Salivary 1,5-Anhydroglucitol and its Correlation with Postprandial Hyperglycemia: Development and Validation of a Novel Assay

Abstract

Background: Saliva has the potential to be used as a noninvasive sample for testing hyperglycemia in diabetes mellitus. Serum 1,5-anhydroglucitol (1,5-AG) decreases with an increase in blood sugar >180 mg/dl. We hypothesized that salivary 1,5-AG can be used to identify blood sugar higher than 180 mg/dl using a novel biochemical method. **Aim:** This study aimed to develop a novel biochemical method for serum and salivary assessment of 1,5-AG and assess its correlation with postprandial blood sugar (PPBS) >180 mg/dl. **Methodology:** The study comprised 45 controls (healthy individuals) and 45 cases (type 2 diabetic patients with PPBS >180 mg/dl). Blood and salivary samples were collected according to the study protocol. A new method was developed for the quantification of 1,5-AG in serum and saliva using liquid chromatography–mass spectrometry. **Results:** The value of serum (mean –22.19 µg/ml and median –22.12 µg/ml) and salivary (mean –0.124 µg/ml and median –0.088 µg/ml) 1,5-AG was higher in healthy individuals compared to corresponding serum (mean –3.89 µg/ml and median –2.52 µg/ml) and salivary (mean –0.025 µg/ml and median – 0.025 µg/ml) levels in diabetics with PPBS >180 mg/dl. In diabetics, a significant negative correlation was noticed with PPBS levels and 1,5-AG levels in serum and saliva. Salivary 1,5-AG level <0.054 µg/ml had an 86.4% sensitivity and 87.2% specificity in predicting a blood sugar value >180 mg/dl. **Conclusion:** The results of our study suggest that the short-term glycemic marker 1,5-AG can be detected in saliva and can be useful as an adjunct marker in monitoring of glycemic status in diabetic patients.

Keywords: *Liquid chromatography–mass spectrometry, salivary 1,5-anhydroglucitol, salivary biomarker, short-term glycemic marker, tandem mass spectrometry, type II diabetes mellitus*

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Introduction

Whole saliva has the potential for diagnosing and monitoring systemic diseases as its constituents are similar to plasma.[1,2] The monitoring of short-term glycemic control in diabetes mellitus (DM) is done using capillary blood obtained by finger prick and assessed using portable glucometer. Consistent short-term glucose control is essential for a long-term favorable glycemia and the prevention of end-organ complications due to DM. In real-world practice, patients with DM show hesitancy in accepting self-monitored blood glucose assessment due to the requirement of finger prick for sampling. Short-term glycemia can also be assessed using the serum glycated albumin or fructosamine which can assess the glycemic control over the previous 2–3 weeks[3,4] and 1,5-anhydroglucitol (1,5-AG) which can reflect the glycemic level over the previous 48 h–2 weeks.[4]

Of particular interest is 1,5-AG which is a naturally occurring dietary polyol that is proposed as a serum marker for postprandial hyperglycemia.[5] Serum 1,5-AG initially was extensively studied in Japan, and an automated assay using an enzymatic method is commercially available in Japan since 1991.[6] A commercial version of this assay in the name GlycoMark has been approved for marketing by the Food and Drug Administration and has been evaluated by clinical trials in the United States which has concluded that serum 1,5-AG responds rapidly and sensitively to glycemic changes and monitors glycemic control in accordance with established markers.[7] Quantification of 1,5-AG in saliva was also evaluated by the GlycoMark assay kit and concluded that the salivary sample served

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as a stable matrix for biochemical assay.[8] The compound 1,5-AG can reflect the postprandial glycemic excursions and identifying the same in saliva will be beneficial to patients and clinicians for monitoring blood glucose levels. We assessed if a correlation existed between serum and salivary 1,5-AG levels and if salivary levels could be used to predict blood sugar >180 mg/dl.

Methodology

We compared two groups of participants attending the outpatient department of Sri Ramachandra Hospital, Porur, Chennai, for the serum and salivary 1,5-AG levels. The study was approved by the Institutional ethics committee. The study comprised 90 subjects. Group I (controls –45) was individuals without any underlying systemic illness and Group II (cases –45) was patients with DM who have postprandial blood glucose value >180 mg/dl. The study excluded (a) diabetic patients with postprandial blood sugar (PPBS) ≤180 mg/dl, (b) chronic liver disease, (c) renal impairment (serum creatinine more than 2 mg/dl), (d) hypertriglyceridemia (more than 30 mmol/l), (e) patients with cystic fibrosis, and (f) pregnancy.

Sampling procedures

The details of the patient were recorded in a structured data entry sheet designed for the study. Blood samples were collected for fasting and PPBS, lipid profile, liver, and renal function tests. Both blood and saliva samples were collected on the same day.

Salivary sample collection

Patients were advised to refrain from eating 30 min before salivary sample collection. Around 3 ml of whole, unstimulated saliva was collected by asking the patient to spit the accumulated saliva into a graduated tube with a lid. Within 30 min of sample collection, both blood and salivary samples were centrifuged at 5000 rotations per min (RPM) for 10 min at 4°C and stored at −80°C. The blood reports of the study population were reviewed to check whether all the study samples were conforming to the inclusion and exclusion criteria. The samples falling under exclusion criteria were discarded. All the samples were stored at −80°C until laboratory analysis.

Quantification of 1,5-anhydroglucitol in serum and saliva using liquid chromatography–mass spectrometry

A sensitive and selective method were developed for the determination of 1,5-AG in human serum and saliva using the Shimadzu liquid chromatography–mass spectrometry (LCMS)-8040 triple quadrupole system coupled with ultra-high performance liquid chromatography (UHPLC) NEXERA. A synergi hydro-reversed phase (RP) column (150 mm × 4.6 mm, 4.0 µm) was used with a mobile phase consisting of water: acetonitrile (90:10 v/v) in an isocratic flow of flow rate 0.700 mL/min was used for the analysis of serum sample. The column oven temperature was 40°C. For the analysis of saliva samples and mobile phase consisting of water: acetonitrile (80:20 v/v) in an isocratic flow rate of 0.700 mL/min was used. The analysis was carried out in negative ionization mode using electrospray ionization (ESI) as an interface with the following conditions: the nebulizer gas as nitrogen at 3.0 L/min, heat block temperature at 250°C, desolvation temperature at 250°C, and drying gas as nitrogen at 17.0 L/min. Argon gas was used as collision-induced dissociation gas for mass spectrometry cum mass spectrometry (MS-MS) experiments. The multi-reaction monitoring (MRM) transitions monitored for 1,5-AG was 163.20 >100.90. Collision energy was 14V. The dwell time was kept as 200 m. The runtime of 4.0 min with ESI interface voltage ±3.5 kV. The serum and saliva sample volume as low as 100 µL is used. The 1,5-AG is being endogenous, requires neat calibration standards and quality control (QC) samples which were accomplished by stripping of serum and saliva. All the data acquired were processed by Shimadzu laboratory solutions version 5.80 software. The concentration curves were analyzed using a linear fit with a $1/C^2$ weighting.

1,5-AG standard stock solution -1,5-AG standard stock solution were prepared at 1 mg/mL in water. This standard stock solution is further diluted for obtaining the appropriate calibration working standard.

Sample preparation for serum samples

One hundred and microliters of serum (drawn from intended −80°C storage area and kept for thawing at room temperature) containing 1,5-AG (standards, QC, and subject samples) aliquoted into radioimmunoassay (RIA) vials, 1.600 mL of acetonitrile was added and vortexed immediately for precipitation. Then, 0.400 mL of water was added, vortexed, and centrifuged in a refrigerated centrifuge for 10 min, 4000 RPM at 4°C. 1 mL supernatant taken, mixed with 1 mL of acetonitrile: water (50:50 v/v), vortexed and 200 µL was transferred into autosampler vials before analyze by LCMS-MS.

Sample preparation for saliva samples

One hundred and microliters of saliva (drawn from intended − 80°C storage area and kept for thawing at room temperature) containing 1,5-AG (standards, QC, and subject samples) aliquoted into RIA vials, 1.600 mL of acetonitrile was added and vortexed immediately for precipitation. Then, 0.400 mL of water was added, vortexed, and centrifuged in a refrigerated centrifuge for 10 min, 4000 RPM at 4°C. 2.000 mL of dichloromethane was added, vortexed and centrifuged at 4°C for 4000 RPM, 10 min. The upper supernatant containing water was transferred to autosampler vials and loaded into LCMS-MS for analysis.

Liquid chromatography–mass spectrometry and liquid chromatography–mass spectrometry cum mass spectrometry condition optimization

1,5-AG was not found to be retained well on traditional RP (C18, C8, and phenyl-Hexyl) high-performance liquid chromatography (HPLC) columns because of its highly water soluble and polar character. Luna hydrophilic interaction liquid chromatography and synergy Hydro-RP column were tried and were successful in retaining 1,5-AG. Further, the synergy Hydro-RP column was selected because of best peak shape and good retention. 1,5-AG was found to be ionized in ESI-negative ionization rather than positive ionization. ESI-negative ionization of 1,5-AG results in the loss of hydrogen atom from one of its hydroxyl (−OH) group resulting in the molecular ion of m/z 163 (M-H). Further, this molecular ion m/z 163 was subjected to 12 different collision energies ranging from 5V to 38V to identify the marker ion (daughter ion) for quantification. The marker ion m/z 101 was stable in all the applied collision energies, so this ion was used for quantifying the levels of 1,5-AG in saliva and serum.

Sample preparation conditions optimization

For 100 µL of samples, it is necessary to provide adequate sensitive, simple and fast sample preparation procedure for both serum and saliva samples. Therefore, the protein precipitation method was adopted for serum analysis and liquid–liquid extraction method was adopted for saliva analysis. It is also necessary to produce sufficiently clean extracts to ensure method ruggedness and long-term performance. For serum sample analysis, precipitation with acetonitrile provided better peak shape and recovery than that of methanol. Further dilution with acetonitrile: water, 50:50 v/v provided less matrix effect. For the analysis of 1,5-AG in saliva, liquid–liquid extraction method provided the best result using dichloromethane to separate water from acetonitrile: water mixture. Thus, a clean extract necessary for LCMS analysis was obtained.

Calculation of 1,5-anhydroglucitol using calibration curve

A calibration graph has been drawn for the 1,5-AG standards using the concentration of 1,5-AG and its corresponding mass counts obtained through MS-MS analysis. The values of 1,5-AG were calculated based on the area (mass count) obtained from the MRM mass chromatogram for each sample. From the graph, the mass count has been derived for a known concentration of 1,5-AG (considered to be corrected mass count) and the same has been applied to calculate the unknown concentration of 1,5-AG in the serum and saliva samples.

$$\text{Unknown } 1,5\text{-AG}\,(\mu g/mL) = \frac{\text{Mass count of unknown sample} \times \text{Known conc. of } 1,5\text{-AG}\,(\mu g/mL)}{\text{Corrected mass count of known conc. of } 1,5\text{-AG}}$$

Statistical analysis

Values of blood sugar, serum, and salivary 1,5-AG were expressed as mean, standard deviation. Correlation between serum and salivary 1,5-AG levels as well as with blood sugar level was assessed using the Pearson correlation followed by the test of significance. Diagnostic accuracy of salivary 1,5-AG levels in predicting a PPBS >180 mg/dl was assessed with sensitivity, specificity, and plotting of the receiver operating characteristics. A $P < 0.05$ was considered statistically significant. The analysis was done with statistical package for the social sciences (SPSS) statistics for Windows, version 16.0. (SPSS Inc.,Chicago, Ill.,USA). The software used for line art was GraphPad Prism version 9.

Results

The standard stock, working standard, and QC standard solutions were prepared, following the International Council on Harmonization Guidelines and a typical pharmacopeia procedure. The assay method has used an external calibration approach, where the limit of detection and limit of quantification have been determined. Further, a five-point linear calibration has been established with accuracy and precision determination. The recovery has been found out to be >65%. The method has been applied for assay after determining these partial validations. This method can be used with the help of liquid chromatography cum triple quad mass spectrometer. The PPBS in cases was 278 mg/dl (mean) and 276 mg/dl (median). Among controls, the blood sugar was 91 mg/dl (mean) and 85 mg/dl (median). The serum 1,5-AG level in cases was 3.89 µg/ml (mean) and 2.52 µg/ml (median) which was significantly lower ($P < 0.001$) than normoglycemic controls; 22.19 µg/ml (mean) and 22.12 µg/ml (median). The salivary 1,5-AG level in diabetic patients was 0.024 µg/ml (mean) and 0.025 µg/ml (median) was significantly lower ($P < 0.001$) than normoglycemic controls; 0.124 µg/ml (mean) and 0.088 µg/ml (median).

There was a significant positive correlation between serum and salivary 1,5-AG level (salivary level increased as serum value increased); $r = 0.648$, $P = 0.0005$ [Figure 1]. A significant negative correlation was observed between serum 1,5-AG level and blood sugar (serum 1,5-AG level decreased with increasing blood sugar level); $r = -0.514$, $P = 0.0005$ [Figure 2]. Furthermore, there was a significant negative correlation between salivary 1,5-AG in Group II with the PPBS levels (increase in the PPBS levels, was associated with decrease in the levels of 1,5-AG); $r = -0.539$ and $P = 0.0005$ [Figure 3]. Salivary 1,5-AG level < 0.054 µg/ml had a 86.4% sensitivity and 87.2% specificity in predicting a blood sugar value >180 mg/dl [Figure 4].

Discussion

Our study observed that the salivary 1,5-AG levels have the potential to be a diagnostic tool to assess hyperglycemia

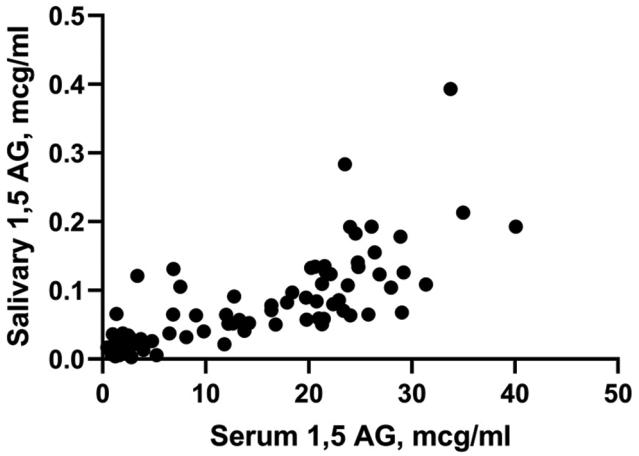

Figure 1: Correlation between serum and salivary 1,5-AG with X-axis plotted with serum 1,5-AG levels and Y-axis plotted with salivary 1,5-AG levels. 1,5-AG: 1,5-anhydroglucitol

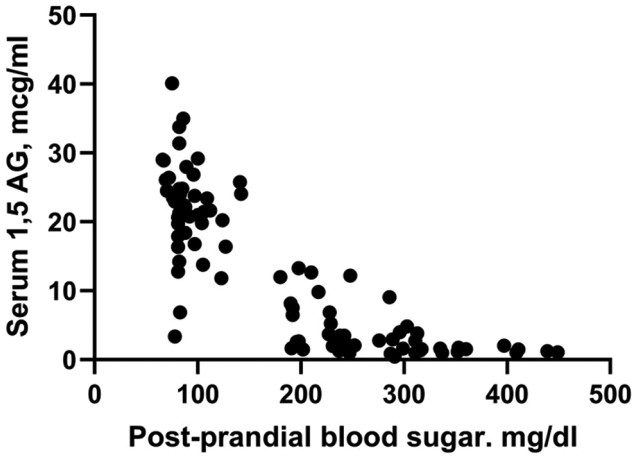

Figure 2: Correlation between serum 1,5-AG levels and PPBS values. X-axis is plotted with PPBS values and Y-axis with serum 1,5-AG levels. 1,5-AG: 1,5-anhydroglucitol. PPBS: Postprandial blood sugar

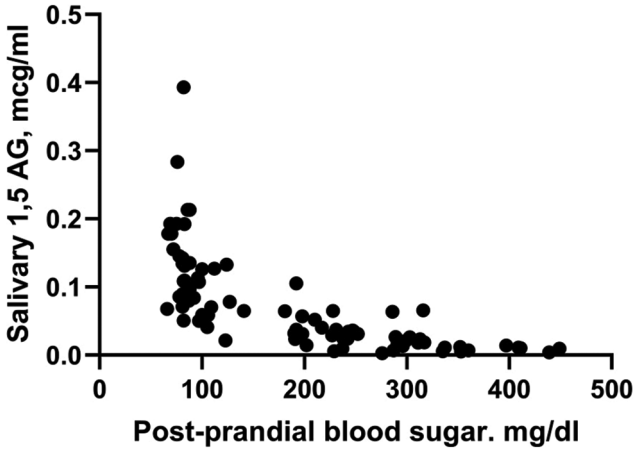

Figure 3: Correlation between salivary 1,5-AG levels and PPBS values. X-axis plotted with PPBS values and Y-axis with salivary 1,5-AG levels. 1,5-AG: 1,5-anhydroglucitol. PPBS: Postprandial blood sugar

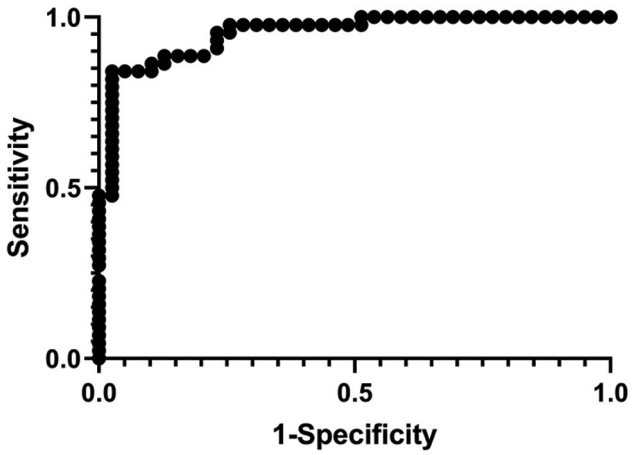

Figure 4: Receiver operating characteristics of diagnostic accuracy parameters of salivary 1,5-AG compared with serum 1,5-AG as reference standard. 1,5-AG: 1,5-anhydroglucitol

higher than 180 mg/dl. Previous studies have evaluated 1,5-AG using the enzymatic method and HPLC, gas chromatography, and coupled with MS and it has been proven that HPLC with MS is a more superior and sensitive method for the quantification of 1,5-AG compared to the enzymatic method.[9] In the current study, a novel method has been used for the quantification of 1,5-AG in saliva and serum using UHPLC with MS/MS.

The molecular weight of 1,5-AG is 164Da. The mass spectrometer detects the molecular weight of the compound through the LCMS interface called ESI which ionizes the 1,5-AG molecule. Since 1,5-AG was detected in negative ionization rather than positive ionization, the ESI used was ESI negative ionization. ESI-negative ionization of 1,5-AG results in the loss of one hydrogen from one of its Hydroxyl (−OH) group resulting in molecular ion of m/z 163 (M-H). Further, this molecular ion m/z 163 is subjected to different collision energies to identify the marker ion (daughter ion) for quantification. The marker ion m/z 101 was stable in all the applied collision energies, so this ion m/z 101 was used for quantifying the levels of 1,5-AG in saliva and serum.

In the case of glycosuria when blood glucose exceeds 180 mg/dl, the reabsorption of 1,5-AG by the kidney is inhibited by competition with glucose excretion.[10] 1,5-AG seems to be of little use in detecting glucose fluctuations below this renal threshold of glycosuria. Hence, study subjects with blood glucose values of more than 180 mg/dl were included in the study. The expression of sodium-glucose cotransporter 4 which is the suggested glucose transporter for 1,5-AG is increased when blood glucose levels attain 160–180 mg/dl.[11,12] Thus, the cutoff value of 180 mg/dl was chosen to ensure the positive detection of the marker molecule of 1,5-AG under LCMS conditions. We excluded other confounding factors that can also decrease the levels of 1,5-AG which are extreme

hypertriglyceridemia, severe renal impairment, chronic liver disease, pregnancy, and cystic fibrosis.[11]

In correlating the values of PPBS with the serum 1,5-AG values of Group II, it was found that as the levels of PPBS increased the levels of serum 1,5-AG decreased. Our study findings on the relationship between levels of hyperglycemia and serum 1,5-AG level were consistent with study conducted by Yamanouchi *et al.,* in 1987, and various other studies which have proved that the levels of serum 1,5-AG in diabetic patients decreases depending upon the severity of diabetes.[13-19] Goto *et al.* in 2011, observed that serum 1,5-AG value of <14 μg/ml is a predictor of PPBS ≥200 mg/dl.[20] In the present study, also the levels of serum 1,5-AG of all the diabetic patients were <14μg/ml and out of 45 serum samples in Group II, only two values of serum 1,5-AG were not predictive of a PPBS level of ≥200 mg/dl, which may be because of the individual variations in the level of serum 1,5-AG.

In correlating the values of salivary 1,5-AG in Group II with the PPBS levels, it was noted that as there is increase in the PPBS levels, there was decrease in the levels of 1,5-AG showing a significant negative correlation. Our finding of the negative correlation of the blood sugar level and salivary 1,5-AG level is consistent with previous studies conducted by Mook-Kanamori *et al*. in 2011,[10] Barnes *et al*. in 2014,[21] Asha *et al*. in 2019.,[22] and Ying *et al*. in 2021.[23] which observed that the salivary levels of 1,5-AG were low in patients who had significantly high levels of blood glucose.

In this study, the sensitivity and specificity of saliva is 86.4% and 87.2%, respectively, which suggests that saliva can be used for the noninvasive monitoring of glycemic status in diabetic patients using salivary 1,5-AG. In a previous study conducted by Mook-Kanamori *et al*. in 2011, the sensitivity of saliva for the detection of 1,5-AG in the evaluation of diabetic patients was 90.3%.[10] The slightly lower values compared to the previous study may be due to racial difference in the levels of 1,5-AG, and limited number of samples. Since the present study has developed the principles for the salivary 1, 5-AG estimation, further optimization of the testing method to suit cost-effective point-of-care utilization is likely to be achievable. However, the effort will need support from research and funding agencies working toward glycemic monitoring in diabetic patients. Future research on the topic can assess a similar correlation between salivary 1,5-AG and glycemia in multiple samples at different phases of dietary intake in varied groups of diabetic patients classified based on complications.

Conclusion

The results of our study suggest that the short-term glycemic marker 1,5-AG can be detected in saliva and can be useful as an adjunct marker in frequent monitoring of glycemic status in diabetic patients.

Ethical statement

Approval was obtained from the Institutional ethics committee Sri Ramachandra University. Reference number – CSP/15/JUL/42/02.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Kaufman E, Lamster IB. The diagnostic applications of saliva – A review. Crit Rev Oral Biol Med 2002;13:197-212.
2. Pink R, Simek J, Vondrakova J, Faber E, Michl P, Pazdera J, *et al.* Saliva as a diagnostic medium. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2009;153:103-10.
3. Armbruster DA. Fructosamine: Structure, analysis, and clinical usefulness. Clin Chem 1987;33:2153-63.
4. Wright LA, Hirsch IB. The challenge of the use of glycemic biomarkers in diabetes: Reflecting on hemoglobin A1C, 1,5-anhydroglucitol, and the glycated proteins fructosamine and glycated albumin. Diabetes Spectr 2012;25:141-8.
5. Dungan KM, Buse JB, Largay J, Kelly MM, Button EA, Kato S, *et al.* 1,5-anhydroglucitol and postprandial hyperglycemia as measured by continuous glucose monitoring system in moderately controlled patients with diabetes. Diabetes Care 2006;29:1214-9.
6. Fukumura Y, Tajima S, Oshitani S, Ushijima Y, Kobayashi I, Hara F, *et al.* Fully enzymatic method for determining 1,5-anhydro-D-glucitol in serum. Clin Chem 1994;40:2013-6.
7. McGill JB, Cole TG, Nowatzke W, Houghton S, Ammirati EB, Gautille T, *et al.* Circulating 1,5-anhydroglucitol levels in adult patients with diabetes reflect longitudinal changes of glycemia: A U.S. trial of the GlycoMark assay. Diabetes Care 2004;27:1859-65.
8. Halama A, Kulinski M, Kader SA, Satheesh NJ, Abou-Samra AB, Suhre K, *et al.* Measurement of 1,5-anhydroglucitol in blood and saliva: From non-targeted metabolomics to biochemical assay. J Transl Med 2016;14:140.
9. Niwa T, Dewald L, Sone J, Miyazaki T, Kajita M. Quantification of serum 1,5-anhydroglucitol in uremic and diabetic patients by liquid chromatography/mass spectrometry. Clin Chem 1994;40:260-4.
10. Mook-Kanamori DO, Selim MM, Takiddin AH, Al-Homsi H, Al-Mahmoud KA, Al-Obaidli A, *et al.* 1,5-Anhydroglucitol in saliva is a noninvasive marker of short-term glycemic control. J Clin Endocrinol Metab 2014;99:E479-83.
11. Kim WJ, Park CY. 1,5-Anhydroglucitol in diabetes mellitus. Endocrine 2013;43:33-40.
12. Rahmoune H, Thompson PW, Ward JM, Smith CD, Hong G, Brown J. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. Diabetes 2005;54:3427-34.
13. Yamanouchi T, Akanuma H, Asano T, Konishi C, Akaoka I, Akanuma Y. Reduction and recovery of plasma 1,5-anhydro-D-glucitol level in diabetes mellitus. Diabetes 1987;36:709-15.

14. Akanuma Y, Morita M, Fukuzawa N, Yamanouchi T, Akanuma H. Urinary excretion of 1,5-anhydro-D-glucitol accompanying glucose excretion in diabetic patients. Diabetologia 1988;31:831-5.
15. Yamanouchi T, Minoda S, Yabuuchi M, Akanuma Y, Akanuma H, Miyashita H, *et al.* Plasma 1,5-anhydro-D-glucitol as new clinical marker of glycemic control in NIDDM patients. Diabetes 1989;38:723-9.
16. Yamanouchi T, Akanuma Y, Toyota T, Kuzuya T, Kawai T, Kawazu S, *et al.* Comparison of 1,5-anhydroglucitol, HbA1c, and fructosamine for detection of diabetes mellitus. Diabetes 1991;40:52-7.
17. Umeda F, Yamauchi T, Ishii H, Nakashima N, Hisatomi A, Nawata H. Serum 1,5-anhydro-D-glucitol and glycemic control in patients with non-insulin-dependent diabetes mellitus. Tohoku J Exp Med 1991;163:93-100.
18. Yamanouchi T, Moromizato H, Shinohara T, Minoda S, Miyashita H, Akaoka I. Estimation of plasma glucose fluctuation with a combination test of hemoglobin A1c and 1,5-anhydroglucitol. Metabolism 1992;41:862-7.
19. Kishimoto M, Yamasaki Y, Kubota M, Arai K, Morishima T, Kawamori R, *et al.* 1,5-Anhydro-D-glucitol evaluates daily glycemic excursions in well-controlled NIDDM. Diabetes Care 1995;18:1156-9.
20. Goto M, Yamamoto-Honda R, Shimbo T, Goto A, Terauchi Y, Kanazawa Y, *et al.* Correlation between baseline serum 1,5-anhydroglucitol levels and 2-hour post-challenge glucose levels during oral glucose tolerance tests. Endocr J 2011;58:13-7.
21. Barnes VM, Kennedy AD, Panagakos F, Devizio W, Trivedi HM, Jönsson T, *et al.* Global metabolomic analysis of human saliva and plasma from healthy and diabetic subjects, with and without periodontal disease. PLoS One 2014;9:e105181.
22. Asha M, Rajarathnam BN, Vinutha N, Kumar H, Premkumar A, Azmi R. Evaluation of 1,5-anhydroglucitol as a salivary biomarker in type 2 diabetes mellitus patients. J Adv Clin Res Insights 2019;6: 67-71.
23. Ying L, Jian C, Ma X, Ge K, Zhu W, Wang Y, *et al.* Saliva 1,5-anhydroglucitol is associated with early-phase insulin secretion in Chinese patients with type 2 diabetes. BMJ Open Diabetes Res Care 2021;9:e002199.