

# The Role of 8-Hydroxy-2'-Deoxyguanosine and Total Antioxidant Capacity in Women with Preterm Birth: A Preliminary Study

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## ABSTRACT

**Objective:** Pregnancy is associated with increased oxidative stress, and exaggeration of oxidative damage is significant in occurrence of pregnancy complications. The preterm or premature births are linked at least in part with oxidative stress (OS) and DNA damage. Therefore, the study was conducted with the aim to assess the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and total antioxidant capacity (TAC) in the women with or without Preterm birth (PTB).

**Material & Methods:** A total of 77 women comprising full term (controls; n=39) and preterm (cases; n=38) birth were enrolled. Predesigned proforma were filled to collect demographic, personal, medical and reproductive history of the subjects. The concentrations of 8-OHdG and TAC were determined in serum of subjects by ELISA technique.

**Results:** A higher 8-OHdG concentrations were found in the serum of women with PTB compared to control group i.e. full-term birth while subjects with PTB had slightly lower level of TAC with respect to the control. Further, a significant negative correlation ( $p < 0.05$ ) between 8-OHdG and TAC levels was observed. This indicates that as the DNA damage increases, there is a decrease in total antioxidant capacity. Risk factors analysis by logistic regression demonstrated that the risk factors such as mental stress (OR 5.63, 95 CI 1.35 to 23.47,  $p < 0.05$ ) and 8-OHdG (OR 1.14, 95 CI 1.00 to 1.30,  $p < 0.05$ ) were associated with preterm birth.

**Conclusion:** The data obtained so far reveals that the DNA damage marker (8-OHdG) and mental stress were might be associated with preterm birth.

**Keywords:** Antioxidant capacity, DNA damage, Mental stress, Oxidative stress, Preterm birth.

## INTRODUCTION

Premature birth (PTB) is defined as live babies born not after 37 weeks of gestation. PTB is a worldwide reproductive public health problem, its cause may be multi factorial in origin, encountered more among lower income countries and it is associated with elevated mortality and morbidity. About 9 % of infants are born preterm in prosperous countries with respect to 12% of infants are born pre-term in lesser income countries. Further, weaker families are at more risk of manifestation of PTB

then affluent families within countries. [1] There might be several causes for the manifestation of PTB. Numerous factors i.e. emotional, racial, social, stress, multiple pregnancies, maternal anxiety, diabetes, high blood pressure, infections during pregnancy etc. shown to be linked with PTB. [2] The oxidative stress (imbalance between oxidant and antioxidant) might also have some role in causation of PTB or preterm labour along with other causes. Ahamed *et al.* reported that impaired oxidant/antioxidant status is connected in

several pregnancy associated difficulties and its outcome. They reported that oxidative stress (OS) induced by lead may be the primary causes of preterm delivery (PTD), indicating the role of OS in PTD. [3] Recently Moore *et al.* reviewed the data with regards to OS and PTB. They reported that an impairment within oxidants and antioxidants status may be related with PTB. The measurements and results to date limit understanding and interpretation. Additional, research is necessary to advance knowledge on this issue of reproduction. [4]

Oxidative stress, can be described as an inequity among antioxidant capacity status and reactive oxygen species or free radicals' generation, has obtained insufficient attention with respect to PTB. The excessive creation of free radicals and inadequate antioxidant availability in the body may play vital role through various pathways in several pregnancy related complications. Reactive oxygen species are formed because of both normal metabolism and through exogenous environmental sources. It has been reported that unstable free radicals attack cellular components which causes harm to lipids, proteins, DNA etc. and this can initiate a chain of procedures resulting various diseases and disorders. [5] Further, Agarwal *et al.* reported that in order to counter ROS and reduce cellular damage, all the organisms have developed composite antioxidant system in the body. The total of food-derived and endogenous antioxidants represents the total antioxidant activity. Thus, the assessment of total antioxidant capacity offers more pertinent biological information with respect to the assessment of individual antioxidant components. [6] Several biomarkers are available to assess oxidative stress in the body, and each biomarker is formed through an exclusive mechanism and has the potential for divergent downstream physiological effects. The 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidized nucleoside is released upon repair of damaged DNA, is exploited frequently as a marker of OS. [7] The 8-

OHdG has been utilized extensively as a biomarker to assess endogenous oxidative DNA damage but also utilized as a risk factor for numerous diseases, disorders and cancer. [8] Therefore, 8-OHdG and TAC level was measured in serum of subjects with full term and preterm to understand their role in PTB.

## MATERIALS & METHODS

The study is basically a hospital-based case-control study in which a total of 77 women (39 with full term and 38 with PTB) were enrolled from the Gynecology ward, civil hospital, Ahmedabad. This paper is a part of a major project entitled "Relationship if any between oxidative stress and lifestyle factors with respect to preterm delivery" for which ethical approval was taken from the institutional human ethical committee, National Institute of Occupational Health, Ahmedabad. A written consensus was also attained from each participant after explaining the aims and objectives of the study as well as ethical issues involved. The subjects with the following criteria were included: subjects with gestational age <37, absence of general diseases, without any symptoms of infection, and without systemic use of medications that could influence immuno-inflammatory reactions or oxidative stress. The subjects included were of homogenous group and were comparable with regards to demographical aspects such as age, food habits and socioeconomic status. Subjects of full term birth were also included as control for comparison purpose.

Pre-designed and pretested proformas were filled through questionnaire interview of the mothers who delivered pre and full-term babies to collect the details of personal, demographic, occupational, lifestyle, reproductive history etc., of the enrolled subjects. Based upon date of last menstrual cycle and ultrasound records, the gestational age was confirmed. The biological sample (~ 4 ml blood) was collected in vacutainers from each subject within 36 hours of delivery and serum was separated by

centrifugation using centrifuge machine at 3000 rpm for 5 minutes. The separated serum was utilized for the estimation of 8-OHdG, TAC concentrations etc. The 8-OHdG and TAC level were measured by commercially available enzyme-linked immunosorbent assay kit using Multimode analyser (BioTek, USA) as per the protocol provided by the manufacturer of the kits (Cayman chemical, USA).

#### Statistical analysis:

The data were examined by statistical software SPSS version 16 (SPSS Inc., Chicago, USA). The data of cases and controls were defined by frequencies and % for the categorical variables and the mean  $\pm$  standard error for continuous variables. The significance between groups was determined at 0.05 levels using  $\chi^2$  test, Student's unpaired t test for normally distributed data whereas Man Whitney 'U' test was applied for data not following normal distribution. The Pearson correlation

was performed, and scatter graph was also plotted to check the association between 8-OHdG and TAC. Logistic regression model with different variables was applied to check risk factors associated for PTB.

## RESULTS

The mean maternal age was slightly higher while BMI was marginally lower in PTB subjects compared to controls (full term birth) subjects. Further, the data of percentage distribution with respect to BMI, dietary habits, area of residence, educational status, socioeconomic status and occupational status displayed marginal differences between PTB cases and full-term birth subjects. These differences were statistically non-significant. However, the numbers of women with chewing habits (tobacco and areca-nut) were significantly ( $p < 0.05$ ) more in PTB cases than controls women with full term infants (table-1).

Table 1: General characteristic of women with Preterm and Full-term birth

Variables	Full term (n=39)	Preterm (n=38)	Significance
Mean maternal Age	24.97 $\pm$ 0.57	25.47 $\pm$ 0.80	NS
Mean maternal BMI	20.54 $\pm$ 0.40	19.29 $\pm$ 0.51	NS
<b>BMI categories</b>			
Under weight	6(15.38%)	13(34.21%)	NS
Healthy	31(79.48%)	24(63.15%)	NS
Overweight	2(5.12%)	1(2.63%)	NS
<b>Area of Residence</b>			
Residential	33(84.61%)	26(68.42%)	NS
Industrial	5(12.82%)	7(18.42%)	NS
Agricultural	1(2.56%)	5(13.15%)	NS
<b>Educational Status</b>			
Illiterate	11(28.20%)	17(44.73%)	NS
Primary/ Secondary	23(58.97%)	16(42.10%)	NS
Graduate to higher	5(12.82%)	5(13.15%)	NS
<b>Dietary Habits</b>			
Vegetarian	15(38.46%)	18(47.36%)	NS
Mixed	24(61.53%)	20(52.63%)	NS
<b>Chewing habit</b>			
Chewer	3(7.63%)	10(26.31%)	<0.05
Non-chewer	36(92.30%)	28(73.68%)	NS
<b>Socioeconomic status</b>			
Lower/Upper lower	29(74.35%)	33(86.84%)	NS
Middle/Upper middle	10(25.64%)	5(13.15%)	NS
<b>Occupational Status</b>			
Employed/Past employed	10(25.64%)	12(31.57%)	NS
Unemployed	29(74.35%)	26(68.42%)	NS

p value calculated as per  $\chi^2$  tests; NS=Nonsignificant

The data related to reproductive variables of the subjects are depicted in table- 2. The mean gestational age was 38.89 $\pm$ 0.17 and 29.34 $\pm$ 0.77 weeks in full

and preterm subjects respectively. The data regarding mode of delivery showed that the rate of caesarian section was statistically significantly ( $p < 0.001$ ) higher in women

delivered PTB as compared to women delivered full term birth. Similarly, the rate of normal delivery was more in number in women with full term birth than PTB cases which was also statistically significant (<0.001). The data with respect to history of adverse pregnancy outcome indicated that there were no subjects with history of PTB in control group whereas ~15.8% women

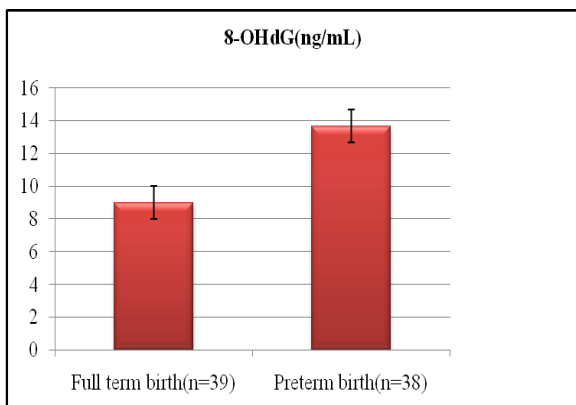
had history of preterm birth in PTB cases. This difference was statistically significant. Additionally, there was no significant difference in the proportion of PTB and full-term birth with respect to history of abortion. Further, it was found that there were higher (p<0.01) number of women with mental stress in PTB cases as compared to control group (table 2).

**Table 2: Reproductive variables among Preterm and full-term birth**

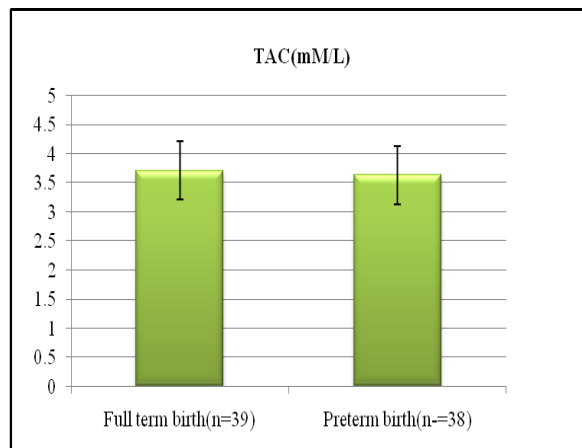
Reproductive variable	Full term (n=39)	Preterm (n=38)	Significance
<b>Mean Gestational Age</b>	38.89±0.17	29.34±0.77	NA
<b>Mode of delivery</b>			
Normal/Vaginal	37(94.87%)	28(73.68%)	<0.001
Caesarian (c-section)	2(5.12%)	10(26.31%)	<0.001
<b>Gravida</b>			
Primigravida	12(30.76%)	15(39.47%)	NS
Multigravida	27(69.23%)	23(60.52%)	NS
<b>Types of PTB</b>			
Spontaneous PTB	NA	8(21.00%)	NA
Induced PTB	NA	30(78.94%)	NA
<b>Reproductive history</b>			
Sub. with H/o Abortion	1(2.56%)	4(10.52%)	NS
Sub. with H/o PTB	0 (0%)	6(15.78%)	<0.05
<b>Maternal Stress</b>	7(17.94%)	20(52.63%)	<0.01

p value calculated as per  $\chi^2$  tests; NS=Nonsignificant; NA=not applicable

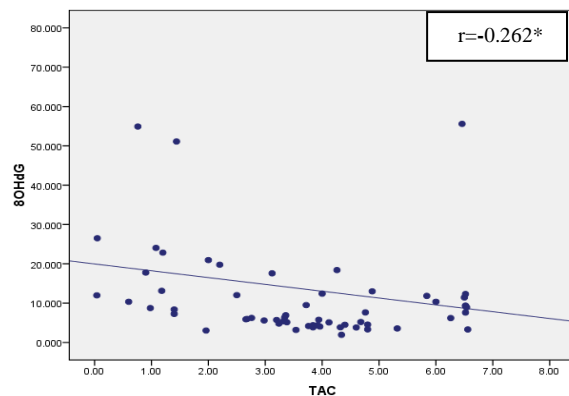
The data of 8-OHdG, TAC levels and their correlation are presented in figure 1, 2, 3 respectively. The data showed marginally higher concentration of 8-OHdG marker in serum of mothers who delivered PTB than mothers who delivered full term babies (Figure 1). While the level of TAC was slightly lower in mothers of PTB cases with respect to TAC level in mothers of full-term birth cases (Figure 2). However, the changes were statistically nonsignificant. The Pearson correlation analysis showed a significant negative correlation ( $r=-0.262$ ) between 8-OHdG and TAC levels (Figure 3).



**Figure 1: Oxidative DNA damage marker in women with Preterm and Full term birth**



**Figure 2: Total Antioxidant Capacity in women with Preterm and Full term birth**



\*p<0.05

**Figure 3: Correlation between 8-OHdG marker and TAC in study subjects**

Further, on application of binary logistic regression analysis with respect to different variables studied, it was found that an increase of one unit of 8-OHdG, elevates the risk of manifestation of PTB by 1.14 times.

In addition, the mental stress also raises this risk of PTB by 5.6 times where as other variables did not show any significant impact related to the occurrence of PTB (Table 3).

**Table 3: Binary logistic regression showing risk factors for Preterm birth**

Risk factors	Exp(B)	Significance (p value)	95.0% C.I.for EXP(B)	
			Lower	Upper
Maternal Age	1.054	0.530	0.894	1.244
Maternal BMI	0.838	0.150	0.659	1.066
Chewing habits	1.746	0.576	0.248	12.281
<b>Mental stress</b>	<b>5.635</b>	<b>0.018</b>	<b>1.352</b>	<b>23.478</b>
H/O abortion	1.997	0.468	0.308	12.945
Total antioxidant capacity	1.160	0.475	0.771	1.745
<b>8-hydroxy-2'-deoxyguanosine</b>	<b>1.142</b>	<b>0.048</b>	<b>1.001</b>	<b>1.303</b>

Variable(s) entered on step 1: maternal age, BMI, Chewing habits, Mental stress, H/O Abortion, Total antioxidant capacity, 8-hydroxy-2' – deoxyguanosine

## DISCUSSION

The 8-OHdG concentration was higher while level of TAC was marginally lower in serum of mothers who delivered preterm birth as compared to mothers who has delivered full term birth. This corroborates with the earlier observations of Kim *et al.* They also found elevated concentrations of urinary 8-OHdG and MDA level in the women who delivered preterm infants than those women with full-term offspring. [9] Further, Peter Stein *et al.* concluded that 8-OHdG associated with lower infant birth weight, shortened gestation duration and birth defects. [10] These are also suggesting role of 8-OHdG in PTB. Later, Ferguson *et al.* reported that maternal OS may be a vital contributor to PTB, irrespective of subtype as well as timing of occurrence of OS during pregnancy. [7] However, the maternal 8-OHdG level was found to be higher in the present study even after the pre-term delivery indicating the role of OS in occurrence of PTB.

Earlier, Cinkaya *et al.* found that preterm labor women have reduced total antioxidant status as compared in women with normal pregnancies in comparable gestational weeks. [11] A slightly lower TAC level was observed in PTB subjects as compared to subjects with full term birth in the present study also. Recently, Moore *et al.* also reported reduce levels of antioxidants in PTB subjects compared to

full-term. Further, in preterm infants insufficient antioxidant defenses may be associated to the pathogenesis of some of the complications of prematurity. [4] Earlier, Turgut *et al.* also hypothesized that low antioxidants in pre-term children may predispose to elevated oxidative stress and cause hyper bilirubinaemia. [12] Later, Chakravarty and Sontakke observed that the concentrations of antioxidants (reduced glutathione, superoxide dismutase) are found to be lower and lipid peroxidation was higher in both preterm mother as well as their offspring. [13] Thus, appropriate antioxidant might have significant role in maintaining of pregnancy or its outcome.

A significant negative relationship was observed between 8-OHdG and TAC in the present study. Earlier, Hung *et al.* also observed a reciprocal correlation amongst the urinary 8-OHdG levels and the level of plasma TAC. [14] Further logistic regression model indicated that risk factors such as mental stress and 8-OHdG were also significantly associated with the manifestation of PTB in the present study signifying the role of mental stress in creating oxidative stress which may cause damage in DNA in the subjects with preterm birth. Recently, Sultana *et al.* reported that the buildup of OS in the body causes damage to lipids, proteins and DNA in the placental tissue that persuades a form of acceleration of ageing. Early ageing of the placenta is related with placental



inadequacy that prevents needs of the foetus, and consequently, the capability of the foetus is compromised. [15] Further, recently Lu *et al.* reviewed the data on the role of OS in female reproduction. They summarized that the OS is involved in the progress of diseases of the reproductive system, and OS plays the double-edged role. [16] In addition, Menon mentioned that the physiologic (full term infants) and pathophysiologic (preterm babies) pathways of labor are not facilitated by OS only but through OS-induced injury to intrauterine tissues, particularly fetal membranes of the placenta. Cellular OS impairment encourages fetal cell senescence (aging). Senescing cells produce biomolecular signals that are initiating labor process. The fetal cell aging is normal in full term, while aging is early in PTB cases, where elements of redox inequalities and OS damage are pronounced. He has hypothesized that fetal cell senescence signals produced by OS damage are probably triggers for early labor. [17] In the present study, 8-OHdG was found to be higher in PTB cases than full term subjects indicating the possible role of maternal OS in causation of PTB. Further, Lee and Davis reviewed that the premature newborn is susceptible to ROS-induced impairments because of deficient antioxidant present at birth, as well as upregulation of impairments in response to OS. Therefore, the premature baby is at elevated risk for ROS-induced damage in the newborn also. [18]

The reproductive history of subjects showed a greater number of preterm births among subjects who were having earlier history of spontaneous abortion and PTB. This corroborates with earlier finding of Sherf *et al.* They also mentioned that the hazard for PTD was greater if their mother had history of PTB and concluded that family history of PTD is an independent risk factor for PTB. [19] Earlier, Wilcox *et al.* also reported that the elevated risk of preterm delivery was more pronounced among mothers who have history of offspring born preterm. [20]

The data gained so far suggests the role of oxidative stress at least in part in causation of preterm birth, as higher concentration of 8-hydroxy-2'-deoxyguanosine and lower level of Total antioxidant capacity was found in serum of mothers with preterm birth as compared to level of these in mother with full term birth even though the data are statistically non-significant. The supplementation of antioxidants in the management of PTB may also be explored by conducting more studies on this important issue of human reproduction.

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#### Conflict of Interest Statement

The research was conducted without commercial or financial relationships with any organization that could be a conflict of interest.

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