Utility of HbA assessment in people with diabetes awaiting liver transplantation

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DOI: 10.1111/dme.13870

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Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Link to publication on Research at Birmingham portal

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Download date: 04. Aug. 2019
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Utility of HbA1c assessment in people with diabetes awaiting liver transplantation


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Accepted 22 November 2018

Abstract

Aims To investigate the relationship between HbA1c and glucose in people with co-existing liver disease and diabetes awaiting transplant, and in those with diabetes but no liver disease.

Methods HbA1c and random plasma glucose data were collected for 125 people with diabetes without liver disease and for 29 people awaiting liver transplant with diabetes and cirrhosis. Cirrhosis was caused by non-alcoholic fatty liver disease, hepatitis C, alcoholic liver disease, hereditary haemochromatosis, polycystic liver/kidneys, cryptogenic/non-cirrhotic portal hypertension and α1-antitrypsin-related disease.

Results The median (interquartile range) age of the diabetes with cirrhosis group was 55 (49–63) years compared to 60 (50–71) years (P=0.13) in the group without cirrhosis. In the diabetes with cirrhosis group there were 21 men (72%) compared with 86 men (69%) in the group with diabetes and no cirrhosis (P=0.82). Of the group with diabetes and cirrhosis, 27 people (93%) were of white European ethnicity, two (7%) were South Asian and none was of Afro-Caribbean/other ethnicity compared with 94 (75%), 16 (13%), 10 (8%)/5 (4%), respectively, in the group with diabetes and no cirrhosis (P=0.20). Median (interquartile range) HbA1c was 41 (32–56) mmol/mol [5.9 (5.1–7.3)%] vs 61 (52–70) mmol/mol [7.7 (6.9–8.6)%] (P<0.001), respectively, in the diabetes with cirrhosis group vs the diabetes without cirrhosis group. The glucose concentrations were 8.4 (7.0–11.2) mmol/l vs 7.3 (5.2–11.5) mmol/l (P=0.17), HbA1c was depressed by 20 mmol/mol (1.8%; P<0.001) in 28 participants with cirrhosis but elevated by 28 mmol/mol (2.6%) in the participant with α1-antitrypsin disorder. Those with cirrhosis and depressed HbA1c had fewer larger erythrocytes, and higher red cell distribution width and reticulocyte count. This was reflected in the positive association of glucose with mean cell volume (r=0.39) and haemoglobin level (r=0.49) and the negative association for HbA1c (r=−0.28 and r=−0.26, respectively) in the diabetes group with cirrhosis.

Conclusion HbA1c is not an appropriate test for blood glucose in people with cirrhosis and diabetes awaiting transplant as it reflects altered erythrocyte presentation.

Diabet. Med. 00: 1–9 (2018)

Introduction

Diabetes is a leading cause of liver disease, with cirrhosis responsible for a considerable number of deaths in people with diabetes in the USA [1]. The association is mediated by multiple mechanisms including dyslipidaemia and altered hepatic fatty acid processing [2]. Peripheral insulin resistance may contribute to the development of diabetes in people with hepatitis...
What’s new?

- HbA1c may not be an accurate reflection of blood glucose for the diagnosis/monitoring of diabetes in people with other illnesses or on certain drugs; people with diabetes and liver disease awaiting transplantation are one such group.
- HbA1c was found to be depressed relative to random plasma glucose by 20 mmol/mol in people with diabetes and cirrhosis (n = 28) compared to people with diabetes but no liver disease (n = 125); however, HbA1c was elevated in one person with cirrhosis attributable to α-1-antitrypsin disorder.
- Compromised HbA1c may be related to haematological differences associated with liver disease involving erythrocyte half-life, with shorter/longer times giving less/more opportunity for glycation of haemoglobin.

C [3] and cirrhosis [4]. Post-transplant diabetes is well recognized, with HbA1c testing not being appropriate immediately afterwards as a result of post-transplant anaemia [5] and also rendered inaccurate by some drugs such as ribavirin which is used for hepatitis C treatment [6].

In 2011, the WHO introduced HbA1c assessment for the diagnosis of diabetes mellitus [7]. HbA1c is now widely used for this purpose in primary care, resulting in a doubling of the number of HbA1c assessments requested, and a corresponding decrease in glucose measurement [8]. Since 2014, the use of HbA1c testing has been included in the American Diabetes Association guidelines for the diagnosis of diabetes in hospital [9]. This recommendation has been confirmed by assessment of undiagnosed diabetes in white European people admitted to an Irish hospital [10]. However, whilst the WHO bulletin lists medical conditions and drugs that may affect HbA1c, it provides no references to quantitative evidence [7].

Our hospital laboratory has reviewed HbA1c test results, referring values below the reference range or very high values in people without a previous diagnosis of diabetes for urgent medical attention [8,11]. Evidence is accumulating that various co-existing conditions affect HbA1c and result in misdiagnosis or mismanagement of diabetes [12]. Recently, HbA1c was measured in 200 people with decompensated cirrhosis referred for liver transplantation. Measured HbA1c values were significantly lower when compared with HbA1c calculated from three previous glucose values [13].

Given these concerns, we investigated random plasma glucose and HbA1c in people recruited for research into the relationships between glycaemic markers when attending diabetes clinics at the hospital, and in people with co-existing cirrhosis and diabetes awaiting liver transplant who had available data on glycaemic markers and Model for End Stage Liver Disease (MELD) scores [14].

Participants and methods

Ethics

The West Midlands Local Research Ethics Committee confirmed ethical approval for the Glucose Fructosamine and HbA1c research study investigating the relationships between glycaemic markers in people attending the diabetes clinic at University Hospitals Birmingham NHS Foundation Trust. This study met the requirements of the current revision of the Declaration of Helsinki.

For people with diabetes attending liver clinics between June and September 2012, data were obtained from the electronic patient record for a registered, internal clinical audit (CAB-05641-13) at University Hospitals Birmingham NHS Foundation Trust.

Study cohort

The people with diabetes without liver cirrhosis included adults with no variant haemoglobin (n=125) who were recruited from the diabetes clinic at Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, UK, between June 2007 and June 2009.

The people with co-existing liver cirrhosis and diabetes comprised people from different parts of the UK with cirrhosis of the liver and diabetes, attending day clinics in the Liver Department at Queen Elizabeth Hospital Birmingham, UK, who were being considered for liver transplantation. Those attending between October 2008 and June 2012 were included in the clinical audit; in total, 240 people were reviewed. HbA1c and random plasma glucose measurements, performed at Queen Elizabeth Hospital Birmingham laboratories, were available for 29 out of the 50 transplant candidates with both cirrhosis and diabetes. No other measure of glycaemic control was available to the study. Indications for transplant included one or more of the following complications of cirrhosis: spontaneous bacterial peritonitis; ascites; variceal bleed; and hepatic encephalopathy. Of the 29 participants in this cohort, 15 (52%) had non-alcoholic fatty liver disease, six (21%) had hepatitis C, three (10%) had alcoholic liver disease, two (7%) had hereditary haemochromatosis, one (3%) had polycystic liver and kidneys, one (3%) had α-1-antitrypsin-related liver disease and one (3%) had cryptogenic/non cirrhotic portal hypertension. Data were collected for this preliminary, clinical audit from the Birmingham Systems Prescribing Information and Communications System and the CDS Telepath Systems Ltd databases.

Measurements

All measurements were performed at University Hospitals Birmingham NHS Foundation Trust, with single measurements of HbA1c, random plasma glucose, serum bilirubin and creatinine, and full blood count. Blood was collected...
into fluoride oxalate vacutainers for glucose measurement. Biochemical variables were measured on Roche c8000 analysers (Roche Diagnostics Ltd, Burgess Hill, UK) and full blood count on Beckman DxH800 analysers (Beckman Coulter Ltd, High Wycombe, UK).

HbA1c was measured in EDTA blood using an International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) aligned TOSOH G8 ion exchange high performance liquid chromatography analysers (Tosoh, Reading, UK) before realignment of their calibrator downwards by the manufacturer in September 2013 [15]. People with abnormal haemoglobin were excluded because HbA1c is not reported by the laboratory in its presence, as were people with a total chromatogram area <500 as specified in the manufacturer’s protocol for HbA1c measurement (~80 g/l haemoglobin).

The MELD score was calculated using the formula: \[0.957 \times \ln(\text{serum creatinine}) + 0.378 \times \ln(\text{serum bilirubin}) + 1.120 \times \ln(\text{INR}) + 0.643\] \times 10, with creatinine set to 4.06 for participants on haemodialysis [14]. The normal range for the MELD score is 0 to 6, with a score of 40 defined as gravely ill.

Statistical analysis

Data on participants without liver disease were entered into an Excel spreadsheet with robust quality assurance. Biochemical and haematological data were downloaded directly from the laboratory Telepath database. Clinical audit data for participants with diabetes and liver disease were accessed in the electronic patient record and entered into a pre-prepared Excel spreadsheet. Data were analysed with Microsoft Excel, Analyse-it Version 2.22 (Analyse-it Software Ltd, Leeds, UK), SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA) and R version 3.4.0 [16].

The characteristics of the study cohort are presented in Table 1 as median and interquartile range (IQR), count or percentage, with Mann–Whitney or Fisher’s exact tests used to compare the groups. Reference ranges were obtained from the hospital laboratory. Simple linear regression was used to assess the relationships between HbA1c and random plasma glucose, with Fig. 1 showing regression lines for both groups. Reference ranges were obtained from the Fisher Z-score equivalents of the two correlation matrices when divided by the standard error of the difference between the two Z-scores. To confirm the assumption that the groups are two distinct populations, separable by the variables measured, a test of equivalence of the Fisher Z-score equivalents of the two correlation matrices was performed, which indicated two distinct groups (\(P<1.2e^{-06}, \text{Z-score of differences} = 4.98\)).

The profile of the participant with α-1-antitrypsin-related liver disease was summarized graphically by expressing each value as a multiple of the median value for that variable in the group of people with diabetes without liver cirrhosis. The median values for the people with co-existing cirrhosis and diabetes disease (excluding the person with α-1-antitrypsin-related liver disease) were plotted similarly, Fig. 3.

Results

Characteristics of participants

There were no significant differences in age, gender or ethnicity, but serum creatinine was significantly lower in people with diabetes and cirrhosis (\(P=0.001, \text{Table 1}\)). Two distinct populations were identified when all the variables were considered (\(P<0.001\)).

Glucose and HbA1c

Random plasma glucose concentrations did not differ, but HbA1c was substantially lower in people with liver disease.
median (IQR) 41 (32–56) mmol/mol [5.9 (5.1–7.3)%] vs 61 (52–70) mmol/mol [7.7(6.9–8.6)%]; \(P<0.001\), Table 1 and Fig. 1).

HbA1c and glucose were positively correlated: \(r^2=0.34\) in those with liver disease and \(r^2=0.30\) in those without (\(P<0.001\)). Linear regression equations are cited in mmol/m mol (IFCC) and % (Diabetes Control and Complications Trial/UK Prospective Diabetes Study) units:

- liver disease: (mmol/mol) \(\text{HbA1c} = 3.0 \times \text{RPG} + 15.5\); or
- (%) \(\text{HbA1c} = 0.27 \times \text{RPG} + 3.6\)

- no liver disease: (mmol/mol) \(\text{HbA1c} = 1.8 \times \text{RPG} + 46.3\); or
- (%) \(\text{HbA1c} = 0.17 \times \text{RPG} + 6.4\),

where RPG is random plasma glucose. There was a significant difference of 20 mmol/mol (1.8%) HbA1c \((P<0.001)\) between the intercepts, assuming the slopes to be equal \((P=0.12)\). A similar result was obtained when the data were restricted to white European people.

**Haematology**

There were major haematological differences between the groups, with fewer red blood cells, and lower haemoglobin and haematocrit levels in the group with diabetes and cirrhosis, with the median values lower than the reference ranges (Table 1). The red blood cell distribution width was higher in those with liver disease and above the reference range. The equivalent values for those with diabetes but no cirrhosis were within the reference ranges. Higher values for mean red blood cell volume and mean red blood cell haemoglobin were found in the group with diabetes and cirrhosis, indicating larger red blood cells, and also a higher reticulocyte count, indicating a shorter half-life. People with

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<th>Table 1 Characteristics of people with liver cirrhosis and diabetes awaiting transplant vs people with diabetes but no liver disease</th>
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Median (IQR) interquartile range; otherwise \(n\) or %.

*Median higher than reference range. †Creatinine reference ranges dependent on age and gender. ‡Median lower than reference range.
diabetes and cirrhosis had fewer white blood cells, platelets and lymphocytes with no difference in eosinophils; all these counts were within the reference ranges in both groups.

**Associations among variables**

Further investigation was undertaken to determine the factors related to the depression of HbA1c in those with cirrhosis using a correlation grid (Fig. 2).

There were significant differences in the magnitude and direction of correlation coefficients for glucose between the groups: with mean cell haemoglobin: $r=-0.092$ (95% CI $-0.265, 0.064$) vs $r=0.488$ (95% CI $0.133, 0.732$; $P=0.010$) in the diabetes without cirrhosis group vs the diabetes and cirrhosis group, respectively; mean cell haemoglobin concentration: $r=-0.110$ (95% CI $-0.281, 0.069$) vs $r=0.363$ (95% CI $-0.018, 0.653$), respectively ($P=0.003$, Fig. 2).

The correlation coefficients for HbA1c or glucose with the haematological variables showed statistically significant differences in the group with diabetes and cirrhosis. The correlation coefficients were positive for glucose, and negative or near zero for HbA1c in (1) mean cell volume: HbA1c, $r=-0.278$ (95% CI $-0.595, 0.114$); glucose, $r=0.387$ (95% CI $0.225, 0.528$; $P=0.020$); (2) mean cell haemoglobin: HbA1c, $r=-0.260$ (95% CI $-0.583, 0.132$); glucose, $r = 0.488$ (95% CI $0.341, 0.612$; $P = 0.010$); (3) mean cell haemoglobin concentration: HbA1c, $r=-0.095$ (95% CI $-0.458, 0.296$); glucose, $r=0.363$ (95% CI $-0.018, 0.653$); ($P =0.049$, Fig. 2).

When stepwise regression models were applied in those with diabetes and liver disease, red blood cell count and eosinophils had an $R^2$ value of 45.7% for HbA1c, and mean cell haemoglobin and eosinophils an $R^2$ value of 39.9% for glucose. The most important factor determining HbA1c in people with diabetes but no liver disease was glucose.

**Severity of liver disease**

The median (interquartile range) Model for End Stage Liver Disease (MELD) score for the study cohort was calculated as 12 (9–17), (normal <6) in those with cirrhosis. The MELD score in people with co-existing liver cirrhosis and diabetes was negatively correlated with HbA1c ($r=-0.56$) and red blood cell count ($r=-0.60$). The correlation with glucose was $r=-0.12$, but MELD was positively correlated with mean cell haemoglobin, ($r=0.32$) and red cell distribution width ($r=0.41$).

**a-1-antitrypsin disorder**

The person with cirrhosis and diabetes related to a-1-antitrypsin disorder had high HbA1c relative to glucose, with HbA1c elevated by 28 mmol/mol (2.6%), (Fig 1). Their
haematological profile was different from that of the other people with cirrhosis and those without cirrhosis. The plot of haematological data for the person with α-1-antitrypsin disorder and for others awaiting transplant (as a multiple of the median for the group without liver disease) shows the differences in their anaemic profiles (Fig. 3).

Discussion

Cirrhosis of the liver in people with diabetes awaiting a liver transplant renders HbA1c unsuitable for assessing blood glucose. In all but one person, it was associated with fewer, larger, more irregular red blood cells. A substantial depression in HbA1c [20 mmol/mol (2%)] was observed relative to those with diabetes but no cirrhosis across a wide range of glucose values. This probably reflects a shorter red blood cell half-life and less exposure of haemoglobin to glucose. In contrast, the person with cirrhosis related to α-1-antitrypsin disorder had a higher HbA1c level relative to glucose, with no factors indicating anaemia, suggesting the red blood cell half-life might be longer with more exposure to glucose.

This effect on HbA1c in people with cirrhotic liver disease will cause misdiagnosis of diabetes and inappropriate clinical care. In our routine clinical practice, many more depressed
than elevated HbA1c results have been noticed. We previously reported overtreatment resulting in hospital admission in one individual with known thalassaemia as a result of elevated HbA1c relative to glucose levels [12]. HbA1c assays do not identify thalassaemia, although some HbA1c analysers identify variant haemoglobins (e.g. S, F, C, D, E or rarer types) on chromatograms.

A recent US study in 200 people (62 with diabetes) referred for liver transplantation with decompensated cirrhosis showed similar depression in HbA1c relative to glucose levels [13]. HbA1c assays do not identify thalassaemia, although some HbA1c analysers identify variant haemoglobins (e.g. S, F, C, D, E or rarer types) on chromatograms.

A recent US study in 200 people (62 with diabetes) referred for liver transplantation with decompensated cirrhosis showed similar depression in HbA1c relative to glucose levels [13]. HbA1c calculated from previous glucose results and compared to measured HbA1c [19], was found to be discordant by >0.5% in 49% of participants and >1.5% in 12% overall. Multivariate model analysis found haemoglobin to be the only independent predictor of the larger HbA1c discrepancies. More evidence is required regarding the extent of the effects of liver disease on the accuracy of HbA1c for clinical guidelines to improve on the diagnosis of diabetes and its management.

The groups differed distinctly when their biochemistry and haematology were compared (Table 1 and Figs 2 and 3). The relationships of glucose and HbA1c to red blood cell haematology in people with diabetes and cirrhosis were markedly different from those in people with diabetes but no cirrhosis (Fig. 2). Low haemoglobin and macrocytosis evident in those with cirrhosis and diabetes were associated with depression in HbA1c. The exception being the person with α1-antitrypsin disorder whose erythrocytes did not display these features and whose HbA1c was elevated relative to glucose level. Anaemia can result in either shorter or longer erythrocyte lifespans and even differences in normal red blood cell morphology have been shown to affect the accuracy of HbA1c [20].

Any suspected inaccuracy in HbA1c can be confirmed using fructosamine, unless proteinuria is present [21], and point-of-care blood glucose testing or non-invasive continuous blood glucose devices. The data presented on >100 people attending the diabetes centre (along with corresponding fructosamine results) are used in our hospital to identify any outliers in glycaemic markers. As such, an elevated HbA1c relative to glucose level shows when additional testing, such as fructosamine/continuous blood glucose monitoring, should be organized by clinicians to confirm whether HbA1c is suitable for assessing glycaemic status. Monitoring glycaemia during the post-liver-transplant period is also an issue, as it is well known that post-transplant anaemia renders HbA1c unsuitable for clinical interpretation for ~6 months [5,22]. It is not known if this problem is resolved after liver transplantation.

Limitations of this study include the small number of people (29) studied with cirrhosis and diabetes compared to the available sample with diabetes but no cirrhosis (125). This sample size may hinder its ability to demonstrate statistical differences between the slopes of the regression lines. HbA1c was depressed by 25 mmol/mol (2.3%), (P<0.001), when the study was limited to age-matched white
European people with liver disease (mean age 55.6 years) compared to those without liver disease (mean age 55.3 years). As most of the participants were white European, it cannot throw any light on the current discussion about the relationship of HbA1c to glucose by ethnicity [23]. Although random plasma glucose was measured rather than fasting, this reflects routine hospital practice as is evident in other studies [10]. Its measurement on glucose meters or blood gas machines quality-assured by the laboratory, or measured in the laboratory, is a quality indicator at the hospital. The number of people studied pre-transplant was small but it should be noted that the clinical audit was generated by observations of inaccurate HbA1c in people with liver disease by experts in glycaemic markers over several years of routine clinical practice. Meta-analyses of small studies are common, with confirmatory studies required for clinical guidelines. Future research by our group will include more people with conditions that affect HbA1c as outlined by WHO on more than one clinic visit [6].

In conclusion, cirrhosis of the liver affects the accuracy of HbA1c results, leading to unreliable estimates of blood glucose over the previous 2 to 3 months. Anaemia in people with cirrhosis awaiting liver transplant is associated with altered red blood cell morphology. Significantly depressed HbA1c was observed in all but one person with cirrhosis, along with lower haemoglobin level and fewer, larger, less uniform red blood cells. Visual representation of HbA1c and random plasma glucose, along with haematology, is useful for assessing whether HbA1c is accurate in individuals with coexisting illnesses or on drug regimens that affect red blood cells. Treatment targets for HbA1c arising from clinical trials in diabetes [24,25] and cut-off values for diagnosis [7,23,26] rely on the provision of HbA1c values that reflect circulating glucose.

Funding sources
The G. A. Roberts Research Fund, Queen Elizabeth Hospital Birmingham Charity, Tosoh Europe and Novo Nordisk UK Research Foundation provided funding for the Diabetes Translational Research Group based at Queen Elizabeth Hospital Birmingham. The Arthur Thompson Trust at the University of Birmingham Medical School and the Queen Elizabeth Hospital Birmingham Charity provided financial support for conference costs for D.B. and S.V. when these data were presented at the American Diabetes Association 74th Scientific Sessions, 2014 and Diabetes UK, 2015. Research by J.A.W. reported in this publication was supported by the National Human Genome Research Institute of the National Institutes of Health under Award Number UM1HG006370. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. G.V.G. acknowledges support from H2020-EINFRA (731075) and the National Science Foundation (IOS:1340112) as well as support from the NIHR Birmingham ECMC, NIHR Birmingham SRMRC and the NIHR Birmingham Biomedical Research Centre and the MRC HDR UK. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research, the Medical Research Council or the Department of Health. The funding organizations had no role in the design of this study, data collection, analysis or interpretation, or preparation of the manuscript, and did not approve or disapprove of, or delay publication of the work.

Competing interests
None declared.

Acknowledgements
The laboratory measurements were produced by the biomedical scientists in Clinical Laboratory Sciences at Queen Elizabeth Hospital Birmingham. We would like to thank Dr Radhika Susarla (Research Scientist, Diabetes Translational Research Group) for her assistance with the manuscript. We would also like to thank Dr Paul Cockwell (Consultant Nephrologist, Renal Medicine, Queen Elizabeth Hospital Birmingham) and Professor Wasim Hanif (Professor of Diabetes and Endocrinology, Diabetes Centre, Queen Elizabeth Hospital Birmingham) for their support and encouragement.

Data access statement
The datasets generated during and/or analysed during the study are not publicly available. The dataset contains clinical data which cannot be shared publicly as a result of UK data protection legislation.

References