

Canadian Journal of Applied Sciences. Volume 2: p-184-191; October, 2012
ISSN 1925-7430; Available online <http://www.canajas.ca>

Leading Article

**THE EFFECT OF ASCORBIC ACID ON GLUCOSE URINE ASSAY BY COPPER
SULPHATE REDUCING SYSTEM AND HEXOKINASE METHOD.**

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ABSTRACT:

Background: The objective of the present study was to compare the results obtained by different methods used in glucose monitoring. Diabetic patients are known to use urine glucose strips or dipsticks tests or glucose pads for urine analysis. Blood glucose monitoring instruments such as different types of blood gluco-meters and digital blood glucose monitoring devices are also available in the local market for home glucose testing.

Methodology: Some of those tests are officially used in the laboratories of different hospitals, where routine analysis is mainly done on advanced Chemical Analyzers.

Results: Some discrepancies were observed in the results of glucose monitoring by different methods. A serious interference was recorded by ascorbic acid in reagent-strip reactions for assay of urinary glucose and hemoglobin. The test methods are known to be based on different chemical reactions including enzymatic and color reactions.

Conclusion: Mostly, the urine concentration of ascorbic acid, uric acid, mercury, copper and iron were labeled to be the main cause of interference. In the present study 2991 urine samples having detectable ascorbic acid levels (≤ 10 mg/dL) were tested by different methods and the results were compared with standard Dimension Chemical Analyzer (Dimension Dade Behring). Ascorbic acid in various concentrations was found to cause serious interference in glucose monitoring, however, hexokinase method was found more reliable.

Key Words: Urinalysis, Glucose monitoring, Strip tests, Ascorbic acid interference, Copper reduction test; Hexokinase method.

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INTRODUCTION:

The presence of glucose in urine is an early and sensitive indication of *Diabetes mellitus* and a part of the physiological condition “Renal Glycosuria”, and glycosuria is always pathological. According to the globally collected data during the year 2000 about 171 million people were reported suffering from diabetes and it was alarmed that the number of diabetic patients will become double 366 million, by the year 2030 [1,2]. Several global authorities and researchers are trying to find methods for early and easy detection of various types of Diabetes and help curing this killer disease [3,4,5]. Attempts have also been made for the cost effective easy detection of sugar to combat the disease and its complications [5-9].

Monitoring with copper reduction test (Clinitest), finger-stick blood glucose devices, blood glucose meters and strip tests made an easy way for the detection and checking of glucose in urine or blood. More advances have been made to avoid ascorbic acid interference with glucose oxidase strips and to achieve better results [10,11]. It was interesting to notice that common urine constituents such as ascorbic acid and uric acid could seriously interfere with deferent strip tests, giving rise to false positive or false negative indication. The observation was not confined only to strip tests, rather the results for the determination of commonly used drug products and several other target compounds were also affected by the indigenous compounds in urine, hence new procedures were developed to obtain better results [12-15]. On the other side, due to several reasons, including the variations in results of different glucose tests and options of using antidiabetic drugs in a complex situation, different Scientific Societies worldwide revised and improved their management plans [1-4, 16-18]. The storage of Diabetes test strips and handling was also assessed to get the maximum surety [18].

Ascorbic acid commonly known as vitamin C is being consumed either therapeutically or unconsciously as it is being widely applied in the food industry either as a preservative, or as a constituent of many foods and drinks. Generally, ascorbic acid treatment was found to lower glucose level in diabetic patients by changing body insulin mechanism [19]. The presence of ascorbic acid in urine was also held responsible for false results in plastic strip testing of glucose [20]. The therapeutic doses of 250 mg and 500 mg of Vitamin C produce a mean urinary ascorbate (ascorbic acid) values of 31 mg/dL and 62 mg/dL respectively [21]. High concentration of ascorbic acid was also reported to hamper test systems with: a peroxide-generating system, peroxidase, and a benzidine-type indicator. As a result, false negative results were obtained for glucose estimation in urine. The presence of Hg^{+2} in reagent strips or diagnostic test solutions were noticed to reduce interference with ascorbic acid [22]. The effect of pH and other elements such as copper in urine needed more explanation [23].

The acidification of urine by ascorbic acid or hippuric acid is common because it facilitates the conversion of therapeutic methenamine mandelate to urinary formaldehyde. After treatment, the copper-reduction procedure showed no glucose in urine samples. The in vitro false positive reactions reported earlier could not be found in vivo [23,24]. Vitamin C was demonstrated to interfere with peroxidase redox indicator systems, such as those used in reagent-strip tests for urinary glucose and hemoglobin [25,26]. Furthermore, it was well described that ascorbic acid when given orally or parenterally, it gave rise to a false-positive reaction in the copper reduction glucose test ‘Clinitest’ [23].

Bayer and Roche took measures to make their strip test more resistant to ascorbic acid concentration and they were marketed with different names to avoid confusion [27,28]. The presence of ascorbic acid in urine at a concentration of > 50 mg/dL could affect the result of

urinary glucose in a urinalysis, and especially when the urine glucose was low, as in the early cases of Diabetes mellitus. It is worth mentioning that: different concentrations ascorbic acid might also interfere with the assay of other analytes namely, bilirubin, nitrite, leukocytes, blood and urobilinogen [29].

In an earlier study it was noticed that 23 of 30 urine samples produced false negative results for glucose at a concentration of 50 mg/dL in the presence of ascorbate at a level of 30 mg/dL [30]. Hence, it was recommended that in the presence of inhibiting quantities of ascorbic acid in a sample, the urine specimen voided at least 10 hours after the last administration of vitamin C should be retested [31]. The negative influence of ascorbic acid on glucose results was also demonstrated quantitatively by using the Reflectometric urine analyzer 'Urotron RL9' [32]. Keeping in view the significance and discrepancies in different test results, current study was designed to compare routinely used strip test results with those obtained by using an advanced clinical laboratory analyzer technique and the results are presented in the present communication.

MATERIALS AND METHODS

In the present study, 2991 urine samples collected for routine urinalysis, were tested by using two methods namely: laboratory analyzer Miditron Junior II, and by using Combur Test M strips (Roche, Germany). A re-test was performed manually with different test strips manufactured by Biomedical Products Company (BMP), Saudi Arabia. BMP tests are also in used to detect the presence of urinary ascorbic acid. In each case, the test comparison was done by using the given comparison procedure by the manufacturer.

All the specimens which were positive for a detectable ascorbic acid level (≤ 10 mg/dL), and samples positive or negative for glucose, were tested with Dimension Chemical Analyzer (Dimension Dade Behring). The target was to get the exact value for glucose concentration. The cut-off point for the Dimension Chemical Analyzer was at and above: 2.8 mmol/L for any positive specimen, and it was same for the strip tests (2.8 mmol/L) as well.

RESULTS:

The results of the present study conducted in King Saud Medical City, Ministry of Health, Riyadh (Saudi Arabia) are depicted in Table 1 and 2. A total of 2991 urine samples were tested routinely during the study period from 2/2/2011 to 31/3/2011 (2 months). Among the samples 212 (7.1%) were positive for Ascorbic acid with a concentration ranging from 10 mg/dL (0.56 mmol/L) to 40 mg/dL (2.28 mmol/L) and a mean = 8.7 ± 0.55 . Other, 400 (13.4%) samples were positive for glucose as detected by the Miditron Junior II and Combur 10 Test M strips and the concentrations ranged between 2.8 to 55 mmol/L (50-1000 mg/dL).

Of the 212 samples positive for ascorbic acid, 23 (10.8%) were positive for glucose as detected with two different test strips. They were confirmed by the test readings from Dimension Chemical Analyzer. However, 188 specimens were found negative with the test strips, while 3 of them could not be run on Dimension Chemical Analyzer. Of the remaining 'negative' 185 samples, 12 samples were found positive when tested with Dimension Chemical Analyzer with the test range from 2.8 mmol/L (50 mg/dL) to 6.8 mmol/L (122 mg/dL). In copper reduction test false positive results were also obtained.

Table 1: Showing the Positive Concentrations of Ascorbic acid and Glucose in mmoles/L.

Patients ID	Ascorbic Acid BMP Strips	Glucose BMP Strips	Glucose Roche Combur 10 Strips	Glucose Dimension Analyzer
62-4/4/1430	1.14	57	6	73.5
74-12/2/1430	0.56	28	6	62.2
09-21/3/1430	0.56	14	6	6.3
06-11/3/1430	0.56	14	6	20.9
16-12/3/1430	0.56	14	6	15.4
63-19/3/1430	0.56	5.5	6	14.8
18-1/4/1430	0.56	14	6	13.7
30-18/3/1430	1.14	28	56	73.1
45-5/3/1430	0.56	56	56	72.9
77-12/2/1430	2.28	58	56	70.3
62-13/2/1430	0.56	60	56	68.9
02-13/3/1430	1.14	56	56	67.8
75-6/3/1430	0.56	56	56	47.2
35-7/2/1430	0.56	59	56	37.8
90-14/2/1430	0.56	5.6	3	7
65-18/3/1430	0.56	14	3	6.8
13-25/3/1430	0.56	5.6	3	5.6
54-9/2/1430	0.56	2.8	3	4.1
07-1/4/1430	0.56	14	3	3.6
62-26/3/1430	2.28	28	18	73.4
51-4/4/1430	0.56	61	18	67.2
74-13/3/1430	2.28	56	18	25.3
15-11/3/1430	0.56	28	18	22.4

DISCUSSION:

The results of the present study clearly demonstrated the interference of ascorbic acid present in urine samples under analysis for glucose by different strip tests. Chemical compounds such as ascorbic acid and uric acid are indigenous compounds in urine; therefore, in the test procedure special attention is essential to be given to the levels of such compounds in order to avoid the possibility of any false result. Our results are full in agreement with earlier findings where high urine ascorbic acid concentration produced unreliable results for the presence of glucose or hemoglobin [25,26]. In addition, the results of the present study added support to the earlier reports that ascorbic acid could affect the result of urine glucose test, carried out by using different routine urine strips, plastic strips, or the Clinitest [7]. In Clinitest (copper reduction test), sometimes 'false positive' results were observed without the presence of glucose due to interaction with other drug products present in urine such as: aspirin, ascorbic acid, isoniazid, and some antibiotics [14].

The dip stick glycosuria test commonly used in different countries was found to be having similar sensitivity and specificity as observed in standard hexokinase method. In general, at higher concentration of ascorbic acid a marked decrease in colour reaction was observed, and at low glycosuria 'false negatives' appeared [33]. False negative results for

urine glucose in the presence of ascorbic acid were well documented and patients with low glycosuria showed false negative results in dipstick method. Hence, it was suggested that the use of such method might not be useful in prenatal management [33]. If the test was carried out by using improved CombuTest product from Roche, then up to 50 mg/dL glucose could be detected successfully and the said test show resistant to ascorbic acid concentration up to 400 mg/L. In cases of high analyte concentration, the test was sensitive and devoid of any interference by ascorbic acid even up to 1000 mg/L concentration [26,33]. However, glycosuria is not only due to high blood glucose but it is also influenced by diastolic blood pressure. As a result it cannot be considered absolute method and routine laboratory measurements must be conducted [35]. Based on the results obtained in the present study and earlier reports, present study results added support to the earlier observations and provide the renewed insight in to the mechanism. It might now be concluded that vitamin C concentrations can alter color reaction leading to misleading results. Such findings suggested that in case of pregnant ladies, glycosuria/proteinuria screening by glucose dipstick method were not much appreciable [33-37].

Table 2: Showing the Ascorbic acid and the Glucose (Test strips Negative and Analyzer Positive) Results.

Patients ID	Ascorbic Acid BMP Strips	Glucose BMP Strips	Glucose Roche Combur 10 Strips	Glucose Dimension
54-18/3/1430	0.56	-	-	6.8
02-5/4/1430	0.56	-	-	5.3
11-14/2/1430	1.14	-	-	5
76-19/3/1430	0.56	-	-	4.9
21-8/2/1430	0.56	-	-	4.3
29-11/3/1430	0.56	-	-	4.3
74-4/3/1430	0.56	-	-	4.3
33-8/2/1430	0.56	-	-	3.8
44-21/3/1430	0.56	-	-	3.6
42-26/3/1430	0.56	-	-	3.2
42-8/2/1430	0.56	-	-	3.1
54-18/3/1430	0.56	-	-	2.8

Clinistix (glucose oxidase test) use toluidine and glucose oxidase which is known to convert glucose in to hydrogen peroxide and gluconic acid. The reaction of hydrogen peroxide with toluidine produces interfering color. Clinistix method is sensitive to aspirin, iron, ascorbic acid, levodopa, and antibiotics such as tetracycline which are responsible for unwanted colors. In pregnancy, due to increased glomerular filtration rate, glycosuria was found in 50% women despite having normal blood sugar levels. Both high intakes of vitamin C and high ketone levels in urine were found to give false positive results. It was well explained that renal thresh hold for glucose was highly variable in those cases, and routine dip stick check up for monitoring glucose was not much valuable at each prenatal visit. There is a need for producing more accurate tests which have no interference with the urine indigenous compounds [11-13].

But on the other hand, when urinary glucose was assayed with the Dimension Analyzer, urinary ascorbic acid did not interfere with the result of glucose assay. It was so

because Dimension analyzer uses the hexokinase method which differs from copper sulphate reducing system used in the Clinitest method (38-41). The Clinitest and the glucose oxidase peroxidase method used in the test strips might be considered alternate method used to detect the presence of ascorbic acid in urine.

CONCLUSION:

Estimation of urinary glucose using the urine test strip alone might not give valid results in certain circumstances especially in early cases of mild Diabetes Mellitus.

RECOMMENDATIONS:

It is recommended that urine test strips with a pad for ascorbic acid screening should be used for routine urine analysis. If there is a doubt of having a positive urinary ascorbic acid sample, then another chemical method for testing urinary glucose should be adopted.

The doubtful cases of nitrite, leukocytes and blood, then microscopic examination should be used which would show urinary sediments of leukocytes and red blood cells. As regards the presence of bilirubin and urobilinogen is concerned, the urine colour usually indicates their presence. In such cases, an alternative method and more reliable laboratory techniques should be used; in more complex cases serum level may be assayed for confirmation.

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