Reitamo S. High serum total IgE predicts poor long-term outcome in atopic dermatitis. Acta DermVenereol 2015;95:943–7.
[38] Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990;249:1431–3.
[39] Vimercati L, Gatti MF, Baldassarre A, Nettis E, Favia N, Palma M, Martina GL, Di Leo E, Musti M. Occupational exposure to urban air pollution and allergic diseases. Int J Environ Res Public Health 2015;12:12977–87.