

Clinical application of 1,5-anhydroglucitol measurements in patients with HNF-1 α MODY.

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Objective: 1, 5-anhydroglucitol (1,5-AG) is a short-term marker of metabolic control in diabetes. Its renal loss is stimulated in hyperglycemic conditions by glycosuria, which results in lowered plasma concentration. As low renal threshold for glucose has been described in HNF-1 α MODY, 1,5-AG level may be altered in these patients. The purpose of this study was to assess the 1,5-AG levels in HNF-1 α MODY patients and in type 2 diabetes (T2DM) subjects with a similar degree of metabolic control. In addition, we aimed to evaluate this particle as a biomarker for HNF-1 α MODY.

Research Design and Methods: We included 33 diabetic patients from the Polish Nationwide Registry of MODY. In addition, we examined 43 T2DM patients and 47 non-diabetic controls. 1,5-AG concentration was measured with an enzymatic assay (GlycoMark). The ROC (receiver operation characteristic) analysis was utilized to evaluate 1,5-AG as a screening marker for HNF-1 α MODY.

Results: The mean 1, 5-AG plasma concentration in diabetic HNF-1 α mutation carriers was 5.9 $\mu\text{g/ml}$ and it was lower than in T2DM patients (11.0 $\mu\text{g/ml}$, $p=0.003$), and in non-diabetic controls (23.9 $\mu\text{g/ml}$, $p<0.00005$). The ROC analysis revealed 85.7% sensitivity and 80.0% specificity of 1,5-AG in screening for HNF-1 α MODY at the criterion of <6.5 $\mu\text{g/ml}$ in patients with HbA_{1c} level between 6.5% and 9.0%.

Conclusions: 1, 5-AG may be a useful biomarker for differential diagnosis of HNF-1 α MODY patients with a specific range of HbA_{1c}, although it requires further investigation. However, the clinical use of this particle in diabetic HNF-1 α mutation carriers for metabolic control has substantial limitations.

Abbreviations: 1,5-AG – 1,5-anhydroglucitol; AUC – area under the curve; BMI – body mass index; CGMS – continuous glucose monitoring system; CI – confidence interval; EDTA – Ethylenediamine Tetraacetic Acid; FPG – fasting plasma glucose; GOD/PAP – glucose oxidase / p-aminophenazone; HbA_{1c} – hemoglobin A_{1c}; HNF-1 α – hepatocyte nuclear factor 1 α ; HNF-4 α – hepatocyte nuclear factor 4 α ; MODY – maturity onset diabetes of the young; OGTT – oral glucose tolerance test; PPG – postprandial glucose; QD - quartile deviation; ROC – receiver operating characteristic; rpm – rounds per minute; SGLT2 – Sodium-Glucose Linked Transporter 2; T1DM – type 1 diabetes mellitus; T2DM – type 2 diabetes mellitus

1,5-anhydroglucitol (1,5-AG) is a monosaccharide that shows a structural similarity to glucose. Its main source in humans is dietary ingestion, particularly meats and cereals [1]. Additionally, about 10% of 1,5-AG is derived from endogenous synthesis. 1,5-AG is generally not metabolized and in healthy subjects it achieves stable plasma concentration that reflects a steady balance between ingestion and urinary excretion [1].

1,5-AG is reabsorbed in renal tubules by an AG/fructose/mannose-common transport system that is distinct from the major glucose reabsorption system [2, 3]. In hyperglycemic conditions, the excess of glucose is reabsorbed due to chemical similarity not only by its own specific active transporters, but also by the AG/fructose/mannose transporter; therefore it competes with 1,5-AG. Subsequently, 1,5-AG urinary excretion is increased during hyperglycemia and it results in lowered plasma concentration [4]. This explains its low plasma level in patients with poorly controlled diabetes. This particle was established in the clinical practice as a short-term marker of metabolic control, and recently it has been investigated as a marker of post-prandial hyperglycemia (PPG) [5, 6]. In contrast to HbA_{1c}, it reflects changes in glycemic control over a period of 1-2 weeks [5].

1,5-AG excretion rate depends on the renal threshold for glucose [7]. Thus, it has obvious limitations in its clinical usefulness in evaluation of some groups of patients, for example pregnant women and subjects with end-stage renal disease [7, 8]. Interestingly, the decreased renal threshold for glucose was observed in HNF-1 α MODY patients, formerly classified as MODY3, and in non-diabetic mutation carriers of this gene [9, 10]. Diabetes mellitus that results from HNF-1 α mutations is usually accompanied by

extrapancreatic features. One of them is a tubulopathy that results in low renal threshold for glucose. An animal model suggests that it is caused by decreased expression of sodium-glucose co-transporter 2 (SGLT2), a low-affinity, high-capacity transporter in proximal renal tubules [11]. Thus, hypothetically, the increased glucose load in renal tubules in these patients may cause stronger competition with 1,5-AG for reabsorption and subsequently increased urinary loss. Moreover, one cannot exclude the impact of HNF-1 α mutations on the expression of the AG/fructose/mannose transporter. Therefore, in HNF-1 α MODY lower plasma concentrations of 1,5-AG compared to other diabetes mellitus types can be expected at a similar HbA_{1c}. In case of a scenario of a very low 1,5-AG level, this particle should also be considered a candidate biochemical marker for this monogenic type of diabetes. This may have considerable clinical implications and enable screening for HNF-1 α MODY patients in large cohorts, potentially avoiding the expensive and laborious technique of direct gene sequencing. In this study, we compared 1,5-AG plasma concentration in diabetic HNF-1 α mutation carriers to patients with type 2 diabetes mellitus (T2DM) and non-diabetic subjects.

PATIENTS AND METHODS

The Nationwide Registry of MODY has been established at the Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland. The details of the inclusion criteria and the examination were previously described [12]. So far, 57 HNF-1 α mutation carriers have been identified. We contacted 47 adult subjects from this group and 41 agreed to enter this study. The following exclusion criteria were used: pregnancy, liver cirrhosis, hypo- or hyperalimentation, steroid therapy, gastrectomy and elevated serum creatinine

level. Based on these criteria, we excluded 4 patients due to increased serum creatinine concentration (i.e. > 97 $\mu\text{mol/l}$, the upper reference limit for the assay). Thus, the examined study group consisted of 37 subjects. The number of HNF-1 α MODY patients diagnosed with diabetes according to WHO criteria was 33, while 4 subjects were normoglycemic in fasting condition and they were classified as non-diabetics, which is also supported by the fact, that they all had HbA_{1c} level within the normal range (<6.0 %). Two more groups were examined. First, 47 apparently healthy controls that were normoglycemic on fasting condition. This group contained spouses of MODY patients and volunteers from the medical personnel of the Department of Metabolic Diseases. We also included 43 patients with T2DM having an age of diagnosis of 35 years or above and for whom no insulin therapy was used for at least two years after diagnosis, ascertained as previously described [13]. The same exclusion criteria were used as for the MODY group. The study protocol and informed consent procedures were approved by the Ethical Committee of the Jagiellonian University, Medical College, and were concordant with the Helsinki Declaration.

The blood samples were collected in fasting conditions for biochemical evaluation. Serum and EDTA-plasma were obtained by spinning at 3500 rpm, and stored in -80°C. The 1,5-AG concentration was measured in EDTA-plasma samples with an enzymatic, colorimetric method utilizing GlycoMark chemicals (Tomen America) by Nippon Kayaku Tokyo Co. Ltd., Tokyo, Japan [14]. HbA_{1c} was measured with high performance liquid chromatography (BioRad). Fasting glucose level was determined by the enzymatic technique (Automated Analysis Boehringer Mannheim Glucose GOD/PAP Method). Serum creatinine concentration was measured with the Jaffe method.

Comparisons were made with the χ^2 or Fisher exact test where applicable for frequencies of qualitative traits. For the quantitative traits, Student t test or analysis of variance (ANOVA) was used with Tukey post hoc test, when data fit into a normal distribution, otherwise the Mann-Whitney or the Kruskal-Wallis tests were used. The data, for which Kruskal-Wallis test was significant, were further analyzed with nonparametric multiple comparison Steel test. Normality was tested with the Kolmogorow-Smirnow test. Spearman's rank correlation was utilized to test the relationships between quantitative traits. Multiple linear regression was applied to determine independent predictors for 1,5-AG level. These predictors were included as covariates in a general linear model analysis performed both in all study groups and in diabetic groups. Computations were performed with MiniTab 14.20 statistical software and the R Language and Environment, version 2.4.1. The values of p estimate smaller than 0.05 were considered significant. In analysis of plasma 1,5-AG measurements as a biochemical test for HNF-1 α MODY, the test parameters and ROC (receiver operating characteristic) curve analysis were calculated with MedCalc 9.3.8.0 statistical software.

RESULTS

The clinical characteristics of the study groups is presented in Table 1. Both diabetic groups were similar for traditional measures of metabolic control such as fasting glucose and HbA_{1c}. They varied, however, for age, BMI and diabetes duration, which is in line with the way both groups were defined. Subjects from all groups also had an almost identical serum creatinine level.

The mean 1,5-AG plasma concentration in the diabetic HNF-1 α mutation carriers group was 5.9 $\mu\text{g/ml}$ (median 2.6 $\mu\text{g/ml}$, quartile deviation (QD) 4.3), while in the T2DM group it was 11.0

$\mu\text{g/ml}$ (median 11.5 $\mu\text{g/ml}$, QD 6.7), and 23.9 $\mu\text{g/ml}$ (median 24.5 $\mu\text{g/ml}$, QD 4.9) in the non-diabetic control group. There was a highly significant difference between MODY and the control group ($p < 0.00005$), as well as between MODY and T2DM ($p = 0.003$). To justify application of a parametric approach in further analysis, the data were re-evaluated with ANOVA and Tukey test, which was also significant ($p < 0.00005$ and $p = 0.013$, respectively). In 4 HNF-1 α normoglycemic mutation carriers, mean 1,5-AG level was 21.1 $\mu\text{g/ml}$, range 16.7-32.9 $\mu\text{g/ml}$, and was not different from the level in the control group ($p = 0.32$). The mean age of the 4 subjects was 24.7 yrs, mean BMI was 21.0 kg/m^2 , and mean HbA_{1c} was 5.6 %.

The 1,5-AG concentration was lower in females than in males in the control group (21.5 $\mu\text{g/ml}$ vs. 27.7 $\mu\text{g/ml}$, $p = 0.003$), while there were no significant differences between sexes in MODY and T2DM groups ($p = 0.71$ and $p = 0.91$, respectively). In the MODY group, the 1,5-AG level significantly correlated only with HbA_{1c} level ($\rho = -0.72$, $p < 0.0005$), while in the T2DM group there was significant correlation of 1,5-AG level with HbA_{1c} ($\rho = -0.72$, $p < 0.0005$) and age of diabetes diagnosis ($\rho = 0.36$, $p = 0.018$). In multiple linear regression HbA_{1c} level remained the only significant predictor for 1,5-AG ($p < 0.0005$). In the non-diabetic control group, 1,5-AG concentration significantly correlated with age ($\rho = -0.52$, $p < 0.0005$) and BMI ($\rho = -0.34$, $p = 0.02$). In the multiple regression model, the only significant predictor was age ($p = 0.004$).

The general linear model for 1,5-AG concentration as a dependent variable was applied in the three study groups combined ($R^2 = 52.1\%$). The differences in 1,5-AG level between the study groups remained significant ($p < 0.0005$), and age was a significant covariable ($p = 0.044$). In the diabetic study groups ($R^2 = 53.2\%$), the general linear model revealed a significant difference

in 1,5-AG level between T2DM and HNF-1 α MODY ($p = 0.006$), with HbA_{1c} level as a significant covariable ($p < 0.0005$).

The ROC curve analysis was performed in diabetic patients: 33 HNF-1 α MODY and 43 T2DM subjects. Area under the ROC curve (AUC) was 0.732 (95% CI: 0.618-0.827). The optimal sensitivity and specificity to test for MODY were: 72.7% (95% CI: 54.5-86.7%) and 65.1% (95% CI: 49.1-79.0%), respectively, at a criterion of $< 6.6 \mu\text{g/ml}$ of plasma 1,5-AG. To further improve the parameters of the test, we performed additional analyses with narrowed HbA_{1c} range based on the plot showing relationships between HbA_{1c} and 1,5-AG in diabetic groups (Figure 1). Since T2DM patients with an HbA_{1c} level $> 9.0\%$ had a very low 1,5-AG level, similar to HNF-1 α MODY subjects, we first excluded all individuals with glycated hemoglobin above this value (5 MODY and 10 T2DM individuals, respectively). This resulted in better ROC analysis outcomes, especially improvement of the test specificity. The AUC was 0.823 (95% CI: 0.704-0.909), and optimal sensitivity and specificity at the criterion of $< 6.6 \mu\text{g/ml}$ 1,5-AG were 71.4% (95% CI: 51.3-86.7%) and 84.85% (95% CI: 68.1-94.8%), respectively. Additionally, we excluded 7 MODY and 13 T2DM patients with an HbA_{1c} $< 6.5\%$, due to a lack of correlation of 1,5-AG with HbA_{1c} in both groups ($p = 0.34$ for MODY and $p = 0.44$ for T2DM). This improved the test specificity, and resulted in the following parameters: AUC 0.887 (95% CI: 0.749-0.964), optimal sensitivity at the criterion for MODY of $< 6.5 \mu\text{g/ml}$ 1,5-AG 85.7% (95% CI: 63.6-96.8%) and specificity 80.0% (95% CI: 56.3-94.1%). Figure 2 shows this ROC curve analysis in the group of patients with an HbA_{1c} range between 6.5% and 9.0%.

DISCUSSION

Here the plasma 1,5-AG levels in HNF-1 α MODY patients are reported for the first time. We observed that its levels in this group were significantly lower than in T2DM subjects with similar glycemic control as assessed by HbA_{1c} levels and fasting glucose level. We were prompted to perform this study by an earlier description of decreased renal threshold and increased glycosuria in HNF-1 α mutation carriers [9, 10]. The major cause of low 1,5-AG levels in diabetic HNF-1 α mutation carriers is most likely, as in other diabetic patients, its competition in the renal tubule with a large amount of glucose for the AG/fructose/mannose transporter [3]. However, in HNF-1 α patients, the threshold for glucosuria is lower, and therefore the excessive glucose in the renal tubule would be expected to result in greater decrements in 1,5-AG. One cannot rule out, however, the additional mechanism associated with the impaired expression of this transporter as a direct result of a mutation in the HNF-1 α gene encoding a transcription factor present in kidney. The confirmation of this later hypothesis will require expression gene experiments.

Our observation may have two important clinical implications. First, 1,5-AG measurements have a limited usefulness in HNF-1 α MODY as a marker of metabolic control. 1,5-AG is accepted by the FDA as a tool of monitoring glycemic control of diabetic patients. There were already some groups of patients described, in whom 1,5-AG should not be recommended as a diabetes control measure due to a low renal threshold for glucose, such as pregnant women and patients with end-stage renal disease [7, 8], although some promising attempts of its use in gestational diabetes were described [15]. In addition, there were some efforts to use 1,5-AG as a screening tool in diabetes [16, 17]. It should be emphasized, however, that no scientific diabetes organization recommended it for this purpose. If,

however, at any time in the future 1,5-AG is accepted as a screening tool for diabetes, this should not include the situations where the renal threshold for glucose is altered, such as HNF-1 α MODY.

At the same time, our observation points to 1,5-AG as a potential differential diagnosis biomarker for MODY associated with HNF-1 α mutations. The measurement of plasma 1,5-AG level seems to have high sensitivity and specificity to differentiate between HNF-1 α MODY and T2DM as long as HbA_{1c} values are between 6.5% and 9.0%. It should be noted that most of the patients seen in everyday clinical practice fall into this range. Our study should be perhaps criticized for dissecting the data to improve the ROC curve and for choosing an arbitrary range of HbA_{1c} for this analysis. As shown in Figure 1, all individuals, MODY patients and T2DM subjects, with HbA_{1c} above 9.0% had very low 1,5-AG levels probably due to a massive overload of renal tubules by glucose and thus the marker is not specific in this range of HbA_{1c}. The exclusion of subjects with low HbA_{1c} values is justified by the fact that some degree of hyperglycemia is necessary to cause glycosuria and a subsequent fall in 1,5-AG level. The renal threshold in HNF-1 α MODY patients was measured at a mean whole blood glucose level of 6.5 mmol/l, (the equivalent of 7.4 mmol/l in plasma) [9, 10]. In another study, during OGTT, glycosuria was detected only in non-diabetic subjects with HNF-1 α mutations and with impaired glucose tolerance [10]. An additional argument constitutes a lack of correlation between HbA_{1c} and 1,5-AG levels among subjects with well-controlled diabetes in our study and in an earlier report [18]. Nevertheless, the specific range of HbA_{1c} where 1,5-AG is a useful biomarker for differential diagnosis will require further confirmation. Interestingly, all non-diabetic HNF-1 α mutation carriers had levels of examined marker in the range of 10th to 90th percentile of the controls and thus 1,5-

AG measurement is not useful for predictive testing.

Lack of PPG data in the examined subjects is a limitation of our study. As a consequence, we cannot entirely rule out that a higher degree of PPG in HNF-1 α MODY patients might have partially contributed to the observed large, almost two fold difference, in 1,5-AG level between both diabetic groups. There are, however, important indirect arguments on the key effect of renal tubulopathy. First, it was shown in two other clinical groups, pregnant women and patients with end-stage renal disease, that renal threshold for glucose, substantially different from normal, seriously influenced circulating 1,5-AG level [7, 8]. Since renal tubulopathy in HNF-1 α MODY has been previously well documented [9, 10], its influence on 1,5-AG seems quite inevitable. Second, our diabetic study groups were identical not only for HbA_{1c}, but also very similar for fasting glucose. Since, HbA_{1c} level is influenced by both PPG and FPG [19], it is very unlikely that putative significantly higher PPG had substantial, if any, contribution to this huge difference in the 1,5-AG level. HNF-1 α gene mutation carriers were previously reported as having higher postchallenge hyperglycemia compared to glucokinase mutation carriers (MODY2) [20]. The latter group, however, is very specific, unusual with its own very low post-challenge glucose rise (2-3 mmol at 2 hours). No published data support a notion that HNF-1 α MODY is characterized by larger PPG than T2DM patients. Previously, particularly high contribution of PPG as assessed by CGMS on 1,5-AG level was described in a group of diabetic patients, with moderately controlled diabetes (with HbA_{1c} below 8%), most of them with T1DM, a type of diabetes characterized by an absolute insulin deficiency [6]. The phenomenon of larger PPG contribution to overall glycemic control in such moderately controlled patients

was earlier described [19]. The comparison done between our diabetic groups with respect to 1,5-AG level included patients with a variable degree of glycemic control, also those with HbA_{1c} values above 8% (more than 30% of MODY subjects) where FPG impact is larger. Our study should also be perhaps criticized for choosing T2DM patients for the comparison as their clinical characteristics were different from that of patients with HNF-1 α mutations and thus, some variables could have an impact on individual glucose profiles and therefore contribute to lower 1,5-AG despite seemingly equal HbA_{1c} values. Two more groups of patients should be considered. First, subjects with other forms of MODY. Among those, the carriers of HNF-4 α (MODY1) seem to be the most appropriate for the comparison due to clinical similarity to HNF-1 α MODY, relatively high frequency and no data on the altered glucose renal threshold [21, 22]. However, the families from The Polish Nationwide Registry of MODY have not been systematically tested for mutations in this gene and we do not have a cohort of an appropriate size in this ethnic group. A second group that might be considered for the comparison of 1,5-AG with diabetic HNF-1 α mutation carriers were T1DM patients. There are, however, already biomarkers available to differentiate between MODY and T1DM such as islet specific antibodies and C-peptide. Additionally, it is difficult to provide positive and negative predictive value of the test based on serum 1,5-AG measurements. It would require a different study design where testing was prospective and not, as in our study, involve testing two discrete groups. Thus, we propose this as a future study. We believe that 1,5-AG will find its eventual application as a marker for differentiation between other MODY subtypes or in young T2DM subjects. The relative prevalence of HNF-1 α MODY may constitute from one third to two thirds of families with early onset autosomal dominant

diabetes [23, 24] and about 8% of young patients with T2DM [25]. This should give a 1,5-AG based test appropriate positive and negative predictive values. Finally, we should acknowledge that a relatively small number of HNF-1 α MODY patients were examined.

Some general observations should also be emphasized. First, as expected, the 1,5-AG concentration was strongly inversely correlated with the HbA_{1c} level in diabetic patients from both groups. The influence of sex and age on 1,5-AG level has been already observed in controls [14, 18], but, as with our data, not in the diabetic study groups, as reported from the other populations [18]. The creatinine level did not influence plasma 1,5-AG concentration in these study groups, all three with normal creatinine levels. The mean level of 1,5-AG in the control group was slightly higher than in an American reference population [14], which may be

caused by different nutritional habits in the Polish population, especially higher consumption of cereal food.

In summary, we conclude that 1,5-AG may be a useful biomarker for the differential diagnosis of HNF-1 α MODY patients with a specific range of HbA_{1c}, although it requires further investigation in larger sets of patients and other diabetic groups, as well as direct assessment of the relative contribution of low renal threshold for glucose and PPG. However, the clinical use of this particle in diabetic HNF-1 α mutation carriers for metabolic control has substantial limitations.

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Table 1. Clinical characteristics of T2DM, HNF-1 α MODY patients and non-diabetic controls.

	T2DM	p (post hoc*)	HNF-1 α MODY	p (post hoc*)	Controls	p
No. of individuals	43	NA	33	NA	47	NA
% of females	65.1	NA	75.8	NA	61.7	0.41 ¹
Age [yrs]	56.2; 9.3	<0.0005 ²	42.9; 16.1	0.68 ²	45.5; 15.3	<0.0005 ³
BMI [kg/m ²]	35.1; 7.9	<0.0005 ²	23.0; 3.4	0.13 ²	25.5; 4.2	<0.0005 ³
Diabetes duration [yrs]	8.8; 6.0; 6.0	NA	17.3; 16.0; 12.2	NA	NA	0.004 ⁴
Fasting glucose [mmol/l]	7.6; 7.5; 1.7	0.62 ⁵	7.9; 7.4; 1.9	<0.00005 ⁵	4.7; 4.8; 0.6	<0.00005 ⁶
A1c [%]	7.6; 7.1; 1.4	NA	7.6; 7.7; 1.1	NA	NA	0.77 ⁴
% on OHA	62.8	NA	45.5	NA	NA	0.13 ¹
Creatinine [μ mol/l]	70.7; 12.7	NA	69.2; 12.6	NA	69.8; 10.6	0.86 ³
1,5-AG [μ g/ml]	11.0; 11.5; 6.7	0.003 ⁵	5.9; 2.6; 4.3	<0.00005 ⁵	23.9; 24.5; 4.9	<0.00005 ⁶

The sex is presented as percentage of females. % on OHA is the proportion of patients treated with oral hypoglycemic agents; the rest of the subjects were insulin treated in monotherapy or in combination with OHA. The age, BMI and the serum creatinine concentration, which fit into normal distribution, are presented as mean and standard deviation. The other quantitative traits are presented as mean, median and quartile deviation. NA – not applicable.

* Non-normally distributed data, for which Kruskal-Wallis test was significant, were further analyzed with Steel's nonparametric multiple comparison test

¹ Chi² test

² Tukey post hoc test

³ One-way ANOVA

⁴ Mann-Whitney test

⁵ Steel test

⁶ Kruskal-Wallis test

Legends to Figures

Figure 1. Individual value plot of 1,5-AG concentrations against HbA_{1c} levels in HNF-1 α MODY and T2DM groups. Open and closed squares represent adult MODY and T2DM patients respectively. Non-diabetic HNF-1 α mutation carriers indicated by circles.

Figure 2. ROC curve analysis for the diabetic patients with normal serum creatinine concentration, and HbA_{1c} level $\geq 6,5\%$ and $\leq 9,0\%$. Panel A) The ROC curve. Marker represents optimal value. Panel B) Sensitivity and specificity plotted against cut-off value of plasma 1,5-AG concentration. Additional vertical gridlines represent the optimal cut-off value of 6.5 $\mu\text{g/ml}$.

