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Effect of heterozygous beta thalassemia on HbA1c levels in individuals without diabetes mellitus: A cross sectional study

D. Tsilingiris[a,*], K. Makrilakis[a], E. Voskaridou[b], S. Pagkrati[c], M. Dalamaga[d], S. Liatis[a]

[a] First Department of Propaedeutic Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[b] Reference Center of Thalassemia and Sickle Cell Disease, «Laiko» General Hospital, Athens, Greece
[c] Hematology Laboratory, «Laiko» General Hospital, Athens, Greece
[d] Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, Athens, Greece

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ABSTRACT

Aims: To investigate the effect of heterozygous β-thalassemia on HbA1c levels in a population without diabetes mellitus (DM).

Methods: Using a cross-sectional design, HbA1c levels were compared between two groups of 100 consecutive carriers of β-thalassemia and 100 healthy controls matched for age, gender and BMI, taking into account fasting serum glucose and fructosamine levels. The effect of hemoglobin concentration on HbA1c was also examined.

Results: The mean HbA1c level was almost identical in the two groups (33.6 mmol/mol [5.23%] vs. 33.6 mmol/mol [5.22%], $p = 0.857$). Within the group of β-thalassemia, there was a positive correlation between HbA1c and hemoglobin concentration ($r = 0.455$, $p < 0.001$), which was not observed in controls. β-thalassemia carriers without anemia had slightly higher HbA1c levels compared to those with anemia (34.9 mmol/mol [5.35%] vs. 32.5 mmol/mol [5.12%] $p < 0.001$, absolute difference (2.4 mmol/mol [0.23%]). In multivariable analysis, hemoglobin concentration, BMI and 1st degree family history of T2DM were significant predictors of HbA1c, while β-thalassemia carrier state was non-significant ($p = 0.07$).

Conclusions: In individuals without DM, heterozygous β-thalassemia has a borderline effect on HbA1c levels, while the impact of β-thalassemia trait-associated anemia on HbA1c is of negligible clinical significance. These findings advocate for the clinical use of HbA1c as a diagnostic criterion for diabetes mellitus in this population.

1. Introduction

Since its discovery in the 1960s, glycated hemoglobin (HbA1c) has emerged as a valuable tool for diagnosing and monitoring individuals with diabetes mellitus (DM) in clinical practice. HbA1c was recently introduced as a novel diagnostic criterion for identifying new cases of DM, with values of ≥48 mmol/mol (6.5%) establishing the diagnosis [1].

Nevertheless, HbA1c as an indicator of recent glycemic levels may be compromised in certain clinical scenarios. Since the interpretation of HbA1c is based on a typical average erythrocyte lifespan of 120 days, conditions associated with a reduced red blood cell survival tend to lower its levels [2]. Typical conditions associated with reduced erythrocyte survival are included in the spectrum of congenital or acquired hemolytic conditions.

Thalassemia consists of a group of hereditary conditions associated with ineffective erythropoiesis and chronic hemolysis. Among thalassemias, heterozygous β-thalassemia (frequently referred to as β-thalassemia minor, β-thalassemia carrier state or β-thalassemia trait) is estimated by the World Health Organization to affect 1 to 5% of the world population. It is frequently encountered within the Mediterranean region, Middle and Far East, central and south East Asia [3]. Nevertheless, migratory flows from areas with inherently high prevalence of the condition to those with a low prevalence (e.g. North America, Northern Europe, Australia) during recent years have begun to alter the epidemiology of β-thalassemia with an increasingly widespread distribution worldwide [4].

Affected individuals usually present with elevated HbA_2 in hemoglobin electrophoresis, hemoglobin levels/hematocrit ranging from normal to mildly reduced, as well as hypochromy and microcytosis in the peripheral blood smear. Gene mapping of the HBB gene can reveal the specific underlying mutation and aid to the diagnosis of cases with ambiguous screening results [5].

The presence of β-thalassemia trait could affect HbA1c levels.

* Corresponding author at: Th. Kairi Str. 14, Nea Smyrni, Athens, Greece.
E-mail address: tsilingirisd@gmail.com (D. Tsilingiris).

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Ineffective erythropoiesis as well as peripheral hemolysis may decrease HbA1c levels due to the reduced erythrocyte lifespan in β-thalassemia carriers. Additionally, extremely elevated fetal hemoglobin (HbF) levels (> 10–15%), occasionally encountered in β-thalassemia carriers [6], could have an effect on the reported result for HbA1c in certain laboratory methods of HbA1c determination, resulting in falsely low HbA1c values [7,8].

There has been a paucity of published papers to address the effect of β-thalassemia trait on HbA1c [9–12]. In the case of alpha-thalassemia that shares a similar pathophysiologic background with β-thalassemia, HbA1c levels are affected in a degree dependent upon the number of missing α-chain genes and the severity of the resulting hemolytic condition [13]. In sickle-cell trait, HbA1c levels appear lower, with potential clinical significance, presumably due to the lower than normal erythrocyte lifespan associated with the trait [14].

The aim of the present study was to investigate whether HbA1c levels are affected by the presence of heterozygous β-thalassemia in a population of individuals without known diabetes mellitus, which is the population used for DM screening purposes.

2. Participants and methods

2.1. Study design and eligibility criteria

Two groups of consecutive healthy individuals were examined, one consisting of β-thalassemia carriers and another of non-carriers (controls), who were matched for age (± 1 year), gender and BMI (± 1 kg/m^2). None of the participants were suffering from DM.

The study population was recruited from two sites, belonging to the same University hospital (Laiko General Hospital, Athens, Greece):

1. The outpatient Internal Medicine clinic.
2. The Reference Center of Thalassemia and Sickle Cell Disease, where individuals of the general population are referred for screening.

The following conditions were considered as criteria of exclusion from the study:

- DM of any duration, based on medical history or fasting serum glucose (FSG) ≥ 7 mmol/L
- Age lower than 18 years or > 65 years
- Any hospitalization or voluntary blood donation during the previous 4 months
- Iron deficiency causing anemia or abnormal erythrocyte indices
- A history of iron supplementation during the previous 4 months
- Other acute or chronic hemolytic conditions
- Pregnancy of any age and the 4-month postpartum period
- Chronic Kidney Disease stage III (eGFR < 60 ml/min/1.73 m^2) or more advanced
- Hemoglobinopathies (S, C, etc), other thalassemias (alpha-thalassemia, homozygous β-thalassemia) and extreme elevations of HbF (> 10% of total hemoglobin).
- Major co-morbidities (malignancies, heart failure of clinical stage NYHA ≥ II, chronic hypoxemic states, chronic liver disease, chronic inflammatory states).
- Any other clinical condition that could potentially affect hemopoiesis and/or erythrocyte turnover.

Written informed consent was obtained from all participants. The study was carried out in accordance with the principles of the Declaration of Helsinki [15] and approved by the hospital's ethics committee.

2.2. Screening for heterozygous β-thalassemia

All participants underwent screening for β-thalassemia trait. Diagnosis of β-thalassemia trait was based on the finding of elevated HbA2 (> 3.5% of total hemoglobin) along with typical hematologic abnormalities in the complete blood count (mean corpuscular volume - MCV < 75 fl and mean corpuscular hemoglobin - MCH < 27 pg) and the peripheral blood smear (hypochromy, microcytosis, anisopoikilocytosis) [16]. HbA2 was quantified by high resolution liquid chromatography (HPLC) using a Bio-Rad Variant II analyzer (Bio-Rad Laboratories, Hercules, California, USA) [17].

2.3. Laboratory measurements

A venous blood sample after overnight fasting (> 10 h) was collected. If the fasting condition was not satisfied at the time of enrollment, a new visit was rescheduled.

A 5 ml fraction of the collected sample was centrifuged after allowing 15–20 min for clotting, and serum samples were obtained for FSG, fructosamine, ferritin, creatinine and high sensitivity C-reactive protein (hsCRP). HbA1c determination was carried out in 2 ml whole-blood EDTA specimens using the Tina-quant® HbA1c 2nd Generation Turbidimetric Inhibition Immunoassay (TINIA) on a Cobas Integra 800 Analyzer (overall precision and repeatability CV for whole-blood determination is < 2%, F. Hoffman-La Roche AG, Basel, Switzerland [18]). The analytical principle of TINIA includes the use of monoclonal antibodies targeted against the pepsin-cleaved glycated N-terminus of the β-globin chain. The excess unbound antibody reacts with polyhaptins to form insoluble complexes that change the solution turbidity, which is then measured to indirectly quantify HbA1c. The result is reported as a ratio to total hemoglobin concentration which is determined colorimetrically [19,20].

FSG and fructosamine measurements were utilized as indicators of glycemia. Creatinine was measured to exclude kidney disease, while ferritin levels were obtained to assess iron stores. hsCRP levels were measured in order to ensure the accuracy of ferritin levels as an indicator of iron stores, by excluding the presence of a potential inflammatory state.

2.4. Statistical analysis

2.4.1. Data were analyzed using IBM SPSS statistical Package, version 21

Qualitative parameters were compared between groups by X^2-square analysis. Group means of continuous normally distributed variables were compared by unpaired two-sided *t*-test. For comparisons of non-normally distributed variables, the Mann-Whitney non-parametric test was used. The estimated marginal means were calculated using general linear models in order to adjust the means for possible confounders. Subsequently, a multivariable linear regression model was used to explore the independent contribution of an individual predictor to HbA1c variability.

A priori power analysis was conducted to calculate the required population size with an estimated power of 80% to detect a true difference in HbA1c of 0.3% (3.3 mmol/mol) between the groups, at the 5% level of statistical significance.

3. Results

3.1. Study participants

A total of 233 individuals were screened. Thirty-three participants were excluded (19 due to heterozygous alpha-thalassemia, 2 due to extremely elevated HbF levels [> 10%], 2 had Lepore hemoglobinopathy, 4 had extreme iron deficiency with affected erythrocyte indices or anemia attributed to iron deficiency, 1 admitted blood donation, 1 had a metallic aortic valve which was considered a potential site of hemolysis and 4 had anemia of unknown cause). Ultimately, 200 individuals (100 with the β-thalassemia trait - group A, and 100 without it - group B) were included in the analysis.

Table 1
Demographic and somatometric characteristics of the study participants. Group A: β-thalassemia trait, Group B: Control. Data are shown as mean ± SD or n (%).

	Group A (n = 100)	Group B (n = 100)	P value
Age (Years)	32.5 ± 8.0	32.0 ± 7.6	0.640
Gender (Males, n [%])	45 (45.0)	47(47.0)	0.777
Body Mass Index (kg/m^2)	25.1 ± 4.0	24.5 ± 3.9	0.290
Smoking (Yes, %)	22 (22)	22 (22)	1.000
Family History of T2DM (Yes, %)	24 (24)	23 (23)	0.868

Table 2
Key laboratory measurements in the two study groups. Data are shown as mean ± SD or median (25–75 interquartile range) or n (%).

	Group A (n = 100)	Group B (n = 100)	P value
HbA1c [mmol/mol (%)]	33.6 ± 3.2 (5.23 ± 0.30)	33.6 ± 2.6 (5.22 ± 0.25)	0.857
Glucose (mmol/L)	4.79 ± 0.61	4.88 ± 0.42	0.221
Fructosamine (μmol/L)	221.2 ± 17.2	221.8 ± 17.4	0.798
Hemoglobin concentration (g/L)	123 ± 13	142 ± 13	< 0.001
MCV (fl)	63.8 ± 4.9	86.3 ± 3.3	< 0.001
MCH (pg)	20.6 ± 1.6	29.3 ± 1.2	< 0.001
MCHC (g/dl)	32.3 ± 0.7	33.9 ± 1.0	< 0.001
HbA2 (% of total Hb)	5.0 (4.6–5.4)	2.7 (2.6–2.8)	< 0.001
HbF (% of total Hb)	1.0 (0.5–1.2)	0.3 (0.2–0.65)	< 0.001
Creatinine (μmol/L)	64.27 ± 13.08	69.75 ± 13.97	0.005
hsCRP (mg/l)	0.95 (0.43–1.78)	0.8 (0.3–1.7)	0.345
Ferritin (pmol/L)	72.0 (35.5–137.8)	55 (29.5–126.3)	0.183

The two groups were comparable in terms of their demographic and somatometric characteristics (Table 1).

3.2. Laboratory results

The key laboratory variables, including hematologic indices are shown in Table 2. The mean HbA1c value was almost identical in the two groups (group A: 33.6 ± 3.2 mmol/mol [5.23 ± 0.30%] vs. group B: 33.6 ± 2.7 mmol/mol [5.22 ± 0.25%], $p = 0.857$). Serum glucose, fructosamine, ferritin and hsCRP did not differ between the two groups, whereas serum creatinine levels were lower in the β-thalassemia group. As expected, the mean hemoglobin concentration was significantly lower in the β-thalassemia group compared to controls (Table 2). Accordingly, the erythrocyte indices, HbA2 and HbF percentage were significantly different between the two groups.

3.3. Hemoglobin concentration and HbA1c levels

Since alterations in the erythrocyte turnover that are reflected upon hemoglobin concentration are considered to have a significant impact on HbA1c levels [21], the association of hemoglobin levels with HbA1c was further investigated. There was a significant positive correlation between HbA1c and hemoglobin concentration within the β-thalassemia group ($r = 0.455$, $p < 0.001$). There was no correlation between HbA1c and hemoglobin levels in the control group ($r = 0.024$, $p = 0.815$).

The group of β-thalassemia carriers was further subdivided according to the presence of anemia, as defined by the World Health Organization (hemoglobin concentration < 120 g/L for women and < 130 g/L for men [22]). Subsequently, HbA1c was compared between individuals with β-thalassemia trait and anemia (group A1) and β-thalassemia trait without anemia (group A2) (Table 3). Mean HbA1c was slightly but significantly higher in Group A2 compared to group A1 (34.9 ± 2.6 mmol/mol [5.35 ± 0.24%] vs. 32.5 ± 3.3 mmol/mol [5.12 ± 0.30%], absolute difference 2.4 mmol/mol [0.23%], $p < 0.001$, Table 3). The two subgroups also differed in terms of gender and certain laboratory parameters, other than hemoglobin concentration (Table 3). The difference, however, in HbA1c between the subgroups remained statistically significant after adjustment for gender, BMI, FSG and serum ferritin levels.

Table 3
Comparison of HbA1c and potential confounders among β-thalassemia carriers with and without anemia (groups A1 and A2 respectively). Data are shown as mean ± SD, mean (95% confidence interval), median (25, 75 percentile) or n (%).

	Group A1	Group A2	P value
N	53	47	
HbA1c [mmol/mol, (%)]	32.5 ± 3.3 (5.12 ± 0.30)	34.9 ± 2.6 (5.35 ± 0.24)	< 0.001
Adjusted HbA1c [mmol/mol, (%)][a]	32.6 (31.9–33.4) [5.14 (5.07–5.21)]	34.8 (33.9–35.6) [5.33 (5.26–5.41)]	0.001
Gender [males, n (%)]	14 (26.4)	31 (65.9)	< 0.001
Hemoglobin concentration (g/L)	113 ± 8.0	134 ± 8.0	< 0.001
Age (years)	32.9 ± 7.9	32.1 ± 8.3	0.606
BMI (kg/m^2)	24.4 ± 3.8	25.9 ± 4.3	0.079
Serum ferritin (pmol/L)[#]	119.1 (60.7, 258.4)	235.9 (110.1, 429.2)	0.008
Fasting Serum Glucose (mmol/L)	4.76 ± 0.59	4.88 ± 0.62	0.201

As mean (95% confidence interval).
[#] Presented as median (25th, 75th percentile).
[a] Mean HbA1c adjusted for age, gender, BMI, FSG and serum ferritin. Presented.

3.4. Association of HbA1c with β-thalassemia carrier status and other predictors

As depicted in Table 4, from the multivariable linear regression model applied to all study participants, the only significant independent predictors of HbA1c (dependent variable) were hemoglobin levels, BMI and 1st degree family history of T2DM (all $p < 0.05$) while β-thalassemia carrier state was of borderline statistical significance ($p = 0.07$). No significant associations emerged regarding age, gender, FSG and serum ferritin levels.

4. Discussion

4.1. Chief study findings

To our knowledge, this is the first study to investigate the effect of β-thalassemia trait on HbA1c, comparing two matched populations of pre-calculated size. The study was carried out in a sample of individuals without known diabetes mellitus, who constitute the population in which HbA1c is used for the purpose of screening for diabetes mellitus. It is furthermore the first attempt to attribute any effect to the distinct unique laboratory features of the trait.

In the present study, non-diabetic individuals with heterozygous β-thalassemia presented a similar mean HbA1c value to those without the thalassemia trait. However, carriers of the trait exhibited a greater variance of HbA1c values, the latter being significantly associated with their hemoglobin levels.

A review of the literature regarding the effect of β-thalassemia trait on HbA1c levels reveals a paucity of relevant published papers. Existing data primarily focus on the differential effect of the trait on various HbA1c laboratory measuring methods, comparing small and likely heterogenous populations. There has been a case report of extremely low HbA1c in an individual with DM and β-thalassemia trait with excessively diminished erythrocyte lifespan [9]. By contrast, elevations of HbA1c to diagnostic ranges of DM have been demonstrated among β-thalassemia carriers, when a Synchron LX20 immunoassay was used for

Table 4
Model of multiple linear regression analysis portraying independent predictors of HbA1c (dependent variable) in 100 β-thalassemia carriers and 100 controls; regression coefficients with corresponding *p*-values.

Independent Predictors	Unstandardized B Coefficient (95% CI)	Standardized beta coefficient	*P* value
Age (years)	0.004 [0.000, 0.009]	0.119	0.077
Gender (female vs. male)	0.040 [−0.074, 0.153]	0.072	0.493
β-thalassemia trait (yes vs. no)	0.091 [−0.008, 0.191]	0.167	0.072
BMI (kg/m^2)	0.021 [0.011, 0.031]	0.314	< 0.001
Hemoglobin concentration (g/l)	0.005 [0.001, 0.008]	0.278	0.015
Fasting Serum Glucose (mmol/L)	0.051 [−0.023, 0.126]	0.089	0.176
Serum ferritin (pmol/L)	0.000 [0.000, 0.000]	−0.098	0.201
1st degree family history of T2DM (yes vs. no)	0.119 [0.037, 0.200]	0.184	0.004

measurement [10,11] due to a positive bias for the analyzer at lower hemoglobin concentrations [11]. A positive bias among carriers has also been demonstrated when using the Variant II turbo HPLC analyzer for HbA1c estimation [12].

In the present study, carriers and non-carriers had the same FSG and fructosamine levels, findings that are strongly suggestive of similar glycemic levels between the two groups. In addition, there were no differences between the compared groups in a number of potentially important (with respect to their effect on HbA1c) parameters (i.e. age, BMI, gender, smoking habits and family history of T2DM). Trends and differences between the two groups in certain laboratory tests are highly attributable to the presence of the β-thalassemia trait per se; hence, hemoglobin concentration, MCV, MCH and MCHC were higher in controls as compared to the carriers, while the opposite was true for the HbF percentage. Serum creatinine levels were lower in the β-thalassemia carriers' group, a finding that has been previously reported [23].

Fructosamine concentration, an alternative glycemic indicator for hemoglobinopathies, was not different between the two groups. Noteworthy, fructosamine determination is not standardized compared to HbA1c. No guidelines or goals are established on glycated protein values in these populations that can be followed by clinicians or individuals with DM. These methods assess the degree of glycemia over a period of approximately 2 to 3 weeks, as opposed to 2 to 3 months for HbA1c and they have not been correlated with the development of long-term diabetic complications, as was shown for HbA1c in the DCCT or UKPDS [24].

4.2. β-thalassemia trait, hemoglobin concentration and HbA1c

The presence of β-thalassemia trait may be implicated in causing both biological and analytical interference on HbA1c measurement. In the case of biological interference, the increased erythrocyte turnover due to the slightly diminished erythrocyte lifespan associated with the trait has the potential to affect HbA1c levels in a downward manner in affected individuals. Since the lower erythrocyte survival is also reflected upon hemoglobin concentration, the degree of anemia would be expected to correlate with the deviation of HbA1c, irrespectively of the laboratory method used for HbA1c measurement. There is also the possibility that alterations in erythrocyte metabolism, as observed in β-thalassemia trait (e.g. differentiated intracellular oxidative status or 2,3-biphosphoglycerate levels [25,26]) may have an impact on HbA1c formation compared to non-carriers [27], although the magnitude of these phenomena is difficult to be quantified in everyday clinical practice. As per analytical interference, β-thalassemia trait represents a quantitative, rather than a qualitative defect of beta globin synthesis. As such, it is not related to hemoglobin structural abnormalities which could potentially bias certain measurement methods. HbF levels are occasionally elevated in affected individuals, albeit rarely at levels as high as those expected to have a significant effect on HbA1c measurement [6–8]. Therefore, optimally a National Glycohemoglobin Standardization Program (NGSP) -certified method that would lack interference from HbF elevations may be a more reasonable choice when measuring HbA1c in carriers of β-thalassemia trait [7].

According to the principal hypothesis of this study, hemoglobin concentration in β-thalassemia carriers might, at least partly, reflect the degree of mild hemolysis, being thereby linked to lower HbA1c levels. The ascertainment of the independent significant positive correlation of hemoglobin concentration and HbA1c values among thalassemia carriers in our study seems to strengthen that hypothesis. From a different point of view, significantly lower HbA1c levels are found in carriers with true anemia compared to those without anemia (Table 2). The absence of a corresponding correlation in the control group is probably the result of a more homogenous erythrocyte turnover among normal subjects.

Nevertheless, this phenomenon did not suffice to translate into lower mean HbA1c levels for the carrier group as a whole when compared to controls. Furthermore, in a multivariable linear regression model that included key determinants of HbA1c, such as FSG, BMI, hemoglobin concentration and family history of T2DM, the presence of β-thalassemia trait was associated with higher HbA1c levels, although the association did not reach statistical significance (standardized beta coefficient: 0.167, $p = 0.07$) (Table 4). Therefore, additional factors, other than anemia and serum glucose, may affect HbA1c in an upward manner in the population of β-thalassemia carriers.

A possible explanation would implicate interference of altered HbF levels on the HbA1c laboratory measurement. However, elevations of HbF are known to influence HbA1c in a downward rather than an upward manner [7,8]. Consequently, an attempt to attribute the elevated HbA1c values to a potential laboratory interference of HbF would, by itself, be contradictory. Alterations in erythrocyte metabolism in β-thalassemia trait may provide an alternative explanation; elevated levels of 2,3-biphosphoglycerate ([2,3]-BPG) have been consistently observed among carriers [26,28]. The principal physiologic role of this molecule is shifting the hemoglobin-oxygen dissociation curve to the right. Increases of [2,3]-BPG are also known to accelerate hemoglobin glycation for a given level of glucose concentration [29] and furthermore, elevated levels of this compound are encountered in “high” compared to “low” glycators [30]. Thus, elevated [2,3]-BPG levels in β-thalassemia trait could provide a pathophysiologic mechanism “pushing” the HbA1c towards higher levels and counteracting the HbA1c lowering effect of hemolysis. However, mechanistic studies are required to investigate this hypothesis.

4.3. Strengths and limitations

The present study has a number of strengths. It is an appropriately powered study, which was sufficient to document significant associations with HbA1c. The two compared groups originate from the same population and are carefully weighed in terms of a number of major clinical and demographic parameters that could potentially confound the targeted comparisons. Additionally, the study design was oriented towards excluding individuals with causes of anemia other than β-thalassemia trait (including iron deficiency anemia, but not isolated

iron store deficiency) or other conditions that could affect erythrocyte turnover. However, this study presents noteworthy limitations. No data on reticulocyte counts are available, which could serve as a surrogate for erythrocyte turnover rate; however, this approach has not been previously shown to yield meaningful information on the effect of erythrocyte turnover on HbA1c among normoglycemic individuals [31]. HbA1c was measured using a single method, namely TINIA which is currently the most frequently used method among the laboratories participating in the College of American Pathology (CAP) proficiency testing program for HbA1c [24] and second only to HPLC among the reported HbA1c methods in the European HbA1c trial (EurA1c) [32]. There is an excellent concordance between HbA1c results yielded from TINIA and other available methods [33,34]. Despite the adequate statistical power to detect significant differences between the groups, minor existing differences during post-hoc analysis may not have surfaced due to small sample size. Therefore, the possibility exists that observed correlation trends could potentially reach statistical significance in a larger sample regarding the independent association of β-thalassemia trait with higher HbA1c levels. However, the small magnitude of the actual quantitative effect of this phenomenon (± 2.4 mmol/mol or ± 0.23%, less than the standard deviation of HbA1c in the sub-population of β- thalassemia carriers, Table 2) would render its clinical significance negligible.

5. Conclusion

In summary, it was shown for the first time that the presence of β-thalassemia trait per se displays a borderline significant impact on HbA1c levels. In carriers of the trait, the HbA1c value seems to depend on their hemoglobin levels, with anemic individuals presenting slightly lower HbA1c values than non-anemic (− 2.4 mmol/mol or − 0.23%, less than the standard deviation of HbA1c in this population). Though statistically significant, this magnitude of difference would be of small impact from a clinical point of view. Consequently, until further research is carried out, the findings of the current study advocate against the exclusion of HbA1c for the diagnosis of DM and long-term monitoring of glycemia in individuals with heterozygosity for β-thalassemia.

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Conflicts of interest

None declared.

Author contributions

All authors have made substantial contributions to the conception and design of the study or acquisition, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and have approved of the final version to be submitted.

References

[1] Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation, Geneva, 2011.
[2] D.B. Sacks, M. Arnold, G.L. Bakris, D.E. Bruns, A.R. Horvath, M.S. Kirkman, A. Lernmark, B.E. Metzger, D.M. Nathan, Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus, Clin. Chem. 57 (6) (2011) e1–e47, https://doi.org/10.1373/clinchem.2010.161596.
[3] D.R. Higgs, J.D. Engel, G. Stamatoyannopoulos, Thalassaemia, Lancet 379 (9813) (2012) 373–383, https://doi.org/10.1016/S0140-6736(11)60283-3.
[4] C.K. Li, New trend in the epidemiology of thalassaemia, Best Pract. Res. Clin. Obstet Gynaecol. 39 (2017) 16–26, https://doi.org/10.1016/j.bpobgyn.2016.10.013.
[5] D.E. Sabath, Molecular diagnosis of Thalassemias and Hemoglobinopathies an ACLPS critical review, Am. J. Clin. Pathol. 148 (1) (2017) 6–15, https://doi.org/10.1093/ajcp/aqx047.
[6] A. Mosca, R. Paleari, D. Leone, G. Ivaldi, The relevance of hemoglobin F measurement in the diagnosis of thalassemias and related hemoglobinopathies, Clin. Biochem. 42 (18) (2009) 1797–1801, https://doi.org/10.1016/j.clinbiochem.2009.06.023.
[7] HbA1c Assay Interferences, Updated October 2018, Accessed on February 15, 2019. http://www.ngsp.org/interf.asp.
[8] C.L. Rohlfing, S.M. Connolly, J.D. England, S.E. Hanson, C.M. Moellering, J.R. Bachelder, R.R. Little, 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 (5) (2008) 811–814, https://doi.org/10.1309/YFVTUD0GHJF7D16H.
[9] L.G. Danescu, S. Levy, J. Levy, Markedly low hemoglobin A1c in a patient with an unusual presentation of beta-thalassemia minor, Endocr. Pract. 16 (1) (2010) 89–92, https://doi.org/10.4158/EP09160.CR.
[10] S.M. Al-Fadhli, A.A. Ahmad, H.A. Al-Jafar, Effect of sickle cell trait and B-thalassemia minor on determinations of HbA1c by an immunoassay method, Saudi. Med. J. 22 (8) (2001) 686–689.
[11] C. Polage, R.R. Little, C.L. Rohlfing, T.G. Cole, W.L. Roberts, Effects of beta thalassemia minor on results of six glycated hemoglobin methods, Clin. Chim. Acta 350 (1–2) (2004) 123–128, https://doi.org/10.1016/j.cccn.2004.07.015.
[12] L. Ji, J. Yu, Y. Zhou, Y. Xia, A. Xu, W. Li, L. Li, Erroneous HbA1c measurements in the presence of beta-thalassemia and common Chinese hemoglobin variants, Clin. Chem. Lab. Med. 53 (9) (2015) 1451–1458, https://doi.org/10.1515/cclm-2014-0598.
[13] A. Xu, L. Ji, W. Chen, Y. Xia, Y. Zhou, Effects of alpha-thalassemia on HbA1c measurement, J. Clin. Lab. Anal. 30 (6) (2016) 1078–1080, https://doi.org/10.1002/jcla.21983.
[14] M.E. Lacy, G.A. Wellenius, A.E. Sumner, A. Correa, M.R. Carnethon, R.I. Liem, J.G. Wilson, D.B. Sacks, D.R. Jacobs Jr., A.P. Carson, X. Luo, A. Gjelsvik, A.P. Reiner, R.P. Naik, S. Liu, S.K. Musani, C.B. Eaton, W.C. Wu, Association of sickle cell trait with Hemoglobin A1c in African Americans, JAMA 317 (5) (2017) 507–515, https://doi.org/10.1001/jama.2016.21035.
[15] J.R. Williams, The declaration of Helsinki and public health, Bull. World Health Organ. 86 (8) (2008) 650–652.
[16] A. Giambona, C. Passarello, D. Renda, A. Maggio, The significance of the hemoglobin a(2) value in screening for hemoglobinopathies, Clin. Biochem. 42 (18) (2009) 1786–1796, https://doi.org/10.1016/j.clinbiochem.2009.06.026.
[17] J. Riou, C. Godart, M. Mathis, D. Hurtrel, H. Wajcman, C. Prehu, J. Bardakdjian, Evaluation of the bio-rad VARIANT II HbA2/HbA1C dual program for measurement of hemoglobin concentrations and detection of variants, Clin. Chem. Lab. Med. 43 (2) (2005) 237–243, https://doi.org/10.1515/CCLM.2005.040.
[18] J.K. Fleming, Evaluation of HbA1c on the Roche COBAS Integra 800 closed tube system, Clin. Biochem. 40 (11) (2007) 822–827, https://doi.org/10.1016/j.clinbiochem.2007.03.017.
[19] F. Braga, A. Dolci, M. Montagnana, F. Pagani, R. Paleari, G.C. Guidi, A. Mosca, M. Panteghini, Revaluation of biological variation of glycated hemoglobin (HbA(1c)) using an accurately designed protocol and an assay traceable to the IFCC reference system, Clin. Chim. Acta 412 (15–16) (2011) 1412–1416, https://doi.org/10.1016/j.cca.2011.04.014.
[20] J.M. Abadie, A.A. Koelsch, Performance of the Roche second generation hemoglobin A1c immunoassay in the presence of HB-S or HB-C traits, Ann. Clin. Lab. Sci. 38 (1) (2008) 31–36.
[21] M.S. Radin, Pitfalls in hemoglobin A1c measurement: when results may be misleading, J. Gen. Intern. Med. 29 (2) (2014) 388–394, https://doi.org/10.1007/s11606-013-2595-x.
[22] Nutritional anaemias. Report of a WHO scientific group, World Health Organization Technical Support Series 405, (1968), pp. 1–40.
[23] A.I. Triantafyllou, G.P. Vyssoulis, E.A. Karpanou, P.L. Karkalousos, E.A. Triantafyllou, A. Aessopos, D.T. Farmakis, Impact of beta-thalassemia trait carrier state on cardiovascular risk factors and metabolic profile in patients with newly diagnosed hypertension, J. Hum. Hypertens. 28 (5) (2014) 328–332, https://doi.org/10.1038/jhh.2013.102.
[24] R.J. Molinaro, Targeting HbA1c: standardization and clinical laboratory measurement, MLO Med. Lab. Obs. 40 (1) (2008) 16–19 10–4.
[25] G.C. Gerli, L. Beretta, M. Bianchi, A. Pellegatta, A. Agostoni, Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activities in beta-thalassaemia (major and minor), Scand. J. Haematol. 25 (1) (1980) 87–92.
[26] H.A. Pearson, E. Motoyama, M. Genel, M. Kramer, C.J. Zigas, Intraerythrocytic adaptation (2,3 DPG,P50) in thalassemia minor, Blood 49 (3) (1977) 463–465.
[27] S.A. Chalew, R.J. McCarter, J.M. Hempe, Biological variation and hemoglobin A1c: relevance to diabetes management and complications, Pediatr. Diabetes 14 (6) (2013) 391–398, https://doi.org/10.1111/pedi.12055.
[28] G. Ricci, G. Castaldi, G. Zavagli, G. Lupi, A. Turati, T. Bezzi, Red cell 2,3-diphosphoglycerate contents and oxygen affinity in heterozygous beta-thalassaemia, Acta Haematol. 68 (1) (1982) 63–64.
[29] C.H. Lowrey, S.J. Lyness, J.S. Soeldner, The effect of hemoglobin ligands on the kinetics of human hemoglobin A1c formation, J. Biol. Chem. 260 (21) (1985) 11611–11618.
[30] B.J. Gould, S.J. Davie, J.S. Yudkin, Investigation of the mechanism underlying the

variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia, Clin. Chim. Acta 260 (1) (1997) 49–64.
[31] F.Q. Nuttall, M.C. Gannon, W.R. Swaim, M.J. Adams, Stability over time of glycohemoglobin, glucose, and red blood cell survival in hematologically stable people without diabetes, Metabolism 53 (11) (2004) 1399–1404.
[32] A.C.T.G. Eur, EurA1c: the European HbA1c trial to investigate the performance of HbA1c assays in 2166 laboratories across 17 countries and 24 manufacturers by use of the IFCC model for quality targets, Clin. Chem. 64 (8) (2018) 1183–1192, https://doi.org/10.1373/clinchem.2018.288795.
[33] F.B. Aksungar, M. Serteser, A. Coskun, I. Unsal, A comparison between turbidimetric inhibition immunoassay and capillary electrophoresis in glycated hemoglobin (HbA1c) measurement, Clin. Chem. Lab. Med. 51 (8) (2013) e191–e193, https://doi.org/10.1515/cclm-2013-0033.
[34] G.F. Gencs, M. Kanmaz-Ozer, N. Ince, F. Ozcelik, et al., The analytical performances of four different Glycated Hemoglobin methods, Med. Chem. 4 (2014) 501–505, https://doi.org/10.4172/2161-0444.1000185.