Changes in Serum Adenosine Deaminase Activity during Normal Pregnancy

Adenosine deaminase (ADA), an enzyme essential for the differentiation of lymphoid cells, has been used for monitoring diseases with altered immunity. The purpose of this study was to investigate the changes in serum ADA activity throughout normal pregnancy. We measured the catalytic values of serum ADA from 202 normal pregnant women using a commercial kit. Subjects were divided into four groups according to the gestational age in weeks (Gwks) (Group I: 5-9 Gwks [n=58]; Group II: 15-20 Gwks [n= 63]; Group III: 24-30 Gwks [n=34]; Group IV: 30-39 Gwks [n=47]). The serum ADA levels for the Groups I, II, III, and IV were as follows: 20.1 ± 6.9 IU/L, 20.0 ± 7.6 IU/L, 37.9 ± 19.9 IU/L, and 24.5 ± 8.6 IU/L, respectively. The serum ADA activity of group III was significantly higher than the other groups ($p<0.05$). However, there was no significant correlation between the Gwks and the serum ADA activity. Furthermore, other parameters, such as maternal age ($p=0.29$), gestational age at delivery ($p=0.07$), delivery mode ($p=0.39$), and birth weight ($p=0.59$) had no correlation with ADA activity. Reference values of serum ADA in normal pregnancy may provide important database for making clinical decisions in pregnancies complicated by conditions where cellular immunity has been altered.

Key Words : *Adenosine Deaminase; Immunity; Cellular; Pregnancy*

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INTRODUCTION

Adenosine deaminase (ADA), an enzyme essential for the differentiation of lymphoid cells, has been used for monitoring several diseases in which immunity has been altered (1). As an indicator of cellular immunity, the serum activity of this enzyme has been suggested to be altered in diseases that cause a cell-mediated immune response such as rheumatoid arthritis, systemic lupus erythematosus, and tuberculosis (2, 3).

ADA catalyses the hydrolytic deamination of adenosine to inosine and 2'-deoxyadenosine to 2'-deoxyinosine (4, 5). This enzyme is widely distributed in human tissues, especially in the lymphoid tissues, and is essential for the maturation and function of human blood monocytes and macrophages (6, 7). It has been considered as an indicator of a nonspecific marker of T-cell activation (8). Human ADA exists in at least three molecular isoforms, ADA1, ADA2, and ADA1 and ADA-complexing protein (9). The homeostasis of these isoforms and the activity of the isoenzymes ADA1 and ADA2 in human cells are of extreme importance in maintaining the homeostasis between adenosine and 2'-deoxyadenosine in monocytes and macrophages (4, 5). There have been several reports on the increase in serum ADA activity in diseases where cellular immunity is activated (6). Lymphocytes or the

monocyte-macrophage cell system have been assumed to be responsible for the changes in serum ADA activity; however, the precise mechanisms by which serum ADA activity is altered has not been clarified (6, 11).

Normal pregnancy has been associated with depressed cell immunity (12), and thus, serum ADA activity may play a significant role (13). There have been conflicting reports about the changes in the activity of serum ADA in normal pregnancy compared to the age-matched non-pregnant women (14-16). However, to our knowledge, only few studies have been performed investigating the changes in serum ADA activity according to gestational age, and the clinical significance of changes in ADA activity throughout normal pregnancy has not been elucidated (14, 15). Several studies have shown that serum ADA activity is significantly increased in women with recurrent spontaneous abortions, preeclampsia, and hyperemesis gravidarum where enhanced cell-mediated immunity is thought to be an important pathogenesis (17-19). There have also been reports showing low levels of both maternal serum and placental ADA activity in anembryonic pregnancies and missed abortions (20).

In this study, we measured the serum ADA activity in normal pregnant women and examined the changes of serum ADA activity according to the gestational age.

MATERIALS AND METHODS

Patients who received continuous antenatal care and underwent either normal spontaneous vaginal delivery or cesarean section at the Yonsei University Medical Center from January of 2004 until March of 2006 were recruited for this study. The criteria for eligibility were as follows: 1) well established gestational age confirmed by ultrasonography, 2) singleton fetus, 3) no fetal anomaly, 4) nonsmoker, 5) normal response to glucose tolerance testing, 6) no evidence of recent infection, 7) no prescribed medications, and 8) no maternal medical complications. Each patient was examined monthly in the outpatient department until the 24th week of gestation, then bimonthly until the 36th week of gestation and subsequently weekly until delivery. If the women developed any complications throughout the pregnancy, they were excluded from the study. All deliveries resulted in live infants with an 1-min Apgar score ≥7 and each infant's birth weight was appropriate for gestational age.

Total of 202 women met the inclusion criteria and were included in this study. All patients gave informed consent after having been informed that they would not benefit directly from the measurement of the serum ADA. In order to reduce the inconvenience to the participants, the blood specimens were collected when the participants came for their routine examinations; first-visit laboratory tests (5-10 gestational weeks), serum α-fetoprotein screening (15-20 gestational weeks), glucose tolerance test (24-28 gestational weeks), and pre-delivery laboratory tests (30-39 weeks). The study groups were divided into four groups as follows: Group I-from 5 to 10 gestational weeks (n=58); Group II-from 15-20 gestational weeks (n=63); Group III-from 24-30 gestational weeks (n=34); and Group IV: from 30 to 39 gestational weeks (n=47).

The measurements were taken after an overnight fast. Venous blood specimens were collected and the serum obtained was stored at -80°C until further analysis. The activity of the ADA was assayed using an automatic analyzer (TBA-80FR; Toshiba, Tokyo, Japan) with a commercial kit according to the instructions of the manufacturer (Toyobo, Osaka, Japan). The enzyme activity was determined by quantifying inosine liberated from the substrate, adenosine (10 mM). Inosine was converted by purine nucleoside phosphorylase into hypoxanthine, which was further converted by xanthine oxidase into uric acid and hydrogen peroxide. The hydrogen peroxide was then converted into the quinone dyes by peroxidase, 4-aminoantipyrine, and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine, and the absorbance at 548/700 nm was measured. The detection limit of the assay was 0-200 IU/L with intra- and interassay coefficients of variable less than 5% and 10%, respectively.

Data were shown as Median ± SEM (standard error of the median). Statistical analysis of the results was carried out using the one-way ANOVA (analysis of variance) for comparison between the four groups. Multiple logistic regression was also performed to evaluate the independent effects of variables on ADA activity. Differences were considered significant at $p<0.05$.

RESULTS

The clinical description of the participants in this study is given in Table 1. There was no significant difference among these four groups in terms of maternal age, parity, gestational age at delivery, birth weight of the infants, or delivery mode. All deliveries resulted in live infants with an 1-min Apgar score ≥7. Each infant's birth weight was appropriate for ges-
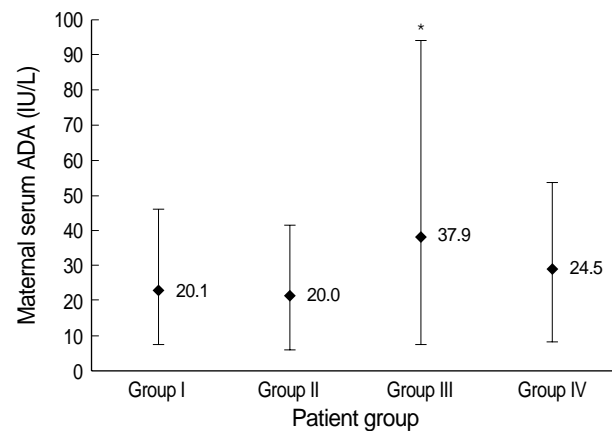

Fig. 1. Changes in serum ADA activity during normal pregnancies. *$p<0.05$ vs. Group I, II, and IV. Data represent median ± standard error of median.

Table 1. Clinical characteristics of patients in each group

	Group I (n=58)	Group II (n=63)	Group III (n=34)	Group IV (n=47)
Maternal age (yr)	29.2±3.4	29.4±2.8	29.9±2.5	29.6±3.8
Gestational age (wks) at study	7.6±3.2	15.8±4.1	26.2±2.6	36.1±3.2
Gestational age (wks) at delivery	39.0±2.2	39.1±3.1	38.9±2.9	39.4±3.1
Parity	0.5±0.2	0.4±0.3	0.3±0.2	0.5±0.3
Birth weight (g)	3,261±723	3,219±897	3,268±567	3,257±891
Delivery mode (%)				
Vaginal delivery	75.9	81	73.5	76.7
Cesarean section	24.1	19	26.5	23.3

Data represented as mean ± STD (standard deviation) value.

tational age.

Changes in the serum ADA activities are shown in Fig. 1. The median serum ADA levels in the Groups I, II, III and IV were as follows; 20.1 ± 6.9 IU/ L, 20.0 ± 7.6 IU/L, 37.9 ± 19.9 IU/L, and 24.5 ± 8.6 IU/L, respectively. The median serum ADA activity of Group III (24-30 gestational weeks) was significantly ($p<0.05$) higher than those of Groups I, II and IV. There was no significant correlation between the serum ADA activity and other measured variables such as maternal age ($p=0.29$), gestational age at delivery ($p=0.07$), delivery mode ($p=0.39$), and birth weight ($p=0.59$).

DISCUSSION

ADA is an essential enzyme for the differentiation of lymphoid cells, and changes in ADA activity reflect alteration in immunity (6-8). However, to have a clear diagnostic significance in infectious diseases, the value of ADA activity should be at least 2.5 times the normal value (21). Alterations in the serum ADA activity during pregnancy are likely to reflect, at least in part, changes in the immunological status that occurs throughout normal pregnancy (12). However, to our current knowledge, there have only been few reports providing the reference values of serum ADA activity throughout normal pregnancy (14, 15).

Yoneyama et al. have measured serum activities of total ADA, ADA1, and ADA2 in normal pregnant women in the third trimester and age-matched healthy nonpregnant women (16). In normal pregnant women, serum total ADA and ADA2 activities were lower than those of the nonpregnant women, while there was no difference in ADA1 activity. These results suggest that reduced serum total ADA activity reflects decreased ADA2 activity, which may be in part associated with depressed cell-mediated immunity during normal pregnancy (16). One possible explanation for decreased ADA activity in normal pregnancy is due to the increase in pregnancy-related hormones such as estradiol and cortisol which tends to inhibit ADA activity (22). Another possible reason is the deamination of adenosine to inosine by ADA, which favors increased adenosine levels (23). Adenosine in turn exhibits inhibitory effect in platelet activation during pregnancy (24). Thus, the observed elevation of adenosine and decreased ADA activity in late normal pregnancy may be thought to be a compensatory mechanism that tends to maintain the vascular integrity to increase the uterine and placental blood flow.

On the contrary, in pregnancies complicated by preeclampsia which is characterized by enhanced cell-mediated immunity (25), ADA activities of both the maternal serum and fetal cord blood were increased (18, 26). An increase in ADA activity in preeclampsia may indirectly contribute to the maintenance of immune response in preeclampsia by controlling adenosine levels. Another study demonstrated an increase in serum total ADA and ADA2 activities in women with hyperemesis gravidarum, which was accompanied by high lymphocyte and monocyte counts (19). The clinical significance and the regulatory mechanism of the increased ADA activity in complicated pregnancies with altered cellular immunity remain to be elucidated.

In the present study, no significant correlation was shown between the serum ADA activity and gestational age in normal pregnant women. Serum ADA activity of Group III was significantly higher than the other three groups, which is somewhat different from an earlier study that showed that serum ADA values did not differ among each trimester of pregnancy (15); however, the study population differed from that of the present study, which may account for the discrepancy in the results. The significant increase in ADA activity seen in our study during 24 to 30 gestational weeks may be associated with the fact that the increase in maternal cardiac output reaches its peak during the late second trimester to early third trimester and decreases in late pregnancy (27). Moreover, maternal blood volume expands most rapidly during the late second trimester (28). The major source of circulating adenosine in the bloodstream is the platelet, vascular endothelium, and red blood cells (29). We can postulate that the rapid increase in red blood cells and vascular endothelium during the late second to early third trimester may release adenosine into the bloodstream, elevate the concentration, and thereby exert its vasodilator actions to increase blood supply to the uterine and placental unit. Thus, the activity of ADA may increase in order to degrade the elevated adenosine and to maintain the level of adenosine at its kinetic equilibrium. However, the clinical significance of the increase in serum ADA in Group III is not fully understood, and further studies are needed to elucidate the possible mechanisms involved.

A number of limitations of the present study should be noted. First, instead of dividing the participants into four different groups according to the gestational age, the serum ADA activity should be prospectively measured from the same set of normal pregnant women from early 1st trimester to delivery. Second, to our knowledge, we have not found any studies in the literature reporting the changes in the activities of ADA and its isoforms throughout the normal pregnancy. Thus, it would be interesting to see if the change in the serum ADA activity is accompanied by similar changes in ADA isoforms, especially ADA2. Despite the limitations of the this study, we were able to provide serum ADA values throughout normal pregnancy from early first trimester to third trimester which was different from previous studies that only compared the serum ADA activity of pregnant women in their third trimester to the non-pregnant controls (16).

We conclude that serum ADA activity may be a marker of altered cell-mediated immunity in pregnancy, and the significance of the regulatory mechanisms that alter the activity of serum ADA and its isoenzymes need further studies.

Reference values of serum ADA in normal pregnancy may provide important database for making clinical decisions in high-risk pregnancies where cellular immunity has been altered.

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