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Nitrogen Dioxide pollution and hazardous household environment: What impacts more congenital malformations

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HIGHLIGHTS

- We show that only the minor malformations are associated with exposure to NO_2.
- Major malformations are associated with hazardous exposures in a household.
- Exposure to close hazardous environment has a more profound impact on health.

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ABSTRACT

Nitrogen Dioxide (NO_2) is a product of fuel combustion originating mainly from industry and transportation. Studies suggest an association between NO_2 and congenital malformations (CM). We investigated an independent effect of NO_2 on CM by adjusting to individual factors and household environment in 1024 Bedouin-Arab pregnant women in southern Israel. This population is characterised by high rates of CMs, frequent consanguineous marriages, paternal smoking, temporary housing and usage of open fire for heat cooking.

Information on household risk factors was collected during an interview. Ambient measurements of 24-h average NO_2 and meteorological conditions were obtained from 13 local monitors.

Median value of daily NO_2 measured in the area was 6.78 ppb. CM was diagnosed in 8.0% (82) of offspring. Maternal NO_2 exposure during the 1st trimester >8.6 ppb was significantly associated with minor CM (RR = 2.68, p = 0.029). Major CM were independently associated with maternal juvenile diabetes (RR = 9.97, p-value = 0.002) and heating by open fire (RR = 2.00, p-value = 0.049), but not NO_2 exposure.

We found that NO_2 emissions had an independent impact only on minor malformations, whereas major malformations depended mostly on the household environment. Antepartum deaths were associated by maternal morbidity.

Abbreviations: APD, ante-partum death; ATD, Admission–Transfer–Discharge; CM, congenital malformations; GIS, geographic information system; ICD, International Classification of Diseases; IP, industrial park; IQR, interquartile range; NO_2, Nitrogen Dioxide; NOx, gases including mix of nitrogen dioxide and nitric oxide; O_3, Ozone; OED, Obstetric Emergency Department; OR, Odds Ratio; $PM_{2.5}$, Particulate Matter $\leqslant$ 2.5 µm; PM_{10}, Particulate Matter $\leqslant$ 10 µm; PPD, Peri-Partum Death; PS, permanent settlements; SES, socio-economic status; SO_2, Sulfur Dioxide; SUMC, Soroka University Medical Center; TTS, traditional tribal settlements.

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1. Background

1.1. Congenital malformations

Congenital malformations (CM) present a major risk of fetal and neonatal morbidity and mortality. According to the WHO estimations, this condition occurs in 1 out of 33 infants, worldwide (WHO, 2014).

The most sensitive period for inducing development of birth malformations is assigned to 3–8 weeks of gestation (Mattison, 2010; Vrijheid et al., 2011). In approximately half of the cases the etiology is believed to be a product of an independent influence or a combination of genetic factors, maternal medications and infections, behavioral exposures and maternal low socioeconomic status, older age, consanguinity, malnutrition (Kalter, 2003; Wigle et al., 2008) and environmental factors, e.g. proximity to industrial facilities, hazardous waste sites and agricultural areas (WHO, 2014).

1.2. An impact of ambient pollution on congenital malformations

Over the last decade there is a growing evidence of an adverse impact of exposure to ambient air pollution on fetal development. Infant mortality, birth weight, prematurity and immune system disorders have all been reported as associated with higher ambient levels of Particulate Matter $\leqslant 10\ \mu m$ (PM_{10}), Nitrogen Dioxide (NO_2), Sulfur Dioxide (SO_2) and other pollutants. (Srám et al., 2005; Glinianaia et al., 2004; Proietti et al., 2013; Stieb et al., 2012; Curtis et al., 2006)

A significant amount of research links ambient air-pollutants with CMs. (Vrijheid et al., 2011; Mattison, 2010; Wang and Pinkerton, 2007; Chen et al., 2014; Proietti et al., 2013; Agay-Shay et al., 2013) The meta-analysis conducted by Vrieheid et al. of 10 epidemiologic studies reported an adverse impact of NO_2 and SO_2 on development of coarctation of the aorta and tetralogy of Fallot, and PM_{10} was found related to an increased risk of atrial septal defects (Vrijheid et al., 2011). The meta-analysis conducted by Chen et al. (2014) reported a significant association found between NO_2 concentrations and coarctation of the aorta. The recent report from a research in Barcelona indicated an association between interquartile range (IQR) increase in NO_2 (12.2 μg/m3) and coarctation of the aorta (Odds Ratio (OR) = 1.15; 95% CI: 1.01, 1.31) and digestive system defects (OR = 1.11; 95% CI: 1.00, 1.23). (Schembari et al., 2014). Israeli researchers found a similar adverse impact of exposure to NOx on specific malformations in the circulatory system and genital organs. (Farhi et al., 2014)

Thus, researchers repeatedly find CM related to NO_2 exposure, whereas its ambient levels remain relatively stable over time, as opposed to the levels of SO_2 and PM_{10} – both constantly decreasing as shown in multiple studies worldwide (Chen et al., 2011; Querol et al., 2014; Karanasiou et al., 2014; Turalioğlu, 2005).

Furthermore, despite an extensive research of an impact of ambient air pollution on neonates, adjustment to individual confounding factors has been problematic in many of the studies focusing on this type of exposure, as pointed out by Chen et al. (2014). While estimating the risk of exposure at a population level, very few confounders can be taken into account in the analysis and is usually limited to parental smoking, occupation, maternal age and season of conception.

A study by Bentov et al. (2006) indicated an increased rate of major CMs among Arab-Bedouin newborns in the Negev region (Israel), whose mothers live within 20 km from the Negev industrial park (IP) (5.6%), compared to mothers residing in remote localities (4.8%). Variation in major CM by distance from IP was unlikely to be explained by a difference in utilization of health care services or varying distribution of consanguineous marriages.

The study by Bentov et al., however, as well as the rest of the published reports on the association on NO_2 with CMs, usually do not account for the exposures measured in immediate subjects proximity and individual risk factors. In the presented study, we aimed to estimate an independent effect of an ambient exposure to NO_2 emissions on congenital malformations and fetal mortality in the Bedouin population. Our estimates were adjusted to individually recorded risk factors related to household environment and parental background, as well as other pollutants and meteorological conditions.

Assuming an increasing severity of an outcome on a scale from healthy subjects to children with minor anomalies, then – to children with major anomalies and finally, ante-partum death (APD) cases – we hypothesized that mothers to neonates with highest degree of morbidity are more likely to be exposed to maternal risk factors and environmental hazardous surroundings compared to others.

1.3. Arab-Bedouin population

Bedouins in the Negev dwell in either recently established permanent settlements (PS) or traditional tribal settlements (TTS). In the area, the Bedouin population shows an increased tendency to cease practicing a nomadic life style and move from TTS to PS. Residents of PS have an access to modern municipal infrastructure (running water, electricity, telephone service, garbage disposal, sewage treatment, paved roads) and live in modern houses. Residents of TTS live in temporary pre-fabricated housing, shacks, or tents without access to municipal infrastructure. Cooking and heating, in both types of residence, is often provided by open fires, causing exposure to pollution from bonfires. The Negev Bedouin population is of low socio-economic level with a high rate of unemployment (up to 20%) and low educational level (Statistical Yearbook of the Negev, 2010). According to Abu-Saad (2002) smoking is very common among Bedouin men (55–74%). Consanguineous marriages are very frequent within Bedouin population-60%, where 36% of the marriages are between first cousins (Zlotogora et al., 2003).

The Bedouin-Arab population is potentially exposed to the emissions of the local industrial park, which consists of 24 chemical and pharmaceutical facilities, has an incinerator and serves as the national industrial waste disposal site. The list of emissions from the IP chemical plants and evaporation pools includes a variety of aliphatic, aromatic and polycyclic hydrocarbons and a few dozen nonorganic agents and may be a significant source of NO_2 in the area. Traffic, on the other hand, usually attributed to high levels of NO_2 (Grundström and Pleijel, 2014) is negligibly scarce in the study area (Fig. 1).

2. Methods

2.1. Study procedures

We enrolled women of Arab-Bedouin origin at the time they were admitted for a delivery at Obstetric Emergency Department (OED) of the Soroka University Medical Center (SUMC). Upon their arrival women were provided with explanations about the study and were invited to consent to participate in the study. After the delivery the enrolled women were approached by Arab-speaking interviewers with an extensive questionnaire on the personal risk factors and the exact address during the pregnancy. The diagnoses of the neonates and the women were collected from the medical charts filled out at the hospitalization. The data on ambient exposure was further verified based on the residence locations confirmed during the interview.

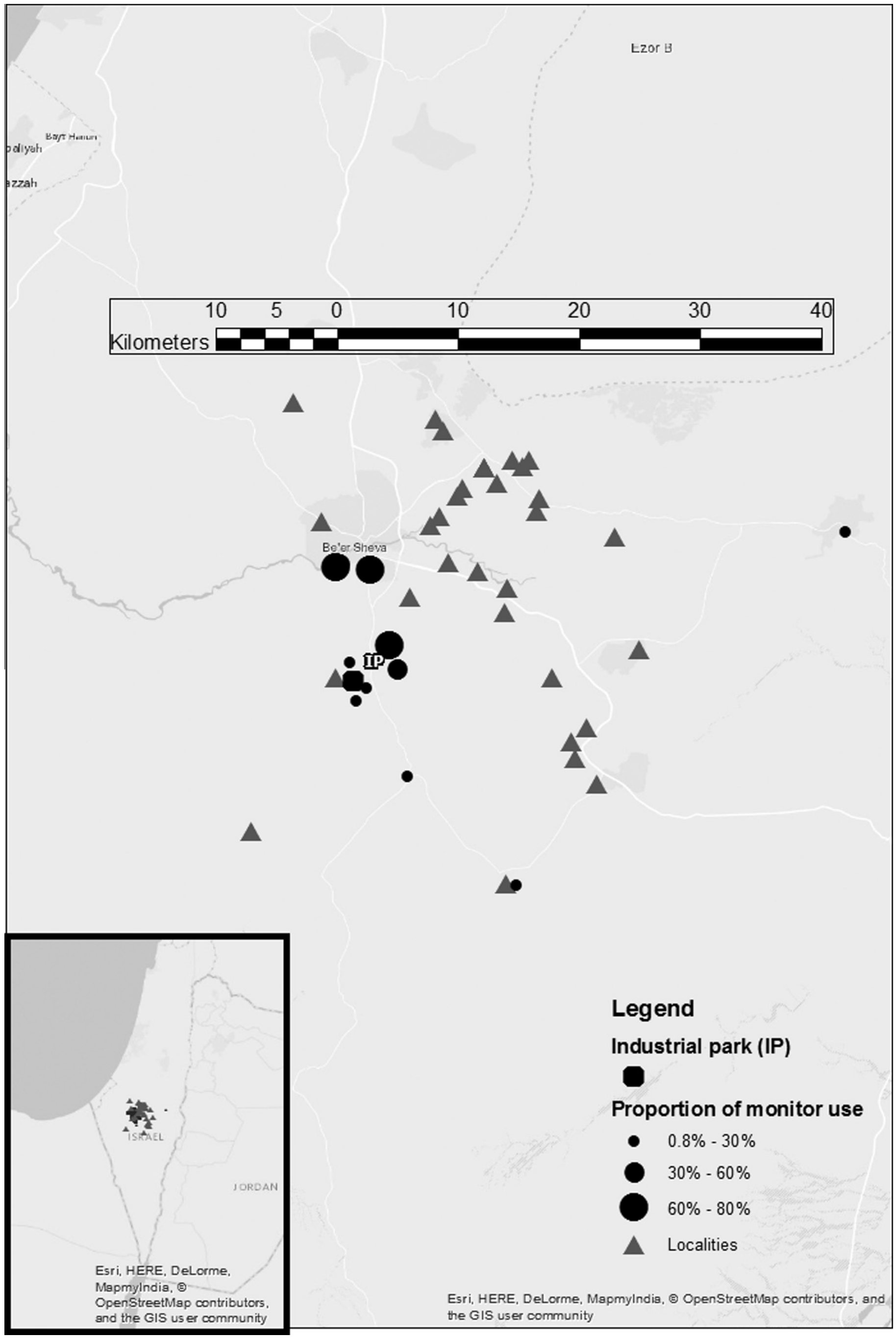

Fig. 1. Map of the study area and monitors' locations.

2.2. Study population

We enrolled women admitted to delivery during December 2010–March 2013. The SUMC is a tertiary medical center serving the population in the Negev region (Southern Israel), with around 15,000 births taking place there annually. To be enrolled in the study the mothers had to be of Arab-Bedouin origin, above 18 years of age and to reside in the area (see specifications of maximal distance of residence from monitoring stations in section "Ambient exposure assessment"). The exclusion criteria were mainly related to potential confounders in association between air pollution and childhood morbidity, e.g. low birth weight and gestational age, which have been shown related to both CM and air pollution exposure (Bobak, 2000; Wang and Pinkerton, 2007).

Thus, mothers to neonates were excluded if: (a) their gestational age was under 22 weeks; (b) birth weight was under 500 g; if (c) delivery took place on the way to the hospital; (d) maternal age < 18 years; or if (e) a result of a multiple pregnancy.

Eligible women were approached by trained Arabic-speaking interviewer in the OED during the day shift on working days.

2.3. Data collection

2.3.1. Parental characteristics

An interview performed after delivery included questions on exact address during pregnancy, socio-economic status, family history of malformations, consanguineous marriages, parental exposure to environmental or occupational hazards, parental health behavior during pregnancy and recent medical problems.

2.3.2. Diagnosis of congenital malformations and other neonatal outcomes

Assessment of neonatal clinical characteristics was available for analysis based on the data collected in an Admission–Transfer–Discharge (ATD) database of the SUMC.

Diagnosis of congenital malformations in a newborn or stillbirth fetus was made upon delivery, or at birth according to International Classification of Diseases (ICD-9) and defined as major or minor depending on a clinical definition of a defect and its impact on the child's health and based on European Surveillance Congenital Anomalies (EUROCAT) (Anomalies, n.d.) as presented in the Appendix (Table 1). The classification into major and minor CMs grouped functionally and etiologically similar malformations. Ante-Partum Death (APD) and Peri-Partum Death (PPD) were classified as the highest level of health outcome severity. The category of major malformations was assigned to non-APD and non-PPD cases with at least one major malformation.

2.4. Exposure assessment during pregnancy

2.4.1. Household hazardous environment

Our questionnaire required description of the household and the list of possible daily exposures of the women. As most of the study participants reported spending most of the time in close proximity of their house – the household represented the main environmental exposure. In more detail, we asked women if they lived in a temporary house (tent or shack) or a permanent building; if the women were disturbed by any environmental factors around their house (e.g., dust – the frequent exposure in the arid and semi-arid area of southern Israel, noise – possibly from a nearby road or power station, waste or sewage – often seen in close proximity of temporary settlements, and transport – as family cars are usually parked close by the house).We inquired on the usual way of heating the house (by open fire, or chimney or any electric device) and cooking (on open fire or in a conventional stove). As traditional cooking is based on open fire – it is prevalent also among the residents in permanent settlements. Finally we asked if the women are using tap water and/or hold it in water containers, possibly exposed to chemical contaminants in its previous usage or were produced out of plastic containing hazardous chemicals. Some Arab-Bedouin families grow plants for their own use around the house, which explains the question on the fertilizer being used in the household and its frequency.

2.4.2. Ambient exposures

The exact addresses during pregnancy were geocoded using the Arcview 3.2 (ESRI, Redlands, CA, USA) geographic information system (GIS). The study area consists of majorly rural settlements and covers approximately 2500 km^2 (Fig. 1). As most of the study subjects resided in small towns or small areas of tribe settlements, the spatial resolution of the address used in the study corresponds to the center of the town/village localities. The address of each participant was further linked to one of the 13 permanent monitoring stations in the study area. Two stations are operated by the Israel Ministry of Environmental protection, 2 stations are operated by the local electrical company and additional (9) are supervised by the Neot-Hovav industrial park located 12 km from Beer-Sheva city (Protection, n.d.). Measurements of pollutants' concentrations, such as NO_2, SO_2, O_3, CO, PM_{10} and $PM_{2.5}$ were reported every 5 min, as well as a daily data on meteorological conditions (temperature, humidity and precipitations). The linkage between the maternal address and a monitoring station depended on the availability of the 6 pollutants mentioned above and limited by the radius of 20 km. An assessment of exposure to a certain pollutant was defined as missing if not available from any of the relevant monitoring stations during the exposure period. All pollutant measurements were averaged over each day and further interpolated to the study areas using the inverse distance weighting method as described by Hwang and Jaakkola (2008) by which every address was assigned a weighted average of the observed values. The weights of the pollutants' concentrations were expressed by a function decreasing with distance to the monitoring station from the participant's residence, specifically:

$$w = 1/d^p$$
with $p > 2$

as mostly commonly used in this field, and d – representing the distance. All the calculated values of pollutants were averaged over a day, week, month and trimester. The potential exposure window times were defined as the first trimester (12 weeks of gestation) and 3 months prior to conception (computed using date of birth and gestational age).

2.5. Statistical analysis

Questionnaire Data were entered using EpiData software and analyzed using the SAS 9.2 package (SAS Institute). Continuous variables were presented as mean ± standard deviation (sd), median, extreme values and were compared using *t*-test, analysis of variance or the Wilcoxon test, depending on the distribution type of the variable. Categorical variables were presented as proportions of non-missing values and compared by Chi-square or Fisher Exact test. Correlations between pollutants and meteorological factors were calculated by Spearman correlation. Trends were estimated by comparison of Row Mean Score difference based on the Cochran–Mantel–Haenszel Statistics or by linear univariate regression. The significance level used in the exploratory analysis was set at 10% due to relatively small sample of patients with outcomes.

Multivariate log-binomial regression was used for adjustment to individual characteristics of the study participants and their delivery. Logistic regression was used in cases when the log-binomial regression did not converge. Analysis of multiple environmental estimates (e.g. collected daily, weekly or by month) utilized generalized estimates equation models for a proper adjustment to clusters of the same subjects. Independent factors used in the model were chosen based on the univariate analysis and significance level below 0.2. The covariates remained in the model if the significance level was below 0.1 or had a potential for confounding associations. Sensitivity analysis was performed with respect to the resolution of exposure estimates, e.g. daily, weekly or over a trimester, as well as for the exposure window, e.g. 3 months prior conception, early pregnancy (up to 8 weeks), weeks 8–13 of gestation, 1st trimester, etc. As a part of the sensitivity analysis, the exposure to air pollutants was tested in single and multiple exposure models. Decision on final presentation of the continuous pollutants in the model, as well as the final list of covariates used for adjustment, was made using the goodness-of-fit measures.

3. Results

3.1. Air pollutants in the study area

The deliveries in the study occurred between December 2011 and April 2013, while the relevant period of exposure including the gestation age and 3 months prior to conception fell on November 2010–April 2013. The median value of daily NO_2 measured in the area during the exposure period was 6.78 ppb (IQR: 5.00–9.29), with maximal value reaching 46.2 ppb. The study area was featured by relatively high Ozone levels – the median value O_3 in the study area was 15.08 (IQR: 8.31–19.83) and hot weather – with maximal average temperature reaching 32.2 °C and minimal – minus 9.8 °C (Table 1a). The Spearman correlations between different air pollutants varied between 0.29 and 0.41 (Table 1b).

3.2. Study population

We enrolled 1024 women during their admission to the delivery room of the SUMC. Interviews were performed in 80.5% (824/1024) of the women enrolled in the study. Women, for whom the questionnaire was not collected, were usually discharged within 48 h after delivery or frequently discharged during weekends. They were not different from the rest of the population in maternal and neonatal characteristics.

Congenital malformations or Ante-Partum Death occurred in 8.0% (82/1024) of neonates – 1% (10) were APD cases, 4% (41) were diagnosed with major and 3% (31) – with only minor malformation (Table 2). There were no PPD cases enrolled in the study. The women in the study population were on average 28.0 ± 6.0 years old (median 27.0), with 25.7% giving birth for the first time and 26.8% at their 6th+ delivery. Over 25% of the study sample resided in shacks or tents (TTS). Around half of the study population reported being in a consanguineous marriage (47.9%), and this factor was not different for mothers to healthy children and women in CM and APD groups (p-value = 0.898). Paternal smoking was reported for approximately 90% of the sample, with comparable rates between the groups. Lack of perinatal care was frequently reported in the study population (10.3%), and occurred more frequently among women in CM and APD groups (up to 50% in the APD group).

Smoking was reported by 9 women (0.9%). Most of the study participants were unemployed and only 51 reported working outside their house (6.2%). Only 6 women (0.8%) reported working out at least once a week. Women reported spending most of their time in close proximity of their house (data not shown).

3.3. Exposure to parental and environmental risk factors

As severity of the birth outcome increased, mothers were more exposed to juvenile diabetes (p-value = 0.017) and gestational diabetes (p-value = 0.084). Besides these factors, no dose–response association was observed in other maternal parameters.

Household environmental factors varied between healthy neonates and CM and APD cases, however many of the differences did not reach the significance level of 0.10 (Table 3). Residence in TTS (shack or tents) was not found associated with the study outcome, although the usage of open fire for heating and cooking was more likely in these households compared to PS (45.9% vs 4.7%, p-value < 0.001 and 30.6% vs 4.1%, p-value < 0.001, respectively). Additionally, the most of the factors prevalent in the group with minor malformations were different from factors featuring the group of major malformations, or APD. For instance, mothers in the group with minor CM reported dust (100%, 23/23, p-value = 0.080) and complained on family cars parked close to their house (65.2%, p-value = 0.065) more frequently than mothers to healthy newborns. In contrast, mothers to newborns with major CMs reported being more exposed to noise (23.7%, p-value = 0.056), using open fire for heating (26.3%, p-value = 0.046), cooking (21.1%, p-value = 0.032) and using chemical fertilizers (15.8%, p-value = 0.081) compared to the healthy population. Of note, some risk factors were less present in the group of the APD cases compared to less severe outcomes or healthy subjects, e.g. using of water containers (p-value = 0.044), or paternal exposure to noise or vibration at work (p-value = 0.078 and p-value = 0.026, respectively).

Nevertheless, the trend of increased exposure coming along with an increased severity was observed for some of the factors,

Table 1a
Pollutants' concentrations and meteorological factors during the study period.

Daily average	Mean ± SD	Median (25th, 75th percentile)	Min; Max	IQR	National Israeli annual standards set for 2015[a]	Current annual WHO standards[b]
NO_2, ppb	7.47 ± 3.52	6.78 (5.00; 9.29)	0.00; 46.18	4.29	40 μg/m^3 (or 21.3 ppb[b])	40 μg/m^3 (or 21.3 ppb[b])
SO_2, ppb	0.69 ± 0.56	0.58 (0.34; 0.94)	0.00; 16.15	0.60	50 μg/m^{3}daily (or 19,1 ppb[b])	20 μg/m^3 daily (or 7.6 ppb)
O_3, ppb	14.20 ± 8.53	15.08 (8.31; 19.83)	0.01; 48.44	11.52	140 μg/m^3 in 8 h (or 71.4 ppb)	100 μg/m^3 in 8 h (or 51.0 ppb)
CO, μg/m^3	0.03 ± 0.06	0.02 (0.01; 0.03)	0.02; 1.01	0.02	na	na
PM10, μg/m^3	17.16 ± 21.13	11.44 (8.41; 17.14)	2.24; 467.29	8.74	130	50 (daily)
PM2.5, μg/m^3	6.79 ± 5.82	5.52 (4.17; 7.49)	0.94; 124.67	3.32	37.5	25 (daily)
Temperature, °C	17.94 ± 4.97	18.23 (15.97; 20.61)	−9.76; 32.31	4.65	–	–
Humidity, %	16.92 ± 7.57	15.28 (13.46; 17.67)	2.44; 62.81	4.22	–	–
Rain, mm	0.0003 ± 0.0016	0.0000 (0.0000; 0.0000)	0.0000; 0.0490	0.0000	–	–

[a] Environmental Health in Israel 2014. Berman T., Karakis I., Reicher S., 2014. Israel Ministry of Health and Environment and Health Fund. http://www.dmag.co.il/pub/ehf/ehf2014/index.html, last accessed on March, 2015.
[b] Was divided by the conversion coefficient depending on the height above the ground level (http://www.svivaaqm.net).

Table 1b
Spearman correlations of pollutants and meteorological factors.[a]

	NO_2, ppb	SO_2, ppb	O_3, ppb	CO, μg/m^3	PM_{10}, μg/m^3	$PM_{2.5}$, μg/m^3	Temperature, °C	Humidity, %	Rain, mm
NO_2, ppb									
SO_2, ppb	0.41								
O_3, ppb	−0.29	0.24							
CO, μg/m^3	0.05	0.05	0.15						
PM_{10}, μg/m^3	0.09	0.24	0.12	0.08					
$PM_{2.5}$, μg/m^3	0.13	0.17	0.16	0.08	0.84				
Temperature, °C	0.15	−0.10	−0.37	0.06	0.07	0.04			
Humidity, %	−0.14	−0.09	0.33	0.25	0.03	0.22	−0.05		
Rain, mm	−0.22	−0.23	−0.06	0.02	−0.14	−0.17	−0.35	0.21	

[a] All the correlations were found statistically significant at p-value < 0.001.

Table 2
Demographical and clinical characteristics of women.

Patients characteristics	Healthy Patients $N = 942$	Patients with minor anomalies		Patients with major anomalies		APD cases		p-value for trend
		$N = 31$	p-value in comparison with healthy patients	$N = 41$	p-value in comparison with healthy patients	$N = 10$	p-value in comparison with healthy patients	
Maternal age at delivery, years								
Mean ± SD (n)	28.0 ± 6.0 (942)	27.7 ± 5.9 (31)	0.781	28.6 ± 6.8 (41)	0.581	27.6 ± 4.1 (10)	0.772	0.813
Median	27.0	26.0		27.0		28.5		
Parity								
1st delivery	25.8% (243/942)	25.8% (8/31)	0.869	24.4% (10/41)	0.739	20.0% (2/10)	0.545	0.945
2–5 Deliveries	47.3% (446/942)	45.2% (14/31)		46.3% (19/41)		70.0% (7/10)		
6+ Deliveries	26.8% (252/942)	29.0% (9/31)		29.3% (12/41)		10.0% (1/10)		
History of repeated abortions	5.9% (56/942)	3.2% (1/31)	1.000	12.2% (5/41)	0.104	0.0% (0/10)	1.000	0.547
Gestational diabetes	3.2% (30/942)	3.2% (1/31)	0.283	7.3% (3/41)	0.789	10.0% (1/10)	0.283	0.084
Juvenile diabetes	0.1% (1/942)	0.0% (0/31)	1.000	2.4% (1/41)	0.082	0.0% (0/10)	1.000	0.017
Mild/Moderate preeclampsia	3.0% (28/942)	0.0% (0/31)	1.000	4.9% (2/41)	0.359	10.0% (1/10)	0.267	0.339
Severe preeclampsia	0.8% (8/942)	3.2% (1/31)	0.254	0.0% (0/41)	1.000	10.0% (1/10)	0.091	0.104
Lack of prenatal care	10.3% (97/942)	3.2% (1/31)	0.198	4.9% (2/41)	0.259	50.0% (5/10)	<0.001	0.280
Consanguineous marriage	47.8% (346/724)	54.5% (12/22)	0.532	41.7% (15/36)	0.473	62.5% (5/8)	0.408	0.942
1st degree	72.8% (238/327)	83.3% (10/12)	0.869	57.1% (8/14)	0.269	40.0% (2/5)	0.022	0.290
2nd degree	15.9% (52/327)	8.3% (1/12)		35.7% (5/14)		0.0% (0/5)		
Distant relatives	11.3% (37/327)	8.3% (1/12)		7.1% (1/14)		60.0% (3/5)		
Maternal smoking in pregnancy	0.1% (1/754)	0.0% (0/23)	1.000	0.0% (0/38)	1.000	0.0% (0/9)	1.000	0.775
Paternal smoking	88.8% (629/708)	81.8% (18/22)	0.307	94.3% (33/35)	0.313	87.5% (7/8)	1.000	0.681

i.e. exposure to noise, open fire for heating and cooking (p-value = 0.027, p-value = 0.020 and p-value = 0.050, respectively).

In general, the cases of APD were typically preterm born (p-value < 0.001), diagnosed by small-for-gestational age (p-value < 0.001) weighting significantly less than the rest of the sample (<0.001) (Table 4).

From the univariate analysis showing the divergent associations between environment and the malformations, it became evident that an effect of the ambient pollution exposure should be inspected separately for minor and major CMs and the APD cases.

Based on a multivariate analysis (Table 5), APD cases were more explained by maternal health conditions and socio-economic status. Major anomalies were associated with maternal morbidity (juvenile diabetes, RR = 9.97, p-value = 0.002) and household factors (i.e. using open fire for heating, RR = 2.00, p-value = 0.049). NO_2 fluctuations had an impact only on minor malformations, with an RR = 2.68 (p-value = 0.029) for an exposure to NO_2 levels above 2 IQR averaged over the 1st trimester of pregnancy. Increase in IQR increments of O_3 was adversely associated with minor CM with RR = 1.63, p-value = 0.097. Analysis of a window period of exposure to NO_2 showed no significant associations with the pre-conception window or for early pregnancy window, as well as for estimates of exposure collected with higher resolution of days or weeks.

Other pollutants, such as Ozone (O_3) and Particulate Matter (PM), were not associated with the malformations or fetal mortality in single and multiple air-pollutant models at different exposure windows during gestation, after accounting for household environment and clinical background.

4. Discussion

We aimed to estimate an association between the exposure to NO_2 emissions during pregnancy and birth outcomes: CMs at birth and/or fetal mortality. In the analysis we were able to account for an immediate environment of a pregnant woman, in addition to an extensive list of clinical conditions and demographical factors which could be attributed to the outcome at study.

A close inspection of data revealed unique features of the study groups, which precluded combined analysis of the study outcomes, which would meaninglessly average the magnitude of the associations. For instance, *APD cases* were more likely to occur in women with severe preeclampsia or diabetes, while half of them were lacking a prenatal care; the group with *major malformations* was characterized by chronic maternal morbidity (history of repeated abortions and juvenile diabetes) and frequently reported exposure to household hazardous factors like noise, transport, usage of open fire and chemical fertilizer; the health status and immediate residential environment of mothers in the group with *minor CMs* was similar to the healthy population.

In our study only minor CMs were found associated with ambient air pollution, specifically with NO_2, whereas exposure was defined as NO_2 level above 2 IQR averaged over the 1st trimester of pregnancy – relatively high for the region and sustained during the period of the first trimester. The finding is in line with previous reports indicating an adverse effect of exposure to NOx, and specifically to NO_2, on malformations frequently of cardiovascular system (Chen et al., 2014; Farhi et al., 2014; Vrijheid et al., 2011). In our study the effect was shown for a group of minor anomalies, rather than for a specific malformation. Furthermore, the relatively similar evidence of an effect was obtained in the study population radically different from other reports, both – in their genetic origin and socio-economic status. This fact reinforces the conclusion on the adverse effect of NO_2, since obtained for diverse population strata.

Table 3
Household environment of the study population.

Household factors	Healthy patients $N = 942$ (754[a])	Patients with minor anomalies		Patients with major anomalies		APD cases		*p*-value for trend
		$N = 31$ (23[a])	*p*-value vs. healthy patients	$N = 41$ (38[a])	*p*-value vs. healthy patients	$N = 10$ (9[a])	*p*-value vs. healthy patients	
Resides in tent or shack	25.1% (189/754)	30.4% (7/23)	0.559	34.2% (13/38)	0.207	0.0% (0/9)	0.084	0.877
Employed	6.6% (50/754)	4.3% (1/23)	0.663	5.3% (2/38)	0.740	11.1% (1/9)	0.465	0.946
Disturbing environmental factors								
Dust	88.2% (665/754)	100.0% (23/23)	0.080	86.8% (33/38)	0.801	77.8% (7/9)	0.338	0.789
Noise	12.9% (97/754)	21.7% (5/23)	0.215	23.7% (9/38)	0.056	22.2% (2/9)	0.407	0.027
Waste	12.9% (97/754)	8.7% (2/23)	0.555	10.5% (4/38)	0.673	11.1% (1/9)	0.876	0.570
Sewage	28.6% (216/754)	43.5% (10/23)	0.123	13.2% (5/38)	0.038	22.2% (2/9)	0.671	0.165
Transport	45.8% (345/754)	65.2% (15/23)	0.065	31.6% (12/38)	0.087	33.3% (3/9)	0.457	0.223
Heating by open fire	14.5% (109/754)	13.0% (3/23)	0.849	26.3% (10/38)	0.046	33.3% (3/9)	0.112	0.020
Cooking on open fire (usually)	10.1% (76/754)	17.4% (4/23)	0.256	21.1% (8/38)	0.032	11.1% (1/9)	1.000	0.050
Using chemical fertilizer	7.8% (59/754)	8.7% (2/23)	0.879	15.8% (6/38)	0.081	0.0% (0/9)	1.000	0.372
Using water containers	81.8% (617/754)	91.3% (21/23)	0.243	78.9% (30/38)	0.654	55.6% (5/9)	0.044	0.246
Husband employed	81.6% (615/754)	87.0% (20/23)	0.510	81.6% (31/38)	0.998	66.7% (6/9)	0.254	0.644
Paternal exposure at work to adverse factors								
Noise	62.1% (468/754)	73.9% (17/23)	0.248	57.9% (22/38)	0.605	33.3% (3/9)	0.078	0.286
Vibration	48.5% (366/754)	56.5% (13/23)	0.451	36.8% (14/38)	0.159	11.1% (1/9)	0.026	0.035
Excessive Heat	8.5% (64/754)	13.0% (3/23)	0.444	13.2% (5/38)	0.320	0.0% (0/9)	1.000	0.647

[a] Verified based on the questionnaire.

It is noteworthy that the exposure period found associated with minor malformations – an outcome with least genetic burden and therefore more sensitive to external factors – was in line with previously defined exposure period relevant for environmental factors (3–8 weeks). (Vrijheid et al., 2011)

The possible pathological pathway of its impact on minor CM may involve an oxidative stress (Karakis et al., 2014) and depend on toxicokinetics and toxicodynamics of NO_2, and its capability of crossing the placental barriers during the sensitive period of the fetus development (Proietti et al., 2013). It is believed that the efficacy of the barriers is influenced by inflammatory processes or a compound effect of different biological processes, which decrease the barriers' efficacy.

The two most prevalent minor malformations in our study were minor congenital malformations in genital organs, e.g. hypospadias, and anomalies of limbs, e.g. polydactyly of fingers or toes. Noteworthy, these types of anomalies were the ones found or assumed to be associated with environmental factors by other researchers. As pointed out by Marrocco et al. (2015) in a review of risk factors for hypospadias, this anomaly as an isolated minor birth defect, may be a consequence of occupational exposure of the parents (e.g., mothers working in leather industry) or use of cosmetics, or fathers' occupation in vehicle mechanics or manufacturers, police officers or fire fighters, or paternal exposure to dust from grinding metals. It has been demonstrated that some gene polymorphisms are associated with an increased risk of hypospadias, and some polymorphisms may cause hypospadias only occasionally. The authors suggest that this phenomenon can be partially explained by an interaction between genotype and environment, i.e. an epigenetic mechanism contributing to this condition.

Polydactyly, another frequent anomaly diagnosed in our study, has been already noted as the one associated with environment in a retrospective cohort study based on Washington State birth records for the years 1980–1993 (Engel et al., 2000). The authors showed that maternal occupational exposure to agricultural chemicals possibly contributed to the risk of giving birth to a child with limb defects including syndactyly, polydactyly, adactyly, and other limb reductions defects.

We did not find an independent effect of NO_2 on major malformations, similarly to few other studies (Lin et al., 2014; Marshall et al., 2010), which can be explained by a possibly different etiology of major malformations, mostly impacted from household environment and maternal morbidity. However, our findings might be biased at some extent, due to exclusion of neonates with very low birth weight (<500 g) and/or gestational age under 22 weeks, who would potentially contribute to the pool of neonates with major malformations. In addition, we could not assess the window period of exposure to household, as women were exposed to the household risk factors during the entire pregnancy and also before conception.

Interaction between genetic factors and air pollutants may be relevant to specific types of major CM. The relatively small number of newborns with the different types of major CM in our study precluded from a more extensive analysis of a possible attributable risk of major CM following an NO_2 exposure.

The levels of NO_2 emissions were not high (median 6.78 ppb (12.4 $\mu g/m^3$), IQR (in ppb): 5.00–9.29) and fall below the standard levels of this pollutant established in Israel and by WHO (Berman et al., 2014). This might be explained by a very low density of motorized traffic in the study area, considered to be one of the main sources of NO_2 exposure. Detecting an independent adverse effect even for low exposure levels, implies that even the low levels of NO_2 emission might jeopardize health, especially if assessed at a population-based level, independently of socio-economic status (SES).

Table 4
Newborn characteristics in the study population.

Patients characteristics	Healthy patients $N = 942$	Patients with minor anomalies		Patients with major anomalies		APD cases		*p*-value for trend
		$N = 31$	*p*-value vs. healthy patients	$N = 41$	*p*-value vs. healthy patients	$N = 10$	*p*-value vs. healthy patients	
Male gender	51.7% (487/942)	64.5% (20/31)	0.160	56.1% (23/41)	0.581	30.0% (3/10)	0.172	0.944
LGA	5.8% (54/924)	16.1% (5/31)	0.017	14.6% (6/41)	0.020	0.0% (0/10)	1.000	0.053
SGA	3.5% (32/924)	9.7% (3/31)	0.065	9.8% (4/41)	0.034	40.0% (4/10)	<0.001	<0.001
Gestational age, days								
Mean ± SD (*n*)	274.5 ± 13.7(941)	267.2 ± 28.3 (31)	0.163	270.3 ± 20.9 (41)	0.209	229.0 ± 27.8 (10)	<0.001	<0.001
Median	277.0	273.0		273.0		213.50		
Birth weight, g								
Mean ± SD (*n*)	3206 ± 525 (942)	3065 ± 926 (31)	0.406	3060 ± 846 (41)	0.279	1585 ± 951(10)	<0.001	<0.001
Median	3240	3275		3025		1200		
Preterm Delivery	5.0% (47/942)	9.7% (3/31)	0.245	17.1% (7/41)	0.001	80.0% (8/10)	<0.001	<0.001

Table 5
An effect of NO_2 exposure on the birth outcomes.

Independent factor	Minor CMs		Major CMs		APD cases	
	RR[a] (90% CI)	*p*-value	RR[a](90% CI)	*p*-value	OR[b](90% CI)	*p*-value
Lack of prenatal care					9.33 (3.50; 24.88)	<0.001
Preeclampsia					4.51 (1.18; 17.29)	0.065
Gestational diabetes					4.19 (0.67; 26.07)	0.197
Juvenile diabetes			9.97 (2.98; 33.40)	0.002		
Heating by open fire			2.00 (1.12; 3.57)	0.049		
O_3, IQR (ppb)	1.63 (1.00; 2.63)	0.097				
NO_2 > 2 IQR (>8.58 ppb or 16.13 μg/m^3) **during the 1st trimester**	**2.68 (1.27; 5.64)**	**0.029**	**1.43 (0.81; 2.53)**	**0.299**	**2.15 (0.71; 6.54)**	**0.256**

Text and numbers in bold show the results for the main exposure of interest in the study – NO_2 pollution.
[a] Based on results of a log-binomial regression.
[b] Based on results of a logistic regression.

Furthermore, the effect assessed in the current investigation was limited to the child morbidity recorded at birth, while it might be of higher importance for future child development. Indications of this long-term effect can be found in the recent report from the 6 European birth cohorts study, which showed a delayed psychomotor development during childhood following exposure to NO_2 during gestation. (Guxens et al., 2014)

The main factor associated with major CMs is possibly the compounds of the household environment, which might be comprised of NO_2, produced by certain fuels used for cooking.

The study methodology has its *limitations*.

- Even though we consequently enrolled every woman coming for a delivery in our hospital, the study cannot be labeled as population-based, as we could not collect information on the household risk factors for around 20% of the questionnaires. In spite of similar maternal and neonatal characteristics between the interviewed participants and those who did not fill out the questionnaire, we cannot expect this similarity in their household exposure or proximity to IP.
- The questionnaire used for description of the women's household is a result of a field study by the members of our team conducted in 2004 and collaboration with the representatives of Arab-Bedouin community (Gorodischer et al., 1995; Naggan et al., 1991) therefore was expected to reflect the actual hazardous factors in the environment of the Arab-Bedouin women. However, we were not able to test for validity of measuring these factors. If in fact the validity is low, the household factors would be misclassified, which would decrease the risk estimates in the study.
- The place of residence used in geocoding could not be verified to a resolution of an exact address for population living in traditional tribal settlements, but frequently within a vicinity of around 500 m. This uncertainty in exposure assessment, in addition to extrapolation method of air pollution measurements of monitoring stations, might have resulted in a non-differential misclassification bias in the exposure, and invariably – in an underestimated effect magnitude.
- An assessment of PM_{10} and $PM_{2.5}$ was performed by only one monitor out of the 13 used in the study, and therefore could not be reliably extrapolated. This was especially critical, when the distance from the participants' residence was close to 20 km, making these populations especially prone to underestimation of the real effects. As a result, our analyses showed no effect between exposure to particles and malformations, as opposed to few other studies indicating this association (Agay-Shay et al., 2013; Padula et al., 2013).
- We could not account for abortions, frequently associated with congenital malformations, as this information was not available in the current analysis.

5. Conclusions

The question of whether or not indoor hazardous exposures or outdoor air pollution contribute to independent or joint effect of air pollution on the risk of congenital malformations is yet not resolved and the current study may contribute to this debate.

Our investigation showed an independent effect of NO_2 emissions on minor congenital malformations, e.g. hypospadias and polydactyly of fingers or toes, whereas major malformations depended mostly on the household environment and APD – were mainly affected by maternal morbidity.

As our findings are consistent with the other studies reporting an adverse effect for relatively low levels of NO_2, the established standards should be possibly revised. An intervention of this kind might serve as an effective primary prevention of childhood morbidity and neonatal mortality.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.06.091.

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