Interindividual Divergence in the Relationship between the Values of Plasma Glucose and Hemoglobin A1c in Type 2 Diabetes

Michiyo Fukudome, Mitsuhiro Nakazaki, Eriko Fukushige, Nobuyuki Koriyama, Yuko Ikeda, Kaori Kato, Takashi Kimura and Chuwa Tei

Abstract

Objective To verify the relationships between the values of plasma glucose (PG) and hemoglobin A1c (HbA1c) in type 2 diabetic outpatients.

Methods The pre- and postbreakfast PG and HbA1c values were monitored every month for 44-90 months. The single regression lines between the values of PG and HbA1c were compared for the slopes and intercepts on the designated ordinates of the regression lines.

Patients or Materials Nine patients of type 2 diabetes not treated with insulin: three males and six females, aged 43-79 years participated.

Results The HbA1c level was combined with the pre- and postbreakfast PG values obtained at one month prior to its determination, because the combinations were correlated most strongly. The slopes of the regression line ranged from 0.33-0.50%/mmol/L and the intercepts at PG level equal to 9.6 mmol/L ranged from 6.95-9.77% in the relationship between the values of 1-month earlier prebreakfast PG and HbA1c. Twenty-eight pairs had significantly different intercepts. Meanwhile, there was no pair that had significantly different slopes. Similar results were obtained in the relationship between the values of 1-month earlier postbreakfast PG and HbA1c.

Conclusion There was interindividual divergence of the regression lines which was due to the difference in the intercepts but not the slopes.

Key words: HbA1c, blood glucose, glycation, type 2 diabetes

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Introduction

Glycemic control is fundamental to the management of diabetes. Prospective randomized clinical trials have shown that achieving glycemic control is associated with decreased rate of diabetes complications. Glycemic control is best judged by the combination of the results of the patient’s self-monitoring of blood glucose testing (as performed) and the current hemoglobin A1c (HbA1c) result. The measurement of the HbA1c value is meaningful not only to assess the patient’s control but also as a check of the accuracy of the meter (of the patient’s self-reported results) and the adequacy of the self-monitoring of blood glucose (1). Therefore, the knowledge regarding the relationship between the plasma glucose (PG) and HbA1c concentrations appears to alter the behavior of healthcare providers and patients, in turn improving glycemic control and lowering the HbA1c values (2).

The correlation between the HbA1c and the mean PG levels on multiple testing over 2-3 months has been presented based on data from the Diabetes Control and Complications Trial (1). The correlation of the population study may be useful if between-subject variation in the HbA1c level is minimal as has been reported in nondiabetic subjects (3). On the other hand, several studies have suggested that the HbA1c value fluctuates widely between individuals having the same
glycemic status, thus indicating the existence of both low glycators and high glycators. Several methods have been used to identify the individual differences in the glycation rate of HbA\textsubscript{lc}: 1) a glycosylation index calculated as a ratio of the HbA\textsubscript{lc} value to the mean blood glucose level preceding several weeks (4, 5); 2) the disparity between the percentile ranking of the HbA\textsubscript{lc} value and the blood glucose level after an oral glucose tolerance test (6); 3) a nondiabetic index of individuality calculated as the square root of the ratio of the intra- to interindividual variance of the HbA\textsubscript{lc} level (7); 4) a hemoglobin glycation index calculated by subtraction of the predicted HbA\textsubscript{lc} level from the observed one (8, 9). However, an analysis of the regression line regarding the relationship between the PG and HbA\textsubscript{lc} values has yet to be performed.

It has been generally accepted that the best correlation is observed between the HbA\textsubscript{lc} level and the mean daily glucose concentrations in both type 1 and type 2 diabetes (1). However, it is not practical to take several samples a day in ambulatory type 2 diabetic patients. We measured the pre- and postbreakfast PG and HbA\textsubscript{lc} values every month in 9 non-insulin treated type 2 diabetic outpatients for 44 to 90 months in order to verify the relationships between the plasma glucose level and hemoglobin A\textsubscript{lc} value. The present study was intended to provide simple explanation of the relationship using data in clinical practice.

**Patients and Methods**

We recruited type 2 diabetic patients without insulin treatment who agreed on coming to Kagoshima University Hospital twice in 1 day (before and after breakfast) a month to measure plasma glucose. Twelve patients participated in the protocol and 9 patients continued the protocol for more than 3 years. The profiles of the patients were investigated regarding sex, age, body mass index, diabetic duration before the start of this study, medications at the beginning of this study and ones added, and the mean interval of the clinic visits and follow-up period. The data of cases 1 to 4 were obtained from our previous report which analyzed the time lag between the plasma glucose and HbA\textsubscript{lc} levels (10). None of the subjects suffered from alcoholism, anemia or concomitant chronic disease. Changes in the treatment were left to the discretion of each patient’s attending physician.

The methods to monitor the HbA\textsubscript{lc} and pre- and post-breakfast PG levels each month have all been described previously (10). Briefly, venous blood was withdrawn to measure the prebreakfast PG level and HbA\textsubscript{lc} value in the morning (7:30-8:30 A.M.) after a 10- to 14-hour overnight fast. Thereafter the postbreakfast PG level was monitored between 10:00-11:00 A.M. The patients were asked to follow their usual treatment regimen and their usual diet on the day they visited the clinic.

The venous blood was centrifuged within 1 hour after withdrawal, and the plasma was used for an immediate assessment of the PG concentration using the gluco-oxidase method (GAO3U, A & T Corp., Yokohama, Japan). Both the intra- and interassay coefficients of variation (CVs) were \( \leq 1.8\% \) at values <10 mmol/L. The HbA\textsubscript{lc} value was measured using an automated high-performance liquid chromatography (HPLC) analyzer (Hi-AUTO A\textsubscript{lc} HA-8121, ARKRAY Inc., Kyoto, Japan), which had both a precaimation system of venous blood with tetrapolyphosphoric acid for removing labile HbA\textsubscript{lc} and a HPLC system for the specific measurement of the stable fraction using the calibration standard established by the Japan Diabetes Society (normal range, 4.3%-5.8%). Both the intra- and interassay CVs were <2.6% at values <11%.

The statistical calculations were performed using the GraphPad Prism 4.0 software program (GraphPad Software Inc., San Diego CA, USA). The data are expressed as the mean ± SD. The difference of the follow-up period was analyzed by ANOVA, followed by a Tukey post-hoc test.

Pearson’s correlation coefficients between the PG and HbA\textsubscript{lc} levels were analyzed by the F test (linear regression analysis of variance) to determine whether the values were zero. The differences of the correlation coefficients in each patient were analyzed by ANOVA, followed by a Tukey post-hoc test. The relationships of the PG and HbA\textsubscript{lc} levels showed linear regressions and single regression lines were obtained using the least square method. The slopes of the single regression lines were analyzed by F test to determine whether the values were zero. The differences in the slopes and intercepts on the ordinates which were the mean values of the individual middle point of the PG range were compared directly. To analyze the above determination factors by ANOVA, followed by the Tukey post-hoc test, their values of the mean, the standard deviation and the number of samples were used.

To analyze the influence of the additional agent on the relationships between PG and HbA\textsubscript{lc} levels (intraindividual difference), the slopes and intercepts before and after addition of the medicine were compared by unpaired t test with Welch’s correction.

For the analysis of the factor of sex related to the individual intercept, the Mann-Whitney test was used. As for the analysis of the factors of age, BMI and duration of diabetes, Spearman’s rank correlation test was used.

In all tests, a p value of <0.05 was considered to indicate significance.

**Results**

The profiles of the patients are summarized in Table 1. There was no significant difference in the intervals of clinic visits among the subjects (\( p \geq 0.05, \) ANOVA). Simple correlations between the HbA\textsubscript{lc} and PG levels before and after breakfast are shown in Table 2. To seek the best correlation, the HbA\textsubscript{lc} value was matched with the PG level obtained at 0, 1 and 2 month(s) prior to its determination. The F test revealed that the values of PG and HbA\textsubscript{lc} in all pairs showed a statistically significant correlation (\( p < 0.01 \)). When the cor-
Table 1. Patients’ Profiles

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>BMI</th>
<th>Duration (yrs)</th>
<th>Diabetic Medications (mg/day)</th>
<th>Follow-up Interval (days)</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>male</td>
<td>76</td>
<td>25.2</td>
<td>11</td>
<td>SU + AGI</td>
<td>30.4 ± 3.7</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>female</td>
<td>74</td>
<td>22.7</td>
<td>14</td>
<td>SU + AGI</td>
<td>30.3 ± 4.2</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>female</td>
<td>68</td>
<td>24.7</td>
<td>10</td>
<td>SU</td>
<td>30.2 ± 4.5</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>male</td>
<td>43</td>
<td>23.5</td>
<td>0</td>
<td>diet SU, BG</td>
<td>30.5 ± 4.5</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>female</td>
<td>68</td>
<td>20.1</td>
<td>9</td>
<td>SU + AGI, TZD</td>
<td>31.0 ± 4.7</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>female</td>
<td>61</td>
<td>25.8</td>
<td>9</td>
<td>AGI</td>
<td>30.4 ± 5.0</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>male</td>
<td>68</td>
<td>23.5</td>
<td>11</td>
<td>SU AGI, BG</td>
<td>30.8 ± 5.2</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>female</td>
<td>79</td>
<td>21.1</td>
<td>6</td>
<td>AGI, SU</td>
<td>29.6 ± 4.2</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>female</td>
<td>59</td>
<td>20.7</td>
<td>0</td>
<td>SU AGI, TZD</td>
<td>30.1 ± 3.8</td>
<td>86</td>
</tr>
</tbody>
</table>

BMI: body mass index; SU: sulphonyl urea; AGI: alpha-glucosidase inhibitor; BG: biguanide; TZD: thiazolidine

Table 2. Simple Correlations between Pre-breakfast Plasma Glucose and HbA1c, and between Post-breakfast Plasma Glucose and HbA1c

<table>
<thead>
<tr>
<th>Case</th>
<th>FPG(-2)</th>
<th>FPG(-1)</th>
<th>FPG(0)</th>
<th>PPG(-2)</th>
<th>PPG(-1)</th>
<th>PPG(0)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.75</td>
<td>0.77</td>
<td>0.77</td>
<td>0.45</td>
<td>0.46</td>
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<tr>
<td>2</td>
<td>0.69</td>
<td>0.80</td>
<td>0.76</td>
<td>0.53</td>
<td>0.64</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.76</td>
<td>0.86</td>
<td>0.73</td>
<td>0.65</td>
<td>0.74</td>
<td>0.62</td>
<td>p &lt; 0.05 to FPG(-1)</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>0.89</td>
<td>0.84</td>
<td>0.70</td>
<td>0.84</td>
<td>0.83</td>
<td>p &lt; 0.05 to FPG(-1)</td>
</tr>
<tr>
<td>5</td>
<td>0.61</td>
<td>0.77</td>
<td>0.66</td>
<td>0.57</td>
<td>0.69</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.76</td>
<td>0.80</td>
<td>0.81</td>
<td>0.56</td>
<td>0.61</td>
<td>0.57</td>
<td>p &lt; 0.05 to FPG(-1)</td>
</tr>
<tr>
<td>7</td>
<td>0.71</td>
<td>0.86</td>
<td>0.71</td>
<td>0.55</td>
<td>0.67</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.77</td>
<td>0.89</td>
<td>0.79</td>
<td>0.60</td>
<td>0.69</td>
<td>0.66</td>
<td>p &lt; 0.05 to FPG(-1)</td>
</tr>
<tr>
<td>9</td>
<td>0.65</td>
<td>0.89</td>
<td>0.72</td>
<td>0.63</td>
<td>0.64</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>

Correlations of HbA1c for PG levels obtained at 0 (the same day), 1 and 2 month(s) prior to HbA1c measurement. The period when plasma glucose was obtained is indicated in parentheses. Each asterisk means statistical significance compared to the indicated PG within the individual data. FPG, pre-breakfast plasma glucose; PPG, post-breakfast plasma glucose.

The relation coefficients were compared within the same period in each patient, the pre-breakfast PG level correlated more strongly than the postbreakfast PG level in every patient. In four patients (cases 3, 4, 6, and 8) the correlation between the values of 2-month earlier postbreakfast PG and HbA1c was significantly weaker than the correlation between the values of 1-month earlier prebreakfast PG and HbA1c (p < 0.05, Tukey). The results indicated that the pre- and post-breakfast PG levels are reliable predictors of the 1-month later HbA1c values, and the postbreakfast PG level may be the more precise one.

The major aim in this paper was to analyze the relationship between the PG and the HbA1c levels. Because of the reason mentioned above, the best relationships between the 1-month earlier PG levels and the HbA1c values were cited...
in this analysis (Figs. 1, 2). The relationship between the PG and HbA₁c levels showed linear regressions and the slopes of all regression lines in Figs. 1, 2 were significantly different than zero (p<0.01, F test). The slope and the intercept val-
Figure 3. The regression lines between the values of PG and HbA1c. (A) A simple linear regression of 1-month-earlier prebreakfast PG and HbA1c levels. The parameters in the list are summarized as the slope and intercept on the ordinate when PG level is equal to 9.6 mmol/L. (B) A simple linear regression of 1-month-earlier postbreakfast PG and HbA1c levels. The parameters in the list are summarized as the slope and intercept on the ordinate when PG level is equal to 14.0 mmol/L. The values are expressed as the mean±SD.

ues of the regression line between the 1-month earlier prebreakfast PG and HbA1c levels ranged from 0.33 to 0.50%/mmol/L (mean 0.43±0.06%/mmol/L) and from 6.95 to 9.77% at PG equal to 9.6 mmol/L which was the mean value of the individual middle point of the PG range (mean 8.18±0.97%), respectively. There was no pair among the 36 combinations whose slopes were significantly different from each other (p=0.061, ANOVA, Fig. 3A). Therefore, next we compared the distributions of HbA1c values at the intercept. Twenty-eight pairs had significantly different intercepts. In the two pairs in parenthesis, (1, 3) and (1, 7), the P values were less than 0.05 and in the pair (2, 9) less than 0.01 (Tukey). The P values of the other 25 pairs were less than 0.001 (Tukey). Statistically significant distributions of HbA1c values were detected in the eight pairs, namely, (1, 6), (3, 6), (3, 7), (3, 8), (4, 8), (5, 9), (6, 7) and (7, 8) (p<0.05, Tukey). The slope and the intercept values of the regression line between the values of 1-month earlier postbreakfast PG and HbA1c ranged from 0.18 to 0.26%/mmol/L (mean 0.22±0.03%/mmol/L) and from 7.21 to 10.04% at PG equal to 14.0 mmol/L calculated by the same method mentioned above (mean 8.12±0.87%), respectively. Similarly, there was no pair among the 36 combinations whose slopes were significantly different from each other (p=0.584, ANOVA, Fig. 3B). The distributions of the HbA1c values at the intercept were compared. Twenty-three pairs had significantly different intercepts from each other (p<0.001, Tukey). Thirteen pairs therefore had statistically insignificant distributions of the HbA1c values. The pairs in parenthesis were (1, 5), (1, 6), (1, 7), (1, 9), (3, 4), (3, 8), (4, 8), (5, 6), (5, 7), (5, 9), (6, 7), (6, 9) and (7, 9) (p>0.05, Tukey). The increased number of the pairs with statistical insignificance in this analysis might reflect the wider standard deviations in the distributions of HbA1c values than those in the relationship between the values of 1-month earlier prebreakfast PG and HbA1c (Figs. 3A, 3B). Interestingly, the regression lines
between the values of 1-month earlier postbreakfast PG and HbA1c were divided into three groups based on the difference of intercept: (1, 5, 6, 7, 9), (2) and (3, 4, 8). Taken together, there was interindividual divergence in the regression lines between the values of PG and HbA1c, which was not due to the difference of slopes but of intercepts, namely the lines were parallel in each relationship.

In cases 4, 5, 7, 8 and 9 which required the addition of antihyperglycemic agents, the correlation coefficients between the values of PG and HbA1c were equivalent to those of other cases (Table 2). The analysis of the additional agent’s influence on the relationships between PG and HbA1c levels was available in case 5 and 8. The numbers of data points before and after the addition of the agent were 39 and 42 in case 5, and 50 and 38 in case 8, respectively. In both cases, the two regression lines before and after addition of the medicine corresponded in both the relationships between the 1-month earlier prebreakfast PG and HbA1c levels and between the 1-month earlier postbreakfast PG and HbA1c levels (p > 0.05, unpaired t test with Welch’s correction). Therefore, changes in the treatment during this study did not seem to have an influence on the relationship. We also attempted to seek the association of the sex, age, BMI, and duration of diabetes with the individual divergence of intercepts. There was no significant correlation in sex (p > 0.05, Mann-Whitney test), age, BMI, or duration of diabetes (p > 0.05, Spearman’s rank correlation test).

Discussion

The present study supported our previous result that the 1-month earlier pre- and postbreakfast PG levels showed the best correlation with the HbA1c level (10). The following previous studies were consistent with our findings. Schultz et al reported that the increase of the HbA1c concentration previous studies were consistent with our findings. Schultz et al reported that the increase of the HbA1c concentration was not due to the difference of slopes but of intercepts, namely the lines were parallel in each relationship.

In cases 4, 5, 7, 8 and 9 which required the addition of antihyperglycemic agents, the correlation coefficients between the values of PG and HbA1c were equivalent to those of other cases (Table 2). The analysis of the additional agent’s influence on the relationships between PG and HbA1c levels was available in case 5 and 8. The numbers of data points before and after the addition of the agent were 39 and 42 in case 5, and 50 and 38 in case 8, respectively. In both cases, the two regression lines before and after addition of the medicine corresponded in both the relationships between the 1-month earlier prebreakfast PG and HbA1c levels and between the 1-month earlier postbreakfast PG and HbA1c levels (p > 0.05, unpaired t test with Welch’s correction). Therefore, changes in the treatment during this study did not seem to have an influence on the relationship. We also attempted to seek the association of the sex, age, BMI, and duration of diabetes with the individual divergence of intercepts. There was no significant correlation in sex (p > 0.05, Mann-Whitney test), age, BMI, or duration of diabetes (p > 0.05, Spearman’s rank correlation test).

In conclusion, the change of the HbA1c value lagged behind the PG level by around one month and the relationships between both variables fitted linear regressions. Significant differences exist among the patients in their relationships between the PG and HbA1c levels in type 2 diabetes mellitus. The analysis of the regression lines revealed that the interindividual divergence was not due to the difference of slopes but of intercepts. This study may illustrate the problems in applying reference ranges derived from population studies to interpret the individual relationship between the PG and HbA1c levels.

References

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