TEST EVALUATION
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TEST

Glycosylated hemoglobin: methodological considerations

PRINCIPLES OF TEST

Glycosylated hemoglobin measurement is a means of monitoring chronic glucose control in both type I and type II diabetics. It provides a time-averaged index of serum glucose levels over the entire life span of the red cell, free from daily fluctuations. Since even mild diabetes is characterized by marked variability of serum glucose levels, a single random glucose determination may not accurately assess the patient's general status. Glycosylated hemoglobin values show a good correlation with clinical estimation of metabolic control based on multiple serum and urine glucose determinations, and an excellent correlation with mean serum glucose values calculated from multiple tests of diabetic individuals performed over several months (4,5).

Hemoglobin glycosylation occurs via a non-enzymatic posttranslational two-stage condensation of glucose and various amino groups (e.g., amino terminal valine fo Hb chain) on the hemoglobin molecule. The first glycosylation step occurs quickly and involves formation of a labile Schiff base. This process is affected by acute fluctuations in serum glucose. Depending on the assay used, this labile component may have to be removed in order to get an accurate indication of chronic metabolic control (see limitations). The labile molecule undergoes a slow process called Amadori rearrangement to form a stable ketoamine glycosylated hemoglobin. Three "fast" glycosylated hemoglobin fractions have been resolved using cation exchange chromatography and are designated HbA1a, HbA1b, and HbA1c. All elute prior to the native HbA0 molecule. HbA 1c is present in the greatest quantity in both normal and diabetic individuals and is the most commonly measured fraction, although some assays quantify total HbA1.

METHOD

There are two main methods for glycosylated hemoglobin assessment currently in use. The older method is cation-exchange chromatography (macrocolumn, or microcolumn) (1). With the minicolumn technique, a negatively charged resin will bind the non-glycosylated (slow) hemoglobin fraction with more avidity than the glycosylated (fast) fraction at a selected ionic strength and acid pH. After addition of an initial buffer, the fast fraction will elute while the slow fraction remains. Fast fraction contains HgA1a, 1b, and 1c. With addition of a second buffer, the slow fraction is eluted. The absorbance of each elution is read spectrophotometrically at 415 nm, and percentage of glycosylated hemoglobin calculated. The macrocolumn technique allows separation of HbA1c from the other "fast" hemoglobins, but the method is time-consuming.
The newer method is boronate affinity chromatography (2,3). This is based on the specific interaction between glycosylated hemoglobin and boronate anion immobilized within an agarose gel. This gel reversibly binds only glycosylated proteins. After elution of the non-glycosylated fraction, glycosylated hemoglobin is dissociated by a sorbitol counterligand. The absorbance of each fraction is measured at 415 nm, and percentage glycosylated hemoglobin calculated.

Other assays less commonly used include agar gel electrophoresis, isoelectric focusing, high performance liquid chromatography, and radioimmunoassay. Colorimetric assays also exist, but are limited by poor specificity and difficulty with standardization.

Normal values vary according to the method employed. By the affinity method, a normal range of between 4.2% and 6.5%, with mean of 4.96% has been reported, but normal ranges should be established by each individual laboratory (2).

TECHNICAL LIMITATIONS OF TEST

The microcolumn cation-exchange assay for glycosylated hemoglobin has several significant limitations which have prevented its common usage (3,6). Sample stability is poor at room temperature or -20°C, and results are extremely sensitive to slight variations in column load, temperature and pH and ionic strength of the buffer system. The method is non-linear due to variable overlap with non-glycosylated hemoglobins, and measures only the "fast" fraction, which represent only hemoglobin glycosylated at the terminal valine whereas hemoglobins glycosylated at the N-terminus of the α chain and certain lysine residues are also formed. This method requires elimination of the "labile" glycosylated hemoglobin, which reflects short-term blood glucose fluctuations, by pretreatment for accurate glucose control assessment. Various factors which alter the charge properties of the hemoglobin molecule may cause spurious results. False positive results have been reported with HbF which coelutes with HbA_{1c} and uremia (in which carboxylated hemoglobin coelutes with HbA_{1a} and 1b), with alcoholism, aspirin, and lead poisoning (3,4,5). False negative results have been reported with HbS, HbC, and other variant hemoglobins. Both increased and decreased levels have been reported after specimen storage (4,6).

The boronate affinity method purportedly eliminates many of these difficulties, although, because the method is new, extensive clinical testing has not yet been performed. There is good sample stability at room temperature and -20°C, only slight pH and temperature variation, errors due to sample size have been reduced, and the method is linear due to complete separation of glycosylated and non-glycosylated fractions (2,3). Pre-treatment is not required since the labile form of HbA_{1c} is not measured. Total glycosylated hemoglobin is measured by this assay, not just HgA_{1a}, 1b and 1c. Hemoglobin F does not give falsely high results. Any condition that shortens the red cell life span may affect glycosylated hemoglobin levels independent of mean blood glucose. Thus patients with hemolytic anemia or sickle-cell disease will have lower percent glycosylation.
In summary, the main utility of glycosylated hemoglobin measurement is an indicator of chronic metabolic control of glucose. With improved assay methods, (e.g., the boronate affinity column) increased clinical use of this test will probably occur. A detailed evaluation of the clinical use of this test is beyond the scope of this review.

REFERENCES


