

# Change of salivary stress marker concentrations during pregnancy: Maternal depressive status suppress changes of those levels

Hiroaki Tsubouchi<sup>1</sup>, Yuichiro Nakai<sup>2</sup>, Masahiro Toda<sup>3</sup>, Kanehisa Morimoto<sup>4</sup>,  
Yang Sil Chang<sup>2</sup>, Norichika Ushioda<sup>2</sup>, Shoji Kaku<sup>2</sup>, Takafumi Nakamura<sup>2</sup>,  
Tadashi Kimura<sup>5</sup> and Koichiro Shimoya<sup>2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Aizenbashi Hospital, Osaka, <sup>2</sup>Department of Obstetrics and Gynecology, Kawasaki Medical School, Kurashiki, <sup>3</sup>Department of Pharmacology, Osaka Dental University, Hirakata, and Departments of <sup>4</sup>Social and Environmental Medicine and <sup>5</sup>Obstetrics and Gynecology, Osaka University Graduate School of Medicine, Osaka, Japan

## Abstract

**Aim:** The aim of the present study was to show changes in salivary cortisol and chromogranin A/protein concentrations as stress markers during pregnancy and to clarify the effect of chronic stress on stress markers.

**Material and Methods:** Salivary samples were collected from 69 pregnant women during pregnancy. Salivary cortisol levels and chromogranin A/protein titers were determined. We surveyed the women's chronic stress using the Zung self-rating depression scale and General Health Questionnaire-28.

**Results:** Cortisol levels in the saliva of pregnant women showed biphasic change during pregnancy. Chromogranin A/protein levels in the saliva of pregnant women increased in the second and the early third trimesters and decreased to the puerperal period. Salivary cortisol concentrations of the chronic high stress group were significantly lower compared with those of the normal group. Salivary chromogranin A/protein concentrations of the chronic high stress group were also significantly lower than those of the normal group.

**Conclusion:** The titration of salivary cortisol concentrations and chromogranin A/protein levels is a useful tool to determine maternal stress levels. The elevation of cortisol and chromogranin A/protein in the saliva was suppressed in the chronic high stress group during pregnancy.

**Key words:** chromogranin A, depressive status, maternal stress, pregnancy, salivary cortisol.

## Introduction

Psycho-mental health potentials were evaluated by stress-related hormonal levels. Data indicate that individuals with good lifestyles showed much younger health ages calculated based on health check-up data, and lower risks for developing lifestyle-related diseases than those with poor lifestyles. Comprehensive health potentials were significantly lower in poor-lifestyle individuals than those with a good lifestyle.<sup>1</sup>

Maternal psycho-mental stress has an adverse effect on pregnancy, such as preterm labor,<sup>2</sup> intrauterine growth restriction<sup>3</sup> and fetal anomalies.<sup>4</sup> Recently, it was reported that maternal cortisol levels in the urine were associated with early pregnancy loss.<sup>5</sup> As shown in Figure 1, the stressor has an effect on the hypothalamus and affects both the hypothalamus-pituitary gland-adrenal cortex axis and sympathetic adrenal medulla system. The markers secreted in the saliva are useful because they are easy to measure without

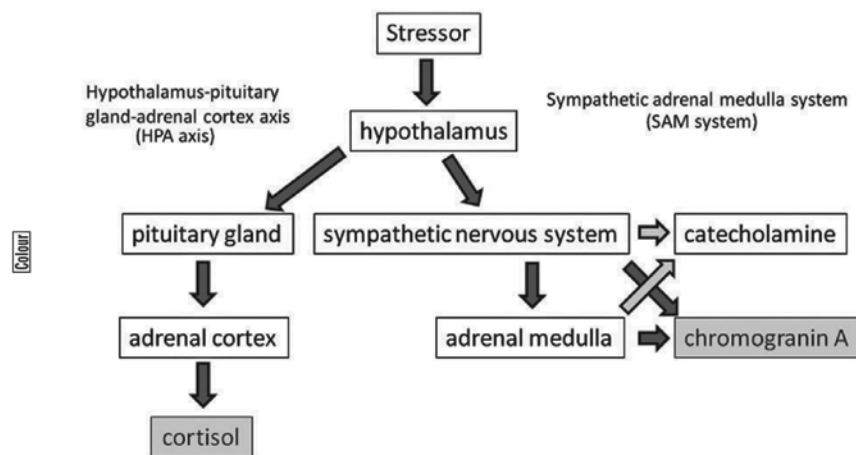
Received: ••.

Accepted: ••.

Reprint request to: Dr Koichiro Shimoya, Department of Obstetrics and Gynecology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan. Email: shimoya@med.kawasaki-m.ac.jp

The authors indicate no potential conflicts of interest.

H. Tsubouchi *et al.*



**Figure 1** Mechanism of induction of cortisol and chromogranin A by stressor.

causing sampling stress or pain. Evaluation of physiological stress markers in the saliva, such as cortisol and catecholamine, is a very useful method for objectively assessing stress. Salivary cortisol is often used as a stress index because its measurement has various advantages, such as non-invasive collection procedure. However, the measurement of salivary catecholamine is difficult because of its low concentration and rapid degradation.<sup>6</sup> Chromogranin A (CgA) is a good marker of psychological stress response, because it reflects only psychological stress, not physiological stress.<sup>7,8</sup> CgA is an acidic-soluble glycoprotein and its concentration can be measured in the saliva. It is known to localize in the secretory granules of a wide variety of endocrine and neuronal tissues.<sup>9</sup> The level of salivary CgA provides a sensitive and reliable index for evaluating psychological stress.<sup>7</sup> CgA is considered to be a substitute for catecholamine as CgA and catecholamine are co-released into the extracellular environment. Therefore, it is considered to be a measure of the activity of the sympathetic/adrenomedullary system, and salivary CgA has begun to be used as a stress marker.<sup>6</sup> We reported that salivary cortisol levels were significantly reduced after coffee intake, but not salivary chromogranin A concentration during pregnancy.<sup>10</sup> Nierop *et al.* demonstrated that salivary cortisol recovery was significantly prolonged in second-trimester pregnant women and prolonged cortisol recovery during the beginning of second-trimester pregnancy might be associated with vulnerability to stress-related pregnancy complications during this period of time.<sup>11</sup> The reaction of stress response during pregnancy was different from subjects of non-pregnant

status. However, there is little information regarding the detailed changes of salivary stress markers during pregnancy.

The aim of the present study was to show changes in salivary cortisol and CgA concentrations as stress markers during pregnancy and to clarify the difference of changes in these stress markers during pregnancy between chronic high stress and low stress groups.

## Material and Methods

### Samples

Saliva samples were obtained at 09.00 to 13.00 hours in the first trimester (10–12 w), second trimester (20–22 w), early third trimester (30–32 w), late third trimester (37–39 w), and puerperal period (1 month after delivery) from 69 normal pregnant women at Osaka University Hospital. Their average age was 32 years (20–40 years). Thirty one of the women were primipara. 34 had only one child and four had two children among the multiparas. We excluded patients who were taking steroid orally, or had complications or a fetus with an anomaly. The study was approved by the local ethics committee of the Department of Obstetrics and Gynecology, Osaka University Graduate School of Medicine. Informed consent was obtained from each patient. Examinations were performed from 10.00 to 11.00 hours, starting more than 2 h after consumption of the morning meal, because no food should be consumed for at least 90 min before salivary sampling for cortisol determination.<sup>12</sup> All saliva samplings were performed in the hospital under the researcher's guidance.

## Measures

We surveyed the women's chronic stress using Zung's Self-rating Depression Scale (SDS) and General Health Questionnaire-28 (GHQ28) at the second trimester and categorized the women into chronic high stress and normal groups. The SDS includes 20 questions that qualify the severity of depression symptoms. Each item ranges from 1 (none or a little of the time) to 4 (more or all of the time). The raw SDS score is the sum of all 20 items and ranges from 20 to 80.<sup>13</sup> This test is sensitive enough to detect depressive symptoms in normal populations. Its Japanese version has been ascertained to possess good internal consistency and test-retest reliability, and has been used in many research projects, such as population surveys.<sup>14</sup> The General Health Questionnaire (GHQ) is a 60-item self-administered screening tool designed to detect non-psychotic psychiatric illnesses. The GHQ28, a shorter version of the original GHQ, has been shown to be reliable and valid.<sup>14</sup> The GHQ28 was used to assess general health problems.<sup>15</sup> It is used in the general population and within community or non-psychiatric clinical settings, such as primary care or general medical outpatients.<sup>16</sup> The use of both the GHQ28 and SDS can enable the assessment of a variety of aspects of the mental health status in a normal population.<sup>14</sup> Using this questionnaire, we classified patients into chronic high stress and normal groups and made a comparison of the changes in stress markers between the two groups.

## Determination of cortisol levels in the saliva

To collect a sufficient quantity of saliva, we used Salivette sampling devices (Sarstedt, Rommelsdorf, Germany). The Salivette includes a small cotton swab and stimulates saliva flow to a rate that enables a sufficient amount to be collected within 1 min. After centrifugation at 7000 g for 15 min, saliva was stored at -80°C until assay. Saliva cortisol levels were determined with a commercial enzyme immunoassay kit (CIRON, Tokyo, Japan). The intra- and interassay coefficient variabilities (CV) were <10%.<sup>17</sup>

## Determination of CgA levels in the saliva

Salivary CgA levels were determined by ELISA using a previously described method.<sup>18</sup> The concentration of CgA in the saliva was determined using a YK070 Chromogranin A (Human) electro-immunoassay kit (Yanaihara Institute, Shizuoka, Japan) and the intra- and interassay CV were <5%.<sup>19</sup>

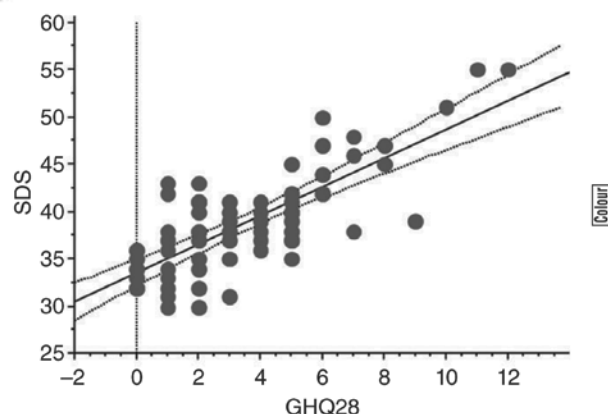
## Statistical analysis

Data are given as mean  $\pm$  SEM. The data were analyzed with StatView version 5.0 (SAS Institute, Cary, NC, USA). Statistical significance was assessed using Wilcoxon signed-rank test with a 5% significance level.

## Results

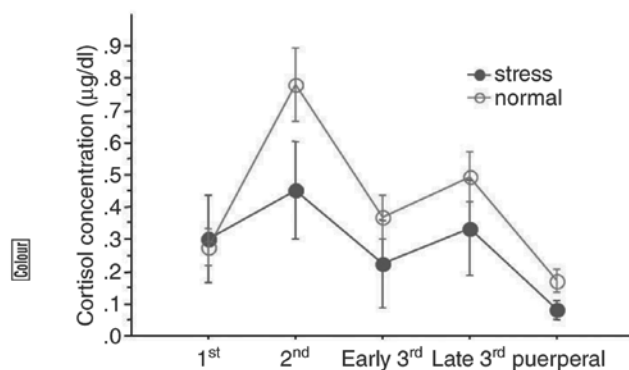
The mean salivary cortisol levels ( $\mu\text{g/dL} \pm \text{SEM}$ ) were  $0.282 \pm 0.054$  (first trimester),  $0.693 \pm 0.094$  (second trimester),  $0.330 \pm 0.061$  (early third trimester),  $0.452 \pm 0.069$  (late third trimester), and  $0.147 \pm 0.028$  (puerperal period). A repeat measure analysis of variance confirmed a significant effect of gestational age in salivary cortisol level ( $P < 0.0001$ ). Cortisol levels in the saliva of pregnant women showed biphasic change during pregnancy. Mean salivary CgA/protein levels ( $\text{pmol/mg} \pm \text{SEM}$ ) were  $1.919 \pm 0.543$  (first trimester),  $4.203 \pm 0.975$  (second trimester),  $3.617 \pm 0.715$  (early third trimester),  $2.619 \pm 0.481$  (late third trimester), and  $1.460 \pm 0.262$  (puerperal period). A repeat measure analysis of variance confirmed a significant effect of gestational age in salivary CgA/protein ( $P = 0.0005$ ). CgA/protein levels in the saliva of pregnant women increased in the second and the early third trimesters and decreased to the puerperal period.

Figure 2 demonstrated that SDS score and general health questionnaire GHQ28 score were significantly correlated ( $P < 0.005$ ). Patients in the chronic high stress

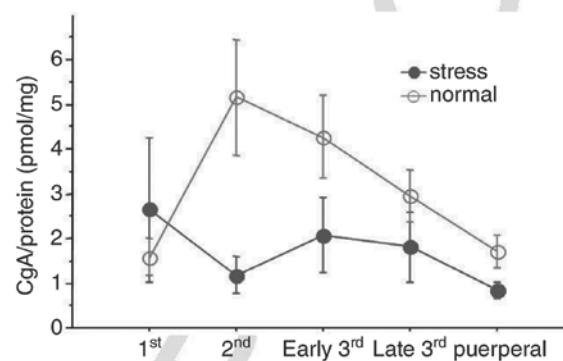


**Figure 2** Correlation between the Zung self-rating depression scale (SDS) and General Health Questionnaire-28 (GHQ28) scores. SDS score and general health questionnaire GHQ28 score were significantly correlated.  $\text{SDS} = 33.527 + 1.516 \times \text{GHQ28}$ ;  $r^2 = 0.58$  ( $P < 0.005$ ).

H. Tsubouchi *et al.*



**Figure 3** Difference in salivary cortisol concentrations between chronic high stress group (Zung self-rating depression scale [SDS] > 42) and normal group (SDS ≤ 42). Salivary cortisol concentrations of the chronic high stress group were significantly lower compared with those of the normal group at the second trimester, early third trimester, and late third trimester ( $P < 0.01$ ).



**Figure 4** Difference in salivary chromogranin A (CgA) concentrations between the chronic high stress group (Zung self-rating depression scale [SDS] > 42) and normal group (SDS ≤ 42). Salivary CgA/protein concentrations of the chronic high stress group were significantly lower than those of the normal group ( $P < 0.01$ ).

group (SDS > 42) were 17 of 69 pregnant women. Characteristics between the chronic high stress group and the normal group, such as maternal age, body weight, body mass index, smoking rate, parity, gravidity, delivery week, neonatal body weight and Apgar score were similar (data not shown). As shown in Figure 3, salivary cortisol concentrations of the chronic high stress group were significantly lower compared with those of the normal group at the second trimester, early third trimester, and late third trimester ( $P < 0.01$ ). However, titers of salivary cortisol of the chronic high stress group were equal to those of the normal group at the first trimester and puerperal period. Salivary CgA/protein concentrations of the chronic high stress group were significantly lower than those of the normal group ( $P < 0.01$ ) (Fig. 4). The elevation of CgA/protein in the saliva was suppressed in the chronic high stress group during pregnancy.

## Discussion

Investigation of physiological stress markers in the saliva is a very useful method for objectively measuring stress. This method is non-invasive and non-stressful. Recent study demonstrated that music therapy during colonoscopy markedly reduces fear-related stress, as indicated by changes in salivary cortisol levels.<sup>20</sup> In the present study, the titration of salivary cortisol concentrations and CgA levels is a

useful tool to determine maternal stress levels during pregnancy. Cortisol levels in the saliva of pregnant women showed biphasic change during pregnancy. Mean salivary cortisol levels increased from the first trimester to the second trimester. Those levels decreased from the second trimester to the early third trimester and increased at the late third trimester. Titers of salivary cortisol at the puerperal period returned to the levels at the first trimester. Serum concentration of circulating cortisol is increased during pregnancy.<sup>21</sup> The reason for the discrepancy between serum and saliva is unknown. The CgA/protein levels in the saliva of pregnant women increased in the second and the early third trimesters and decreased to the puerperal period. The mechanism of the change of stress marker levels during pregnancy is unknown. We speculated that the titers of those stress markers might be affected by hormonal changes, such as progesterone and immunological changes during pregnancy. Further investigations are necessary to determine the mechanism of the change of stress levels during pregnancy and the usefulness of cortisol and CgA as stress markers during pregnancy.

Psycho-mental health potentials were evaluated by both quality-of-life-related questionnaires and stress-related hormonal and cytokine levels, such as cortisol and interleukins. Self-reporting instruments represent an efficient and cost-effective way to identify individuals who should be evaluated additionally for the presence of a depressive disorder. The Zung

Self-rating Depression Scale (SDS) has 10 positively worded and 10 negatively worded items that cover affective, psychological, and somatic symptoms. The overall score represents the severity of the depressive symptoms.<sup>22</sup> GHQ28 represented the general health problems of pregnant women. To determine psychological health potentials, several questionnaires have been used. Zung SDS and GHQ28 are useful tools because in Japan, the scale has been ascertained to have good internal consistency and test-retest reliability, and construct validity has been demonstrated in many research projects, including population surveys.<sup>23</sup>

In a population of Japanese women (mean age 46.3 years), mean SDS score was 37.8, 40.7, and 47.8 in the good lifestyle group, average lifestyle group, and poor lifestyle group, respectively.<sup>24</sup> We noticed that there was a significant correlation between SDS and GHQ28. These results suggest that maternal depressive status represents general health during pregnancy. Several studies demonstrated maternal stress had adverse effects on pregnancy outcome.<sup>2-5</sup> The betterment of SDS and GHQ28 might affect the improvement of maternal and neonatal outcomes.

Maternal salivary cortisol and CgA of the chronic high stress group were significantly suppressed in comparison with those of the normal group. These results suggest that maternal chronic stress suppress the function or activity of the hypothalamic-pituitary-adrenal axis and sympatho-adrenomedullary system. Angelika *et al.* demonstrated that in children with allergic feature of atopic dermatitis under chronic psychosocial stress, the reaction of cortisol level to the stress test were suppressed.<sup>25</sup> Furthermore, Seng *et al.* found that for pregnant woman affected by posttraumatic stress disorder, the circadian rhythm of cortisol titer was suppressed and they had lower peak basal salivary cortisol concentrations.<sup>26</sup> Our results do not contradict those reports. The suppression of maternal response at the adrenal gland might affect the maternal immune defense system and endocrine function. This would be one possible reason why maternal stress has adverse effects on pregnancy outcome.

## Acknowledgments

This work was supported, in part, by Grants-in-Aid for Scientific Research (Nos. 21592118) from the Ministry of Education, Science, and Culture of Japan (Tokyo, Japan), Health Labor Sciences Research Grant of Research on Child and Families (Tokyo, Japan), and

grants of the Kawasaki Medical School Project 21-411 (Kurashiki, Japan).

## References

- Morimoto K. [Lifestyle and health]. *Nippon Eiseigaku Zasshi* 2000; **54**: 572-591.
- Holzman C, Senagore P, Tian Y *et al.* Maternal catecholamine levels in midpregnancy and risk of preterm delivery. *Am J Epidemiol* 2009; **170**: 1014-1024.
- Maric NP, Dunjic B, Stojiljkovic DJ, Britvic D, Jasovic-Gasic M. Prenatal stress during the 1999 bombing associated with lower birth weight - a study of 3815 births from Belgrade. *Arch Womens Ment Health* 2010; **13**: 83-89.
- Hansen D, Lou HC, Olsen J. Serious life events and congenital malformations: A national study with complete follow-up. *Lancet* 2000; **356**: 875-880.
- Nepomnaschy PA, Welch KB, McConnell DS, Low BS, Strassmann BI, England BG. Cortisol levels and very early pregnancy loss in humans. *Proc Natl Acad Sci USA* 2006; **103**: 3938-3942.
- Miyakawa M, Matsui T, Kishikawa H *et al.* Salivary chromogranin A as a measure of stress response to noise. *Noise Health* 2006; **8**: 108-113.
- Nakane H, Asami O, Yamada Y *et al.* Salivary chromogranin A as an index of psychosomatic stress response. *Biomed Res* 1998; **19**: 401-406.
- Nakane H, Asami O, Yamada Y, Ohira H. Effect of negative air ions on computer operation, anxiety, and salivary chromogranin A-like immunoreactivity. *Int J Psychophysiol* 2002; **46**: 85-89.
- Winkler H, Fischer-Colbrie R. The chromogranin A and B: The first 25 years and future perspectives. *Neuroscience* 1992; **49**: 497-528.
- Tsubouchi H, Shimoya K, Hayashi S, Toda M, Morimoto K, Murata Y. Effect of coffee intake on blood flow and maternal stress during the third trimester of pregnancy. *Int J Gynaecol Obstet* 2006; **92**: 19-22.
- Nierop A, Bratsikas A, Klinkenberg A, Nater UM, Zimmermann R, Ehlert U. Prolonged salivary cortisol recovery in second-trimester pregnant women and attenuated salivary alpha-amylase responses to psychosocial stress in human pregnancy. *J Clin Endocrinol Metab* 2006; **91**: 1329-1335.
- Toda M, Morimoto K, Nagasawa S, Kitamura K. Effect of snack eating on sensitive salivary stress markers cortisol and chromogranin A. *Environ Health Prev Med* 2004; **9**: 27-29.
- Zung WW. A self-rating depression scale. *Arch Gen Psychiatry* 1965; **12**: 63-70.
- Suda M, Nakayama K, Morimoto K. Relationship between behavioral lifestyle and mental health status evaluated using the GHQ-28 and SDS questionnaires in Japanese factory workers. *Ind Health* 2007; **45**: 467-473.
- Goldberg DP, Hillier VF. A scaled version of the General Health Questionnaire. *Psychol Med* 1979; **9**: 139-145.
- Vallejo MA, Jordan CM, Diaz MI, Comeche MI, Ortega J. Psychological assessment via internet: A reliability and validity study of online (vs paper-and-pencil) versions of the general health questionnaire-28 (GHQ-28) and the symptoms check-list-90-revised (SCL-90-R). *J Med Internet Res* 2007; **9**:

H. Tsubouchi *et al.*

- e2.
17. Shimada M, Takahashi K, Ohkawa T, Segawa M, Higurashi M. Determination of salivary cortisol by ELISA and its application to the assessment of the circadian rhythm in children. *Horm Res* 1995; **44**: 213–217.
18. Shingo N, Yasuko N, Li J *et al.* Simple enzyme immunoassay for the measurement of immunoreactive chromogranin A in human plasma, urine and saliva. *Biomed Res* 1998; **19**: 407–410.
19. Hong R-H, Yang Y-J, Kim S-Y, Lee W-Y, Hong Y-P. Determination of appropriate sampling time for job stress assessment: The salivary chromogranin A and cortisol in adult females. *J Prev Public Health* 2009; **42**: 231–236.
20. Uedo N, Ishikawa H, Morimoto K *et al.* Reduction in salivary cortisol level by music therapy during colonoscopic examination. *Hepatogastroenterology* 2004; **51**: 451–453.
21. Carr BR, Parker CR Jr, Madden JD, MacDonald PC, Porter JC. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. *Am J Obstet Gynecol* 1981; **139**: 416–422.
22. Nelson CJ, Cho C, Berk AR, Holland J, Roth AJ. Are gold standard depression measures appropriate for use in geriatric cancer patients? A systematic evaluation of self-report depression instruments used with geriatric, cancer, and geriatric cancer samples. *J Clin Oncol* 2010; **28**: 348–356.
23. Sarai K. Epidemiology of depression. *Jpn Psychiatry Neurol* 1979; **82**: 777–784.
24. Maruyama S, Morimoto K. The effects of lifestyle and type A behavior on the life-stress process. *Environ Health Prev Med* 1997; **2**: 28–34.
25. Angelika BK, Kristin VA, Dilke K, Stefan W, Wolfgang R, Dirk H. Blunted cortisol responses to psychosocial stress in asthmatic children: A general feature of atopic disease? *Psychosom Med* 2003; **65**: 806–810.
26. Seng JS, Low LK, Ben-Ami D, Liberzon I. Cortisol level and perinatal outcome in pregnant women with posttraumatic stress disorder: A pilot study. *J Midwifery Womens Health* 2005; **50**: 392–398.