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ORIGINAL ARTICLE

Comparative Study of Two Different Methods for Determination of Blood Glucose

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Highlights:
1. Difference in blood glucose monitoring from different methods.
2. Accuracy for blood glucose monitoring to prevent diseases.
3. It does not meet the criteria for comparison between laboratory and self-monitoring tests.

ABSTRACT

Self-monitoring in diabetic patients prevents erroneous doses of insulin from being applied, in order to avoid complications of recurrent hypoglycemia and hyperglycemia that lead to more serious damage. The objective of this study was to evaluate the quality of a capillary blood glucose self-monitoring test (blood glucose test, HGT), comparing its results to a glucose test using the enzymatic method. Method: blood was collected for the capillary and venous method from 90 volunteers, under fasting conditions and diagnosed with diabetes. The samples were collected at the same time from the volunteers. Results presented as mean ± standard deviation. For HGT the glycemia was 132.99 ± 44.65 mg/dL and for the laboratory method 108.3 ± 43.59 mg/dL. The results showed disagreement, corroborated by the Kappa coefficient method (κ) with a value of 0.181. Only 30.681% were within zone A and zone B stipulated by ISO 15197/2013 as 99%. Conclusion: There is a difference between the values of capillary blood glucose performed by digital blood glucose meter and the values of venous blood glucose performed in traditional laboratory tests, so that the equipment under test did not meet the reference values given by resolution 3.161, of 16/11/2018, normative number 24, which governs Brazil, which was based on the international standards of ISO 15197/2013, and therefore does not meet international standards.

Keywords: glucose; self-monitoring; Diabetes *Mellitus*.

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INTRODUCTION

Diabetes *mellitus* is a disease characterized by failure in the regulation of circulating glucose (glycemia), which can be caused due to dysfunction in insulin production (type 1 diabetes *mellitus*, DM1) or in its action (type 2 diabetes *mellitus*, DM2), as well as in both mechanisms. This failure in glycemic homeostasis leads to an excess of glucose in the blood (hyperglycemia), which stands out as a signal for the diagnosis and monitoring of this disease[1].

DM2 is the most common, and in its pathophysiological process it is caused by resistance to the action of the hormone insulin and chronic hyperglycemia[2]. For the proper monitoring of this disease, it is necessary to carry out a number of tests that are not yet standardized, but it is advised to do so after the main meals to learn the status of the metabolic control of glucose.[3-5]

According to the World Health Organization (WHO), there are approximately 422 million adult people with diabetes in the world, with an annual mortality of 1.6 million respectively, which is attributed to complications caused by poorly controlled hyperglycemia[2]. According to the International Diabetes Federation (2017), 26 million adults are affected by persistent hyperglycemia in Brazil and, in 2045, the estimate of people with diabetes worldwide will be approximately 629 million[6].

Laboratory tests are of great importance for the diagnosis[7], monitoring and control of various types of diseases. According to Campana, Oplustil and Faro (2011) in 2008, only private assistance performed 450 million laboratory tests[8]. In addition, 80% of medical decisions are made based on the results of laboratory tests, so in recent years a large amount of equipment is being marketed for the monitoring and treatment of diabetes[5].

The monitoring of capillary glycemia is very important for the patient, since hyperglycemia can lead to severe damage in various parts of the body, resulting in frequent hospitalizations, such as chronic kidney disease (CKD) and even premature death[6]. The self-monitoring test has the following advantages: patient comfort because it is performed at home, low invasiveness and low cost. The test with the reactive tape is essential to determine the correct dose of insulin to be applied, and for the patient to know the reaction that certain foods provoke in the glycemia and even control the hypoglycemia after physical exercise[9].

According to the Guidelines of the Brazilian Diabetes Society (2019), the gold standard method for determining plasma glucose is the end-time colorimetric enzyme. In this methodology, the reference values for plasma glucose are: fasting glucose <100mg/dL, glucose 2 hours after the oral glucose tolerance test (OGTT) <140mg/dL; for the diagnosis of diabetes, the criteria of the American Diabetes Association (ADA), which are also followed by the Brazilian Diabetes Society, are random blood glucose (collected at any time of day) above 200 mg/dL associated with classic diabetes symptoms, such as excessive thirst, increased frequency of urination and unintentional weight loss(9). In addition, fasting glucose greater than or equal to 126 mg/dL or 2-hour glucose result after receiving 75 grams of glucose (OGTT) greater than or equal to 200 mg/dL, glycated hemoglobin (HbAc >6.4%) are also criteria. However, to close the diagnosis, it is necessary to confirm by repeating the test on another day[10].

All methods have methodological limitations. Fasting glycemia requires fasting, suffers interference due to acute conditions and has a lower reproducibility rate when compared to HbA1c. The OGTT is costly, uncomfortable, and more time consuming. HbA1c has a higher cost and does not take into account individual variability in the phenomenon of protein glycation, in addition to having lower diagnostic sensitivity than other methods. In addition, the laboratory analysis of HbA1c has been standardized worldwide by the high performance liquid chromatography (HPLC) method, and its validation needs to be certified by the National Glycohemoglobin Standardization Program (NGSP), established for applicability in the Diabetes Control and Complications Trial (DCCT) study. In the

presence of unequivocal symptoms of hyperglycemia, it is recommended that the diagnosis be made by means of random blood glucose ≥ 200 mg/dl[10].

Thus, the present study aims to evaluate the quality of a capillary glycemia self-monitoring test (CGMT), comparing its results to a glucose test using the enzymatic method in volunteers diagnosed with DM2.

MATERIALS AND METHODS

Characterization of the research

The present study was characterized as a cross-sectional study, in which plasma and capillary glucose values were compared using two analytical methods.

Population and sample

The population consisted of 90 patients (17 men, 18.89% and 73 women, 81.11%), with a mean age of about 62 (± 12.31) years, diagnosed with DM2, undergoing monitoring in the diabetic patient programs attended by the Family Health Strategies (FHS) teams in the municipality of Santo Ângelo (RS). The volunteers underwent laboratory evaluation only after they were informed about the study, and signed the Informed Consent Form (ICF). For analysis of these parameters, the population was selected based on the inclusion and exclusion criteria.

Inclusion criteria:

Volunteers included in the research aged over 40 years who were under medical monitoring through the groups of diabetic patients of the FHSs, in the municipality of Santo Ângelo-RS and who wished to participate in the study.

Exclusion criteria:

All volunteers who did not wish to participate in the study or who failed to perform the laboratory evaluation were excluded. Also, volunteers who had any disease that may interfere with the results of analytical methods, such as changes in dehydration, anemia and polyglobulia. During laboratory analysis, samples that presented lipemia, hemolysis, and/or jaundice were excluded due to interferences in the blood glucose detection method.

Procedures

Initially, the volunteers received all the information about the research and those who agreed to participate signed the Informed Consent Form (ICF). After signing the ICF, the dates for the blood collection for laboratory analysis were scheduled with the volunteers. The procedures were carried out in special rooms, contemplating the aspects of biosafety, in an environment that protected the privacy of each and every participant.

All laboratory procedures, from the collection to the final analysis for the reports, were performed by professionals, researchers trained in the execution of activities so that there is no injury or discomfort to any participant. It is also important to note that for the execution of the study, the samples were collected in the morning, with the volunteers fasting and at a temperature of 23ºC. After a screening, the team of researchers performed the blood collection, and a single trained professional performed capillary blood glucose determination in order to maintain the standardization of procedures. The collection dates were scheduled with the research participants with the FHS, as well as at the same time the evaluation of capillary blood glucose (glucometer) was performed in the

digital pulp of the index finger of the right arm and then the venipuncture (also in the right arm). Then the samples were transported, according to all biosafety standards, to the laboratory in which they were processed, screened and separated for later laboratory evaluation.

Determination of Capillary Glycemia

The capillary self-monitoring method was based on the collection of a drop of blood with a disposable lancet, which was placed on the disposable biosensor tape, which contains glucose dehydrogenase and glucose oxidase, and then connected to the tape on the glucometer (reflectometer). The amperometric method uses the measurement of electronic light that is reflected from the dipstick, and quantification occurs by determining the current that is produced when glucose oxidase catalyzes the oxidation of glucose and gluconic acid or when glucose dehydrogenase catalyzes the oxidation of glucose to gluconolactone. The electrons generated during the reactions are transferred to electrodes, the magnitude of the current is proportional to the glucose concentration in the capillary blood and is converted to the reading in the glucometer[9]. In the determination of capillary glycemia, the commercial control, of the same brand and batch of reagent strips, was first used to assess whether the performance of the glucometer was within the ranges recommended by the manufacturer. After this procedure performed in triplicate, capillary blood glucose determinations were initialized.

The manufacturer of the glucometer describes that its devices are calibrated for plasma and that the difference of its result for the laboratory is 12% higher, presenting an interval margin of ±20% compared to the laboratory. In addition, the manufacturer mentions that results greater than ±20% may occur in cases of postprandial evaluation, with hematocrit above 55% or below 30% or if the ambient temperature is less than 6ºC.

Determination of Venous Blood Glucose

Venous blood collection: collection was performed in volunteers fasting for 10 hours through puncture in the median vein of the right arm, in which 4.0 mL of venous blood was collected in a tube with fluoride anticoagulant. The blood was processed to obtain fluoridated plasma, obtained by centrifugation (15 minutes AT 3,500 RPM, AT a temperature of 25ºC, and then the determination of venous glycemia was already performed.

Determination of blood glucose: performed by BS200 Mindray automated colorimetric method. In the colorimetric enzymatic method, the glucose found in the plasma is enzymatically oxidized by glucose oxidase, producing hydrogen peroxide, which in the presence of peroxidase reacts with 4-Aminoantipyrine and Phenol, thus generating a cherry red color that its intensity is proportional to the amount of glucose present[11].

Statistical Analysis

Descriptive analysis is presented as mean ± standard deviation. Data normality was tested using the Kolmogorov Smirnov test, followed by analysis using the Mann Whitney test, with a significance level of 5% ($P<0.05$). In addition, the agreement between the glycemia analyses performed from the blood glucose test (HGT) and venous glycemia/colorimetric enzymatic method (CENM) was evaluated by the Kappa coefficient test (κ)[12], through an organizational matrix, with the categorization of the evaluated data, from the proportion of the observed frequency (Σ fa) by the random frequency (Σ fe), followed by the ratio of this value by subtracting the total number of individuals evaluated by the proportion of Σ fe[13]. The following values were used to categorize the glycemia of diabetic patients[14]:

- Blood glucose up to 99 mg/dL;
- Blood glucose between 100 and 125 mg/dL;
- Glycemia ≥126 mg/dL

Based on this categorization, an organizational matrix was prepared (Table 1). Then, the coefficient κ was calculated with the mathematical formula through the frequency:

$\kappa = (\Sigma fa - \Sigma fe)/(N - \Sigma fe)$.

Ethical Aspects

This study was designed in accordance with the Guidelines and Regulatory Standards for Research Involving Human Subjects according to the Resolution of the National Health Council (CNS) number 466/2012, and was approved by the Ethics Committee of Unijuí under number 1,173,158.

RESULTS

Our study observed an elevation in peripheral capillary glycemia (HGT) in relation to venous (CENM), with a mean glycemia by the HGT method of 132.99 ± 44.65 mg/dL (men 156.06 ± 70.03 mg/dL and women 127.46 ± 34.50 mg/dL), while in the CENM method the mean was 108.3 ± 43.59 mg/dL (men 133.12 ± 64.30 mg/dL and women 102.5 ± 35.36 mg/dL).

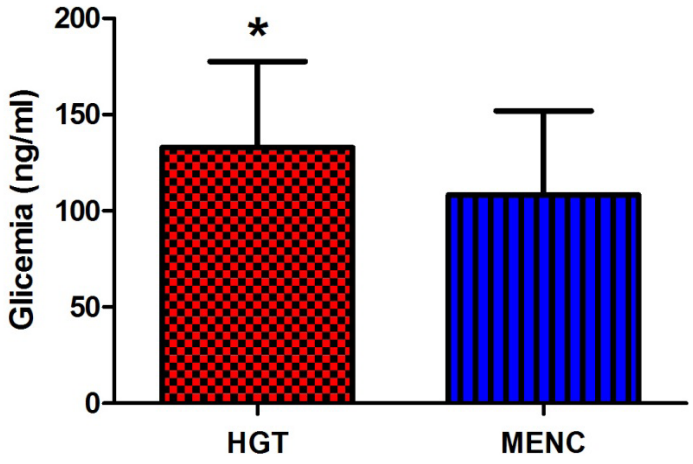

Figure 1 – Blood glucose measurement from blood glucose test (HGT) and venous blood glucose/colorimetric enzymatic method (CENM). Statistical analysis performed with Mann Whitney test (*P < 0.0001)

In the analysis of the results, the κ coefficient value obtained was 0.181, considered unsatisfactory for the agreement between the blood glucose measurements by the different methods of analysis (Table 1) (13).

Table 1 – κ coefficient for the test of agreement between blood glucose assessment methods

Venous glycemia/colorimetric enzymatic method				
HGT	≤ 99 mg/dL	100–125 mg/dL	≥126 mg/dL	Total
≤ 99 mg/dL	16	0	0	16
100–125 mg/dL	24	4	1	29
≥126 mg/dL	9	18	16	43
Total	49	22	17	88

Legend: The observed total agreement (Σ fa) was obtained by summing all values contained in the diagonal cells. According to chance (Σ fe) it was obtained by summing the multiplications between the total of the row by the total of the corresponding column divided by the total of observations:

Σ fa = 16+4+16

Σ fa = 36

Σ fe = ([49x16]/88+[22x29]/88+[17X43]/88)

Σ fe = 8.909 + 7.25 + 8.306

Σ fe = 24.459

Then the coefficient κ was calculated:

κ = (Σ fa – Σ fe)/N – Σ fe

κ = (36 – 24.459)/(88-24.459)

κ = 11.541/63.541

κ = 0.181

In addition, in Brazil, the resolution that regulates the use of blood glucose tests is 3,161, of 16/11/2018 with normative number 24, which was based on the international standards of ISO 15197/2013. Such resolutions regulate the following criteria for comparing a laboratory test with self--monitoring: self-monitoring must have at least 95% of tests within ±15mg/dL at glucose concentrations <100mg/dL and within ±15% at glucose concentrations >100mg/dL[15]

Of the results found in the glycemic zone below 100mg/dL, of the 50 tests, 7 (14%) were within the recommended limits and 43(86%) exceeded the recommended value. In the glycemic zone above 100mg/dL of the 38 tests, 20 (52.63%) were within the recommended limits and 18 (47.37%) exceeded the recommended value. Also, according to ISO15197/2013 of the total tests performed, 99% must fall within zone A (<100 mg/dL) and zone B (>100 mg/dL). However, in our study we did not obtain satisfactory results, since of the 88 tests evaluated only 30.681% correlated adequately within zone A and B in both methods (Table 2).

Table 2 – Distribution of glycemic results in Zones A and B

Mean blood glucose ≤100 mg/dL			
Venous Blood Glucose	**Capillary Glycemia**	**Mean difference**	
: 82 mg / dL	92.58 mg /dL	10.5 mg/dL (12.79%)	50 tests, 7 (14%) within the recommended limits 43(86%) exceeded the limit.
Mean blood glucose >100 mg/dL			
Venous Blood Glucose	**Capillary Glycemia**	**Mean difference**	
142.56 mg/dL	144.12 mg/dL	1.56 mg/dL (1.09%)	38 tests 20 (52.63%) within limits 18 (47.37%) exceeded the limit.

DISCUSSION

The analysis of our study showed lower blood glucose in the analysis of venous blood from laboratory collection. According to previous studies, the result can be justified by the variability in the analyses in different types of samples, since there is a difference in the time required for changes in venous glucose to reach capillary blood levels[16]. This difference in glucose concentration suggests disagreement in the methods for evaluating this parameter, which was shown by the κ coefficient test, which showed disagreement between the analyses (Table 1).

On the other hand, agreement between laboratory evaluations in venous blood and peripheral capillary glycemia can already be observed from the Kappa test[17]. In research by Garingarao, Buenaluz-Sedurante and Jimeno (2014) on the devices in which they did the study, 79% did not comply with the standards established by ISO 15197:2003[18]. Karon et al. (2014) presented a study similar to ours, in which the AccuChek brand did not obtain results in accordance with the standards established by ISO[19]. In the study by Pereira et al. (2015), the values obtained by the two glucometers tested also showed inaccuracy and low accuracy in comparison with the reference method. Van Hooijdonk et al. (2015) described results different from those established by ISO 15197:2003 and noted that the values of glucometers become more reliable when calibrated frequently[20].

The evaluation of the reliability and rigor of different devices to measure peripheral capillary glycemia has already shown variability, as in the study by Pariente et al. (2017)[21]. According to a previous study, different glucometers showed a difference to the laboratory method, and none of the glucometers tested met the precision and accuracy criteria stipulated by ISSO, as the results showed that the glucometers tested were more reliable in blood glucose ranges above 100mg/dL[22].

The recommended values of glucose for fasting diabetics for the Brazilian Diabetes Society (BDS) is up to 100 mg/dL and for International Diabetes Federation (IDF) is up to 115 mg/dL and postprandial <160 mg/dL for both institutions. If the glucometer shows deviations in the accuracy of the results, patients will not have the necessary safety for the application of insulin, failing to apply the drug or applying an exaggerated amount due to erroneous values[23].

The differences in peripheral capillary glycemia compared to the laboratory method demonstrate concern, especially regarding the insulin dose to be applied, because if the insulin dose is above that required by the patient, it can trigger hypoglycemia. On the other hand, if hyperglycemia values are erroneously identified, the doses of insulin stipulated and administered will be lower than what should be administered, which will cause disorders to the patient, such as a condition of greater hyperglycemia, ketoacidosis as well as predisposing earlier complications of diabetes[9].

By definition, veins and capillaries are different in the body, with capillaries responsible for the exchange of substances between blood and tissues[24], while veins carry oxygen-poor blood towards the heart. As glycemic self-monitoring is performed in the capillaries it is normal that the upper value, but within already established limits[25]. Even if the characteristics between these blood vessels are different, the results obtained with this study are not approved by the legislation that ensures the quality of the devices in Brazil.

The manufacturer of the glucometer used in our study showed that its variation could be up to 20% in some situations, however in our performance evaluation of the equipment we found in several tests a higher variation between venous and capillary glycemia. This fact causes us concern, as the results of capillary glycemia are very important both for the treatment of the patients and for their knowledge of the glycemic index of foods in response to feeding[26,27]. Daily monitoring of blood glucose makes it so that the diabetic does not have off-target blood glucose and that it is possible to treat it more easily when outside, it is also possible to monitor the relationship between carbohydrates and

the insulin that will be applied, so the carbohydrate count will be optimized and it is also simpler to adjust for carbohydrate sensitivity not generating a hyperglycemic crisis[28].

We understand that one way to minimize the identified problem would be for the Ministry of Health through Anvisa to follow the same regulation proposed by the American National Institute of Health, in which the results obtained in the glucometer should be between 15% of the results of an established reference method for the equipment to be considered clinically useful. Bearing in mind that the work was carried out with a specific device, we have the perspective to continue this study to evaluate different manufacturers/brands/models of glucometers, different collection times as well as to increase the number of glycemic ranges to analyze the performance of these equipment in order to improve the results for diabetic patients.

CONCLUSION

There is a difference between the values of capillary glycemia performed by digital glucometer and the values of venous glycemia performed in traditional laboratory tests, so that the equipment under test did not meet the reference values given by resolution 3.161, of 16/11/2018, normative number 24, which governs Brazil, which was based on the international standards of ISO 15197/2013, and therefore does not meet international standards.

PERSPECTIVES

A perspective for new studies would be to evaluate the performance of several glycemic self-monitoring tests, with fasting and random blood glucose throughout the day, to lower whether they are reproducing results within the ranges established by Anvisa.

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Pauline Brendler Goettems Fiorin: Methodology.

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Matias Nunes Frizzo: Conceptualization; Data curation; Methodology; Supervision; Writing – original draft; Writing – review & editing.

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