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Differential Diagnosis of Glycosuria Using Raman Spectroscopy

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Differential Diagnosis of Glycosuria Using Raman Spectroscopy

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Abstract - **The aim of this research was to detect spectral differences in glycemic components. Urine samples were collected from 40 patients who were divided into a control group and a diabetic and hypertensive group. The samples were obtained in the morning, fasting, and stored a freezer at -80°C until spectral analysis. Spectral data collection was performed using a dispersive Raman spectrometer (Dimension P-1 model, Lambda Solutions, Inc., MA, USA). The equipment uses a stabilized multimode diode laser operating at 830 nm, with about 300 mW power output, and time integration to collect the Raman signal was adjusted to 5s. The mean Raman spectra displaced from the urine of patients in the study groups (CT and DM & HBP) were identified at the range of 516 and 1127cm^{-1}. Comparative analysis of mean urine spectra showed a significant difference ($p < 0.05$) between the groups, the Student's t-test was used to compare the mean Raman spectra of the groups. The comparative analysis of peak intensities at 516 and 1127 cm^{-1} in the urine of diabetic control and hypertensive patients revealed that it was higher in the DM & HBP group than in the CT group, however, with no significant difference ($p > 0.05$). To quantify the glucose in urine and discriminate the groups, a model was developed to estimate the concentration using a quantitative regression model based on partial least squares (PLS). According to the data obtained, there was an excellent correlation ($r = 0.98$) between the concentrations estimated by the model and the concentrations determined by colorimetric analysis. Discriminant analysis (DA) based on a regression model (PLS) proved to be promising as it discriminated the control group without errors, and the rate in the DM & HBP group was 89.1%. Raman spectroscopy can be a potentially useful tool for testing glucose in urine.**

Keywords – **Glucose, Diabetes Mellitus, Raman spectroscopy, Glycosuria, Urine.**

I. Introduction

Diabetes mellitus is a complex metabolic condition, and its classification and diagnosis has been the object of intense research for decades. It is categorized into four types: type 1 and type 2 diabetes (most recurrent), hyperglycemia in pregnancy (including gestational diabetes) and diabetes that has a specific etiology due to genetics, secondary to drugs, pancreatic factors, or other diseases. The International Diabetes Federation (IDF) ranked Brazil as the third country with the highest incidence of new cases of diabetes, second only to the USA and India [1]

The diagnosis of diabetes is based on the concentration of glucose in blood and urine. The traditional invasive methods for blood collection, such as the colorimetric biochemical method [2], have been replaced by non-invasive strategies such as the use of photonics [3, 4, 5]

Since 1841, urine has been used as a diagnostic fluid for diabetes, which has been extensively studied, as it can be collected easily and non-invasively. Urine is composed of metabolites, such as glucose, proteins, and nitrates, in addition to other dissolved salts, such as sodium and potassium. Glucose can be found in urine when it is excreted from blood in elevated levels and as a result, this fluid has been used for the diagnosis of diabetes [6]

Glycosuria means the presence of glucose in urine. It can be measured by using reagent strips that make a semiquantitative measurement of glucose in urine, which is easy to perform at low cost. In positive glycosuria, the serum concentration is greater than 180mg/dl in patients with normal renal function and higher values are found in patients with diabetic nephropathy. Several interferences may occur when measuring glucose in urine, such as volume. Despite these limitations, the measurement of glycosuria can be used for both the diagnosis and monitoring of glucose and for patients on insulin treatment who are unable to measure capillary glucose before meals and at bedtime [7]

The concentration of glucose in urine is also an important indicator for many other diseases, which makes the development of non-invasive or minimally invasive methods for frequent monitoring of glucose in urine imperative [8]

Raman spectroscopy is the study of the spectrum obtained by the interaction of electromagnetic radiation with matter. One of its main objectives is to determine the energy levels of atoms or molecules. This analysis technique uses electromagnetic radiation to test the vibrational behavior of molecules by observing the absorption or scattering of radiation.

This innovation has been used successfully in the diagnosis of pathologies in biological materials for the biochemical characterization of the sample. The Raman spectrum provides chemical and structural information of organic and inorganic

compounds, thus allowing their identification in a few seconds, with little or no need for sample preparation [9]

RS can be performed in real time in *in vivo* materials to monitor the analytes in biological fluids, including the non-invasive analysis of urine, dispensing the need for chemicals and reagents. In addition, as it uses an optical technique, it offers more benefits than traditional biochemical techniques due to its molecular specificity and capacity for quantitative analysis [10]. It may be used for *in vitro* and *in vivo* urine tests, offering early diagnosis of diseases, with the benefit of providing a rapid analysis in individual samples [11]. The main advantages for using RS over other techniques, such as mass spectroscopy and chromatography, are that it allows the analysis process to be developed in real time, at a lower cost, without tissue extraction or use of dyes and other contrast agents [12]. In addition to the above-mentioned aspects, it is important to note that techniques based on the Raman effect require minimal or no sample preparation, offer reduced artifact production, may be an alternative to techniques that require extensive preparation, provide non-destructive analysis of the sample [13], and multiple biochemical components can be analyzed in a single spectral collection.

The research conducted by [14] aimed to develop a model for quantifying biomarkers, such as creatinine, urea and glucose. By using selected peaks of these compounds, it was possible to obtain quantitative information on important biomarkers in urine for the assessment of renal failure in patients with diabetes and hypertension. The information obtained could be correlated with clinical criteria for the diagnosis of chronic kidney disease.

Based on this information, the peaks in glucose in the urine of healthy volunteers (CT) and diabetic and hypertensive patients (DM & HBP) were identified in the present study. The use of RS allowed to quantify, analyze, and monitor glucose in urine in real time as well as verify significant differences in the mean spectra of the groups studied.

II. Materials and Methods

This study was approved by the Research Ethics Committee of Universidade Anhembi Morumbi (report No. 2,717,746), in accordance with the guidelines and regulatory standards for research involving human beings (resolution No. 466/12). A total of 40 patients (24 women and 16 men) were recruited and divided as follows: 20 normoglycemic and normotensive volunteers in a control group (CT) and 20 diabetic and hypertensive individuals (DM & HBP). Urine samples were collected in a sterile vial provided by the team. The volunteers were instructed to collect the medium jet in spontaneous urination, after previous hygiene of the external genitalia, that is, the first jet had to be discarded and urination was to be deposited into the sterile vial.

The biological samples were obtained in the morning on an empty stomach, stored in 2.0 ml cryogenic tubes and kept frozen in a freezer at -80°C until spectral analysis. The samples of normoglycemic and normotensive volunteers in the control (CT) and diabetics and hypertension (DM & HBP) groups were obtained from the Hiperdia Group organized by the Municipal Secretary of Health (Santarém/Pará).

The collection of spectral data was performed using a dispersive Raman spectrometer (Dimension P-1 model, Lambda Solutions, Inc., MA, USA). The equipment uses a stabilized multimode diode laser operating at 830 nm, with about 300-mW power output and the time integration to collect the Raman signal was adjusted to 5 s. A Raman fiber optic probe cable was used to deliver radiation to the sample and collect the signal emitted by the sample. The urine samples were placed in an aluminum sample holder with 4-mm diameter holes and approximately 80 µL in volume. The Raman probe was placed at a distance of 10 mm from the sample holder. Thus, the spectral changes in the urine samples were accessed via optical fiber, repetition of excitation geometry and signal collection. Therefore, it was possible to study the spectral differences related to the differences in the biochemical constitution of the urine from different individuals. Six spectra were collected randomly from each samples, totaling 232 spectra for 40 samples (Fig.1).The collected Raman spectra were processed in this order: manual removing the cosmic ray spikes; removal of the background fluorescence by fitting and subtracting a 5th order polynomial, providing baseline correction and normalization by spectrum of water.

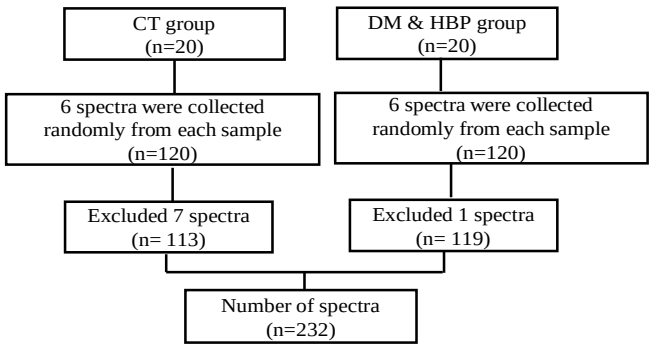

Fig. 1 Illustrative diagram to demonstrate number of spectra that were included and excluded of study

The mean normalized spectra of urine of the groups studied were compared and statistical analysis was performed using the Student's t-test ($p < 0.05$) and the GraphPad Instat® software, version 3.0. The algorithm used for multivariate data analysis was based on partial least squares (PLS) [15], employing the spectra obtained for each sample and the concentrations of the glucose in urine obtained by colorimetry. The routine from Chemoface (http://www.ufla.br/chemoface) [16] was used to discriminate

between CT and DM & HBP (PLS-DA) and also to predict the concentration of the glucose, the leave-one-out cross-validation method was used.

III. Results

Raman spectrum of urine samples and identification of glucose peaks

Figure 2 shows the mean Raman spectra displaced from the urine of patients in the study groups: Control and DM & HBP. The arrows in Figure 2 highlight the glucose peaks at 516 and 1127cm^{-1} in urine. According to the scientific literature, peaks at 527 and 1129 cm^{-1} can be attributed in part to glucose overlapping protein bands [17, 18, 19, 20, 21]. The comparative analysis of the mean urine spectra showed that there was a significant difference ($p < 0.05$) between the CT and DM & HBP groups. The Student's t-test was applied by comparing the mean Raman spectra of the CT and DM & HBP groups, and the statistical analysis showed a significant difference ($p < 0.05$).

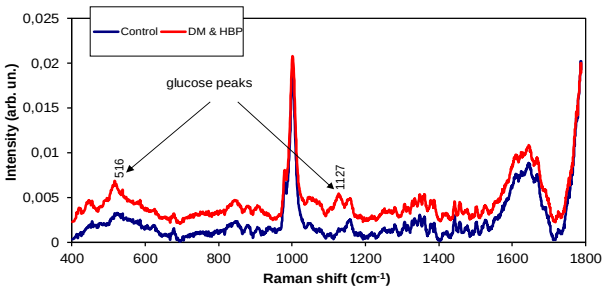

Fig. 2 Mean spectrum of glucose in urine of the control group in comparison with DM & HBP groups

A comparative analysis of the peak intensity at 516 and 1127 cm^{-1} was also performed in the urine of control patients and hypertensive diabetics. The data in Figure 3 show that the peak intensity at 516 and 1127cm^{-1} was greater in the DM & HBP group than in the CT group, however, there was no significant difference ($p > 0.05$) in peak intensity.

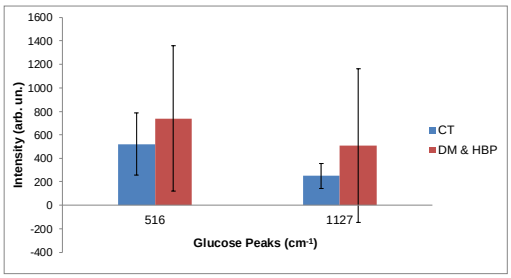

Fig. 3 Mean peak intensity at 516 and 1127cm^{-1} in urine of the CT and DM & HBP groups

Quantitative model based on the partial least squares regression analysis (PLS)

To quantify the glucose in urine and discriminate the groups, a model was developed to estimate the concentration using a quantitative regression model based on partial least squares (PLS) (Fig. 4). The leave-one-out cross-validation method was used, one prediction model, in which a sample is removed. The model (discriminating or quantitative) is built and it is tested on that sample left out, acquiring the predicted group or concentration in this sample. The process of obtention of the model and testing is repeated to all samples. The Table 1 presents the parameters of the PLS-based regression model obtained by Chemoface routine. According to the data obtained, there was an excellent correlation ($r = 0.98$) between the concentrations estimated by the model and the concentrations determined by colorimetric analysis.

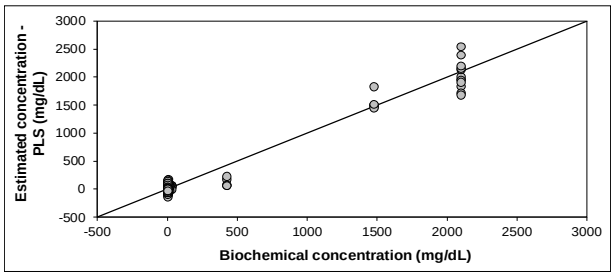

Fig. 4 Estimated concentration of glucose (mg/dL) in urine according to the PLS regression analysis versus the concentration determined by spectrophotometry (colorimetric analysis). RMSEcv (mean square error of cross-validation)

Table 1 Parameters of the PLS-based regression model obtained by Chemoface routine.

Parameters of the PLS-based regression model					
No. of latent variables	RMSEcv (mg/dL)	R^2cv	rcv	Error (%)	Accuracy (%)
8	92	0.97	0.98	5.6	94.4

The Table 2 shows the number of spectra classified correctly using PLS discrimination model in comparison with the clinical classification of patients in the Control and DM & HBP groups. The data showed that the model employed classified correctly at 100%, 89.1%, respectively, the patients in the Control and DM & HBP group.

Table 2 Results of the discriminant analysis (DA) based on the PLS regression model included the analysis of 8 latent variables (LVs)

Classification according to the clinical criteria	Predicted Classification according to PLS-DA		
	Successes	Unsuccesses	Correct classification (%)

Control (n=113)	113	0	100
DM & HBP (n=119)	106	13	89.1

IV. Discussion

In this research, we investigated the viability of RS to identify and quantify glucose in urine based on two study groups: CT and DM & HBP patients. Based on the results obtained, the mean Raman spectra of urine of patients for the study groups (Fig. 2) showed peaks in glucose at 516 and 1127cm^{-1} in urine. These data are in agreement with the data of [22], who highlighted that the peak at 1128cm^{-1} in urine was one of the biomarkers for diabetic and hypertensive patients. Their study pointed out that the Raman technique is a fast and reliable method for the qualitative assessment of urine in patients with diabetes and hypertension and it can be useful to diagnose complications associated with these diseases.

A study that reports the successful analysis of the Raman spectroscopy for non-invasive (transcutaneous) quantification of blood analytes, using glucose as an example, was conducted by Enejder and colleagues [23]. The authors used a standard glucose tolerance test protocol for the transcutaneous measurement of glucose in 17 healthy individuals whose glucose levels in the blood were elevated over a period of 2 to 3 h. The mean absolute errors for each individual were 7.8% ± 1.8% (mean ± standard) with R^2 values of 0.83 ± 0.10. The difference in our study is that we carried out the analysis in a control group and a group with pathologies, expanding the possibilities of applying the technique. The data from our research revealed that the PLS regression analysis, using the model adopted the R^2 value was 0.97 (Table 1).

Figure 3 shows the peak intensity analysis that, despite being higher, revealed no significant differences between the groups, probably due to the high standard deviation. These data are in agreement with [24], that evaluated peak intensity at 1128 cm^{-1} in urine of diabetic patients. One justification for this fact would be that not all patients perform an adequate glycemic control, even receiving the treatment prescribed by the medical team. Other possibility can be related with the number of samples, we intend to conduct further research, increasing the number of patients for a more robust analysis. Thus, was applied a model based on partial least squares (PLS) regression to correlate the concentrations of glucose in urine through Raman spectroscopy using the biochemical concentrations evaluated by the colorimetric method as the real concentrations of the sample.

Table 2 shows that the capacity of glucose discrimination in patients in the CT group was 100% and 89.1% in the HBP group, which suggests that the Raman Spectroscopy may be an efficient low-cost technique for rapid population screening of glucose.

In some countries, diagnostic tests for glucose in urine have been carried out using urinary biochemical analysis. In the work conducted by [25], a screening test using glucose in urine detected that 97.5% of the population were diagnosed with asymptomatic DM and were classified as DM2. The technique has been described as a powerful new diagnostic tool compared to routine biochemical tests, as it has many advantages, such as: rapid analysis, little or no sample preparation, use of small sample volume, detection of a wide variety of parameters in a single spectrum and no need for reagents. The advantages of the Raman spectroscopy technique as an instrument for analysis include the rapid speed of processing, reduced processing time and precision in the results to monitor and control glycemic changes in patients. The data presented in the present study show that the RS may be a useful tool in the future for population screening, in order to identify asymptomatic patients e that are without adequate treatment and to monitor patients being treated. In addition, the proposed model was able to discriminate healthy patients with 100% success when compared to diabetics and hypertensive patients.

V. Conclusions

In the present study, we evaluated the performance of Raman spectroscopy as a screening tool for testing glucose in urine. The comparative analysis of the mean urine spectra showed that there is a significant difference between the groups studied. The comparative analysis of the peak intensities at 516 and 1127 cm^{-1} in urine of the control and hypertensive diabetic patients, showed that it was greater in the DM & HBP group than in the CT group, with no significant difference. The PLS method discriminated 100% of the patients in the DM & HBP group, and the R^2 value was 0.97. The discriminant analysis (DA) based on the regression model (PLS) proved to be promising, as it managed to discriminate the control group without errors, and the hit rate in the DM & HBP group was 89.1%. The prediction errors indicated that Raman spectroscopy is a promising technique that can be used to quantify glucose in urine complementing or replacing conventional biochemical analysis techniques, particularly for population screenings.

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