Letter to the Editor
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Ascorbate Oxidase Minimizes Interference by High-Concentration Ascorbic Acid in Total Cholesterol Assays

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Dear Editor,

Ascorbic acid interferes with certain clinical chemistry assays based on peroxidase and redox indicators [1]. Ascorbic acid is reported to interfere with the measurement of glucose, total cholesterol, triglycerides, and uric acid [2-5]. We recently encountered several cases of interference from ascorbic acid in serum total cholesterol assays based on colorimetric enzymatic reactions.

A 48-yr-old-female with recurrent ovarian cancer underwent palliative segmental resection surgery of the small intestine. The patient showed progressive renal impairment following surgery, and total cholesterol level was <3 mg/dL, as measured using the OSR6516 total cholesterol assay (Beckman Coulter, Brea, CA, USA) on an AU5800 analyzer (Beckman Coulter). We repeated the test with the same sample, using the Pureauto S CHO-N total cholesterol assay (Sekisui Medical Co., Tokyo, Japan) on a Hitachi7600 analyzer (Hitachi Co., Tokyo, Japan) and observed a concentration of 121 mg/dL. We did not repeat the OSR6516 assay after a certain period. The specimen was checked for lipemic, hemolytic, and icteric indices to rule out other possible interferents. The patient was administered 30 g of ascorbic acid intravenously daily for 22 days prior to total cholesterol measurements. Dipstick analysis of the patient's urine was performed by using an URiSCAN SUPER analyzer (YD Diagnostics, Seoul, Korea), which showed a value of 2+ (equivalent to 25 mg/dL) for ascorbic acid. Three additional cases characterized by spuriously low total cholesterol values owing to ascorbic acid interference are summarized in Table 1. Although we could not determine serum ascorbic acid levels, we detected its presence by using a urine dipstick assay in three of the four cases.

Several studies recommend the addition of ascorbate oxidase to minimize ascorbic acid interference in assays to assess uric acid, triglyceride, oxalate, and cholesterol [5-8]. Most cholesterol assays in clinical laboratories use an enzymatic colorimetric method with the Trinder end-point reaction, in which hydrogen peroxide reacts with a chromogen via peroxidase to form a colored product; absorbance of this product is proportional to the concentration of total cholesterol in the sample [1]. Ascorbic acid interferes with peroxidase-based oxidation of the chromogen [6]. Since dehydroascorbic acid is already oxidized and has lost its reducing power, interference via ascorbic acid can be successfully prevented. Commercially available total cholesterol assays are summarized in Table 2. Ascorbate oxidase included in the Sekisui total cholesterol assay effectively converts serum

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Table 1. Summary of the four cases with inappropriately low total cholesterol level

Case No.	Age (yr)	Sex	Diagnosis	eGFR* (mL/min/1.73 m^2)	ascorbic acid supplementation history	Initial total cholesterol assay	Initial serum total cholesterol level (mg/dL)	Subsequent total cholesterol assay	Subsequent serum total cholesterol level (mg/dL)	Urine dipstick analysis for ascorbic acid
1	48	F	Ovarian cancer	11	30 g IV one time per day for 22 days	Beckman Coulter/ AU5800†	<3 (0.5 hr§)	Sekisui/Hitachi 7600‖	121	2+¶ (1.5 hr**)
2	91	M	Bladder cancer	23	40 mg PO 4 times per day for 2 weeks	Roche/ Cobas c701‡	<4 (6 hr§)	Sekisui/Hitachi 7600‖	94	N/A†† (N/A**)
3	89	M	Diabetes mellitus	85	20 g IV one time per day for 19 days	Beckman Coulter/ AU5800†	<3 (3 hr§)	Sekisui/Hitachi 7600‖	97	2+¶ (3 days**)
4	80	M	Pancreatic cancer	14	N/A	Beckman Coulter/ AU5800†	<3 (N/A§)	Sekisui/Hitachi 7600‖	95	3+¶ (4 hr**)

*eGFR was calculated by using 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equations currently recommended by The National Kidney Foundation. No patients had dialysis history; †OSR6516 Cholesterol assay (Beckman Coulter, Inc., Brea, CA, USA) on the AU5800 Clinical Chemistry System (Beckman Coulter, Inc.); ‡CHOL2 (Roche Diagnostics GmbH, Mannheim, Germany) assay on the Cobas c701 Analyzer (Roche Diagnostics GmbH); §Time interval between the most recent ascorbic acid administration and total cholesterol measurement; ‖Pureauto S CHO-N (Sekisui Medical Co., Ltd., Tokyo, Japan) assay on the Hitachi 7600 Analyzer (Hitachi Co., Tokyo, Japan); ¶The urine dipstick analyses for cases 1, 3, and 4 were performed using the URiSCAN SUPER analyzer (YD Diagnostics, Seoul, Korea). In the urine dipstick analysis for ascorbic acid, -, 1+, 2+, and 3+ are equivalent to ascorbic acid concentrations of negative, 10 mg/dL, 25 mg/dL, and 50 mg/dL, respectively; **Time interval between total cholesterol measurement and urine analysis; ††Since the ascorbic acid item is not included in the CLINITEK Novus (Siemens Healthcare Diagnostics Inc., Erlangen, Germany) urine dipstick analysis strip, the urine ascorbic acid concentration of the patient from case 2 could not be measured.
Abbreviations: eGFR, estimated glomerular filtration rate; F, female; M, male; PO, *per os* or oral administration; IV, intravenously; N/A, not available.

Table 2. Summary of commercially available total cholesterol assays with respect to the presence of ascorbate oxidase

Total cholesterol assays*	Ascorbate oxidase in the list of ingredients	Description of ascorbic acid interference by manufacturer
OSR6516 Cholesterol (Beckman Coulter, Inc., Brea, CA, USA)	No	No significant interference (within ± 10.0 mg/dL) up to 3 mg/dL
CHOL (Beckman Coulter, Inc., Brea, CA, USA)	No	No significant interference (within ± 10.0 mg/dL) up to 3 mg/dL
CHOL2 (Roche Diagnostics GmbH, Mannheim, Germany)	No	Not shown
CHOLESTEROL (Abbott Laboratories, Chicago, IL, USA)	No	Observed % of target: 97.6% at 3 mg/dL of ascorbic acid
Pureauto S CHO-N (Sekisui Medical Co., Ltd., Tokyo, Japan)	Yes	No ascorbic acid interference up to 50 mg/dL
Wako L Type CHO M (Wako Pure Chemical Industries, Ltd., Osaka, Japan)	Yes	No significant effects
SEIKEN T-CHO (S) (Denka Seiken Co., Ltd., Niigata, Japan)	No	No interference up to 50 mg/dL
Determiner C-TC (Kyowa Medex Co., Ltd., Tokyo, Japan)	No	May negatively affect measurement if their concentrations in blood are high

*The total cholesterol assays listed are all based on colorimetric enzymatic reactions with the Trinder reaction end-point.

ascorbic acid (up to concentrations of 50 mg/dL) to dehydroascorbic acid.

Since ascorbic acid rapidly auto-oxidizes over time, it is difficult to detect its interference in cholesterol measurements [3, 9]. Reviewing medical records can also be problematic; some ascorbic acid supplements are not physician-prescribed but are readily available over-the-counter. Furthermore, it is difficult to predict serum ascorbic acid concentrations based on ingested doses of ascorbic acid in patients with impaired renal function.

Artiss *et al*. [9] suggested that we can exclude ascorbate oxidase in wet-chemistry reagent systems for cholesterol determination because solvated ascorbic acid is subject to air oxidation. Thus, the interfering substance is oxidized prior to analysis by laboratory instruments. This is partly true for most samples from patients whose renal function is intact and for those consuming typical ascorbic acid doses. It might be particularly problematic for patients with impaired renal function or for those taking high ascorbic acid doses. In the second case (Table 1), we presumed that this low cholesterol level was likely due to ascorbic acid accumulation in the blood owing to impaired renal function, even

though the prescribed dose of ascorbic acid was not high.

As all of our cases showed extremely low total cholesterol levels, the laboratory technician easily noticed analytical errors and tried to troubleshoot them before reporting the results. These problems occurred in approximately one case bimonthly and were reported to managers and documented. However, interference from low concentrations of ascorbic acid causes a small negative bias in total cholesterol levels, which may go unnoticed. Ascorbic acid is one of the most widely consumed nutritional supplements, and some physicians prescribe megadoses for cancer therapy [10]. Consequently, managers of clinical laboratories should consider the use of cholesterol assays that include ascorbate oxidase.

In conclusion, when colorimetric enzymatic assays detect inappropriately low levels of total cholesterol, we should suspect ascorbic acid interference. Addition of ascorbate oxidase to cholesterol reagents can minimize the inference of ascorbic acid in total cholesterol assays.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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