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Glycated hemoglobin fundamentals. Value and advantages in practical clinical

Fundamentos de la hemoglobina glicada. Valor y ventajas en la práctica clínica

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Palabras clave:
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ABSTRACT

The glycated hemoglobin (HbA1c) test is a useful, economic, and practical clinical tool for long-term glycemic control in patients with diabetes mellitus (DM). Historically, since 1955, the HbA1c was described for the first time by Kunkel and Wallenius as a minor fraction of human hemoglobin. However, until the 70s, the molecule was recognized as a glycemic control marker. The HbA1c is a conjugated protein (heteroprotein, hemoglobin-glucose) formed through the non-enzymatic and post-translational process called glycation (Maillard reaction) as a stable Amadori product. If the reaction continues, the final results are irreversible products called advanced glycation end products (AGEs). AGEs are responsible for modifying proteins of the whole tissues and contribute to inflammatory reactions mediated by the AGE receptor and complications of DM. Additionally, HbA1c levels of less than 7% have been associated with reducing microvascular and macrovascular lesions. An adequate evaluation and monitoring routinely of HbA1c levels would allow adequa te glycemic control and help to reduce the risk of future complications.

RESUMEN

La prueba de hemoglobina glucosilada (HbA1c) es una herramienta clínica útil, económica y práctica para el control glucémico a largo plazo en pacientes con diabetes mellitus (DM). Históricamente, desde 1955, la HbA1c fue descrita por primera vez por Kunkel y Wallenius como una fracción menor de la hemoglobina humana. Sin embargo, hasta la década de los 70, la molécula fue reconocida como un marcador de control glucémico. La HbA1c es una proteína conjugada (heteroproteína, hemoglobina-glucosa) formada a través de un proceso no enzimático y postraduccional llamado glicación (reacción de Maillard) como un producto estable de Amadori. Si la reacción continúa, los resultados finales son productos irreversibles llamados productos finales de glicación (AGE, por sus siglas en inglés). Los AGE son responsables de modificar las proteínas de todos los tejidos y contribuyen a las reacciones inflamatorias mediadas por el receptor AGE y las complicaciones de la DM. También, los niveles de HbA1c inferiores a 7% se han asociado con la reducción de lesiones microvasculares y macrovasculares. Una adecuada evaluación y monitorización rutinaria de los niveles de HbA1c permitiría un adecuado control glucémico y ayudaría a reducir el riesgo de futuras complicaciones.

INTRODUCTION

Glycated hemoglobin (HbA1c), still in recent times, is the most useful, economical, and practical clinical tool for long-term glycemic control in patients with diabetes mellitus (DM).[1,2] Unfortunately, many aspects regarding its basic biology, assay and standardization techniques, sensitivity, pitfalls, and shortcomings, and its correlation with micro and macrovascular lesions in DM, remain not fully understood by many practitioners. This review aims to make this important clinical instrument's basic and clinical foundations available to caregivers, especially in medical care's first and second levels. After this brief introduction, the paper is organized as follows. The HbA1c history is presented.

The Hb variants section presents the principal features of hemoglobin, different types of Hb, and Hb variants caused by genetic alterations. The glycation and glycosylation

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reactions are detailed in the following sections (glycation, glycated hemoglobin, glycation vs glycosylation). Some important points about glycemic control are presented in the measurement of HbA1c, HbA1c in the initial diagnosis of diabetes mellitus, and shortcomings of HbA1c sections. Next, HbA1c and the microvascular and microvascular diabetic complications are supported. Finally, this paper is concluded.

HBA1C HISTORY

The history of HbA1c started in 1955 when Kunkel and Wallenius reported the separation of minor fractions of human hemoglobin (Hb) by electrophoresis.[3] Subsequent studies using chromatographic techniques confirmed the presence of adult and fetal types of Hb,[4] and five minor subtypes of adult Hb (HbA1c) were named a, b, c, d, and e.[4,5] In 1962, Huisman,[6] through cellulose acetate electrophoresis, found an HbA minor variant in diabetic patients, while Rahbar, in 1969, observed that one of these Hb bands generated a rapid positional movement, later described as a «fast-moving abnormal hemoglobin band», and recognized it as the subfraction HbA1c.[7,8] Since the 70s, the molecule has been recognized as an excellent marker of glycemic control and micro and macrovascular diabetic complications.[9]

HB VARIANTS

Hb is a protein found in red blood cells, composed of two globin dimers associated with a heme group whose primary role is oxygen transport.[10] The two αβ dimers (named α1β1 and α2β2) are arranged around a 2-fold axis of symmetry resulting in a large central water cavity (deoxygenated structure) and a thinner cavity oxygenated structure.[11] Through electrophoresis, different types of Hb were identified, as already stated, allowing a classification according to subunits conformation (a, b, or g dimers)[12] in three main groups: hemoglobin A1 (HbA), which is the most abundant type in adults (~96%), hemoglobin A2 (HbA2, frequency about 2.3-2.8%), generally found in small amounts in adults and hemoglobin F (HbF < 2%), mainly found in fetuses and newborns.[5] In addition, there are abnormal genetic hemoglobinopathies such as α- and β-thalassemia syndromes and structural Hb variants (HbS found in sickle cell anaemia, and the variants of HbE and HbC diseases, among others).[13-15]

Furthermore, many Hb variants are secondary to gene deletions, insertions, polymorphisms, or mutations, which are the basis of different types of hemoglobinopathies.[16,17]

More recently, the interactions among genes, certain pathologic conditions, and environments have been described that potentially can influence the glycemic control of patients with type 2 DM (DM2). In other cases, these can affect the production and structure of Hb, the lifespan of red blood cells, iron metabolism, resistance to malaria, and many other functions and traits.[16,17] Additionally, several subfractions are recognized, according to their migrating velocity: slower or faster, depending on the sugar bound: HbA1a1 (glycation with fructose 1-bisphosphate); HbA1a2 (glycation with glucose 6-phosphate); HbA1b (glycation with pyruvic acid), and HbA1c (glycation with glucose).[18]

GLYCATION

Glycation is a non-enzymatic chemical reaction in which some sugar is directly added to proteins, lipids, or nucleic acids biomolecules *(Figure 1)*. It is involved in the so-called Maillard reaction or non-enzymatic browning process, resulting from adding amino groups and reducing sugars, which produces a discoloration of food exposed to thermal effect.[19] The Maillard reaction occurs not only in food or beverage processing but also in other industrial conditions and even in the metabolism of mammals.[20] *In vivo*, the early step of the Maillard reaction is an autoxidative reaction involving the addition of oxidized glucose with other biomolecules, mainly amino groups, such as lysine or arginine residues. The second phase occurs when the glucose-amino adducts form the so-called Schiff bases (the condensation of primary amines and carbonyl functional groups), such as glycosylamine, that are naturally unstable.[21] Then, the base suffers a molecular rearrangement. This phenomenon first generates a large series of

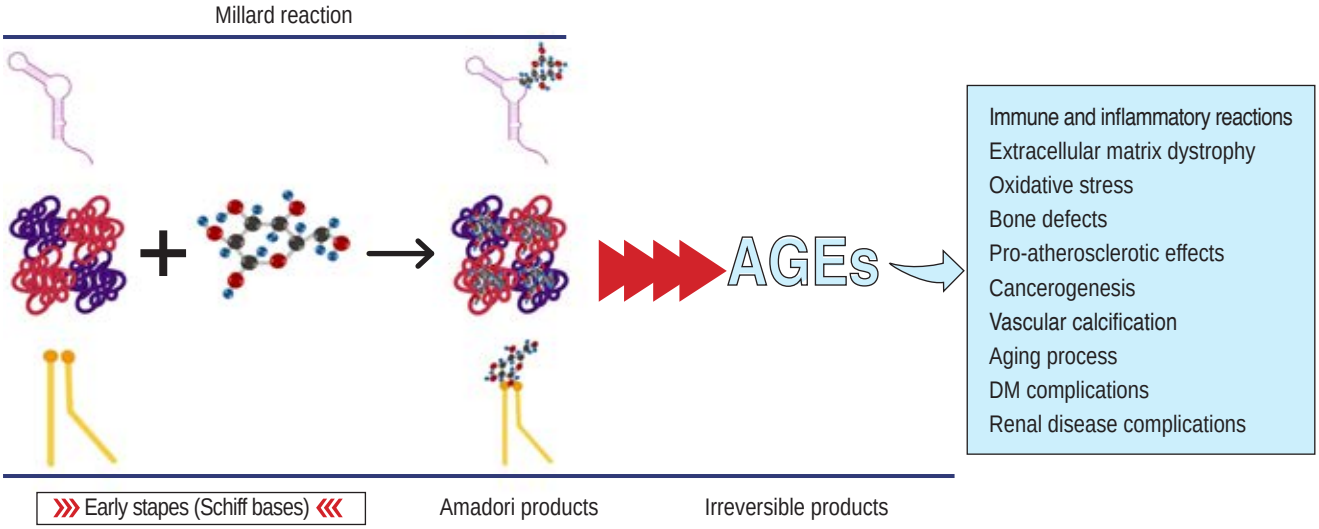

Figure 1: Glycation reaction, products and their effects.
AGEs = advanced glycated end products.

intermediate molecules and, finally, the more stable Amadori products (such as HbA1c and fructosamine, among others). In the last phase of the Maillard reaction, these early adducts are further transformed into more glycated compounds.[22,23] These final irreversible products are called advanced glycated end products (AGEs) and have crucial importance in the genesis of tissue damage in disorders like DM *(Figure 1)*.[24] AGEs are not solely generated endogenously but are components of processed food and beverages and are also generated by aging, ultraviolet radiation, tobacco smoking, diverse chemical agents and air pollution, among other conditions.[19] In the advanced glycation process, some proteins are modified by oxoaldehydes, mainly glyoxal, methylglyoxal, and 3-deoxyglucosone.[25,26] These proteins modified by AGEs can harm every body cell and tissue, eliciting an inflammatory reaction mediated by the so-called AGE receptor,[27,28] which recognizes as ligands not only AGEs products but also a vast set of molecules as S100/calgranulins, high mobility group box one (HMGB1), a chromatin-associated protein family, and specific amyloid molecules, among many others. All these substances can detonate complex functional and structural phenomena of immune and inflammatory reactions, extracellular matrix dystrophy, oxidative stress, bone defects, pro-atherosclerotic effects, cancerogenesis, and vascular calcification, among other catastrophic consequences.[28-30] Precisely, the increment of AGEs precursors, the carbonyl highly reactive compounds, named carbonyl stress,[31] contributes to the aging process, too many complications of DM and renal disease, and some of the derangements of dysmetabolic obesity, among many other severe pathological events *(Figure 1)*.[31] The concept of carbonyl stress signals that the excess oxidation of sugars and lipids, associated with a poor removal (as it happens in renal failure) of carbonyls, by itself or generating AGEs, exerts a powerful deleterious effect in several tissues.[31,32] One of the therapeutic benefits of metformin is the drastic decrease of serum glycating agents dicarbonyls in patients with dysmetabolic obesity, treated with an even small dose of the drug.[33]

GLYCATED HEMOGLOBIN

The HbA1c is a conjugated protein (hemoglobin-glucose), a heteroprotein. It is formed through the non-enzymatic and post-translational processes described above. The union of glucose to the β-N-terminal valine residues of globin forms the Amadori product named HbA1c. Hence, as the

amount of plasma glucose increases, it also raises HbA1c.[34] As this process is irreversible throughout the 120-day lifespan of non-transfused erythrocytes,[35,36] there is a direct relationship between the mean concentration of glucose and the amount of HbA1c. This fact makes Hba1c an excellent long-range marker of glycemic control.[36]

GLYCATION VS GLYCOSYLATION

Even though there are striking differences between glycosylation and glycation, both chemical processes are misunderstood and frequently misused.[18,37] As stated above, protein glycation is an irreversible, non-enzymatic reaction where the amino groups of proteins are conjugated with reduced sugars, forming brown polymers (browning o Maillard reaction).[23] Such a reaction depends on the substrate concentration (free glucose) and is characterized by forming a ketoamine at the N end of the beta chain of Hb. Similarly, nucleic acids, lipids, and intracellular and extracellular proteins can be modified by glycation.

On the other hand, glycosylation refers to a post-translational modification in which carbohydrates combine with other biomolecules (proteins, lipids, or nucleic acids) under the effect of multiple enzymes, with strict control in binding sugars to residues such as serine, asparagine, and hydroxylysine in enzymatic glycosylation. This phenomenon is necessary for certain normal functions, such as protein folding and stabilization. Abnormalities of glycosylation can result in inflammation processes, altered immunity, extracellular matrix dysfunction, stimulation of malignant metastasis, and other severe health problems. This process will allow them to fulfill a wide variety of functions, such as a longer protein survival, facilitation of protein secretion from its cell of origin, molecular traffic, cell signalization, the formation of specific receptors for hormones and other humoral substances, and the provision of a barrier or protective layer, and others.[35,37] Although use makes customs and customs make laws, from the scientific point of view, the terms glycation and glycosylation cannot be used as synonyms since they indicate totally different biological and biochemical processes.[37]

THE MEASUREMENT OF HBA1C

When the assay of HbA1c is certified by the National Glycohemoglobin Standardization Program (NGSP),[38,39] which describes the measurement techniques (using a high-performance liquid chromatography [HPLC] system and a BioRex 70 CE resin column) and standardization follows the specifications derived from the diabetes control and complications trial (DCCT);[40] the main use is to assess the glycemic control in the last three months.[38] In general, it is accepted that among diabetic patients, a value of HbA1c less than 7% signals a good control of the disease. The ADA counsels the measurements of HBA1c twice or thrice a year in stable patients but more frequently in patients with labile or uncontrolled glycemia.[38] The ADA recommends achieving a value of Hba1c < 7% in diabetic, non-pregnant patients as a good marker of good glycemic control, with a low risk of hypoglycemia.[38] Later, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) introduced a new method for measuring the concentration of a single molecular species of glycated A1c.[36,41] The reason was the lack of uniformity of HbA1c results of many laboratories, using different assay methods and kits from different manufacturers. So, the task group of the IFCC established international reference methods and obtained pure samples of HbA1c for calibration purposes.[42,43] Anyhow, the percentage values of HbA1c continue to be the most widely used method. Both methods, however, correlate well with the estimated average glucose *(Table 1)*.

Until recently, glycemic control in patients with DM was based on frequent self-monitoring of blood glucose (SMBG) and the periodical estimation of HbA1c, so far, the gold standard for long-term glycemic monitoring. The so-called «Point-Of-Care» tests (POCT, also called extra-laboratory or close-patient glucose measurements) have clear advantages over the more time-consuming central analytical laboratory glucose measurements regarding feasibility, promptness of results, and probably costs in both the intrahospital and home milieus. Modern POCT devices are light, economical, and accurate, allowing better

Table 1: Relations among DCCT and IFCC values of HbA1c and estimated average glycemia.

HbA1c (NGSP/DCCT) %	HbA1c (IFCC) mmol/mol	Estimated average glucose (eAG) mg/dL
5	31	97
6	42	126
7	53	154
8	64	183
9	75	212
10	83	240
11	96	269
12	107	298
13	118	355

Adapted from: American Diabetes Association Professional Practice Committee[38] and Agrawal SN.[59]

control of hyperglycemia.[44] In this context, the measurement in capillary blood of HbA1c with high-sensitivity POCT devices also allows better long-term glycemic control.[45] Continuous glucose monitoring (CGM) is another tool that allows better long-term glycemic control in insulin-treated patients or those with great variability of glycemic values, making glycemic control more difficult. HbA1c can be derived from the average glycemia, although its value occasionally differs from the compound's lab assay. CGM apparatus detects interstitial and no intravascular glucose concentrations, so the provided glucose values could also differ from the capillary glycemia measured with oxidase reagents, generating hydrogen peroxide, by the POCT devices.[46,47] CGM has some advantages over HbA1c. The latter cannot detect the abrupt glycemia oscillations observed in patients with type 1 DM (DM1) or those under intensive antidiabetic treatment, mainly with complex associations of different insulins. Some of these important fluctuations can provoke threatening hypoglycemic episodes. It is evident that HbA1c measurement cannot detect this kind of event.[46] Nevertheless, it also seems that in most patients with stable DM2, non-insulin users, and with low risk of hypoglycemia, it is mainly treated with modern incretins, glucagon-like peptide analogues, or sodium-glucose cotransporter (SGLT2) inhibitors, the higher cost of CGM is not rewarded with better clinical outcomes.[48]

HBA1C IN THE INITIAL DIAGNOSIS OF DM

Most clinicians, together with fasting plasma glucose (FPG), use the HbA1c value for the initial diagnosis of DM, given that it is easier and faster than determining blood glucose 2 hours after an oral intake of a 75 g load (2-h PG). However, the latter is a more accurate and earlier marker of DM. Both glucose measurements show marked variability depending on diet, exercise, medications, and mental and social stress, among other factors.[49,50] The technique of obtaining and transporting the sample and the delay in performing the analysis can also influence the result.[50]

In contrast, HbA1c is more stable, providing, by inference, the estimated average glycemia in a long lapse. Nevertheless, HbA1c does not represent the direct measure of serum glucose, only the glycation phenomenon. The results deserve less credibility if the assay is not performed according to international standardization norms (as in many Mexican laboratories of private and institutional hospitals and clinics). The abundant sources of errors and shortcomings (see below) in the assessment of HbA1c require that the result be taken with caution and accompanied by careful clinical criteria and other laboratory techniques (FPG and 2-h PG). The result of the HbA1c assay is usually expressed as the glycated percentage of the total Hb content. It has been established that people without prolonged hyperglycemia have HbA1c values of less than 5.6%, while persons with uncontrolled DM exhibit values ≥ of 6.5%.[38] Between these two limits are those with a high probability of developing DM («pre-diabetes»).[38,51]

SHORTCOMINGS OF HBA1C

Along with advantages and virtues, HbA1c has a lot of pitfalls and shortcomings, both as a diagnostic marker for diabetes and as an indicator of glycemic control.[38,50,51] A set of conditions can influence the results of the HbA1c assay. Among those falsely increasing HBa1c

value are deficient anemias (iron, vitamin B12), alcohol abuse, some hemoglobinopathies, advanced renal failure, and splenectomy. In the opposite situation, normal pregnancy, erythropoietic-stimulating drugs, vitamins E and C, certain hemoglobinopathies, hypersplenism, and use of drugs like aspirin (in great doses), opiates, and antiretrovirals, among others, can decrease HbA1c values.

HBA1C AS A MARKER OF MICROVASCULAR DIABETIC COMPLICATIONS

A long time ago, it was confirmed that in patients with DM1, HbA1c is an excellent risk marker that predicts the development and aggravation not only of microvascular diabetic lesions but also of macrovascular ones, well.[52,53] According to the DCCT,[40] lowering HBa1c in young patients with DM1 less than 7% was associated with a 50-76% reduction of microvascular diabetic lesions,[54] while the UKPDS trial showed that a value of HbA1c of 7% diminished the risk of all diabetes-related endpoints by 12-32%.[55] Diabetic microangiopathy includes the classical retinal, renal, and peripheral nerve lesions and those affecting the brain, the skin, and myocardial microcirculation. The so-called «therapeutic legacy» describes the fact that patients with better HbA1c at the beginning, many years later, continue to obtain benefits and reduced outcomes due to micro and macroangiopathy.[56] However, since diabetic vascular lesions are not entirely due to persistent hyperglycemia, regardless of the value of HbA1c, not all diabetic patients exhibit the same incidence and extent of vascular damage. Genetic, metabolic, and nutritional factors can exert a protective role. Moreover, some trials testing the effect of intensive treatment to get tight glycemic control failed to show a substantial reduction in cardiovascular outcomes.[57]

HBA1C AND MACROVASCULAR DIABETIC COMPLICATIONS

A graded relationship between HbA1c and the occurrence of coronary syndromes and mortality has been established since normal values of the variable.[58] There is also a relation with ischemic stroke but not with hemorrhagic cerebral events.[58,59] An important message is that hyperglycemia also has a pathogenic role in macrovascular diabetic lesions, dyslipidemia, and HBP, underlying the holistic approach in the diabetic patient, with the obligatory reduction of all risk factors.

CONCLUSIONS

Hba1c is a conjugated protein formed through the glycation process termed and recognized as a glycemic control marker in diabetic patients since the last century. The glycation should not be confused or used as a synonym for glycosylation. Hba1c is a remarkable clinical tool useful in the management of diabetes mellitus and the prediction of microvascular and macrovascular lesions. The clinician must know its value, limitations, and advantages to use it wisely in the initial diagnosis of DM and the follow-up of the disease as an excellent marker of long-term control and the prevention of vascular complications.

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