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SYSTEMATIC REVIEW

The effect of anaemia and abnormalities of erythrocyte indices on HbA_{1c} analysis: a systematic review

Emma English[1] · Iskandar Idris[1] · Georgina Smith[1] · Ketan Dhatariya[2] · Eric S. Kilpatrick[3] · W. Garry John[4]

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Abstract
Aims/hypothesis The use of HbA_{1c} for the diagnosis of diabetes is now widely advocated despite caveats to its use. Anaemia is cited as a major confounder to this use; however, the effect of erythrocyte indices and to what degree anaemia influences HbA_{1c} levels is not known.
Methods A systematic electronic database search of MEDLINE, EMBASE, the Cumulative Index to Nursing & Allied Health Literature (CINAHL) and the Cochrane Library was conducted for relevant articles published between January 1990 and May 2014. Included studies had at least one measurement of HbA_{1c} and glucose, and a least one index of haematinic deficiency, involving non-pregnant adults, not known to have diabetes.
Results A total of 12 articles from 544 were included. The majority of studies focused on iron deficiency anaemia (IDA) and, in general, demonstrated that the presence of iron deficiency with or without anaemia led to an increase in HbA_{1c} values compared with controls, with no concomitant rise in glucose indices. Data on the effects of other indices of erythrocyte abnormalities on HbA_{1c} are limited but show a possible decrease in HbA_{1c} values with non-iron deficiency forms of anaemia.
Conclusions/interpretation HbA_{1c} is likely to be affected by iron deficiency and IDA with a spurious increase in HbA_{1c} values; conversely, non-IDA may lead to a decreased HbA_{1c} value. This may lead to confusion when diagnosing diabetes using HbA_{1c}. This review clearly identifies the need for more evidence, especially in identifying the types and degrees of anaemia likely to have significant impact on the reliability of HbA_{1c}.

Keywords Anaemia · Diabetes · HbA_{1c} · Iron deficiency · Systematic review

Abbreviations

CINAHL	Cumulative Index to Nursing & Allied Health Literature
FPG	Fasting plasma glucose
IDA	Iron deficiency anaemia
IFCC	International Federation for Clinical Chemistry and Laboratory Medicine
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NHANES	National Health and Nutrition Examination Survey
PCV	Packed cell volume
RDW	Red cell distribution width
SIGN	Scottish Intercollegiate Guidelines Network
TSAT	Transferrin saturation

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✉ Emma English
emma.english@nottingham.ac.uk

[1] School of Medicine, University of Nottingham, Derby DE22 3DT, UK

[2] Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK

[3] Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK

[4] Department of Clinical Biochemistry, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK

Introduction

The traditional role of HbA_{1c} analysis has been for assessing glycaemic control in patients with diabetes. The results of seminal studies [1, 2] demonstrated that early, intensive glycaemic control could significantly reduce the risk of a range of diabetes-related complications, and permitted the establishment of precise HbA_{1c} target values for treatment goals [3].

More recently, there has been a move towards the use of HbA_{1c} for the diagnosis of type 2 diabetes. The WHO and the ADA have both advocated the use of HbA_{1c} for diagnosing type 2 diabetes, at a value of 6.5% (48 mmol/mol) [4, 5].

Further to the recommendations of the WHO, the UK issued an expert position statement on the application of these recommendations in clinical practice in the UK [6]. One key factor thought to be a confounder in the use of HbA_{1c} is an altered erythrocyte lifespan, in particular due to anaemia. The WHO defines anaemia in adults as 120 g/l Hb in non-pregnant women and 130 g/l in men [7]. It is widely purported that haemolytic anaemia can lead to decreased HbA_{1c} values due to reduced erythrocyte lifespan, and iron deficiency anaemia (IDA) may result in increased HbA_{1c} values due to an elongation of the erythrocyte lifespan. However, it is not known to what degree alterations in erythrocyte indices affect HbA_{1c} values especially around the diagnostic cut point of 6.5% (48 mmol/mol) or the degree of abnormality severity required to result in a significant change. With approximately 29% of non-pregnant women worldwide having anaemia in 2011 [8], this translates to a significant number of people where the use of HbA_{1c} for diagnosis of diabetes may be precluded.

Since the publication of the recommendations, there has been a demand for clarity on this topic. The key questions asked are: 'At what level of anaemia should I not use HbA_{1c} for diagnosis?' and 'Should I routinely screen patients for anaemia when using HbA_{1c} for diagnosis and if so, what test should I use?'

This systematic review aims to address the above questions by assessing the available evidence on the impact of abnormalities of erythrocyte indices and anaemia, on HbA_{1c} levels around the diagnostic cut off point of 6.5% (48 mmol/mol).

Methods

This systematic review is registered on Prospero (registration no. CRD42013005251). Approval of an ethics committee was not required.

Study identification

An electronic database search for relevant articles published between January 1990 and May 2014 was conducted using the following databases: MEDLINE, EMBASE, Cumulative Index to Nursing & Allied Health Literature (CINAHL) and The Cochrane Library.

The search was carried out using a combination of keywords and MESH terms or EMtree terms depending on the particular database (see Electronic Supplementary Material [ESM] Methods for details).

In general, the search strategy consisted of: (1) HbA_{1c}/glycated haemoglobin; (2) iron deficiency; (3) folate, B12 deficiency; and (4) anaemia.

Study selection

Results from all searches were combined and duplicates were removed. Two investigators (EE and GS) evaluated the title and abstract of each reference identified by the search. Inclusion criteria are fully detailed in Table 1; for inclusion, all studies required at least one HbA_{1c} value with fasting plasma glucose (FPG) or OGTT values and erythrocyte and/or iron indices measured. This was to ensure that any changes in HbA_{1c} value were due to changes in the erythrocytes and not due to changes in glycaemia. Any that were clearly irrelevant were removed and the full texts of remaining articles were retrieved. Upon further scrutiny of the full texts, those that did not meet the inclusion criteria were subsequently excluded. The reference lists of included articles were reviewed in order to identify any further articles of relevance to the subject area, and that met the inclusion criteria.

Data extraction

Two investigators extracted the results from each article using a data extraction form based on the pre-defined study inclusion criteria (Table 1). Any disagreement regarding study selection was resolved by means of consensus, involving a third investigator, according to a-priori agreed criteria. The main categories extracted included: author, year, title, study overview, patient characteristics, definition of diabetes, definition of anaemia and key conclusions, as detailed in Table 2.

Quality assessment of included articles

The methodological quality of each study was independently rated by two investigators (EE and GS—who were not blinded to author, journal or institution) according to the Scottish Intercollegiate Guidelines Network (SIGN) criteria [9]. Quality assessment was not used as an exclusion criterion, though articles conducted to high standards in minimising the risk of confounding were noted (ESM Table 1).

Table 1 Inclusion criteria for the systematic review

Inclusion criteria	Description
Population	All non-pregnant adults (≥18 years), without known diabetes (HbA_{1c} should not be used for diagnosis of diabetes in children or pregnancy). Participants should have at least one measurement of HbA_{1c} and measure of glucose, as well as at least one index of anaemia or haematinic deficiency. A range of definitions of anaemia were used in the studies detailed in this review, with some basing the diagnosis on Hb levels and others refining this further based on indices such as MCV, MCH, ferritin and TSAT levels.
Intervention	The use of HbA_{1c} for the diagnosis of diabetes or comparison of HbA_{1c} in participants with and without anaemia or other abnormalities of erythrocyte indices.
Comparisons	HbA_{1c} values ideally will be compared with FPG or OGTTs and values compared between participants with and without anaemia or erythrocyte indices abnormalities. Whilst some studies may include glucose values, others only report that all participants had values below a set point.
Outcomes	Changes in HbA_{1c} values, compared with glucose indices, with decreased haemoglobin concentration. Changes in HbA_{1c}, compared with glucose indices, with other erythrocyte indices.
Study design	Cohort studies evaluating the association between HbA_{1c} levels and anaemia or other erythrocyte indices with or without anaemia were included. Case report, case–control, case series studies, letters and commentaries were excluded.
Search limits	Adults (aged ≥18 years); English language; humans; publication year 1990–current. HbA_{1c} was not widely used in routine clinical practice and methods showed very high inter and intra-laboratory variation prior to 1990. Identification of grey literature including unpublished data, conference reports and presentations was not attempted.

Data analysis

Variation within study designs, and heterogeneity of results, meant that the data was unsuitable for meta-analysis. Instead, the existing analyses described in the included articles were extracted and reported in a systematic format, as a narrative synthesis of the main outcomes and results of each study. In line with current recommendations [10, 11], the HbA_{1c} units in the main text have been converted to SI units using the master equation.

Results

The electronic database searches identified 451 potentially relevant, unique articles. Titles/abstracts of the 451 articles were reviewed and 408 were excluded based on evaluation of the title and/or abstract. The remaining 43 full text articles were reviewed, 31 were excluded upon further reading. The residual 12 were approved and quality assessed using the SIGN criteria. The main reasons for exclusion were irrelevant or incomplete data when compared with the inclusion criteria. No additional articles were identified through review of the reference lists (see Fig. 1 for flow chart).

Narrative analysis of included studies

Cross-sectional studies in patients with and without diabetes

Kim et al [12] investigated the influence of iron deficiency on HbA_{1c} distribution among adults who were not known to have diabetes, over 7 years of the National Health and Nutrition Examination Survey (NHANES). Of the 6,666 female participants, 13.7% had iron deficiency and 30% of these had IDA. A much lower proportion of males (1.6%) had iron deficiency and 33% of these had IDA. When HbA_{1c} values in women were adjusted for age and ethnicity, the difference between iron deficient and non-iron deficient became significant (5.33% vs 5.27% [35 mmol/mol vs 34 mmol/mol], p= 0.002). The authors found that iron deficiency in women of reproductive age was associated with a shift in HbA_{1c} from <5.5% to 5.5–6.0% (<37 mmol/mol to 37–42 mmol/mol), although no association was found at higher levels, possibly owing to the lower number of participants in those groups. After adjusting for age and ethnicity, the authors concluded that HbA_{1c} was higher in iron-deficient individuals and was likely to result in an upward shift of HbA_{1c} distributions.

Ford et al [13] evaluated 1999–2002 NHANES data sets but included analysis of both IDA and non-IDA in participants both with and without diabetes. They found that non-diabetic participants with low Hb but normal iron levels had lower HbA_{1c} values (5.16% [33 mmol/mol]) than those with normal Hb and normal iron levels (5.31% [35 mmol/mol], p<0.001). In contrast, those with normal Hb but low iron values had borderline higher HbA_{1c} values than normal participants (5.39% [35 mmol/mol], p=0.061). In addition, in all participants without diabetes, the HbA_{1c} values increased with increasing Hb levels ranging from a mean HbA_{1c} of 5.18% (33 mmol/mol) at Hb <100 g/l through to 5.50% (37 mmol/mol) with Hb >170 g/l. The authors advocate caution when interpreting HbA_{1c} values near diagnostic cut points when Hb levels are high or low. The study was limited by low numbers of iron deficiency and/or anaemia cases and few severe cases but the authors suggest that the likely impact of haemoglobin concentration on HbA_{1c} values was an approximate change of HbA_{1c} 0.2% (2.2 mmol/mol), between the extremes of Hb levels.

Table 2 Data extraction form and key data from included studies

Author, year and title	*n* (male)	Study	Diabetes, anaemia or erythrocyte indices	Mean Hb	Mean HbA_{1c}	Other key findings	Conclusion
Behan, 2006 [21]	169 (0)	Comparison of HbA_{1c} in premenopausal (n=100) and postmenopausal (n=69) women without diabetes. Authors correlate HbA_{1c} to FPG in both groups. They also introduce absolute A_{1c}, (total Hb×HbA_{1c}) and test its correlation to FPG in both groups.	Diabetes determined by known diabetes or FPG ≥7.0 mmol/l (126 mg/dl).	Premenopausal 136 g/l Postmenopausal 139 g/l (p=0.009)	Premenopausal 5.4% Postmenopausal 5.4%, (p=0.081).	FPG showed weak correlation with HbA_{1c} value overall r 0.251 (p=0.001), which was higher in postmenopausal women indicating there is haemodynamic variability that renders HbA_{1c} values less reliable in terms of representing past glycaemia in premenopausal women.	This study suggests that menstruation during middle age can have a significant effect on the accuracy of HbA_{1c} analysis and that effect is nullified after cessation of menstruation. This is a significant finding because half of women who develop type 2 diabetes do so during the peri-menopause. The authors suggest the use of absolute A_{1c} in order to account for haematinic variation.
Coban et al, 2004 [17]	100 (40)	Study consists of 50 patients with IDA and 50 healthy, age- and sex-matched controls. Hb, PCV, MCV, MCH, ferritin, serum fasting, and postprandial glucose and HbA_{1c} levels measured. Patients with IDA were treated with oral ferrous sulphate 100 mg a day for 3 months.	IDA determined by low ferritin levels (<9 ng/ml for women and <15 ng/ml for men) associated with microcytic hypochromic anaemia.	Pre treatment 108±12 g/l Post treatment 127±9.6 g/l Controls 136±9 g/l	Pre treatment 7.4±0.8% Post treatment 6.2±0.6% Controls 5.2±0.2%	In patients with IDA, HbA_{1c} decreased significantly after iron treatment (p<0.001). No significant difference across the three groups in FPG or postprandial glucose.	This study suggests correcting iron deficiency before making a diagnostic or therapeutic decision based on HbA_{1c}.
El-Agouza et al, 2002 [18]	81 (NK)	University students (n=81) identified with microcytic, hypochromic anaemia of which 47 were iron deficient, 26 were β-thalassaemia carriers and four had both conditions. The participants with anaemia were treated with oral ferrous sulphate (325 mg/day) for 20 weeks and repeat analyses performed.	IDA determined as microcytic (<80 fl MCV), hypochromic (<26 pg MCH) and low ferritin (undefined).	Pre treatment 109.6±11.2 g/l Post 20 weeks treatment 132±6.5 g/l (p<0.001)	Pre treatment 6.15±0.62% Post 20 weeks treatment 5.25±0.45% (p<0.001).	HbA_2 % Pre treatment 1.89±0.45% Post 20 weeks treatment: 2.19±0.53% (p<0.001) HbF % Pre treatment: 0.94±0.18% Post 20 weeks treatment: 0.95±0.17% (p>0.05)	7% of university students in Gaza had iron deficiency. HbA_{1c} falls significantly with iron replacement therapy but results did not plateau at 20 weeks. The authors also demonstrate an increase in HbA_2 post treatment indicating that diagnoses of β-thalassaemia carriers may be missed in iron-deficient anaemia.
Ford et al, 2011 [13]	8,296 (NK)	Data from the NHANES, 1999–2002. HbA_{1c} and complete blood count were used to examine whether there was an association between anaemia and HbA_{1c} and whether associations between concentrations of glucose and HbA_{1c} differed according to Hb and iron status.	IDA determined by: Low Hb (<120 g/l in women aged 20-69, <118 g/l in women aged ≥70, <137 g/l in men aged 20-49, <133 g/l in men aged 50-69 and <124 g/l in men ≥70) and two or more abnormalities: low TSAT (<15%), low serum ferritin (<26.96 pmol/l) and elevated erythrocyte protoporpyrin (>1.24 μmol/l).	Prevalence of anaemia in total cohort: IDA 2.3±0.2% Non-IDA 3.2 ±0.3% Normal Hb but iron deficient 4.4±0.3% Normal Hb and normal iron 90.1±0.4%	Total population mean HbA_{1c} 5.28% with Hb <100 g/l to 5.72% with Hb>170 g/l The adjusted mean concentrations of HbA_{1c} were 5.56% and 5.46% among participants with and without iron deficiency (p=0.095).	Low Hb but normal iron vs normal Hb and normal iron had significantly lower HbA_{1c} (p=0.001) Normal Hb but low iron vs normal Hb and normal iron had borderline higher HbA_{1c} (p=0.061) Greatest effect seen in HbA_{1c} <5.0% group; also highest number of low Hb normal iron seen in this group.	Hb concentration appears to be positively correlated with HbA_{1c} value. HbA_{1c} appears higher in patients with iron deficiency and similar to values in patients with normal iron and normal Hb. The authors suggest caution should be taken when diagnosing diabetes and prediabetes in people with a high or low Hb when the HbA_{1c} level is close to the 6.5% or 5.7% cut off.

Table 2 (continued)

Author, year and title	*n* (male)	Study	Diabetes, anaemia or erythrocyte indices	Mean Hb	Mean HbA_{1c}	Other key findings	Conclusion
Gram-Hansen et al, 1990 [19]	30 (NK)	Ten patients with IDA, ten with vitamin B12 deficiency anaemia and ten healthy controls were studied. HbA_{1c}, Hb and erythrocyte variables were measured at 0, 3, 6 and 9 weeks after initiation of treatment. Effect of iron and B12 therapy on HbA_{1c} over 9 weeks was assessed.	No variables given for diabetes, IDA or vitamin B12 deficiency. Haematinic data for controls also not presented.	Median Hb IDA pre treatment: 5.1 mmol/l B12A pre treatment: 6.1 mmol/l Controls not presented; assumed within quoted ref range of 7.5-10.4 mmol/l IDA at 3 weeks: 7.2 mmol/l B12A at 3 weeks: 8.3 mmol/l IDA and B12A at 9 weeks not presented.	Median HbA_{1c} IDA pre treatment 4.9% B12A pre treatment 5.1% Controls 5.0% IDA at 9 weeks 4.6% B12A at 9 weeks 4.8%.	No initial significant difference between the IDA group 4.9% and vitamin B12 deficiency group 5.1% ($p<0.4$). After 3 weeks of therapy, HbA_{1c} in IDA 4.3% and vitamin B12 deficiency anaemia 4.4% decreased significantly ($p<0.01$). Significance not stated at 9 weeks.	The authors conclude that, in patients with iron and vitamin B12 deficiency, HbA_{1c} is an important marker of the changes in the erythrocyte population that occur when therapy is initiated. This paper is widely cited by other articles on the subject of anaemia and HbA_{1c} measurement.
Hardikar et al, 2012 [14]	243 (136)	Study included young adults from the Pune Children's Study cohort in India. The authors investigated the diagnostic performance of HbA_{1c} against a standard OGTT, and looked at the haematological, nutritional and other factors influencing HbA_{1c} concentration.	Diabetes determined by OGTT WHO criteria. HbA_{1c} ADA criteria; prediabetes 5.7–6.4%); diabetes ≥6.5%. Anaemia determined by Hb <120 g/l in females and <130 g/l in males.	Mean Hb 130±20 g/l Prevalence of anaemia: Approx. one third had anaemia, 43.6% had microcytosis, 2.5% had macrocytosis. 66.7% were iron deficient, 30.8% were B12 deficient and 15.4% were folate deficient.	Mean HbA_{1c} 5.4%, range 4.4-6.7%.	OGTT showed that 7.8% of participants were prediabetic and 2.6% were diabetic. The HbA_{1c} values showed that 23.3% were prediabetic and 2.6% were diabetic. This increased to 33% having diabetes or prediabetes in the anaemic group.	There was higher prevalence of iron deficiency in the participants classified by HbA_{1c} as prediabetic or diabetic. Serum ferritin concentrations were significantly lower in prediabetes and diabetes compared with the normal group. The authors suggest that diagnosing diabetes and prediabetes in iron-deficient populations may lead to spuriously high HbA_{1c} concentrations and potential for increased mis-diagnosis of diabetes.
Kim et al, 2010 [12]	10,535 (3869)	Data from the NHANES 1999–2006 surveys. The distribution of HbA_{1c} values were examined at a cut point of <5.5% vs ≥5.5% and because of the recommended cut point of <6.5% vs ≥6.5%. Study looks at the influence of iron deficiency on HbA_{1c} distribution among adults without diabetes.	Iron deficiency determined by at least two abnormalities including free erythrocyte protoporphyrin >1.24 μmol/l erythrocytes, TSAT <16%, or serum ferritin ≤15 μg/l. IDA determined by: as above with Hb <135 g/l in men and <120 g/l in women.	Mean Hb data not provided. Prevalence data: 13.7% (n=1150) women had iron deficiency, and of these 30% had anaemia (n=345). 1.6% (n=75) men had iron deficiency, and of these 33% had anaemia (n=33). 2.3% (n=127) women and 3.3% (n=125) men had anaemia but not iron deficiency.	Mean HbA_{1c} in women with iron deficiency was 5.31±0.02% and without iron deficiency 5.27±0.01% (p=0.127) Mean HbA_{1c} in men with iron deficiency was 5.43±0.06% and without iron deficiency 5.29±0.02% (p=0.035).	Although in women the mean HbA_{1c} did not differ between the iron deficient and iron sufficient there was an upward shift in distribution of HbA_{1c} from ≤5.4% to 5.5-6.0%. 316 women had iron deficiency and HbA_{1c} ≥5.5% and 32 had IDA and HbA_{1c} ≥6.5%. 13 men had iron deficiency and HbA_{1c} ≥5.5% and only 12 had IDA and HbA_{1c} ≥6.5%.	The authors concluded that iron deficiency was common among women; this iron deficiency was not necessarily accompanied by anaemia. Iron deficiency in the female cohort led to a re-distribution of HbA_{1c} values. The shift in levels at the higher end of HbA_{1c} (6.0–6.5%) was not significant but only a small number of participants had higher values of HbA_{1c} (n=13).

Table 2 (continued)

Author, year and title	*n* (male)	Study	Diabetes, anaemia or erythrocyte indices	Mean Hb	Mean HbA_{1c}	Other key findings	Conclusion
Koga et al, 2007 [22]	423 (0)	HbA_{1c}, erythrocyte count, PCV, Hb, MCV and MCH in 423 women with normal glucose tolerance were taken. 180 of the patients were premenopausal and the other 243 were postmenopausal. They examined the relationship between HbA_{1c} and erythrocyte indices in pre and postmenopausal participants.	Anaemia determined by Hb <114 g/l. Impaired glucose metabolism and diabetes diagnosed using OGTT and WHO criteria.	Hb in premenopausal women: 126 g/l vs postmenopausal: 130 g/l (p<0.0001).	FPG in pre- vs postmenopausal women: 5.1±0.3 mmol/l and 5.3±0.3 mmol/l (p<0.0001). HbA_{1c} in pre- vs postmenopausal women: 4.9±0.2% and 5.1±0.3% (p<0.0001).	Erythrocyte counts ($4.30\pm0.30\times10^6$/μl) of premenopausal women were positively associated with HbA_{1c} (4.9±0.2%), whereas Hb (126±12 g/l), MCV (89.9±6.1 fl) and MCH (29.4±2.7 pg/cell) showed negative association. In contrast, no significant association of any indices with HbA_{1c} (5.1±0.3%) was detected in the postmenopausal women.	The authors concluded that erythrocyte indices are associated with HbA_{1c}, independently of plasma glucose levels, in premenopausal women even when they are not anaemic. The authors highlight that MCH and MCV are early indicators of iron deficiency and may change prior to a fall in Hb levels. They recommend this be considered when interpreting HbA_{1c} in premenopausal patients with diabetes.
Koga et al, 2010 [23]	104(0)	Study included 57 patients with normal iron state (NIS), 30 with IDS and 17 with IDA. Measurements of FPG, erythrocyte count, GA, Hb, PCV MCV, serum iron, STAT and serum ferritin were included.	IDA determined by Hb <114 g/l and serum ferritin <15 ng/ml. IDS determined by Hb ≥114 g/l and serum ferritin <15 ng/ml. NIS determined by Hb ≥114 g/l and serum ferritin ≥15 ng/ml.	NIS Hb 131±8 g/l IDS 124±8 g/l IDA 103±6 g/l p <0.001 for NIS vs IDS and also for IDA vs NIS and vs IDS.	NIS 4.8±0.2% IDS 5.0±0.2% IDA 5.1±0.2% p<0.001 for NIS vs IDA and p<0.05 for NIS vs IDS.	HbA_{1c} showed significant inverse association with serum iron, TSAT and serum ferritin. HbA_{1c} levels in IDA and IDS were slightly but significantly higher than NIS participants. There were no significantly different values of GA between groups. Serum ferritin (decreased) was identified as a significant risk factor for increased HbA_{1c}.	The authors concluded that iron metabolism indices influence HbA_{1c} levels, but not serum GA levels, in premenopausal women. The authors state that they found no evidence of IDA causing increased lifespan of erythrocytes.
Shanthi et al, 2013 [16]	100 (38)	50 non-diabetic IDA patients and 50 healthy age matched participants. Hb, PCV MCV, MCH, ferritin, FPG and HbA_{1c} were analysed, to determine the effects of IDA on HbA_{1c} levels in nondiabetics.	Anaemia determined by: Hb<110 g/l, ferritin levels <9 ng/ml for women and <15 ng/ml for men and on their peripheral blood smears mostly microcytic hypochromic, which suggests IDA.	The mean Hb (106±14 g/l) level in the patients with IDA was lower than those in the control group (134±9.6 g/l) (p<0.05).	Mean HbA_{1c} (7.6±0.5%) level in patients with IDA was higher than those in the control group (5.5±0.8%) (p<0.001).	All iron indices were lower in IDA compared with controls; however, FPG was not significantly different.	The authors conclude that HbA_{1c} is not affected by blood sugar levels alone, and that iron deficiency has a substantial effect on HbA_{1c}. The authors suggest that anaemia may lead to a shortened half-life of erythrocytes rather than elongation. They also suggest that IDA changes the quaternary structure of Hb leading to increased glycation.

Table 2 (continued)

Author, year and title	*n* (male)	Study	Diabetes, anaemia or erythrocyte indices	Mean Hb	Mean HbA_{1c}	Other key findings	Conclusion
Sinha et al, 2012 [20]	50 (16) Controls 50 (29)	This study investigated the effects of IDA on HbA_{1c} levels and whether treatment of IDA influenced HbA_{1c} levels. 50 patients confirmed to have IDA were treated with iron supplements. Hb, MCH, PCV, MCV and MCHC at baseline, at 1 month and at 2 months were analysed; the controls were only analysed once.	Diabetes determined by - FPG >5.6 mmol/l Mild anaemia determined by Hb 120-129 g/l in males and 110-119 g/l in females Moderate anaemia determined by Hb 90-119 g/l in males and 80-109 g/l in females. Severe anaemia determined by Hb <90 g/l in males and <80 g/l in females. ID determined by predominantly microcytic indices (MCV <80 fl) and hypochromic indices (MCH <26 pg/cell), confirmed by low serum ferritin (<10 ng/ml in females and <29 ng/ml in males).	The mean Hb at baseline in anaemic patients (62±21 g/l) was significantly lower than that in the control group (134±6 g/l) ($p<0.01$). The mean Hb after 2 months (125±10 g/l) was significantly higher than at baseline ($p<0.01$). However, this was still lower than controls ($p<0.01$).	Mean HbA_{1c} at baseline in anaemic patients (4.6%) was significantly lower than that in the control group (5.5%) ($p<0.05$). Mean HbA_{1c} after 2 months (5.9±0.6%) was significantly higher than at baseline ($p<0.01$).	A significant increase was observed in the absolute HbA_{1c} levels at 2 months after treatment (0.29 g/dl vs 0.73 g/dl, $p<0.01$).	This study found decreased HbA_{1c} levels at baseline and a rise in HbA_{1c} with iron supplementation; these results are in complete contrast to the majority of other studies. No explanation of the results was presented.
Son et al, 2013 [15]	329 (111)	This study examined anaemic individuals who were drug naive and suspected of having diabetes and compared with age- and sex-matched controls. Participants underwent an OGTT and HbA_{1c} values were simultaneously assessed.	Diabetes determined by FPG ≥7.0 mmol/l or ≥11.1 mmol/l, 2 h after glucose load Pre diabetes: FPG 5.6-69 mmol/l or 2 h glucose 7.8-11.0 mmol/l Anaemia determined by Hb<130 g/l in men and <120 g/l in women.	Anaemic group Hb: 113±11 g/l Control group 142±13 g/l ($p<0.001$)	In the normoglycaemic groups (by FPG) there was no difference in HbA_{1c} values between anaemic and controls. In prediabetes groups, HbA_{1c} was 6.4±1.0% vs 6.1±0.7% in anaemic vs controls ($p=0.05$).	The comparisons of HbA_{1c} in anaemic and controls for 2 h glucose levels were similar but showed borderline significance. Glucose-based distributions were not significantly different between anaemic and non-anaemic groups.	Stratifying patients by HbA_{1c} showed an increased sensitivity in anaemic group but decreased specificity. The authors suggest that a diagnosis of DM by HbA_{1c} in anaemia may have decreased diagnostic significance but acknowledge that study numbers are small and the types of anaemia were not defined.

ESM Table 2 provides a detailed version of this table, with inclusion and exclusion criteria and dual reporting of HbA_{1c} values (% and mmol/mol)

CRF chronic renal failure, *DM* diabetes mellitus, *GA* glycated albumin, *ID* iron deficiency, *IDS* iron-deficient state, *IGT* impaired glucose tolerance, *NIS* normal iron state, *NK* not known

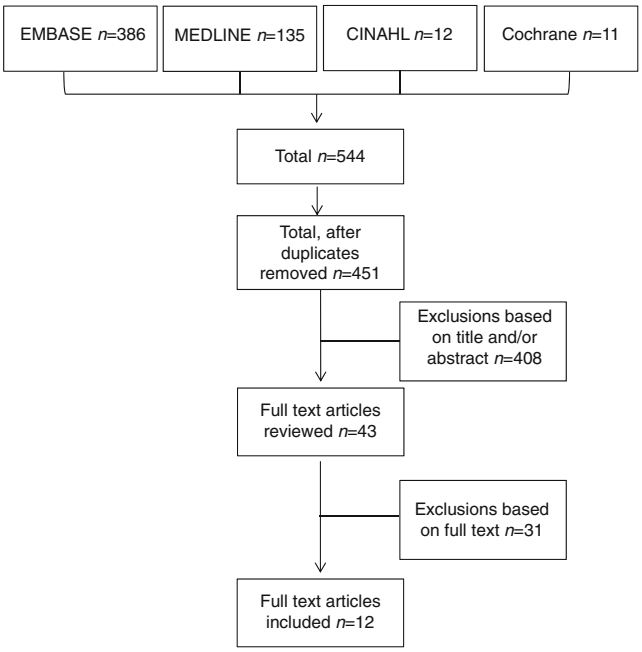

Fig. 1 Flow chart detailing the search strategy employed

Studies comparing prevalence of diabetes and prediabetes diagnosed by glucose or HbA_{1c}

Two studies [14, 15] compared the prevalence of diabetes/prediabetes determined by glucose-based criteria and HbA_{1c}-based criteria. Hardikar et al [14] compared HbA_{1c} and OGTT determined diabetes rates in a cohort of young adults in India. The authors found that the prevalence of diabetes or prediabetes was higher with HbA_{1c} criteria than by standard OGTT (25.9% vs 10.4%). Within a subgroup of anaemic (IDA, B12 and folate deficiency) patients, the discordance was even greater with 33% classed as prediabetes or diabetes by HbA_{1c} compared with 12% by OGTT.

Son et al [15] grouped 329 Korean participants, not previously known to have diabetes, by diabetes status according to OGTT results. Participants were grouped as normal, prediabetes or diabetes, HbA_{1c} levels were then compared in each group for both anaemic and non-anaemic participants. In the normoglycaemic group, HbA_{1c} values were found to be the same in both anaemic and non-anaemic groups. In the prediabetes group, HbA_{1c} values were found to be higher in the anaemic group compared with controls ($p=0.05$) and borderline significant in the diabetes group. The authors acknowledge that small sample sizes and a lack of definition of types of anaemia are confounding factors in their study.

Studies comparing HbA_{1c} and glucose values in patients without diabetes, with and without IDA

One study [16] compared the HbA_{1c} values in 50 individuals with IDA, not previously known to have diabetes, with non-anaemic healthy controls. This was a short article with limited description or discussion but essentially both FPG and postprandial glucose values were not significantly different between the IDA and control groups. HbA_{1c} values however were significantly higher in the IDA group (mean 7.6±0.5% [60±5.5 mmol/mol]) compared with the control group (5.5±0.8% [37±8.7 mmol/mol]; $p<0.001$).

Studies evaluating the effects of treatment, to resolve anaemia, on HbA_{1c}

Four studies [17–20] evaluated HbA_{1c} levels in non-diabetic patients, pre and post treatment for anaemia. Each study included patients treated with oral iron replacement therapy, three studies [17–19] reported a significant fall in HbA_{1c} values after treatment with iron replacement over periods of 9–20 weeks.

Coban et al [17] saw a fall from an HbA_{1c} of 7.2±0.8% (55±8.7 mmol/mol) pre treatment to 6.2±0.6% (44±6.6 mmol/mol) post 3 months treatment but this was still not as low as the non-anaemic control group 5.2±0.2% (33±2.2 mmol/mol); however, the iron indices had not fully normalised compared with controls at that point in time either. The same pattern was demonstrated by El-Agouza et al [18] where patients were followed for 20 weeks and HbA_{1c} fell steadily over that period from 6.15±0.62% to 5.25±0.45% (44±6.8 mmol/mol to 34±4.9 mmol/mol). However, there were no control participants included for comparison and it would be of interest to know the point at which HbA_{1c} values plateaued post treatment. Similarly, a small study ($n=10$) [19], showed a significant decline ($p<0.01$) in HbA_{1c} values by 3 weeks of treatment and the values remained below baseline values after 9 weeks, despite an upward rebound.

Interestingly, a study by Sinha et al [20] showed the converse pattern of results in 50 patients with moderate to severe IDA (mean±SD Hb, 62±1 g/l). The mean HbA_{1c} was significantly lower in the anaemic group compared with the non-anaemic controls (4.6±0.6% vs 5.5±0.6%; 27±6.6 mmol/mol vs 37±6.6 mmol/mol). After 2 months of treatment for IDA, the HbA_{1c} values were significantly higher than the controls raising the question of whether severe iron deficiency has the same effect on glycation as more mild anaemia.

Studies investigating the effect of menstruation on HbA_{1c} levels

Three studies [21–23] investigated the impact of the premenopausal state on HbA_{1c} values. Behan [21] compared Hb values in non-diabetic, non-anaemic premenopausal women with postmenopausal white women. The study found that Hb levels were significantly lower in premenopausal women, despite no reported history of anaemia. HbA_{1c} values were not significantly different between the two groups but the correlation between HbA_{1c} and FPG was weaker in the

premenopausal group. The author suggests that the varying effects of menstruation on iron metabolism could lead to less reliable HbA_{1c} and FPG correlations.

Koga [22, 23] and colleagues also looked at the effect of the menopause on HbA_{1c} values. The initial study identified a significant difference in HbA_{1c} levels between pre- and postmenopausal women (4.9±0.2% vs 5.1±0.3% [30±2.2 mmol/mol vs 32±2.2 mmol/mol] $p<0.0001$). In premenopausal women, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were inversely correlated with HbA_{1c} but this association was not significant in postmenopausal women. The authors suggest that every 1 pg decrease in MCH correlated with a 0.03% (0.3 mmol/mol) increase in HbA_{1c} value and that erythrocyte indices influence HbA_{1c} values in premenopausal women in the absence of overt anaemia. Koga et al [23] also looked at the effect of iron deficiency and IDA compared with iron sufficiency on HbA_{1c} and glycated albumin values in premenopausal women. Iron deficiency and IDA were both associated with a significant increase in HbA_{1c} levels with iron metabolism indices being significantly negatively associated with HbA_{1c}. This association was not present in glycated albumin levels across the three iron status groups, essentially indicating that iron metabolism rather than glycaemic changes influence changes in HbA_{1c} in premenopausal women.

Studies evaluating erythrocyte indices and markers of iron status

All studies were reviewed for data on erythrocyte indices and markers of iron status as these provide further insight into which adjunct tests may support the use of HbA_{1c} for diagnosis. Data extracted are detailed in Table 3. Combinations of the following indices were measured: Hb, packed cell volume (PCV), MCV, MCH, MCH concentration (MCHC), ferritin, transferrin saturation (TSAT), reticulocytes, red cell distribution width (RDW) and erythrocyte protoporphyrin. Some studies only used the values to identify or exclude patients with iron deficiency or anaemia whereas others correlated changes in erythrocyte indices with changes in HbA_{1c} value. Of the studies that focused on IDA, three studies compared values pre and post treatment with iron replacement and two compared values in patients with anaemia against control participants. All studies demonstrated an increase in MCV and MCH with treatment or higher levels in controls compared with anaemia patients. Two studies [14, 22] demonstrated an inverse correlation between HbA_{1c} and Hb, MCV and MCH levels. Overall, the data indicate that iron deficiency, demonstrated by low Hb, low MCV and low MCH, is associated with increased HbA_{1c} levels both with and without overt anaemia. In addition, normal MCV and MCH with low Hb would not be associated with increased HbA_{1c} levels but rather a decrease in values.

Ferritin was measured in nine studies [12–14, 16–18, 20, 21, 23]. Of these, most showed an increase in ferritin levels post treatment for anaemia and also showed that ferritin levels were lower in iron-deficient participants compared with controls. Three studies [14, 16, 23] showed that ferritin was a negative predictor of HbA_{1c} and one study [13] showed a small but significant positive correlation between HbA_{1c} and ferritin. Generally, ferritin was utilised as a marker of iron deficiency rather than analysed as an independent predictor of HbA_{1c} values; where the latter was done, the results were mixed.

Discussion

Does anaemia affect HbA_{1c}?

Generally, the studies investigating the effect of anaemia and abnormalities of erythrocyte indices on HbA_{1c} analysis are limited to small sample groups and two studies that both analysed the same large cross-sectional population study. It is apparent that IDA can have a significant impact on HbA_{1c} values with most studies suggesting a spuriously high HbA_{1c} in IDA compared with other markers of glycaemia. The exception to this is the study by Sinha et al [20], which found low values of HbA_{1c} in severe cases of IDA that increased with iron replacement therapy. The authors did not discuss why their data conflicts with other studies but it may be due to the severity of anaemia as the participants in this study had low mean Hb levels (62 g/l); the duration of anaemia was not given. Together, these data support the hypothesis that iron deficiency per se may cause elevated HbA_{1c} values, irrespective of anaemia.

Limited data indicate that non-IDAs also affect HbA_{1c} values to a varying degree [13, 14, 19]. Ford et al [13] showed that in patients without diabetes, with low Hb but normal iron levels had significantly lower HbA_{1c} values than those with normal iron and normal Hb (see Table 2). The difference between the non-IDA and control values was greater than was observed between IDA and iron deficiency values and controls, suggesting that HbA_{1c} may be spuriously elevated in iron deficiency and spuriously depressed in non-IDAs. Ideally, any further studies would separate out iron deficiency and non-iron deficiency cases prior to analysis as there is the potential to null the data by combing the two pathologies in one evaluation.

Hardikar et al [14] investigated a population where of those with anaemia 30.8% had vitamin B12 deficiency, 15% had folate deficiency and 30% had multiple nutrient deficiencies. In multivariate analysis, B12 and folate were not significantly related to HbA_{1c} levels but this may be due to the small sample size analysed. Where mixed nutrient deficiencies are observed, the combination of pathologies will lead to variable and unpredictable effects on HbA_{1c} levels depending on the relative degree of each nutrient deficiency.

Table 3 Correlations of erythrocyte indices and markers of iron status with HbA_{1c}

Study	Erythrocyte indices measured	Main observations	Conclusions	Notes
Behan, 2006 [21]	Hb, MCV and RDW	Hb significantly lower in premenopausal women, no difference for MCV and RDW	No conclusions drawn regarding erythrocyte indices.	Whilst Hb was lower, none of the patients had low ferritin, to rule out iron deficiency.
Coban et al, 2004 [17]	Hb, PCV, MCV, MCH, ferritin	Hb, PCV, MCV, MCH and ferritin ↓in the IDA group compared with controls. Post treatment no significant differences between groups.	Treatment with iron appears to normalise the erythrocyte indices along with a reduction in HbA_{1c} values at 3 months.	Erythrocyte indices used to demonstrate normalisation in patients with anaemia. RDW values may have assisted in assessing the heterogeneity of the erythrocyte population.
El-Agouza et al, 2002 [18]	Hb, ferritin, PCV, MCV, MCH, MCHC, RDW	Participants had microcytic, microchromic indices—not all had ↓ ferritin levels—not all ID. Post iron replacement in 51 iron-deficient participants—all indices significantly increased except MCHC (small decrease).	Treatment with iron appears to normalise the erythrocyte indices along with a reduction in HbA_{1c} values. Erythrocyte indices used to demonstrate normalisation of iron status.	RDW mean was significantly higher than the normal range pre treatment and even higher post treatment showing a very mixed cell type profile at 20 weeks of treatment. This would indicate that erythrocyte indices may not stabilise even at 20 weeks.
Ford et al, 2011 [13]	Hb, PCV, MCV, MCH, MCHC, ferritin, TSAT	Pearson correlations: HbA_{1c} ↑as ferritin ↑ HbA_{1c} ↑ as MCH and MCV ↓	Not as expected in the face of IDA: ↓Ferritin, MCH and MCV would be expected with ↑$HbA_{1c.}$	MCV and MCH may be better markers for ID and IDA than ferritin.
Gram-Hansen et al, 1990 [19]	Hb, MCV, reticulocytes	Hb ↑ and MCV ↑, HbA_{1c} ↓with iron replacement Hb ↑, MCV ↓ and HbA_{1c} ↓ with B12 replacement	MCV used to differentiate between IDA and non-IDA.	Very limited study and duration of follow-up.
Hardikar et al, 2012 [14]	Hb, PCV, MCV, MCH, MCHC, ferritin	Hb, MCV, MCH, MCHC negatively correlated with HbA_{1c}	Markers of microchromic anaemia were associated with increased HbA_{1c} values in people who were normal by OGTT.	MCV did not show a significant change in pre and post treatment groups, despite negative correlations to HbA_{1c}.
Kim et al, 2010 [12]	Erythrocyte protoporphyrin, ferritin, TSAT	Indices measured but only used to identify IDA	None can be drawn.	
Koga et al, 2007 [22]	RBC, Hb, PCV, MCH, MCV	Hb, MCH, MCV had a statistically significant negative correlation with HbA_{1c} in premenopausal women only	↓ Hb, MCH, MCV associated with ↑HbA_{1c}, indicates ID assessed by MCH and MCV, correlates to ↑HbA_{1c} with no change in glucose levels.	No history of diabetes or anaemia in cohort.
Koga et al, 2010 [23]	Iron, ferritin, TSAT	Serum iron, TSAT and log ferritin inversely associated with HbA_{1c}	Low iron indices associated with ↑$HbA_{1c.}$	MCH and PCV measured and decreased with IDA but not correlated to $HbA_{1c.}$
Shanthi et al, 2013 [16]	Hb, PCV, MCV, MCH, Ferritin	All ↓ in IDA group, HbA_{1c} ↑in this group	Statistical significance is unclear.	Very poorly described results.
Sinha et al, 2012 [20]	Ferritin	↓Hb and ↓ferritin associated with ↓HbA_{1c}, all increased with iron replacement therapy	HbA_{1c} appear to correlate with Hb and ferritin.	MCV, MCH, Hb used to differentiate type of anaemia but values not reported.
Son et al, 2013 [15]	Hb, MCV, MCH,	Hb, MCV and MCH all ↓ in 'anaemic' group defined only by Hb	No correlations made to $HbA_{1c.}$	No differentiation between types of anaemia in the study although mean MCV and MCH suggest predominantly IDA.

ID, iron deficiency

To what degree do abnormalities of erythrocyte indices affect HbA_{1c} values?

The two evaluations of the cross-sectional NHANES data showed that having iron deficiency or IDA increased the odds of having an HbA_{1c} value shift upwards from <5.5% (<37 mmol/mol) to 5.5–5.9% (37–41 mmol/mol) [12, 13]. Hardikar et al [14] demonstrated that markers of microchromic anaemia were associated with increased HbA_{1c} values and also more patients diagnosed with prediabetes or diabetes by HbA_{1c} compared with glucose indices. In patients with non-IDA, there was a marked shift downwards in apparent distribution of HbA_{1c} results [13]. Son et al [15] demonstrated that IDA in the patients with prediabetes defined by glucose levels had a mean HbA_{1c} of 6.4% vs 6.1% (46 mmol/mol vs 43 mmol/mol) in controls; this is sufficient to re-categorise some patients from prediabetes to diabetes in the anaemic group.

Other studies which looked more specifically at HbA_{1c} levels in patients with or without anaemia showed differences of up to 2.1% (23 mmol/mol) HbA_{1c} increase with IDA [16] and studies measuring HbA_{1c} pre and post treatment for anaemia showed changes as large as −1.2% (−13 mmol/mol) HbA_{1c} post treatment [17].

What does this mean on a wider scale?

Recent data has shown a surge in the incidence and prevalence of young onset obesity, many of whom are premenopausal females, at increased risk of iron deficiency. There has been a concomitant increase in HbA_{1c} values in these participants considered at high risk of diabetes, owing to factors such as obesity [24]. Data from England shows that the prevalence of prediabetes rose from 11.6% in 2003 to 35.3% in 2011 [25] and in view of the rising prevalence of obesity, we would anticipate to identify a larger proportion of patients, with higher HbA_{1c} values. If the estimated upward shift in HbA_{1c} values seen with iron deficiency is combined with the apparent increased prevalence of prediabetes, it may result in a significant number of patients where the combination of the two will be sufficient to shift HbA_{1c} values to move from a diagnosis of prediabetes to diabetes.

Clinical use and relevance

One of the main questions still to answer is how to apply this information to everyday clinical practice. In addition to published guidance [4–6], we suggest the following:

1. During monitoring of people with diabetes, when glucose and HbA_{1c} are discordant, consider abnormalities of erythrocyte indices.
2. When HbA_{1c} is normal/elevated but Hb is low, do not assume that HbA_{1c} is falsely elevated—check erythrocyte indices, in particular MCV and MCH; if low, consider iron deficiency by TSAT or ferritin. If MCV and MCH are not low then consider other forms of anaemia—HbA_{1c} may be falsely decreased in these cases.
3. Iron deficiency, as well as IDA, may be sufficient to cause a change in HbA_{1c} values; this is highly relevant in women of childbearing age.
4. If abnormalities of erythrocyte indices or anaemia are identified, consider correction of the abnormality before using HbA_{1c} for diagnosis or monitoring. The studies included in this review suggest that it may take up to 6 months after treatment is initiated to normalise erythrocyte indices. RDW will provide an additional indicator of normalisation of the erythrocyte population and erythrocyte lifespan.

Ferritin as a marker of iron status is useful if it is low but would not rule out iron deficiency if it was found to be normal or elevated as ferritin is an acute phase reactant and inflammation may mask a true low ferritin. Raj and Rajan [26] investigated 86 patients with type 2 diabetes mellitus and demonstrated that serum ferritin positively correlated with HbA_{1c} and was increased with increasing duration of disease, indicating that poor glycaemic control can contribute to elevated ferritin levels independent of iron status. In situations of increased inflammation, alternative measures of iron status such as TSAT or total iron binding capacity should be considered.

Changes in erythrocyte lifespan even with normal haematological indices can affect HbA_{1c} values [27], therefore, it is important to show that the erythrocyte population is stable after treatment for abnormalities before using HbA_{1c}. Erythrocyte indices including RDW may assist with this.

Further research questions

Although it is clear that anaemia may influence HbA_{1c} results, further studies to identify the roles of erythrocyte indices as appropriate adjunct analyses to identify patients where this is an issue, are needed. Future studies should consider the effect of erythrocyte indices rather than anaemia alone. In particular, studies should aim to include sufficient participants to differentiate between the effects of the type and severity of erythrocyte abnormalities/anaemia on HbA_{1c} values. This may be achieved through a combination of population based studies and intervention studies where changes in HbA_{1c} values are assessed in relation to therapy for erythrocyte abnormalities and anaemias.

Strengths and limitations of this study

This review is limited to a selection of small study groups and two separate analyses of the NHANES survey data from overlapping time periods. The conclusions drawn are limited by a lack of robust evidence from a significant number of the included studies. The quality analysis of the included studies has highlighted shortfalls and confounders in most of the cases, which would need to be addressed in future studies—in particular, a lack of power to confer significance on many of the findings.

Notes on standardisation of HbA_{1c} methods

International standardisation of HbA_{1c} was achieved in 2002 [28–30]. An International HbA_{1c} Consensus Committee was formed which agreed that HbA_{1c} values should be reported in both SI units (mmol/mol) and converted to % units via the master equations established by the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) and designated comparison methods [10, 11]. All studies reported in this review either stated that they were aligned to the NGSP or did not state any performance information for the HbA_{1c} analysis. In light of this, even if there was sufficient data for meta-analysis, it is likely that a lack of standardisation of results would be a major confounder. Any further studies should endeavour to ensure that all HbA_{1c} measurements are performed in alignment with the IFCC and clear quality data should be provided in the reports.

Conclusion

It is clear from the limited number of studies, many with low participant numbers, that the subject of anaemia and HbA_{1c} warrants further investigation. Generally, the studies described demonstrate that abnormalities of erythrocyte indices are a considerable confounder in the analysis of HbA_{1c} and there is currently insufficient data to fully inform clinicians and scientists on how to address this in clinical practice. However, we have made some suggestions to facilitate its use whilst clarity is awaited.

The key questions that are still to be answered are whether anaemia and erythrocyte abnormalities will have a significant impact on the diagnosis of diabetes using HbA_{1c} in the general population—something that is now widely performed.

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References

1. The Diabetes Control and Complications Trial Research Group (1993) 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
2. UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–853
3. Inzucchi SE, Bergenstal RM, Buse JB et al (2012) Management of hyperglycemia in type 2 diabetes: a patient-centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care 35:1364–1379
4. World Health Organization (2011) Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus (abbreviated report of a WHO consultation)
5. American Diabetes Association (2013) Standards of medical care in diabetes–2013. Diabetes Care 36(Suppl 1):S11–S66
6. John WG (2012) Use of HbA1c in the diagnosis of diabetes mellitus in the UK. The implementation of World Health Organization guidance 2011. Diabet Med 29:1350–1357
7. United Nations Children's Fund, United Nations University, World Health Organization (2001) Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers. Available from www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf
8. Stevens GA, Finucane MM, De-Regil LM et al (2013) Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob Health 1:e16–25
9. Lowe G, Twaddle S (2005) The Scottish Intercollegiate Guidelines Network (SIGN): an update. Scot Med J 50:51–52
10. Consensus Committee (2007) Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care 30:2399–2400
11. Hanas R, John G, International Hb ACC (2010) 2010 consensus statement on the worldwide standardization of the hemoglobin A1c measurement. Clin Chem 56:1362–1364
12. Kim C, Bullard KM, Herman WH, Beckles GL (2010) 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
13. Ford ES, Cowie CC, Li C, Handelsman Y, Bloomgarden ZT (2011) Iron-deficiency anemia, non-iron-deficiency anemia and HbA1c among adults in the US. J Diabetes 3:67–73

14. Hardikar PS, Joshi SM, Bhat DS et al (2012) Spuriously high prevalence of prediabetes diagnosed by HbA(1c) in young Indians partly explained by hematological factors and iron deficiency anemia. Diabetes Care 35:797–802
15. Son JI, Rhee SY, Woo JT et al (2013) Hemoglobin A1c may be an inadequate diagnostic tool for diabetes mellitus in anemic subjects. Diabetes Metab J 37:343–348
16. Shanthi B, Revathy C, Manjula Devi AJ, Subhashree (2013) Effect of iron deficiency on glycation of haemoglobin in nondiabetics. J Clin Diagn Res 7:15–17
17. Coban E, Ozdogan M, Timuragaoglu A (2004) Effect of iron deficiency anemia on the levels of hemoglobin A1c in nondiabetic patients. Acta Haematol 112:126–128
18. El-Agouza I, Abu Shahla A, Sirdah M (2002) The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 24:285–289
19. Gram-Hansen P, Eriksen J, Mourits-Andersen T, Olesen L (1990) Glycosylated haemoglobin (HbA1c) in iron- and vitamin B12 deficiency. J Intern Med 227:133–136
20. Sinha N, Mishra TK, Singh T, Gupta N (2012) Effect of iron deficiency anemia on hemoglobin A1c levels. Ann Lab Med 32:17–22
21. Behan KJ (2006) Cessation of menstruation improves the correlation of FPG to hemoglobin A1c in Caucasian women. Clin Lab Sci 19:225–230
22. Koga M, Morita S, Saito H, Mukai M, Kasayama S (2007) Association of erythrocyte indices with glycated haemoglobin in pre-menopausal women. Diabet Med 24:843–847
23. Koga M, Saito H, Mukai M, Matsumoto S, Kasayama S (2010) Influence of iron metabolism indices on glycated haemoglobin but not glycated albumin levels in premenopausal women. Acta Diabetol 47(Suppl 1):S65–S69
24. Selvin E, Parrinello CM, Sacks DB, Coresh J (2014) Trends in prevalence and control of diabetes in the United States, 1988–1994 and 1999–2010. Ann Intern Med 160:517–525
25. Mainous AG 3rd, Tanner RJ, Baker R, Zayas CE, Harle CA (2014) Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. BMJ Open 4:e005002
26. Raj S, Rajan GV (2013) Correlation between elevated serum ferritin and HbA1c in type 2 diabetes mellitus. Int J Res Med Sci 1: 12–15
27. Cohen RM, Franco RS, Khera PK et al (2008) Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. Blood 112:4284–4291
28. Hoelzel W, Weykamp C, Jeppsson JO et al (2004) IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 50:166–174
29. Jeppsson JO, Kobold U, Barr J et al (2002) Approved IFCC reference method for the measurement of HbA(1c) in human blood. Clin Chem Lab Med 40:78–89
30. Weykamp C, John WG, Mosca A et al (2008) The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem 54:240–248