A1C but Not Serum Glycated Albumin Is Elevated in Late Pregnancy Owing to Iron Deficiency

KUNIHIKO HASHIMOTO, MD, PHD[1]
SANAI NOGUCHI, MD, PHD[1]
YASUHIKO MORIMOTO, MD, PHD[1]
SHINICHI HAMADA, MD[2]
KENSHI WASADA, MD[2]
SHIRO IMAI, MD, PHD[2]
YUJI MURATA, MD, PHD[2]
SOJI KASAYAMA, MD, PHD[3]
MASAFUMI KOGA, MD, PHD[4]

OBJECTIVE — A1C levels have been shown to be elevated in relation to glycemia in late pregnancy, although the precise mechanisms remain undetermined. We hypothesized that iron deficiency is involved in the A1C increase in late pregnancy.

RESEARCH DESIGN AND METHODS — In study 1, A1C, serum glycated albumin, erythrocyte indexes, and iron metabolism indexes were determined in 47 nondiabetic pregnant women not receiving iron supplementation who were divided into four groups according to gestational period (group I, 21–24 weeks; group II, 25–28 weeks; group III, 29–32 weeks; and group IV, 33–36 weeks). In study 2, these determinants were obtained at two gestational periods (20–23 weeks and 32–33 weeks) in 17 nondiabetic pregnant women.

RESULTS — In study 1, A1C levels were higher in groups III and IV than those in groups I and II, whereas serum glycated albumin levels were not different among these four groups. Hemoglobin, mean corpuscular hemoglobin (MCH), serum transferrin saturation, and serum ferritin were lower in groups III and IV. A1C levels were negatively correlated with MCH, serum transferrin saturation, and serum ferritin. In study 2, A1C levels were significantly increased at gestational weeks 32–33 from those at weeks 20–23, whereas serum glycated albumin levels did not differ between the two gestational periods. MCH, serum transferrin saturation, and serum ferritin were decreased at gestational weeks 32–33. A1C levels showed a negative correlation with MCH, serum transferrin saturation, and serum ferritin.

CONCLUSIONS — A1C levels were elevated in late pregnancy owing to iron deficiency. Serum glycated albumin may offer a better index for monitoring glycemic control in pregnancy.

Diabetes Care **31:1945–1948, 2008**

In pregnant women displaying diabetes and women with gestational diabetes mellitus, intensive glycemic control during pregnancy is needed to lower the risk of intrauterine fetal death, fetal growth disorders, and maternal complications (1,2). The extent of nonenzymatic glycation of proteins increases in diabetic patients. Of these glycated proteins, A1C is widely used as the current standard marker for monitoring chronic glycemic control (3,4) and represents an important target for treatment of diabetic patients (5). Phelps et al. (6) showed biphasic changes in A1C levels during pregnancy, with A1C levels being lowest at gestational week 24. A longitudinal study also demonstrated similar biphasic changes in A1C levels (7).

A1C measurements are known to be profoundly affected by erythrocyte turnover, as are plasma glucose levels (8,9). Blood dilution–related anemia is known to be frequently observed in pregnancy. In late pregnancy, iron deficiency anemia is also often observed, caused by the increased demands for iron (10). A1C levels have been shown to be higher in relation to glycemia in patients with iron deficiency anemia (11–13). We have recently shown that A1C levels are higher in premenopausal women with an iron-deficient state, even in the absence of anemia (14). We therefore hypothesized that A1C levels are set higher in relation to glycemia in late pregnancy, during which most women are iron deficient. To confirm this possibility, we studied the relationship between A1C and iron metabolism in nondiabetic pregnant women. For clinical issues, a study performed in pregnant diabetic women is important. However, in diabetic women fluctuations of plasma glucose may directly influence A1C levels beyond the effect of iron metabolism, making it difficult to analyze the direct effects of gestational course on A1C levels. Thus, in this study, we aimed to examine the relationship between A1C and iron metabolism in nondiabetic pregnant women, in whom the influence of plasma glucose levels is minimal. Serum glycated albumin, a different indicator for chronic glycemia, was also studied in these subjects.

RESEARCH DESIGN AND METHODS

— In a cross-sectional study (study 1), we studied 47 pregnant Japanese women at gestational weeks 21–36. All subjects had been seen at Aizenbashi Hospital from February to July 2007, and ambulatory plasma glucose levels were <100 mg/dl. Mean ± SD age was 29.5 ± 5.7 years. All subjects had not been and were not receiving iron and vitamin supplementations during pregnancy. Subjects were divided into four groups according to gestational period: group I (n = 20), gestational weeks 21–24; group II (n = 9), gestational weeks 25–28; group III (n = 11), gestational weeks 29–32; and group IV (n = 7), gestational weeks 33–36. A1C, erythrocyte (red blood cell [RBC]) count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum iron, serum transferrin saturation, serum ferritin, and glycated albumin were determined.

From the [1]Department of Medicine, Aizenbashi Hospital, Osaka, Japan; the [2]Department of Gynecology and Obstetrics, Aizenbashi Hospital, Osaka, Japan; the [3]Department of Medicine, Nissay Hospital, Osaka, Japan; and the [4]Department of Medicine, Kinki Central Hospital, Hyogo, Japan.
Corresponding author: Masafumi Koga, koga_m@kich.itami.hyogo.jp.
Received 18 February 2008 and accepted 26 June 2008.
Published ahead of print at http://care.diabetesjournals.org on 3 July 2008. DOI: 10.2337/dc08-0352.

In a longitudinal study (study 2), we studied 17 nondiabetic pregnant women who had been seen at Aizenbashi Hospital between February and July 2007. Their age was 28.0 ± 5.7 years. A1C, RBC count, hematocrit, hemoglobin, MCV, MCH, serum iron, serum transferrin saturation, serum ferritin, and serum glycated albumin were determined at two periods (gestational weeks 20–23 and 32–33).

For control subjects, 19 age-matched nonpregnant healthy women whose age was 27.7 ± 2.0 years were also studied.

The investigations reported here were performed in accordance with the principles of the Declaration of Helsinki as revised in 2000. The institutional review board approved this study, and all patients provided written informed consent.

Laboratory methods

A1C was measured by latex aggregation immunoassay using Determiner HbA_{1C} (Kyowa Medix, Tokyo, Japan), which was found not to be influenced by hemoglobin F and other minor hemoglobin species (15), with calibration using Japan Diabetes Society Lot 2 (16). Inter- and intra-assay coefficients of variation were 0.98 and 0.97%, respectively. Serum glycated albumin was determined by enzymatic methods using albumin-specific protease, ketoamine oxidase, and albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan) (17). Blood cell counts, hematocrit, hemoglobin, MCV, and MCH were measured by an automated hematology system. Serum iron and unsaturated iron-binding capacity were determined by a calorimetric method. Serum ferritin concentrations were measured by a chemiluminescent immunoassay method. Total iron-binding capacity and serum transferrin saturation were calculated by adding unsaturated iron-binding capacity to serum iron and dividing serum iron by total iron-binding capacity, respectively. All tests were performed in a central laboratory at Aizenbashi Hospital.

Statistical analyses

Data are shown as means ± SD for continuous variables and as numbers for categorical variables. Unadjusted comparisons for continuous variables were performed among groups I–IV using ANOVA, and unpaired *t* tests were used to estimate the level of significance of differences between means. To evaluate relationships between A1C levels and different variables, single linear univariate regression analyses were performed. In study 2, paired *t* tests were used to compare two groups. The StatView computer program (version 5.0 for Windows; Abacus Concepts, Berkeley, CA) was used for all statistical analyses. Values of $P < 0.05$ were considered statistically significant.

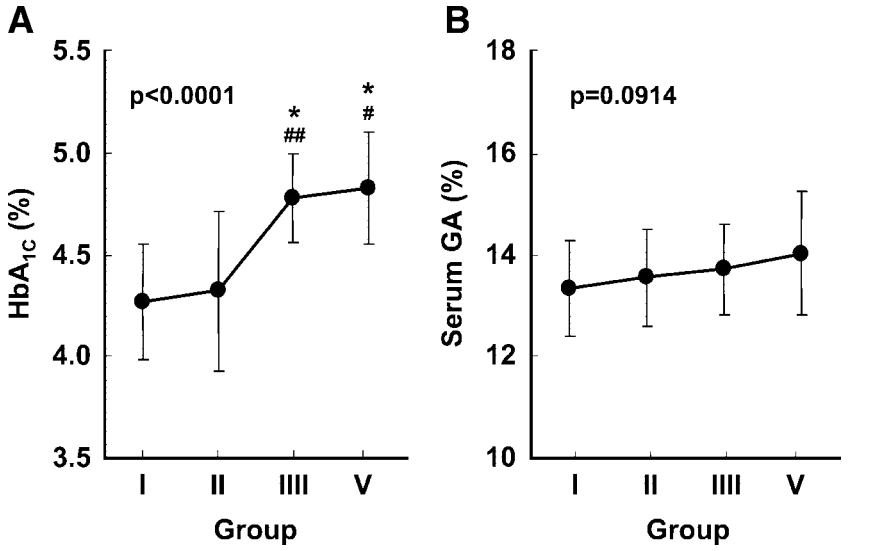

Figure 1— *A1C* (A) *and serum glycated albumin (GA)* (B) *levels in 47 pregnant women (study 1) divided according to gestational period into group I (21–24 weeks;* n = *20), group II (25–28 weeks;* n = *9), group III (29–32 weeks;* n = *11), and group IV (33–36 weeks;* n = *7).* *P < *0.001 versus group I;* #P < *0.05 versus group II;* ##P < *0.01 versus group II.*

RESULTS — Figure 1 shows A1C and glycated albumin levels in pregnant women divided into four groups according to gestational period. The results show that A1C levels were higher in groups III (29–32 weeks) and IV (33–36 weeks) than in groups I (21–24 weeks) and II (25–28 weeks). Glycated albumin

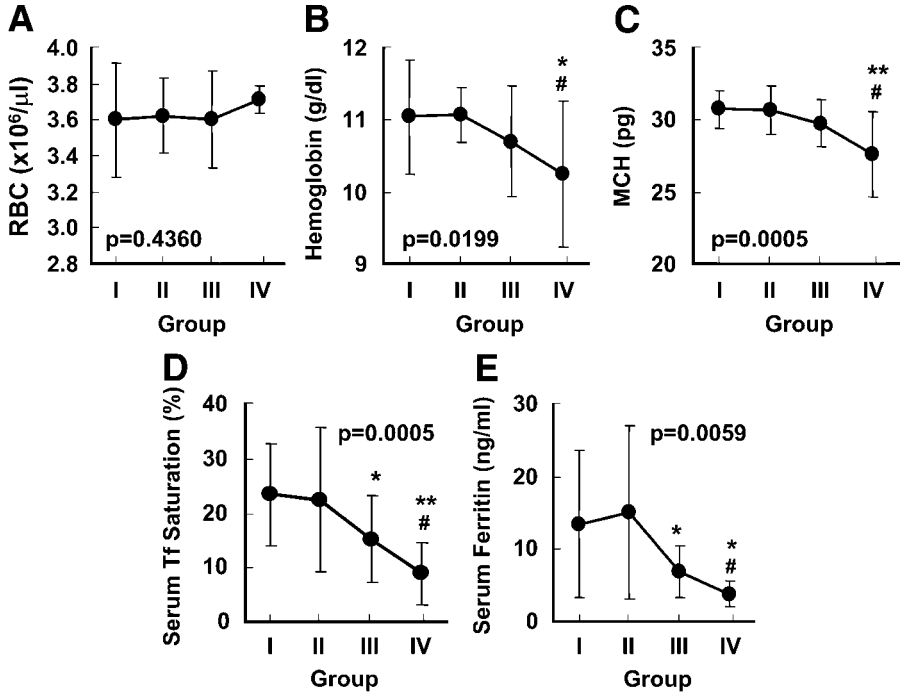

Figure 2— *RBC counts* (A)*, hemoglobin* (B)*, MCH* (C)*, serum transferrin (Tf) saturation* (D)*, and serum ferritin* (E) *in 47 pregnant women (study 1) divided according to gestational period into group I (21–24 weeks;* n = *20), group II (25–28 weeks;* n = *9), group III (29–32 weeks;* n = *11), and group IV (33–36 weeks;* n = *7).* *P < *0.05 versus group I;* **P < *0.001 versus group I;* #P < *0.05 versus group II.*

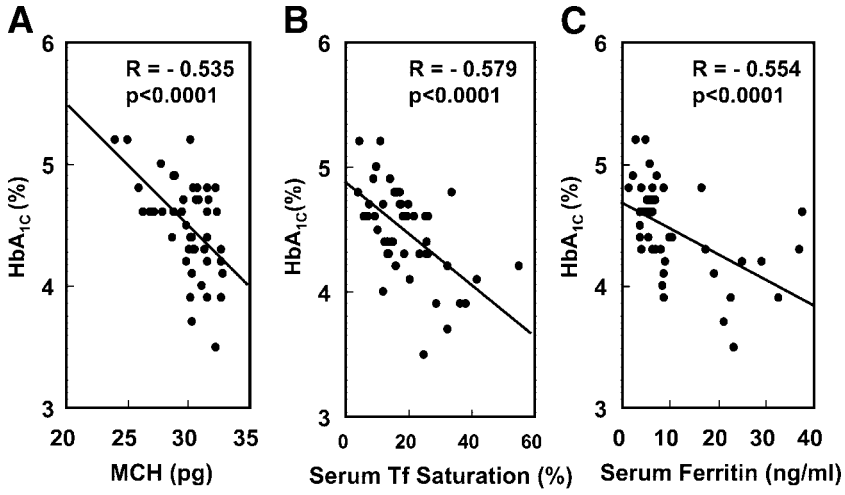

Figure 3— *Association of A1C levels with MCH* (A)*, serum transferrin (Tf) saturation* (B)*, and serum ferritin (C) in 47 pregnant women (study 1).*

levels remained constant in these four groups. RBC counts did not differ among the four groups of pregnant women. However, hemoglobin, MCH, transferrin saturation, and serum ferritin levels were lower in groups III and IV (Fig. 2). A1C levels were negatively correlated with MCH, serum transferrin saturation, and serum ferritin (Fig. 3).

Next, we studied 17 pregnant individuals at two periods during middle pregnancy (20–23 weeks) and late pregnancy (32–33 weeks). A1C levels significantly increased from middle pregnancy (4.4 ± 0.2%) to late pregnancy (4.8 ± 0.2%; $P < 0.0001$), whereas serum glycated albumin levels did not change (from 13.9 ± 1.2 to 13.9 ± 1.0%; $P = 0.7029$) (Fig. 4). RBC counts were unchanged during both periods (365 ± 26 × 10^6/μl in middle pregnancy vs. 367 ± 20 × 10^6/μl in late pregnancy; $P = 0.6630$), whereas MCH (30.2 ± 1.5 vs. 28.8 ± 2.4 pg; $P = 0.0016$), transferrin saturation (21.7 ± 10.4 vs. 12.5 ± 7.9%; $P = 0.0011$), and serum ferritin (17.4 ± 14.3 vs. 5.8 ± 3.5 ng/ml; $P = 0.0022$) were decreased in late pregnancy compared with values in middle pregnancy. Hemoglobin levels were also decreased but of borderline significance (from 11.0 ± 0.5 to 10.6 ± 0.9 g/dl; $P = 0.0555$). When iron deficiency was defined as serum ferritin <15 ng/ml, this condition was present in 35% of women in middle pregnancy and 95% in late pregnancy. A1C levels were negatively correlated with MCH, transferrin saturation, and serum ferritin (Fig. 5).

In our study 2, the mean A1C level of 17 women in late pregnancy (32–33 weeks) was not significantly different from that of 19 age-matched nonpregnant women (4.8 ± 0.2 vs. 4.8 ± 0.2%).

CONCLUSIONS — We hypothesized that changes in A1C levels during pregnancy are at least partially attributable to iron deficiency, as pregnant women are often iron deficient and iron deficiency is known to influence A1C levels (14). In studies 1 and 2, MCH, serum transferrin saturation, and serum transferrin were found to be lower in pregnant women at later stages of gestation. In addition, A1C levels showed a negative correlation with MCH, serum transferrin saturation, and serum transferrin. On the basis of these observations, the increase in A1C levels in late pregnancy seems to be mainly attributable to an iron-deficient status at this period. To the best of our knowledge, this is the first study to demonstrate the involvement of iron deficiency in increased A1C levels in late pregnancy.

In both the cross-sectional study (study 1) and longitudinal study (study 2), we found that A1C levels were increased in late pregnancy. Serum glycated albumin levels, by contrast, were unchanged during the gestational course. These results suggest that the increase in A1C levels in late pregnancy is unrelated to changes in plasma glucose levels. Phelps et al. (6) have shown biphasic changes in A1C levels during pregnancy, with a nadir at gestational week 24. They also demonstrated biphasic changes in 1-h glucose levels for the 50-g oral glucose tolerance test during pregnancy, with a nadir at 20 weeks. Those results suggest that changes in plasma glucose levels are followed by changes in A1C levels during pregnancy. However, changes in plasma glucose levels were relatively small compared with changes in A1C levels. Changes in A1C levels during pregnancy may thus result from factors other than plasma glucose levels alone. In this regard, Cousins et al. (18) showed that plasma glucose levels were unchanged from middle to late pregnancy.

Supplementation with iron is recommended for pregnant women with iron deficiency anemia (10). In patients with iron deficiency anemia, A1C levels have been shown to temporarily decrease after treatment with iron (19). Thus, in patients with iron deficiency anemia, whether treated or not, A1C is inadequate as an indicator to accurately reflect glycemic control. Whether A1C levels are relatively stable in pregnant women who are continuously receiving iron supplementation from early pregnancy should be investigated.

In contrast with the results of Nielsen et al. (20) demonstrating that A1C levels were decreased early in pregnancy and

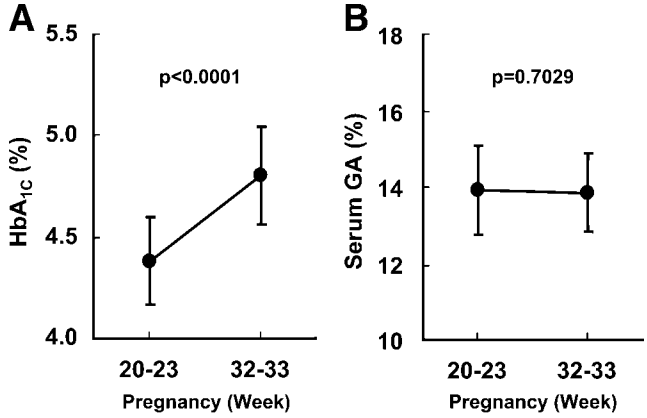

Figure 4— *A1C* (A) *and serum glycated albumin (GA)* (B) *levels in 17 pregnant women (study 2) studied in middle pregnancy (20–23 weeks) and late pregnancy (32–33 weeks).*

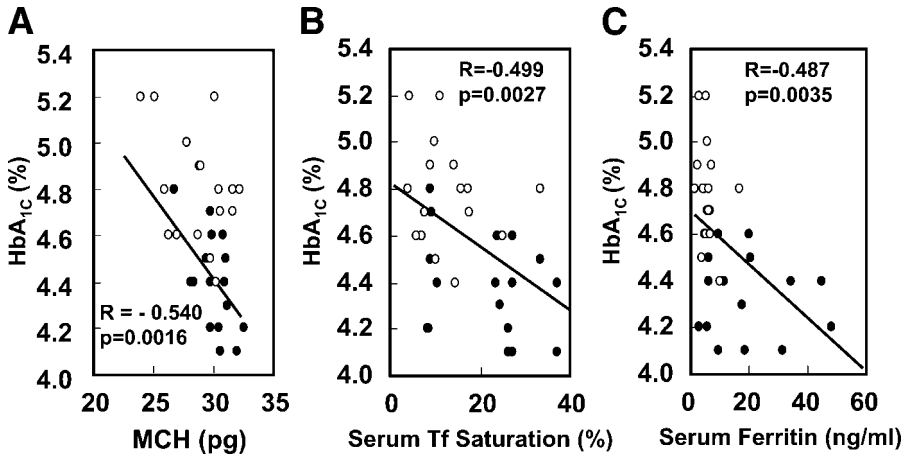

Figure 5— *Association of A1C levels with MCH (A), serum transferrin (Tf) saturation (B), and serum ferritin (C) in 17 pregnant women (study 2). ●, middle pregnancy (20–23 weeks); ○, late pregnancy (32–33 weeks).*

further decreased in late pregnancy compared with those in nonpregnant women, we showed that A1C levels increased in that period. The reasons for the differences from our results are unclear. Our Japanese women may behave differently during pregnancy than the Danish women with regard to iron metabolism. Differences in iron supplementation status, which was not demonstrated in the article by Nielsen et al. (20), may cause the different results.

Serum fructosamine levels reportedly decrease as gestation progresses (21,22). Serum fructosamine reflects the total amount of glycated serum proteins. Measurement is thus influenced when serum protein levels are altered. In pregnant women, serum protein levels decrease by dilution, resulting in decreased serum fructosamine levels. Serum fructosamine is thus also inadequate for monitoring chronic glucose control in pregnancy. In contrast, because serum glycated albumin is measured as the ratio of serum glycated albumin to serum albumin, measurement is not influenced by serum albumin levels. Serum glycated albumin levels decrease slightly from early to middle pregnancy (21). These changes resemble those of A1C levels, probably reflecting changes in plasma glucose levels during these periods. However, we found that serum glycated albumin levels were unaltered from middle to late pregnancy.

The present study reveals that iron deficiency is involved in increased A1C levels in late pregnancy. Our results suggest that caution is warranted in interpretation of A1C in late pregnancy. Conversely, serum glycated albumin may offer a better index for monitoring glycemic control in pregnancy. The present study was performed in pregnant women without diabetes, although gestational diabetes mellitus was not completely ruled out. Further studies on pregnant women with diabetes will make our observations useful for clinical management of these patients.

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