

HbA_{1C}, but not serum glycated albumin, is elevated in late pregnancy due to iron deficiency

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Objective: HbA_{1C} 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 HbA_{1C} increase in late pregnancy.

Methods: In Study 1, HbA_{1C}, serum glycated albumin (GA), erythrocyte indices and iron metabolism indices were determined in 47 non-diabetic pregnant women not supplemented with iron divided into 4 groups according to gestational period (Group I, 21-24 weeks, Group II, 25-28 weeks; Group III, 29-32 weeks; 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 non-diabetic pregnant women.

Results: In Study 1, HbA_{1C} levels were higher in Groups III and IV than those Groups I and II, while serum GA levels were not different between these four groups. Hemoglobin, mean corpuscular hemoglobin (MCH), serum transferrin saturation and serum ferritin were lower in Groups III and IV. HbA_{1C} levels were negatively correlated with MCH, serum transferrin saturation and serum ferritin. In Study 2, HbA_{1C} levels were significantly increased at gestational week 32-33 from those at week 20-23, while serum GA levels did not differ between the two gestational periods. MCH, serum transferrin saturation and serum ferritin were decreased at gestational week 32-33. HbA_{1C} levels showed a negative correlation with MCH, serum transferrin saturation and serum ferritin.

Conclusions: HbA_{1C} levels were elevated in late pregnancy due to iron deficiency. Serum GA may offer a better index for monitoring glycemic control in pregnancy.

In pregnant women displaying diabetes mellitus and women with gestational diabetes, 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 non-enzymatic glycation of proteins increases in diabetic patients. Of these glycated proteins, HbA_{1C} 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 HbA_{1C} levels during pregnancy, with HbA_{1C} levels lowest at gestational week 24. A longitudinal study also demonstrated similar biphasic changes in HbA_{1C} levels (7).

HbA_{1C} measurements are known to be profoundly affected by erythrocytes turnover, in addition to 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). HbA_{1C} levels have been shown to be higher in relation to glycemia in patients with iron-deficiency anemia (11-13). We have recently shown that HbA_{1C} levels are higher in pre-menopausal women with an iron-deficient state, even in the absence of anemia (14). We therefore hypothesized that HbA_{1C} levels are set higher in relation to glycemia in late pregnancy, in which most women are iron-deficient. To confirm this possibility, we studied the relationship between HbA_{1C} and iron metabolism in non-diabetic pregnant women. In clinical issues, the study performed in pregnant diabetic women is important. However, in diabetic women fluctuations of plasma glucose may directly influence HbA_{1C} levels beyond the effect of iron metabolism, making it difficult to analyze the direct effects of pregnant course on HbA_{1C} levels. Thus, in

this study, we aimed to examine relationship between HbA_{1C} and iron metabolism in non-diabetic pregnant women, in whom the influence of plasma glucose levels is minimal. Serum glycated albumin (GA), a different indicator for chronic glycemia, was also studied in these subjects.

RESEARCH DESIGN AND METHODS

Study subjects: In cross-sectional study (Study 1), we studied 47 pregnant Japanese women at gestational week 21-36. All subjects had attended Aizenbashi Hospital from February 2007 to July 2007 and ambulatory plasma glucose levels were <100 mg/dl. Mean age was 29.5±5.7 years. All subjects had not been and were not receiving iron and vitamins supplementations during pregnant periods. Subjects were divided into 4 groups according to the gestational period: Group I (n=20), gestational week 21-24; Group II (n=9), gestational week 25-28; Group III (n=11), gestational week 29-32; Group IV (n=7), gestational week 33-36. HbA_{1C}, red blood cell (RBC) count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), serum iron, serum transferrin (Tf) saturation, serum ferritin and GA were determined.

In longitudinal study (Study 2), we studied 17 non-diabetic pregnant women who had attended Aizenbashi Hospital between February and July 2007. Mean age was 28.0±5.7 years. HbA_{1C}, RBC count, hematocrit, hemoglobin, MCV, MCH, serum iron, serum transferrin saturation, serum ferritin and serum GA were determined at two periods (gestational weeks 20-23 and 32-33).

For control, 19 age-matched non-pregnant healthy women whose mean age was 27.7±2.0 years old were also studied.

The reported investigations 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: HbA_{1C} was measured by latex aggregation immunoassay using Determiner HbA_{1C} (Kyowa Medix, Tokyo, Japan) which were found not influenced by HbF and other minor Hb species (15), with calibration using Japan Diabetes Society (JDS) Lot 2 (16). Inter- and intra-assay coefficients of variations were 0.98% and 0.97%, respectively. Serum GA 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 (UIBC) were determined by calorimetric method. Serum ferritin concentrations were measured by chemiluminescent immunoassay (CLIA) method. Total iron-binding capacity (TIBC) and serum transferrin saturation were calculated by adding UIBC to serum iron and dividing serum iron by TIBC, respectively. All tests were measured at a central laboratory at Aizenbashi Hospital.

Statistical analyses: Data are shown as mean \pm standard deviation for continuous variables and as numbers for categorical variables. Unadjusted comparisons for continuous variables were performed among Groups I-IV using analysis of variance, and unpaired *t* tests were used to estimate the level of significance of differences between means. To evaluate relationships between HbA_{1C} 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.

RESULTS

Figure 1 shows HbA_{1C} and GA levels in pregnant women divided into 4 groups according to gestational period. The results show that HbA_{1C} 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). GA levels remained constant in these 4 groups. RBC counts did not differ among the 4 groups of pregnant women. However, hemoglobin, MCH, transferrin saturation and serum ferritin levels were lower in Groups III and IV (Fig. 2). HbA_{1C} levels were negatively correlated with MCH, serum transferrin saturation and serum ferritin (Fig. 3).

Next, we studied 17 pregnant individuals at two periods of middle pregnancy (20-23 weeks) and late pregnancy (32-33 weeks). HbA_{1C} levels significantly increased from middle pregnancy ($4.4 \pm 0.2\%$) to late pregnancy ($4.8 \pm 0.2\%$; $p < 0.0001$), whereas serum GA levels did not change (from $13.9 \pm 1.2\%$ to $13.9 \pm 1.0\%$; $p = 0.7029$) (Fig. 4). RBC counts were unchanged at both periods ($365 \pm 26 \times 10^6/\mu\text{l}$ in middle pregnancy vs. $367 \pm 20 \times 10^6/\mu\text{l}$ in late pregnancy; $p = 0.6630$), while MCH (30.2 ± 1.5 pg vs. 28.8 ± 2.4 pg; $p = 0.0016$), transferrin saturation ($21.7 \pm 10.4\%$ vs. $12.5 \pm 7.9\%$; $p = 0.0011$) and serum ferritin (17.4 ± 14.3 ng/ml vs. 5.8 ± 3.5 ng/ml; $p = 0.0022$) were decreased in late pregnancy compared to middle pregnancy. Hemoglobin levels were also decreased of borderline significance (from 11.0 ± 0.5 g/dl 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. HbA_{1C} levels were negatively correlated with MCH, transferrin saturation and serum ferritin (Fig. 5).

In our Study 2, mean HbA_{1C} level of 17 women in late pregnancy (32-33 weeks) was not significantly different from that of 19

age-matched non-pregnant women ($4.8 \pm 0.2\%$ vs. $4.8 \pm 0.2\%$).

CONCLUSIONS

We hypothesized that changes in HbA_{1C} levels during pregnancy are at least partially attributable to iron deficiency, since pregnant women are often iron deficient and iron deficiency is known to influence HbA_{1C} levels (14). In Study 1 and Study 2, MCH, serum transferrin saturation and serum transferrin were found to be lower in pregnant women with progressed gestation. In addition, HbA_{1C} levels showed a negative correlation with MCH, serum transferrin saturation and serum transferrin. Based on these observations, the increase in HbA_{1C} levels in late pregnancy appears mainly attributable to 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 HbA_{1C} levels in late pregnancy.

In both the cross-sectional study (Study 1) and longitudinal study (Study 2), we found that HbA_{1C} levels were increased in late pregnancy. Serum GA levels, by contrast, were unchanged during the gestational course. These results suggest that the increase in HbA_{1C} levels in late pregnancy is unrelated to changes in plasma glucose levels. Phelps et al. (6) have shown biphasic changes in HbA_{1C} 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 (OGTT) during pregnancy, with a nadir at 20 weeks. Those results suggest that changes in plasma glucose levels are followed by those of HbA_{1C} levels during pregnancy. However, changes in plasma glucose levels were relatively small compared with changes in HbA_{1C} levels. Changes in HbA_{1C} 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, HbA_{1C} levels have been shown to temporarily decrease after treatment with iron (19). Thus, in patients with iron-deficiency anemia, whether treated or not, HbA_{1C} is inadequate as an indicator to accurately reflect glycemic control. It should be investigated whether HbA_{1C} levels are relatively stable in pregnant women who are continuously supplemented with iron from early pregnancy.

In contrast to the results by Nielsen et al. (20) demonstrating that HbA_{1C} was decreased early in pregnancy and further decreased in late pregnancy compared with non-pregnant women, we showed that HbA_{1C} levels did increase 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 as to iron metabolism. Differences in iron supplementation status, which is not demonstrated in the paper by Nielsen et al. (20), may cause the different results.

Serum fructosamine levels reportedly decrease as gestation progresses (21, 22). Serum fructosamine reflects 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, as serum GA is measured as the ratio of serum glycated albumin to serum albumin, measurement is not influenced by serum albumin levels. Serum GA levels decrease slightly from early to middle pregnancy (21). These changes resemble those of HbA_{1C} levels, probably reflecting changes in plasma glucose levels

during these periods. However, we found that serum GA levels were unaltered from middle to late pregnancy.

The present study reveals that iron deficiency is involved in increased HbA_{1C} levels in late pregnancy. It suggests that interpretation of HbA_{1C} in late pregnancy thus warrants caution. Conversely, serum GA may

offer a better index for monitoring glycemic control in pregnancy. The present study was performed in pregnant women without diabetes, although gestational diabetes was not completely ruled out. Further studies on pregnant women with diabetes mellitus will make our observations to be useful for clinical management of these patients.

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FIGURE LEGENDS

Figure 1: HbA_{1c} (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 vs. Group I; #P<0.05 vs. Group II; ##P<0.01 vs. Group II.

Figure 2: RBC counts (A), hemoglobin (B), MCH (C), serum transferrin 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 vs. Group I; **P<0.001 vs. Group I; #P<0.05 vs. Group II.

Figure 3: Association of HbA_{1c} levels with MCH (A), serum transferrin saturation (B) and serum ferritin (C) in 47 pregnant women (Study 1).

Figure 4: HbA_{1c} (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 HbA_{1c} levels with MCH (A), serum transferrin saturation (B) and serum ferritin (C) in 17 pregnant women (Study 2). ●, middle pregnancy (20-23 weeks); ○, late pregnancy (32-33 weeks).

Fig. 1

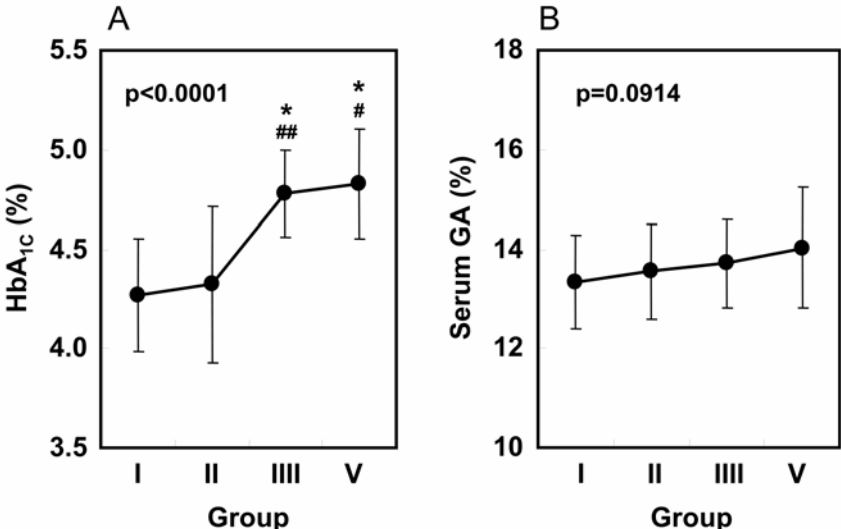


Fig. 2

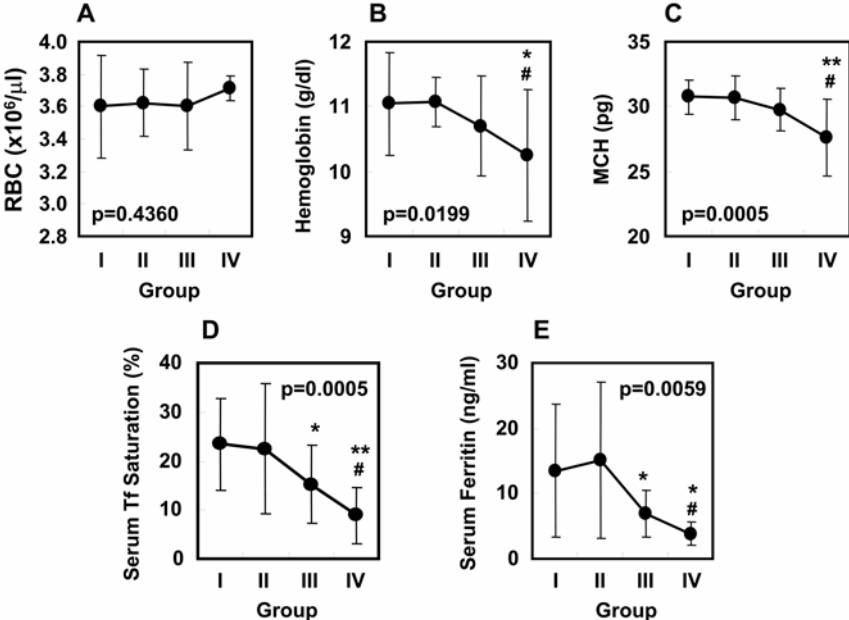


Fig. 3

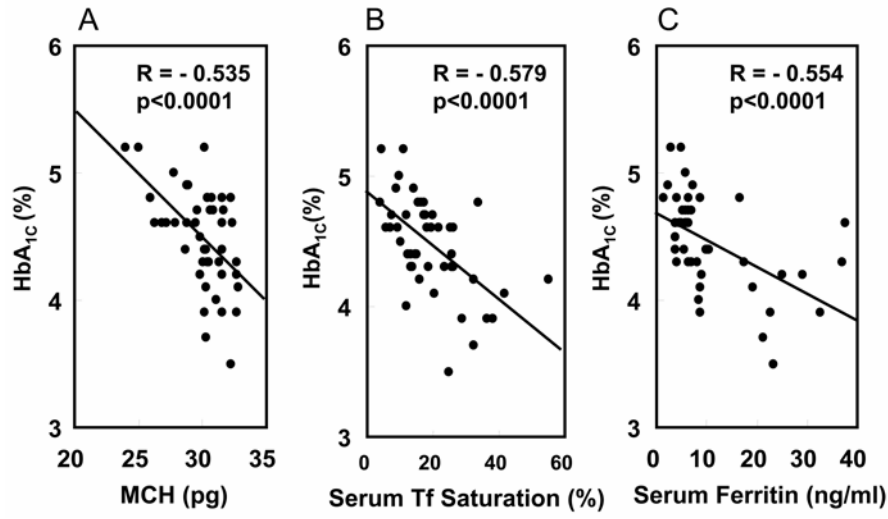


Fig. 4

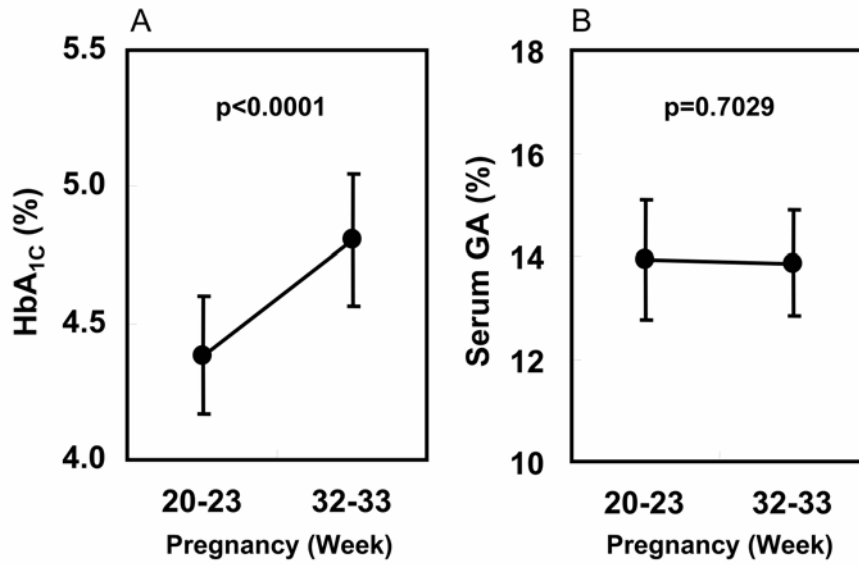


Fig. 5

