

Measure of glycosylated hemoglobin

Lorenza Calisti, Simona Tognetti

Department of Pediatrics, University of Pisa, Italy

Abstract. Glycosylated hemoglobin (HbA1c) is a marker of evaluation of long-term glycemic control in diabetic patients and predicts risks for the development and/or progression of diabetic complications. Glycosylation process depends on the exposure to glucose, so on the half-life of erythrocyte. It was demonstrated, however that metabolic control concerning the last 90-120 days had only a 10% effect on the result of HbA1c; mean blood glucose of the last 30 days contributes for 50% of HbA1c value. Blood glucose value in the afternoon and in the evening better correlate with HbA1c levels if compared with blood glucose values in the morning. It is important to know that there may be, in the evaluation of HbA1c, interference in the dosage, due to a condition of uremia, hiperlipemia, bad conservation and hemolysis of the sample, increase of leucocytes and presence of anomalous hemoglobins. Moreover the use of different methods, the lack of a common calibration concerning the same methods and the variability of instrumentation do not make reproducible results yet, in different laboratories. (www.actabiomedica.it)

Key words: Glycosylated hemoglobin, glycemic control, diabetes, glycosylation

Introduction

Important perspective studies on chronic complications of Diabetes mellitus allowed us to establish with absolute certainty the role of glycosylated hemoglobin (HbA1c) as a marker of evaluation of long-term glycaemic control in diabetic patients and the strict relationship between the risk for chronic complications and HbA1c levels. Diabetes Control and Complication Trial (DCCT), a great extent study, has demonstrated that the 10% stable reduction in HbA1c determines a 35% risk reduction for retinopathy, a 25-44% risk reduction for nephropathy and a 30% risk reduction for neuropathy (1-3).

HbA1c is the product of non-enzymatic reaction between glucose and free amino groups of hemoglobin. This reaction, called glycosylation, involves lots of other proteins, too and it is the principal mechanism through which glucotoxicity is formed. Other invol-

ved mechanisms are: oxidative stress, activation of the polyols pathway, activation of protein kinase-C, endothelial damage, haemodynamic and coagulative changes.

Glycosylation

Glycosylation is a non-enzymatic reaction between free aldehydic group of glucose and free amino groups of proteins. A labile aldiminic adduct (Schiff base) forms at first, then, through a molecular rearrangement, a stable ketoaminic product slowly accumulates.

Concerning the hemoglobin, the preferential glycosylation site is the amino-terminal valine of the beta chain of the globin (about 60% of glycosylated globin). Other sites are: lysin 66 and 17 of the beta chain, valine 1 of the alpha chain.

The term HbA1c refers to the hemoglobin fraction of the glucose bound stably (ketoamine) to beta terminal valines. Other proteins with a possible glycosylation are: albumine, alfa 2 macroglobulin, antithrombin III, erythrocyte enzymes, fibrinogen, ferritin, HDL-LDL, transferrin; all of them are short half-life proteins. Instead, actin, collagen, fibronectin, myelin, nucleoproteins, spectrin, tubulin can also be glycosylated and have a long half-life.

The glycosylation process of short half-life proteins stops at the formation of the stable ketoamine adduct. Instead, long half-life proteins (myelin and collagen) undergo a complex and irreversible rearrangement process, with the formation of Advanced Glycosylation End products (AGE). AGE form a family with many compounds, only partially identified; they accumulate in the structural proteins modifying the function of them. They bind to specific macrophage receptors inducing a release of hydrolytic enzymes, cytokines and growth factors able to promote the synthesis of fundamental substance and, acting at intracellular level, to determine a damage of the nucleic acids. Hemoglobin and plasmatic proteins (4-7) are the product of non enzymatic glycosylation that can be dosed in a diabetic patient.

Methods of measurement of HbA1c

The addition of fructose group to the hemoglobin molecule changes some of its physical-chemical properties; those properties are utilized for electrophoretic and chromatographic measurement methods. In particular HbA1c differs from native hemoglobin for total electrical charge, regional electric affinity by the fructose group and antigenic properties. In the last 20 years improved techniques in laboratory and new electrophoretic, chromatographic and immunological methods available, gave us a greater reliability on our results. However the use of different methods, the lack of a common calibration concerning the same method and the variability of instrumentation do not make reproducible results yet in different laboratories (8). For this reason studies and procedures of standardization are going on (9-11). In Italy an external check service of quality is permanently active,

supported by scientific firms - SID and AMD. Participation in the study is the best way to value the reliability in the future of their own measures of HbA1c. The program is on Internet www.glicata.org (12). It is important to know there may be, in the evaluation of HbA1c, interferences in the dosage, due to a condition of uraemia, hyperlipemia, bad conservation and hemolysis of the sample, increase of leucocytes and presence of anomalous hemoglobins (13, 14).

The liquid chromatography ionic exchange is now the most reliable methodology. It is based on the difference in electric charge with numerous advantages. It is completely automatic and presents an excellent reproducibility in different laboratories. It allows to measure with precision all sub-fractions of HbA1c and anomalous hemoglobins. Its cost is high and it is not available in all the laboratories (15, 16).

The immunochemical method DCA 2000 is the most popular and utilizes antigenic properties. It allows to dose the HbA1c in 6 minutes, it requires a small amount of blood (1 microliter), it is done with sample of capillary blood, it presents a strict correlation ($r=97$) with the HPLC method and it distinguishes neither the normal hemoglobin nor the labile fraction of glycohemoglobin.

At present there are available instruments for domiciliary measurement of HbA1c and fructosamine. Their application is still being studied (17, 18).

Clinical significance of HbA1c

The determination of the glycosylated hemoglobin in the management of a diabetic patient was proposed for the first time by Gabbay and Koenig in 1976 (19, 20). It has become the parameter of election concerning the retrospective evaluation of the glycemic control. It is in fact strictly correlated with the mean plasmatic glycemia (21, 22). Glycosylation process depends on the exposure to glucose, so on the half-life of erythrocyte. It was demonstrated, however that metabolic control concerning the last 90-120 days had only a 10% effect on the result of HbA1c. Mean blood glucose of the last 30 days contributes for 50% of HbA1c value. HbA1c levels can vary quite rapidly in relation to striking fluctuations of blood glucose (23).

The study of Rholging on the glyceimic profiles collected in DCCT, 1439 Type 1 diabetic patients aged 13-39, beyond confirming a strict correlation between mean plasmatic glycemia and HbA1c, demonstrated that:

- 35 mg/dl increases of mean plasma glucose cause a rise of 1% in HbA1c.
- Blood glucose values in the afternoon and in the evening (postlunch, predinner, postdinner, bedtime) better correlate with HbA1c levels if compared with blood glucose values in the morning (prebreakfast, postbreakfast, prelunch).
- The greatest correlation is with postlunch glycemia and bedtime glycemia.
- Fasting glucose tends to underestimate HbA1c values.

The strong correlation of postlunch glycemia with the levels of HbA1c can be explained by the fact that a subject in condition of normal life is a postlunch stage or postassorbing stage and in a fasting condition only in the second part of the night (24, 25).

Some algorithms were worked out and they allowed us to value the mean glycemia of the 6-8 weeks before taking from the result of the HbA1c measure.

The line equation for the estimate of the mean value of glycemia is:

$$\text{MBG} = 33.3 \times \text{HbA1c} - 86$$

Similarly, it is possible to go back to the value of HbA1c knowing the mean plasmatic glycemia; HbA1c presumed is:

$$\text{HbA1c} = (\text{MBG} + 86)/33.3$$

Criteria of evaluation of the metabolic control for (the value of) HbA1c were also defined as:

- HbA1c <6.3%: very good glyceimic control.
- HbA1c between 6.3 and 7.1%: good glyceimic control.
- HbA1c between 7.1 and 9%: poor glyceimic control.
- HbA1c >9%: bad glyceimic control.

Protective values of HbA1c to chronic complications are considered <7%. Italian or foreign studies concerning the evaluation of the metabolic control in pediatric age are still far from this objective.

Recently the first results of a wide italian study on the metabolic compensation in children with IDDM,

have been expressed. The mean value of HbA1c in the examined population is $8.87 \pm 1.7\%$, without significant differences between males and females. Patients with the best metabolic control (HbA1c <8%) had more than 3 glyceimic controls a day. No significant differences came out concerning the administrations of insulin, the intensified therapy would not seem to guarantee an optimus metabolic compensation.

The italian situation, even compared with other studies, seems to be rather good.

Conclusions

Concentration of HbA1c is an indicator of average blood glucose concentration over the preceding 2-3 months. HbA1c is currently considered the best index of metabolic control for diabetic patients in clinical setting and participants in epidemiological studies as well as a measure of risk for the development of micro and macrovascular complications.

However, the clinical utility of this measure is compromised by limitation inherent in most assay methods. Standardization Program was developed in an attempt to standardize these methods so that HbA1c results can be related to the candidate reference method used in the DCCT.

References

1. Dahl-Jorghensen K, Brichmann-Hanssen O, Hansenn KF, et al. Effect of near normoglycemia for two years on progression of early diabetic retinopathy, nephropathy and neuropathy. The Oslo Study. *Br Med J* 1986; 293: 1195-9.
2. Lauritzen T, Forst-Larsen K, Larsen HV, Deckert T. Steno Study group: Two-year experience with continuous subcutaneous infusion in relation to retinopathy and nephropathy. *Diabetes* 1985; 34 (suppl. 3): 74-9.
3. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of Diabetes on the development and progression of long term complications in the diabetes control in insulin diabetes mellitus. *N Engl J Med* 1993; 329: 977-86.
4. Bunn HF, Gabbay KH, Gallop PM. The glycosylation of hemoglobin: relevance to diabetes mellitus. *Science* 1978; 200: 21.
5. Brownlee M, Cerami A, Vlassara H. Advanced products glycosilation and the pathogenesis of diabetic vascular disease. *Diabetes/Metabolism Reviews* 1988; 4: 437-51.

6. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Engl J Med* 1988; 318: 1315.
7. Johnson RN, Metcalf PA, Baker JR. Fructosamine: a new approach to the estimation of serum glycosyl protein. An index of diabetic control. *Clin Chim Acta* 1982; 127: 87-95.
8. Mosca A, Lapolla A, Franzini C. La determinazione della emoglobina glicata (HbA1c) nel sangue: raccomandazioni. *Biochim Clin* 2000; 24: 183-7.
9. Little RR, et al. The National glycoemoglobin standardization program: a five year progress report. *Clin Chem* 2001; 47: 1985-992.
10. NGSP Steering Committee: Implementation of the National Glycohemoglobin standardization Program (NGSP). *Diabetes* 1997; 46 (Suppl.1): 151A.
11. Mosca A, Paleari R. Standardizzazione dell'emoglobina glicata rapporto sugli ultimi tre anni di attività. *Biochim Clin* 1998; 22: 645-9.
12. www.glicata.org
13. Roberts WL, McCraw M, Cook CB. Effects of sickle cell trait and hemoglobin C trait on determinations of HbA1c by an immunoassay method. *Diabetes Care* 21: 983-6.
14. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW, Krejs GJ. Evaluation of HbA1c determination methods in patients with hemoglobinopathies. *Diabetes Care* 23: 339-44.
15. Schifreen RS, Hickingbotham JM, Bowers GN. Accuracy, precision and stability in measurement of hemoglobin A1c by high performance cation exchange chromatography. *Clin Chem* 1980; 26: 3-8.
16. Matteucci E, Milioni C, Biasci E, Bertoni C, Boldrini E, Giampiero O. With regard to glycohemoglobin measurement: are we sure that high-performance liquid chromatography currently works in the clinical routine? *Acta Diabetologica* 1998; 35: 41-7.
17. Vanelli M, De Fanti A, Avantageggiato S, et al. Performance e utilità di un metodo immunologico rapido per il dosaggio dell'HbA1c nell'ambulatorio diabetologico. *Minerva Pediatrica* 1993; 45: 373-7.
18. Marrero DG, Vandagriff JL, Fineberg SE, Fineberg NS, Hiar CE, Crowley LE. Immediate HbA1c Results: Performance of the new HbA1c system in pediatric outpatient population. *Diabetes Care* 1992; 15: 1045-8.
19. Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM. Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus. *J Clin Endocrinol Metab* 1977; 44: 859-64.
20. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lerman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. *N Engl J Med* 1976; 295: 417-20.
21. Bun FH. Evaluation of glycosylated hemoglobin in diabetic patients. *Diabetes* 1981; 30: 613-7.
22. Gillery P, Dumont G, Vassault A. Evaluation of GHb assays in France by national quality surveys and controls. *Diabetes Care* 21: 265-70.
23. Goldstein DE, Peth SB, England JD, et al. Effects of acute changes in blood glucose on HbA1c. *Diabetes* 1980; 29: 623-8.
24. Rohlfing CL, Wiedmeyer H-M, Little RR, England JD, Tennill A, Goldstein DE. Defining the Relationship Between Plasma Glucose and HbA_{1c}: Analysis of glucose profiles and HbA_{1c} in the Diabetes Control and Complications Trial. *Diabetes Care* 25: 275-8.
25. Rose E, Ketchell MLS. Does daily monitoring of blood glucose predict hemoglobin A1c levels? *J Fam Pract* 2003; 52: 485-90.
26. Monnier L, Lapinski H, Claude C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of Type 2 diabetic patients. Variations with increasing levels of HbA1c. *Diabetes Care* 2003; 26: 881-5.

Correspondence: Dr. Lorenza Calisti
Dept. of Pediatrics
University of Pisa, Italy