ORIGINAL ARTICLE

Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study

Maysaa Nemer,[1,2] Liv I B Sikkeland,[3] Mayes Kasem,[4] Petter Kristensen,[2,5] Khaldoun Nijem,[1] Espen Bjertness,[2] Øivind Skare,[5] Berit Bakke,[4] Johny Kongerud,[3,6] Marit Skogstad[5]

For numbered affiliations see end of article.

Correspondence to
Maysaa Nemer, Occupational Epidemiology and Biological Research Lab, Department of Biology, Hebron University, P.O.Box 40, Hebron, Palestine; maysa.nemer@gmail.com

Received 3 July 2014
Revised 15 January 2015
Accepted 18 January 2015
Published Online First 4 February 2015

ABSTRACT

Objectives Little is known about the working conditions and airway inflammation in hairdressers in Palestine. We aimed to investigate if hairdressers in Palestine have a higher level of airway inflammation as compared to a control group. We also assessed the hairdressers' physical working conditions and exposure to ammonia gases at the hair salons. Lastly, we investigated the association between ammonia levels and inflammation markers in the airways and the blood.

Methods Our study participants were 33 non-smoking hairdressers (aged 19–50 years) and 35 non-smoking control subjects (aged 18–49 years). Both groups answered a questionnaire on respiratory symptoms, and performed lung function and exhaled nitric oxide (eNO) tests. Blood and sputum samples were collected from all participants and air concentration levels of ammonia were measured in 13 salons.

Results Hairdressers had a higher level of sputum neutrophil count (absolute numbers/mg sputum (median (25th–75th centiles)) compared to controls, 376 (183–980) and 182 (96–358), respectively. Hairdressers also had significantly elevated eNO and blood C reactive protein (CRP) levels compared to the control subjects, controlled for age and body mass index. Exposure measurements showed that the hairdressers in salons with scarce ventilation were exposed to ammonia concentration, ranging from 3 to 61 mg/m^3.

Conclusions Compared to unexposed controls, the hairdressers had signs of neutrophilic airway inflammation, higher eNO levels and higher CRP. The hairdressers were exposed to high concentrations of ammonia from hairdressing chemicals and their working conditions were unsatisfactory.

What this paper adds

- Exposure to chemicals at the workplace causes respiratory problems and airway inflammation for hairdressers.
- Increase the limited knowledge base regarding airway inflammation among female hairdressers.
- This study found that female Palestinian hairdressers have a higher neutrophilic airway inflammation compared to non-exposed control group, and are exposed to high levels of ammonia at the workplace.
- Improving working conditions like ventilation and use of respiratory protective equipment is of importance for the workers' respiratory health.

INTRODUCTION

Several studies have assessed occupational health among hairdressers[1–7] and many have reported occupational asthma among hairdressers caused by chemical exposure at the workplace.[4 8–10]

Induced sputum, a non-invasive method for collecting samples from the central airways, may be used to study airway inflammation among occupationally exposed workers. To our knowledge, no previous studies have carried out induced sputum tests among hairdressers in an occupational setting. Only two studies have previously published results using this sputum technique for studying asthmatic hairdressers in a clinical setting.[9 11] One was a study of 47 asthmatic hairdressers referred for clinical examinations. Seven responded positively to specific inhalation of ammonium persulfate, which suggests they had eosinophilic airway inflammation.[9] The other study reported high counts of sputum eosinophils in one out of five non-atopic hairdressers with asthma.[11]

Nitric oxide (NO) is produced by several cell types in the lungs and plays a role in asthma pathogenesis.[12] An exhaled NO (eNO) test is a simple and safe way of measuring NO gas in a person's breath and it has been applied in several occupational settings, including that of hairdressers.[13–18] Higher levels of eNO and a higher prevalence of asthma have been found among hairdressers compared to other groups of workers.[18]

Hairdressers are exposed to irritants such as vapours, solvents, perfumes and dust in salons that often have high temperatures. Hairdressing products contain several potentially hazardous chemicals that may cause respiratory problems, such as airway inflammation.[9 11] One of the chemicals that has been under study as an irritating agent in hairdressers is ammonia.[1] It is released into the atmosphere from permanent hair dyes and permanent wave preparations.

To cite: Nemer M, Sikkeland LIB, Kasem M, *et al*. *Occup Environ Med* 2015;**72**:428–434.

In a previous cross-sectional study, we found that female hairdressers in Palestine had higher levels of respiratory symptoms and lower lung function, as measured by forced respiratory volume in 1 s (FEV_1) compared to a control group.[19] The present study is based on a subsample of the same group of hairdressers and unexposed control group of female university students and staff. We sought to investigate differences in inflammatory cells in the sputum between the groups. Second, we wanted to test if there were any differences in eNO levels, C reactive protein (CRP) and white cell count (WCC) between hairdressers and the control group. Additionally, we aimed to explore workplace exposure to ammonia in the hairdressing salons and any association between air concentration levels of ammonia and measured outcomes.

METHODS

Study design and subjects

A cross-sectional study was carried out between October 2012 and March 2013. The participants included 33 non-smoking female hairdressers aged 19–50 years (mean 38 years), who had been selected out of a cohort of 200 hairdressers who had been studied previously.[19] Every sixth participant from a list, sorted according to salon names, was invited. Replacement hairdressers were randomly selected from the cohort for every invitee who failed to show up or declined to participate (n=7). Furthermore, some hairdressers were included in a non-random fashion because they showed up with an invited coworker (n=4). The control group was recruited through advertisements, including 35 non-smoking female students (n=27) and staff (n=8) at Hebron University aged 18–49 years (mean 24 years).

On the day of the examination at the laboratory at Hebron University, all participants answered a modified version of a standardised respiratory questionnaire from American Thoracic Society,[20] including questions about respiratory symptoms, such as chest tightness, shortness of breath, wheezing, coughing and phlegm production during the past 12 months and doctors' diagnosed asthma. Additionally, a lung function test (LFT) was performed according to American Thoracic Society/European Respiratory Standards guidelines,[21] using a PC spirometer (ML2525, Micro Medical Limited, UK). All participants had FEV_1 values above 75% of the predicted values. Participants were advised not to consume food or beverages 1 h before the measurements. Participants were not tested within 3 weeks of a respiratory infection. All participants (hairdressers and the controls) were living in Hebron City.

General characteristics, reported respiratory symptoms and lung function measurements of the hairdressers and controls are given in table 1.

Table 1 General characteristics, respiratory symptoms and lung function of hairdressers and controls in Hebron city, 2013

	Hairdressers (n=33)	Controls (n=35)
Age	38 (8.2)	24 (7.1)*
Years of education	11 (2.2)	15 (1.1)*
Weight (kg)	68 (10)	62 (11)*
Height (cm)	159 (5)	159 (5)
BMI	27 (4.3)	25 (4.8)
Years of employment in hairdressing	12 (7)	–
Wheezing or whistling in the chest, in the past 12 months	9 (27)	6 (17)
Morning chest tightness, in the past 12 months	19 (58)	7 (20)*
Shortness of breath during the past 12 months	16 (49)	3 (9)*
Cough in the morning	7 (21)	1 (3)
Chest phlegm in the morning	15 (46)	5 (14)*
Asthma (diagnosed by a doctor)	3 (9)	0 (0)
FVC (L)	2.87 (0.30) (92%)	3.39* (0.40) (100%)
FEV_1(L)	2.42 (0.52) (88%)	2.91 (0.34) (97%*)
FEV_1/FVC	0.84 (0.15)	0.86 (0.10)

Data are presented as means (SD) for age, education, BMI and years of employment. Symptoms and asthma are presented as number (%).
Lung function measurements are presented as mean (SD; % of predicted value compared to the European standards).
*Significant difference ($p<0.05$) between hairdressers and controls, symptoms differences are adjusted for age while lung function differences are adjusted for age, BMI and height.
BMI, body mass index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

Induced sputum

Sputum was collected successfully and processed at the lab in Hebron University for 30 hairdressers and 33 controls, out of the 33 hairdressers and 35 controls who participated in the study, according to the procedure described previously by our group.[22] After the participants had taken a baseline spirometry, aerosols of hypertonic saline solutions at concentrations of 3%, 4% and 5% were inhaled, each for 7 min, from the eFlowRapid nebuliser (PARI GmbH, Germany). At the end of each inhalation period the participant's sputum sample was collected.[23] Participants were treated with a bronchodilator before inhalation of hypertonic saline.

The induced sputum was conserved at 4°C and processed within 2 h. Sputum plugs were selected, weighed, treated with phosphate-buffered saline containing 0.1% (w/V) dithiothreitol, diluted and filtered.[22 24] Using optical microscopy, total number of cells was counted and cell viability was assessed using Trypan blue (0.4%; Sigma, St. Louis, USA). Cytospin slides were stained using Diff Quik (Medion Diagnostics, Düdingen, Germany). Differential cell counts were conducted by two technicians each counting 300 cells, not including squamous cells. Of 63 collected sputum samples, 7 were excluded. Four of the samples contained more than 50% squamous cells, and 3 samples had less than 50% viable cells.

Exhaled NO

eNO was measured using the NIOX MINO (Aerocrine AB, Sweden) device, set to a flow rate of 50 mL/s, expressed in parts per billion (ppb). All measurements were performed according to the manufacturer's protocol and according to the American Thoracic Society recommendations,[12] with the exception that only one measurement was taken from each participant each time eNO was measured. All measurements were conducted in the research laboratory at Hebron University. The ambient level of NO in the lab was less than 5 ppb.

Blood parameters

We drew blood samples in the laboratory at Hebron University. The samples were then incubated at room temperature and sent to a local medical laboratory the same day. We obtained two blood parameters as a measure of systemic inflammation: a complete blood count (CBC) test, which included a WCC, and a differential cell count using Sysmex XE-210 haematology system (Sysmex). To determine the concentration level of CRP in the blood an immunoturbidimetric assay (CRP Latex, latex

turbidimetry, vital diagnostics, Italy) was used. The interassay variation (coefficient of variation) was 7.8%.

Working conditions and exposure

All salons (n=13) where the hairdressers worked were visited on randomly selected days. In order to systematically collect information on working conditions and potential predictors of exposure to ammonia, we assessed the following properties in the salon: salon size, number of workers; number of customers; presence and type of ventilation; and use of personal respiratory protective equipment in the salon.

We measured the concentration levels of atmospheric ammonia in the salons using an electrochemical sensor instrument (pac7000 DrägerAktiengesellschaft, Lübeck, Germany), which was affixed to one hairdresser in each of the salons. The sampling duration ranged from 45 to 305 min. The variation in the sampling duration among salons is due to the variation in the number of customers the hairdressers were dyeing or cutting during attaching of the device as the number of customers varied between salons at the time of sampling. We set the instrument to average readings of concentration levels over a 30 s period (time weighted average). The instrument allowed for both a direct reading of concentration levels and logging of the data. The instrument's limit of detection of ammonia was 1.4 mg/m^3. We calibrated the instrument's response factor by using certified calibration gas obtained from Yara Praxair ASA, Oslo, Norway.

Statistical methods

Analyses were performed using both SPSS V.21.0 for Windows (SPSS Inc, Chicago, USA) and Stata SE V.13.0 (StataCorp, Texas, USA). Standard descriptive statistics (arithmetic means (AMs) and SDs) were computed for exposure data and outcome variables. The sputum differential cell data, blood parameters and exhaled NO data were highly skewed and were, therefore, expressed as medians and 25th–75th centiles. Air concentrations of ammonia for each measurement session at each salon were presented as median, minimum (min) and maximum (max) concentrations. The overall concentrations were aggregated using AM, geometric mean and geometric SD (GSD). Peak exposure was evaluated by counting the number of times ammonia levels exceeded 15 ppm (10.5 mg/m^3) since this value is the threshold limit value (TLV) of ammonia exposure at a workplace[25] and by summing the total time spent above this exposure level.

In order to compare levels of inflammatory cells in the sputum, eNO levels and blood parameters between hairdressers and the control group, we used median regression (in Stata). Crude and adjusted results were presented with point estimates (differences) and 95% CIs.

The association between exposure to ammonia and the different outcomes was also analysed. To assess associations with lung function we used linear regression; for sputum data, eNO data and blood parameters we used median regression. Similarly, the associations between years of employment in hairdressing and the different outcomes were analysed using linear or median regression.

We adjusted for potential confounders considered to affect the association between exposure and study outcomes. Sputum data differences were adjusted for age, blood parameters were adjusted for age and body mass index (BMI), eNO data were adjusted for age and height and LFT data were adjusted for age, BMI and height.

Ethics

Since there is no ethical committee in Palestine, the study protocol was approved by the Regional Committee for Research Ethics, Oslo, Norway (reference: 2012/344/REK). Written informed consent was obtained from all participants and they were informed about the opportunity to withdraw from the study at any time.

RESULTS

The hairdressers and controls were significantly different in age, years of education and weight. The hairdressers reported more chest tightness, shortness of breath and phlegm compared to the controls. Adjusted for age and height, the hairdressers had significantly lower forced vital capacity (FVC) than the controls. They also had significantly lower percent of predicted value of FEV_1 than the controls.

Differential cell count in induced sputum

Table 2 shows the differential cell counts in induced sputum samples among hairdressers and controls. A significantly higher median neutrophil count (neutrophils/mg sputum) in the sputum was observed in the hairdressers compared to the controls: counts were 376 (183–980; 25th–75th centiles) and 182 (96–358), respectively. The sputum eosinophils and lymphocytes counts were very low in both groups, with less than 2% in all samples.

Exhaled NO

All the hairdressers (n=33) and the controls (n=35) performed the eNO test. eNO levels were significantly elevated among the hairdressers compared to the control group, with values of 18 ppb (12–22; median (25th–75th centiles)) and 12 ppb (9–17), respectively. The difference in the medians of the two groups, after controlling for age and height, was 5.9 with 95% CI 0.6 to 11.1, (p=0.02). Similar results were found when asthmatics were excluded.

Blood parameters

Blood parameters showed that both hairdressers and controls have a similar number of WCC and percentage of blood neutrophils. The percentage of eosinophils and basophils in the blood were too low to be detected. The median level of CRP was higher among hairdressers compared to the controls (p<0.001), at 8 mg/L (6–10 (25th–75th centiles)) and 3 mg/L (1–5), respectively. The difference in median levels was also significant even after adjusting for age and BMI, as shown in table 3.

Working conditions and exposure

The results from the exposure assessment from all 13 salons are presented in table 4. The salons differ with respect to size as well as to the number of hairdressers at work and number of customers. With respect to ventilation, none of the salons had mechanical ventilation where the air is exchanged or filtered. Six salons had no windows, three of which had air conditioning; two salons had one or two holes in the wall (approximately 20 cm in diameter each) allowing for fresh air ventilation; and the remaining five salons had one window. Respiratory protective masks with only particle filter were available in two salons, but were reported to rarely be in use.

One direct reading of atmospheric ammonia concentrations was obtained from each salon (n=13). All measurements had a time-weighted average concentration level above the limit of detection of 1.4 mg/m^3. The concentration levels in the salons varied from 3 to 61 mg/m^3, with a GM of 7.3 and GSD of 3. In two of the salons (2 and 4), the mean concentration levels of ammonia were very high (38 and 61 mg/m^3, respectively; table 4).

Table 2 Differential cell count in induced sputum from hairdressers and controls in Hebron city, 2013

			Crude			Adjusted†		
				95% CI			95% CI	
	Hairdressers (n=27)	Controls (n=29)	Difference‡	Lower	Upper	Difference‡	Lower	Upper
Total cells/mg sputum	852 (478 to 1714)	615 (380 to 1045)*	+237	+160	+733	+268	+85	+972
Neutrophil granulocytes								
Per cent	55 (27 to 75)	41 (20 to 67)	+14	−11	+39	+11	−26	+48
Absolute numbers/mg sputum	376 (183 to 980)	182 (96 to 358)*	+194	+80	+469	+93	+37	+515
Airway macrophage								
Per cent	51 (33 to 72)	58 (33 to 78)	−7	−29	+16	−5	−39	+28
Absolute numbers/mg sputum	344 (186 to 701)	250 (176 to 517)	+94	−130	+318	+92	−233	+418
Lymphocytes								
Per cent	2 (0.8 to 4)	1 (0.8 to 2)	+1	−0.3	+2	+0.9	−0.4	+3
Absolute numbers/mg sputum	16 (6 to 39)	8 (3 to 15)	+8	−4	+20	+1	−15	+18
Eosinophils								
Per cent	1 (0.4 to 3)	1 (0.2 to 1)	+0.2	−0.8	+1	+0.1	−1.3	+1.5
Absolute numbers/mg sputum	13 (3 to 32)	4 (2 to 13)	+9	−2	+19	+8	−7	+24

Data are presented as median (25th–75th centiles).
*p<0.05.
†Adjusted for age.
‡Differences: median differences between hairdressers and controls.

Peak exposure to ammonia was evaluated by obtaining the number and duration of peaks above 10.5 mg/m^3 for each measurement session in a salon. The percentage of total sampling time when ammonia levels exceeded 10.5 mg/m^3 was very high in three salons (2, 4 and 6), with percentages at 92%, 99.6% and 75%, respectively (results not shown).

Within the exposure data, we found correlation between the size of the salon and NH_3 level (p=0.02), between number of workers in the salon and NH_3 level (p=0.008), and between number of customers on the day of measurement and NH_3 (p=0.02).

Associations between exposures and outcomes

The association between measured atmospheric ammonia in the 13 different hairdressing salons and the different outcomes among the hairdressers is shown in table 5. No significant association was found between levels of ammonia, including peak exposure, and lung function measurements, sputum neutrophils count or eNO levels.

We also tested the association between the outcomes and length of employment. Compared to hairdressers who had been employed for a shorter period, hairdressers who had been employed for a long period had lower FVC, −0.014 (L), 95% CI (−0.026 to −0.001), p=0.04, and FEV_1, −0.031 (L), 95% CI (−0.052 to −0.010), p=0.005, after adjusting for age and height. Additionally, WCC was higher; +0.105 (×10^3/μL), 95% CI (+0.002 to +0.208), p=0.04, among hairdressers who had worked in hairdressing for a longer period after controlling for age and BMI.

Hairdressers who reported asthma-like symptoms were working in different salons of different sizes and ventilation possibilities. No association was found between exposure variables including measured ammonia, size of the salon, ventilation of the salon or number of working years and reported asthma-like symptoms or airway inflammation variables.

DISCUSSION

In this study we found that female hairdressers in Hebron had significantly higher number of neutrophils in the sputum, elevated levels of eNO and CRP, compared to the control group. These hairdressers were working in relatively small sized salons with no or poor ventilation, lacked respiratory protective equipment and were exposed to high levels of ammonia above TLV.

Working in hairdressing has been found to cause airway inflammation and asthma.[4 9 10 26] Elevated levels of sputum neutrophils have been found in several occupational groups.[27–29]

Table 3 Blood parameters among hairdressers and controls in Hebron city, 2013

			Crude			Adjusted*		
				95% CI			95% CI	
	Hairdressers (n=28)	Controls (n=32)	Difference†	Lower	Upper	Difference†	Lower	Upper
CRP (mg/L)	8 (6 to 10)	3 (1 to 5)	+5.0	+2.7	+6.3	+5.0	+2.5	+7.5
WCC (×10^3/μL)	6 (5 to 7)	7 (6 to 8)	−1.0	−1.2	+0.2	−0.6	−1.9	+0.6
Neutrophils %	64 (60 to 67)	60 (52 to 67)	+4.0	−1.8	+9.8	+6.3	−0.5	+13.3
Lymphocytes %	36 (34 to 41)	33 (30 to 36)	+4.0	+0.8	+7.1	+2.6	−1.3	+6.6
Monocytes %	7 (6 to 9)	5 (3 to 7)	+2.0	+0.4	+3.5	+2.8	+0.5	+5.1

Data are presented as median (25th–75th centiles).
*Adjusted for age and BMI.
†Differences: median differences between hairdressers and controls.
BMI, body mass index; CRP, C reactive protein; WCC, white cell count.

Table 4 Exposure assessment in 13 female hairdressing salons in Hebron city, 2013

Salon ID	Floor area (m^2)	Air volume (m^3)	Number of workers	Number of customers on the measurement day	Duration of NH_3 measurement in the salon (minutes)	Median NH_3 (mg/m^3)	Min–Max NH_3 (mg/m^3)	Tasks at peak value	Number of hairdressers tested
1	48	86.4	4	18	45	9	0–51.6	Colouring	3
2	12	21.6	8	21	195	38	0–48.1	Colouring	4
3	9	22.5	3	6	160	4	0–18.8	Colouring	3
4	8	14.4	10	20	265	61	6.3–202.1	Colouring, bleaching	3
5	3	6.0	4	15	228	8	3.5–29.3	Colouring, cutting	3
6	40	76.0	6	11	70	8	5.6–13.9	Colouring	2
7	10	20.0	3	5	205	4	0–10.5	Spraying	2
8	6	10.8	5	5	212	4	0–6.3	Cutting, spraying	3
9	25	47.5	6	26	305	4	0–11.2	Colouring	2
10	30	75.0	4	5	180	5	3.5–9.1	Colouring, spraying	2
11	6	11.4	4	5	285	7	0–14.6	Spraying, cutting	2
12	4	8.0	3	14	285	5	0–8.4	Spraying, cutting	2
13	16	32.0	2	11	45	3	0–12.5	Cutting	2

Overall (n=13): NH_3 (mg/m^3).
Arithmetic mean=12.3.
Geometric mean=7.3.
Geometric SD=3.

The only previous report on different levels of neutrophils in sputum among hairdressers was conducted on a group of hairdressers with persulfate-induced asthma.[9] This group had sputum neutrophil levels in normal range. However, the hairdressers who did not respond to ammonium persulfate had elevated numbers and elevated percentage of neutrophils compared to the hairdressers with a positive response. An increased risk of asthma has been found among these hairdressers.[4 8 9 26] In the present study, the percentage of eosinophils in sputum is less than 2% for all participants. This does not support an increased risk for atopic asthma because having more than 2.5% eosinophils is the cut-off point for sputum eosinophila which could be present in both atopic and non-atopic asthma.[30] Furthermore, hairdressers in our study population who reported having doctor's diagnosed asthma (n=3) did not have higher levels of eosinophils in sputum. The presence of high sputum neutrophil count among hairdressers could be occurring in response to these workers' exposure to different types of irritants in the work atmosphere. The presence of high sputum neutrophil count neither excludes nor confirms an asthma diagnosis among the hairdressers. Sputum neutrophilia is known to be influenced by age.[31] Since the hairdressers and control group are of different age, we have adjusted the comparison of the sputum data for age.

The mean level of eNO was significantly elevated among the hairdressers compared to the controls (17.1 ppb vs 10.9 ppb). Similarly, a study from Greece found a significantly higher level of eNO among hairdressers compared to office workers.[17] These results, however, have not been confirmed in other studies of hairdressers.[13 16 18] eNO levels are elevated among asthmatic participants with atopy and with higher levels of sputum eosinophils. We found that the eNO levels were within the normal range among the controls (2–25 ppb) as well as among the hairdressers (6–35 ppb), with the exception of one hairdresser who had an eNO value of 74 ppb. This participant had a more obstructive lung function (FEV_1=73% of predicted) than the other hairdressers and a high percentage of neutrophils in sputum (80%), but surprisingly a normal percentage of eosinophils (<2%). eNO values in the normal range are in accordance with the differential cell count results from the sputum, since the participants did not have eosinophilic inflammation.

Table 5 Association between measured ammonia and outcomes among 33 hairdressers in Hebron city

	Measured NH_3(mg/m^3)					
	Crude			Adjusted*		
		95% CI			95% CI	
Outcomes	Coefficient†	Lower	Upper	Coefficient†	Lower	Upper
FVC (L; n=33)	−0.003	−0.007	+0.001	−0.003	−0.007	+0.001
FEV_1(L; n=33)	−0.003	−0.010	+0.003	−0.002	−0.008	+0.003
Total number of cells/mg sputum (n=27)	−3.593	−20.664	+13.478	−3.998	−21.663	+13.665
Number of neutrophils/mg sputum(n=27)	+2.259	−9.444	+13.962	−8.710	−12.663	+13.665
Number of eosinophils/mg sputum(n=27)	−0.031	−0.448	+0.385	−0.032	−0.567	+0.502
eNO (ppb; n=33)	−0.067	−0.224	+0.090	−0.041	−0.192	+0.109
WCC (×103/μL; n=28)	+0.009	−0.028	+0.046	+0.011	−0.022	+0.043
CRP (mg/L; n=28)	−0.047	−0.098	+0.003	−0.060	−0.105	+0.015

*Adjusted for age and BMI in WCC and CRP, for age in inflammatory cells in sputum, for age and height in eNO and for age, BMI and height in FVC, FEV_1.
†Coefficient is the estimated mean/median effect in association with 1 mg/m^3 increased ammonia level.
CRP, C reactive Protein; eNO, exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; WCC, white cell count.

As eNO levels have been shown to be highly affected by age and height,[32] we adjusted our models for these variables.

The higher levels of eNO among hairdressers compared to controls could be due to unmeasured factors other than age and height which were controlled for in our study. It may be possible that hairdressers had a slight increase in sputum eosinophils, but since the values of sputum eosinophils were low a difference would be difficult to detect. On the other hand, the increased eNO level among hairdressers could be due to the fact that the hairdressers have more tendency to develop asthma because of their occupational exposure, which is still not very high but higher than the controls.

With respect to the blood parameters, we found an increase in WCC count among hairdressers who had worked in hairdressing for a longer period. This could be due to chronic inflammation caused by exposure. The CRP level was significantly higher among hairdressers compared to controls. Moreover, hairdressers' CRP levels were above the normal level of 5 mg/L. CRP is a substance produced by the liver that increases in the presence of inflammation in the body. An elevated CRP level is considered an indicator for the presence and intensity of inflammation, for example, viral and bacterial infections, but also physical trauma, excess weight, exposure to environmental toxins and air pollutants at work may increase the CRP level.[33 34] Previous research has found that occupational exposure to chemicals in hair salons can lead to an increase in serum CRP.[34] Serum CRP is highly associated with BMI[35] and as our group of hairdressers had a higher BMI than the controls this could explain the elevated levels of CRP found among hairdressers. However, this difference remained significant after adjusting for BMI.

We found hazardously high levels of atmospheric ammonia in some of the salons, reaching 61 mg/m^3 which exceeds safety guidelines from the developed world of short-term exposure levels (15 min) in the range from 10.5 to 24.4 mg/m^3[25 36] and the LOAEL (lowest observed adverse effect level) for irritation of 18 mg/m^3. Ammonia levels in the hairdressing salons could give an indication of the levels of the chemical exposure in the salons since ammonia is emitted from several hair treatment products, including permanent hair dye, permanent wave and permanent fix.[1] Previous studies have shown that the levels of persulfates and ethanol correlate with the levels of ammonia.[3 5 6] Workplace exposure to ammonia could cause severe irritation of the nose and throat if inhaled, and inhalation of high concentrations of ammonia cause acute respiratory injury and chronic lung disease.[37]

The mean levels of ammonia in the salons in the present study ranged from 3 to 61 mg/m^3, which differs markedly from what was found in a similar study in Norway—where concentrations of ammonia in the salon ranged from 0.1 to 1.2 mg/m^3, with the highest levels of ammonia measured during bleaching.[1] The highest recorded levels of ammonia also occurred during bleaching in the present study (salon 4). Our results do not correspond with results from other European countries, where ammonia levels in the salons ranged from 0.9 to 3.5 mg/m^3.[3 5]

We have found that the hairdressers have neutrophilic airway inflammation and the amount of ammonia in most salons is higher than the moderate irritating levels. However, we found no correlation between inflammation and ammonia exposure. This could be due to few exposure measurements, misclassification of exposure or two independent factors. So it would have been of interest to measure other occupational factors known to cause inflammation, such as persulfates.

The device we used for exposure measurements had a limited specificity for measuring ammonia as compared to other gases. Thus the elevated levels of ammonia that we found in our sample could be due to other gases in the work atmosphere which could have interfered and led to an increase in the measured levels. These gases could mainly be other chemicals emitted from products like permanent dyes and bleaches. Some of them can also cause irritation or other respiratory problems, such as persulfates and paraphenylenediamine. However, the measurements are likely to reflect the true exposure to harmful gases, including ammonia, generated at the salons since the salons in the present study had limited or no proper ventilation. Similarly, previous exposure studies have found high levels of ammonia and ethanol in the absence of mechanical ventilation.[38] The salons in our sample are small in size. This could also explain the high levels of ammonia and the detected correlations between the size of the salon and the mean level of ammonia.

In the present cross-sectional study, we planned to select participants at random; however, as some hairdressers failed to show up, we selected additional participants at random from our original list. Some hairdressers who showed up with coworkers who had been preselected also participated in the study. The control group was self-selected based on students' and staff's interest in participation. These differences in recruitment of the study samples might have caused a selection bias. On the other hand, hairdressers with a high workload might have been too busy to participate in the study. This might have affected our assumption of having selected representative samples. The selection bias could have both directions, as the sick might be more encouraged to participate resulting in a higher airway inflammation than the true one. It could also be the opposite as the healthier and the lower exposed might be more encouraged to participate resulting in a lower estimate than the true one.

To reduce bias related to data collection, all tests were performed using the same devices and carried out by the same researchers. Standard instructions were followed for the induced sputum and spirometry tests, and blood samples were analysed by individuals blinded to exposure information. Standard instructions were also followed when performing the eNO test, with the exception that only one measurement was taken from each participant. Studies have shown that one measurement is adequate when using the NIOX MINO device due to high reproducibility of this method.[39 40]

The control group we selected was similar to the hairdressers group with respect to smoking habits (all non-smokers), but differed with respect to age, weight, BMI, years of education and probably socioeconomic status. These differences might affect the findings as some measurements were found to be highly affected by age and BMI, namely CRP, sputum neutrophilia, eNO and WCC. Age as a main difference between the two groups is considered as the most important confounder that might affect the comparisons between the two groups; therefore, all our comparisons were adjusted for age. We also adjusted for height and BMI as potential confounders. Confounders that could not be adequately controlled might include unknown respiratory irritants outside the workplace.

If the healthy worker effect affected our study, workers leaving hairdressing due to ill health would have led to an underestimation of true differences between the two groups. In this occupational setting, however, we believe that the healthy worker effect did not highly affect our results. The absence of a social security system in Palestine might force hairdressers into keeping their jobs despite health problems.

We only carried out a single measurement of ammonia at each salon. These samples might not be representative, as potential differences in the number of customers, working tasks and

weather conditions could be present. This in combination with the small sample size could explain the lack of association between ammonia and outcomes. The internal comparisons results might be affected by reverse causation. If this was the case, hairdressers in greater health may have been more strongly encouraged to use chemicals without precautions and to perform the tasks that included more extensive use of dangerous chemicals, such as bleaching and colouring.

CONCLUSIONS

To the best of our knowledge this is the first study of a group of hairdressers in an occupational setting which has investigated airway inflammation using sputum analysis together with exposure measurements in the salons. This study reveals that female hairdressers showed signs of neutrophilic airway inflammation, possibly caused by irritating chemical properties, and had elevated levels of CRP and eNO. The present hairdressers work in small places with limited ventilation and respiratory protective equipment, with exposure levels of ammonia even reaching hazardous levels of 61 mg/m^3.

Internal comparison within the hairdressers group showed significantly lower level of lung function measurements and elevated level of WCC among the hairdressers with a greater number of working years.

Author affiliations
[1]Occupational Epidemiology and Biological Research Lab, Department of Biology, Hebron University, Hebron, Palestine
[2]Section for Preventive Medicine and Epidemiology, Institute of Health and Society, University of Oslo, Oslo, Norway
[3]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.
[4]Department of Chemical and Biological Work Environment, National Institute of Occupational Health, Oslo, Norway
[5]Department of Occupational Medicine and Epidemiology, National Institute of Occupational Health, Oslo, Norway
[6]Department of Respiratory Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Acknowledgements The authors would like to thank the financial support provided by the Norwegian State Education Loan Funds. We also thank NUFU for funding this study. Karina Corbett is gratefully acknowledged for linguistic support.

Contributors MN, MK, LIBS, BB, ØS and MS performed the data collection, the experiments and analysis. MN, LIBS, PK, BB, KN, EB, ØS JK and MS designed the study, interpreted the data and prepared the manuscript. All the authors have seen, reviewed and approved the final version

Funding This study was funded by NUFU Building Competence in Epidemiology in Palestine- Pro 50-2002 and supportive measures 2008–2009.

Competing interests None.

Patient consent Obtained.

Ethics approval The study was approved by the Regional Committee for Research Ethics, Oslo, Norway (reference: 2012/344/REK).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Hollund BE, Moen BE. Chemical exposure in hairdresser salons: effect of local exhaust ventilation. *Ann Occup Hyg* 1998;42:277–82.
2 Labreche F, Forest J, Trottier M, *et al*. Characterization of chemical exposures in hairdressing salons. *Appl Occup Environ Hyg* 2003;18:1014–21.
3 Leino T, Kahkonen E, Saarinen L, *et al*. Working conditions and health in hairdressing salons. *Appl Occup Environ Hyg* 1999;14:26–33.
4 Moscato G, Galdi E. Asthma and hairdressers. *Curr Opin Allergy Clin Immunol* 2006;6:91–5.
5 Mounier-Geyssant E, Oury V, Mouchot L, *et al*. Exposure of hairdressing apprentices to airborne hazardous substances. *Environ Health* 2006;5:23.
6 Oikawa D, Takeuchi W, Murata S, *et al*. Measurement of concentrations of thioglycolic acid, dithiodiglycolic acid and ammonia in indoor air of a beauty salon. *J Occup Health* 2012;54:370–5.
7 van Muiswinkel WJ, Kromhout H, Onos T, *et al*. Monitoring and modelling of exposure to ethanol in hairdressing salons. *Ann Occup Hyg* 1997;41:235–47.
8 Leino T, Tammilehto L, Hytönen M, *et al*. Occupational skin and respiratory diseases among hairdressers. *Scand J Work Environ Health* 1998;24:398–406.
9 Moscato G, Pignatti P, Yacoub MR, *et al*. Occupational asthma and occupational rhinitis in hairdressers. *Chest* 2005;128:3590–8.
10 Espuga M, Munoz X, Plana E, *et al*. Prevalence of possible occupational asthma in hairdressers working in hair salons for women. *Int Arch Allergy Immunol* 2011;155:379–88.
11 Macchioni P, Kotopulos C, Talini D, *et al*. [Asthma in hairdressers: a report of 5 cases]. *Med Lav* 1999;90:776–85.
12 Dweik RA, Boggs PB, Erzurum SC, *et al*.; American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–15.
13 Demange V, Wild P, Zmirou-Navier D, *et al*. Associations of airway inflammation and responsiveness markers in non asthmatic subjects at start of apprenticeship. *BMC Pulm Med* 2010;10:37.
14 Fell AK, Noto H, Skogstad M, *et al*. A cross-shift study of lung function, exhaled nitric oxide and inflammatory markers in blood in Norwegian cement production workers. *Occup Environ Med* 2011;68:799–805.
15 Lund MB, Oksne PI, Hamre R, *et al*. Increased nitric oxide in exhaled air: an early marker of asthma in non-smoking aluminium potroom workers? *Occup Environ Med* 2000;57:274–8.
16 Remen T, Coevoet V, Acouetey DS, *et al*. Early incidence of occupational asthma among young bakers, pastry-makers and hairdressers: design of a retrospective cohort study. *BMC Public Health* 2010;10:206.
17 Skoufi GI, Nena E, Kostikas K, *et al*. Work-related respiratory symptoms and airway disease in hairdressers. *Int J Occup Environ Med* 2013;4:53–60.
18 Tossa P, Paris C, Zmirou-Navier D, *et al*. Increase in exhaled nitric oxide is associated with bronchial hyperresponsiveness among apprentices. *Am J Respir Crit Care Med* 2010;182:738–44.
19 Nemer M, Kristensen P, Nijem K, *et al*. Respiratory function and chemical exposures among female hairdressers in Palestine. *Occup Med (Lond)* 2013;63:73–6.
20 Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978;118(6 Pt 2):1–120.
21 Miller MR, Hankinson J, Brusasco V, *et al*. Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
22 Sikkeland LI, Haug T, Stangeland AM, *et al*. Airway inflammation in paper mill workers. *J Occup Environ Med* 2007;49:1135–42.
23 Sikkeland LI, Lund MB, Kongerud J. Immunological analysis of induced sputum in work exposures. *J Environ Immunol Toxicol* 2014;2:79–85.
24 Iwatsubo Y, Matrat M, Brochard P, *et al*. Healthy worker effect and changes in respiratory symptoms and lung function in hairdressing apprentices. *Occup Environ Med* 2003;60:831–40.
25 ACGIH. TLVs and BEIs based on the documentation of the Threshold Limit Values for Chemical Substances and Physical Agents & Biological Exposure Indices. *ACGIH worldwide*. 2005:12.
26 Albin M, Rylander L, Mikoczy Z, *et al*. Incidence of asthma in female Swedish hairdressers. *Occup Environ Med* 2002;59:119–23.
27 Fell AK, Sikkeland LI, Svendsen MV, *et al*. Airway inflammation in cement production workers. *Occup Environ Med* 2010;67:395–400.
28 Heldal KK, Halstensen AS, Thorn J, *et al*. Airway inflammation in waste handlers exposed to bioaerosols assessed by induced sputum. *Eur Respir J* 2003;21:641–5.
29 Sikkeland LI, Eduard W, Skogstad M, *et al*. Recovery from workplace-induced airway inflammation 1 year after cessation of exposure. *Occup Environ Med* 2012;69:721–6.
30 Kips JC, Inman MD, Jayaram L, *et al*. The use of induced sputum in clinical trials. *Eur Respir J Suppl* 2002;37:47s–50.
31 Thomas RA, Green RH, Brightling CE, *et al*. The influence of age on induced sputum differential cell counts in normal subjects. *Chest* 2004;126:1811–14.
32 Olin AC, Rosengren A, Thelle DS, *et al*. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. *Chest* 2006;130:1319–25.
33 Li Y, Rittenhouse-Olson K, Scheider WL, *et al*. Effect of particulate matter air pollution on C-reactive protein: a review of epidemiologic studies. *Rev Environ Health* 2012;27:133–49.
34 Ma CM, Lin LY, Chen HW, *et al*. Volatile organic compounds exposure and cardiovascular effects in hair salons. *Occup Med (Lond)* 2010;60:624–30.
35 Kao TW, Lu IS, Liao KC, *et al*. Associations between body mass index and serum levels of C-reactive protein. *S Afr Med J* 2009;99:326–30.
36 Air-Liquide. Safety Data Sheet/Ammonia. 2005. http://mfc.engr.arizona.edu/safety/MSDS%20FOLDER/Ammonia_MSDS.pdf
37 Leduc D, Gris P, Lheureux P, *et al*. Acute and long term respiratory damage following inhalation of ammonia. *Thorax* 1992;47:755–7.
38 van der Wal JF, Hoogeveen AW, Moons AM, *et al*. Investigation on the exposure of hairdressers to chemical agents. *Environ Int* 1997;23:433–9.
39 Chen W, Purohit A, Barnig C, *et al*. Niox and Niox Mino: comparison of exhaled NO in grass pollen allergic adult volunteers. *Allergy* 2007;62:571–2.
40 Khalili B, Boggs PB, Bahna SL. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 2007;62:1171–4.

Airway inflammation and ammonia exposure among female Palestinian hairdressers: a cross-sectional study

Maysaa Nemer, Liv I B Sikkeland, Mayes Kasem, Petter Kristensen, Khaldoun Nijem, Espen Bjertness, Øivind Skare, Berit Bakke, Johny Kongerud and Marit Skogstad

Occup Environ Med 2015 72: 428-434 originally published online February 4, 2015
doi: 10.1136/oemed-2014-102437