academicJournals

Vol. 5(3), pp. 52-59, March, 2013
DOI 10.5897/JTEHS2013.0255
ISSN 2006-9820 ©2013 Academic Journals
http://www.academicjournals.org/JTEHS

Journal of Toxicology and Environmental Health Sciences

Full Length Research Paper

Correlation between glutathione S-transferase Mu 1 (*GSTM1*) and glutathione S-transferase pi gene (*GSTP1*) polymorphisms and markers of inflammatory stress in pregnant females

Poovendhree Reddy[1]*, Rajen N. Naidoo[2], Anil Chuturgoon[3], Kareshma Asharam[2], Dhaneshree Naidoo[3], Alisa Phulukdaree[3] and Shivona Gounden[3]

[1]Department of Community Health Studies, Durban University of Technology, Durban, South Africa.
[2]Discipline of Occupational and Environmental Health, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa.
[3]Discipline of Medical Biochemistry, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa.

Accepted 13 March, 2013

The Mother and Child Environmental Cohort (MACE) study piloted in South Africa in 2010 to 2011, collected genetic, biochemical and clinical data from pregnant females residing in south and north Durban. We evaluated birth outcomes and the influence of *GSTM1*pos→*GSTM1*null and the *GSTP1* (Ile105Val; AA→AG/GG) polymorphisms on the extent of DNA damage and with biomarkers [glutathione (GSH) and malondialdehyde (MDA)] related to oxidative stress in mothers with different levels of pollutant exposure. There was no significant difference in adverse birth outcomes or genotype distribution between mothers from the exposed and lower exposed areas. Mean GSH and comet tail length did not differ significantly between *GSTM1*pos and *GSTM1*null genotypes. When stratified by genotype, mean MDA levels was higher among *GSTM1* null mothers compared to the *GSTM1*pos mothers (p = 0.01). When each of the genotypes was stratified by exposure, mean GSH concentration was significantly higher in north Durban for the *GSTM1*pos, *GSTM1*null and *GSTP1*AG+GG genotypes (p < 0.05), and mean comet tail length was significantly increased in south Durban among participants with the *GSTM1*pos, *GSTM1*null, and the *GSTP1*AG+GG genotypes. The expression of *GSTM1* and *GSTP1* polymorphic genotypes may lead to varying susceptibility to the adverse effects of pollutants by modifying the response to oxidative stress.

Key words: Glutathione S-transferase Mu 1 (*GSTM1*), glutathione S-transferase pi gene (*GSTP1*), oxidative stress, birth cohort, glutathione, gene polymorphism, DNA damage.

INTRODUCTION

Evidence suggests that environmental air pollution is associated with an elevated risk of adverse pregnancy outcomes (Glinianaia et al., 2004; Maisionet et al., 2004). Fetuses are considered to be highly susceptible to a

*Corresponding author. E-mail: PoovieR@dut.ac.za. Tel: +27-31-3732808. Fax: +27-31-3732038.

variety of toxicants because their developing organ systems can be more vulnerable to environmental toxicants during critical periods of growth (Perera et al., 1999; Sram et al., 1999, 2005), therefore prenatal exposure to pollution may result in adverse birth outcomes and have implications for poorer health at later stages.

Genetic factors interact with environmental exposures to determine disease risk (Adeyemo and Rotimi, 2010). Africa's genetic diversity, coupled with environmental exposures linked to disease burdens remains with relatively unexplored. Currently, most existing birth cohorts are in high income countries, relatively few in low- and middle-income countries (Lawlor et al., 2009). A systematic review by Campbell and Rudan (2011) revealed that of the 28 birth cohorts in sub Saharan Africa, 14 collected biological data while only one collected DNA for storage.

The Mother and Child Environmental Cohort (MACE) study piloted in South Africa in 2010 to 2011, collected genetic, biochemical and clinical data from communities in Durban, South Africa that have been previously studied for pollution-health outcome associations. The study area, south Durban, is recognised as one of the most highly industrialized and heavily polluted areas in Southern Africa (Matooane, 2000) while a comparison area with relatively lower exposure levels in the north of Durban was used. Previous studies have shown increased levels of sulphur dioxide (SO_2) and oxides of nitrogen in south Durban compared to the north while particulate matter < 10 microns (PM_{10}) were similar in both areas (Naidoo et al., 2007).

Transmission of genetic information in cells requires both accurate replication and the ability to repair DNA damage. There are many enzymes involved in the DNA damage response mechanism and in cellular defense against toxicant induced damage. Enzymes of the glutathione S-transferase (GST) family, such as glutathione S-transferase Mu 1 (*GSTM1*) and glutathione S-transferase pi gene (*GSTP1*) are involved in the detoxification of electrophilic compounds and protect against oxidative stress by free radical scavenging (Wlodarczyk and Nowicka, 2012). Quantitatively, conjugation to glutathione (GSH) catalysed by GSTs is the major detoxification pathway in humans (Dusinka et al., 2012).

Polymorphisms in the genes coding for GSTs that alter expression of GSH can modify risk in individuals exposed to toxins such as pollutants. Additionally, biomarkers such as malonaldehyde (MDA), a carbonyl compound generated by lipid peroxidation which plays a vital role in the pathogenesis of several diseases and inflammatory processes, is a marker of oxidative stress. (Bathi et al., 2009). In the present paper, the influence of polymorphisms in *GSTM1* and *GSTP1* on the extent of DNA damage and the presence of biomarkers related to oxidative stress in mothers exposed and unexposed to pollution were evaluated.

MATERIALS AND METHODS

Selection of Ante-natal clinics and participants

eThekwini Municipality is located on the east coast of South Africa in the province of Kwazulu-Natal (KZN). It spans an area of approximately 2297 km^2 and has a population of 3.5 million people. Four ante-natal public sector clinics of similar socio-economic profiles were selected based on their proximity to eThekwini Municipality Air Quality Monitoring (AQMS) station. Two were in the industrially polluted south (high exposure) and a further two in the lesser polluted north of the city (low exposure). One hundred pregnant women (50 from the south Durban area and 50 from north Durban) were selected from the clinics. The participants had to be residents in the geographical area within which the clinic and AQMS station were located. They had to have been living in this area for the full duration of the pregnancy, and had no plans to move out of the area during the remainder of the pregnancy. The participants had to be living within a 5 km radius of a monitoring station.

Women were excluded if: (1) they experienced the following complications like hypertension, diabetes, placenta previa genital tract infection; (2) the first antenatal visit occurred > 34 weeks and; (3) this was a multiple pregnancy. Human immunodeficiency virus (HIV) status assessed during ante-natal testing was not an exclusion criterion. All pregnant females that met the inclusion criteria were recruited into the study. Participation was on a voluntary basis and only consenting women were enrolled.The Biomedical and Research Ethics Committee at the University of Kwazulu-Natal (UKZN) approved the research protocol. Pregnant women answered a questionnaire collecting demographic, clinical and exposure data. Prenatal clinical data was obtained from patient records. Postnatal data such as gestational age and birth weight were done by the hospital nursery staff using standard World Health Organisation guidelines as per the hospital protocol.

Genetic analysis

Genomic DNA from mothers was extracted from whole blood as described by Sambrook (2001). The *GSTM1* deletion polymorphism was detected via differential polymerase chain reaction (PCR) using β-globin as the positive control (Bell et al., 1993). Primers used were: *GSTM1* (5'GAA CTC CCT GAA AAG CTA AAG C3'; 5'GTT GGG CTC AAA TAT ACG GTG G3'), β-globin (5'CAA CTT CAT CCA CGT TCA CC3'; 5' GAA GAG CCA AGG ACA GGT AC3'). To determine the presence or absence of the *GSTM1* gene, the 268 base-pair (β-globin) and 215 base-pair (*GSTM1*) PCR products were amplified using 30 pmol of forward and reverse primers for the *GSTM1* gene and 10 pmol forward and reverse primers for the β-globin gene in a 25 µl reaction containing 200 µM of each dNTP, 3.3 mM $MgCl_2$, 1× Green GoTaq Flexi buffer (Promega), 1 U GoTaq DNA polymerase (Promega) and 100 ng genomic DNA template. A lack of amplification of the 215 base-pair product in the presence of a 268 base-pair product was indicative of a gene deletion. The presence of both the 268 and 215 base-pair products was indicative of either a homozygous or heterozygous state. The Ileucine (Ile)/Valine (Val) polymorphism in codon 105 of the *GSTP1* gene was determined by restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) (Vibhuti et al., 2007) analysis using the following primers: *GSTP1* (5'ACCCCAGGGCTCTATGGGAA 3'; 5'TGAGGGCACAAGAAGCCCCT-3'). A PCR product was amplified using 15 pmol of forward and reverse primer in a 25 µl reaction containing 200 µM of each dNTP, 1.5 mM $MgCl_2$, 1× Green GoTaq

Flexi buffer (Promega), 0.5 U GoTaq DNA polymerase (Promega) and 100 ng genomic DNA template. Overnight digestion (37°C) was performed in 25 µL (15 µL PCR product, 4.5 µL Buffer-R and 0.5 µL (5 U) BsmAI). Amplicons homozygous for the G allele (Ile) were completely digested and resulted in two restriction fragments (91 and 85 bp). Restriction fragments were electrophoresed on an agarose gel (3%, 0.5 mg/ml ethidium bromide) and visualized.

Biomarker analysis

Quantification of glutathione

The GSH-Glo™ assay (Promega, USA) was used according to manufacturer's guidelines to quantify glutathione.

Comet assay

The comet assay was used to assess DNA tail length as a measure of DNA damage. A layer of low melting point agarose (LMPA) (0.5%) was mixed with whole blood and sandwiched between two 1% LMPA layers on a glass slide. The slides were immersed in freshly prepared lysing solution [2.5 M NaCl, 100 mM Ethylenediaminetetraacetic acid (EDTA), 1 M Tris (pH10), 1% Triton ×100, 10% dimethyl sulfoxide (DMSO)] for 1 h at 4°C and thereafter placed in electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH- pH 13) for 20 min to equilibrate. Slides were then electrophoresed at room temperature (RT) for 35 min at 25 V. After washing slides with 0.4 M Tris (3 ml), pH 7.5 for 5 min, they were stained with 50 µl ethidium bromide (50 µg/ml). Slides were viewed with a Nikon ultra violet (UV) fluorescent microscope with an excitation filter of 515 to 560 nm. Five random groups of 10 cells each were counted for each sample. Nuclear damage was characterized according to the 2 features observed: an intact core of DNA and DNA migration out of the cell (comet effect).

Lipid peroxidation assay (MDA)

The lipid peroxidation assay was used to assess oxidative damage in serum. The thiobarbituric acid (TBA) assay measured the lipid peroxidation end-product, malondialdehyde (MDA). 200 µl of serum was transferred into clean glass tubes. The positive control was 1% malondialdehyde bis (dimethyl acetyl). To this, 400 µl TBA (1%, w/v)/0.1 mM butylated hydroxytoluene (BHT) mixture was added. The solution was adjusted to pH 1.5 and heated to 100°C for 15 min. Butanol (1.5 ml) was added to the mixture after cooling and thereafter centrifuged (10,000 ×*g*) for 6 min to separate organic phases. Aliquots (300 µl) of the butanol phase were transferred into microtitre plates and optical density was measured at 532 nm.

Statistical analyses

Statistical analysis was done using STATA version 11. Data was expressed as mean ± standard deviation (SD) and frequency distributions. The court-lab calculator was used to assess the frequency of alleles in the sample population to determine Hardy-Weinberg statistics. Normality was evaluated graphically and the t-test and analysis of variance (ANOVA) was used for analysis of continuous variables. The Pearsons chi-squared test was used to evaluate bivariate associations between demographic and clinical variables stratified by pollutant exposure and genotype. All p values ≤ 0.05 were considered statistically significant.

RESULTS

Demographic and socioeconomic characteristics of the study population were grouped according to exposure status in Table 1, 83% were of African origin and approximately 87% of the women were single. Although bivariate testing showed a significant difference between ethnicity (Africans and Whites) and exposure (Table 1), this was due to the population residential demographic in south and north Durban. With respect to age, marital status, education, paternal and maternal income, the exposed and low exposed groups were comparable and showed a relatively poor socioeconomic status. The proportion of women who smoked during pregnancy in this pilot was too small (4%) to do any further comparisons. However, environmental exposure to tobacco smoke by a family member smoking was significantly higher in the south of Durban compared to the north of Durban ($p = 0.05$). There were more babies born at less than 36 weeks gestation in the south compared to the north (16 versus 10), and there were 7 babies born with birth weights lower than 2500 g in south Durban compared to 2 innorth Durban. However, there was no statistically significant difference between birth outcomes such as gestational age and birth weight and exposure to environmental pollutants. Three stillbirths were reported.

Genotypic distribution did not deviate from that predicted by the Hardy-Weinberg equilibrium in the cohort. With the maternal genotypes, there were no significant differences in genotypic distribution of both genes between the patients from the low and high exposure areas. Thirty four percent of participants carried the *GSTM1*null genotype in the low exposure area compared with 28% in the high exposure area; while 80% carried the polymorphic *GSTP1* AG+GG genotype in the low exposure area compared to 82% in the high exposure area (Table 1).

Biomarkers related to oxidative stress and DNA damage were quantified and stratified by genotype. The mean GSH (µM) concentration among mothers did not differ significantly between *GSTM1*pos and *GSTM1*null genotypes (5.8 versus 6.3 µM), nor between *GSTP1*AA and *GSTP1* AG+GG (6.5 versus 5.9 µM). The mean comet tail length did not differ between the wild type and the polymorphic genotype for both GSTM1 and GSTP1 (Table 2). Mean MDA levels was significantly higher in *GSTM1*null than in *GSTM1*pos individuals. This remained significant among low exposed participants when genotype was further stratified by area. The GSH and comet tail length did not differ by region with any of the genotypes tested (Table 3). However, when genotype was stratified by exposure, the mean GSH concentration was significantly higher in the lower exposure area for the *GSTM1*pos, *GSTM1*null and GSTP1AG+GG genotypes. Similarly, consistent with increased oxidative stress, the

Table 1. Summary characteristics of study population stratified by high and low pollution area (N = 100).

Characteristic	North Durban	South Durban
Age (mean ± SD)	25.1 (5.7)	25.8 (6.4)
Race		
African	47 (94.0)	36 (72.0)*
White	0	6 (12.0)*
Indian	0	1 (2)
Mixed ethnicity	3 (6.0)	7 (14.0)
Marital Status		
Married	3 (5.9)	8 (15.7)
Living together	1 (2.0)	1 (1.9)
Single	46 (92.1)	41 (82.4)
Education		
Grades 1 through 8	3 (6.0)	3 (6.0)
Grades 9 through 11	11 (22.0)	17 (34.0)
Graduated high school	30 (60.0)	29 (58.0)
College/technikon/university	2 (4.0)	1 (2.0)
Other education	4 (8.0)	0
Gross annual income (mother)		
No income	31 (60.8)	36 (72.0)
Less than R5000	5 (9.8)	2 (3.9)
R5000-R10000	2 (3.9)	2 (3.9)
R10000-R20000	2 (3.9)	2 (3.9)
R30000-R50000	5 (9.8)	4 (7.8)
R50000-R100000	3 (2.2)	4 (7.8)
Smoking		
Maternal smoking	1 (2)	4 (8.0)
Paternal smoking	8 (15.6)	17 (33.3)
Family member smoking	11 (21.6)	20 (39.2)
Maternal Genotyping		
GSTM1pos	31 (65.9)	37 (72.5)
GSTM1null	16 (34.1)	14 (27.5)
^{2}*GSTP1 AA*	10 (20.4)	9(18.0)
GSTP1 AG+GG	39 (79.5)	41 (82.0)
Birth outcome		
Normal	45 (95.7)	46 (97.8)
Stillbirth	2 (4.3)	1 (2.1)
Gestational age		
>37 weeks	36 (78.2)	38 (84.4)
32-36 weeks	9 (19.5)	14 (15.2)
<32 weeks	1 (2.1)	2 (4.4)

Table 1. Contd.

Birth Weight (BW)		
Normal (>2500 g)	42 (93.3)	40 (85.1)
Low BW (1500-2500 g)	3 (6.4)	5 (10.6)
Very low BW (<1500 g)	0	2 (4.3)

Missing data in each category was a refusal to participate. Values in parentheses are percentages of total sample. Income is measured in South African Rands (ZAR). *p-value < 0.05.

Table 2. Maternal biomarkers (mean ± SD) stratified by *GSTM1* and *GSTP1* genotypes.

Genotype	GSH (µM)	MDA (µM)	Comet tail length
GSTM1 pos	5.8 (1.9)	0.06 (0.04)	0.5 (0.1)
GSTM1 null	6.3 (1.9)	0.09 (0.08)*	0.5 (0.1)
*GSTP1*AA	6.5 (2.3)	0.08 (0.05)	0.4 (0.1)
GSTP1 AG+GG	5.9 (1.4)	0.06 (0.05)	0.5 (0.1)

* = p-value < 0.05, the students T-test was used to evaluate differences for all biomarkers.

mean comet tail length was significantly increased in the higher exposure area among participants with the *GSTM1*pos, *GSTM1*null and the *GSTP1*AG+GG genotypes (Table 3).

DISCUSSION

To our knowledge, this is the first report in sub-Saharan Africa to integrate genetic, biochemical and exposure data from a birth cohort. In this pilot study of 100 women for the MACE cohort, it was found that while birth outcome, gestational age and birth weight were not associated with *GSTM1* or *GSTP1* genotypes, biomarkers of oxidative stress were modified by genotype and exposure to air pollutants. The genotoxic and biochemical damage induced by exposure to air pollutants may indicate inter-individual variability which may be due to polymorphic genotypes. These include genes, such as *GSTM1* and *GSTP1*, which are involved in the detoxification of reactive oxygen species (ROS) and other xenobiotics (Sram et al., 2005).

Descriptive analysis of the exposure data from a previous South Durban Health Study (SDHS) revealed that NO_2 concentrations were lowest in the north (mean of 11 ppb), highest in the Durban city centre (19 to 24 ppb), and between 12 to 14 ppb in south Durban. Average SO_2 concentrations varied widely; with low concentrations (1 to 3 ppb) at sites in the north; medium to high concentrations (6 to 10 ppb) at central Durban and (12 to 20 ppb) in the south.

Average PM_{10}concentrations measured using the TEOM method were nearly identical, 38 to 39 µg/m^3 in both north and south communities, and slightly elevated (46 µg/m^3) at one southern site (Naidoo et al., 2007).

Race, marital status education, income and parental smoking were not significantly different between the two areas.

Since the prevalence of smoking in this sample of women was very low (1 in the low exposure and 4 in the high exposure area), it was not considered a significant confounder to the effects of pollutants. However, mothers from south durban were significantly more exposed to environmental tobacco smoke (ETS) by a family member smoking than mothers from the north ($p = 0.05$).

Exposure to ETS, coupled with the high pollutant exposure in south Durban may increase vulnerability to adverse birth outcomes. However, the sample size in this pilot was too low to explore this further. Evidence implicating the adverse effects of pollution on birth outcomes has been reported previously (Sram et al., 2005) and some authors have suggested a biological mechanism linked to impaired placental function and increased DNA adducts in blood and placentas of women exposed to pollutants (Bobak and Leon, 1999).

In this study, there were more adverse outcomes (gestational age and low birthweight) reported for the mothers from the south than north durban but there was no significant association with environmental exposure.

The polymorphism frequency for the *GSTM1* genotype, as determined in our sample, was typical of similar populations described previously (Hanene et al., 2007). A

Table 3. Maternal biomarkers (mean ± SD) stratified by GST genotypes and exposure area.

Stratified by	GSH (uM) n = 100	MDA (uM) n = 99	Comet tail length n = 100
Low exposure			
GSTM1pos	6.48 (1.12)	0.05 (0.02)*****	0.42 (0.08)
GSTM1null	7.19 (2.08)	0.09 (0.07)	0.47(0.10)
GSTP1AA	7.49 (2.86)	0.07 (0.02)	0.48 (0.08)
GSTP1 AG+GG	6.75 (1.49)	0.06 (0.05)	0.46 (0.10)
High exposure			
GSTM1pos	5.24 (0.94)	0.06 (0.05)	0.56 (0.13)
GSTM1null	5.30 (1.14)	0.09 (0.09)	0.52 (0.12)
GSTP1AA	5.39 (0.77)	0.09 (0.07)	0.52 (0.09)
GSTP1 AG+GG	5.23 (1.03)	0.06 (0.06)	0.56 (0.14)
***GSTM1*pos**			
Low exposure	6.61 (1.31)*****	0.05 (0.02)	0.47 (0.09)*****
High exposure	5.25 (0.94)	0.06 (0.05)	0.54 (0.11)
***GSTM1*null**			
Low exposure	7.19 (2.08)*****	0.07 (0.03)	0.46 (0.08)*****
High exposure	5.31 (1.14)	0.08 (0.07)	0.54 (0.11)
***GSTP1*AA**			
Low exposure	6.23 (1.11)	0.08 (0.02)	0.42 (0.08)
High exposure	5.39 (0.77)	0.07 (0.04)	0.50 (0.06)
***GSTP1*AG+GG**			
Low exposure	6.75 (1.49)*****	0.06 (0.02)	0.47 (0.10)*****
High exposure	5.23 (1.03)	0.06 (0.05)	0.55 (0.12)

* = p-value < 0.05, the students T-test was used to evaluate differences for all biomarkers.

*GSTM1*null frequency of 40 to 60% is common in both Caucasians and Asians, while a relatively lower frequency is usually found in the African populations (16 to 36%) (Garte et al., 2001). While fewer studies have been done on *GSTP1* and populations from Africa, we found that 66% of the mothers carried the *GSTP1*AG+GG genotype which was comparable to a previous study among South Africans and Tunisians (Reddy et al., 2010; Hanene et al., 2007).

We hypothesized that maternal exposure to pollution induces DNA damage and affects biomarkers which may be modulated by *GSTM1* and *GSTP1* which are involved in detoxification metabolism and repair. Results from numerous studies on genotoxic exposures as modulated by these genes are conflicting (Dusinska et al., 2012). Some studies have shown that exposure to air pollutants increases the levels of oxidative stress markers (Loft et al., 2008; Sorenson et al., 2003). These effects may be mediated through the formation of cell damaging lipid peroxides and from a decrease in maternal antioxidant reserves (Maroziene and Grazuleviciene, 2002). GSTs are important in antioxidant protection in that GSH plays a central role in the defense against oxidative damage and toxins. When present extracellularly, GSH is able to react directly with cytotoxic aldehydes produced during lipid peroxidation (Barrera, 2012). The mean GSH concentration was significantly higher among participants from the low exposure area which was consistent with the notion that these mothers had reduced oxidative stress compared to the mothers from the high exposure area.

MDA, the end product of lipid peroxidation, is also a useful marker for oxidative stress (Manoj et al., 2011). MDA plays a vital role in the pathogenesis of several diseases and inflammatory conditions such as chronic

obstructive pulmonary disease (COPD) and asthma (Bartoli et al., 2011). Increased levels of MDA have also been found in cancer patients (Bathi et al., 2009). MDA levels were significantly higher among the *GSTM1*null mothers when stratified by area and genotype. Since mothers with the null genotype have a reduced capacity to deal with oxidative attack, elevated MDA levels in these mothers may be related to increased environmental exposures. Persistent environmental onslaught may cause DNA damage which may be influenced by polymorphisms in genes encoding for the proteins involved in the metabolism of xenobiotics (Wlodarczyk and Nowicka, 2012).

DNA damage, as measured by the comet assay in the present study, is a useful marker for the biological effects of toxicant exposure and DNA repair ability (Wlodarczyk and Nowicka, 2012). Increased DNA damage was evident among participants from the high exposure area for the *GSTM1*pos, *GSTM1*null and the *GSTP1*AG+GG genotypes. While Abhishek et al. (2010) showed that *GSTM1*pos was better protected against DNA damage than the *GSTM1*null (4.75 versus 6.01, $p < 0.005$) and Topinka et al. (1997) found that DNA adducts from pregnant Czech women living in a heavily polluted area was significantly increased in individuals with the *GSTM1* null genotype, our study did not show any difference by *GSTM1* genotype. However, there was a statistically significant difference in comet tail length with *GSTP1*AG+GG genotypes between the high and low exposure areas. This concurs with a study by Dusinska et al. (2012) who found an association between GST polymorphisms and DNA damage, with *GSTP1AG*+GG genotypes presenting with the most DNA damage. This may be due to the reduced capacity by this polymorphic genotype to protect against cell damage.

Conclusion

While our pilot study, with a small sample size of 100 mothers, lacked the power to detect associations between exposure and adverse birth and biochemical outcomes, it provided important initial data for future studies. The expression of *GSTM1* and *GSTP1* polymorphic genotypes may lead to varying susceptibility to the adverse effects of pollutants by modifying the response to oxidative stress. Initial findings from this pilot birth cohort indicate that biomarkers related to oxidative stress and DNA damage are adversely affected by increased pollution. *GSTM1* and *GSTP1* may be suitable biomarkers for identifying genetically susceptible mothers, particularly those exposed to pollutants who may require stricter intervention. Future work with the large prospective MACE cohort will include investigating the effects of these genotypes on adverse birth outcomes and exploring the gene-environment interaction in relation to birth outcomes.

REFERENCES

Abhishek S, Kaur N, Lata M, Sharma JK, Sharma A (2010). Association of *GSTM1* and *GSTT1* gene deletions with susceptibility to DNA damage in the pesticide-exposed workers of Punjab. Rejuvenation Res. 13(2-3):281-284.

Adeyemo A, Rotimi C (2010). Genetic variants associated with complex human diseases show wide variation across multiple populations. Pub. Health Genom. 13:72–79.

Barrera G (2012). Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. ISRN Oncology. Vol 2012. Article ID:137289. p. 21.

Bartoli ML, Novelli F, Costa L, Malagrino L, Melosini L, Bacci E, Cianchetti S, Dente FL, Di Franco A, Vagaggini B, Paggiaro PL (2011). Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. Mediators Inflamm. Vol 2011. doi:10.1155/2011/891752.

Bathi RJ, Rao R, Mutalik S (2009). GST null genotype and antioxidants: Risk indicators for oral pre-cancer and cancer. Ind. J. Dental Res. 20(3):298-303.

Bell DA, Taylor JA, Paulson DF, Robertson CN, Mohler JL, Lucier GW (1993). Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene Glutathione-S-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. J .Natl. Cancer Ins. 85(14):1159-1164.

Bobak M, Leon DA (1999). Pregnancy outcomes and outdoor air pollution:an ecological study in districts of the Czech Republic 1986-8. Occup. Environ. Med. 56:539-543.

Dusinska M, Staruchova M, Horska A, Smolkova B, Collins A, Bonassi S, Volkovova K (2012). Are glutathione S transferases involved in DNA damage signalling? Interactions with DNA damage and repair revealed from molecular epidemiology studies. Mutation Res. 736(1-2):130-137.

Garte S, Gaspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL, Baranova H, Bathum L, Benhamou S, Boffetta P, Bouchardy C, Breskvar K, Brockmoller J, Cascorbi I, Clapper ML, Coutelle C, Daly A, Dell'Omo M, Dolzan V, Dresler CM, Fryer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hsieh LL, Ingelman-Sundberg M, Kalina I, Kang D, Kihara M, Kiyohara C, Kremers P, Lazarus P, Le Marchand L, Lechner MC, van Lieshout EM, London S, Manni JJ, Maugard CM, Morita S, Nazar-Stewart V, Noda K, Oda Y, Parl FF, Pastorelli R, Persson I, Peters WH, Rannug A, Rebbeck T, Risch A, Roelandt L, Romkes M, Ryberg D, Salagovic J, Schoket B, Seidegard J, Shields PG, Sim E, Sinnet D, Strange RC, Stücker I, Sugimura H, To-Figueras J, Vineis P, Yu MC, Taioli E (2001). Metabolic gene polymorphism frequencies in control populations. Can epidemiol, Biomark. Prev. 10:1239-1248.

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.

Hanene C, Jihene L, Jamel A, Kamel H, Agnes H (2007). Association of GST polymorphisms with asthma in Tunisian children. Mediators Inflamm. Vol 2007.Article ID 19564, PMC185288.

Lawlor DA, Andersen AN, Batty GA (2009). Birth cohort studies: past, present and future. Int. J. Epidemiol. 38(4):897-902.

Loft S, Hogh-Danielsen P, Mikkelsen L, Risom L, Forchhammer L, Moller P (2008). Biomarkers of oxidative damage to DNA and repair. Biochem. Soc. Trans. 36(5):1071-1076.

Maisonet M, Correa A, Misra D, Jaakkola JJ (2004). A review of the literature on the effects of ambient air pollution on fetal growth. Environ. Res. 95:106–115.

Manoj A, Rao KR, Bhat BV, Venkatesh C, Bobby Z (2011). Oxidative stress induced DNA damage in perinatal asphyxia. Curr. Pediatr. Res. 15(1):19-23.

Maroziene L, Grazuleviciene R (2002). Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environ. Health: A Global Access Science Source, 1:6. Available from: http://www.ehjournal.net/content/1/1/6.

Matooane M (2000). Air pollution and health effects in South Durban - a review of existing data. CSIR Report. ENV-D-C 2002-011. June 2000.

Naidoo RN, Gqaleni N, Robins T, Batterman S (2007) The South Durban Health Study: Final Project Report. University of KwaZulu-Natal, Durban, South Africa; 2007. Available at: http://doeh.ukzn.ac.za/Libraries/Documents/SDHS_FINAL_Report_revision_February_2007.sflb.ashx. Accessed May 2012.

Perera FP, Jedrychowski W, Rauh V, Whyatt RM (1999). Molecular epidemiologic research on the effect of environmental pollutants on the fetus. Environ. Health Perspect . 107:451–460.

Reddy P, Naidoo RN, Robins TG, Mentz G, London SJ, Li H, Naidoo R (2010). *GSTM1*, *GSTP1* and NQO1 polymorphisms in relation to susceptibility to adverse respiratory outcomes among South African schoolchildren. Lung 188(5):409-414.

Sambrook J (2001). Molecular Cloning: A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, New York.

Sorenson M, Autrup H, Hertel O, Wallin H, Knudsen LE, Loft S (2003). Personal expsoure to $PM_{2.5}$ and biomarkers of DNA damage. Cancer Epidemiol. Biomark. Prev. 12(3):191-196.

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.

Šrám RJ, Binková B, Rössner P, Rubeš J, Topinka J, Dejmek J (1999). Adverse reproductive outcomes from exposure to environmental mutagens. Mutat. Res. 428:203–215.

Topinka J, Binková B, Mračková G, Stávková Z, Beneš I, Dejmek J, et al. (1997). DNA adducts in human placenta as related to air pollution and to *GSTM1* genotype. Mutat. Res. 390:59–68.

Vibhuti A, Arif E, Deepak D, Singh B, Qadar Pasha MA (2007) Genetic polymorphisms of *GSTP1* and mEPHX correlate with oxidative stress markers and lung function in COPD. Biochem. Biophy. Res. Comm. 359(1):136-42.

Wlodarczyk M, Nowicka G (2012). Common polymorphisms in CYP1A1, *GSTM1*, GSTT1, *GSTP1* and XPD genes and endogenous DNA damage. Mol. Biol. Rep. 39:5699-5704.