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The application of plasma 1,5-anhydro-D-glucitol for monitoring type 2 diabetic patients

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Abstract. *Aim:* Recent data have suggested that effective control of postprandial blood glucose can reduce the risk of macroangiopathic complications of diabetes, especially cardiovascular risk. 1,5-Anhydro-D-glucitol (1,5-AG) has been proposed as a marker of short-term hyperglycaemic excursions. We aimed to evaluate its usefulness in patients with type 2 diabetes and have attempted to indicate when 1,5-AG monitoring should be used in ordinary diabetes care settings.
Methods: The study group consisted of 130 type 2 diabetic patients aged 36–69 years. 1,5-AG plasma level, HbA_1c concentrations and daily glucose profile were measured. Mean blood glucose (MBG), M-value were calculated and maximal daily glycaemia (MxG) was established as indicators of short-term hyperglycaemic episodes.
Results: 1,5-AG plasma level was negatively and HbA_1c was positively correlated with fasting glycaemia (FG), MBG, M-value and MxG. Multivariate regression analysis revealed that 1,5-AG plasma level is determined by MxG only, while FG determined HbA_1c concentration in blood. The analysis of 1,5-AG level and HbA_1c distributions in well and poorly controlled patients revealed that persons with low HbA_1c values may have decreased 1,5-AG plasma level.
Conclusion: 1,5-AG plasma level monitoring is the useful method to identify well controlled, exclusively based on HbA_1c levels type 2 diabetic patients with transient hyperglycaemia, accordingly patients at high risk of macroangiopathic complications.

Keywords: 1,5-anhydro-D-glucitol, glycated haemoglobin, postprandial hyperglycaemia, diabetes mellitus type 2

1. Introduction

The polyol 1,5-anhydro-D-glucitol (1,5-AG), 1-deoxy form of glucopyranose, was found to be present in most human tissues including plasma. The amounts of 1,5-AG produced endogenously are not significant and the main source of 1,5-AG in human body is food [1]. The most characteristic features of 1,5-AG are: 1) inert metabolism; the contribution of 1,5-AG to metabolic pathways is negligible; the portion of 1,5-AG ingested with food within a day is balanced by the portion which is eliminated from the body in the same time 2) 1,5-AG is eliminated by the kidneys; its reabsorption in renal tubules occurs by glucose transporting mechanisms [1–3]. The first property supplies a stable concentration of 1,5-AG in plasma of healthy persons within 24 hours, the second one – rapid elimination of 1,5-AG by the kidneys when renal threshold for glucose is exceeded. 1,5-AG competes with glucose for transporter mechanism binding sites in renal tubules. The appearance of glucose in urine results in saturation of binding sites by glucose and automatic elimination of 1,5-AG with urine. Rapid 1,5-AG elimination with urine leads to prompt drop in the plasma 1,5-AG level [2]. Therefore falls in 1,5-AG plasma levels are determined and closely related to hyperglycaemia, even when very short-lasting episodes appear.

For several years 1,5-AG has been suggested as an indicator of metabolic control [4–6]. It was established that changes in 1,5-AG plasma level reflect hyperglycaemic episodes appearing 1–2 days before the

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assay [4]. Decreased plasma 1,5-AG is strongly correlated with poor metabolic control in diabetic patients [3]. The total body pool of 1,5-AG can be depleted rapidly by glucosuric hyperglycaemia on the time scale of days in the presence of overt hyperglycaemia [2,4]. Because 1,5-AG is derived from food and based on the fact that normal daily intake of 1,5-AG represents only a small fraction of total body pool of 1,5-AG in the replete normoglycaemic steady state, recovery from a significantly depleted state is slow (takes about 5 weeks), even under continuously normoglycaemic conditions [1,4]. Thus, decreased plasma 1,5-AG in various patients reflects hyperglycaemia within time scale of days or weeks.

Currently, when epidemiological data revealed that postprandial hyperglycaemia is the cardiovascular risk factor much more significant than fasting glycaemia and HbA_1c are [7,8], the clinical benefits of 1,5-AG monitoring seems to be obvious. However, the interpretation of 1,5-AG plasma changes in relation to standard parameters, like fasting glycaemia, postprandial glycaemia, mean blood glucose and HbA_1c, is needed. We have attempted to indicate when 1,5-AG monitoring is especially useful in ordinary diabetic care setting.

2. Patients

The subjects were 130 (61 men, 69 women, 36–69 years old white Caucasians) with type 2 diabetes outpatients and inpatients of University Hospital in Poznan, Poland. Diabetes mellitus was diagnosed according to EDPG criteria [9]. Patients were treated with gliclazide (Diaprel; Servier, twice daily 80 mg – 90 persons) or with conventional insulin regimens (twice a day ready-to-use mixtures of insulins: Mixtard 30; Novo Nordisk or Humalog Mix 25; Elli Lilly – 40 persons). Therapeutic schedule for each patient was started at least 3 months before beginning of the investigation. Patients with concomitant diseases (liver diseases, renal diseases, anaemia – haemoglobin < 13 g/dl) or with severe diabetic complications (nephropathy – serum creatinine > 0.2 mmol/l, neuropathy, retinopathy), as well as patients taking drugs that might affect HbA_1c assay, such as ascorbic acid or aspirin were excluded from the study. In gliclazide-treated group 51 patients suffered from macrovascular complications (coronary heart disease, peripheral blood vessels disease, cerebral vascular disease). In insulin-treated group 28 patients were diagnosed with macrovascular complications.

Prior approval for all studies was given by the local Ethical Committee of the Poznan University of Medical Sciences and all participants signed informed consent.

3. Measurements

Glucose concentration was measured 8 times a day (at fasting, 2 hours after each meal, at 10 p.m. and at 2 a.m.) in 95 patients from a venous blood sample by the glucose oxidase method using Cormay analyser (PZ Cormay).

HbA_1c (normal range: 4.1–6.0%) was assayed by HPLC method (Variant^{TM} Hemoglobin A_1c, BIO-RAD) standardised according to DCCT/NGSP [10,11].

The plasma concentration of 1,5-AG was measured using a modified column enzymatic method [12,13]. Briefly, 100 μl of plasma samples deproteinised with trichloroacetic acid were passed through a two-layer microcolums packed with ion-exchange resins (cationite Dowex 50WX8; anionite Dowex 1X8, Sigma) to remove glucose. 1,5-AG was efficiently recovered in the flow-through fraction. Hydrogen peroxide formed in the enzymatic oxidation of 1,5-AG with pyranose oxidase was detected by a standard method utilising an enzymatic colour-developing system. The intra-assay CV was 4.9% and inter-assay CV – 3.7%. The mean recovery was 96.6%. Reference range was between 14.4–30.2 mg/l.

Based on 24-hours glucose profile, MBG (mean blood glucose) and M-value by Schlichtkrull [14] were calculated. M-value is a measure of overall diurnal variability in blood glucose within a day and is calculated on the basis of the patient's glycaemic profile. M-value is a parameter modified by both hyperglycaemic spikes and hypoglycaemic troughs. The mean maximal daily glycaemia (MxG) was established as the mean of the maximum daily plasma glucose values of all patients.

Considering the glucose metabolism, the patients were thought to be well or unsatisfactorily controlled according to HbA_1c and 1,5-AG. For HbA_1c values, the patients were considered as well controlled for the value less or equal 6.5%, and poorly controlled for the value more than 6.5%. For the 1,5-AG value, the patients were defined as well controlled with 1,5-AG value equal or higher 14.0 mg/l and poorly controlled for the value less than 14.0 mg/l. Because the appropriate 1,5-AG levels preventing hyperglycaemia-dependent complications have not been sufficiently evaluated we established the "near reference" range for healthy human as necessary to achieve good metabolic control.

4. Statistical analysis

All results were expressed as means $\pm$ SD and medians. Regression analysis, multivariate non-linear re-

Table 1
Clinical characteristics of type 2 diabetic patients

	N	Mean ± SD	Median
Age (years)	130	56.1 ± 8.7	54.0
Diabetes duration (years)	130	7.8 ± 1.1	5.0
Fasting glycaemia (mmol/l)	95	9.2 ± 4.4	8.0
MBG (mmol/l)	95	10.7 ± 3.6	10.2
M-value	95	48.1 ± 40.7	38.2
MxG (mmol/l)	95	14.8 ± 4.3	14.8
HbA_1c (%)	130	7.0 ± 2.3	6.1
1,5-AG (mg/l)	130	10.2 ± 6.3	8.4

Table 2
Linear multiple regression analyses

Independent values	Dependent value – 1,5-AG Coefficient β	$R^2 = 0.2$ $p < 0.00032$ P-level	Dependent value – HbA_1c Coefficient β	$R^2 = 0.25$ $p < 0.00002$ P-level
FG	0.0115	0.94	0.50	0.003*
MBG	−0.128	0.59	0.19	0.42
M-value	0.238	0.39	−0.29	0.27
MxG	−0.55	0.015*	0.1	0.65

*Statististically significant.

gression analysis were used for statistical analysis and p value <0.05 was considered significant. Statistical analyses were performed using Statistica 6.0 (StatSoft, Inc.).

5. Results

Clinical data on studied group are shown in Table 1. In the cross-sectional study plasma 1,5-AG concentrations were distributed over a wide range (0.9–29.9 mg/l), with a mean value 10.2 ± 6.3 mg/l. HbA_1c levels were variable too, ranged between 4.0–13.7% (6.9 ± 2.2%).

Significant negative correlations were found between 1,5-AG and fasting glycaemia (FG); $r = [-0.31],\ p \leqslant 0.05$, MBG; $r = [-0.35],\ p \leqslant 0.05$, M-value; $r = [-0.35],\ p \leqslant 0.05$ and MxG; $r = [-0.40],\ p \leqslant 0.05$. Similarly HbA_1c was correlated, but positively with FG, $r = 0.51, p \leqslant 0.05$, MBG, $r = 0.46,\ p \leqslant 0.05$; M-value, $r = 0.39, p \leqslant 0.05$ and MxG, $r = 0.43,\ p \leqslant 0.05$.

The multiple regression analysis was used to evaluate which one from glycaemia-dependent factors (FG, MGB, M-value, MxG) independently determines 1,5-AG plasma level and HbA_1c. It was found out that 1,5-AG plasma concentration was dependent only on MxG (standardised coefficient $\beta = [-0.55],\ p < 0.00032$), while HbA_1c was primarily determined by FG (standardised coefficient $\beta = 0.50,\ p < 0.00002$).

Relationship between 1,5-AG and HbA_1c levels.

Patients were subdivided into 2 subgroups according to HbA_1c levels (values $\leqslant$ 6.5% and $>$ 6.5%) (Fig. 1). In the well controlled subgroup ($HbA_1c \leqslant 6.5\%$) the range of 1,5-AG plasma level was rather wide from 2.0 to 29.9 mg/l. In the subgroup with poor metabolic control ($HbA_1c > 6.5\%$) 1,5-AG plasma level was low and distributed between 0.9–14.7 mg/l.

Patients were then subsequently again subdivided into 2 subgroups according to 1,5-AG plasma level: $\geqslant$ 14.0 mg/l and $<$ 14.0 mg/l (Fig. 2). It was revealed that in well controlled group (according to 1,5-AG) HbA_1c levels were not higher than 6.5%, but in poorly controlled group HbA_1c ranged between 4.0–13.7%.

6. Discussion

Recently an important role has been advocated for 1,5-AG in the assessment and ongoing management of diabetes mellitus, where the hyperglycaemic state is associated with marked decrease in plasma 1,5-AG levels [3,4]. 1,5-AG and HbA_1c both are the retrospective markers of metabolic control. In general HbA_1c levels reflect average glucose levels of 1–2 month before the assay [15]. HbA_1c and 1,5-AG values are caused by different factors participating in overall metabolic compensation. The relationship between HbA_1c and various categories of glycaemia is complex and efforts to link them with fasting or postprandial glycaemia have produced conflicting conclusions [16,17]. Fasting glycaemia (premeal and interprandial) is rather chronic

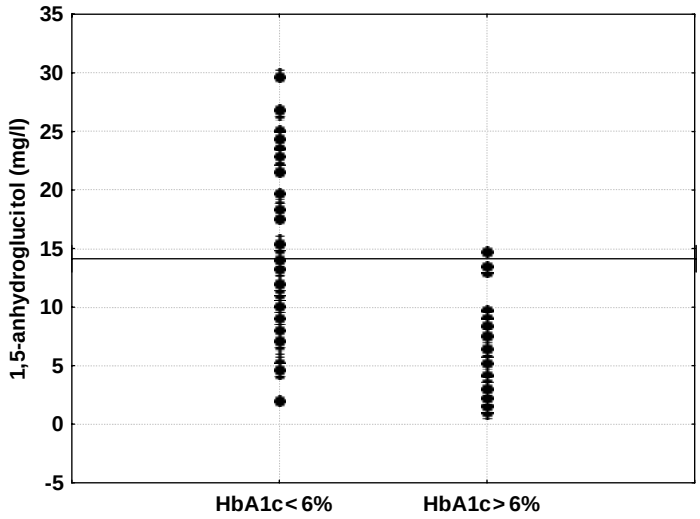

Fig. 1. The distribution of 1,5-AG in well controlled ($n = 80$) and in poorly controlled group ($n = 50$) according to HbA_1c levels.

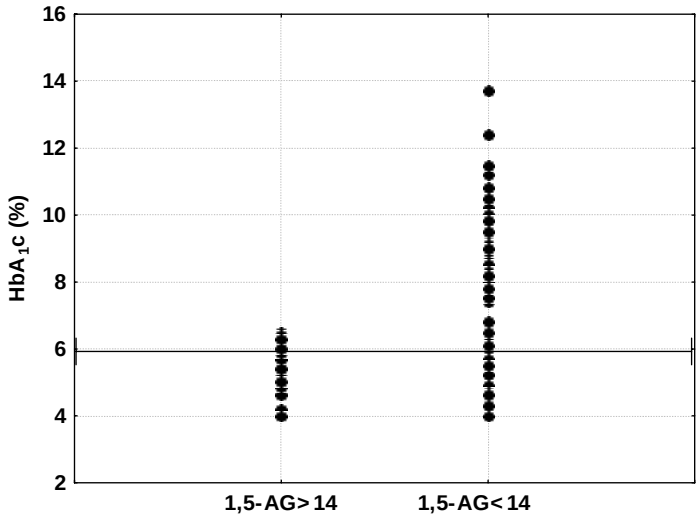

Fig. 2. The distribution of HbA_1c in well controlled ($n = 29$) and in poorly controlled ($n = 101$) group according to 1,5-AG levels.

while postprandial glycaemia is usually short-lasting episode.We found that in type 2 diabetic patients HbA_1c levels were correlated with FG, MBG, M-values and MxG, but multiple regression analysis revealed that the only category of glycaemia determining HbA_1c levels independently on others glycaemic parameters, was FG. Moreover, it was well established previously that although postprandial glycaemia can influence HbA_1c concentration, glycated haemoglobin is not sensitive for short-lasting, transient hyperglycaemia. The capability of HbA_1c to capture a hike in blood glucose level immediately after meals is weak [18]. The formation of HbA_1c is 2-step chemical process. The first reaction leading do Schiff base formation is almost completely reversible and its rate is much higher than the rate of the second reaction, irreversibly leading to the real HbA_1c (ketoamine) formation [19]. Most of currently used test for HbA_1c estimation eliminates Schiff base, thus transient hyperglycaemia, postprandial or whichever acute one, is not able to change HbA_1c level.

Alternatively, 1,5-AG concentration fall in the plasma reflects not only chronic, but also short-lasting hyperglycaemic episodes. However we found correlations between 1,5-AG level and FG, MBG, M-value, MxG, but independently MxG only determined 1,5-AG levels in plasma. Therefore, 1,5-AG plasma level

reflects the highest hyperglycaemic peaks observed within day long. Considering correlation coefficient (between 1,5-AG and MxG, $r = [-0.40]$), it seems to be a little low. It should be kept in mind that the real highest daily glucose levels that appeared during our observation could be not detected in some patients and it is the possible reason of weaker statistical correlation. Moreover, in poorly controlled patients ($HbA_1c > 6.5\%$) hyperglycaemia appeared much earlier than 1–2 days before our measurement. It means that hyperglycaemic peaks were repeated within last 6–5 weeks and 1,5-AG plasma level was affected several times. In these patients 1,5-AG levels were low but not directly dependent on maximal glucose levels observed at the day before. Such results may lead to conclusion that 1,5-AG level is dependent on maximal daily glycaemia, but has to be interpreted for each patient individually.

What can we conclude from the distribution of 1,5-AG values in well controlled and unsatisfactory controlled patients exclusively based on HbA_1c levels? It's clear that in poorly controlled patients, with high HbA_1c levels we should ever expect low 1,5-AG values, while in patients with low HbA_1c we found not only persons with high 1,5-AG levels (well controlled), but also those with low 1,5-AG and hyperglycaemic spikes not reflected by HbA_1c (Fig. 1). The opposite analysis confirmed this observation. We found that group with low 1,5-AG values presented good or poor metabolic control as regards HbA_1c, while in patients with high 1,5-AG levels HbA_1c values were low (Fig. 2).

In summary, 1,5-AG estimation is required especially for patients with satisfactory HbA_1c levels to detect transient hyperglycaemic peaks.

7. Conclusion

Recent epidemiological, clinical and experimental data have suggested that controlling blood glucose in the nonfasting state, especially the postprandial period, can reduce the risk of macroangiopathic complications of diabetes [7,8,20]. Monitoring of HbA_1c allows to diminish risk of microvascular complications. Unfortunately, low HbA_1c level is not sufficient to decrease risk of macrovascular complications, especially risk of coronary heart disease. Coronary heart disease is known as the main reason of high morbity and mortality among patients with diabetes type 2. 1,5-AG level in plasma reflects short-term (postprandial especially) changes in serum glucose and could be an excellent tool to achieve optimal glycemic control as an adjunct to HbA_1c. 1,5-AG level monitoring is the useful method to identify otherwise well controlled patients with transient hyperglycaemia – patients at high risk of macroangiopathic complications.

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