Interpretation of HbA1c lies at the intersection of analytical methodology, clinical biochemistry and hematology (Review)

ZIXIN CHEN[1], LIMEI SHAO[1], MINGFENG JIANG[2], XUEJIAO BA[1], BINGJIE MA[1] and TAO ZHOU[1]

[1]Department of Clinical Laboratory, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650500; [2]Institute of Microbiological Detection and Analyses, Sichuan Center for Disease Control and Prevention, Chengdu, Sichuan, 610044, P.R. China

Abstract. Over past few decades, diabetes has become widespread on a global scale. Hemoglobin A1c (HbA1c) assessment is crucial for diabetes care, since it allows for the monitoring of an individual's level of glycemic control over the course of 2 to 3 months and risk assessment to determine any possible complications. Numerous methods, including cation-exchange chromatography, electrophoresis, immunoassays and affinity chromatography, can be used to determine the HbA1c level. Each method has its limitations, however. The amount of HbA1c in patient samples is not only dependent on blood glucose levels, but is also strongly influenced by changes in red blood cell lifespan and globin chain structure. Consequently, hematological, clinical biochemistry and analytical methods all intertwine when interpreting HbA1c. There are numerous reports on the interactions of HbA1c with inherited and acquired diseases. Some of these impacts are inconsistent and difficult to explain. The present review article aimed to summarize and classify these effects and evaluate their clinical relevance. The findings discussed herein may serve as a reminder that clinical HbA1c values need to be analyzed with caution.

Contents

1. Introduction
2. Data collection methods
3. Biochemistry of hemoglobin A1c
4. HbA1c detection methods
5. Interfering factors
6. Summary
7. Conclusions and future perspectives

Correspondence to: Professor Tao Zhou, Department of Clinical Laboratory, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Kunming, Yunnan 650500, P.R. China
E-mail: 18468035043@139.com

1. Introduction

Poor long-term glycemic control is a hallmark of diabetes mellitus, which increases the risk of complications, including microvascular complications, such as nerve damage, diabetic nephropathy and renal failure, as well as macrovascular complications, such as coronary heart disease, stroke and peripheral arterial disease (1). Glycated hemoglobin (Hb) A1c (HbA1c) is the product of the non-enzymatic interaction between the N-terminal valine of the Hb β chain and glucose, and it can be used to assess glycemic control over a period of 2-3 months. Furthermore, since its successful standardization, HbA1c is currently used to monitor long-term glycemic control, make treatment decisions and evaluate the risk of developing complications (2,3). Since 2010, HbA1c has been used in the diagnosis of diabetes. Therefore, a glycated hemoglobin value of >6.5% (48 mmol/mol), is indicative of diabetes (4). HbA1c levels between 5.7-6.4% (39-46 mmol/mol) indicate that an individual is at a high risk of developing diabetes (4). The reference range for HbA1c is 4-6% (20-42 mmol/mol) (2). The National Glycohemoglobin Standardization Program (NGSP) units or mmol/mol are different ways with which to express HbA1c [International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units]. The formula used to express the association between % NGSP HbA1c and IFCC mmol/mol is the following: NGSP=(0.09148 IFCC) + 2.152 (5). However, HbA1c values may be unreliable in particular circumstances. Therefore, the present review article aimed to summarize and discuss the common obstacles encountered in determining HbA1c values, as well as their consequences.

2. Data collection methods

For the purposes of the present review article, a search of the literature was performed using the PubMed Embase, Web of Science, Cochrane Library and CNKI databases using the following search terms: HbA1c methods; HbA1c interferences; HbA1c interpretation; hemoglobin A, glycosylated; glycated hemoglobin; hemoglobin variant; fetal hemoglobin;

Hb derivatives; carbamylated hemoglobin; acetylated hemoglobin; aspirin; vitamins C and E; dapsone; sulfasalazine; HIV; iron deficiency anemia. Language was limited primarily to English.

3. Biochemistry of hemoglobin A1c

Hb is a tetramer composed of four (two pairs) globin peptide chains. The common globin peptide chains are termed α, β, δ and γ. Comprising two α and two β (α2β2) chains, HbA is the most abundant Hb in adults, accounting for 95-98% of all Hb types. HbF (α2γ2), which is the most dominant form of Hb found in fetuses, and HbA2 (α2δ2), accounting for ~2% of total Hb in adults, are the other forms of normal Hb. In the presence of glucose, amino acids interact with Hb, which in turn undergoes a non-enzymatic glycation reaction, a process known as 'glycation'. There are two steps in this process. The first step is the reaction of the aldehyde group of glucose with the NH_2 group of the amino acid to form a Schiff base or aldodiamine, also known as 'labile HbA1c' or LA1c. In the second step, LA1c undergoes the Amadori rearrangement to form 1-amino-1-deoxyfructose, which contains a more stable and irreversible ketoamine bond, namely HbA1c. In HbA1c, valine at the N-terminus of the β chain of Hb is linked to amino-1-deoxyfructose via a ketoamine linkage. However, the glycation process can also occur between the N-terminal valine of the α-polypeptide chain and the ε-amino group of the lysine side chain on the globin peptide chain (6-8). It is worth noting that HbA1c does not contain LA1c or α-globin subunits glycated on the N-terminal valine and α- or β-globin subunits glycated on lysine side chains. HbA0 refers to non-glycated Hb or glycated at positions other than the N-terminus of the β chain. In addition, HbA is also glycated to form HbA1a1, containing fructose-1,6-bisphosphate, HbA1a2 containing glucose-6-phosphate and HbA1b containing a pyruvate at the N-terminal valine (9). Therefore, the concepts of 'glycated Hb' and 'HbA1c' need to be clarified.

4. HbA1c detection methods

The most common method for detecting HbA1c levels is cation/ion-exchange chromatography (IEC). During this method, Hb molecules are separated based on charge differences. Therefore, each positively charged ion in the sample interacts with a negatively charged column, where positively charged Hb molecules travel slower than negatively charged ones. Eventually, each component of HbA is eluted at different time points due to charge differences. Glycated Hb gains an extra negative charge when glucose attaches to the N-terminal valine of the chain, causing it to be accelerated in the cation exchange resin and to be eluted earlier. To measure the concentration of Hb, the area beneath each peak of the chromatogram is calculated and compared with the standardized chromatogram using a spectrometer. Currently, IEC systems can already generate high-resolution separation curves that can distinguish HbA1c from LA1c and other common variants, such as Hb S, C and D (10,11).

The boronate affinity chromatography (BAC) method is often used as a reference method in several studies, since it can reveal the presence of Hb variants with minimal analytical interference. BAC is based on the ability of the *cis*-diol group of glycosylated Hb to interact and bind with m-aminophenylboronic acid immobilized on the carrier, in an alkaline environment (pH >8.0). While other non-glycated Hb species pass through, the trapped glycated Hb molecules are released from the filter using an acid reagent. Therefore, Hb can be divided into two parts, namely the glycated and non-glycated forms. Finally, the total glycated Hb is converted to %HbA1c according to an empirical formula. However, as BAC only recognizes the presence of total glycated Hb, it is unable to detect the existence of hemoglobin variations (12).

During capillary electrophoresis (CE), which is used to separate proteins, different protein molecules are encouraged to move from the anode to the cathode through the capillaries by an electric field produced using a high-voltage power source. Using CE, Hb variants are divided based on their rate of diffusion, which is defined by their charge and mass. Therefore, positively charged substances migrate through the capillaries more rapidly than neutral and negatively charged ones. The disadvantage of this method is that for high efficiency operation, parallel capillaries are required. Furthermore, the consistency of the results across all capillaries remains a challenge (12,13).

Immunoassay is an immunoturbidimetric inhibitory assay that uses antibodies precisely binding to HbA1c by recognizing the N-terminal glycosylated amino acids. Polyhapten lectins, synthetic molecules with different HbA1 epitopes, agglutinate with anti-HbA1c antibodies to create insoluble antibody-polyhapten complexes. Therefore, a considerable light scattering is developed via the antibody-polyhapten complexes in the absence of HbA1c. A soluble antigen-antibody combination is generated when HbA1c is combined with its corresponding anti-HbA1c antibody, thus reducing light scattering. Increased %HbA1c indicates attenuated agglutination reactions. The HbA1c value is then calculated by dividing the amount of total Hb. In addition, chemical spectroscopic analysis is used to determine the quantity of total Hb. However, the aforementioned chemical procedures, immunoassay and enzymatic analysis, require two independent tests, namely HbA1c and total Hb assays, which may negatively affect the analytical quality (11,12).

The enzymatic method is based on the protease-mediated release of the N-terminal glycosylated valine of the HbA1c molecule from the blood sample and red blood cell (RBC) lysate of the patient. Glycosylated valine is oxidized by fructosyl valine oxidases to generate hydrogen peroxide, which is in turn used to quantify HbA1c levels. Therefore, the total Hb concentration is simultaneously determined using an optical method. Hb variations have no effect on the enzymatic approach in terms of analysis (12,14).

5. Interfering factors

HbA1c assays are mainly affected by three factors: i) Methodological-specific interference, commonly associated with the effect of several Hb variants, HbF and Hb derivatives, on the detection method (15-19); ii) biochemical effects: For example, an inconsistent glycation rate of Hb variants and HbA can lead to biased results, particularly when affinity chromatography is used (20); and iii) abnormal results due
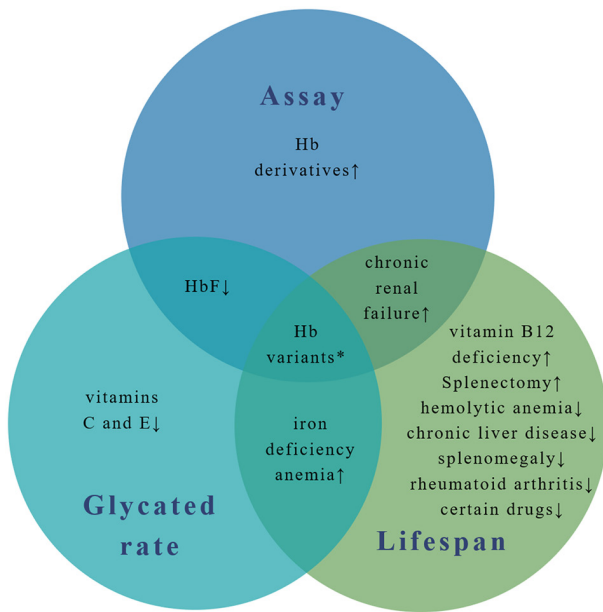
Figure 1. Factors influencing A1c. Downward arrows (↓) indicate a decreased HbA1c value; upward arrows (↑) indicate an increased HbA1c value; the asterisk (*) indicates variable changes in the HbA1c value. HbA1c, hemoglobin A1c.

to changes in the life cycle of RBCs. For instance, hemolytic anemia can result in a reduced RBC lifespan, while iron deficiency anemia can cause the opposite effect (21-23). Such factors are summarized in Fig. 1.

Hb variants. Hb variants are a group of prevailing inherited genetic defects caused by point mutations in the globin gene, eventually resulting in amino acid substitution (24). Previous studies have demonstrated that Hb variations can result in HbA1c values that do not correspond to blood glucose levels in the same patient, while the degree of interference is dependent on the method used and the specificity of the variation (15-17,25). Therefore, for each variant, this interference can be divided into method-specific, where some, but not all HbA1c determination methods are affected and variant-specific, where HbA1c levels are affected by an altered erythrocyte lifespan and glycation rate.

Method-specific interference of Hb variant. The charge and mass of Hb can change when an amino acid at a particular location on the globin peptide chain is altered. Therefore, detection methods based on the physical properties of Hb, such as IEC and CE, are vulnerable to interference, since variants interfere with the elution of the peak of interest, thus resulting in false to glycated Hb values and even invalid HbA1c measurements. For the IEC method, the %A1c is measured by excluding the A1c peak area, divided by the area sum of A1a, A1b, LA1c, A1c, A0 and HbX (both HbX1c and unmodified HbX) from the calculation. The effect of Hb variants on IEC is mainly due to the inability of IEC to completely separate the HbA from HbA1c variants. Therefore, when the peak of HbA1c cannot be completely separated from that of HbX, a proportion of HbX value is calculated into that of HbA1c, thus resulting in falsely elevated HbA1c values (Fig. 2C). Conversely, when HbX is calculated into the denominator of the formula, a spuriously

reduced HbA1c value is obtained (Fig. 2B). However, the IEC-generated interference can vary in different systems and is mainly dependent on the position of the elution peaks of HbX, HbA, HbX1c and HbA1c, and whether or not a clear separation occurs. IEC, the most commonly used method for HbA1c detection, is also susceptible to interference from Hb variants. From another perspective, IEC exhibits more advantages compared with other methods, since it is easier to identify abnormal Hb by examining the chromatogram. Therefore, it is of utmost importance for the laboratory to routinely inspect the chromatogram prior to issuing the report. In terms of CE, the reported HbA1c is derived from the ratio (A1c)/(A0+A1c), where A1c indicates HbA glycated at the N-terminal valine of the β chain and A0 the not glycated one. Consistent with the IEC method, any mutations that can cause changes in charge and mass, can potentially co-migrate with A1c or A0, thus resulting in erroneous results or even invalid HbA1c measurements. However, CE runs considerably longer and is more capable of distinguishing between HbX and HbA, as well as between HbX1c and HbA1c, compared with IEC, when dealing with the majority of variants. Yun *et al* (16) suggested that the changes in the glycosylation rate can be estimated by comparing the results of the CE method with those obtained using immunoassay or BAC. Since immunoassays rely on antibodies that specifically recognize and bind to the first 4-10 amino acids of the N-terminal of the β chain, the use of this technique can be limited when bases at this site are mutated. However, the main disadvantage of the immunoassay is its inability to distinguish between HbA and HbX (26). By using an immunoassay, %HbA1c is calculated by dividing A1c + X1c by total HbA + HbX. Therefore, when HbA is absent or erythrocyte biology is altered, using an immunoassay may lead to errors in clinically reported HbA1 levels. This issue is discussed in further detail below. Ideally, repeated analyses using methods based on different analytical principles need to be performed, since the effect of a particular Hb variation on HbA1c readings could be associated with the method sensitivity. The benefits and challenges of each method are summarized in Table I.

Variant-specific erythrocyte lifespan changes. Interpretation of HbA1c values in terms of glycemic control is affected by a combination of factors. For example, a HbA1c value of 7%, generally corresponds to an average plasma glucose value of 154 mg/dl in the majority of individuals (27). However, for individuals with a shorter RBC lifespan or higher glycation rates, a HbA1c value of 7% may be associated with different average glucose levels. A normal RBC has a lifespan of ~100-115 days on average, although the range is much wider, ~70-140 days (28). It has been reported that the levels of A1c are most commonly affected by the levels of blood glucose over the past 30 days, accounting for ~50% of the total A1c levels. However, only 10% of A1c levels are influenced by blood glucose levels from the past 90-120 days (29). When the glycation phase of RBCs changes, and more specifically their lifespan, HbA1c can no longer accurately represent glycemic control. HbS (β6Glu>Val) and HbC (β6 Glu>Lys) are the most common Hb variants (30,31). It has been demonstrated that ~75% of patients with sickle cell disease are found in Sub-Saharan Africa (32). Additionally, significant prevalence rates have been also recorded in the Middle East, India and
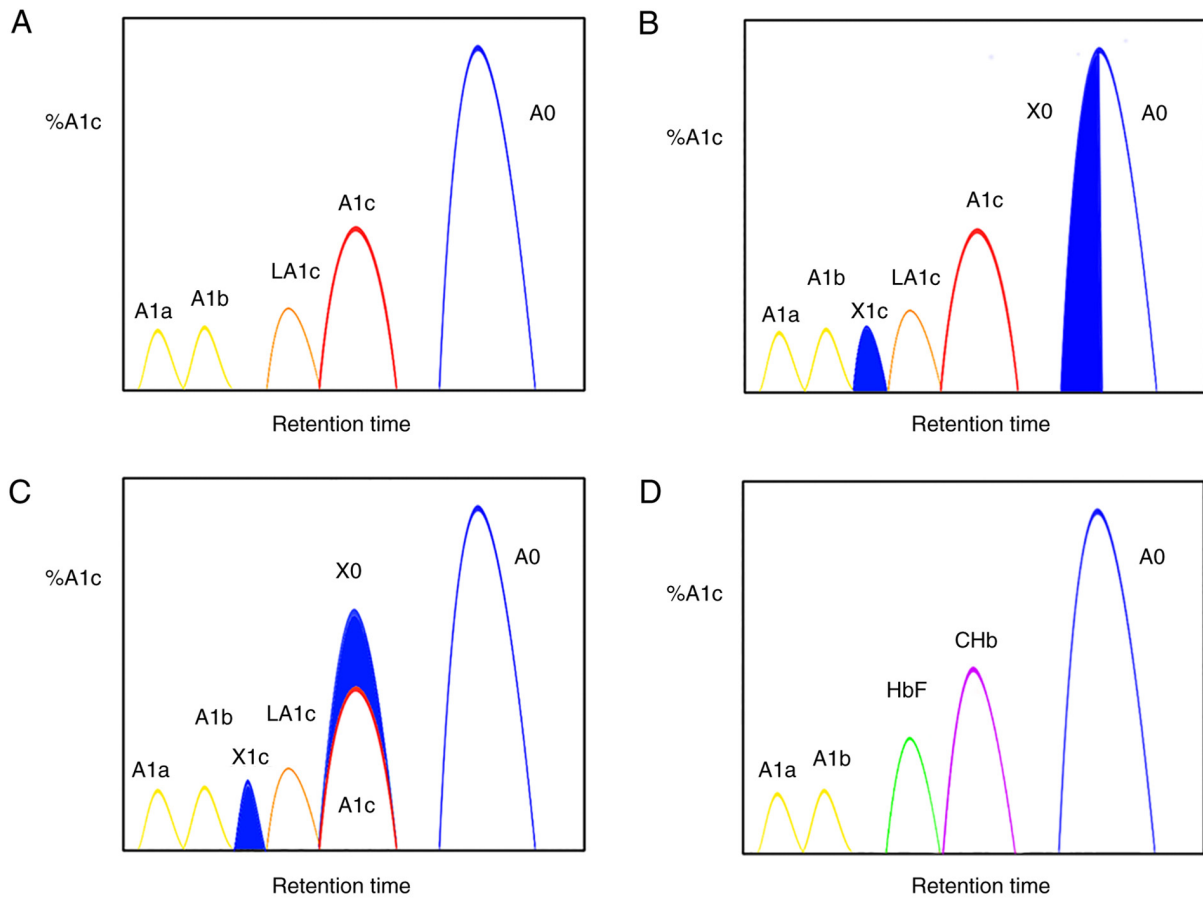
Figure 2. Schematic representation of cation-exchange chromatography. (A) Normal chromatogram. (B and C) The majority of the systems fail to separate Hb A from HbX (location of HbX1c only indicates that HbX1 is separated from HbA1c, it can also be in other locations). (D) Potential overlap and interference from elevated HbF and Hb derivatives. Hb, hemoglobin.

the Mediterranean area (33). In West Africa, the prevalence of HbC has reached 40-50%, while that in Benin, the United States and North Africa is estimated to 20, 3 and 1-10%, respectively (31). Since heterozygous forms of both variants do not cause hemolytic disease, they cannot, therefore, affect glycosylation. Sickle cell disease, and more particularly its homozygous clinically severe condition (HbSS), where the lifetime of RBCs is decreased to <20 days, is accompanied by severe hemolysis (12). Therefore, HbA1c values in those patients need to be interpreted with caution, taking into consideration factors, such as anemia, an enhanced RBC turnover, increased blood transfusion needs and increased HbF levels, which may all have a negative impact on HbA1c as a long-term glycemic control indicator. Additionally, the heterozygous type of the disease, HbSC, exhibits the same confounding issues as HbSS when it comes to determining HbA1c values. However, HbSC causes less severe anemia compared with sickle cell disease. In a large cohort study published by Lacy *et al* (34) in 2017, African-Americans with sickle trait had a decrease of 0.3% in HbA1c values compared to those without the sickle trait. In addition, patients with the sickle trait had a decrease of 0.29% in HbA1c levels at the same fasting blood glucose levels.

Variant-specific glycation rate alterations. The biological question is whether the glycation rates of HbA and Hb variations are equivalent. When they are not equal, the results of the method used to measure total glycated Hb (affinity chromatography) may be biased. Therefore, the interpretation of glycemic control based on the glycated Hb levels results may be incorrect. Although none of the first codon variants in the β-globin gene can cause any major clinical condition, such mutations are of interest due to their potential interference with co-translational modifications, such as acetylation at the same site during β-globin synthesis. The type of N-terminal amino acid can determine the degree of acetylation. Therefore, valine can substantially inhibit this process, resulting in the slight acetylation of α- and β-globin. However, it has been reported that the N-terminal glycine of γ-globin is less inhibitory, thus leading to ~15% acetylation (35). A previous study demonstrated that in Hb Raleigh (β1Val>Ala), the N-terminal amino acid of its β chain could be substituted to produce acetylated alanine, thus producing a large amount of acetylated Hb, which could not be glycosylated normally (36). It was hypothesized that the glycation process occurred close to the N-terminal valine and the 140th amino acid in the β chain (37). Mutants located near valine at the N-terminus of the β chain could cause changes in the glycation rate, such as reduced Hb Görwihl (β5Pro→Ala) levels, thus suggesting attenuated glycation reaction (38). Hb Himeji (β140Ala→Asp), a rare variant occurring on the β chain, is characterized by an enhanced glycation (37). In heterozygous carriers, HbA1c values determined by immunoassay or BAC have been found to be significantly higher compared with those determined

Table I. The advantages and challenges for each Hb determination method.

Method	Principle	Advantages	Challenges
IE-HPLC	Separates Hb species based on charge	Ability to detect the most Hb variants	Susceptible to interference from Hb variants, Hb derivatives and HbF
Boronate affinity	Glycohemoglobin binds affinity resin while non-glycated hemoglobin pass through the column	Strong anti-interference ability from Hb variant and Hb adducts	Interfered by rare Hb variants; unable to detect Hb variants; measures total glycated Hb; HbF at higher levels
Capillary electrophoresis	Separates Hb species based on charge and mass	High chromatographic resolution and resulting ability to detect many Hb variants	Throughput; consistency of results across all capillaries
Immunoassay	Uses an antibody targeted against the glycated N-terminus of the β chain	No analytical interference from the most common Hb variants	Susceptible to interference from rare Hb variants; unable to detect Hb variants; HbF at higher levels; two independent tests may affect analytical quality
Enzymatic	Uses an enzyme that specifically cleaves the N-terminal valine	No analytical interference from the Hb variants	Unable to detect Hb variants; two independent tests may affect analytical quality

Hb, hemoglobin; IE-HPLC, ion exchange-high-performance liquid chromatography.

using cation exchange chromatography (37). Mutations in this region can either increase or decrease glycosylation. For example, a previous study demonstrated that individuals with Hb Sagami (β139Asn→Lys) exhibited low HbA1c levels, as assessed using immunoassay, thus indicating a reduction in the glycation response (39). Glycosylation rates can even have an effect on HbA1c measurements of more common variants, such as those associated with sickle traits. Therefore, the glycosylation rate of $β^S$ appears to be higher than that of $β^A$. However, Kabytaev et al (40) concluded that the clinical interpretation was relatively unaffected by the tiny net difference between HbAS (sickle phenotype) and uncharacterized total glycosylation. A summary of the altered glycosylation rates presented in mutants located in the first 10 amino acids of the β chain and at amino acid positions 139-140 is presented in Table II (41-44).

HbF. Elevated HbF can cause related problems. Certain thalassemias and genetic persistence of fetal Hb (HPFH) can lead to elevated HbF. Increased HbF levels can be also caused by hydroxyurea, a medication commonly used to treat sickle cell disease and several hematological malignancies (45). In the IEC measurement method, the HbF and A1c peaks are eluted adjacently. Therefore, high HbF may overlap and distort the shape of the A1c peak, thus resulting in falsely high values (Fig. 2D). Elevated HbF can also cause other effects in immunoassay and BAC (10,18). This interference could be caused by the slower rate of glycation compared with that of HbA. HbF lacks the β chain and encompasses glycine instead of valine at the N-terminus of the γ chain. HbF can only be glycosylated on the lysine residue at the N-terminus of α chain. A previous study showed that the rate of glycosylation at the N-terminus of the α chain was indeed 8-10 times lower compared with that of the β chain's N-terminus (6). Therefore, for the boronate affinity assay, the glycation fraction in individuals with elevated HbF levels would be lower compared with those without increased HbF value due to the lower degree of glycation of HbF. During immunoassay, HbA1c antibodies cannot bind with the glycated portion of HbF and, therefore, total Hb measurements also include HbF value, eventually leading to a significant reduction in the measured HbA1c. From a clinical perspective, in patients with HbF levels of <20%, the use of immunoassays or BAC could reduce HbA1c levels by 1-2% (18). The Diabetes Control and Complications Trial clearly showed that a 1% reduction in HbA1c levels was equivalent to a reduction in the risk of diabetes complications by approximately 30% (2). A spurious reduction of 1-2% in HbA1c value could result to undertreatment of hyperglycemia, which in turn could lead to a significantly increased risk of complications.

Hb derivatives. When separation techniques based on charge differences are utilized, HbA1c measurements may also be affected by the chemical changes of Hb, which may physically and chemically imitate HbA1c, thus leading to an incorrect assessment of HbA1c. Carbamylated Hb is more common in uremic patients and is the most common derivative (46). This is due to the decomposition of urea nitrogen into ammonia and cyanate in the body. In turn, cyanate is protonated to form isocyanic acid, which combines with the α and ε amino groups of proteins to form carbamoyl moieties (46,47). Valine at the N-terminus of the Hb β chain reacts specifically with

Table II. Hb mutations relative to the altered glycosylation rates for the first 10 amino acids of the β chain and at amino acid positions 139-140.

Name	Mutation	Clinical significance	Glycation rate
Hb Niigata	β1Val>Leu	Clinically silent	↓
Hb South Florida	β1Val>Met	Heterozygote clinically silent	↓
Hb Raleigh	β1Val>Ala	Heterozygote clinically silent	↓
Hb Görwihl	β5Pro>Ala	Heterozygote clinically silent	↓
Hb Tyne	β5Pro>Ser	Heterozygote clinically silent	↓
Hb Aix-les-Bains	β5Pro>Leu	Heterozygote clinically silent	↓
Hb Sagami	β139Asn>Ly	produced β-thalassemia carrier phenotype when combined with β⁺-thalassemia allele	↓
Hb Himeji	β140Ala>Asp	Heterozygote clinically silent	↑

Downward arrows (↓) indicate a decreased glycation rate; upward arrows (↑) indicate an increased glycation rate. Hb, hemoglobin.

isocyanic acid to form stable carbamyl-Hb (CHb). The isoelectric points of CHb and HbA1c are similar. Therefore, the peak times of both CHb and HbA1c are close in the IEC system based on the detection principle of Hb species with different charges. When CHb reaches a certain concentration, the peak time of LA1c/CHb is delayed or the peak shape increases, thus resulting in the overlap of the LA1c/CHb peak with that of HbA1c. The overlap increases with the enhanced CHb concentration (Fig. 2D). If the peak is large, depending on the system, it can lead to biased results in the HbA1c levels (either increase or decrease) (19,48). *In vitro*, the carbamylation of Hb at concentrations up to 5.4% can result in erroneous readings in glycated Hb levels, when different cation-exchange techniques are used (19). However, studies evaluating the *in vivo* effects of carbamoyl Hb have revealed several differences, ranging from insignificant to significant (49-51). The studies by Little *et al* (50) and Dolscheid-Pommerich *et al* (51) demonstrated that the effects of CHb were statistically, yet not clinically significant. However, the assessment of HbA1c in this population should be always performed using the same measuring technique to ensure longitudinal comparability and produce comparable readings. Furthermore, the association between chronic kidney disease (CKD) and A1c is complex. Therefore, previous studies have demonstrated that patients with CKD exhibit lower levels of erythropoietin, possible increased glycation and enhanced carbamylated Hb levels, while dialysis in such patients may shorten the RBC lifespan and decrease A1c levels (50-53). The study by Little *et al* (50), comparing the levels of glycated albumin (GA) with HbA1c in patients with chronic renal failure, demonstrated that the levels of HbA1c in such patients were decreased by ~1.5% compared with those of GA. Additionally, the results of a clinical trial revealed that the treatment of 15 individuals with type 2 diabetes mellitus (T2DM) and CKD (3B/4) with erythropoietin resulted in a clinically meaningful decrease of ~0.7% in HbA1C readings (52). Therefore, HbA1c testing should be interpreted cautiously in patients with renal failure. Long-term aspirin use can also induce the acetylation of Hb, thus resulting in falsely elevated levels of HbA1c, due to its interference with some of the assays used (54,55). More specifically, aspirin promotes the acetylation of Hb to change its surface charge. In turn, this acetylated product can co-migrate with A1c, eventually leading to a falsely elevated HbA1c value (56). Although the exposure of normal Hb to aspirin *in vitro* results in falsely high acetylated Hb values, as verified using different cation-exchange methods, the results of *in vivo* studies have contradicted this finding (56,57). Camargo *et al* (56) demonstrated that treatment with lower doses of aspirin (200 mg/day) for 4 months did not result in a clinically relevant increase in HbA1c levels. In clinical practice, the effect of aspirin on HbA1c levels could not be palpable until long-term and high-dose therapy (55). However, it has been reported that the BAC method is not affected by carbamylated and acetylated Hb (50,57).

Drugs. High doses of vitamins C and E have also been shown to be associated with decreased A1c levels mediated by the inhibition of Hb glycosylation. Vitamin C can form ionic bonds with several biomolecules. In turn, ionic interactions of ascorbate and its free radical with proteins can affect the reactivity of molecular complexes via altering the local redox potentials and charge transfer reactions. It has been suggested that the potential for non-enzymatic glycosylation is reduced when vitamin C reacts directly with the glucose-binding site (lysine residue) (58). A previous study revealed that vitamin E supplements could prevent the glycosylation of Hb by blocking glycation in the early stages of the Maillard reaction or by partially preventing the development of advanced glycosylation end-products (59). However, the inhibition of Hb glycosylation by vitamins C and E is clinically controversial. An *in vitro* study demonstrated that vitamins C and E could prevent the development of protein glycosylation (60). However, the outcomes of *in vivo* experiments are contradictory with the aforementioned finding. While Ceriello *et al* (61) demonstrated the inverse association between vitamin E consumption and glycated Hb levels, a later meta-analysis (59) was unable to reveal a discernible difference. However, further subgroup analysis revealed that following vitamin E supplementation, HbA1c was noticeably reduced in patients with T2DM in the group with low vitamin E levels. However, the small datasets used in this subgroup analysis, suggested that further research should be conducted to support this conclusion (59). Likewise, conflicting information on vitamin C intake and glycated Hb

levels can be found in the literature (56,62,63). Additionally, other drugs, such as dapsone, sulfasalazine, antiretrovirals and ribavirin can increase the hemolysis rate, thus reducing glycated Hb levels (64-68). In a retrospective review of 49 individuals with T2DM and Hansen's illness, 35 patients (71%) had HbA1c readings lower than the mean blood glucose levels (65). At the same fasting blood glucose concentration, the HbA1c discordant group had a significantly lower hemoglobin A1c value (mean HbA1c, 4.4±1.8%) compared with the HbA1c consistent group (mean HbA1c, 7.9±2.1%). During the first 3 months of dapsone therapy, the HbA1c levels decreased considerably (65).

Illness-related factors. Particular pathologies can alter the lifespan of RBCs, thus affecting the HbA1c levels. The average lifetime of RBC increases when erythropoiesis is suppressed, due to the lack of iron and vitamin B12, leading to high HbA1c levels (21-23,69). Kim *et al* (70) demonstrated that women with an iron deficiency without anemia (n=1,150) exhibited a small increase in HbA1c levels (<5.5% to ≥5.5%), independent of fasting glucose levels. In another study, comparing the use of both HbA1c and fasting blood glucose as diagnostic criteria, Attard *et al* (71) demonstrated that males with an iron deficiency alone or with iron deficiency anemia (IDA) exhibited a greater relative risk of developing pre-diabetes. However, other studies have yielded inconclusive results. According to a meta-analysis, IDA and iron deficiency had no effect on the HbA1c levels (72). In addition, patients with IDA have been found to have a higher glycation rate, which may be due to the higher malondialdehyde levels, a lipid peroxidation metabolite, observed in this population, thus enhancing Hb glycation (73,74). Additionally, it has been reported that splenectomy can promote RBC survival, thus enhancing glycated Hb levels (75). Conversely, a decrease in the mean age of RBCs can reduce glycated Hb levels. However, in the absence of fibrosis and splenomegaly, this can be observed in chronic liver disease, although the cause remains unknown (76). Furthermore, splenomegaly and rheumatoid arthritis can also increase the rate of hemolysis, thus resulting in a decrease in glycated Hb value (77).

Age and race. It has been reported that HbA1c levels can be affected by age and race. Previous studies have indicated that after the age of 30, the glycated Hb value can be increased by ~0.1% every 10 years (78,79). The effects of race on HbA1c values are controversial, however. Accumulating evidence has suggested that differences in HbA1c levels can be observed among different races. Therefore, several studies have demonstrated that African Americans and Hispanics have higher glycated Hb levels compared with Caucasians at the same blood glucose levels (79-81). Additionally, Selvin *et al* (82) demonstrated that the mean HbA1c value of African Americans was 0.4% higher compared with that of non-Hispanic whites. However, race did not alter the association between HbA1c concentrations and adverse cardiovascular outcomes or mortality (82).

6. Overview

In summary, various factors influence the determination of HbA1c values. These are discussed below and are summarized in Table III.

i) The effect of rare Hb variations, often observed in various national populations, on HbA1c measurement varies, as the prevalence and types of Hb variants also vary across different nations. A study on 114 patients with rare Hb variants, common in the Korean population revealed that the proportion of 'unacceptable' HbA1c results (relative bias, >±7%) in samples significantly varied depending on the assay used. More specifically, the rates recorded were 16% for CE (17/109 patients), 7% for immunoassay (8/114 patients), 51% for IEC1 (58/114 patients), 80% for IEC2 (108/114 patients) and 89% for IEC3 (66/74 patients). CE and IEC3 assays yielded no results, in 5 and 40 samples with Hb variations, respectively. However, IEC revealed a considerable deviation compared with immunoassay and CE (16).

ii) Another study demonstrated that African-Americans with sickle phenotype exhibited somewhat decreased the HbA1c readings, while having an apparently normal RBC lifetime (34). As shown in Table II, the variations with different glycation rates were mostly clustered at positions 1, 5, 139 and 140. Therefore, greater caution should be taken for the changes in the glycation rate in the presence of variations with alterations in these two locations.

iii) The association between CKD and A1c is complex (50-53). HbA1c can be still used to track therapy in patients with stage 1-3 CKD. However, various biomarkers need to be employed in patients suffering from stage 4/5 CKD or in those treated with erythropoietin (50,52).

iv) Vitamins C and E can block Hb glycation over time, thus reducing A1c levels. However, this result is not always observed (59-63). In addition, it has been reported that acetylated Hb does not interfere with the measurement of HbA1c under the conventional dosage of aspirin (56). Other drugs, such as sulfasalazine, antiretroviral drugs and ribavirin have been less studied in recent years (66-68). Therefore, caution should be exercised when applying HbA1c to this population.

v) The threshold value of HbA1c for the diagnosis of hyperglycemia associated with T2DM in patients with IDA remains debatable. Misinterpretation may result in misdiagnosis or under-diagnosis, thus having severe implications (83).

7. Conclusions and future perspectives

Over the past 30 years, there has been a marked increase in the prevalence of diabetes worldwide. Diabetes affects ~420 million individuals worldwide, accounting for >6% of the world's population (84). The self-monitoring of blood glucose and the measurement of HbA1c levels are essential for the management of diabetes. Glycated Hb testing has become straightforward and convenient, since it does not require overnight fasting or the ingestion of a standard glucose dose and it can be performed at any time of the day. However, the ease of measuring HbA1c belies its biochemical complexity. It is widely accepted that HbA1c is a hematological parameter whose interpretation is affected by numerous factors, including methodology, clinical biochemistry and hematological factors. Any sequence that affects the globin polypeptide chain, the biochemical properties of erythrocytes and their lifespan may interfere with HbA1c values. Therefore, the more accurate recording of HbA1c levels could enhance the effective management of diabetes. Laboratories need to be aware of

Table III. Summary of the influence of various interference factors on the determination of HbA1c values.

Author, year of publication	Variable	Influencing factor	Effect upon HbA1c	Comment	(Refs.)
Lacy *et al*, 2017	Sickle trait	L	Possible decrease of 0.3%	Used only to assess long-term glycemic control and should use the same measurement technique to ensure longitudinal comparability	(34)
Harris *et al*, 2021	Sickle disease	L	Unable to utilize due to ↓↓ RBC lifespan clinically	Consider the use of additional biomarkers	(9)
Rohlfing *et al*, 2008	↑↑ HbF to 15-30%	M&G	↓ Hb A1c of 1-2% and/or distorted chromatogram	Consider the use of additional biomarkers	(18)
Little *et al*, 2013; Dolscheid-Pommerich *et al*, 2015	CHb (when eGFR <11 ml/min or BUN >80 mg/dl)	M	Statistically but not clinically significant bias in HbA1c	Other issues with CKD need to be considered (see below)	(50,51)
Little *et al*, 2013; Ng *et al*, 2010	CKD (stages 4-5)	M&L	Possible decrease of 0.7-1.5% after treatment	Stages 4-5 do not use HbA1c	(50,52)
Xu *et al*, 2014	Vitamin E (>400 mg/day)	G	Possible decrease of 0.35%	Insufficient evidence at present, further research required	(59)
Basavaraj *et al*, 2022	Dapsone	L	Clinically significant reduction in HbA1	Consider the use of additional biomarkers	(65)
Kim *et al*, 2010	Iron deficiency	L&G	Statistically, but not clinically significant increase in HbA1c	Consider the use of additional biomarkers until red cell indices become stable	(70)

L, erythocyte lifespan; G, glycated rate; M, methodological interference; Hb, hemoglobin; CKD, chronic kidney disease.

common HbA1c assay interferences, that should be taken into consideration when glycated Hb testing does not match clinical perceptions or other metabolic indicators. For cases suspected of possible interference, and particularly for factors involving both methodological interference and altered RBC properties, HbA1c levels need to be determined using instruments with different analytical principles to obtain more useful clinical information. Clinicians need to be advised of the limitations of HbA1c testing used in patients, particularly as regards analytical interference or changes in RBC properties, in order to help them better understand HbA1c. For any situation that can cause an abnormal lifespan of RBCs, the American Diabetes Association (ADA) has recommended the use of glucose criteria for the diagnosis of diabetes (85). When the interpretation of HbA1c is negatively affected by factors affecting erythrocyte lifespan and their glycation rate, alternative non-Hb-based tests, such as GA and serum fructosamine should be used to assess long-term blood glucose levels. However, clinicians need to be aware that GA and serum fructosamine can only assess plasma glucose levels of the previous 2 weeks (86).

The present review article summarized the interference of glycated hemoglobin detection in terms of methodology, glycation rate, and erythrocyte lifespan. To better grasp the principle of diverse interference factors, the biochemical concept and typical HbA1c detection methods were briefly described at the beginning of the manuscript. The methodological component explains the aspects influencing HbA1c detection, whereas the RBC biological properties (glycation rate and erythrocyte lifespan) summarize the factors influencing glycated hemoglobin interpretation. However, unlike Campbell *et al* (87), the authors consider that certain factors (such as hemoglobinopathies, HbF, IDA and chronic renal disease) can have numerous effects. In hemoglobinopathies, for example, there may be changes in the erythrocyte lifespan and/or glycation rate, while the globin peptide chain varies. Rather than merely categorizing hemoglobinopathies based on RBC longevity, as Campbell *et al* (87). A summary of the factors influencing HbA1c values is illustrated in Fig. 1. In addition, hemoglobinopathies are a common glycated hemoglobin interference factor, a large part of which are asymptomatic. They frequently appear when chromatograms and/or HbA1c levels are abnormal (16,88). Therefore, the present review mainly discussed the interference of hemoglobinopathies in an effort to remind laboratories to consider hemoglobinopathies when they meet abnormal HbA1c readings, disparities between blood glucose and HbA1c levels, and abnormal chromatograms. The

present review also summarized the changes in the glycation rate caused by rare hemoglobinopathies at some loci, which are rare in the literature.

Availability of data and materials

Not applicable.

Authors' contributions

ZC and TZ were involved in the conception of the study and in data interpretation, as well as in the writing and critical revision of the manuscript. LS and MJ wrote the manuscript. BM and XB were involved in the conception of the study, and in the design of the figures. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Emerging Risk Factors Collaboration; Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, *et al*: Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. Lancet 375: 2215-2222, 2010.
2. Diabetes Control and Complications Trial Research Group; Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L and Siebert C: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329: 977-986, 1993.
3. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK prospective diabetes study (UKPDS) group. Lancet 352: 837-853, 1998.
4. American Diabetes Association: 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2021. Diabetes Care 44 (Suppl 1): S15-S33, 2021.
5. Weykamp C, John WG, Mosca A, Hoshino T, Little R, Jeppsson JO, Goodall I, Miedema K, Myers G, Reinauer H, *et al*: The IFCC reference measurement system for HbA1c: A 6-year progress report. Clin Chem 54: 240-248, 2008.
6. Bunn HF: Evaluation of glycosylated hemoglobin diabetic patients. Diabetes 30: 613-617, 1981.
7. Mossine VV and Mawhinney TP: 1-Amino-1-deoxy-D-fructose ('fructosamine') and its derivatives. Adv Carbohydr Chem Biochem 64: 291-402, 2010.
8. Shapiro R, McManus MJ, Zalut C and Bunn HF: Sites of nonenzymatic glycosylation of human hemoglobin A. J Biol Chem 255: 3120-3127, 1980.
9. Harris NS, Weaver KD, Beal SG and Winter WE: The interaction between Hb A1C and selected genetic factors in the African American population in the USA. J Appl Lab Med 6: 167-179, 2021.
10. Weykamp C: HbA1c: A review of analytical and clinical aspects. Ann Lab Med 33: 393-400, 2013.
11. Ang SH, Thevarajah M, Alias Y and Khor SM: Current aspects in hemoglobin A1c detection: A review. Clin Chim Acta 439: 202-211, 2015.
12. Rhea JM and Molinaro R: Pathology consultation on HbA(1c) methods and interferences. Am J Clin Pathol 141: 5-16, 2014.
13. Jaisson S, Leroy N, Meurice J, Guillard E and Gillery P: First evaluation of Capillarys 2 Flex Piercing® (Sebia) as a new analyzer for HbA1c assay by capillary electrophoresis. Clin Chem Lab Med 50: 1769-1775, 2012.
14. Teodoro-Morrison T, Janssen MJ, Mols J, Hendrickx BH, Velmans MH, Lotz J, Lackner K, Lennartz L, Armbruster D, Maine G and Yip PM: Evaluation of a next generation direct whole blood enzymatic assay for hemoglobin A1c on the ARCHITECT c8000 chemistry system. Clin Chem Lab Med 53: 125-132, 2015.
15. Little RR, Rohlfing CL, Hanson S, Connolly S, Higgins T, Weykamp CW, D'Costa M, Luzzi V, Owen WE and Roberts WL: Effects of hemoglobin (Hb) E and HbD traits on measurements of glycated Hb (HbA1c) by 23 methods. Clin Chem 54: 1277-1282, 2008.
16. Yun YM, Ji M, Ko DH, Chun S, Kwon GC, Lee K, Song SH, Seong MW, Park SS and Song J: Hb variants in Korea: Effect on HbA1c using five routine methods. Clin Chem Lab Med 55: 1234-1242, 2017.
17. Xu A, Chen W, Xia Y, Zhou Y and Ji L: Effects of common hemoglobin variants on HbA1c measurements in China: Results for α- and β-globin variants measured by six methods. Clin Chem Lab Med 56: 1353-1361, 2018.
18. Rohlfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR and Little RR: The effect of elevated fetal hemoglobin on hemoglobin A1c results: Five common hemoglobin A1c methods compared with the IFCC reference method. Am J Clin Pathol 129: 811-814, 2008.
19. Chachou A, Randoux C, Millart H, Chanard J and Gillery P: Influence of in vivo hemoglobin carbamylation on HbA1c measurements by various methods. Clin Chem Lab Med 38: 321-326, 2000.
20. Weykamp C, Kemna E, Leppink S and Siebelder C: Glycation rate of haemoglobins S, C, D, E, J and G, and analytical interference on the measurement of HbA1c with affinity chromatography and capillary electrophoresis. Clin Chem Lab Med 53: e207-e210, 2015.
21. Coban E, Ozdogan M and Timuragaoglu A: Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 112: 126-128, 2004.
22. Koga M, Morita S, Saito H, Mukai M and Kasayama S: Association of erythrocyte indices with glycated haemoglobin in pre-menopausal women. Diabet Med 24: 843-847, 2007.
23. Son JI, Rhee SY, Woo JT, Hwang JK, Chin SO, Chon S, Oh S, Kim SW and Kim YS: Hemoglobin a1c may be an inadequate diagnostic tool for diabetes mellitus in anemic subjects. Diabetes Metab J 37: 343-348, 2013.
24. Thom CS, Dickson CF, Gell DA and Weiss MJ: Hemoglobin variants: Biochemical properties and clinical correlates. Cold Spring Harb Perspect Med 3: a011858, 2013.
25. Dessi M, Pieri M, Pignalosa S, Martino FG and Zenobi R: Performances of capillary electrophoresis and HPLC methods in HbA1c determination: Diagnostic accuracy in HbS and HbD-Iran variants' presence. J Clin Lab Anal 29: 57-60, 2015.
26. Suo M, Wen D, Wang W, Zhang D, Xu S, Wang X and Hu T: False measurement of glycated hemoglobin in patients without hemoglobin A. Biosci Rep 39: BSR20180128, 2019.
27. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D and Heine RJ; A1c-Derived Average Glucose Study Group: Translating the A1C assay into estimated average glucose values. Diabetes Care 31: 1473-1478, 2008.
28. Franco RS: Measurement of red cell lifespan and aging. Transfus Med Hemother 39: 302-307, 2012.

29. Goldstein DE, Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM and Sacks DB: Tests of glycemia in diabetes. Diabetes Care 27: 1761-1773, 2004.
30. Piel FB, Howes RE, Patil AP, Nyangiri OA, Gething PW, Bhatt S, Williams TN, Weatherall DJ and Hay SI: The distribution of haemoglobin C and its prevalence in newborns in Africa. Sci Rep 3: 1671, 2013.
31. Ouzzif Z, El Maataoui A, Oukhedda N, Messaoudi N, Mikdam M, Abdellatifi M and Doghmi K: Hemoglobinosis C in Morocco : A report of 111 cas. Tunis Med 95: 229-233, 2017.
32. Kato GJ, Piel FB, Reid CD, Gaston MH, Ohene-Frempong K, Krishnamurti L, Smith WR, Panepinto JA, Weatherall DJ, Costa FF and Vichinsky EP: Sickle cell disease. Nat Rev Dis Primers 4: 18010, 2018.
33. Piel FB, Steinberg MH and Rees DC: Sickle cell disease. N Engl J Med 376: 1561-1573, 2017.
34. Lacy ME, Wellenius GA, Sumner AE, Correa A, Carnethon MR, Liem RI, Wilson JG, Sacks DB, Jacobs DR Jr, Carson AP, *et al*: Association of sickle cell trait with hemoglobin A1c in African Americans. JAMA 317: 507-515, 2017.
35. Qiu CC, Kasten-Jolly J and Abraham EC: Human red cell acetyl-transferase. Life Sci 42: 2739-2748, 1988.
36. Chen D, Crimmins DL, Hsu FF, Lindberg FP and Scott MG: Hemoglobin Raleigh as the cause of a falsely increased hemo-globin A1C in an automated ion-exchange HPLC method. Clin Chem 44: 1296-1301, 1998.
37. Koga M, Inada S, Shimizu S, Hatazaki M, Umayahara Y and Nishihara E: Aldimine formation reaction, the first step of the maillard early-phase reaction, might be enhanced in variant Hemoglobin, Hb Himeji. Ann Clin Lab Sci 45: 643-649, 2015.
38. Bissé E, Schauber C, Zorn N, Epting T, Eigel A, Van Dorsselaer A, Wieland H, Kister J and Kiger L: Hemoglobin Görwihl [alpha2beta(2)5(A2)Pro->Ala], an electrophoretically silent variant with impaired glycation. Clin Chem 49: 137-143, 2003.
39. Nakanishi T, Miyazaki A, Kishikawa M, Shimizu A, Aoki K and Kikuchi M: Hb Sagami [beta 139(H17)Asn->Thr]: A new hemoglobin variant not detected by isoelectrofocusing and propan-2-ol test, was detected by electrospray ionization mass spectrometry. J Mass Spectrom 33: 565-569, 1998.
40. Kabytaev K, Connolly S, Rohlfing CL, Sacks DB, Stoyanov AV and Little RR: Higher degree of glycation of hemoglobin S compared to hemoglobin A measured by mass spectrometry: Potential impact on HbA1c testing. Clin Chim Acta 458: 40-43, 2016.
41. Watanabe T, Kato K, Yamada D, Midorikawa S, Sato W, Shiga M, Otsuka Y, Miura M, Harano K and Harano T: A nondiabetic case of hemoglobin variant (Hb Niigata) with inappropriately high and low HbA1c titers detected by different methods. Clin Chem 44: 1562-1564, 1998.
42. Shah SC, Malone JI, Boissel JP and Kasper TJ: Hemoglobin South Florida. New variant with normal electrophoretic pattern mistaken for glycosylated hemoglobin. Diabetes 35: 1073-1076, 1986.
43. Langdown JV, Williamson D, Beresford CH, Gibb I, Taylor R and Deacon-Smith R: A new beta chain variant, Hb Tyne [beta 5(A2)Pro->Ser]. Hemoglobin 18: 333-336, 1994.
44. Joly P, Garcia C, Lacan P, Couprie N and Francina A: Two new hemoglobin variants: Hb Aix-Les-Bains [β5(A2) Pro→Leu; HBB:c.17 C>T] and Hb Dubai [α122(H5) His→Leu (α2); HBA2:c.368 A>T]. Hemoglobin 35: 147-151, 2011.
45. Karsegard J, Wicky J, Mensi N, Caulfield A and Philippe J: Spurious glycohemoglobin values associated with hydroxyurea treatment. Diabetes Care 20: 1211-1212, 1997.
46. Bry L, Chen PC and Sacks DB: Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemo-globin. Clin Chem 47: 153-163, 2001.
47. Stim J, Shaykh M, Anwar F, Ansari A, Arruda JA and Dunea G: Factors determining hemoglobin carbamylation in renal failure. Kidney Int 48: 1605-1610, 1995.
48. Jaisson S, Leroy N, Guillard E, Desmons A and Gillery P: Analytical performances of the D-100TM hemoglobin testing system (Bio-Rad) for HbA1c assay. Clin Chem Lab Med 53: 1473-1479, 2015.
49. Weykamp CW, Miedema K, de Haan T and Doelman CJ: Carbamylated hemoglobin interference in glycohemoglobin assays. Clin Chem 45: 438-440, 1999.
50. Little RR, Rohlfing CL, Tennill AL, Hanson SE, Connolly S, Higgins T, Wiedmeyer CE, Weykamp CW, Krause R and Roberts W: Measurement of Hba(1C) in patients with chronic renal failure. Clin Chim Acta 418: 73-76, 2013.
51. Dolscheid-Pommerich RC, Kirchner S, Weigel C, Eichhorn L, Conrad R, Stoffel-Wagner B and Zur B: Impact of carbamylation on three different methods, HPLC, capillary electrophoresis and TINIA of measuring HbA1c levels in patients with kidney disease. Diabetes Res Clin Pract 108: 15-22, 2015.
52. Ng JM, Cooke M, Bhandari S, Atkin SL and Kilpatrick ES: The effect of iron and erythropoietin treatment on the A1C of patients with diabetes and chronic kidney disease. Diabetes Care 33: 2310-2313, 2010.
53. Kilpatrick ES and Atkin SL: Using haemoglobin A(1c) to diag-nose type 2 diabetes or to identify people at high risk of diabetes. BMJ 348: g2867, 2014.
54. Bridges KR, Schmidt GJ, Jensen M, Cerami A and Bunn HF: The acetylation of hemoglobin by aspirin. In vitro and in vivo. J Clin Invest 56: 201-207, 1975.
55. Unnikrishnan R, Anjana RM and Mohan V: Drugs affecting HbA1c levels. Indian J Endocrinol Metab 16: 528-531, 2012.
56. Camargo JL, Stifft J and Gross JL: The effect of aspirin and vita-mins C and E on HbA1c assays. Clin Chim Acta 372: 206-209, 2006.
57. Weykamp CW, Penders TJ, Siebelder CW, Muskiet FA and van der Slik W: Interference of carbamylated and acetylated hemoglobins in assays of glycohemoglobin by HPLC, electro-phoresis, affinity chromatography, and enzyme immunoassay. Clin Chem 39: 138-142, 1993.
58. Davie SJ, Gould BJ and Yudkin JS: Effect of vitamin C on glyco-sylation of proteins. Diabetes 41: 167-173, 1992.
59. Xu R, Zhang S, Tao A, Chen G and Zhang M: Influence of vitamin E supplementation on glycaemic control: A meta-analysis of randomised controlled trials. PLoS One 9: e95008, 2014.
60. Jain SK and Palmer M: The effect of oxygen radicals metabolites and vitamin E on glycosylation of proteins. Free Radic Biol Med 22: 593-596, 1997.
61. Ceriello A, Giugliano D, Quatraro A, Donzella C, Dipalo G and Lefebvre PJ: Vitamin E reduction of protein glycosylation in diabetes. New prospect for prevention of diabetic complications? Diabetes Care 14: 68-72, 1991.
62. Shoff SM, Mares-Perlman JA, Cruickshanks KJ, Klein R, Klein BE and Ritter LL: Glycosylated hemoglobin concentrations and vitamin E, vitamin C, and beta-carotene intake in diabetic and nondiabetic older adults. Am J Clin Nutr 58: 412-416, 1993.
63. Weykamp CW, Penders TJ, Baadenhuijsen H, Muskiet FA, Martina W and van der Slik W: Vitamin C and glycohemoglobin. Clin Chem 41: 713-716, 1995.
64. Shah AD, Fox RK and Rushakoff RJ: Falsely decreased HbA1c in a type 2 diabetic patient treated with dapsone. Endocr Pract 20: e229-e232, 2014.
65. Basavaraj GS, Gupta RD, Patel B, Jebasingh F, George AA, Peter D, George L, Paul TV and Thomas N: Erroneous reduc-tion of HbA1c levels in patients with type 2 diabetes mellitus on dapsone treatment for Hansen's disease-a single-center retro-spective cohort study. Indian J Dermatol Venereol Leprol 88: 519-522, 2022.
66. Mitchell K and Mukhopadhyay B: Drug-Induced Falsely Low A1C: Report of a case series from a diabetes clinic. Clin Diabetes 36: 80-84, 2018.
67. Diop ME, Bastard JP, Meunier N, Thévenet S, Maachi M, Capeau J, Pialoux G and Vigouroux C: Inappropriately low glycated hemoglobin values and hemolysis in HIV-infected patients. AIDS Res Hum Retroviruses 22: 1242-1247, 2006.
68. Robertson M: Artificially low HbA1c associated with treatment with ribavirin. BMJ 336: 505, 2008.
69. Gram-Hansen P, Eriksen J, Mourits-Andersen T and Olesen L: Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med 227: 133-136, 1990.
70. Kim C, Bullard KM, Herman WH and Beckles GL: Association between iron deficiency and A1C Levels among adults without diabetes in the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care 33: 780-785, 2010.
71. Attard SM, Herring AH, Wang H, Howard AG, Thompson AL, Adair LS, Mayer-Davis EJ and Gordon-Larsen P: Implications of iron deficiency/anemia on the classification of diabetes using HbA1c. Nutr Diabetes 5: e166, 2015.
72. Cavagnolli G, Pimentel AL, Freitas PA, Gross JL and Camargo JL: Factors affecting A1C in non-diabetic individuals: Review and meta-analysis. Clin Chim Acta 445: 107-114, 2015.

73. Sundaram RC, Selvaraj N, Vijayan G, Bobby Z, Hamide A and Rattina Dasse N: Increased plasma malondialdehyde and fructosamine in iron deficiency anemia: Effect of treatment. Biomed Pharmacother 61: 682-685, 2007.
74. Guo W, Zhou Q, Jia Y and Xu J: Increased levels of glycated hemoglobin A1c and iron deficiency Anemia: A review. Med Sci Monit 25: 8371-8378, 2019.
75. Willekens FL, Roerdinkholder-Stoelwinder B, Groenen-Döpp YA, Bos HJ, Bosman GJ, van den Bos AG, Verkleij AJ and Were JM: Hemoglobin loss from erythrocytes in vivo results from spleen-facilitated vesiculation. Blood 101: 747-751, 2003.
76. Schnedl WJ, Wallner SJ, Piswanger C, Krause R and Lipp RW: Glycated hemoglobin and liver disease in diabetes mellitus. Wien Med Wochenschr 155: 411-415, 2005.
77. Bernstein RM, Freedman DB, Liyanage SP and Dandona P: Glycosylated haemoglobin in rheumatoid arthritis. Ann Rheum Dis 41: 604-606, 1982.
78. Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, Sullivan L, D'Agostino RB and Nathan DM: Effect of aging on A1C levels in individuals without diabetes: Evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care 31: 1991-1996, 2008.
79. Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, Narayan KM, Koch DD and Phillips LS: Glucose-independent, black-white differences in hemoglobin A1c levels: A cross-sectional analysis of 2 studies. Ann Intern Med 152: 770-777, 2010.
80. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, Lachin JM, Montez MG, Brenneman T and Barrett-Connor E; Diabetes Prevention Program Research Group: Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the diabetes prevention program. Diabetes Care 30: 2453-2457, 2007.
81. Bergenstal RM, Gal RL, Connor CG, Gubitosi-Klug R, Kruger D, Olson BA, Willi SM, Aleppo G, Weinstock RS, Wood J, et al: Racial differences in the relationship of glucose concentrations and hemoglobin A1c Levels. Ann Intern Med 167: 95-102, 2017.
82. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J and Brancati FL: Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 362: 800-811, 2010.
83. Nakatani R, Murata T, Usui T, Moriyoshi K, Komeda T, Masuda Y, Kakita-Kobayashi M, Tagami T, Imashuku S, Kono S, et al: Importance of the average glucose level and estimated glycated hemoglobin in a diabetic patient with hereditary hemolytic Anemia and liver cirrhosis. Intern Med 57: 537-543, 2018.
84. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, et al: Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International diabetes federation diabetes atlas, 9(th) edition. Diabetes Res Clin Pract 157: 107843, 2019.
85. American Diabetes Association: Standards of medical care in diabetes-2013. Diabetes Care 36 (Suppl 1): S11-S66, 2013.
86. Bergman M, Abdul-Ghani M, DeFronzo RA, Manco M, Sesti G, Fiorentino TV, Ceriello A, Rhee M, Phillips LS, Chung S, et al: Review of methods for detecting glycemic disorders. Diabetes Res Clin Pract 165: 108233, 2020.
87. Campbell L, Pepper T and Shipman K: HbA1c: A review of non-glycaemic variables. J Clin Pathol 72: 12-19, 2019.
88. Xu A, Sun J, Li J, Chen W, Zheng R, Han Z and Ji L: Hb I: A α-globin chain variant causing unexpected HbA_{1c} results. J Clin Lab Anal 33: e22671, 2019.