

EVALUATION OF CHEMICAL COLORIMETRIC METHOD FOR DETERMINATION OF GLYCATED HEMOGLOBIN

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It has been suggested that glycosylated hemoglobin is a reliable index of long-term blood glucose control in diabetes mellitus. Chemical colorimetric method used to measure the amount of glycosylated hemoglobin is less affected by the labile hemoglobin, abnormal hemoglobins and variations in analytical conditions. In our modified chemical colorimetric method, the 5-hydroxymethylfurfuraldehyde was produced by heating glycosylated hemoglobin in an autoclave (115°C, 104 KPa, 50 min) in the presence of weak oxalic acid. The 5-hydroxymethylfurfuraldehyde was reacted with thiobarbituric acid (37°C, 40 min) to form an adduct that was then measured photometrically. Result was expressed as the concentration of hexoses in hemoglobin, with mmol/mol hemoglobin as the unit. For our assay, the precisions (within-run C.V. <5%; between-run C.V. <10%) and linearity ($r=0.997$ between 61.8 and 272.7 mmol/mol hemoglobin) were acceptable. Sample stability was good. Whole blood and hemolysate were stable at room temperature or 4°C. for at least one week, hemolysate was stable over 3 months at -20°C. Our method correlated well with Leeco resin-adsorption method ($r=0.559$, $P<0.01$) and high performance liquid chromatography ($r=0.840$, $P<0.01$). But

Leeco resin-adsorption method was affected by labile hemoglobin (glycosylated hemoglobin values decreased 13.6% after removal of labile hemoglobin). We also found that the amount of glycosylated hemoglobin correlated well with fasting plasma glucose ($r=0.777$, $P<0.01$). There were significant differences between diabetic patients and nondiabetic subjects ($P<0.0001$), and between well controlled and poorly controlled diabetic patients ($P<0.0001$), but no significant difference between nondiabetic subjects and well controlled diabetic patients ($P>0.2$) in their glycosylated hemoglobins. The glycosylated hemoglobin reference interval for Chinese with this method was 78.4-125.3 mmol/mol hemoglobin. From nondiabetic subjects, we found that there were no significant differences in glycosylated hemoglobin between male and female ($P>0.4$), and within all age intervals, except that the age groups of 39 years and under had a small difference from the age groups of 40 years and over ($\alpha=0.05$).

All results revealed that the assessment of this chemical colorimetric method was good and it could be applied in clinical practice. Besides, the method needs neither difficult techniques nor expensive instruments, which make it a suitable method for routine clinical use.

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