

BMJ Open Urinary 8-hydroxy-2'-deoxyguanosine levels and small-for-gestational age infants: a prospective cohort study from the Japan Environment and Children's Study

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ABSTRACT

Objectives To evaluate the association between the urinary 8-hydroxy-2'-deoxyguanosine (U8-OHdG) levels and the incidence of small-for-gestational age (SGA) infants and to assess the utility of U8-OHdG as a biomarker to predict the incidence of SGA infants.

Design Prospective cohort study.

Setting The Japan Environment and Children's Study.

Participants Data of participants enrolled in the Japan Environment and Children's Study, a nationwide birth cohort study, between 2011 and 2014 were analysed; 104 062 fetal records were analysed. Data of women with singleton pregnancies ≥ 22 weeks of gestation were analysed.

Primary and secondary outcome measures U8-OHdG levels were assessed using liquid chromatography-tandem mass spectrometry. Participants were categorised into the following three groups according to the quartile of the distribution of U8-OHdG: low U8-OHdG (<1.95 ng/mgCre), moderate U8-OHdG (the combined second and third quartiles; 1.95 – 2.95 ng/mgCre) and high U8-OHdG (>2.95 ng/mgCre) groups. Additionally, participants in the 90th percentile for U8-OHdG levels were analysed. Odds ratios (ORs) for SGA infants (<-1.5 and <-2.0 SD) were calculated using a logistic regression model while adjusting for confounding factors; the moderate U8-OHdG group was used as a reference. The cut-off value of U8-OHdG to predict the incidence of SGA infants was calculated using a receiver operating characteristic (ROC) curve analysis.

Results Data of 80 212 participants were analysed. The adjusted ORs for SGA infants (<-1.5 and <-2.0 SD) in the high U8-OHdG group were 1.16 (95% CI 1.07 to 1.25) and 1.22 (95% CI 1.07 to 1.38). The cut-off value of U8-OHdG (3.26 ng/mgCre) showed a poor ability to predict SGA infants (sensitivity, 21.9%; specificity, 83.6%; area under the ROC curve, 0.530).

Conclusions Elevated U8-OHdG levels were associated with an increased incidence of SGA infants. However, this parameter would not be a useful screening tool for

Strengths and limitations of this study

- The odds ratios for the incidence of small-for-gestational age infants were calculated using the data from a large Japanese birth cohort.
- The time point of assessing urinary 8-hydroxy-2'-deoxyguanosine levels varied for participants in the second/third trimester.
- Data related to inflammatory cytokines were not considered in the analysis.

predicting SGA infants owing to its low sensitivity and specificity.

INTRODUCTION

Small-for-gestational age (SGA) infants are affected by fetal growth restriction (FGR) mainly because of placental insufficiency,^{1–3} and SGA is closely associated with perinatal morbidity and mortality.^{4,5} Additionally, SGA infants are known to have an increased risk of coronary artery disease, diabetes mellitus and arterial hypertension in adulthood.⁶ However, to date, there is no reliable biomarker to predict the incidence of SGA infants in pregnant women.⁷

Oxidative stress (OS), which is defined as an imbalance between prooxidants, such as reactive oxygen species, and antioxidants, is a concomitant factor with chronic inflammation as its underlying mechanism and a potentiation factor for producing inflammatory cytokines, which cause several obstetric complications.^{8,9} The relationship between OS and the development of pathological processes leading to obstetric complications,

such as recurrent miscarriage, preterm births, chorioamnionitis, pre-eclampsia, gestational diabetes mellitus (GDM), FGR and fetal death, has been reported.^{10–14} However, the methods of evaluating OS in different complications and the time points of measurements in previous studies have been inconsistent. Moreover, there is no standardised protocol to estimate OS in pregnant women.

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most frequently explored products of oxidative DNA damage because of its mutagenic properties and the ease of measurement using an ELISA.¹⁵ Elevated urinary 8-hydroxy-2'-deoxyguanosine (U8-OHdG) levels in pregnant women have been reported to be associated with the development of GDM.¹⁴ Similarly, several studies have reported that elevated U8-OHdG levels are associated with a reduced birth weight^{16–18}; however, these studies had small sample sizes with potential selection bias.

We hypothesised that elevated U8-OHdG levels during pregnancy would be associated with a higher incidence of SGA infants due to chronic placental hypoxia.¹⁷ Therefore, in the present study, we evaluated the association between U8-OHdG levels during pregnancy and the incidence of SGA infants and assessed the utility of U8-OHdG as a biomarker to predict the incidence of SGA infants using data from a nationwide Japanese birth cohort study.

METHODS

Cohort selection

In this study, we analysed the data from the Japan Environment and Children's Study (JECS), which is a nationwide government-funded prospective birth cohort study that was started in January 2011 to investigate the effects of environmental factors on children's health.^{19 20} Briefly, the JECS is funded directly by the Ministry of the Environment, Japan, and involves collaboration among the Programme Office (National Institute for Environmental Studies), the Medical Support Centre (National Centre for Child Health and Development) and 15 Regional Centres (Hokkaido, Miyagi, Fukushima, Chiba, Kanagawa, Koshin, Toyama, Aichi, Kyoto, Osaka, Hyogo, Tottori, Kochi, Fukuoka and South Kyushu/Okinawa).^{19 20} The eligibility criteria for the JECS participants (expectant mothers) were as follows: (1) residing in the study areas at the time of recruitment and expected to continually reside in Japan for the foreseeable future, (2) an expected delivery date between 1 August 2011 and mid-2014 and (3) capable of participating in the study without difficulty (ie, able to comprehend the Japanese language and complete the self-administered questionnaires).

Either or both of the following recruitment protocols were applied: (1) recruitment at the time of the first prenatal examination at the cooperating healthcare centres and (2) recruitment at local government offices issuing a pregnancy journal, called the Maternal and Child Health Handbook, that is given to all expectant mothers in Japan before they receive municipal services

for pregnancy, delivery and childcare. We contacted pregnant women through cooperating healthcare providers and/or local government offices issuing the Maternal and Child Health Handbooks and registered those willing to participate. Self-administered questionnaires, which were completed by the women during the first trimester and second/third trimester, were used to collect information on demographic factors, medical and obstetric history, physical and mental health, lifestyle, occupation, environmental exposure at home and in the workplace, housing conditions and socioeconomic status.^{19 20}

As described in our previous study,¹ the current analysis used the data set released in 2019 (data set: jecta-20190930). Specifically, we used three types of data: (1) M-T1, obtained from a self-report questionnaire that was collected during the first trimester (the first questionnaire) and that included questions regarding maternal medical background, (2) M-T2, obtained from a self-report questionnaire that was collected during the second or third trimester (second questionnaire) and that included questions regarding partner lifestyle and socioeconomic status and (3) Dr-T1 and Dr-0m, which were collected from the medical record transcripts provided by each participant's cooperating healthcare centre and that included data on obstetrical outcomes during the first, second and third trimesters, such as gestational age and birth weight. The medical record transcriptions were performed by physicians, midwives/nurses and/or research coordinators.

Participants with singleton pregnancies at and after 22 weeks of gestation were included in the present study. Women with spontaneous abortion, stillbirth and missing data were excluded from the study. We used a complete case analysis; there were no significant differences in participants' characteristics between those included in and excluded from the study (data not shown), as reported in our previous study.¹

Exposure

U8-OHdG levels were measured from a urine sample collected once during pregnancy using liquid chromatography–tandem mass spectrometry at a single centre, the method of which has been reported elsewhere.²¹ Although urine sampling for U8-OHdG assessment was planned for the second or third trimester in the JECS, there were differences in the actual measurement time points (median, 27.0 weeks of gestation; quartiles at 25.0, 27.0 and 29.0 weeks of gestation). U8-OHdG levels were expressed as a ratio to urinary creatinine levels (ng/mgCre).²² Based on the Shapiro-Wilk test and histogram, the levels of U8-OHdG were not normally distributed; therefore, after combining the second and third groups as the moderate group, the participants were categorised into the following three groups according to the quartile of the distribution of U8-OHdG: low U8-OHdG group (<1.95 ng/mgCre), moderate U8-OHdG group (1.95–2.95 ng/mgCre) and high U8-OHdG group (>2.95 ng/

mgCre). Additionally, participants in the 90th percentile for U8-OHdG levels were analysed.

Outcomes

SGA infants were defined by a birthweight <-1.5 SD, corrected for parity, gestational age and sex according to the 'New Japanese neonatal anthropometric charts for gestational age at birth'.^{1 23} Additionally, SGA infants with a birthweight <-2.0 SD were also analysed.^{24 25}

The following parameters were analysed as potential confounding factors: maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal smoking status, maternal alcohol consumption status, maternal educational status, annual household income, gestational weight gain (GWG), mean daily energy intake (DEI), daily physical activity (DPA) and presence of hypertensive disorders of pregnancy. Based on age, the participants were categorised into three groups (<20 years, 20–34 years and ≥ 35 years).²⁶ BMI before pregnancy was categorised into three groups (<18.5 kg/m², 18.5–24.9 kg/m² and ≥ 25.0 kg/m²).²⁷ Parity was categorised into two groups (nulliparous and multiparous).

As described in our previous study,¹ maternal participants were requested to provide information about their smoking status by choosing one of the following: 'currently smoking', 'never smoked', 'previously did, but quit before realising current pregnancy' and 'previously did, but quit after realising current pregnancy'. Participants who chose 'currently smoking' comprised the smoking category, whereas other participants comprised the non-smoking category.

Maternal participants were requested to provide information about their alcohol consumption status by choosing one of the following: 'never drank', 'quit drinking before pregnancy', 'quit drinking during early pregnancy' and 'kept drinking during pregnancy'.²⁸ Participants who chose 'kept drinking during pregnancy' comprised the drinking category, while other participants comprised the non-drinking category.

Furthermore, as described in our previous study,¹ maternal educational status was categorised into four groups based on the number of years of education (junior high school, <10 years; high school, 10–12 years; technical/vocational school or university, 13–16 years; and graduate school, ≥ 17 years). Annual household income was categorised into four levels (<2 000 000 Japanese yen, 2 000 000–5 999 999 Japanese yen, 6 000 000–9 999 999 Japanese yen and ≥ 10 000 000 Japanese yen).

GWG was defined as the body weight just before delivery (kg) minus the body weight before pregnancy (kg). We defined appropriate GWG as less than 12 kg and excessive GWG as more than 12 kg according to the criteria aimed at an appropriate birth weight, as described by the Ministry of Health, Labour and Welfare, Japan,²⁹ which defines 9–12 kg as the appropriate GWG for women with a prepregnancy BMI below 18.8 kg/m², 7–12 kg for women with a prepregnancy BMI between 18.5 kg/m² and 24.9 kg/m² and specifies individual values for women

with a prepregnancy BMI above 25.0 kg/m². Maternal DEI was evaluated using a semiquantitative food frequency questionnaire that has been validated in another cohort study²⁸ and was calculated based on the Japan Standard Tables of Food Composition (fifth Revised Edition). Maternal DPA was evaluated as metabolic equivalent and duration (minutes).^{30 31} Hypertensive disorder of pregnancy was defined as persistently elevated blood pressure ($\geq 140/90$ mm Hg) after 20 weeks of pregnancy in an otherwise normotensive woman. All confounding factors were chosen on the basis of their clinical importance.

Statistical analysis

The participants' characteristics were summarised based on the groups stratified by U8-OHdG levels. One-way analysis of variance and Kruskal-Wallis test were used to compare continuous variables based on the differences in the distribution of data. The χ^2 test was used to compare the categorical variables among the groups. Crude odds ratios (cORs), adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for SGA infants were calculated using a multiple logistic regression model, with women in the moderate U8-OHdG group considered as the reference. The ORs were adjusted for maternal age, maternal BMI before pregnancy, parity, maternal smoking status, maternal alcohol consumption status, maternal educational status, annual household income, excessive GWG, maternal DEI, DPA and hypertensive disorders of pregnancy. Moreover, maternal age, maternal BMI before pregnancy and GWG were also used as confounding factors for continuous variables.

Additionally, the cut-off value of U8-OHdG to predict the incidence of SGA infants was calculated using a receiver operating characteristic curve analysis. SPSS, V.26 (IBM) was used to perform the statistical analyses. Differences with p values <0.05 were considered statistically significant.

Patient and public involvement

The JECS started recruiting expectant mothers in 2011 to assess environmental factors that affect children's health, which aimed to recruit approximately 100 000 pregnant women and their partners, to collect biological samples and to collect data on their children until they became 13 years old.¹⁹ No cohort members were involved in setting the research question. No cohort members were involved in the interpretation or writing of the results. The results of the research are disseminated to the general public by presentations and press releases.

RESULTS

The total number of fetal records of women who gave birth between 2011 and 2014 during the JECS was 104 062. After applying our inclusion criteria, 80 212 participants were eligible for this study. The details of missing data are shown in figure 1. Among 80 212 participants, 19 815 were in the low U8-OHdG group, 40 466 in the moderate

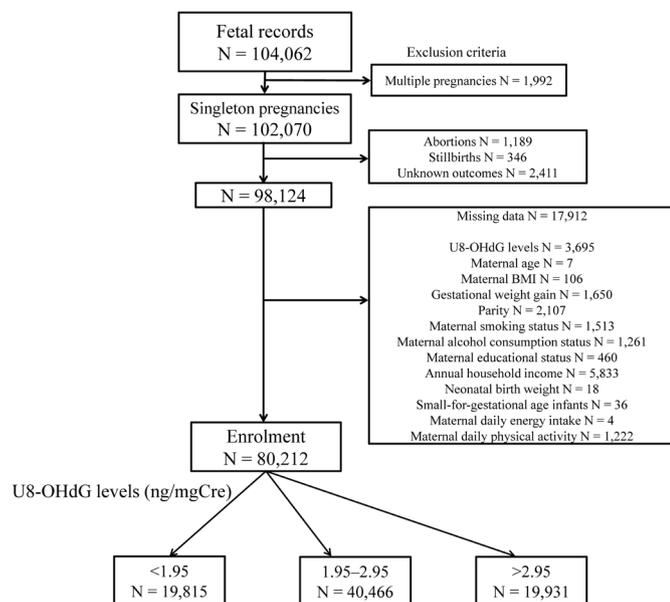


Figure 1 Flowchart showing the enrolment of participants in the study. BMI, body mass index; U8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine.

U8-OHdG group and the remaining 19931 in the high U8-OHdG group; 8067 participants were in the 90th percentile for U8-OHdG levels.

Table 1 summarises the maternal background and obstetric outcomes of the participants stratified by U8-OHdG levels. All variables except the maternal educational status and DPA differed significantly among the groups stratified by U8-OHdG levels. The proportion of pregnant women who currently smoked was the highest in the high U8-OHdG group.

Table 2 shows the cORs and aORs for SGA infants <-1.5 SD among women in the low and high U8-OHdG groups, with women in the moderate U8-OHdG group as the reference. The aORs for SGA infants <-1.5 SD were 1.23 (95% CI 1.14 to 1.32), 1.20 (95% CI 1.10 to 1.29), 1.20 (95% CI 1.11 to 1.29) and 1.16 (95% CI 1.07 to 1.25) among women in the high U8-OHdG group in model 1, model 2, model 3 and model 4, respectively. The aORs for SGA infants <-1.5 SD in participants in the 90th percentile for U8-OHdG levels were also increased.

Table 3 shows the cORs and aORs for SGA infants <-2.0 SD among women in the low and high U8-OHdG groups, with women in the moderate U8-OHdG group as the reference. The aORs for SGA infants <-2.0 SD were 1.30 (95% CI 1.15 to 1.48), 1.27 (95% CI 1.12 to 1.44), 1.27 (95% CI 1.12 to 1.44) and 1.22 (95% CI 1.07 to 1.38) among women in the high U8-OHdG group in model 1, model 2, model 3 and model 4, respectively. The aORs for SGA infants <-2.0 SD in participants in the 90th percentile for U8-OHdG levels were also increased.

The cut-off value of U8-OHdG to predict the incidence of SGA infants was 3.26 ng/mgCre (sensitivity 21.9% and specificity 83.6%). In the receiver operating characteristic curve analysis, the area under the curve for this cut-off value was 0.530.

DISCUSSION

Principal findings

The findings of the present study suggest that high U8-OHdG levels during pregnancy were associated with an increased incidence of SGA infants after adjusting for the confounding factors. Additionally, we estimated the cut-off value of U8-OHdG to predict the incidence of SGA infants; however, it had low sensitivity and specificity, which implies that it is not suitable for universal screening.

Strengths of the study

Among the strengths of the present study is that the ORs for the incidence of SGA infants were calculated using data from a large Japanese birth cohort. This strengthens previous findings regarding the association between high U8-OHdG levels and an increased incidence of SGA infants. The results of the present study may facilitate the clinical management of pregnant women and would be informative to perinatologists. Further studies evaluating the association between U8-OHdG levels and the incidence of SGA infants while accounting for various lifestyle factors and elucidating the mechanisms of OS underlying SGA infants may be instrumental in suggesting modification of lifestyle factors.

Limitations of the data

Despite the aforementioned strengths, the present study had several limitations. First, the time point of assessing U8-OHdG levels was not consistent for all participants in the second/third trimester (median, 27.0 weeks of gestation). This would have influenced the results because the levels of U8-OHdG vary by gestational age.¹⁷ Nevertheless, the large number of participants might have overcome this difference in gestational ages and led to a robust analysis of data in the second/third trimester. Second, the present study did not include data for inflammatory cytokines. Although U8-OHdG levels directly reflect OS, elucidation of the underlying mechanism while accounting for the cascade of inflammatory cytokines would strengthen the association between U8-OHdG levels and obstetric complications. Third, the present data set does not include data on some potential confounders, such as ferritin level, air pollution level and altitude. These factors may influence the association between U8-OHdG levels and obstetric complications, especially in urban and high-altitude settings. Additionally, there was no detailed information about FGR in the JECS, and we could not evaluate the true association between FGR and SGA infants. Finally, a complete case analysis performed in this study might have led to the potentially biased findings. Although this analysis was judged suitable based on the fact that the missingness did not depend jointly on the exposure and the outcome and that there were no significant differences in characteristics between those included in and excluded from the study, careful interpretation of the findings is needed.

Table 1 Maternal medical background and obstetric outcomes of participants based on the urinary 8-hydroxy-2'-deoxyguanosine levels

Variable	Low U8-OHdG <1.95 ng/mgCre n=19815	Moderate U8-OHdG 1.95–2.95 ng/mgCre n=40466	High U8-OHdG >2.95 ng/mgCre n=19931	P value
Maternal age, % (n)				<0.001
<20 years	0.7 (129)	0.6 (240)	0.4 (83)	
20–34 years	71.9 (14,250)	72.1 (29,183)	70.7 (14,092)	
≥35 years	27.4 (5,436)	27.3 (11,043)	28.9 (5,756)	
BMI before pregnancy, % (n)				<0.001
<18.5 kg/m ²	13.6 (2,695)	16.0 (6,461)	17.6 (3,501)	
18.5–24.9 kg/m ²	73.7 (14,601)	73.8 (29,862)	72.5 (14,442)	
≥25.0 kg/m ²	12.7 (2,519)	10.2 (4,143)	10.0 (1,988)	
Nulliparous, % (n)	39.1 (7,742)	38.6 (15,620)	41.9 (8,361)	<0.001
Smoking during pregnancy, % (n)	2.9 (575)	4.3 (1,721)	7.3 (1,455)	<0.001
Alcohol consumption, % (n)	2.6 (519)	2.7 (1,103)	3.4 (678)	<0.001
Maternal education, % (n)				0.199
<10 years	4.3 (858)	4.4 (1,798)	4.7 (931)	
10–12 years	31.1 (6,162)	30.7 (12,435)	31.2 (6,225)	
13–16 years	63.1 (12,503)	63.4 (25,668)	62.5 (12,462)	
>17 years	1.5 (292)	1.4 (565)	1.6 (313)	
Annual household income, % (n)				0.020
<2 000 000 Japanese yen	5.8 (1,159)	5.5 (2,236)	5.6 (1,126)	
2 000,000–5 999 999 Japanese yen	68.2 (13,508)	67.7 (27,411)	67.0 (13,360)	
6 000 000–9 999 999 Japanese yen	21.6 (4,281)	22.6 (9,129)	23.0 (4,581)	
>10 000 000 Japanese yen	4.4 (867)	4.2 (1,690)	4.3 (864)	
Excessive GWG (>12 kg), % (n)	31.9 (6,318)	30.4 (12,319)	27.4 (5,459)	<0.001
Maternal daily energy intake (kcal/day), median (quartile range)	1686.0 (1365.0–2096.0)	1702.0 (1380.0–2116.0)	1694.0 (1375.0–2104.0)	0.003
Maternal daily physical activity (METs*min), median (IQR)	120.0 (28.3–411.4)	118.9 (28.3–424.3)	120.0 (28.3–452.6)	0.604
Hypertensive disorders of pregnancy, % (n)	3.6 (712)	2.8 (1,152)	3.0 (596)	<0.001
SGA infants<–1.5SD, % (n)	4.6 (920)	4.6 (1,879)	5.9 (1,168)	<0.001
SGA infants<–2.0SD, % (n)	1.6 (317)	1.5 (614)	2.1 (414)	<0.001

BMI, body mass index; GWG, gestational weight gain; MET, metabolic equivalent; SGA, small-for-gestational age; U8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine.

Interpretation

The findings of our study, which suggest that high U8-OHdG levels were associated with an increased incidence of SGA infants, are consistent with those of previous studies.^{16–18} Pregnancy itself is a state of OS, and appropriate levels of reactive oxygen species are necessary to maintain the physiological functions; however, OS, especially in higher concentrations, may cause endothelial dysfunction and insufficient uteroplacental perfusion, which result in SGA infants.^{17,32} Several inflammatory cytokines, such as tumour necrosis factor- α and

prostaglandins, are instrumental in causing endothelial dysfunction.³² Additionally, reactive oxygen species generated from nicotinamide adenine dinucleotide phosphate oxidase are critical for vascular endothelial growth factor signalling and angiogenesis, which may be important causative factors for endothelial dysfunction.³² Furthermore, since maternal OS appears to be a reliable proxy assessment of fetal OS,¹⁷ maternal biomarkers of OS can closely correlate with the fetal and neonatal condition.

Conversely, low U8-OHdG levels were not associated with a decreased incidence of SGA infants when compared

Table 2 Crude ORs and adjusted ORs for the incidence of small-for-gestational age infants <-1.5 SD in women with low and high urinary 8-hydroxy-2'-deoxyguanosine levels

U8-OHdG groups	Low U8-OHdG N=19815		Moderate U8-OHdG N=40466	High U8-OHdG N=19931		90th percentile for U8-OHdG N=8067	
	ORs	95% CI		ORs	95% CI	ORs	95% CI
cOR	1.00	(0.92 to 1.08)	Ref	1.28	(1.19 to 1.38)	1.52	(1.38 to 1.68)
aOR (model 1)*	1.03	(0.95 to 1.12)	Ref	1.23	(1.14 to 1.32)	1.43	(1.29 to 1.58)
aOR (model 2)†	1.04	(0.96 to 1.13)	Ref	1.20	(1.11 to 1.29)	1.38	(1.25 to 1.53)
aOR (model 3)‡	1.03	(0.95 to 1.12)	Ref	1.20	(1.11 to 1.29)	1.37	(1.24 to 1.52)
aOR (model 4)§	1.06	(0.97 to 1.15)	Ref	1.16	(1.07 to 1.25)	1.31	(1.18 to 1.44)

*Maternal age, body mass index before pregnancy, parity, maternal smoking status, maternal alcohol consumption status, maternal educational status and annual household income were used as confounding factors in model 1.

†In addition to the confounding factors in model 1, excessive gestational weight gain, maternal daily energy intake, and daily physical activity were used as confounding factors in model 2.

‡In addition to the confounding factors in model 2, the presence of hypertensive disorders of pregnancy was used as a confounding factor in model 3.

§Maternal age, body mass index before pregnancy, and gestational weight gain were used as confounding factors for continuous variables in model 4, and other confounding factors were also used in model 3.

aOR, adjusted OR; cOR, crude OR; Ref, reference; U8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine.

with moderate U8-OHdG levels. Although little is known about the association between low U8-OHdG levels and obstetric outcomes, it can be speculated that low U8-OHdG levels would be associated with low maternal nutrition and low BMI, and high U8-OHdG levels would be associated with high maternal nutrition and high BMI,³³ which frequently affect fetal and neonatal growth. Since low maternal nutrition and insufficient GWG lead to unfavourable obstetric outcomes³⁴ and moderate OS is essential for the maintenance of physiological functions,³² physicians should not suggest that low U8-OHdG levels indicate better obstetric outcomes.

The cut-off value of U8-OHdG to predict the incidence of SGA infants calculated in this study revealed that this parameter may not be useful for universal screening. To date, no biomarker has been established to predict the incidence of SGA infants.^{7 17} Since our calculated cut-off value

of U8-OHdG had low sensitivity, low specificity and low area under the curve, we do not suggest that U8-OHdG is useful as a clinical biomarker for predicting the incidence of SGA infants. Further studies to evaluate the utility of U8-OHdG to predict SGA birth with stratification of the aetiology of SGA infants are needed because this condition is caused by heterogeneous factors. Moreover, future studies should identify and investigate the usefulness of other biomarkers for OS to predict SGA infants.

CONCLUSIONS

The findings of the present study suggest that high U8-OHdG levels were associated with an increased incidence of SGA infants. Further studies to confirm the utility of measuring U8-OHdG levels to predict the

Table 3 Crude ORs and adjusted ORs for the incidence of small-for-gestational age infants <-2.0SD in women with low and high urinary 8-hydroxy-2'-deoxyguanosine levels

U8-OHdG groups	Low U8-OHdG N=19815		Moderate U8-OHdG N=40466	High U8-OHdG N=19931		90th percentile for U8-OHdG N=8067	
	ORs	95% CI		ORs	95% CI	ORs	95% CI
cOR	1.06	(0.92 to 1.21)	Ref	1.38	(1.21 to 1.56)	1.67	(1.42 to 1.96)
aOR (model 1)*	1.09	(0.95 to 1.25)	Ref	1.30	(1.15 to 1.48)	1.53	(1.30 to 1.80)
aOR (model 2)†	1.10	(0.96 to 1.26)	Ref	1.27	(1.12 to 1.44)	1.47	(1.25 to 1.73)
aOR (model 3)‡	1.08	(0.94 to 1.24)	Ref	1.27	(1.12 to 1.44)	1.46	(1.24 to 1.72)
aOR (model 4)§	1.10	(0.96 to 1.26)	Ref	1.22	(1.07 to 1.38)	1.37	(1.17 to 1.62)

*Maternal age, body mass index before pregnancy, parity, maternal smoking status, maternal alcohol consumption status, maternal educational status and annual household income were used as confounding factors in model 1.

†In addition to the confounding factors in model 1, excessive gestational weight gain, maternal daily energy intake, and daily physical activity were used as confounding factors in model 2.

‡In addition to the confounding factors in model 2, the presence of hypertensive disorders of pregnancy was used as a confounding factor in model 3.

§Maternal age, body mass index before pregnancy, and gestational weight gain were used as confounding factors as continuous variables in model 4 and other confounding factors were also used in model 3.

aOR, adjusted OR; cOR, crude OR; Ref, reference; U8-OHdG, urinary 8-hydroxy-2'-deoxyguanosine.

incidence of SGA infants, while accounting for variable aetiology of this condition, are needed.

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REFERENCES

- Murata T, Kyojuzuka H, Fukuda T, *et al*. Maternal sleep duration and neonatal birth weight: the Japan environment and children's study. *BMC Pregnancy Childbirth* 2021;21:295.
- Zur RL, Kingdom JC, Parks WT, *et al*. The placental basis of fetal growth restriction. *Obstet Gynecol Clin North Am* 2020;47:81–98.
- Silver RM. Examining the link between placental pathology, growth restriction, and stillbirth. *Best Pract Res Clin Obstet Gynaecol* 2018;49:89–102.
- Katz J, Lee AC, Kozuki N, *et al*. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet* 2013;382:417–25.
- Pulver LS, Guest-Warnick G, Stoddard GJ, *et al*. Weight for gestational age affects the mortality of late preterm infants. *Pediatrics* 2009;123:e1072–7.
- Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science* 2004;305:1733–6.
- Conde-Agudelo A, Papageorghiou AT, Kennedy SH, *et al*. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. *BJOG* 2013;120:681–94.
- Labouesse MA, Langhans W, Meyer U. Long-term pathological consequences of prenatal infection: beyond brain disorders. *Am J Physiol Regul Integr Comp Physiol* 2015;309:R1–12.
- Kelly AC, Powell TL, Jansson T. Placental function in maternal obesity. *Clin Sci* 2020;134:961–84.
- Gupta S, Agarwal A, Banerjee J, *et al*. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. *Obstet Gynecol Surv* 2007;62:335–47.
- Hadi T, Bardou M, Mace G, *et al*. Glutathione prevents preterm parturition and fetal death by targeting macrophage-induced reactive oxygen species production in the myometrium. *Faseb J* 2015;29:2653–66.
- Temma K, Shimoya K, Zhang Q, *et al*. Effects of 4-hydroxy-2-nonenal, a marker of oxidative stress, on the cyclooxygenase-2 of human placenta in chorioamnionitis. *Mol Hum Reprod* 2004;10:167–71.
- Boutet M, Roland L, Thomas N, *et al*. Specific systemic antioxidant response to preeclampsia in late pregnancy: the study of intracellular glutathione peroxidases in maternal and fetal blood. *Am J Obstet Gynecol* 2009;200:530.e1–530.e7.
- Urbaniak SK, Boguszewska K, Szwczuk M, *et al*. 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine (8-oxodG) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a potential biomarker for gestational diabetes mellitus (GDM) development. *Molecules* 2020;25:202.
- Hirano T, Yamaguchi R, Asami S, *et al*. 8-hydroxyguanine levels in nuclear DNA and its repair activity in rat organs associated with age. *J Gerontol A Biol Sci Med Sci* 1996;51:B303–7.
- Scholl TO, Stein TP. Oxidant damage to DNA and pregnancy outcome. *J Matern Fetal Med* 2001;10:182–5.
- Potdar N, Singh R, Mistry V, *et al*. First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. *BJOG* 2009;116:637–42.
- Kim Y-J, Hong Y-C, Lee K-H, *et al*. Oxidative stress in pregnant women and birth weight reduction. *Reprod Toxicol* 2005;19:487–92.
- Kawamoto T, Nitta H, Murata K, *et al*. Rationale and study design of the Japan environment and children's study (JECS). *BMC Public Health* 2014;14:25.
- Michikawa T, Nitta H, Nakayama SF, *et al*. Baseline profile of participants in the Japan environment and children's study (JECS). *J Epidemiol* 2018;28:99–104.
- Nishihama Y, Nakayama SF, Tabuchi T, *et al*. Determination of Urinary Cotinine Cut-Off Concentrations for Pregnant Women in the Japan Environment and Children's Study (JECS). *Int J Environ Res Public Health* 2020;17:5537.
- Mazlumoglu MR, Ozkan O, Alp HH, *et al*. Measuring oxidative DNA damage with 8-hydroxy-2'-deoxyguanosine levels in patients with laryngeal cancer. *Ann Otol Rhinol Laryngol* 2017;126:103–9.
- Itabashi K, Miura F, Uehara R, *et al*. New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr Int* 2014;56:702–8.
- Saenger P, Czernichow P, Hughes I, *et al*. Small for gestational age: short stature and beyond. *Endocr Rev* 2007;28:219–51.



- 25 Yamakawa T, Itabashi K, Kusuda S, *et al.* Mortality and morbidity risks vary with birth weight standard deviation score in growth restricted extremely preterm infants. *Early Hum Dev* 2016;92:7–11.
- 26 Kyoizuka H, Fujimori K, Hosoya M, *et al.* The effect of maternal age at the first childbirth on gestational age and birth weight: the Japan environment and children's study (JECS). *J Epidemiol* 2019;29:187–91.
- 27 Murata T, Kyoizuka H, Yamaguchi A, *et al.* Maternal pre-pregnancy body mass index and foetal acidosis in vaginal and caesarean deliveries: the Japan environment and children's study. *Sci Rep* 2021;11:4350.
- 28 Yokoyama Y, Takachi R, Ishihara J, *et al.* Validity of short and long self-administered food frequency questionnaires in ranking dietary intake in middle-aged and elderly Japanese in the Japan public health Center-Based prospective study for the next generation (JPHC-NEXT) protocol area. *J Epidemiol* 2016;26:420–32.
- 29 Ministry of Health, Labour and Welfare, Japan. Japan: guidelines for health promotion in pregnant women (in Japanese). Available: <https://www.mhlw.go.jp/houdou/2006/02/h0201-3a.html> [Accessed 20 Feb 2021].
- 30 Craig CL, Marshall AL, Sjöström M, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003;35:1381–95.
- 31 Murase N, Katsumura T, Ueda C. International standardization of physical activity level: reliability and validity study of the Japanese version of the International physical activity questionnaire (IPAQ). *J Health Welfare Stat* 2002;49:1–9.
- 32 Lu J, Wang Z, Cao J, *et al.* A novel and compact review on the role of oxidative stress in female reproduction. *Reprod Biol Endocrinol* 2018;16:80.
- 33 Ballesteros-Guzmán AK, Carrasco-Legleu CE, Levario-Carrillo M, *et al.* Prepregnancy obesity, maternal dietary intake, and oxidative stress biomarkers in the fetomaternal unit. *Biomed Res Int* 2019;2019:5070453
- 34 Ehrenberg HM, Dierker L, Milluzzi C, *et al.* Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol* 2003;189:1726–30.