Lewicki Marcin, Chołuj Łukasz, Molendowska Marta, Czarnota Zuzanna, Mazur Rafał, Tyniec Mateusz, Smoleń Agata. Glycated haemoglobin - when can we trust it? Analysis of factors affecting the HbA1c level. Journal of Education, Health and Sport. 2018;8(12):944-954. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2621067
http://ojs.ukw.edu.pl/index.php/johs/article/view/6776

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper
Received: 03.11.2018. Revised: 10.12.2018. Accepted: 30.12.2018.

Glycated haemoglobin - when can we trust it? Analysis of factors affecting the HbA1c level

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ABSTRACT

Epidemiological data leaves no illusions – diabetes mellitus is a real epidemic of a non-communicable disease. Glycated haemoglobin was isolated with chromatography column as a separate subtype of haemoglobin as early as in 1958. An increase in its concentration in response to hyperglycaemia has already been recognized in 1969. Glycated hemoglobin (HbA1c) is a routinely used marker for long-term glycemic control in patients with diabetes. With HbA1C measurement we are able to retrospectively assess the average blood glucose levels. HbA1c correlates well with the risk of long-term diabetes complications. However, HbA1C levels may sometimes be misleading as a reliable measure of glycemic control. HbA1c values measurements are prone to diagnostic interference with many factors. The aim of this review was to present the numerous clinical scenarios in which the use of HbA1c levels alone for either diagnosis or as a marker of glycemic control, may lead to false assumptions.

Keywords: glycated haemoglobin, HbA1C, diabetes mellitus

Introduction

Diabetes is a complex disorder possessing a broad spectrum of serious metabolic disorders which are rooted in the persistent state of hyperglycaemia. The occurrence of this disorder is associated with abnormal effects or a defect in the secretion of insulin and is dependent on the type of diabetes. As a result, hyperglycaemia occurs, causing the dysfunction of many organs and systemic systems, and possibly even leading to their failure. The most common complications which affect blood vessels, the heart, kidneys as well as sight and the nervous system. Symptoms that may indicate the development of the disorder include: weight loss, polyuria, polydipsia, drowsiness and weakness, recurrent purulent skin lesions, as well as inflammation of the genitourinary organs [1]. The clinical symptoms manifested much quicker in type 1 diabetes mellitus. A more common form of the disorder is type 2 diabetes, which is characterised by an extended preclinical phase during which organ complications develop. The occurrence of slightly more intense symptoms in type 2 does not prompt the patient to seek medical attention. The presence of non-specific prodromal symptoms, or in many cases their complete absence, includes type 2 diabetes mellitus into a group of diseases with an epidemiology consistent with the so-called ‘’iceberg’’ model. In accordance to which, up to half of type 2 diabetes causes remains undiagnosed [2]. Epidemiological data leaves no illusions – This is a real non-communicable disease epidemic.

According to data from the WHO, 422 million adults suffered from diabetes during 2014, compared to 108 million during 1980. The percentage of people suffering from diabetes since 1980 has doubled from 4.7% to 8.5% in the global adult population. During 2012, diabetes was responsible for 1.5 million deaths. Furthermore, higher than the optimal blood glucose concentration associated with cardiovascular diseases resulted in 2.2 million deaths [3]. The Ministry of Health estimates that there are over 2 million people currently suffering from diabetes in Poland, of which about 25% are not aware of this [3]. According to the International Diabetes Federation (IDF), 415 million people suffered from the disorder during 2015. However, according to forecasts, this number may increase up to 642 million patients in 2040. In addition to the 415 million people with diabetes, another 318 million show carbohydrate disorders in the form of Impaired Glucose Tolerance (IGT), which constitutes a risk factor for the development of diabetes [4].

Historical outline and present diagnostic role

Glycated haemoglobin was isolated as a separate subtype of haemoglobin using a chromatography column as early as 1958 [5], 10 years later in 1968, Bookchin and Gallop, determined the structure of its molecule as being that of glycoprotein [6]. An increase in its concentration during the course of diabetes was already noted in 1969 [7]. The exact process of biochemical transformation leading to glycation was described by Bunn and his colleagues in 1975 [8]. Glycated haemoglobin (HbA1c) is formed as a result of the non-enzymatic

glycation of globin, being the main component of haemoglobin. This process occurs without the participation of specialised enzymes and high energy compounds resulting in the HbA1c levels being proportional to blood glucose. In the glycation process, where glycated haemoglobin is a constant and irreversible product, the amino group of the protein is combined with the straight-line aldehyde group. Several glycated fractions of A1 haemoglobin do exist, however, HbA1c accounts for 75-80% of the total glycated haemoglobin pool. It's formed as a result of the link between D-glucose and the N-terminal valine of the β chain. HbA1c, due to its stability and its good correlation with the blood glucose concentration, found its best use as a marker of glycaemic control in patients with diabetes. Due to the specificity of this transformation, determining the level of glycated haemoglobin is a useful method of measuring the average glycaemia during a 120 day period (i.e. the time interval corresponding to the average survival of the erythrocyte). Thus, it provides information on the effectiveness of the implemented treatment, as well as on the level of glycaemia diagnosed in persons with suspected diabetes. It should be noted that the largest share in the result, amounting to as much as 50% of its value, is attributed to glycaemia occurring within 30 days before the measurement, while the glycaemia occurring between 90-120 days before the measurement is characterised by a 10% share in the result [9]. Despite the irrefutable advantages, this method is not free from the following restrictions, which undoubtedly: have lack of a unified scale and the possibility of factors such as the presence of other forms of haemoglobin, being the result of certain toxins, drugs, vitamins or disorders affecting the average survival time of erythrocytes.

Currently, there are two leading methods for the standardisation of the HbA1C result which have been established by the National Glycohaemoglobin Standardisation Program (NGSP) and the International Federation of Clinical Chemistry (IFCC). Currently, the Polskie Towarzystwo Diabetologiczne (PTD) [Polish Diabetes Association] recommends using measurements in accordance with the certified methods of National Glycohaemoglobin Standardisation Program (NGSP). However, it also recommends that laboratories express HbA1c levels in SI units [mmol/mol], in accordance with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) system [1].

HbA1c has become an important marker used as a tool in many studies in which the intensity of hypoglycaemic treatment was compared with the risk of developing chronic vascular complications in diabetes. The results of tests such as ADVANCE (Action in Diabetes and Vascular disease: preterax and diamicron mr Controlled Evaluation), ACCORD (Action to Control Cardiovascular Risk in Diabetes), VADT (Veterans Affairs Diabetes Trial), STENO, STENO-2, UKPDS (UK Prospective Diabetes Study) caused major changes in the approach to diabetes treatment. Currently, when starting hypoglycaemic treatment, one should consider the individualisation of therapeutic methods for each patient. It has been observed that glycosylated haemoglobin> 7% correlates with increases in **TC, LDL-C, TG and LDL-C as well as disorders in HDL-C. Thus, HbA1C can be used as a biomarker for predicting dyslipidaemia in patients with type 2 diabetes. The relation between dyslipidaemia and the increased risk of cardiovascular events is widely known. It has been demonstrated**

that glycosylated haemoglobin may reduce cardiovascular risk up to 7%. It's anticipated that in the event of elevated HbA1c, a 0.2% reduction could reduce mortality by 10% [10]. Due to the fact that HbA1c is a very sensitive indicator that enables us to predict the risk of developing both microangiopathic as well as macroangiopathic complications, determining it should be a daily practice used not only by diabetologists and endocrinologists, but also by General Practitioners who consult patients with diabetes [1]. The most important benefit for the General Practitioners assessing the glycated haemoglobin level is undoubtedly the very rapid detection of incorrect glycaemic levels, which will signal the need for treatment by a diabetologist before there is a clinical manifestation in the form of complications. Unfortunately, there are many obstacles for assessing this and HbA1c has become a diagnostic tool used in the doctor's office. The primary obstacles come in the form of analysis costs, test cards and issues resulting in the standardisation of measurement methods. This is a challenge which must be resolved in the near future [11,12].

HbA1c in daily practice

Criteria for the diagnosis of diabetes according to PTD for 2019[1]:

Random blood glucose — measured in a blood sample collected at any time of the day, regardless of the timing of the last meal	≥ 200 mg/dL (≥ 11.1 mmol/L) A diabetes* (if symptoms of hyperglycemia are present, such as increased thirst, polyuria, fatigue)
Fasting blood glucose — measured in a blood sample collected 8–14 hours after the last meal	• 70–99 mg/dL (3.9–5.5 mmol/L) A normal glucose tolerance (NGT) • 100–125 mg/dL (5.6–6.9 mmol/L) A impaired fasting glucose (IFG) • ≥ 126 mg/dL (≥ 7.0 mmol/L) A diabetes*
Blood glucose at 120 minutes during an oral glucose tolerance test (OGTT) according to WHO	• < 140 mg/dL (7.8 mmol/L) A normal glucose tolerance (NGT) • 140–199 mg/dL (7.8–11.0 mmol/L) A impaired glucose tolerance (IGT) • ≥ 200 mg/dL (≥ 11.1 mmol/L) A diabetes*

*Diagnosis of diabetes requires one abnormal reading except for fasting blood glucose which requires two abnormal readings. A potential effect of factors not related to testing itself should be taken into account when measuring blood glucose (timing of the last meal, exercise, time of the day)

With one exception, the criteria recommended by the American Diabetes Association in the United States for diagnosing diabetes are consistent with the proposed Post transplantation diabetes (PTD). The American criteria allow the diagnosis of diabetes based on HbA1c level> 6.5% [13]. It is worth noting, that the United States was the first country in the world to introduce the determining glycosylated haemoglobin for the diagnosis of diabetes into the criteria [14]. Currently, determining the HbA1c level in accordance with the recommendations of the Polish Diabetes Association (PDA) is not one of the criteria for diagnosing diabetes. It is however recommended as a screening test [1]. Interestingly, the Polish Society of Cardiology, which is modelled of the −European Society of Cardiology (ESC), also recommends indicating the HbA1c level next to the Fasting Blood Glucose measurement level (FPG), in order to confirm or rule out the coexistence of diabetes [15]. However, according to current diagnostic criteria, HbA1c may only be used as a signal to implement further diagnostics and in itself is not sufficient to conclude a diagnosis.. Some restrictions in using the HbA1c measurement as the only test is less so than when compared to the Glucose Tolerance Test (GTT) and the detection of pre-diabetic states and type 2 diabetes, which may prevent the early diagnosis of such disorders [16,17].

Due to the numerous doubts which have accumulated around glycated haemoglobin over the past few years, it seems reasonable to present a clear picture of the most important factors which can result in a false picture of the average glycaemic values when determined using this method. Studies carried out by independent researchers indicate that A1C values in patients differ despite similar concentrations of fructosamine and blood glucose levels, which may indicate a probable individual variability in the level of glycated haemoglobin depending on factors previously unknown [18,19,20].

Factors affecting HbA1c concentration

- Alcohol

It is suspected that persons consuming alcohol may have false negative HbA1c results due to the reaction of aldehyde with amino acids of globin chains, resulting from the formation of acetaldehyde during its metabolism [21]. As presented by Ahmed, Ameena T et al, in adult patients with type 1 or type 2 diabetes, lower HbA1C values can be observed when consuming 2 drinks per day (One drink is approximately 10-15 g of ethanol) [22].

- Age

Studies have shown that there is a general increase in HbA1c levels with age. No pattern was observed which would reflect the rate and magnitude of such an increase dependent on the age group examined [23]. This is most likely due to the increase in the glycation rate relating to age, subject to the absence of simultaneous disturbances related to RBC (red blood cell count) or carbohydrate metabolism [24.25].

- Impact of race

A hypothesis exists which suggests HbA1c levels are relates to race. Studies comparing people with diabetes showed differences in the concentration of glycated haemoglobin in

relation to their ethnicity. Hispanic and African American people have an average of 0.5% higher HbA1c levels when compared to the Caucasian people. This is related not only to genetic factors but also to differences in lifestyle, access and quality of health care as well as socio-economic conditions [26,27,28].

- Iron levels

Researchers found that patients suffering from diabetes whose glucose levels remained at a similar level, showed higher levels of HbA1c related to iron deficiency. Additionally, iron replacement treatment results decreased HbA1c levels in both healthy and diabetic patients, which is why when interpreting HbA1c results one should consider if the amount of iron in the body is at the correct level [29]. The mechanism of such phenomenon lies in the fact that during iron deficiency the production of red blood cells decreases, however, in proportion to the decrease in erythrocyte production, the average age of red blood cells increases. One should consider that glycation of haemoglobin is an irreversible process. Consequently as the cell's age increases, so the level of HbA1c increases [30].

- Pregnancy

In pregnant women as well as those suffering from diabetes, a correlation was observed during late pregnancy between elevated HbA1c levels and iron deficiency. It was simultaneously noted that the albumin concentration of glycated serum in pregnant women with diabetes is a more reliable indicator of glycaemic levels as it does not depend on the iron metabolism [31].

- Chronic liver disease

In the event of developing liver cirrhosis, red blood cells have a shortened life span which consequently lowers the HbA1C level. However, hyperbilirubinemia on its own, is capable of diagnostic interferrence and false elevation of HbA1C [32]. Due to chronic liver disease, plasma albumin levels are reduced. For this reason a high level of glycated albumin is observed resulting from a reduced turnover of proteins and its accumulation in the body. Therefore, in the event of developing cirrhosis, the determining HbA1c and glycated albumin level produces unrealistic results [33].

- Different variants of haemoglobin

The HbA1c test is not a reliable parameter in people with haemoglobinopathy. Mutations of globin genes cause amino acid changes in the globin protein, which contributes to the occurrence of different variants of haemoglobin. Approximately 7% of the world population may have a hemoglobin variant [34]. Haemoglobins S and E are commonly found in people of African, Mediterranean or South Asian origins. Haemoglobinopathy results in false increase of glycated haemoglobin, which can be misinterpreted [35][36]. Hemoglobin variants cause falsely low HbA1c due to hemolysis or a shortened erythrocyte survival. As a matter of fact any condition that decreases red cell survival rate or requires blood transfusions will

falsely lower HbA1c levels. A history of a recent trteatment with blood transfusion renders HbA1c unusable [37].

- Chronic kidney disease (CKD)

In chronic kidney disease, various factors may influence the HbA1c test results. In electrophoretic studies, glycated haemoglobin cannot be distinguished from haemoglobin and carbamate, which is a form of high urea nitrogen concentration. The 1.26% increase in glycated haemoglobin is caused by an increase in the concentration of urea nitrogen in the blood from 3.57-24.9 mol/L. This however is a much smaller increase than in the comparable increase of glucose concentration. [36]. The treatment of anaemia in CKD by means of ESA (erythropoiesis stimulating agents*)* results in a 1.2% reduction in HbA1c in non-diabetic patients with CKD and 0.7% in patients with diabetes and chronic renal disorder. The difference in the reduction of HbA1c is most likely due to variations between research methods. Nakao et al. described the decrease in HbA1c in subjects who received iron in addition to ESA during treatment, whereas Ng, Jen M et al. described the reduction only with ESA treatment [38][39].

- The influence of vitamins C and E

Studies have shown that both vitamin E and C can inhibit the glycation process of proteins, including globin, consequently leading to conditions of hypervitaminosis C and E, as well as excessive supplementation which can lead to a falsely underestimated result [40] [41].

- Impaired glycation

Haemoglobin molecule can present an altered affinity for glucose. In a situation like that, we would observe a discrepancy between patient's HbA1C level (usually low) comparaed with elevated fasting and postprandial blood glucose levels. The glycation of HbA1C predominantly involves valine (position 1) of the N-terminal β-chain. However, other aminoacids in the haemoglobin molecule can also bind glucose, the second most common site being lysine, at position 16 on the α chain. Genetic traits like HbI Philadelphia, which results from AAG → GAG transition on codon 16 of the α2 gene leading to a substitution of lysine by a glutaminic acid, can impaire glycation rate. It is possible to assess glycation separately at both sites of Hb glycation, yet this method is not available for routine testing in every day practice [42].

Conclusions:

The level of glycated haemoglobin is a very common determining parameter used during daily medical practice. This study presents many advantages of this marker. Thanks to it, we are able to retrospectively assess the glycaemic control, as a relationship exists between the concentration of glycolhaemoglobin and the average glycaemia, which has an impact on the formation of chronic vascular complications. Currently, it is suggested not only by Diabetological but also other Societies, that determining HbA1c at a doctor's office has become an everyday practice. However, while citing the above clinical conditions, we should bear in mind that this is not an ideal parameter for every patient. When answering the question posed in the title, we must conclude that we cannot always trust glycated haemoglobin. Its value depends on many factors, such as iron parameters, erythrocyte survival time, hemoglobinopathies, age or other comorbidities. Interpretation of HbA1c is especially difficult in patients with renal function impairment. It should be remembered that signs should be interpreted individually when considering a clinical condition. When a patient has a condition in which HbA1C reults are misleading, the glycameic control should be evaluated based on appropriate blood glucose self-monitoring, performed with adequate frequency.

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