



Can we trust the positivity of semi-quantitative glucose measurement in the urine?

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ABSTRACT

As known, when the blood glucose level exceeds the renal threshold value of 180-200 mg/dL it begins to be excreted with urine. Spot urine analysis is easy to perform and an important test, with false positive or negative test results incompatible with the clinic. Our aim in this study is to investigate the relationship between glucose levels in different sample types with simultaneous measurements. Material and Simultaneous fasting serum glucose, HbA1c levels and urine glucose of 2375 patients were screened retrospectively from the hospital information system between June 1, 2015 and November 30, 2015. Fasting serum glucose was measured by enzymatic hexokinase method in biochemical autoanalyzer, HbA1c was measured by chromatographic method on HPLC and urine glucose was measured semi-quantitatively by urine autoanalyzer by strip glucose oxidase method. It was found that there was a medium-strong relationship between all three results. There was significant correlation between; fasting serum glucose and HbA1c ($r: 0.787, p < 0.001$) and the correlation between urinary glucose and fasting serum glucose and HbA1c were ($r: 635, p < 0.001$) and ($r: 533, p < 0.001$), respectively. In our study, we indicated that there is a strong correlation between the glucose results of different types of samples that we use in our laboratory. The patient's concurrently measured HbA1c and fasting serum glucose results may be helpful to the laboratory specialist to avoid both false positives and false negatives.

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1. Introduction

The tests used to measure glucose in the blood were developed about 100 years ago, and they became the only criteria for the diagnosis of diabetes and hyperglycemia. The most widely accepted glucose-based criteria for the diagnosis of diabetes are fasting plasma glucose (FPG) ≥ 126 mg/dL or 2 hours plasma glucose ≥ 200 mg/dL during the 75 g oral glucose tolerance test (OGTT). In addition, a patient with classic diabetes symptoms, having a single measured random plasma glucose ≥ 200 mg/dL is considered diagnostic (Sacks, 2011; Sacks et al., 2011; ADA, 2016). HbA1c is a marker that is formed by nonenzymatic glycosylation of hemoglobin, indicating glucose regulation and glucose tolerance (Koenig et al, 1976). HbA1c levels of 6.5% or above is also among the diagnostic criteria for diabetes (Sacks, 2011; ADA, 2016) and it is accepted that HbA1c in diabetic patients reflects the risk of developing diabetic complications and the quality of diabetic care (Herman and Fajans, 2010, Karatoprak et al., 2012).

Routine urine screening is a strip test method that includes

chemical tests for, pH, protein, glucose, ketone, erythrocyte, bilirubin, urobilinogen, nitrite, leucocyte esterase and specific gravity. Urine analysis results may change depending on the type of strip, the autoanalyzer or the evaluation method used in the laboratories and provides qualitative (positive or negative) or semiquantitative (eg, negative - 4+ positive) measurement results (Simerville et al., 2005; Mundt and Shanahan, 2010).

There is no glucose in the urine of a healthy person or it can be detected in small quantities (2-20 mg/dl) (Mundt and Shanahan, 2010). The amount of glucose in the urine depends on the blood glucose level, the glomerular filtration rate and the degree of reabsorption from the tubules. Generally, glucose starts to be detected in urine after a blood level of about 180-200 mg/dL, which is the normal renal threshold for glucose (Simerville et al., 2005; Cersosimo et al., 2014). Glycosuria occurs when the blood glucose exceeds the renal threshold since the tubules can not reabsorb all filtered glucose (Mundt and Shanahan, 2010). Diabetes mellitus, Cushing's syndrome, diseases of the liver and pancreas, and

Fanconi syndrome are included in the etiology of glucosuria (Simerville et al., 2005). Urinalysis for glucose can be used to detect diabetic hyperglycemia, including ketoacidosis, but false positives and negatives can occur (Mitchell et al., 2013). It has been reported that urinary glucose level varies depending on the time after the meal which may hence affect the validity of urinary glucose test as a screening test for diabetes (Shinozaki et al., 1999).

Currently, blood and urinary glucose measurement results are routinely used in evaluating the glucose level. However, the reliability of the relationship between existing methods creates a question mark in the minds of physicians. Especially in spot urine analysis, false positive or negative test results incompatible with the clinic are frequently observed. In this study, it was aimed to evaluate the relationship between measured glucose results using different methods in three different sample types and the location of urine glucose in diabetes screening.

2. Materials And Methods

Simultaneous serum glucose, HbA1c levels and urine glucose were scanned retrospectively from the hospital information system of 2375 patients who applied to Ahi Evran University Training and Research Hospital between June 1, 2015 and November 30, 2015. Fasting serum glucose was measured by enzymatic hexokinase method in biochemical autoanalyzer, HbA1c was measured by chromatographic method on HPLC and urine glucose was measured semi-quantitatively by urine autoanalyzer by strip glucose oxidase method. HbA1c levels were converted to estimated glucose values using the formula $EG (mg / dL) = (28.7 \times HbA1c) - 46.7$ (Nathan et al., 2008). Semiquantitative urine results were converted to quantitative results using the values given in the kit brochure (\pm (90 mg / dl), 1+ (252 mg / dl), 2+ (504 mg / dl), 3+ (1980 mg / dl)). Subjects with HbA1c <6.5%, HbA1c \geq 6.5%, serum glucose <126 mg / dl, serum glucose \geq 126 mg / dl, serum glucose \geq 180 mg / dl and serum glucose \geq 200 mg / dl were divided into groups. The mean glucose value, mean HbA1c value and glucose positivity counts in the urine were evaluated in the groups.

Statistical analysis of the data was performed using the SPSS analysis program. SPSS version 17.0 software (SPSS Inc., Chicago, Illinois, USA) and Microsoft Office Excel 2007 programs were used for statistical evaluations. The relationship between sample types was evaluated by Pearson correlation coefficient. $P < 0.05$ was considered statistically significant. The results of the groups were expressed as mean \pm standard deviation.

3. Results

In the analysis of correlation, it was seen that there was a medium-strong relationship between all three results. Correlation between serum glucose and HbA1c was $r: 0.787$, $p < 0.001$, correlation between urine glucose and serum glucose was $r: 0.635$, $p < 0.001$ and correlation between urine glucose and HbA1c was $r: 0.533$, $p < 0.001$ (Table 1).

Table 1: Correlation between serum glucose, urine glucose and HbA1c

Variables	r value	p value
Serum glucose-HbA1c	0.787	<0.001
Serum glucose - Urine glucose	0.635	<0.001
HbA1c-Urine Glucose	0.533	<0.001

878 patients with an HbA1c value <6.5% had a mean serum glucose of 104.8 mg/dl and urine glucose positivity (1+) was detected in only one patient with serum glucose 130 mg/dl and HbA1c value 6.4%. This group had a mean serum glucose of 180.7 mg/dl and urine glucose positivity was detected in 242 of these persons. Urine glucose positivity was found in persons with an HbA1c level of 6.6% or above (Table 2).

Table 2: Mean glucose and HbA1c values of the groups and the number of urine glucose positivity

Groups	Number of subject	Mean glucose value	Mean HbA1c value	Urine glucose positivity
HbA1c value less than 6.5 (%)	878	104.8 \pm 17.83	5.82 \pm 0.38	1 (0.11%)
HbA1c value of 6.5 and above (%)	1497	180.7 \pm 72.46	8.53 \pm 1.79	242 (16.2%)
Serum glucose values below 126 (mg/dl)	1084	102.8 \pm 13.38	6.2 \pm 1.01	7 (0.64%)
Serum glucose values 126 and above (mg/dl)	1291	194.6 \pm 69.1	8.62 \pm 1.88	236 (18.2%)
Serum glucose values 180 and above (mg/dl)	600	248.7 \pm 67.5	9.83 \pm 1.82	218 (36.3) %
Serum glucose values 200 and above (mg/dl)	442	270.2 \pm 66.56	10.27 \pm 1.80	210 (47.5%)

When 1084 subjects with serum glucose <126 mg/dL were analyzed, the mean HbA1c value was found as 6.2% and urine glucose positivity was found in 7 persons. In 1291 subjects with serum glucose \geq 126 mg/dl, the mean HbA1c value was found as 8.62% and urine glucose positivity was found in 236 persons. Urine glucose positivity was found in 218 (36.3%) of 600 subjects with a renal threshold value of 180 mg/dl and above serum glucose values and negative in 382

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4. Discussion

All of the urine strips used for the semiquantitative measurement of urine glucose use glucose oxidase, a glucose-specific enzyme in a chromogenic assay. There is no glucose in

the urine of a healthy person or it can be detected in small quantities (2–20 mg/dl) (Mundt and Shanahan, 2010). Usually, glucose will not be present in the urine until the blood level exceeds 180–200 mg/dL, which is the normal renal threshold for glucose (Mundt and Shanahan, 2010; Simerville et al., 2005; Cersosimo et al., 2014). In routine examinations, the negative result of the strip test is usually interpreted as the absence of glucose in the urine specimen (Altınışık, 2010). However, there are factors that affect the urine test and lead to misjudgment of the result. The increased amount of ketones and the use of levodopa may lead to false positives of the glucose result in the urine, while elevated specific gravity, uric acid and vitamin C can lead to false negativity in the urine (Simerville et al., 2005; Mundt and Shanahan, 2010). The benefit of glucose screening in the urine and the relationship between urinary glucose and diabetes have been shown in various studies (Davies et al. 1993; Yokota et al., 2004; Urakami et al., 2005; Ogawa et al., 2012). The school health law of Japan was passed in 1974 mandating urine screening of elementary and junior high-school students for the detection of renal disease, in 1994 urine glucose screening was also made compulsory (Yokota et al., 2004).

Ogawa E. et al. performed urine glucose screening in Tokyo between 1988 and 2009 and glucosuria was detected in 298 school children. As a result of the application of the oral glucose tolerance test to these children, they detected renal glucosuria in 146 students, diabetes mellitus in 133 students, and impaired glucose tolerance in 19 students (Ogawa et al., 2012).

Urakami T et al. investigated 8,812,356 school-aged children for glucosuria from 1974 to 2002 in Tokyo, when the urine was positive for glucose, an oral glucose tolerance test was carried out to confirm diabetes. In all, 232 students were identified to have type 2 diabetes. Low cost urine glucose screening has been shown to be useful for school children in the detection of diabetes in the early stages of the disease (Urakami et al., 2005).

However, the usefulness of urinary glucose as a screening test for unrecognized diabetes is limited because the urine test was found with specificity of > 98% and low sensitivities (21–64%) (Engelgau et al., 2000). Davies MJ et al. performed OGTT on 330 subjects, who were screened for postprandial glucosuria and detected positive glucose in urine. 99 of these subjects had newly diagnosed diabetes, 56 had impaired glucose tolerance. The test had a sensitivity of 43% and specificity of 98% (Davies et al., 1993). Friderichsen et al. evaluated the urine glucose test by randomly selected 106 test-negative participants and they detected 3 DM and 4 impaired glucose tolerance. They reported that the test had a sensitivity of 21% and specificity of 99% (Friderichsen and Maunsbach, 1997).

Considering the renal threshold value, we found glucose positivity in urine only 36.3% in the group with serum glucose value of 180 mg/dl and above. A randomly measured plasma glucose of ≥ 200 mg/dL is a diagnostic criteria for diabetes. In our study, glucose positivity was detected in the urine of 210 patients (47.5%) in this group, but we observed negativity more than half. This is consistent with the literature and may depend on the timing of the urine sample collection, the difference between the individuals at the renal threshold value, and the low sensitivity of urine glucose sc-

Glycosuria test may give false-negative results in the diagnosis of diabetes mellitus, as age-related increases in renal threshold (Friderichsen and Maunsbach, 1997). The urine sample collection timing for urine glucose screening test should be carefully examined and its performance is usually better with random, postprandial, or glucose-loaded measurements than fasting measurements (Shinozaki et al., 1999; Engelgau et al., 2000).

Glucose measurement in fasting plasma is widely used as a diabetes diagnostic criteria (ADA, 2016). Easily and cost-effectively analysis with automated devices in the laboratories all around the world are the advantages of this test, however, there are some limitations. Fasting glucose concentrations vary significantly from day to day in a single individual. Individual changes in a healthy person are reported between 5.7% and 8.3% (Lacher et al., 2005). Fasting plasma glucose (FPG) can range from 112 to 140 mg/dL in an individual with an FPG of 126 mg/dL, depending on a CV (coefficient of variation) of 5.7%. Plasma glucose concentration can be altered by preanalytical factors such as drugs, foods, long-term fasting, exercise, sample handling (Young and Bermes, 2006; Sacks, 2011).

HbA1c is formed by non-enzymatic binding of N-terminal valine glucose in the β -chain of hemoglobin. HbA1c reflects the long-term mean plasma glucose, representing the mean glucose concentration over the previous 2–3 months. Compared with OGTT, HbA1c measurement is faster and more useful. Many factors that alter fasting blood sugar do not significantly affect HbA1c concentrations. Short-term lifestyle changes such as acute illness, exercise, recent food consumption and sampling conditions do not significantly change HbA1c levels. In non-diabetic individuals, intraindividual variation of HbA1c is minimal with 1% CV (Hu et al., 2003; Sacks, 2011).

Hillman N. et al. reported a correlation between mean blood glucose values and HbA1c values of 146 DM patients ($r: 0.620, p < 0.001$) (Hillman et al., 2004). Ogawa E. et al. reported a high correlation between fasting plasma glucose and HbA1c values ($r: 0.86, p < 0.0001$) (Ogawa et al., 2012). Motor S. et al. observed a correlation between HbA1c levels and mean blood glucose levels in 131 DM patients with chronic renal failure ($r: 0.755, p < 0.001$) (Motor et al., 2013). In our study, there was a significant correlation between serum glucose and HbA1c levels ($r: 0.787, p < 0.001$) and it was found consistent with the previous studies.

The remarkable point in our study is the significant correlation between urinary glucose and serum glucose and HbA1c ($p < 0.001$). As stated by the World Health Organization, semiquantitative urine glucose screening test for diabetes mellitus is not appropriate due to its low sensitivity (WHO, 2003; Wei and Teece, 2006; Altınışık, 2010). However, we believe that when there is no blood sample, without ignoring the false-negative results, urine glucose results can be used in diabetes mellitus screening.

5. Conclusion

In our study, we found a strong correlation between the results of different methods that we used in three different sample types of glucose analysis in our laboratory. While reporting urine glucose results, we think that evaluating concurrently analyzed HbA1c and fasting serum glucose results

may help the laboratory specialist in avoiding both false positives and false negatives. In addition, if there is no evidence of blood glucose and positive urine glucose results are

encountered, it is necessary to perform further examination for diabetes and also with urine glucose negativity clinician physicians should not exclude diabetes risk.

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