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HIV induced nitric oxide and lipid peroxidation, influences neonatal birthweight in a South African population

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ABSTRACT

HIV has been implicated in adverse birth outcomes, due to increased oxidative stress and inflammation. In addition, HIV has been reported to increase nitric oxide levels. Therefore the combined exposures to HIV and traffic-related air pollution, within South Durban, South Africa (SA), may lead to adverse birth outcomes. However, the exact mechanism is still unknown; this study aimed to identify a potential mechanism. First, the influence of HIV on oxidative and nitrosative stress markers in pregnant women was assessed. Secondly, the effect of these stress makers and exposure to oxides of nitrogen (NOx) on neonatal birthweight (BW) was evaluated. Finally, the effect HIV and traffic-related pollution exposure has on the oxidative and endoplasmic profile and epigenetic regulation of Nrf2-Keap1 pathway by miR-144 and miR-28 in pregnant women was determined. Women, in their third trimester with singleton pregnancies, who were HIV+ and HIV−, were recruited from Durban, SA. Biomarker levels of serum nitrites/nitrates (NO) and malondialdehyde (MDA) were analysed and mRNA expression levels of oxidative and endoplasmic stress response genes were assessed. Land regression modelling was performed to determine NOx exposure levels. HIV exposure during pregnancy was associated with increased NO levels. NO was shown to reduce neonatal BW. NO and MDA was found to reciprocally increase each other, with HIV differentially influencing MDA's effect on BW. HIV down-regulated miR-144 which was negatively associated with *Nrf2*, suggesting a potential mechanism for HIV associated chronic oxidative stress. This study proposes that NO plays a key role in neonatal BW reduction in response to HIV and traffic-related air pollution.

1. Introduction

An estimated 36.7 million people in the world are living with the human immunodeficiency virus (HIV). Of these, 7 million are living in South Africa (SA) and women account for more than half of the total HIV infected population in SA (UNAIDS, 2015). This is of major concern, not only due to the associated morbidity and mortality, but due to increased risk of adverse birth outcomes associated with HIV infection (Xiao et al., 2015).

HIV infection in pregnant women has been reported to increase the susceptibility of neonates to shunting, wasting and reduced weight with increased risk of low birth weight (LBW) and preterm births (PTB) (Xiao

Abbreviations: HIV, human immunodeficiency virus; SA, South Africa; NOx, oxides of nitrogen; BW, birthweight; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Keap1, kelch-like ECH-associated protein 1; mi-RNA, microRNA; NO, nitrites/nitrates; MDA, malondialdehyde; ER, endoplasmic reticulum; PTB, preterm-birth; LBW, low birthweight; NOS, NO synthases; nNOS, neural; iNOS, inducible; eNOS, endothelial; Hb, haemoglobin; LP, lipid peroxidation; PUFA, polyunsaturated fatty acids; SD, South Durban; ND, North Durban; AP, air pollution; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; OGG1, human 8-oxoguanine glycosylase 1; ARE, antioxidant response element; UP, unfolded proteins; UPR, UP response pathway; MACE, Mother and Child in the Environment; dH_2O, deionised water; TBARS, thiobarbituric acid reactive substances; RT, room-temperature; RT-PCR, real-time polymerase chain reaction; cDNA, complementary DNA; GA, gestational age; AIDS, acquired immune deficiency syndrome; O_2, molecular oxygen; BiP, binding immunoglobulin protein; eIF2, eukaryotic Initiation Factor 2; ATF4, activating transcription factor 4; CHOP, CCAAT-enhancer-binding protein homologous protein

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et al., 2015). Factors that affect LBW and PTB pathology include oxidative stress, inflammation, endothelial dysfunction, reduced oxygen transport across the placenta and abnormalities of the placenta (Fleischer et al., 2014). HIV is a lentivirus that infects and kills vital cells of the immune system (Ivanov et al., 2016). Upon HIV infection, it triggers the innate immune response activating macrophages to produce nitric oxide (NO), which is a major mediator of inflammation and apoptosis (Torre et al., 2002). Studies have shown increased levels of NO and inflammatory markers in HIV infected patients (Giovannoni et al., 1998; Torre et al., 2002). HIV has also been associated with increased oxidative stress, where decreased antioxidant capacity and increased oxidative damage have been reported (Ivanov et al., 2016; Simão et al., 2015). Therefore HIV could elicit LBW and PTB as a result of increased inflammation and oxidative stress, with NO potentially playing a key role.

Nitric oxide is an inorganic free radical that is produced endogenously by a family of isoenzymes as a reaction by-product, during the catalytic conversion of L-arginine to L-citrulline (Deng and Deitrich, 2007). Three isoenzymes of NO synthases (NOS) have been identified; neural (nNOS), endothelial (eNOS) and inducible (iNOS). NO readily diffuses across cell membranes; at low concentrations it binds to haemoglobin (Hb) and becomes inactive, whilst in excess NO reacts with superoxide and oxygen to form peroxynitrite and dinitrogen trioxide, respectively. NO and its intermediates are highly reactive and result in macromolecular damage; including modifying cysteine amino acids of target proteins, triggering lipid peroxidation (LP), inhibiting the electron transport chain and oxidising biological thiol-containing compounds. Both NO and peroxynitrite are highly unstable and degrade to nitrates and nitrites, which act as markers of NO concentration (Bakan et al., 2002; Calabrese et al., 2009). Increased levels of NO have been associated with PTB (Chadha et al., 2007).

Lipid peroxidation is the oxidative conversion of polyunsaturated fatty acids (PUFA) to products known as malondialdehyde (MDA) or lipid peroxides. The decomposition of NO and peroxynitrite to form hydroxyl radicals and nitrite radicals initiate LP chain reactions, where hydroxyl radicals and nitrite abstract hydrogen ions from the methylene group of PUFA resulting in an unpaired electron on the carbon that reacts with oxygen to form peroxyl radicals. This sets up the chain reaction of LP, which can continue to cause macromolecular damage or is terminated by antioxidants (Gutteridge, 1995). Several studies have associated increased LP with adverse birth outcomes, especially PTB (Tabacova et al., 1998; Walsh and Wang, 1993).

Exogenous sources of NO include vehicle exhaust fumes and cigarette smoke (Castiglione et al., 2012; Kelly, 2003). Durban, in SA, is a rapidly developing city with increased urbanisation and road traffic, all factors associated with an increase in oxides of nitrogen (NOx) pollution within the atmosphere. This in combination with residential areas in close proximity to busy highways and roads makes individuals highly susceptible to adverse effects of NOx exposure, with infants' in utero being highly vulnerable to the negative effects. It has been reported previously that exposure to NOx and other air pollution (AP) have been associated with increased risk of LBW, PTB and reduced gestational age (GA) (Anderson et al., 2018; Kloog et al., 2012; Seo et al., 2007; Tabacova et al., 1998; Vassilev et al., 2001; Wang et al., 1997). Durban, consisting of a highly industrialised South region (SD) and a less industrialised North region (ND), is an ideal location to investigate the effects of traffic-related AP on birth outcomes.

Endogenous and exogenous toxic insult, viz. exposure to HIV or traffic-related AP, results in macromolecular and cellular damage leading to increased stress responses. Cytoprotection against these insults is provided by antioxidant enzymes such as catalase (CAT), superoxide dismutase 2 (SOD2) and glutathione peroxidase (GPx) (Kensler et al., 2007) and repair enzymes including the human 8-oxoguanine glycosylase 1 (OGG1) which is a key component within the DNA repair pathway. OGG1 is highly susceptible to oxidative modification and has been shown to be inhibited by NO (Bravard et al., 2006; Jaiswal et al., 2001). These responses are regulated via the antioxidant response element (ARE) where nuclear factor (erythroid-derived 2)-like 2 (Nrf2) acts as the key transcription factor. Upon increased oxidative damage and subsequent dissociation from kelch-like ECH-associated protein 1 (Keap1), Nrf2 translocates to the nucleus where it binds to the ARE resulting in transcriptional activation of specific target genes, including antioxidants (Kobayashi and Yamamoto, 2005). Epigenetic regulation of Nrf2-Keap1 pathway by microRNA (miR)-144 and miR-28 has been found to be an important determinate of an individual's response to certain adverse conditions (Yamamoto et al., 2013; Yang et al., 2011). The endoplasmic reticulum (ER) stress response is also activated when there is increased oxidative insult resulting in increased unfolded proteins (UP). Increased UP and ER stress is sensed by ER chaperones, including BiP, which activates the UP response pathway (UPR) to mitigate ER stress (Malhotra and Kaufman, 2007a).

A pilot study conducted by Nagiah and colleagues reported increased markers of oxidative stress within pregnant women living in the heavily industrialised South Durban (Nagiah et al., 2015). However, due to the small sample size, this study did not investigate whether HIV influenced stress profiles, nor their subsequent effect on adverse birth outcomes. As a follow on study we aimed to determine whether HIV infection influenced oxidative and nitrosative stress markers in pregnant women, with consideration taken for the NOx exposure and living area of each women. The effect of the stress markers and NOx exposure on neonatal birth weight (BW) was then investigated. To further understand the oxidative profile of HIV and locational effect on these mothers, gene expression for oxidative and ER stress markers, including epigenetic regulation of the Nrf2-Keap1 pathway by miR-144 and miR-28 were also investigated.

2. Methodology

2.1. Study population

The Mother and Child in the Environment (MACE) longitudinal cohort study recruited HIV negative (HIV−) (n = 230) and positive (HIV+) (n = 126) pregnant women, and followed them through to delivery of the neonate.

They were recruited from public sector anti-natal clinics, having similar socio-economic profiles, two located within the heavily-polluted Durban South region and two located within the less-industrialised Durban North region KwaZulu-Natal, SA. The woman participants met the following inclusion criteria: they had to be residents of the geographical area for the full duration of the pregnancy and have a singleton pregnancy. Women with hypertension, diabetes, placenta previa, genital tract infections and other complications which result in adverse growth effects were excluded from the study. The HIV status of the women was not an exclusion criterion.

Ethical approval from the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BF263/12) and informed consent from study participants was obtained.

2.2. NOx pollution

The exposure levels of atmospheric NOx for individual study participants was determined using land use regression modelling, developed following the ESCAPE approach (Beelen and Hoek, 2010). A multivariate regression model was developed by regressing NOx measurements at selected locations against site-specific pre-selected (i.e. direction of effect) geographic predictors, such as land use types, road length, topography, population and housing density. NOx measurements were collected using Ogawa samplers over two, two-week periods during mid-summer and mid-winter. Measurements were taken at 40 randomly selected sites in the North and South Durban areas. Seasonal variation was accounted for, as the sampling periods i.e. mid-