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The Effect of Nitric Oxide Pollution on Oxidative Stress in Pregnant Women Living in Durban, South Africa

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Abstract The purpose of the study was to evaluate the effect nitric oxide (NO_x) pollution had on maternal serum 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) levels and neonatal outcomes in pregnant women living in Durban, South Africa (SA). Women, in their third trimester with singleton pregnancies, were recruited from the heavily industrialised south ($n = 225$) and less industrialised north ($n = 152$). Biomarker levels of serum 8-OHdG concentrations were analysed, and the women were genotyped for glutathione-S-transferases pi 1 (GSTP1) and glutathione-S-transferases mu 1 (GSTM1) polymorphisms. The level of NO_x pollution in the two regions was determined by using land use regression modelling. The serum 8-OHdG was shown to correlate significantly with NO_x levels; this relationship was strengthened in the south ($p < 0.05$). This relationship was still observed after adjusting for maternal characteristics. GSTP1 was significantly associated with the south region, where the variant (AG+GG) genotype was associated with increased 8-OHdG levels as a result of NO_x exposure ($p < 0.05$). GSTM1 null genotype was associated with a positive correlation between NO_x and 8-OHdG levels ($p < 0.05$). NO_x levels were found marginally to reduce gestational age ($p < 0.05$) with mothers carrying male neonates. Variant GSTP1 and living in the north were factors that contributed to gestational age reduction ($p < 0.05$). Our study demonstrated that NO_x exposure resulted in increased 8-OHdG levels in pregnant women living in Durban, SA, which led to gestational age reduction. The GSTP1 variant increased susceptibility of individuals to harmful effects of NO_x.

Durban, South Africa (SA) is a rapidly developing city with increased road traffic and industrial development in close proximity to residential areas. Durban is divided into a heavily industrial south region (also known as the South Durban Industrial Basin) and a less industrial north region; however, both regions are undergoing increased urbanisation and development. This is of major health concern due to increased levels of ambient air pollution (AAP) in both of these areas (Naidoo et al. 2013). Ambient air pollution and associated oxidative stress has been implicated in many pathological conditions, including cancer, asthma, acute respiratory infections, and adverse birth outcomes (Šrám et al. 2005; Kampa and Castanas 2008; Wu et al. 2009; Fleischer et al. 2014; Chen et al. 2015; Moorthy et al. 2015). Pregnant females are highly susceptible to oxidative stress due to increased basal oxygen and changes in energy consumption during placental and foetal development. Infants in utero are highly susceptible to the harmful effects of AAP; exposure has been associated with low birth weight (LBW), inter-utero growth restrictions, preterm birth (PTB), and preeclampsia (Glinianaia et al. 2004; Negi et al. 2012a; Proietti et al. 2013). High levels of pollutants, including sulphur dioxide, carbon monoxide (CO), particulate matter (PM), and nitric oxide (NO_x), have been reported (Kistnasamy et al. 2008). Oxides of nitrogen are of particular interest, because they are by-products of vehicle combustion,

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smoking, and cooking with gas and have a nitrogen-centred free radical. They interact directly with macromolecules (DNA, lipids, and proteins) and often result in a cascade of radical production and compromised cellular antioxidant function (Kelly 2003).

The increase in reactive oxygen species (ROS) and decrease in antioxidants lead to oxidative stress. The premutagenic deoxyguanosine DNA lesion is highly susceptible to oxidative stress, resulting in the hydroxylation of the guanosine residue at position C_8. This produces oxidative 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) and acts as a biomarker for oxidative DNA damage (Kim et al. 2005).

Glutathione-S-transferases (GST) are a family of phase II isoenzymes that protect against oxidative stress. This occurs through the conjugation of glutathione to electrophilic species that can react with and form protein or DNA adducts (Romieu et al. 2006). Two common and highly polymorphic antioxidants that have been implicated in health effects in response to chemical exposure are GST mu (M) and pi (P) 1(Wong et al. 2008). Common single-nucleotide polymorphisms (SNP) in the GSTM1 GSTP1 affect the enzymatic activity of GST and have been associated with AAP-associated health effects and increased oxidative stress (Romieu et al. 2006; Mustafa et al. 2010). A homozygous deletion in the GSTM1 gene results in the complete absence of enzyme activity (Seidegård et al. 1988; Xu et al. 1998), whilst a single nucleotide substitution of adenine (A) for guanine (G) results in the amino acid change of isoleucine for valine in GSTP1, at codon 105. This codon forms part of the active site of the enzyme; therefore, this change results in the alteration of the substrate-specificity of the enzymes' binding site (Johanssona et al. 1998; Moyer et al. 2008). These polymorphisms have been associated with adverse birth outcomes due to AAP, such as increased risk of PTB (Sram et al. 2006; Mustafa et al. 2010; Slama et al. 2010).

To investigate a possible correlation between NO_x and maternal oxidative stress, this study measured serum 8-OHdG in third trimester bloods of women living in the south and north regions of Durban. To determine whether NO_x and maternal oxidative stress impacted neonatal birth weight (BW) and gestational age (GA), associations were investigated between NO_x, 8-OHdG adduct concentration, and neonatal BW and GA. Multivariate analyses were performed to assess whether the relationship held when confounding factors were controlled. The study also investigated the prevalence of GST polymorphisms in South African women and their potential to affect the susceptibility of mothers, who were exposed to AAP, to oxidative stress.

Methodology

Study Population

The Mother and Child in the Environment (MACE) longitudinal cohort study recruited pregnant women from public sector anti-natal clinics in the heavily polluted south Durban ($n = 225$). A comparison sample of women with similar socioeconomic statuses was recruited from the less-industrialised north Durban ($n = 152$). The women were residents of the geographical area for the full duration of the pregnancy. Women with hypertension, multiple pregnancies, diabetes, placenta previa, genital tract infections, and other complications that result in adverse growth effects were excluded from the study.

The study was approved by the Biomedical Research Ethics Committee of the University of Kwa-Zulu Natal (BF263/12). Informed consent from study participants was obtained.

Nitric Oxide Levels

Land use regression modelling was used to determine the exposure levels of NO_x for individual study participants. The method was developed following the ESCAPE approach (Beelen and Hoek 2010). Model development entailed measurement of NO_x at selected locations and regressing these measurements against site-specific a priori defined (i.e., direction of effect) geographic predictors, such as road length, land use types, topography, population, and housing density, in a multivariate regression model. NO_x measurements were conducted over two, 2-week periods during mid-winter and mid-summer using Ogawa samplers, which were deployed at 40 randomly selected sites in the north and south Durban areas. The sampling periods selected are representative of the two distinct seasons to occur in Durban (Tyson and Preston-Whyte 2004; Tularam and Ramsay 2013), thus accounting for seasonal variation. The adjusted NO_x measurements were then used in model development of which the regression coefficients were applied to each participant. This determined individual NO_x exposure levels for each study participant (Muttoo et al. 2017).

Collection and Preparation of Samples

Third-trimester blood was collected from pregnant women during the period between 2013 and 2015. The serum and whole blood was stored (− 80 °C) for analyses. Isolated serum was used for 8-OHdG adduct quantification and whole blood for genotyping of polymorphisms.

Polymorphisms of GSTM1 and GSTP1

DNA was isolated from whole blood using the Qiagen FlexiGene® DNA Kit (as per manufacturer's instructions). Isolated DNA was quantified using the Nanodrop 2000 spectrophotometer and standardised to 10 ng/µl.

Differentiation polymerase chain reaction (PCR) was performed to assess the GSTM1 polymorphism ($n = 372$), using β-globin as a reference gene. GSTM1 (215 base pair (bp)) and *β*-globin (268 bp) PCR products were amplified using 40 pmol of GSTM1 and *β*-globin primers (Inqaba Biotech, SA; Table 1) in a 30-µl reaction (1 × Green GoTaq Flexi buffer, 1.25 mM $MgCl_2$, 0.5 U GoTaq DNA polymerase (Promega), 200 µM of each deoxyribonucleotide (dNTP), 10 ng DNA template). Initial denaturation was applied (96 °C, 5 min), followed by 30 cycles of denaturation (96 °C, 30 s), annealing (57 °C, 30 s), and extension (72 °C, 30 s), concluding with final extension occurring at 72 °C for 5 min. Amplification products were electrophoresed on agarose gel (4%, 2 µl GelRed) and visualised on the Bio-Rad ChemiDoc™ XRS + System, using the Image Lab™ software. The presence of a single 268 bp is indicative of homozygous null genotype, and the presence of 218 bp indicates either a homozygous positive or heterozygous (wild-type (wt)) genotype.

PCR restriction fragment length polymorphism (RFLP) was used to investigate GSTP1 genotypes ($n = 377$). A 176-bp PCR product was amplified using 10 pmol of GSTP1 primers (Inqaba Biotech, SA; Table 1) in a 25-µl reaction (1 × Green GoTaq Flexi buffer, 1.5 mM $MgCl_2$, 0.5 U GoTaq DNA polymerase (Promega), 200 µM of each dNTP, 10 ng DNA template). Initial denaturation was applied (96 °C, 5 min), followed by 30 cycles of denaturation (96 °C, 30 s), annealing (55 °C, 30 s), and extension (72 °C, 30 s), concluding with final extension (72 °C, 5 min). Amplification products were electrophoresed on agarose gel (3%, 1 µl GelRed) and visualised. The PCR amplicon underwent restriction endonuclease digestion to determine the presence of the polymorphic restriction site. An overnight digestion (37 °C) was performed in 28-µl reactions: 10 µl PCR product and 18 µl (18 µl nuclease-free water, 2 µl 10 × Buffer Tango, 1 µl *Alw261 (BsmA1)*; Thermo Fisher Scientific). Amplicons completely digested into two restriction fragments (91 bp and 85 bp) were homozygous for G_{105} allele. The restriction fragments were electrophoresed on an agarose gel (3%, 2 µl GelRed) and visualised on the Bio-Rad ChemiDoc™ XRS + System, using the Image Lab™ software.

Table 1 Primer sequences for PCR

Primer	Primer sequence
GSTM1 forward	5′-GAACTCCCTGAAAAGCTAAAGC-3′
GSTM1 reverse	5′-GTTGGGCTCAAATATACGGTGG-3′
β-globin forward	5′-CAACTTCATCCACGTTCACC-3′
β-globin reverse	5′-GAAGAGCCAAGGACAGGTAC-3′
GSTP1 forward	5′-ACCCCAGGGCTCTATGGGAA-3′
GSTP1 reverse	5′-TGAGGGCACAAGAAGCCCCT-3′

Determination of 8-Hydroxydeoxyguanosine

The amount of serum 8-OHdG adduct was determined using a competitive OxiSelect™ Oxidative DNA Damage ELISA Kit (Cell Biolabs, Inc.), with a sensitivity range of 100 $pgmL^{-1}$–20 $ngmL^{-1}$. Non-haemolysed serum samples ($n = 166$) were chosen at random by region from the study population; diluted (1:5) in assay diluent and assayed as per manufacturer's instructions. A 1:5 dilution was recommended as per manufacturer protocol. An initial experiment was performed using this dilution, and the levels of 8-OHdG fell within the range of the 8-OHdG standards. Therefore, this dilution was used for all subsequent analysis. A standard curve was prepared by using known concentrations of 8-OHdG standards (0–20 ng/ml). The logarithmic equation for the best fit line was used to extrapolate the concentrations of the unknown samples. Each 96-well plate that was used had its own set of standards to ensure that human-error and variation between experiments was accounted for. The final concentration of 8-OHdG adduct was the anti-log multiplied by the dilution factor of 5.

Statistical Analysis

Statistical analyses were performed by using GraphPad Prism V5 Software Package (GraphPad Software Inc., San Diego, CA). Comparisons between north and south groups for maternal and neonatal characteristics, atmospheric NO_x levels, and maternal serum 8-OHdG adduct concentrations were determined by using the Student *t* test. Data were log transformed to ensure normalcy and to allow graphical representation of the data. Correlations among atmospheric NO_x, maternal serum 8-OHdG, and neonatal BW and neonatal GA, and genotypes of GSTM1 and GSTP1 were performed by using the nonparametric Spearman correlation. Chi square and Fischer's exact tests were used to test the significant difference in the prevalence of GSTM1 and GSTP1 genotypes between the north and south groups. One-way ANOVA was performed to determine the level of difference for maternal serum 8-OHdG adduct concentrations among the genotypes of GSTM1 and GSTP1. All statistical tests were two-sided. Multivariate linear regression was used to determine whether the relationship between atmospheric NO_x and maternal serum 8-OHdG was affected by potential confounders, namely: maternal age, maternal body mass index (BMI), HIV status, area, maternal systolic and diastolic blood pressure (BP), and haemoglobin (Hb) levels.

The relationship between neonatal GA and atmospheric NO_x and maternal serum 8-OHdG also was determined by using linear regression, and potential confounders were controlled. The linear regression analyses were performed using STATA version 13.1.

Results

Maternal and neonatal characteristics of study participants are described in Table 2. The maternal age was slightly higher in the north compared with the south ($p = 0.0844$). Mothers in the north were significantly shorter with higher body mass index (BMI) levels compared with the south mothers who were taller and had lower BMI levels ($p < 0.0001$). Maternal Hb levels were higher in the north compared with the south ($p = 0.0002$). The systolic and diastolic BP measurements were significantly lower in the north compared with the south ($p < 0.05$). Mean GA and BW were lower in the north compared with the south ($p = 0.0540$ and 0.0951 (Table 2), respectively) but did not reach significance.

The level of atmospheric NO_x in the south (37.04 ± 7.46) was significantly greater than in the north (33.26 ± 8.51, $p < 0.0001$; Fig. 1a). This corresponds to a significant increase in maternal serum 8-OhdG concentration observed in the south (20.26 ± 40.88 ng/mL) compared with the north (11.51 ± 18.51 ng/mL, $p = 0.0197$; Fig. 1b).

A significant positive correlation (Spearman $r = 0.2173$; $p = 0.0158$) was found between the levels of maternal serum 8-OHdG and atmospheric NO_x (Fig. 2a). When the specific area was taken into consideration, the south (Spearman $r = 0.2337$; $p = 0.0466$) atmospheric NO_x correlated significantly with maternal serum 8-OHdG concentration (Fig. 2c), whereas the north (Spearman $r = 0.1270$; $p = 0.3795$) showed a positive trend, although not significant (Fig. 2b). No relationship was observed between NO_x and neonate BW (Spearman $r = 0.09865$; 95% confidence interval [CI] − 0.016 to 0.210; $p = 0.0814$) and NO_x and neonatal GA (Spearman $r = -0.0716$; 95% CI − 0.185 to 0.043; $p = 0.0814$). When area was considered, a negative trend is suggested for NO_x and BW in both north (Spearman $r = -0.1135$; 95% CI − 0.282 to 0.062; $p = 0.19$) and south (Spearman $r = -0.060$; 95% CI 0.211 to 0.093; $p = 0.43$). A similar negative trend is observed between NO_x and GA in both the north (Spearman $r = -0.1135$; 95% CI − 0.282 to 0.062; $p = 0.19$) and south (Spearman $r = -0.060$; 95% CI − 0.211 to 0.093; $p = 0.43$). Maternal serum 8-OHdG was not shown to correlate with BW (Spearman $r = 0.058$;

Table 2 Maternal and neonate characteristics

	North		South		*p* value
	n	Mean (SD)	*n*	Mean (SD)	
Maternal age (yr)	152	26.38 (5.90)	225	25.32 (5.67)	0.0844
Maternal height (cm)	152	142.3 (19.85)	224	159.0 (6.28)	< 0.0001***
Maternal BMI	152	34.12 (12.41)	224	25.99 (6.64)	< 0.0001***
Haemoglobin (g/dL)	152	10.96 (1.73)	224	7.79 (5.50)	0.0002***
BP systolic (mmHg)	152	109.1 (13.20)	224	111.8 (11.97)	0.0143*
BP diastolic (mmHg)	152	67.75 (9.187)	224	70.34 (8.58)	0.002**
Gestational age (weeks)	137	38.55 (1.81)	202	38.85 (1.76)	0.0540
Birthweight (g)	139	3003 (670.7)	202	3125 (610.1)	0.0951

n sample size, *SD* standard deviation,

Statistical significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$

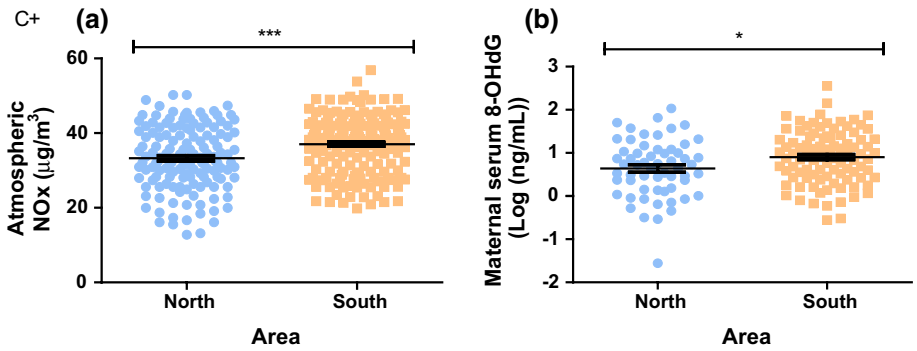

Fig. 1 Concentrations of **a** atmospheric NO_x (µg/cm^3) and **b** maternal serum 8-OHdG (log (ng/mL)) for patients living in the north (**a** $n = 142$; **b** $n = 59$) and south (**a** $n = 185$; **b** $n = 97$). Statistical significance: ***$p < 0.0001$ and *$p < 0.05$

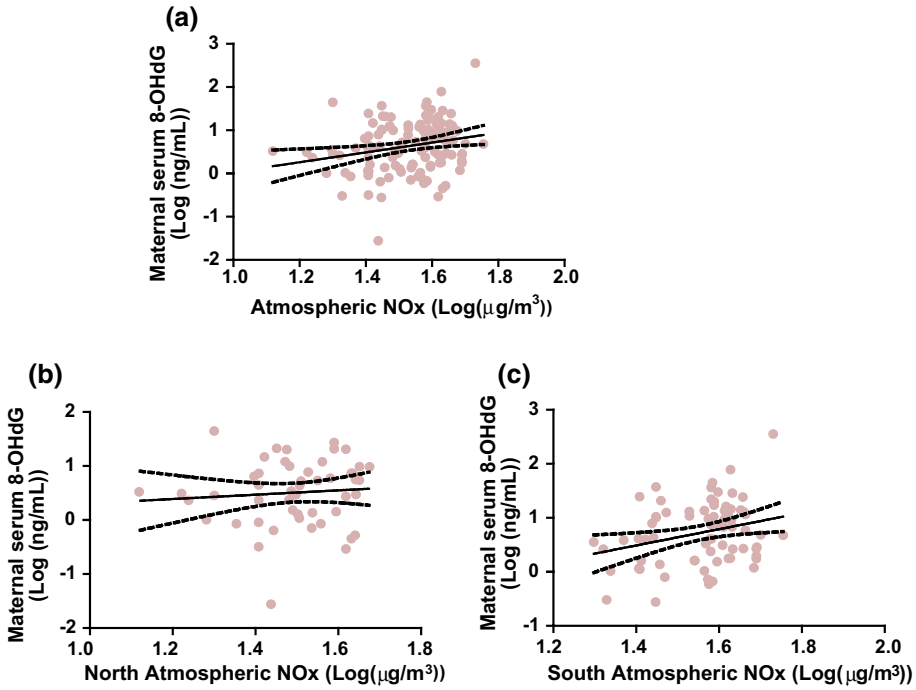

Fig. 2 Relationship between the concentrations of atmospheric NO_x (log (μg/cm^3)) and maternal serum 8-OHdG (log (ng/mL)) for all patients (**a**) (Spearman $r = 0.2173$; 95% CI 0.03657–0.3843; * $p = 0.0158$; $n = 123$), patients living in the north (**b**) (Spearman $r = 0.1270$; 95% CI − 0.1652–0.3987; $p = 0.3795$; $n = 50$) and south (**c**) (Spearman $r = 0.2337$; 95% CI − 0.003195–0.4457; *$p = 0.0466$; $n = 73$). Dotted lines represent 95% CI interval. Statistical significance: *$p < 0.05$

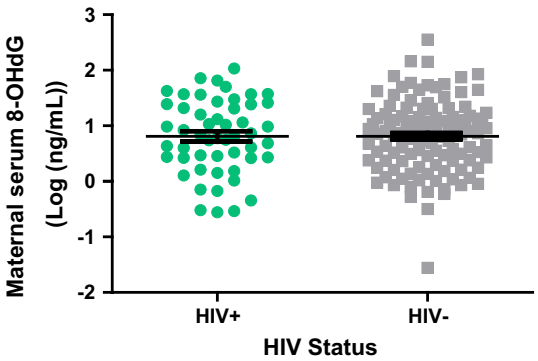

Fig. 3 Maternal serum 8-OHdG adduct [log (ng/mL)] concentration between HIV-positive ($n = 52$) and HIV-negative ($n = 113$) patients in the total sample group

95% CI − 0.1521 to 0.2635; $p = 0.58$) and GA (Spearman $r = 0.044$; 95% CI − 0.121 to 0.207; $p = 0.59$).

Maternal HIV status was thought to influence maternal serum 8-OHdG adduct concentration; however, no difference between HIV-positive (16.02 ± 21.39 ng/mL) and HIV-negative (18.48 ± 40.23 ng/mL) 8-OHdG concentration was observed (Fig. 3).

The genotypic prevalence of GSTM1 and GSTP1 amongst study participants are shown in Tables 3 and 4, respectively. For all subjects, the prevalence of the GSTM1 null (0/0) and wt (0/+ or +/+) type was 25.3 and 75.7%, respectively. The frequencies of the A_{105} and G_{105} allele of GSTP1 were 44.2 and 55.8%, respectively. A significantly greater fold increase ($p = 0.0144$) was observed in the south

Table 3 Genotypic frequency of GSTM1 in patients living in the North and South ($n = 372$)

	Total *n* (%)	North *n* (%)	South *n* (%)	*p* value
Genotype frequency				
GSTM1 0/0	94 (25.3)	39 (41.5)	55 (59.5)	0.9038[a]
GSTM1 0/+ or +/+	278 (75.7)	113 (40.7)	165 (59.3)	

[a]RR (1.021, 95% CI 0.7722–1.349); OR (1.035, 95% CI 0.6438–1.665)

n sample size, *RR* relative risk, *OR* odds ratio, *CI* confidence interval

Table 4 Genotype and allele frequencies of GSTP1 in patients living in the north and south ($n = 377$)

	Total *n* (%)	North *n* (%)	South *n* (%)	*p* value
Genotype frequency				
GSTP1 A_{105}/A_{105}	92 (24.4)	28 (30.4)	64 (69.6)	0.0281[a,*]
GSTP1 A_{105}/G_{105} and G_{105}/G_{105}	285 (75.6)	124 (43.5)	161 (56.5)	
Allelotype frequency				
GSTP1 A_{105}	361 (44.2)	129 (35.7)	232 (64.3)	0.0144[b,*]
GSTP1 G_{105}	456 (55.8)	175 (44.5)	218 (55.5)	

n sample size, *RR* relative risk, *OR* odds ratio, *CI* confidence interval

[a]RR (0.6995; 95% CI 0.4998–0.9790); OR (0.5680; 95% CI 0.3438–0.9386)

[b]RR (0.8025, 95% CI 0.6723–0.9579); OR (0.6927, 95% CI 0.5166–0.9286)

Statistical significance: *$p < 0.05$

for the GSTP1 A_{105} allelotype compared to G_{105} allelotype (1.8 and 1.2, respectively). The prevalence of GSTP1 AA (wt) and AG+GG (variant) ($p = 0.0281$) was significantly different between the north and south; whilst the prevalence of GSTM1 ($p = 0.9038$) did not differ significantly. The GSTP1 AG and GG genotypes were combined for analysis, because subjects with a single GSTP1 G_{105} allele have reduced enzyme activity compared with those with the GSTP1 A_{105} allele (Zimniak et al. 1994).

No difference was observed in the levels of maternal serum 8-OHdG among the GSTM1 genotypes between the north and south (Fig. 4a). Maternal serum 8-OHdG concentration was higher in AA genotyped mothers (29.31 ± 10.10 ng/mL, $n = 38$) compared with AG+GG genotyped mothers (13.16 ± 1.772, $n = 127$, $p = 0.1589$), although not significant. When area was considered, the level of maternal serum 8-OHdG was higher in the north GSTP1 AA (21.11 ± 8.9 ng/mL) mothers compared with the GSTP1 AG+GG (8.70 ± 2.0 ng/mL) mothers but did not reach significance ($p = 0.1421$). The south GSTP1 AG+GG (13.50 ± 2.0 ng/mL) mothers had significantly greater levels of maternal serum 8-OHdG compared with the north (8.70 ± 2.0 ng/mL, $p = 0.0188$; Fig. 4b).

The relationship between the atmospheric NO_x and maternal serum 8-OHdG concentration was investigated among the different genotypes in Table 5. A significant correlation was observed between the atmospheric NO_x and the level of maternal serum 8-OHdG within the GSTM1 null genotype (Spearman $r = 0.4227$, $p = 0.0199$) and the GSTP1 AA + GG genotype (Spearman $r = 0.2105$, $p = 0.0395$). No relationship was observed when north and south was taken into consideration.

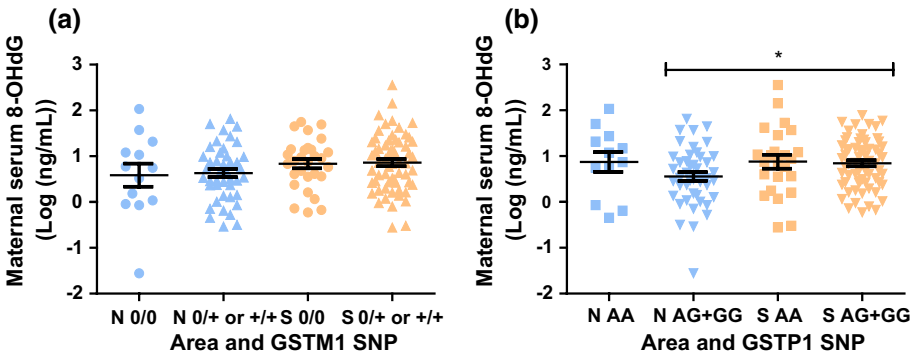

Fig. 4 Maternal serum 8-OHdG adduct [log (ng/mL)] concentration between the GSTM1 (**a**) and GSTP1 (**b**) genotypes for patients living in the North and South. Statistical significance: *$p < 0.05$

Table 5 Relationship between the concentrations of atmospheric NO_x (log (μg/cm^3)) and maternal serum 8-OHdG (log (ng/mL)) among the different SNP genotypes (GSTM1 and GSTP1) for the total patient sample and those living in the north and south

		GSTM1 SNP genotypes	
		0/0	0/+ or +/+
Total	Spearman *r* (95%CI)	0.4227 (0.06250 to 0.6855)	0.1566 (− 0.05478 to 0.3546)
	p value	0.0199*	0.1338
North	Spearman *r* (95%CI)	0.5758 (0.0816)	0.03247 (− 0.2907 to 0.3490)
	p value	ns	0.8424
South	Spearman *r* (95%CI) *p* value	0.3046 (− 0.1732 to 0.6663)	0.2218 (− 0.05980 to 0.4707)
	p value	0.1916	0.1104
		GSTP1 SNP genotypes	
		A_{105}/A_{105}	A_{105}/G_{105} and G_{105}/G_{105}
Total	Spearman *r* (95% CI)	0.2525 (− 0.1527 to 0.5851)	0.2105 (0.004413 to 0.3995)
	p value	0.2038	0.0395*
North	Spearman *r* (95% CI)	− 0.1459 (0.6876)	0.07900 (− 0.2474 to 0.3893)
	p value	ns	0.6280
South	Spearman *r* (95% CI)	0.3887 (− 0.1284 to 0.7397)	0.1864 (− 0.08845 to 0.4348)
	p value	0.1231	0.1690

CI confidence interval, *ns* not significant

Statistical significance: *$p < 0.05$

The relationship between atmospheric NO_x and maternal serum 8-OHdG concentration, controlled for potential confounders, is described in Table 6. The β-coefficient and regression model for the total samples was strengthened when maternal characteristics were controlled (unadjusted = 1.14, adjusted[2] = 1.21, $p < 0.05$). Every 1% increase in NO_x would lead to a 1.21% increase in maternal serum 8-OHdG. This almost doubled when area was taken into consideration: a 1% increase in atmospheric NO_x would result in a 1.99% increase in maternal 8-OHdG in the south ($p = 0.004$). When polymorphisms were considered, a 1% increase in atmospheric NO_x would lead to a 1.14% increase in maternal 8-OHdG for the variant GSTP1 genotype ($p = 0.026$), whereas an almost equal change in percent was observed between NO_x and 8-OHdG for the GSTM1 wt genotype ($p = 0.076$). The neonate gender was shown to influence the concentration of maternal serum 8-OHdG: a 1% increase in NO_x would result in a 2.48% increase in maternal serum 8-OHdG for women carrying female neonates ($p = 0.002$).

The relationship between atmospheric NO_x and GA, controlled for potential confounders, is described in Table 7. Controlling for potential maternal and neonate confounders strengthened the β-coefficient and regression model for the total samples (unadjusted = − 0.464, adjusted[2] = − 1.64, $p < 0.05$). Therefore, for every 1% increase in NO_x, there would be a 0.0164% reduction in GA. The maternal serum 8-OhdG did not significantly impact GA. However, when it was not included in the regression model, NO_x was not found to influence GA. Controlling for 8-OHdG, therefore, was an important factor for NO_x influencing GA. When area was considered, a 1% increase in NO_x would result in a 0.0196%

Table 6 Impact of atmospheric NO_x (log) concentration on maternal serum 8-OHdG adduct (log) concentration—linear regression analysis for total mothers and subdivided into area, GSTM1 and GSTP1 SNP with adjustments for maternal characteristics

	Maternal serum 8-OHdG (log) concentration			
	β-coefficient	(95%CI)	p value	R-squared (p value)
Atmospheric NO_x (log)				
Total				
Unadjusted ($n = 123$)	1.14	(0.251–2.02)	0.012*	0.0507 (0.012*)
Adjusted[1] ($n = 123$)	1.14	(0.256–2.03)	0.012*	0.0517 (0.041*)
Adjusted[2] ($n = 123$)	1.21	(0.244–2.18)	0.015*	0.1423 (0.022*)
Area				
North ($n = 50$)	0.317	(− 1.41 to 2.04)	0.713	0.1061 (0.662)
South ($n = 73$)	1.99	(0.679–3.30)	0.004**	0.1818 (0.060)
GSTP1				
AA ($n = 27$)	1.29	(− 1.70 to 4.28)	0.378	0.3063 (0.350)
AG+GG ($n = 96$)	1.14	(0.137–2.14)	0.026*	0.1195 (0.118)
GSTM1				
0/+ or +/+ ($n = 93$)	1.01	(− 0.108 to 2.13)	0.076	0.1171 (0.144)
0/0 ($n = 30$)	0.824	(− 1.36 to 3.01)	0.442	0.3835 (0.101)
Neonate gender				
Male ($n = 70$)	0.75	(− 0.588 to 2.08)	0.268	0.1445 (0.185)
Female ($n = 48$)	2.48	(1.02–3.95)	0.001**	0.2932 (0.040*)

Adjusted[1]: HIV status; Adjusted[2]: HIV status, area, maternal age, body mass index, haemoglobin, blood pressure systolic and diastolic

Area, GSTP1 and GSTM1 results were all adjusted for HIV status, maternal age, body mass index, haemoglobin, blood pressure systolic and diastolic

CI confidence interval, *n* sample size

Statistical significance: *$p < 0.05$, **$p < 0.01$

Table 7 Impact of atmospheric NO_x (log) concentration on gestational age—linear regression analysis for total mothers and subdivided into area, GSTM1, and GSTP1 SNP with adjustments for maternal characteristics

	Gestational age (weeks)			
	B-coefficient	(95% CI)	*p* value	*R*-squared (*p* value)
Atmospheric NO_x (log)				
Total				
Unadjusted ($n = 311$)	− 0.464	(− 1.12 to 0.194)	0.166	0.2075 (< 0.0001***)
Adjusted[1] ($n = 118$)	− 1.49	(− 2.60 to − 0.377)	0.009**	0.3384 (0.003***)
Adjusted[2] ($n = 64$)	− 1.64	(− 3.21 to − 0.066)	0.042*	0.5597 (0.0008***)
Area				
North ($n = 49$)	− 1.96	(− 3.63 to − 0.287)	0.023*	0.5254 (0.0093**)
South ($n = 69$)	− 0.688	(− 2.59 to 1.21)	0.471	0.3030 (0.0884)
GSTP1				
AA ($n = 27$)	− 1.35	(− 4.15 to 1.46)	0.316	0.7943 (0.0222*)
AG+GG ($n = 91$)	− 1.30	(− 2.52 to − 0.072)	0.038*	0.2553 (0.0445*)
GSTM1				
0/+ or +/+ ($n = 88$)	− 1.26	(− 2.66 to − 0.109)	0.034*	0.3115 (0.0093**)
0/0 ($n = 30$)	− 1.38	(− 4.39 to 1.88)	0.407	0.6875 (0.0554)
Neonate gender				
Male ($n = 70$)	− 1.56	(− 2.95 to − 0.184)	0.027*	0.3541 (0.0135*)
Female ($n = 48$)	− 2.03	(− 4.44 to 0.385)	0.097	0.3550 (0.1922)

Unadjusted: birthweight; Adjusted[1]: neonatal characteristics: birthweight, child gender, Apgar scores: 1 and 5 m, body: brain ratio, ponderal index and maternal characteristics: HIV status, area, maternal age, body mass index, haemoglobin, blood pressure systolic and diastolic, GSTP1 and GSTM1; Adjusted[2]: same as Adjusted[1] with parity included

Area, GSTP1, GSTM1, and neonate gender results were all adjusted for neonatal characteristics: birthweight, child gender, Apgar scores: 1 and 5 m, body: brain ratio, ponderal index, and maternal characteristics: HIV status, maternal age, body mass index, haemoglobin, blood pressure systolic and diastolic

CI confidence interval, *n* sample size

Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$

decrease in GA ($p = 0.023$). The GSTP1 variant caused a 0.013% reduction in GA, whilst the GSTM1 wt genotype caused a 0.0126% decrease in GA if a 1% increase in NO_x is observed ($p < 0.05$; Table 7). Mothers carrying a male foetus were significantly associated with a 0.0156% decrease in GA if NO_x were to increase by 1% ($p = 0.027$). This decrease was almost doubled (0.0203% change) with mothers carrying a female foetus, although not significant ($p = 0.097$).

Discussion

Exposure to NO_x was shown to influence maternal serum 8-OHdG concentrations directly in pregnant women living in Durban, SA. Gestational age of these women also was shown to decrease significantly as a result of increased NO_x exposure. This is the first study in Durban, SA to link the increase in oxidative stress in pregnant women to increased NO_x pollution.

Oxides of nitrogen, a by-product of combustion, have been linked to several adverse health conditions, including respiratory diseases, cardiovascular diseases, low birth weight, and preterm birth (Seo et al. 2007; Wu et al. 2009; César et al. 2015). This nitrogen-centred free radical, upon inhalation, is absorbed in lung fluids producing free radical products that enter the blood stream. These free radicals are then able to react directly with macromolecules (protein, lipids, and DNA) present resulting in ROS production (Tabacova et al. 1998). Guanine, having the lowest redox potential among the nucleic bases, is highly susceptible to oxidation

by ROS, which results in the production of 8-OHdG mutagenic lesions (Kershaw and Hodges 2012; Ba et al. 2015). Therefore, this accounts for the significant increase in maternal serum 8-OHdG adduct concentration as a result of NO_x exposure (Figs. 1 and 2).

The Durban south region has been shown previously to have higher levels of air pollution (Naidoo et al. 2013) and in the present study with significantly increased NO_x concentration (Fig. 1a) compared with the north. Previously, pregnant women in the south region have been shown to exhibit increased markers of oxidative stress compared with the north (Nagiah et al. 2015). This finding was corroborated in our study, where pregnant women in the south had increased levels of serum 8-OHdG compared with the north (Fig. 1b). Our study went further and investigated the relationship between NO_x and 8-OHdG, the influence of GST polymorphisms, as well as linking NO_x to GA.

Several studies have found an association of pollution (polycyclic aromatic hydrocarbon (PAH), diesel-exhaust smoke, and smoking) to increased 8-OHdG and oxidative stress (Risom et al. 2005; Lewtas 2007; Leonardi-Bee et al. 2008; Ren et al. 2010). Studies also have observed that a dose–effect relationship occurs between PAH exposure and levels of urinary 8-OHdG (Kuang et al. 2013; Li et al. 2015). Our study found a significantly positive correlation between atmospheric NO_x and maternal serum 8-OHdG (Fig. 2a, $p = 0.158$). As mentioned above, the south region is considered to have a higher pollution level than the north. When area was taken into consideration, the relationship between NO_x and 8-OHdG was strengthened in the heavily industrialised south (Fig. 2c, $p = 0.0466$) whilst the relationship was lost in the less industrialised north (Fig. 2b). This relationship was further investigated by controlling for potential confounding factors to determine whether this effect was indeed a response to NO_x exposure. The results confirmed that NO_x exposure caused a significant increase in 8-OHdG concentration when controlled for maternal characteristics (Table 7). It was found that a 1% increase in NO_x results in a 1.21% ($p = 0.015$) increase in 8-OHdG, with an even higher increase (1.99%, $p = 0.004$) observed for south living mothers. The results provide evidence that exposure to atmospheric NO_x increases serum 8-OHdG levels in pregnant women.

Antioxidants are an important controller of oxidative stress by helping to reduce and eliminate oxidants to prevent oxidative stress-related damage. However, genetic susceptibility plays an important role in determining the effect and responses an individual has to oxidative damage. Therefore, the risk for cancer, adverse reproductive outcomes, and cardiovascular diseases are a consequence of air pollution exposure and genetic susceptibility (Lewtas 2007; Lagadu et al. 2010). GSTs are antioxidant enzymes that protect against oxidative stress by conjugating electrophilic species, thereby neutralising their effect. These GSTs have two common polymorphisms that influence an individual's genetic susceptibility to oxidants. A homozygous deletion in GSTM1 results in the enzyme inactivation (Mustafa et al. 2010), whereas the substitution of isoleucine for valine at codon 105 in GSTP1 reduces substrate specificity (Wong et al. 2008).

Our results indicate that GSTP1 is associated with the heavily industrialised south (Table 4, $p = 0.0281$), with increased serum 8-OHdG observed in the GSTP1 AG+GG genotypes compared with the AA genotype. This suggests that wt GSTP1 is able to scavenge oxidants more effectively than the variant, which leads to reduced serum 8-OHdG concentrations observed. When subdivided into areas, no difference in 8-OHdG was observed in north and south wt GSTP1 whilst the north variant mothers had significantly lower 8-OHdG levels compared with the south variant mothers (Fig. 4b, $p < 0.05$). This suggests that at low pollution levels (north) the variant GSTP1 enzymes, with its reduced specificity is still able to conjugate electrophiles and reduce their effect whilst at higher pollution levels (south) the variant genotype is overwhelmed and becomes inefficient at scavenging, which leads to increased oxidants present that attack DNA leading to increased 8-OHdG levels.

It has been shown that the variant GSTP1 allele has a sevenfold greater efficacy against PAH diol epoxides than wt allele while threefold less effective against 1-chloro-2,4-dinitrobenzene (Strange et al. 2000). However, this study showed that the variant genotype is less efficient compared with the wt at detoxifying oxidant products. A significant positive correlation was observed between NO_x and 8-OHdG levels in AG+GG GSTP1 genotyped mothers (Table 5; $p = 0.0395$), further confirming that the variant GSTP1 mothers when exposed to NO_x are unable to effectively scavenge oxidants leading to increased DNA damage. No association was observed between GSTM1 and the heavily industrialised south (Table 3), with no difference in serum 8-OHdG levels observed between the null and wt GSTM1 genotypes. A significant positive correlation was observed between NO_x and serum 8-OHdG for null GSTM1 mothers (Table 5; $p = 0.0199$), thus suggesting that the inactive enzyme GSTM1 was unable to neutralise oxidants leading to increased DNA damage. These results were further analysed in multivariate analyses to determine whether the results observed in bivariate correlations remained when controlling for maternal characteristics as potential confounders.

The GSTP1 variant mothers had increased serum 8-OHdG (1.14%) with increasing NO_x (1%, $p = 0.026$; Table 6), whilst in GSTM1 null mothers this relationship was lost. This could be a result of small sample size, once all confounding factors were taken into account, only 30 GSTM1 null mothers remained, which could account for the lack of association between NO_x and 8-OHdG. Other studies have shown no association between GSTM1 null

individuals, DNA damage, and pollution (PAH (Marczynski et al. 2002; Garte et al. 2007) and particulate matter (Sørensen et al. 2003)). The multivariate analysis, however, suggests a parallel increase in 8-OHdG (1.01%) as a result of NO_x (1%) exposure for GSTM1 wt mothers (Table 6, $p = 0.076$). The results provide evidence that GSTP1 variant genotype increases the susceptibility of mothers to NO_x exposure, leading to increased oxidative stress.

Pregnancy, a physiological state characterised by increased basal oxygen demand and high-energy requirement, favours ROS production and has been shown to exhibit increased susceptibility to oxidative stress in normal pregnancies (Saker et al. 2008). This already highly susceptible condition in the presence of high AAP would exasperate antioxidant stores and lead to increased oxidative stress. Exposure to traffic-related air pollution (i.e., NO_x, CO, and primary exhaust particles) have been implicated in deceased foetal growth, LBW, and PTB (Seo et al. 2007; Darrow et al. 2011). Several studies also have reported oxidative stress as a potential mechanism for LBW and PTB, with reports showing increased 8-OHdG in LBW and PTB (Kim et al. 2005; Mustafa et al. 2010; Rossner et al. 2011).

Our study first set out to find an association between NO_x and serum 8-OHdG with neonate BW and GA. Using simple correlations, no association was observed between serum 8-OHdG and BW, with a negative trend suggested between NO_x and BW in north and south. This negative trend was not significant; however, this could be due to our small sample size, because previous studies have shown a link between AAP and BW reduction (Lacasana et al. 2005; Darrow et al. 2011; Wilhelm et al. 2012). Next, our study used bivariate linear regression to determine whether NO_x was associated with reductions in GA. The results from the unadjusted bivariate analysis again suggested a negative trend but remained nonsignificant, which also was observed using simple correlation. We then controlled for maternal and neonatal characteristics, which revealed a small, significant reduction in gestational age as a result of NO_x exposure (Table 7, $p = 0.042$). This relationship was only found to be significant when maternal serum 8-OHdG was controlled for, suggesting 8-OHdG may affect GA.

Previous studies found increased 8-OHdG in mothers who give birth prematurely (Matsubasa et al. 2002; Nassi et al. 2009; Darrow et al. 2011; Negi et al. 2012b). When area was considered, the Durban north mothers showed a significantly higher reduction in GA compared with the total mothers, whilst Durban south mothers showed no reduction. This study only measured the levels of NO_x; however, other pollutants or environmental factors may have been present in high concentrations within the north area. These pollutants or environmental factors may be potent enough to cause the reduction in GA observed within the north. This would be an ideal follow-up study. The GTSP1 variant and GSTM1 wt mothers were susceptible to GA reduction as a consequence of NO_x exposure ($p = 0.034$ and $p = 0.038$, respectively; Table 7). Neonatal gender also was found to be associated with reduced gestational age due to NO_x exposure; mothers carrying male infants exposed to NO_x had a significant reduction in GA. These reductions in GA observed were small (< 0.1 weeks per percent change in NO_x) and, therefore, would not impact clinical significance for individual neonate. However, a negative shift in GA on the population level could result in increased PTB nationwide.

The findings of our study must be interpreted in light of the following limitations. First, upon subdivision, by area and genotype, our sample size becomes relatively small. Second, due to our relatively small sample size, ethnicity was not taken into account; this could be addressed in a future study with increased sample numbers. Although significant results are obtained, increasing population numbers in future studies could give further insight into the conclusions observed. Third, measuring other pollutants in study areas could give insight into their effects on 8-OHdG levels and GA. A combined effect of NO_x and other pollutions, such as particulate matter, also would be interesting to investigate.

Conclusions

This study demonstrated increased maternal serum 8-OHdG in pregnant women exposed to higher levels of NO_x pollution in the south. This increase in DNA damage was found to be a direct consequence of increased NO_x exposure, with increased susceptibility found in GSTP1 variant carriers and GSTM1 wt carriers. Gestational age also was found to be reduced as a consequence of NO_x exposure; male neonates making mothers more susceptible to GA reduction. This study highlights the need for better systems in place to reduce traffic-related air pollution close to residential areas, so that vulnerable individuals are better protected against oxidative stress related injury.

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Compliance with Ethical Standards

Ethical Approval The Biomedical Research Ethics Committee of the University of Kwa-Zulu Natal (BF263/12) approved the study including the use of human subjects.

Informed Consent Informed consent from all study participants was obtained.

References

Ba X, Aguilera-Aguirre L, Sur S, Boldogh I (2015) 8-Oxoguanine DNA glycosylase-1-driven DNA base excision repair. Curr Opin Allergy Clin Immunol 15:89–97. doi:10.1097/ACI.0000000000000135

Beelen R, Hoek G (2010) ESCAPE Exposure assessment manual

César ACG, Carvalho JA, Nascimento LFC (2015) Association between NO_x exposure and deaths caused by respiratory diseases in a medium-sized Brazilian city. Braz J Med Biol Res 48:1130–1135. doi:10.1590/1414-431X20154396

Chen Z, Salam MT, Eckel SP, Breton CV, Gilliland FD (2015) Chronic effects of air pollution on respiratory health in Southern California children: findings from the Southern California Children's Health Study. J Thorac Dis 7:46–58. doi:10.3978/j.issn.2072-1439.2014.12.20

Darrow LA, Klein M, Strickland MJ, Mulholland JA, Tolbert PE (2011) Ambient air pollution and birth weight in full-term infants in Atlanta, 1994–2004. Environ Health Perspect 119:731–737. doi:10.1289/ehp.1002785

Fleischer NL, Merialdi M, Van Donkelaar A, Vadillo-Ortega F, Martin RV, van Donkelaar A, Vadillo-Ortega F, Martin RV, Betran AP, Souza JP, O'Neill MS, Van Donkelaar A, Vadillo-Ortega F, Martin RV, van Donkelaar A, Vadillo-Ortega F, Martin RV, Betran AP, Souza JP, O'Neill MS (2014) Outdoor air pollution, preterm birth, and low birth weight: analysis of the world health organization global survey on maternal and perinatal health. Environ Health Perspect 122:425–430. doi:10.1289/ehp.1306837

Garte S, Taioli E, Raimondi S, Paracchini V, Binkova B, Sram RJ, Kalina I, Popov TA, Singh R, Farmer PB (2007) Effects of metabolic genotypes on intermediary biomarkers in subjects exposed to PAHS: results from the EXPAH study. Mutat Res Fundam Mol Mech Mutagen 620:7–15. doi:10.1016/j.mrfmmm.2007.02.017

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D (2004) Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15:36–45. doi:10.1097/01.ede.0000101023.41844.ac

Johanssona A-S, Stenberga G, Widerstena M, Mannervik B (1998) Structure-activity relationships and thermal stability of human glutathione transferase P1-1 governed by the H-site residue 105. J Mol Biol 278:687–698. doi:10.1006/jmbi.1998.1708

Kampa M, Castanas E (2008) Human health effects of air pollution. Environ Pollut 151:362–367. doi:10.1016/j.envpol.2007.06.012

Kelly FJ (2003) Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 60:612–616. doi:10.1136/oem.60.8.612

Kershaw RM, Hodges NJ (2012) Repair of oxidative DNA damage is delayed in the Ser326Cys polymorphic variant of the base excision repair protein OGG1. Mutagenesis 27:501–510. doi:10.1093/mutage/ges012

Kim Y, Hong Y, Lee K, Park H, Park E, Moon H, Ha E (2005) Oxidative stress in pregnant women and birth weight reduction. Reprod Toxicol 19:487–492. doi:10.1016/j.reprotox.2004.10.007

Kistnasamy EJ, Robins TG, Naidoo R, Batterman S, Mentz GB, Jack C, Irusen E (2008) The relationship between asthma and ambient air pollutants among primary school students in Durban, South Africa. Int J Environ Heal 2:365. doi:10.1504/IJENVH.2008.020929

Kuang D, Zhang W, Deng Q, Zhang X, Huang K, Guan L, Hu D, Wu T, Guo H (2013) Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. Environ Sci Technol 47:7446–7456. doi:10.1021/es401639x

Lacasana M, Esplugues A, Ballester F (2005) Exposure to ambient air pollution and prenatal and early childhood health effects. Eur J Epidemiol 20:183–199. doi:10.1007/s10654-004-3005-9

Lagadu S, Lechevrel M, Sichel F, Breton J, Pottier D, Couderc R, Moussa F, Prevost V (2010) 8-oxo-7,8-dihydro-2′-deoxyguanosine as a biomarker of oxidative damage in oesophageal cancer patients: lack of association with antioxidant vitamins and polymorphism of hOGG1 and GST. J Exp Clin Cancer Res 29:157. doi:10.1186/1756-9966-29-157

Leonardi-Bee J, Smyth A, Britton J, Coleman T (2008) Environmental tobacco smoke and fetal health: systematic review and meta-analysis. Arch Dis Child Fetal Neonatal Ed 93:F351–F361. doi:10.1136/adc.2007.133553

Lewtas J (2007) Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. Mutat Res Rev Mutat Res 636:95–133. doi:10.1016/j.mrrev.2007.08.003

Li J, Lu S, Liu G, Zhou Y, Lv Y, She J, Fan R (2015) Co-exposure to polycyclic aromatic hydrocarbons, benzene and toluene and their dose-effects on oxidative stress damage in kindergarten-aged children in Guangzhou, China. Sci Total Environ 524–525:74–80. doi:10.1016/j.scitotenv.2015.04.020

Marczynski B, Rihs H-P, Rossbach B, Hölzer J, Angerer J, Scherenberg M, Hoffmann G, Brüning T, Wilhelm M (2002) Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. Carcinogenesis 23:273–281. doi:10.1093/carcin/23.2.273

Matsubasa T, Uchino T, Karashima S, Tanimura M, Endo F (2002) Oxidative stress in very low birth weight infants as measured by urinary 8-OHdG. Free Radic Res 36:189–193. doi:10.1080/10715760290006510

Moorthy B, Chu C, Carlin DJ (2015) Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. Toxicol Sci 145:5–15. doi:10.1093/toxsci/kfv040

Moyer AM, Salavaggione OE, Wu TY, Moon I, Eckloff BW, Hildebrandt MA, Schaid DJ, Wieben ED, Weinshilboum RM (2008) Glutathione s-transferase p1: gene sequence variation and functional genomic studies. Cancer Res 68:4791–4801. doi:10.1158/0008-5472.can-07-6724

Mustafa MD, Pathak R, Ahmed T, Ahmed RS, Tripathi AK, Guleria K, Banerjee BD (2010) Association of glutathione S-transferase M1 and T1 gene polymorphisms and oxidative stress markers in preterm labor. Clin Biochem 43:1124–1128. doi:10.1016/j.clinbiochem.2010.06.018

Muttoo S, Ramsay L, Brunekreef B, Beelen R, Meliefste K, Naidoo RN (2017) Land use regression modelling estimating nitrogen oxides exposure in industrial South Durban, South Africa. Sci Total Environ 610–611:1439–1447. doi:10.1016/j.scitotenv.2017.07.278

Nagiah S, Phulukdaree A, Naidoo D, Ramcharan K, Naidoo R, Moodley D, Chuturgoon A (2015) Oxidative stress and air pollution exposure during pregnancy: a molecular assessment. Hum Exp Toxicol 34:838–847. doi:10.1177/0960327114559992

Naidoo RN, Robins T, Batterman S, Mentz G, Jack C (2013) Ambient pollution and respiratory outcomes among schoolchildren in Durban, South Africa. S Afr J Child Health 7:127–134. doi:10.7196/sajch.598

Nassi N, Ponziani V, Becatti M, Galvan P, Donzelli G (2009) Anti-oxidant enzymes and related elements in term and preterm newborns. Pediatr Int 51:183–187. doi:10.1111/j.1442-200X.2008.02662.x

Negi R, Pande D, Kumar A, Khanna RS, Khanna HD (2012a) Evaluation of biomarkers of oxidative stress and antioxidant capacity in the cord blood of preterm low birth weight neonates. J Matern Neonatal Med 25:1338–1341. doi:10.3109/14767058.2011.633672

Negi R, Pande D, Kumar A, Khanna RS, Khanna HD (2012b) In vivo oxidative DNA damage and lipid peroxidation as a biomarker of oxidative stress in preterm low-birthweight infants. J Trop Pediatr 58:326–328. doi:10.1093/tropej/fmr078

Proietti E, Röösli M, Frey U, Latzin P (2013) Air pollution during pregnancy and neonatal outcome: a review. J Aerosol Med Pulm Drug Deliv 26:9–23. doi:10.1089/jamp.2011.0932

Ren C, Vokonas PS, Suh H, Fang S, Christiani DC, Schwartz J (2010) Effect modification of air pollution on urinary 8-hydroxy-2′-deoxyguanosine by genotypes: an application of the multiple testing procedure to identify significant SNP interactions. Environ Health 9:78. doi:10.1186/1476-069X-9-78

Risom L, Møller P, Loft S (2005) Oxidative stress-induced DNA damage by particulate air pollution 592:119–137. doi:10.1016/j.mrfmmm.2005.06.012

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Moreno-Macías H, del Rio-Navarro BE, David G, Marzec J, Hernández-Avila M, London S (2006) GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J 28:953–959. doi:10.1183/09031936.06.00114905

Rossner P, Tabashidze N, Dostal M, Novakova Z, Chvatalova I, Spatova M, Sram RJ (2011) Genetic, biochemical, and environmental factors associated with pregnancy outcomes in newborns from the Czech Republic. Environ Health Perspect 119:265–271. doi:10.1289/ehp.1002470

Saker M, Soulimane Mokhtari N, Merzouk SA, Merzouk H, Belarbi B, Narce M (2008) Oxidant and antioxidant status in mothers and their newborns according to birthweight. Eur J Obstet Gynecol Reprod Biol 141:95–99. doi:10.1016/j.ejogrb.2008.07.013

Seidegård J, Vorachek WR, Pero RW, Pearson WR (1988) Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. Proc Natl Acad Sci USA 85:7293–7297. doi:10.1073/pnas.85.19.7293

Seo J, Ha E, Kim O, Kim B, Park H, Leem J, Hong Y, Kim Y (2007) Environmental health surveillance of low birth weight in Seoul using air monitoring and birth data [in Korean]. J Prev Med Public Heal 40:363–370. doi:10.3961/jpmph.2007.40.5.363

Slama R, Gräbsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S, Herbarth O, Bauer M, Borte M, Wichmann HE, Heinrich J (2010) Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. Reprod Toxicol 30:600–612. doi:10.1016/j.reprotox.2010.07.001

Sørensen M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S (2003) Personal exposure to PM2. 5 and biomarkers of DNA damage. Cancer Epidemiol Biomark Prev 12:191–196

Sram RJ, Binkova B, Dejmek J, Chvatalova I, Solansky I, Topinka J (2006) Association of DNA adducts and genotypes with birth weight. Mutat Res Genet Toxicol Environ Mutagen 608:121–128. doi:10.1016/j.mrgentox.2006.04.022

Šrám RJ, Binková B, Dejmek J, Bobak M (2005) Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect 113:375–382. doi:10.1289/ehp.6362

Strange RC, Jones PW, Fryer AA (2000) Glutathione S-transferase: genetics and role in toxicology. Toxicol Lett 112–113:357–363. doi:10.1016/S0378-4274(99)00230-1

Tabacova S, Baird DD, Balabaeva L (1998) Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. Arch Env Health 53:214–221. doi:10.1080/00039899809605698

Tularam H, Ramsay L (2013) Synoptic influences on air pollution events in the Durban South Basin, 2006 to 2010. University of Kwa-Zulu Natal

Tyson P, Preston-Whyte R (2004) The weather and climate of southern Africa, 2nd edn. Oxford University Press, Oxford, New York

Wilhelm M, Ghosh JK, Su J, Cockburn M, Jerrett M, Ritz B (2012) Traffic-related air toxics and term low birth weight in Los Angeles County, California. Environ Health Perspect 120:132–138. doi:10.1289/ehp.1103408

Wong RH, Hu CW, Yeh CY, Chao MR, Chen CC, Huang JH, Chang SH, Lee SI, Lee HS (2008) Sulfotransferase 1A1 and glutathione S-transferase P1 genetic polymorphisms modulate the levels of urinary 8-hydroxy-2′-deoxyguanosine in betel quid chewers. Arch Toxicol 82:313–321. doi:10.1007/s00204-007-0248-8

Wu J, Ren C, Delfino RJ, Chung J, Wilhelm M, Ritz B (2009) Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the South Coast Air Basin of California. Environ Health Perspect 117:1773–1779. doi:10.1289/ehp.0800334

Xu SJ, Wang YP, Roe B, Pearson WR (1998) Characterization of the human class Mu glutathione S-transferase gene cluster and the GSTM1 deletion. J Biol Chem 273:3517–3527. doi:10.1074/jbc.273.6.3517

Zimniak P, Nanduri B, Pikula S, Bandorowicz-Pikula J, Singhal SS, Srivastava SK, Awasthi S, Awasthi YC (1994) Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur J Biochem 224:893–899. doi:10.1111/j.1432-1033.1994.00893.x