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A Comparative Study on the Methods of Glycosylated Hemoglobin Measurement

당화헤모글로빈의 측정방법에 대한 비교 연구

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The increased glycosylation of hemoglobin in diabetes is well established. Measurement of glycosylated hemoglobin is being used by many centers to monitor diabetic control. There are several methods for the measurement of the glycosylated hemoglobin and in each method, there are several relative merits and drawbacks. We compared the glycosylated hemoglobin measured by cation exchange resin column chromatography (minicolumn method), chemical colorimetry (thiobarbituric acid method [TBA]), and fluorometry (diacetyldihydrolutidine method [DDL]) for the correlation. To know whether the labile form of the glycosylated hemoglobin could be measured by three methods, we incubated the RBC in 1000 mg/dl glucose saline and subsequently in glucose-free saline and measured the glycosylated hemoglobin by the three methods before and after the incubations.

METHODS

Blood specimens were drawn by venipuncture into evacuated tubes containing EDTA. Centrifuged the blood (1000 x g, 10 min, 4°C), removed the plasma, and washed the packed cells at 20°C with five volumes of saline. Repeated this washing procedure two additional times. We used these washed RBC for glycosylated hemoglobin measurement.

1. Column-chromatographic method: We used minicolumns (Bio-Rad, Watford, Herts, U.K., Cat. No. 191~7001) and performed the assay at 22°C in a temperature-controlled chamber to reduce temperature effects (Worth et al., 1980).

This small column of cation exchange resin method depends on the change in the charge of the hemoglobin molecule caused by glycosylation. The glycosylated hemoglobins (HbA_1) are eluted readily from a cation exchange resin and results are expressed as % HbA_1 of total hemoglobin.

2. Colorimetric method: We used Autoclave Hydrolysis method of Parker et al. (1981), a modification of the method of Flückiger and and Winterhalter (1976).

The glucose moieties of glycosylated hemoglobin is converted to 5-hydroxymethylfurfural by heating with oxalic acid in an autoclave. The adduct formed by reacting 2-thiobarbituric acid (TBA) with 5-hydroxymethylfurfural is measured photometrically and results are expressed as fructose equivalents.

3. DDL method: We used the method of fluorometric detection of formaldehyde released upon periodate oxidation of glycosylated hemoglobin described by Gallop et al. (1981).

Formaldehyde is released on periodate ($NaIo_4$) oxidation of the glucose moieties of glycosylated hemoglobin. The formaldehyde product is measured as the fluorescent 3, 5-diacetyl-1, 4-dihydrolutidine (DDL) formed from the condensation of formaldehyde with acetylacetone and ammonia

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and results are expressed as number of glycogroups per alpha, beta-dimer of hemoglobin.

4. RBC incubation: For producing the labile form of glycosylated hemglobin, we incubated the RBC for 20 hours at 37°C in phophate-bufffered saline containing 1000mg/dl glucose and subsequently, for removing the labile form of glycosylated hemoglobin, we incubated the RBC for 5 hours at 37°C in glucose-free normal saline.

We measured the glycosylated hemoglobin by the three methods described above and examine the correlationship between three methods. And we compared the values of glycosylated hemoglobin before and after the incubations by each method, and the differences were tested by paired t-test.

RESULTS

1. Correlation between the three methods was shown in Figures 1, 2, and 3. The three methods correlated well.

2. Comparisons of the glycosylated hemoglobin by each method before (Original, O) and after (Total=Labile+Stable, T) incubation in the 1000 mg/dl glucose phosphate-buffered saline, and after incubation in the glucose-free saline (Stable, S) were shown in Figures 4, 5, and 6. After incubation in 1000 mg/dl glucose phosphate-buffered saline, the values of glycosylated hemoglobin increased and after incubation in glucose-free saline, the values decreased to the lower levels than those before inbubation of 1000 mg/dl glucose phosphate-buffered saline.

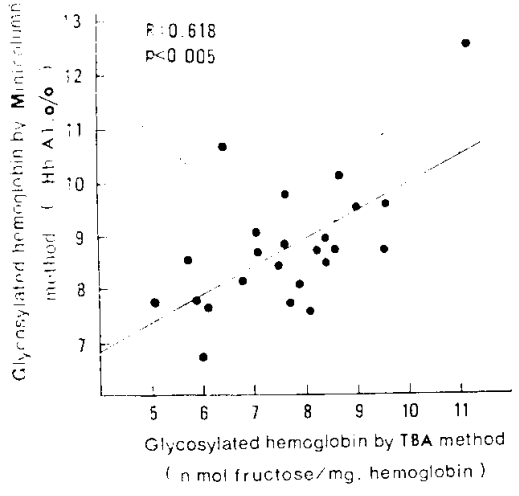

Fig. 1. Comparison of glycosylated hemoglobin measured by the Minicolumn method with glycosylated hemoglobin measured by TBA method.

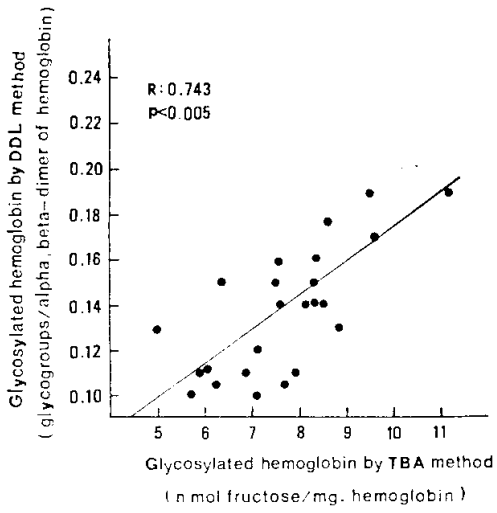

Fig. 2. Comparison of glycosylated hemoglobin measured by the DDL method with glycosylated hemoglobin measured by TBA method.

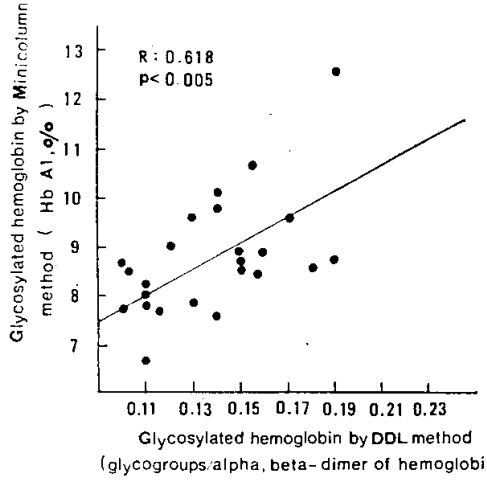

Fig. 3. Comparison of glycosylated hemoglobin measured by the Minicolumn method with glycosylated hemoglobin measured by DDL method.

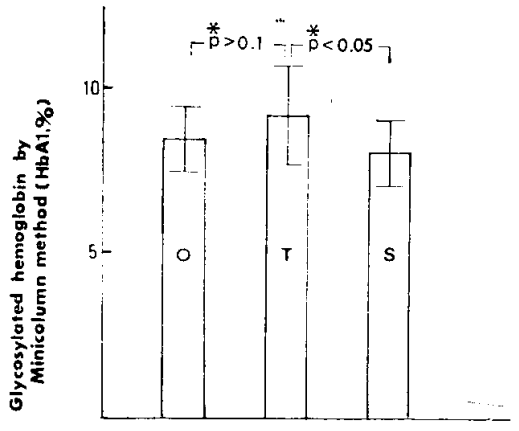

Fig. 4. Comparison of glycosylated hemoglobin measured by minicolumn method before (O), and after (T) incubation of RBC in 1000mg/dl glucose saline and after (S) subsequent incubation of RBC in glucose-free saline.
*; by paired-t test

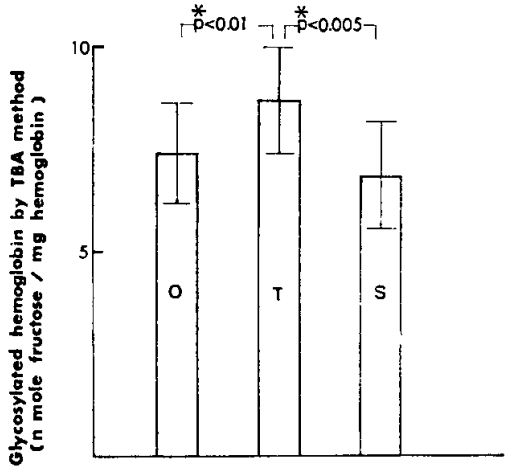

Fig. 5. Comparison of glycosylated hemoglobin measured by TBA method before (O), and after (T) incubation of RBC in 1000mg/dl glucose saline and after (S) subsequent incubation of RBC in glucose-free saline.
*; by paired-t test

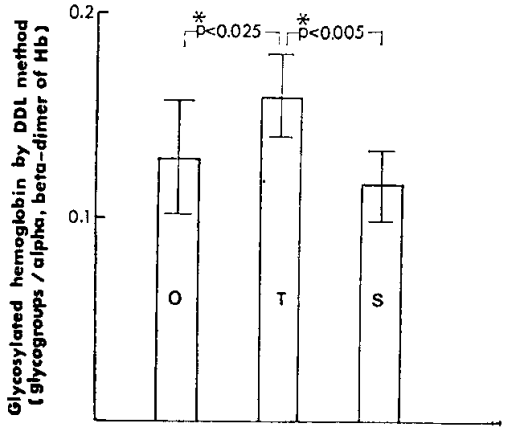

Fig. 6. Comparison of glycosylated hemoglobin measured by DDL method before (O), and after (T) incubation of RBC in 1000mg/dl glucose saline and after(S) subsequent incubation in glucose-free saline.
*; by paired-t test

DISCUSSION

Although minicolumn, thiobarbituric acid (TBA) and diacetyldihydrolutidine(DDL) methods measured the different sites of glycosylation of hemoglobin, the values by the three method correlated well in agreement with other reports (Gabbay et al., 1979; Gallop et al., 1981).

After incubation of RBC in the high glucose saline, the values by the three methods increased and after incubation of RBC in glucose free saline, the values by the three methods decreased. These rapid increasing and decreasing of the values of glycosylated hemoglobin suggested that the differences of glycosylated hemoglobin before and after incubation was ascribed to the labile forms of glycosylated hemoglobin.

In disagreement with other reports (Gallop et al., 1981; Ploybutr et al., 1982), labile from of glycosylated hemoglobin could be measured by the TBA method in the present study. This discrepancy was difficult to explain.

CONCLUSION

1. The values of glycosylated hemoglobin measured by minicolumn, TBA and DDL methods correlated well each other.

2. Labile form of glycosylated hemoglobin could be measured by the TBA method.

3. The new method of glycosylated hemoglobin measurement, the DDL method could also detect both labile amd stable forms of glycosylated hemoglobin.

SUMMARY

We measured the glycosylated hemoglobin (GHB) by cation exchange resin column chromatography (CEC), thiobarbituric acid colorimetry (TBA) and DDL fluorometry (DDL)and compared the results (n=36) for the correlations. Although these methods represent measurements of different sites of glycosylation, they correlated quite well; R values being 0, 81 (CEC vs. TBA), 0. 62 (CEC vs. DDL) and 0. 79 (TBA vs. DDL). By all the three methods, incubation of RBC in phosphate-buffered saline with 10 mg/dl of glucose for 20 hours at 37°C increased the GHB values and subsequent incubation of RBC in normal saline for 5 hours at 37°C decreased the values to initial levels. Contrary to the previous reports that the TBA method can measure only stable form of GHB, this data suggested that TBA method can measure both stable and labile form of GHB together.

—국문 요약—

당화헤모글로빈의 측정방법에 대한 비교 연구

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저자들은 cation exchange resin column chromatography (CEC), thiobarbituric acid colorimetry (TBA)와 DDL fluorometry (DDL)로 당화헤모글로빈을 측정하여 각 방법사이의 상관관계를 관찰하였다.

비록 이 방법들은 당화작용의 다른 부위를 측정하나 그 결과는 CEC와 TBA의 상관계수는 0. 81, CEC와 DDL은 0. 62, TBA와 DDL은 0. 79로서 서로 간에 높은 상관관계를 보였다.

적혈구를 10mg/dl 고포도당농도의 phosphate buffered saline에서 37°C로 20시간 배양후 위의 3가지 방법으로 측정한 당화헤모글로빈치는 증가하였으며 그후 곧이 적혈구를 포도당이 없는 생리식염수에서 37°C로 5시간 배양후에는 당화 헤모글로빈치는 본래 수치로 감소하였다. TBA로는 단지 안정형의 당화헤모글로빈만 측정할 수 있다는 과거 보고와는 달리 이 결과는 TBA가 불안정형과 안정형 당화헤모글로빈 양자를 측정할 수 있음을 보여주고 있다.

REFERENCES

Flückiger, R. and Winterhalter, K.H.: *In vitro synthesis of hemoglobin A_{1c}. FEBS Lett.*, 71:356-360, 1976.

Gabby, K.H., Sosenko, J.M., Banuchi, G.A., Mininsohn M.J. and Flückiger R.: *Glycosylated hemoglobins; increased glycosylation of hemoglobin A in diabetic patients. Diabetes*, 28:*337-340, 1979.*

Gallop, P.M., Flückiger, R., Hanneken, A., Minnsohn, M.M. and Gabbay, K.H.: *Chemical quantitation of hemoglobin glycosylation; fluorometric detection of formaldehyde released on periodate oxiation of glycoglobin. Anal. Biochemistry*, 117:*427-432, 1981.*

Parker, K.M., England, J.D.., Costa, J.D., Hess, R.L. and Goldstein, D.E.: *Improved colorimetric assay for glycosylated hemoglobin. Clin. Chem.*, 27(5): *669-672, 1981.*

Ploybutr, S., Burrin, J.M. and Alberti, K.G.M.M.: *Rapid in vitro glycosylation of hemoglobin; a hazard of columm chromatographic but not colorimetric techniques. Clin. Chem.*, 28(5):*1233-34, 1982.*

Worth, R.C., Ashworth, L., Burrin, J.M., Johnston, D.G., Skillen, A.W., Anderson, J. and Alberti, K.G. M.M.: *Column assay of haemoglobin A_1. Clin. Chim. Acta.*, 104: *401-404, 1980.*