

# A study of oxidative stress in neonates delivered through meconium-stained amniotic fluid

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**Abstract** To estimate the levels of malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OH-dG) in cord blood plasma of newborns born through meconium-stained amniotic fluid (MSAF) and also to find out the correlation between their levels with birth weight and gestation, we measured the cord blood plasma levels of MDA and 8-OH-dG in 59 newborns born through MSAF and 50 newborns born through clear liquor. The levels of cord blood plasma MDA and 8-OH-dG were significantly higher in full-term and late-preterm newborns born through MSAF. On further comparison, it was found that both full-term and late-preterm intrauterine growth restricted (IUGR) neonates had higher levels of these markers as compared to babies born as appropriate for gestational age (AGA) through MSAF. Plasma levels of MDA and 8-OH-dG were significantly correlated with birth weight even after controlling the relationship with gestational age for all cases as well as all full-term cases. These markers are also significantly correlated to each other.

**Conclusions:** The present study suggest that the neonates born through MSAF experience higher degrees of oxidative

stress, as evidenced by increased levels of cord blood plasma MDA and 8-OH-dG.

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#### What is known:

- Aspirated meconium has been found to induce free radical generation and cellular damage in animal studies.
- Its role in free radical generation and oxidative damage in human neonates is scarce.

#### What is new:

- Neonates born through meconium-stained amniotic fluid experience significant oxidative stress.
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**Keywords** Meconium-stained amniotic fluid · Malondialdehyde · 8-Hydroxy-2-deoxyguanosine · Intrauterine growth retardation

#### Abbreviations

8-OH-dG	8-Hydroxy-2-deoxyguanosine
AGA	Appropriate for gestational age
IUGR	Intrauterine growth retardation
MAS	Meconium aspiration syndrome
MDA	Malondialdehyde
MSAF	Meconium-stained amniotic fluid

#### Introduction

Meconium is a sterile, thick, blackish green, odorless material observed in the fetal intestine as early as the third month of gestation composed of water (approximately 80%), mucopolysaccharides, bilirubin, intestinal enzymes, hair, and squamous cells [2]. Meconium-stained amniotic fluid (MSAF) complicates deliveries in about 12 to 16% of cases [21], primarily in situations of advanced fetal maturity or in utero fetal distress. It

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is associated with higher rate of cesarean delivery, increased need for resuscitation, and meconium aspiration syndrome (MAS) [39]. The risk factors for MSAF are both maternal and fetal. The maternal factors are hypertension, gestational diabetes mellitus, maternal chronic respiratory or cardiovascular diseases, post-term pregnancy, preeclampsia, and eclampsia. The fetal factors include oligohydramnios, intrauterine growth restriction, and poor biophysical profile [14].

The rates of MAS have shown a dramatic decline in developed countries largely attributed to the reduction in post-term deliveries, more frequent detection of non reassuring fetal heart rate patterns, and increased use of amnioinfusion [47]. However, it still remains a serious problem in developing countries with incidence from India reported to be as high as 13% [31]. MAS is associated with increased risk of respiratory failure, air leaks, persistent pulmonary hypertension [11], mortality, and long-term pulmonary and neurodevelopmental sequelae [45]. Inflammation and damage to lung tissue can be caused by the aspirated meconium due to the presence of both hydrophilic (consisting of gastrointestinal enzymes including pancreatic phospholipase A2 or bile acids) and hydrophobic (consisting of cholesterol, free fatty acids, etc.) fractions of meconium. In addition, meconium contains variable amounts of cytokines (IL-1b, IL-6, IL-8, TNF $\alpha$ ) and heme [12]. Consequently, components of meconium enhance chemotactic activity of polymorphonuclears (PMNs), particularly of neutrophils, and stimulate their leak through the alveolocapillary membrane [46]. It also indirectly induces inflammation, as it stimulates expression of cytokines (e.g., TNF $\alpha$ , IL-1, IL-6, IL-8), pro-inflammatory enzymes (phospholipase A2, proteases), and other bioactive substances such as derivatives of arachidonic acid, endothelin-1, and platelet activating factor from the activated cells. Furthermore, the cells generate large amounts of reactive oxygen species (ROS) leading to oxidative stress [10, 41]. All the mentioned substances predispose to subsequent surfactant dysfunction, parenchymal damage, edema, pulmonary vasoconstriction, and bronchial smooth muscle contraction [22].

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify it or to repair the resulting damage. It is a physiological event during the normal intrauterine- to extrauterine transition, which greatly increases the production of free radicals and must be controlled by the antioxidant defense system, the maturation of which follows the course of the gestation. Adequately mature and healthy infants are able to tolerate this drastic change in the oxygen concentration. Problem arises when the intrauterine development is incomplete or abnormal. Preterm and intrauterine growth-retarded (IUGR) neonates are typically of this kind [26, 27]. Oxygen free radicals are extremely reactive chemical species that react with several living cell contents, e.g., phospholipids, aminoacids, and nucleic acids leading to lipid peroxidation, DNA strand breaks, and

base methylation. Prime targets of reactive oxygen species are the PUFAs (polyunsaturated fatty acids) in cell membranes causing lipid peroxidation, which may lead to damage of the cell structure and function [19]. Additionally, decomposition of lipid hydroperoxides yields a wide variety of end products, including malondialdehyde (MDA) which can be assessed biologically as a measure of lipid peroxidation. Reactive oxygen species such as superoxide radical ( $O^{\cdot -}$ ) and hydrogen peroxide ( $H_2O_2$ ) do not directly react with DNA [16].

But the  $H_2O_2$  crosses the nuclear membrane and reacts with ions of iron or copper to form highly toxic hydroxyl radicals ( $OH\cdot$ ) which directly react with DNA bases [15]. Among the oxidative bases, 8-OH-dG is the most abundant and accepted as a sensitive marker for oxidative DNA damage [17].

Diseases resulting from oxidative damage are grouped and categorized as *free radical-mediated diseases* (FRD) [33]. FRD in neonates include retinopathy of prematurity, bronchopulmonary dysplasia, periventricular leukomalacia, intraventricular hemorrhage, necrotizing enterocolitis, and patent ductus arteriosus [32–34]. In a study by Arjmand et al. measuring prooxidant-antioxidant balance in umbilical cord blood of neonates born through MSAF versus clear liquor showed that the former are exposed to oxidative stress as compared to the latter [3].

The identification of reliable biomarkers is essential for the characterization of oxidative stress (OS) and probably for the early discovery of OS-associated diseases. Biomarkers evaluate host susceptibility to OS by measuring proteins, lipids, and DNA damage. They can be used as “intermediate endpoints or early-outcome predictors” of disease development [7, 30]. The biomarkers that have been studied in various disorders of oxidative stress in neonates include advanced oxidation protein products [8, 9], total hydroperoxide [7–9], carbonylated proteins [38], acrolein-lysine adduct [42, 43], MDA [25, 28, 29], and 8-OH-dG [25, 28, 29].

There is a paucity of literature on markers of oxidative stress in neonates delivered through MSAF. There is just one study that showed that neonates delivered through MSAF have high concentration of 8-iso-prostaglandin F2 $\alpha$  in neonatal cord blood, as a marker of lipid oxidation, and suggests that these infants were exposed to oxidative stress [20]. We hypothesized that delivery through MSAF is associated with oxidative stress in infants which is responsible for the acute and long-term complications. The present study was conducted to evaluate the oxidative stress markers (MDA, 8-OH-dG) in neonates delivered through MSAF and thus to ascertain their role in this condition.

## Methods

This cross-sectional study was conducted in the Division of Neonatology, Department of Pediatrics, Institute of Medical

Sciences and the Department of Biophysics, Banaras Hindu University, Varanasi, India, between September 2009 and April 2011. The study was approved by the Institute Ethics Committee, and informed consents were taken from parents before inclusion in the study.

## Study population

### *Inclusion criteria*

All term ( $\geq 37$  weeks) and late-preterm neonates (35–36 weeks) born through MSAF were included as cases, whereas those born through clear liquor were included as controls.

### *Exclusion criteria*

Difficulty in collecting sample within 30 min of delivery, severe congenital malformation, and mothers suffering from severe illnesses were part of the exclusion criteria.

In the absence of previous studies in the same topic, a convenient sample size of 109 babies was chosen.

## Clinical data

Detailed base line information of maternal and neonatal variables were recorded including number of antenatal visits (booked pregnancy was defined as  $\geq 4$  and unbooked as  $< 4$  antenatal visits), gestational age at delivery, maternal medical and reproductive history, use of continuous electronic fetal monitoring for detection of fetal distress during active labor, presenting part of the baby at the time of delivery, mode of delivery and Apgar scores at 1 and 5 min. Weight of the newborn was recorded immediately after delivery in Seca weighing scale with an accuracy within 5 g.

IUGR status was defined as discrepancy between gestational age assessment by clinical examination and antenatal scans of  $> 3$  weeks and/or estimated fetal weight  $< 10$ th centile as per standards using antenatal scan.

In the absence of blood gases, we defined perinatal asphyxia as Apgar score of  $\leq 6$  at 1 min which is in accordance with the National Neonatal-Perinatal Database of India [24].

Meconium aspiration syndrome was defined as the presence of any two of the following: [24]

- Meconium staining of liquor or of nails or umbilical cord or skin
- Respiratory distress soon after birth, within 1 h of birth
- Radiological evidence of aspiration pneumonitis (atelectasis and or hyperinflation)

## Laboratory analysis

Ten-milliliterS of free flowing cord blood of all the neonates (cases and controls) were collected in a sterile, heparinized, deionized polyethylene vials within 30 min of delivery. Plasma was separated from the blood samples immediately by centrifugation at 1500g for 10 min and stored in separate deionized vials at  $-20$  °C.

### *Malondialdehyde—marker of lipid peroxidation*

Plasma malondialdehyde (MDA) levels in the samples were assayed by thiobarbituric acid reactive substances (TBARS) technique of Philpot [35]. The plasma (1 ml) was mixed thoroughly with 2 ml of TCA–TBA–HCl (15% w/v TCA and 0.375% w/v TBA in 0.25 N HCl). The mixture was heated in a boiling water bath for 15 min. Thereafter, samples were centrifuged at 750g for 10 min. The absorbance of the sample was determined at 530 nm in a spectrophotometer against a suitable blank. The MDA concentration of each sample was calculated by using extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### *8-OH-dG—marker of oxidative DNA damage*

Plasma of all the samples was used for the measurement of 8-OH-dG levels using competitive in vitro enzyme linked immunosorbent assay (ELISA) kit obtained from Caymen Chemical Company, U.S.A. [40]. 8-OH-dG measurements were performed using microtiter ELISA plate pre-coated with anti-mouse IgG. Fifty microliters plasma, 50  $\mu\text{l}$  8-OH-dG AChE (acetylcholinesterase) tracer, and 50  $\mu\text{l}$  8-OH-dG monoclonal antibody were added to each well and incubated at 4 °C for 18 h. Thereafter, the wells were washed for five times, and 200  $\mu\text{l}$  Ellman's reagent was added to each well. The wells were incubated at room temperature in the dark for 100 min. The absorbance was read at wavelength of 420 nm. ELISA assay displays IC<sub>50</sub> (50% B/B<sub>0</sub>) and IC<sub>80</sub> (80% B/B<sub>0</sub>) values of approximately 100 and 30 pg/ml, respectively.

## Statistical analysis

Statistical analysis was done using the SPSS statistical package (version 16.0). Data were expressed as mean  $\pm$  standard deviation (SD) for continuous variables and as percentages for categorical variables. Student's *t* test (unpaired) was used for analysis of continuous variables. Categorical variables were compared by chi-square test or Fisher's exact test. Correlations between variables were studied by Pearson's correlation test. A value of  $p < 0.05$  was considered statistically significant.

## Results

The flow of participants into the study is presented in Fig. 1. For statistical analysis, the cases were further subclassified into those who were appropriate for gestational age (AGA) and those who were intrauterine growth restricted (IUGR). Table 1 shows the baseline characteristics of study participants. The MSAF group had significantly higher number of unbooked pregnancies, primiparous mothers, neonates having fetal distress during active labor, breech presentation at the time of delivery, and cesarean deliveries. These babies were born at a significantly higher gestational age than the control group (3 babies were post-term in cases and none in control arm). However, the two groups were comparable in birth weight, Apgar score at 1 and 5 min, hypertensive disorders of pregnancy, IUGR status, and IUGR babies born vaginally versus delivered by cesarean section (16 vaginally born vs 17 delivered by cesarean section).

Table 2 shows the concentration of oxidative damage markers in umbilical cord blood of full-term newborn delivered through MSAF as compared to clear liquor including both AGA and IUGR babies. The extent of lipid peroxidation and oxidative DNA damage as evidenced by MDA and 8-OH-dG, respectively, was significantly increased in MSAF group as compared to control subjects ( $2.43 \pm 0.89$  vs  $0.94 \pm 0.24$  for MDA, and  $55.36 \pm 12.4$  vs  $24.60 \pm 13.49$  for 8-OH-dG, respectively). Full-term IUGR and AGA MSAF group had significantly higher levels of MDA and 8-OH-dG levels as compared to their respective controls. It was also found that full-term IUGR MSAF group had significantly higher levels of MDA and 8-OH-dG levels as compared to full-term AGA MSAF group ( $3.24 \pm 1.07$  vs  $2.08 \pm 0.52$  for MDA, and  $67.12 \pm 12.21$  vs  $50.38 \pm 8.77$  for 8-OH-dG, respectively). Further, the levels of 8-OH-dG were significantly higher in

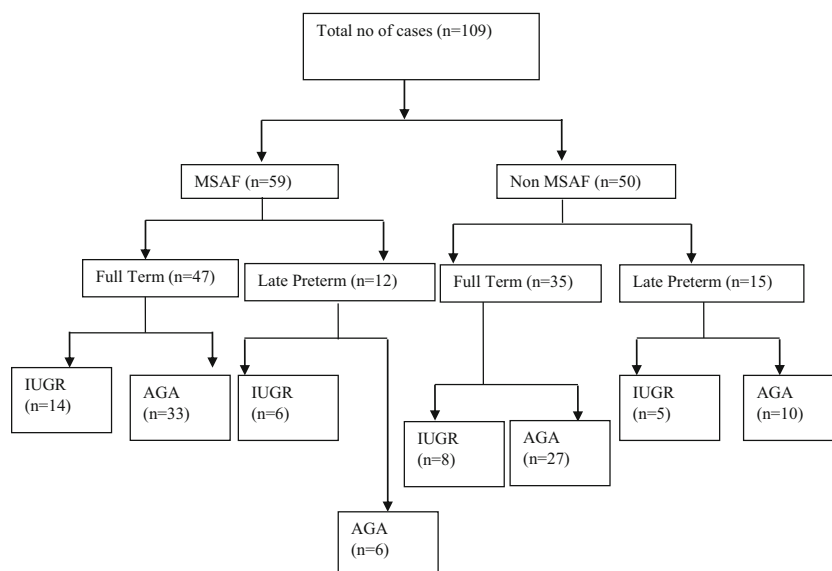
full-term IUGR control as compared to full-term AGA control ( $36.73 \pm 11.02$  vs  $21.01 \pm 12.11$ ). However, the levels of MDA were comparable in both groups.

Table 3 shows the concentration of oxidative damage markers in umbilical cord blood of late-preterm newborn delivered through MSAF as compared to clear liquor including both AGA and IUGR babies. Levels of MDA and 8-OH-dG were significantly higher in MSAF group as compared to control ( $2.83 \pm 0.65$  vs  $1.11 \pm 0.57$  for MDA, and  $56.63 \pm 10.27$  vs  $39.59 \pm 5.39$  for 8-OH-dG, respectively). Late-preterm IUGR and AGA MSAF group had significantly higher levels of MDA and 8-OH-dG levels as compared to their respective controls. It was also found that late-preterm IUGR MSAF group had higher means for MDA and 8-OH-dG levels as compared to late-preterm AGA MSAF group ( $3.38 \pm 0.29$  vs  $2.32 \pm 0.52$  for MDA and  $64.86 \pm 7.77$  vs  $49.36 \pm 6.61$  for 8-OH-dG, respectively). However, the levels of MDA and 8-OH-dG in late-preterm IUGR control was comparable to late-preterm AGA control.

In the present study, 6 babies (5 full term and 1 late pre-term) developed meconium aspiration syndrome. The mean MDA and 8-OH-dG levels in these subgroups of babies were  $3.17 \pm 0.74$  mmol/L and  $70.08 \pm 12.69$  pg/ml, respectively.

Table 4 showed that plasma MDA levels were significantly correlated with birth weight even after controlling the relationship between MDA and gestation, when all MSAF deliveries were considered. Similar trend persisted when all full-term MSAF deliveries were analyzed as a group. The plasma 8-OH-dG levels were significantly correlated with birth weight as well as gestation age; however, the relationship between 8-OH-dG and gestational age became insignificant when relation was controlled for birth weight. All full-term babies delivered through MSAF revealed significant correlation between birth

**Fig. 1** Flow diagram of study subjects and control



**Table 1** Comparison of baseline characteristics of cases with control

	Cases (n = 59)	Control (n = 50)	Chi-square	p value
<b>Demographics</b>				
Booked <sup>a</sup>	27 (45.7%)	33 (66%)	4.48	0.034*
Primiparity	43 (72.8%)	23 (46%)	8.18	0.04*
Hypertensive disorder <sup>c</sup>	20 (33.8%)	17 (34%)		NS
<b>Delivery complications</b>				
Fetal distress				
Present	15 (25.4%)	2 (4%)	9.44	0.02*
<b>Apgar</b>				
1 min {median (IQR)}	8 (6–9)	8 (7–9)		NS}
5 min {median (IQR)}	8 (7–9)	8 (8–9)		NS
<b>Presentation</b>				
Vertex	28 (47.4%)	37 (74%)		
Breech	30 (50.8%)	12 (24%)	8.27	0.006 <sup>#</sup>
Others	1 (1.6%)	1 (2%)		
<b>Mode of delivery<sup>b</sup></b>				
Vaginal	17 (28.8%)	27 (54%)		
Cesarean	41 (69.4%)	22 (44%)	7.79	0.012 <sup>#</sup>
Forceps	1 (1.6%)	2 (4%)		
IUGR	20 (33.8%)	13 (26%)		NS
			<i>t</i> value	<i>p</i> value
Birth weight in grams (mean ± SD)	2540 ± 508	2500 ± 549	0.52	NS
Gestational age in weeks (mean ± SD)	37.91 ± 2.00	37.10 ± 2.15	2.04	0.043*

NS non significant

\*Statistically significant by chi-square test at 5% level of significance

<sup>#</sup> Statistically significant by Fisher’s exact test at 5% level of significance

<sup>a</sup> Defined as ≥4 antenatal visits during pregnancy

<sup>b</sup> Among IUGR babies (16 were born vaginally vs 17 by cesarean)

<sup>c</sup> Hypertensive disorder includes gestational hypertension, preeclampsia, eclampsia, and pregestational hypertension

weight and 8-OH-dG even after controlling the relationship for gestation. Correlation between MDA and 8-OH-dG levels in all subjects was also found to be highly significant (*r* 0.6173 and *p* < 0.0001). However, in the late-preterm MSAF group, no significant correlation was found between these oxidative stress markers with birth weight or gestational age as well as no significant inter-correlation was found between these markers.

**Discussion**

Meconium-stained amniotic fluid (MSAF) complicates about 10 to 20% of deliveries in which enzymatic antioxidant defenses fails and oxidative damage to the tissues occurs [3]. The present study evaluated whether babies born through MSAF show higher degree of oxidative stress in the cord blood as compared to babies born through clear liquor as well as to

**Table 2** Plasma malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OH-dG) levels in full-term babies (gestational age of ≥37 weeks) born through meconium-stained amniotic fluid and controls

	No. of babies	MDA levels (mmol/l)(mean ± SD)	<i>t</i> value <i>p</i> value	8-OH-dG levels (pg/ml)(mean ± SD)	<i>t</i> value <i>p</i> value	
IUGR	MSAF	14	3.24 ± 1.07	5.93	67.12 ± 12.21	5.78
	Controls	8	0.94 ± 0.24	<0.0001	36.73 ± 11.02	<0.0001
AGA	MSAF	33	2.08 ± 0.52	6.003	50.38 ± 8.77	3.75
	Controls	27	0.93 ± 0.25	<0.0001	21.01 ± 12.11	<0.0001
Total	MSAF	47	2.43 ± 0.89	39.165	55.36 ± 12.4	10.669
	Control	35	0.94 ± 0.24	<0.0001	24.60 ± 13.49	<0.0001

**Table 3** Plasma malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OH-dG) levels in late-preterm babies (gestational age of 35–36 weeks) born through meconium-stained amniotic fluid and controls

		No. of babies	MDA levels (mmol/l) (mean ± SD)	<i>t</i> value <i>p</i> value	8-OH-dG levels (pg/ml) (mean ± SD)	<i>t</i> value <i>p</i> value
IUGR	MSAF	6	3.38 ± 0.29	7.72	64.86 ± 7.77	5.30
	Controls	5	0.88 ± 0.13	<0.0001	42.84 ± 5.49	<0.001
AGA	MSAF	6	2.32 ± 0.52	3.36	49.36 ± 6.61	3.99
	Controls	10	1.23 ± 0.68	<0.0004	37.97 ± 4.80	0.0013
Total	MSAF	12	2.83 ± 0.65	7.23	56.63 ± 10.27	4.346
	Control	15	1.11 ± 0.57	<0.0001	39.59 ± 5.39	<0.001

find out the correlation between plasma MDA and 8-OH-dG levels with birth weight and gestation as well as to each other.

In the present study, most of the babies born through MSAF belonged to unbooked primiparous mothers and were born at a higher mean gestational age, which are consistent with the previous studies of Mundhra et al. [23] and Sankhyan N et al. [36].

In our study, the breech presentation was more commonly associated with in utero passage of meconium, which is in agreement with the previous study by Balchin et al. [4].

In the present study, most of the cases had fetal distress during active labor and were born through cesarean section. Saunder et al. also found fetal distress as a significant predictive factor for in utero passage of meconium with cesarean sections being performed twice as frequently which were largely due to increased rates of fetal distress, failure of progression of labor, and aggressiveness of obstetricians in managing a fetus with in utero passage of meconium [37].

Free radical injury has been implicated in the pathogenesis of several neonatal diseases including perinatal asphyxia, but the status of free radical injury in babies born through MSAF remained unexplored. A study on prooxidant-antioxidant balance (PAB) assay in umbilical cord blood of infants delivered through MSAF found significantly increased PAB value in cord blood of these infants suggesting that they are exposed to oxidative stress [3].

We have found significantly higher serum MDA levels in both full-term and late-preterm babies born through MSAF as compared to those born through clear liquor. Even after excluding IUGR from the analysis, serum MDA levels were significantly higher in AGA babies (include both full term and late preterm) born through MSAF as compared to those born through clear liquor. Lipids are the most susceptible to oxidative damage. Estimation of lipid peroxidation products in cord blood has been proposed as a reliable marker of ROS activity in the fetus and as a marker of perinatal outcome [25]. This observation is in agreement with the previous studies by Bhatia et al. [5] and Wang et al. [44]. However, the sample size of both the previous studies was small, and subgroup comparison (IUGR and AGA) was not done.

Our study have also shown that IUGR babies (include both full term and late preterm) born through MSAF have significantly higher levels of MDA as compared to those born

through clear liquor suggesting that they are additionally subjected to further oxidative stress due to meconium. It was further found that serum MDA levels in IUGR babies delivered through MSAF were significantly higher as compared to the AGA babies delivered through MSAF. There is sufficient evidence in literature to conclude that IUGR babies have significantly higher levels of markers of lipid peroxidation (MDA) in cord blood as compared to the AGA babies [6, 13, 18]. Whether they are subjected to further elevation in the levels of MDA due to meconium per se is not known.

We have found significantly higher serum 8-OH-dG levels in both full-term and late-preterm babies born through MSAF as compared to clear liquor. Further, it was additionally found that both IUGR and AGA babies (include both full term and late preterm) born through MSAF had significantly higher levels of 8-OH-dG as compared to those born through clear liquor. It was also found that both full-term and late-preterm IUGR babies born through MSAF had significantly higher levels of 8-OH-dG as compared to the AGA born through MSAF. Currently, 8-OH-dG has been used as a sensitive marker for oxidative DNA damage in various studies [25, 26, 28, 29]. But till now, there is no human study related to 8-OH-dG as a marker of oxidative DNA damage in cord blood of neonates delivered through MSAF. There is a related animal study conducted in Finland on newborn piglets born through MSAF by Minna Aaltonen et al. [1] which showed higher levels of 8-OH-dG in hippocampal neurons at 6 h following meconium instillation in the hippocampus of newborn piglets. The present study suggests that babies delivered through MSAF are exposed to higher degree of oxidative stress in cord blood than those delivered through clear liquor.

Further, we examined whether any correlation exists between these oxidative stress markers with birth weight or and gestational age as well as any intercorrelation between these markers. Our result showed that MDA levels were significantly correlated with birth weight even after controlling the relationship between MDA and gestation, when all MSAF deliveries were considered. Similar trend persisted when all full-term MSAF deliveries were analyzed as a group. The plasma 8-OH-dG levels were significantly correlated with birth weight as well as gestation; however, the relationship between 8-OH-dG and gestation became insignificant when relation was controlled for birth weight. All full-term babies

**Table 4** Correlation coefficients between plasma MDA and 8-hydroxy-2-deoxyguanosine (8-OH-dG) levels with birth weight and gestation in different subgroups of MSAF deliveries

	All cases ( <i>n</i> = 59)	All AGAs ( <i>n</i> = 39)	All IUGR ( <i>n</i> = 20)	All full-term AGA ( <i>n</i> = 33)	All full-term IUGR ( <i>n</i> = 14)	All full term ( <i>n</i> = 47)
rGest, MDA	<i>r</i> −0.154	<i>r</i> −0.191	<i>r</i> 0.016	<i>r</i> 0.086	<i>r</i> 0.0311	<i>r</i> −0.029
rBW, MDA	<i>r</i> −0.447***	<i>r</i> −0.162	<i>r</i> −0.118	<i>r</i> −0.050	<i>r</i> −0.222	<i>r</i> −0.455***
rBW, MDA Gest	<i>r</i> −0.456***	<i>r</i> −0.017	<i>r</i> −0.281	<i>r</i> 0.000	<i>r</i> −0.369	<i>r</i> −0.475
rGest, MDA BW	<i>r</i> 0.184	<i>r</i> −0.605	<i>r</i> 0.257	<i>r</i> −0.070	<i>r</i> 0.305	<i>r</i> 0.158
rGest, 8-OH-dG	<i>r</i> −0.270*	<i>r</i> −0.396**	<i>r</i> −0.074	<i>r</i> −0.235	<i>r</i> −0.172	<i>r</i> −0.125
rBW, 8-OH-dG	<i>r</i> −0.581***	<i>r</i> −0.296*	<i>r</i> −0.227	<i>r</i> −0.037	<i>r</i> −0.369	<i>r</i> −0.607***
rBW, 8-OH-dG Gest	<i>r</i> −0.549***	<i>r</i> −0.034	<i>r</i> −0.342	<i>r</i> −0.124	<i>r</i> −0.409	<i>r</i> −0.606***
rGest, 8-OH-dG BW	<i>r</i> 0.151	<i>r</i> 0.278	<i>r</i> 0.272	<i>r</i> −0.261	<i>r</i> 0.204	<i>r</i> −0.120

Gest gestational age, BW birth weight, *r* correlation coefficient

\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001

delivered through MSAF revealed significant correlation between birth weight and 8-OH-dG even after controlling the relationship for gestation. The correlation between MDA and 8-OH-dG levels in all subjects was also found to be positive and highly significant. This observation is in agreement with the previous studies by Negi et al. [25]. However, in the late-preterm MSAF group, no significant correlation was found between these oxidative stress markers with birth weight or gestational age as well as no significant intercorrelation was found between these markers. This could be explained by the small sample size in late-preterm group (*n* = 12) in our study and needs further evaluation in a larger study.

On the basis of the present study, we suggest that the levels of oxidative stress markers are elevated in neonates born through MSAF. In addition to meconium, activated leukocytes as well as structural cells of the airway induce production of oxygen radicals, proteases, and cytokines leading to lipid peroxidation and oxidative DNA damage. These ROS have important effect on a variety of lung cells as regulator of signal transduction, activators of key transcription factors, and modulators of gene expression and apoptosis. The levels of these free radicals may be high enough to counteract the effect of normally increased levels of antioxidant system in response to inflammation. Therefore, we suggest that the levels of MDA and 8-OH-dG may also be used as predictive parameters at birth for neonates who subsequently develop meconium aspiration syndrome or perinatal asphyxia, but further research is necessary involving biomarkers as well as its clinical validation. One also needs to establish normative gestational age dependent data, along with their definite cutoff values. Our study is limited by small sample size, failure to establish if similar effect is also seen in neonates who subsequently develop MAS in a large sample of babies.

To conclude, although the incidence of MAS has decreased in developed countries, it poses a significant problem in developing nations. Lack of antenatal care, primiparity, fetal

distress during active labor, breech presentation, and advanced gestational age at the time of delivery are the risk factors for in utero passage of meconium. Higher levels of MDA and 8-OH-dG have been documented as markers of oxidative stress in babies born through MSAF. Levels of these oxidative stress markers are higher in IUGR as compared to AGA babies born as either late preterm or full term. Correlation of MDA and 8-OH-dG with birth weight as well as with birth weight even after controlling the relationship with gestation was significantly higher for all cases as well as all full-term cases. These markers are also significantly correlated to each other.

**Authors' contributions** Dr. Tapas Bandyopadhyay collected the data, carried out the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted.

Dr. B.D. Bhatia conceptualized and designed the study and critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

Dr. H.D. Khanna critically reviewed and revised the manuscript, and approved the final manuscript as submitted.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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