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Fetal hemoglobin and hemoglobin A_{1c} level among pediatric patients with type 1 diabetes

Wpływ hemoglobiny płodowej na stężenie hemoglobiny glikowanej w populacji dzieci z cukrzycą typu 1

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Abstract
Introduction. Glycated hemoglobin (HbA1c) is used as a cumulative estimate of mean blood glucose levels from the preceding 5–12 weeks. This is the gold standard in assessing glycemic control in patients with diabetes. The ADA criteria for the diagnosis of diabetes, including HbA1c level, contribute to the importance of recognizing any variation pertaining to the HbA1c measurement. HbA1c is often used as a primary endpoint in the interventional studies among patients with diabetes. Thus, knowledge about factors independently to glycemia, affecting HbA1c is clinically useful. **Aim of study.** Evaluation variability of fetal hemoglobin (HbF) level among Polish children with diabetes and how it may affect the HbA1c level measurement. **Material and methods.** This was a prospective cohort study. A laboratory HbA1c testing was performed for more than 96% of pediatric diabetic patients in the region. In our study we included all consecutive patients aged 2 to 18 years with type 1 diabetes (T1D) and the disease duration longer than one year (555 patients). All patients had HbA1c and HbF measured at three time-points during minimum one-year period. In the same time, clinical data were recorded. The measurements of HbA1c and HbF were performed by means of cation-exchange high-pressure liquid chromatography (HPLC) on a D-10 Dual A2/F/A1c (Bio-Rad Laboratories, Hercules, CA, USA). Statistical analysis was performed using the Statistica 10.0 package (StatSoft, Tulsa, USA). **Results.** An average age in the observed group was 12.9±3.8 years, diabetes duration 5.6±3.4 years, HbA1c was 7.59±1.33% (59±10.65 mmol/mol). In 78 (14%) patients elevated levels of HbF (>0.8%) were found at each time-point, mean value 1.2±0.45%. Elevated HbF was associated with younger age at examination (p=0.03) and younger age of diagnosis (p=0.01). It was not related to diabetes duration (p=0.21). No correlation between HbA1c and HbF was observed in the study (R=-0.09; p=0.43). **Conclusions.** Fetal hemoglobin does not affect HbA1c measurement among pediatric patients with type 1 diabetes older that 2 years.
Key words:
HbA_{1c}, hemoglobin F, type 1 diabetes, children

Streszczenie
Wstęp. Hemoglobina glikowana (HbA1c) jest używana jako parametr odzwierciedlający średnie stężenie glukozy z okresu 5–12 tygodni przed badaniem. Jest ona złotym standardem w ocenie wyrównania metabolicznego u pacjentów z cukrzycą. Kryteria ADA rozpoznania cukrzycy włączają stężenie HbA1c do istotnych czynników w procesie diagnozowania tej choroby. HbA1c jest też często używana jako punkt końcowy w badaniach interwencyjnych u pacjentów z cukrzycą. Z tych przyczyn wiedza na temat czynników niezależnych od glikemii, wpływających na HbA1c, może być klinicznie przydatna. **Cel badania.** Oznaczenie zmienności stężenia

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hemoglobiny płodowej (HbF) wśród dzieci z cukrzycą w Polsce i ocena wpływu tej zmienności na stężenie HbA1c. **Materiały i metody.** Badanie miało charakter prospektywny. Przeprowadzono oznaczenie stężenia HbA1c u ponad 96% dzieci z cukrzycą w regionie łódzkim. Do badania włączono wszystkich pacjentów w wieku od 2 do 18 lat z cukrzycą typu 1 (T1D) i czasem trwania cukrzycy powyżej jednego roku (n=555). U wszystkich tych pacjentów przeprowadzono pomiar stężenia HbA1c i HbF minimum trzykrotnie w ciągu roku. Z tych punktów czasowych zebrano dane kliniczne dotyczące pacjenta. Pomiar HbA1c i HbF przeprowadzono metodą wysokosprawnej chromatografii cieczowej (HPLC) na aparacie D-10 Dual A2/F/A1c (Bio-Rad Laboratories, Hercules, CA, USA). Analizę statystyczną przeprowadzono przy użyciu pakietu Statistica 10.0 (StatSoft, Tulsa, USA). **Wyniki.** Średni wiek pacjentów w obserwowanej grupie wynosił 12,9±3,8 roku, czas trwania cukrzycy 5,6±3,4, stężenie HbA1c 7,59±1,33% (59±10,65 mmol/mol). U 78 (14%) pacjentów stwierdzono podwyższone stężenie HbF (>0,8%) w każdym z obserwowanych punktów czasowych, z średnią wartością 1,2±0,45%. Podwyższone stężenie HbF było związane z młodszym wiekiem pacjentów (p=0,03) i młodszym wiekiem w momencie rozpoznania choroby (p=0,01). Nie zaobserwowano związku z czasem trwania cukrzycy (p=0,21). Nie stwierdzono korelacji pomiędzy stężeniem HbA1c a HbF w obserwowanej grupie (R=-0,09; p=0,43). **Wnioski.** HbF nie wpływa na pomiar HbA1c u starszych niż 2 lata pacjentów pediatrycznych z cukrzycą.

Słowa kluczowe:
HbA1c, Hemoglobina płodowa, cukrzyca typu 1, dzieci

Introduction

Glycated hemoglobin (HbA1c), which is formed through the non-enzymatic glycation of hemoglobin A1, is used as a cumulative estimate of mean blood glucose levels from the preceding 5–12 weeks in healthy people and in patients with diabetes. This is the gold standard in assessing glycemic control in patients with diabetes (1). The ADA criteria for the diagnosis of diabetes including HbA1c level contribute to the importance of recognizing any variation pertaining to the HbA1c measurement. For these reasons, the HbA1c measurement should be precise and replicable and standardized by NGSP protocols (www.ngsp.org). Moreover, HbA1c is often used as a primary endpoint in many interventional studies among patients with diabetes. Thus, knowledge about factors independent of glycemia, affecting HbA1c is clinically useful. Recently, we have revealed a clear seasonal variability in HbA1c among children and adolescents with type 1 diabetes and total bilirubin levels among children with type 1 diabetes (2, 3).

Hemoglobin (Hb) is composed of four globin chains. The most abundant form after the second year of life is hemoglobin A1 (HbA1; $\alpha 2\beta 2$ chains). Fetal hemoglobin (HbF; $\alpha 2\gamma 2$) is the predominant hemoglobin in the prenatal period (4). After birth, it is gradually replaced by the adult Hb (HbA1)(5, 6). In normal conditions, HbF is present in less than 0.8% of the total Hb. This is usually asymptomatic, and is only noticed when screening for hematological disorders. It may be increased in children under 2 years.. For this reason, HbA1c level measurement in this group of patients can be inadequate. Moreover hemoglobinopathy and genetic variants of hemoglobin gene (*BCL11A* and *HBS1L-MYB*) may influenceon the level of HbF (7-9). The main goal of this study is to evaluate the variability of the HbF level among Polish children with diabetes and examine how it may affect the HbA1c level measurement.

Methods

Study participants and design

This was a prospective cohort study conducted in Lodz, an administrative district in central Poland populated by approximately 2.6 million inhabitants, between 1 August 2012 and 30 January 2015. A laboratory HbA1c testing was performed for more than 96% of pediatric diabetic patients in the region, correspondingly to the proportion of patients treated in the study center.

In our study, we included all consecutive patients aged 2 to 18 years with type 1 diabetes (T1D) and the disease duration longer than one year. The diagnosis based on the WHO criteria from 1999 and the presence of at least one anti-islet autoantibody (anti-GAD, anti-IA2, ICA or anti-ZnT8). All patients had HbA1c and HbF measured at three time-points during minimum one-year period. At the same time, patient weight, height and insulin dose and the downloaded data downloadedfrom their glucometers were recorded. Participating patients and/or their parents provided written consent for the participation in the study. The protocol was approved by the University Bioethics Committee at the Medical University in Lodz, Poland (RNN/365/12/KB).

Laboratory methods

The measurement of HbA1c and HbF was performed by cation-exchange high-pressure liquid chromatography (HPLC) on a D-10 Dual A2/F/A1c (Bio-Rad Laboratories, Hercules, CA, USA), using reagents according to the manufacturer's instructions. This method was calibrated using calibrators supplied by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Network and was certified by the National Glycohemoglobin Standarization Program (NGSP) as having documented traceability to the Diabetes Control and Complication Trial (DCCT) reference method (http://www.ngsp.org/docs/methods.pdf; last accessed January 2016). Capillary blood samples were collected using the Hemoglobin Capillary Collection System (Bio-Rad Laboratories GmbH, Munchen, Germany) and stored at the temperature of 8°C for 1-7 days until measurements were performed. Samples prepared using this procedure give reliable HbA1c results after storing for 2 weeks at room temperature, for 4 week at 4-8°C, and for 4 days at 42°C. These procedures ensure stable HbF results after a

week of storing samples in 8°C. Reference values of HbA1c for healthy people estimated by our local laboratory were from 4.7 to 5.7%. The within run coefficient of variation (CV) determined by the manufacturer was 1.05% for normal patients and 0.94% for diabetic patients; the between run CV was 1.61% and 1.16 for normal and for diabetic patients respectively. The cutoff value for the precise HbF level detection was 0.8%. Thus, a detailed result of HbF was given only for the elevated level (in the another case it was given as "<0.8%").

Statistical analysis

A statistical analysis was performed using the Statistica 10.0 package (StatSoft, Tulsa, USA). Univariate analyses were assessed with nonparametric tests: U-Mann Whitney's test for comparisons between groups and Spearman's correlation coefficient for quantitative variables. As statistically significant we considered results with a p value<0.05. Average values of HbA1c and HbF from all measurements in each patient during a one-year observation (at least three measurements for patient) were studied.

Results

In total, we had 819 pediatric patients with diabetes treated in the center during the study entry. Among them, 555 fulfilled the inclusion criteria. All individuals were of Caucasian origin. An average age was 12.9±3.8 years, diabetes duration 5.6±3.4 years, HbA1c was 7.59±1.33% (59±10.65 mmol/mol). The detailed group characteristics are displayed in table I. Average values of HbA1c and HbF strongly correlated with single time-point measurements (see also figure Ia and Ib).

In 78 (14%) patients we found elevated levels of HbF (>0.8%) in each time-point, mean value 1.2±0.45%. Detailed characteristics of these participants compared to the group without any elevated HbF measurement are presented in Table II. Elevated HbFwas associated with younger age at examination (p=0.03) and younger age of diagnosis (p=0.01). It was not related to diabetes duration (p=0.21). Elevated HbF was not significantly different between boys and girls (p=0.84) or between CSII and MDI treatment (p=0.09). It was not related

Table I. Clinical characteristics of the study group
Tabela I. *Charakterystyka kliniczna badanej grupy*

		N	Median or number (%)	25–75% range	SD
Diagnosis age (years) / Wiek w chwili diagnozy (lata)		555	7.3	4.22–10.12	3.71
T1DM duration (years) / T1DM czas trwania (lata)		555	5.6	2.72–7.92	3.38
Observation age (years)		555	12.9	10.22–16.39	3.79
Sex / Płeć	Female / żeńska	260	(46.8)		
	Male / męska	295	(53.2)		
Insulin regimen metoda podawania insuliny	CSII	390	(70.3)		
	MDI	165	(29.7)		
Average HbA1c (%) / Średnia HbA1c (%)		555	7.59	6.74–7.99	1.33
Average HbA1c (mmol/mol) / Średnia HbA1c (mmol/mol)		555	59	50–64	10.65
Average HbF (%) / Średnia HbF (%)		78	1.28	1.0–1.37	0.45
Insulin dose (unit/kg/d) / Dawka insuliny (jednostka/kg/d)		555	0.82	0.68–0.97	0.23
Average glycemia from SMBG from 30 days before observation (mg/dl) Średnia z samokontroli glikemii 30 dni przed obserwacją (mg / dl)		555	157.1	137.13–171.14	33.00
Number of measurements from SMBG from 30 days before observation per day Liczba pomiarów z SMBG z 30 dni przed dniem obserwacji		555	6.65	5.02–7.98	2.53

T1DM – diabetes mellitus type 1; CSII – continuous subcutaneous insulin infusion; MDI – multiple daily injection; HbA1c – glycated hemoglobin; HbF – fetal hemoglobin; SMBG – self-monitoring of blood glucose
T1DM – cukrzyca typu 1; CSII – ciągły podskórny wlew insuliny; MDI – metoda wielokrotnych wstrzyknięć insuliny; HbA1c – hemoglobina glikowana HbF – hemoglobina płodowa; SMBG – samodzielna kontrola glikemii

Table II. Characteristics of the patients with an elevated HbF level at each time-point and patients with normal HbF level at each time-point
Tabela II. *Charakterystyka grupy pacjentów z podwyższonym stężeniem HbF w każdym punkcie obserwacji w porównaniu z grupą z prawidłowym stężeniem HbF w każdym punkcie obserwacji*

		No-elevated HbF group (in each measurement) Grupa z prawidłowym stężeniem HbF (w każdym pomiarze)			Elevated HbF group (in each measurement) Grupa z podwyższonym stężeniem HbF (w każdym pomiarze)			P
		N	Median or number (%) Mediana lub liczba (%)	25–75% range	N	Median or number (%) Mediana lub liczba (%)	25–75% range	
Diagnosis age (yr) Wiek w chwili diagnozy (lata)		403	7.53	4.56–10.33	78	6.57	3.37–9.18	0.03
T1DM duration (yr) T1DM czas trwania (lata)		403	5.7	2.9–8.03	78	5.22	2.11–7.16	0.21
Age at examination (yr) Wiek w chwili badania (lata)		403	13.21	10.76–16.6	78	11.8	8.12–15.08	0.01
Sex / Płeć	Female / żeńska	181	(44.9)		36	(46.1)		0.84
	Male / męska	222	(55.1)		42	(53.9)		
Insulin regimen	CSII	280	(69.5)		59	(75.6)		0.09
	MDI	123	(30.5)		19	(24.4)		
Average HbA1c (%) / Średnia HbA1c (%)		403	7.58	6.73–7.99	78	7.53	6.71–7.94	0.89
Average HbA1c (mmol/mol) Średnia HbA1c (mmol/mol)		403	59	50–64	78	59	50–63	0.89
Insulin dose (unit/kg/d) Dawka insuliny (jednostka/kg/d)		403	0.82	0.68–0.97	78	0.86	0.7–0.99	0.32
Average glycemia from SMBG from 30 days before observation (mg/dl) Średnia glikemia z SMBG z 30 dni przed obserwacją (mg/dl)		403	156	137–170	78	160	137–170	0.66
Number of measurements from SMBG from 30 days before observation per day Liczba pomiarów z SMBG z 30 dni przed dniem obserwacji		403	6.65	5.07–7.95	78	6.48	4.74–8.01	0.69

T1DM – diabetes mellitus type 1; CSII – continuous subcutaneous insulin infusion; MDI – multiple daily injection; HbA1c – glycated hemoglobin; HbF – fetal hemoglobin; SMBG – self-monitoring of blood glucose
T1DM – cukrzyca typu 1; CSII – ciągły podskórny wlew insuliny; MDI – metoda wielokrotnych wstrzyknięć insuliny; HbA1c – hemoglobina glikowana; HbF – hemoglobina płodowa; SMBG – samodzielna kontrola glikemii

to relationship the insulin dose, average glycemia or number of measurements.

No correlation between HbA1 and HbF was observed in the study (R=-0.09; p=0.43) (figure II). Patients with HbF level higher that the 3rd quartile (>1.37%) had a slightly lower level of HbA1c (p=0.12).

Discussion

Our study was designed to evaluate a possible link between fetal hemoglobin and HbA1c measurement among pediatric patients with type 1 diabetes older than two years. It needs to be highlighted that in the study we also excluded children who were in diabetes remission phase (at least 1 year after diagnosis) to reduce this confounder which affects the HbA1c level and might interfere with HbA1c/HbF

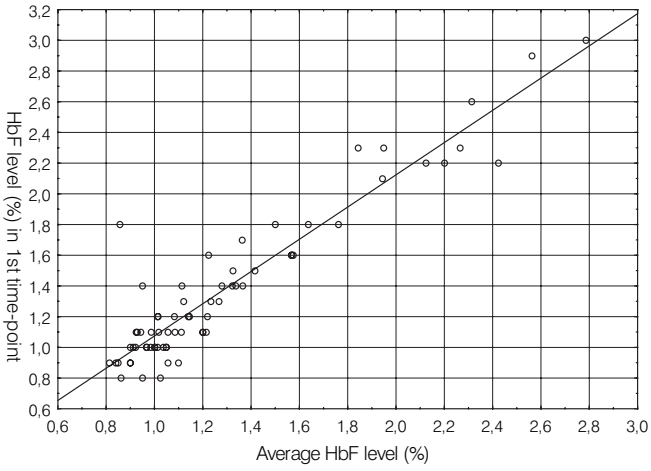

Fig. 1a. Correlation between average HbF level (at least three measurements within one year) and HbF single measurement at study entry

Ryc. 1a. *Korelacja pomiędzy średnim stężeniem HbF (minimum trzy pomiary w trakcie roku) a stężeniem HbF w pierwszym punkcie obserwacji*

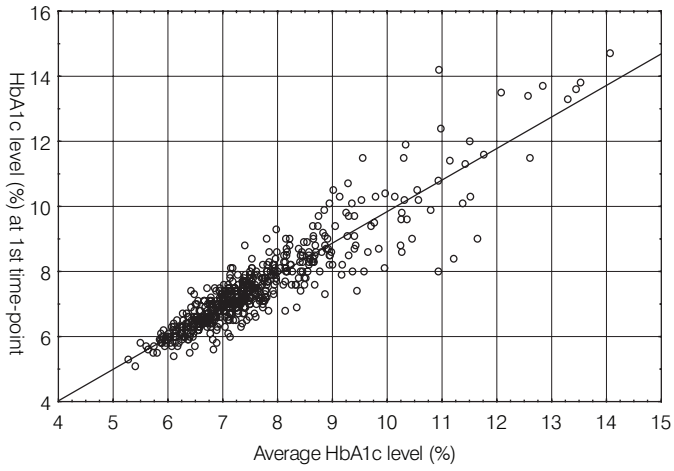

Fig. 1b. Correlation between average HbA1c level (at least three measurements within one year) and HbA1c level single measurement at study entry.

Ryc. 1b. *Korelacja pomiędzy średnim stężeniem HbF (minimum trzy pomiary w trakcie roku) a stężeniem HbF w pierwszym punkcie obserwacji*

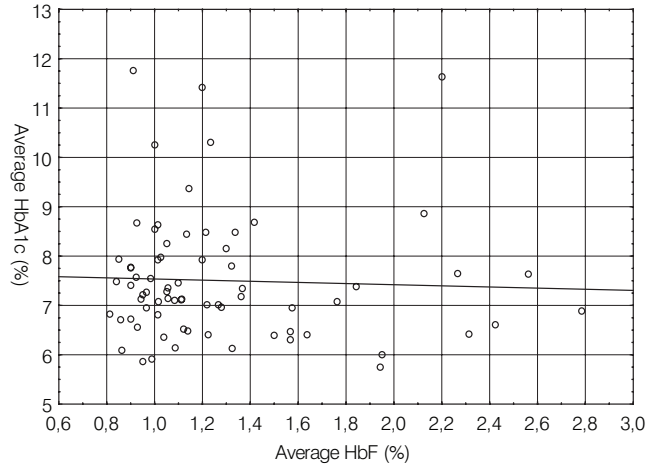

Fig. 2. Correlation between average HbF level and average HbA1c level among children with type 1 diabetes and elevated HbF level (>0.8%)

Ryc. 2. *Korelacja pomiędzy średnim stężeniem HbF (minimum trzy pomiary w trakcie roku) a stężeniem HbF w pierwszym punkcie obserwacji*

relationship (10-12). Additionally, we performed the serial measurements of both HbA1c and HbF. This procedure excludes the variability error coming from single measurement approach.

We were able to confirm a relationship between the HbF level and age at examination and age at diagnosis in the study group. There was no association between the HbF level and the duration of diabetes. Thus, HbF level-patient age at examination and at diabetes diagnosis is probably related to the effect of the different level of HbF during child development. Fortunately, we were not able to find the relationship between the HbF level and the HbA1c level measurements. Thus, it seems that Hb1Ac may serve as a reliable measurement for diabetes metabolic control among pediatric patients older than 2 years. In our study group only 14% of patient had an elevated level of HbF (>0.8%). However, average HbF level was 1.28% of total Hb in the group with elevated fetal hemoglobin. Previous reports have revealed that a significant increase in HbF affects HbA1, especially concerning newborns, neonates and patients with hemoglobinophathies (13, 14). Among these patients, relatively high HbF levels (even greater than 70% in newborns and to 5% in patients with hemoglobinopathathies) were observed (15, 16). In our total group, only slight increase in HbF was observed and this may explain a lack of interference with HbA1c measurements in our study.

In terms of limitations of the study, we have no recorded data on hemoglobinopathy and anemia in the study group, which might explain high HbF in some of the patients and affect HbA1c/HbF relationship (17, 18). Another limitation is unknown exact HbF values for patients with HbF below 0.8%, as a result of methodology. However, since we were not able to find an association between HbA1c and HbF in the group with an elevated HbF level, it is unlikely that HbF values below 0.8% might significantly affect HbA1c levels in the study population.

Conclusions

In general, fetal hemoglobin does not affect HbA1c measurement among pediatric patoents with type 1 diabetes older than 2 years. However, in some groups of diabetic children, for instance neonates, one should be aware that the level of HbF hemoglobin subtypes might interfere with glycated HbA1c values.

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