1,5-Anhydroglucitol Levels in Type 2 Diabetic and Non-Diabetic Subjects in Southern Taiwan

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ABSTRACT

Purposes: To estimate plasma 1,5-anhydroglucitol (AG) in diabetic (DM) and non-DM patients in a Chinese population, and to compare it with fructosamine, glycosylated hemoglobin (HbA1c), and fasting glucose (FG) levels. **Methods:** Case-control study on the significance of AG conducted in a medical center of southern Taiwan, including 356 inpatients (300 non-DM and 56 type 2 DM). Plasma AG, fructosamine, HbA1c and FG were measured on the second day of admission and only those with normal values (except glucose) were enrolled. Glycemic markers of the non-DM patients were examined only once whereas DM patients were sequentially sampled over 3 months. **Results:** Mean plasma AG levels were lower in DM than in non-DM patients (4.02±2.96 vs 26.68±11.33µg/ml, P<0.001), and lower in non-DM females than males (22.90±9.51 vs 29.45±11.7µg/ml, P<0.05). AG showed a good correlation with FG. Mean plasma AG were inversely correlated with FG, fructosamine and HbA1c in DM patients and worked as well as other glycemic markers in detecting short-term changes in glycemic control. AG levels of DM patients demonstrated no difference with or without smoking, hypertension, micro- and macro-vascular complications. **Conclusions:** We recommend clinical application of plasma AG in long-standing DM patients for short-term detection and monitoring glycemic condition. **(Arq Bras Endocrinol Metab 2003;47/6:711-715)**

Keywords: 1, 5-anhydroglucitol; Fructosamine; Glycosylated hemoglobin; Type 2 diabetes mellitus; Southern Taiwan

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RESUMO

Níveis de 1,5-Anidroglucitol em Pacientes Não-Diabéticos e Diabéticos do Tipo 2 no Sul de Taiwan.

Objetivos: Avaliar o 1,5-anidroglucitol (AG) plasmático em pacientes com (DM) e sem (não-DM) diabetes numa população chinesa, e compará-lo à frutosamina, hemoglobina glicada (HbA1c) e glicemia de jejum (GJ). **MÈtodos:** Estudo caso-controle sobre a significância do AG conduzido em centro médico no sul de Taiwan com 356 pacientes selecionados (300 não-DM e 56 DM2). Os níveis de AG, frutosamina, HbA1c e GJ foram avaliados no 2º. dia de admissão, e apenas aqueles com resultados normais (exceto GJ) foram incluídos. Esses marcadores foram examinados apenas uma vez nos não-DM, enquanto os DM foram amostrados sequencialmente por 3 meses. **Resultados:** As concentrações de AG foram mais baixas nos DM do que nos não-DM (4,02±2,96 vs 26,68±11,33µg/ml, P<0,001), e mais baixas nas mulheres do que nos homens não-DM (22,90±9,51 vs 29,45±11,7µg/ml, P<0,05). Houve boa correlação entre AG e GJ. AG plasmático apresentou correlação inversa com a GJ, frutosamina e HbA1c nos pacientes com DM, e mostrou-se tão eficaz quanto outros marcadores na detecção de mudança de curto prazo no controle glicêmico. Os níveis de AG nos pacientes DM não foram diferentes quanto ao tabagismo, hipertensão e complicações micro e macrovasculares. **Conclusões:** O emprego clínico do AG plasmático é recomendado em pacientes com DM de longa

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duração para detecção e monitoração da condição glicêmica a curto prazo. **(Arq Bras Endocrinol Metab 2003;47/6:711-715)**

Descritores: 1,5-anidroglucitol; Frutosamina; Hemoglobina glicada; Diabetes mellitus tipo 2; Sul do Taiwan

1,5-ANHYDROGLUCITOL (AG) IS A six-carbon monosaccharide in 1-deoxy form of glucopyranose. Its 1,5-anhydrohexitol nature was discovered in 1932 and its isomeric structure established in 1943 (1-4). Pitkänen (1) first reported on the existence of AG in human plasma and cerebrospinal fluid (CSF) in diabetic patients in 1975. 1,5-anhydro-D-glucitol, 1-deoxyglucose and 1,5-AG are all synonyms of AG. AG can be found in human CSF and plasma, in rats, and even in soil Pseudomonas (2). The concentration of AG is slightly higher in plasma than in CSF, and higher in males than in females (2).

AG is mainly generated from the diet. Daily intake is about 4.5mg/day, but because of a relatively large body pool of AG (500 to 1000mg), there is minimal daily fluctuation in concentrations. The kidney reabsorbs almost all AG and re-absorption is competitively inhibited by unreabsorbed glucose at the AG specific transporter in renal tubule (4,5). The daily recovery rate of AG in serum is about 0.3µg/ml/day in those who have excellent glycemic control.

Gas-liquid chromatography and an enzymatic method have been developed to measure AG (2,3,6-12). The enzymatic method is simple and fast and has been used in most publications (6).

Thus, blood glucose test, glycosylated hemoglobin (HbA1c), fructosamine and AG may be used to assess the degree of glycemia (13-20). Compared with fructosamine or HbA1c, AG shows a much greater sensitivity to glycemic changes, which makes AG reliable in monitoring daily changes of glycemia (4). Impaired renal function and removal of AG by dialysis may contribute to the decrease of AG concentration in patients with end-stage renal disease (21,22). Plasma AG is inversely correlated with fasting plasma glucose, fructosamine, and HbA1c (19-20,23-26).

In this study, we report AG, plasma glucose and fructosamine in Chinese patients with or without DM, and compare the change of plasma AG over time to other glycemic markers in hospitalized type 2 DM patients.

MATERIALS AND METHODS

Patients: We enrolled 300 adult Chinese inpatients of Kaohsiung Veterans General Hospital as controls, whose routine biochemistry studies were all within normal limits. During the same period, we recruited 56 type 2 DM inpatients, diagnosed in accordance with the World Health Organization criteria (27), with a mean DM duration of 10.1±6.7 years. These patients were admitted mostly due to poor glycemic control. Other biochemical studies were within normal limits, except plasma glucose. The proteinuria of diabetic patients was defined as frank proteinuria. The micro- and macro-vascular complications of diabetic patients were diagnosed by consulting specialists.

Sampling protocol of diabetic patients: Plasma AG concentration, fructosamine, HbA1c and glucose of the diabetic patients were measured on the first sampling day and at the end of the second week, and the first, second and third months. Plasma AG concentrations, fructosamine and glucose of the diabetic patients were checked every other day during the first two weeks of admission. Most of the patients were discharged within 2 weeks from admission and the rest of the blood sampling was carried out at the outpatient department.

Biochemical study: All biochemistry was measured by the ion selective electrode, colorimetric and kinetic enzymatic methods with Hitachi 747 automatic analyzer provided by Boehringer Mannheim/Hitachi (Germany/Japan).

The glycemic marker HbA1c was measured by HPLC with Hi-autoA1c HA-8121 provided by Kyoto Daiichi Kagaku Co. Ltd. (Kyoto, Japan). The reference range is 3.8% to 5.7% (with inter- and intra-assay CVs of 0.6% and 0.71%, respectively). Fructosamine was measured by a colorimetric assay system (nitroblue tetrazolium method) provided by Technicon Co. (Japan) with Technicon RA-1000 analyzer (intra- and inter-assay CVs of 2.57% and 3.94%). The reference range is 1.59mmol/l to 2.81mmol/l. Plasma AG concentration was determined by the pyranose oxidase method through "Lana AG" column-enzyme assay kit (NK-15 kit) (inter- and intra-assay CVs of 4.7% and 2.3%) developed by Nippon Kayaku Co. Ltd. (Tokyo, Japan) (6) (1µmol/l = 0.18µg/ml). All the glucose and AG data of non-diabetic patients and the first sampling of diabetic patients were adopted for analyzing the cutoff value for detecting DM.

Statistical analysis: We used SPSS for Windows (version 7.0, SPSS Inc.) and Excel for Windows (version 2000, Microsoft, Redmond, WA) on an IBM-

compatible personal computer for the statistical analysis. All data were expressed as mean±SD. The statistical significance of any inter-group differences was assessed by Kruskal-Wallis one-way ANOVA, Mann-Whitney-U test, or Student's t test wherever appropriate. A p value <0.05 was considered statistically significant. The cut-off value of AG was estimated by using the receiver operating characteristic (ROC) curve method (28,29).

RESULTS

Clinical characteristics of the diabetic subjects are in table 1. They had lower concentrations of plasma AG than non-diabetics (4.02±2.96 vs 26.68±11.33µg/ml, p<0.001) (table 2). Non-diabetic male patients had higher concentrations of plasma AG than female (29.45± 11.78 vs 22.90±9.51µg/ml, p<0.05), however, there were no sex differences in the diabetic patients (table 2).

The first blood sample was used as the baseline for evaluating the changes in serial measurements of the glycemic markers in diabetic patients. AG detects improvement of plasma glucose before the first end point (mean duration was 6.22±2.84 days, 95% confidence interval 5.21 days to 7.23 days). Plasma AG showed a greater negative correlation to the change of plasma glucose (PG) (r = -0.475, p<0.01) in serial follow-ups than fructosamine and HbA1c to the change of PG (r = 0.360 and 0.399, both p<0.01; respectively) (table 3).

We adopted the receiver operating characteristic (ROC) curve to determine the best cutoff value of plasma AG, distinguishing diabetic from non-diabetic patients. The cutoff value was estimated about 11.50µg/ml with a sensitivity of 95.8% and specificity of 95.7%.

Table 1. Characteristics of the diabetic subjects.

	All	Male	Female
Number	56	39	17
Age (years)	62.0 ± 10.0	64.9 ± 8.9	55.4 ± 9.5
Smoking	21	18	3
DM duration (years)	10.1 ± 6.7	10.5 ± 7.3	9.2 ± 5.5
BMI (kg/m^2)	24.6 ± 4.1	24.3 ± 3.8	25.2 ± 4.8
Hypertension	12	9	3
Retinopathy (B/P) [a]	30 (22/8)	20 (16/4)	10 (6/4)
Neuropathy	14	10	4
Proteinuria	13	10	3
Macro-vascular	8	5	3
Treatment (I/O) [b]	40/16	28/11	12/5

[a] B: background; P: proliferative; [b] I: insulin; O: oral sulfonylurea

Table 2. Glycemic markers in different subgroups.

Group	Number	Age	AG[a]	Fru[b]	FPG[c]
Non-DM	300	54.2 ± 16.7	26.68 ± 11.33	1.97 ± 0.29	5.2 ± 0.6
Male	173	57.5 ± 15.4	29.45 ± 11.78	2.00 ± 0.31	5.3 ± 0.6
Female	127	52.3 ± 16.7	22.90 ± 9.51	1.93 ± 0.26	5.1 ± 0.6
DM[d]	56	62.0 ± 10.0	4.02 ± 2.96	3.44 ± 1.04	10.9 ± 3.2
Male	39	64.9 ± 8.9	3.95 ± 2.02	3.54 ± 0.81	10.5 ± 2.6
Female	17	55.4 ± 9.5	4.19 ± 4.10	3.22 ± 1.45	11.9 ± 4.3
Non-smoker	35	61.1 ± 10.1	4.38 ± 3.40	3.28 ± 1.09	10.9 ± 3.4
Smoker	21	63.6 ± 9.9	3.43 ± 1.98	3.71 ± 0.91	11.0 ± 3.0
No Hypertension	26	61.0 ± 10.5	4.40 ± 3.67	3.18 ± 1.07	10.5 ± 3.6
Hypertension	32	62.9 ± 9.7	3.69 ± 2.18	3.67 ± 0.98	11.3 ± 2.9
No Retinopathy	26	62.0 ± 10.0	4.02 ± 2.96	3.44 ± 1.04	10.9 ± 3.2
Retinopathy	30	52.3 ± 16.7	3.81 ± 2.36	3.45 ± 1.03	11.7 ± 3.1
No Neuropathy	17	62.5 ± 9.9	4.32 ± 3.17	3.40 ± 1.13	10.9 ± 3.5
Neuropathy	39	60.6 ± 10.6	3.14 ± 2.07	3.57 ± 0.73	11.1 ± 2.1
No Proteinuria	43	62.5 ± 10.1	4.12 ± 3.11	3.50 ± 1.09	10.7 ± 3.3
Proteinuria	13	60.3 ± 10.0	3.72 ± 2.48	3.26 ± 0.88	11.7 ± 3.0
No Macro-vascular	48	61.7 ± 10.2	4.16 ± 3.11	3.44 ± 1.02	10.6 ± 3.1
Macro-vascular	8	63.6 ± 9.2	3.20 ± 1.79	3.44 ± 1.24	13.0 ± 3.6
SU[e]	16	61.7 ± 9.9	3.71 ± 1.95	3.47 ± 0.93	11.2 ± 3.2
Insulin	40	62.7 ± 10.6	4.8 ± 4.62	3.3.8 ± 1.31	10.3 ± 3.3

[a] AG: 1,5-anhydroglucitol (mg/ml); [b] Fru: fructosamine (mmol/l); [c] FPG: fasting plasma glucose (mmol/l); [d] Baseline fasting plasma glucose (mmol/l); [e] SU: sulfonylurea.

Table 3. Change of all glycemic markers at different endpoints (percent change from baseline data).

Endpoint	FPG[a] (%)	AG[b] (%)	Fru[c] (%)	HbA1c[d] (%)
Baseline	10.9 ± 3.2	4.02 ± 2.96	3.44 ± 1.04	10.7 ± 2.1
2 weeks	9.2 ± 3.3 (-13.7)	4.97 ± 3.91 (23.6)	3.05 ± 0.94 (-11.3)	9.0 ± 3.2 (-15.89)
1 month	9.9 ± 3.5 (-9.7)	6.61 ± 4.58 (64.5)	2.58 ± 0.86 (-25.0)	8.8 ± 2.0 (-17.8)
2 months	10.7 ± 5.8 (-3.7)	6.58 ± 5.70 (63.7)	2.68 ± 0.75 (-22.1)	7.8 ± 2.7 (-27.1)
3 months	11.5 ± 4.6 (+4.6)	6.37 ± 5.80 (58.5)	3.37 ± 1.51 (-2.0)	8.0 ± 2.3 (-25.2)

[a] FPG: fasting plasma glucose (mmol/l); [b] AG: 1,5-anhydroglucitol (mg/ml); [c] Fru: fructosamine (mmol/l); [d] HbA1c: glycosylated hemoglobin (%).

DISCUSSION

Since Pitkänen (1,2) first reported on the existence and reduction of 1,5-anhydroglucitol (AG) in type 1 DM in 1975, AG has been adopted as a tool for screening DM and for evaluating the glycemic control in DM patients (4,23–25). Similar to previous reports, our study has detected lower plasma concentrations of AG in female non-diabetic patients than in male ones, but no significant differences of plasma fructosamine and glucose (2,21). Our results were similar to those reported by Yabuuchi in Japanese (24.6±7.2 and 7.3±7.1µg/ml in non-diabetic and diabetic patients, respectively) (6). Serum AG concentration did not correlate with age or body mass index (BMI) (30).

Concentrations of AG were identical in diabetic patients with or without smoking, hypertension, micro-vascular complications including retinopathy, neuropathy, proteinuria, and macro-vascular complications. Non-smoking diabetic patients seemed to have higher levels of AG; however, the difference was close to the statistic significance (P= 0.06).

The cutoff value estimated for detecting DM in our subjects is lower than that in the Japanese (14µg/ml) (4,15,20). The poor plasma glucose control, as well as the fact that the subjects were not selected from a community basis, and the long DM duration might explain the discrepancy between the Japanese and our patients. However, a lower level of plasma AG in type 1 than in type 2 DM patients may counteract to some of the findings due to our exclusive selection of type 2 DM patients (2,14,22,24-25).

Our study has demonstrated that AG is as good as HbA1c and fructosamine in short-term evaluation of the glycemic control. Different from HbA1c and fructosamine, AG can be measured directly and, therefore, is not influenced by anemia or hypoalbuminemia, which often exist in long-standing diabetic patients. The mean plasma AG level was no different in diabetic patients with nephropathy with or without proteinuria. Plasma AG has a shorter latent period and a higher degree of fluctuation to express glycemic condition.

The possible reasons why AG is not widely used worldwide are because most of the published literature on the subject were conducted in Japan; recently, to constrain hospital management expenses and observing health policies such as global budget, new laboratory tests and drugs are seldom approved unless they are evidently superior or merely less expensive; there were too few published international multicenter studies on AG and the development of commercial automation is still underway.

In conclusion, plasma AG is a good marker for evaluating glycemic control of type 2 DM patients. No differences in plasma AG levels could be demonstrated in diabetic patients with or without co-morbidities. Plasma AG is as good as other glycemic markers in short-term evaluation of long standing DM patients with fluctuating glycemic control.

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