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Comparison of two plasma urate assays in patients receiving vitamin C supplementation

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Summary

The aim of this study was to compare plasma urate (PU) concentrations using two different assays in patients receiving vitamin C supplementation. PU was measured using two routinely available enzymatic uricase methods: (1) uric acid plus method (ascorbate oxidase assay), and (2) uric acid method (non-ascorbate oxidase assay). Twenty patients receiving allopurinol were randomised to an increase in allopurinol dose or commence vitamin C 500 mg/d on a 1:1 ratio. Twenty patients not receiving allopurinol were randomised to start allopurinol or vitamin C 500 mg/d on a 1:1 ratio. Trough fasting samples for plasma ascorbate and urate were measured weekly until week 8. There was no significant difference in the mean PU measured by the two assays. In patients not receiving supplemental vitamin C the mean PU concentrations were identical for both assays. For patients receiving supplemental vitamin C the mean PU concentrations for the ascorbate oxidase assay was 0.525 mmol/L (SE 0.034) and for the non-ascorbate oxidase assay 0.510 mmol/L (SE 0.033), $p = 0.079$. There is a small non-significant difference in measured PU in patients receiving supplemental vitamin C between the two assays. The assay which does not include ascorbate oxidase results in consistently lower PU concentrations compared to the assay which includes ascorbate oxidase.

Key words: Ascorbic acid, plasma urate, urate assays, vitamin C.

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INTRODUCTION

Hyperuricaemia is the primary biochemical abnormality in gout. The measurement of plasma urate plays an integral part in monitoring of urate lowering treatment, with evidence that sustained plasma urate <0.36 mmol/L is required for improved clinical outcomes.[1] Hyperuricaemia can also be a feature of pre-eclampsia (though not a diagnostic criterion) and tumour lysis syndrome and should be measured in patients undergoing chemotherapy.

Recently, vitamin C supplementation has been associated with lower plasma urate concentrations and a reduced risk of gout.[2] Whether there is a role for vitamin C supplementation in the long-term management of gout requires further investigation.[3] However, a recent study from our group suggests that there is no significant effect of low dose vitamin C (500 mg/d) on serum urate concentrations as compared to allopurinol.[4]

Proclamations of statistically significant changes in plasma urate levels raises the question of how reliable the methods are for measuring urate and, more importantly, whether vitamin C interferes with the urate assay. Given the observed reductions in serum urate with vitamin C supplementation are small (~0.02 mmol/L)[5] interference by vitamin C resulting in lower urate concentrations may explain the observed results.

Historically, several methods of measuring urate have been adapted for automated use. Colourimetric based assays use the formation of a chromogen whereby phosphotungstic acid is reduced by urate in an alkaline solution. This method is subject to interferences from drugs and reducing substances other than urate. Enzymatic based assays use bacterially derived uricase oxidoreductase which oxidises urate to allantoin, hydrogen peroxide and carbon dioxide which can be measured by the Trinder reaction. This method has been further modified by some manufacturers (Roche and Abbott) to include ascorbate oxidase in the reagent mixture to remove interference from other agents, including ascorbate (vitamin C).

As part of a study of the effect of vitamin C on urate levels in patients with gout, we examined the differences between two routinely available enzymatic uricase plasma urate methods, one with and the other without the addition of ascorbic oxidase to the reagents. The aim was to determine whether low dose vitamin C supplementation had any effect on measured plasma urate concentrations.

METHODS

The study design and results of the randomised clinical trial have been published elsewhere.[4] Ethical approval was obtained from the Upper South A Regional Ethics Committee, New Zealand. Written informed consent was obtained from each patient. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000545066). In brief this was an 8 week open-label parallel randomised controlled trial in Christchurch, New Zealand. Patients with gout as defined by American Rheumatism Association criteria[6] and with plasma urate >0.36 mmol/L were recruited. Twenty patients already receiving allopurinol were randomised, 10 received an increase in allopurinol dose and 10 commenced vitamin C 500 mg/d. Twenty patients not receiving allopurinol were randomised, 10 commenced allopurinol and 10 commenced vitamin C 500 mg/d. Trough fasting samples for plasma ascorbate and urate were measured at baseline, day 3, and then weekly until week 8.

Plasma urate measurements

Gel separated lithium heparin plasma samples (Ref 367883, Vacutainer; BD, USA) collected from study participants were separated by centrifugation within 2 h of collection. Plasma urate levels were measured by two methods: (1) uric

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acid plus method (cat. no. 11875426; Roche, USA) (ascorbate oxidase assay), stated intra-assay coefficient of variation (CV) 0.5% and inter-assay CV 1.7%; and (2) uric acid method (list number 7D76; Abbott, Germany) (non-ascorbate oxidase assay) on the Abbott Architect platform. Both methods were calibrated using the same commercial calibrator [Randox (UK): 2 (CAL2350) and 3 (CAL2351)]. The stated total (inter + intra) assay CV is ≤4%. The Canterbury Health Laboratories stated estimation of uncertainty is 5.05% and our % CV on the Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program (QAP) is 2.5%.

Plasma vitamin C determination

An aliquot of the gel separated lithium heparin plasma sample as collected for urate determination was frozen at −80°C for a minimum of 2 months and then analysed for vitamin C using a high-performance liquid chromatography (HPLC) method: ClinRep complete kit for vitamin C in plasma (reference 28000; Recipe, Germany). The package insert states an intra-assay CV of 5.9% and an inter-assay CV of 6.9%. The lower determination limit is 2.8 μmol/L. All of these parameters are in agreement for the assay performance here at Canterbury Health Laboratories.

Vitamin C analysis was performed on completed sets of participant samples to eliminate between batch variation.

Statistical analysis

Repeated measures analysis of variance was used to compare measurements of plasma urate concentrations between assays, allowing for repeated observations on individuals. A linear mixed model was used to test the association between plasma ascorbate concentrations and the difference between assays in plasma urate. This model included patients as a random factor and randomised vitamin C group as a fixed between patient factor.

RESULTS

The demographics of the patients are outlined in Table 1. There was no significant difference in the overall mean plasma urate measurements between the two different assays; Abbott assay (non-ascorbate oxidase assay) mean 0.468 mmol/L (SE 0.025) versus Roche assay (ascorbate oxidase assay) mean 0.460 mmol/L (SE.024), $p = 0.11$.

The mean plasma urate concentrations for both assays were identical in patients without supplemental vitamin C (Table 2), whereas in those patients receiving supplemental vitamin C the mean plasma urate concentrations were higher with the Roche assay (ascorbate oxidase assay) compared to the Abbott assay (non-ascorbate oxidase assay) (Table 2; Fig. 1).

There was a statistically significant positive correlation between measured plasma ascorbate concentrations and the difference between the assays in measured plasma urate concentrations (Roche assay – Abbott assay). For every μmol/L increase in plasma ascorbate there was a 0.000310 mmol/L increase in plasma urate with the Roche assay compared with the Abbott assay $p < 0.001$.

Table 1 Demographics of the patients who received vitamin C and those who did not receive vitamin C

	Vitamin C ($n = 20$)	No vitamin C ($n = 20$)
Male	18 (90%)	18 (90%)
Age, mean years (range)	61.2 (39–86)	55.0 (27–78)
Weight, kg	93.1 ± 3.3	100.3 ± 5.9
BMI	30.4 ± 0.96	32.0 ± 1.5
NZ European	14	11
eGFR (mL/min/1.73 m^2)	65.5 ± 3.5	67.9 ± 4.6

Data are presented as mean ± SEM unless otherwise stated.
BMI, body mass index; eGFR, estimated glomerular filtration rate; SEM, standard error of the mean.

DISCUSSION

We have demonstrated a small non-significant difference in measured plasma urate in patients receiving supplemental vitamin C between the two assays. The Abbott assay, which does not include ascorbate oxidase, results in consistently lower plasma urate concentrations compared to the Roche assay which includes ascorbate oxidase. This lowering effect on plasma urate increases as plasma ascorbate concentrations increase.

A previous study of 18 healthy volunteers examined the effects of vitamin C doses between 250 mg and 4.0 g per daily for 1 week on plasma urate. Plasma urate was determined at 4, 12, and 24 h after vitamin C intake using a Trinder based assay, which is subject to interference by vitamin C. Even at the lowest vitamin C dose of 250 mg/d there was a reduction in measured urate concentration, and the magnitude of the reduction increased as the vitamin C dose increased. Using *in vitro* assays they reported that serum ascorbate concentrations correlated with the percentage of negative interference on the assay 4 h after vitamin C intake. However, the effect of vitamin C on the negative interference decreased progressively in the samples obtained at 12 and 24 h after vitamin C intake and in the *in vitro* studies out to 24 h.[7] On the basis of these results the authors suggested that vitamin C supplements should be stopped 3–4 days prior to measuring plasma urate levels.

The intra-individual coefficient of variation (CVi) for plasma urate measurement has been established at 9.0%, which when combined with analytical imprecision (CVa = 0.7%), gives a reference change value or critical difference (CD) of 25% (approximately 0.07 mmol/L at a level of 0.29 mmol/L) (www.acb.org.uk/docs/NHLM/Uric). This represents the degree of change that cannot be solely attributed to either analytical or biological variation. A serum vitamin C concentration of greater than 225 μmol/L of ascorbate would be required to achieve this degree of method interference from ingested vitamin C. While none of the participants in this study achieved ascorbate levels greater than 225 μmol/L (highest 167 μmol/L), participants were only receiving 500 mg vitamin C per day. However, it would not be unreasonable to assume that plasma ascorbate levels above 225 μmol/L could be achieved at supplementation levels greater than 500 mg/day; this would then have significant clinical implications as the measured urate levels would be reduced by more than the CD.

High levels of ascorbate in the urine have been recognised to cause interferences in the measurement of haemoglobin and glucose in urine and have been addressed by reagent manufacturers with the adaption of urine dipstick methods to overcome interferences to the peroxidase-redox reactions.[8] Ascorbate has also been shown to interfere with glucose meters.[9] There is widespread use of over-the counter vitamin supplements.[10] Clinicians and laboratories should be reminded that many routine plasma methods including urate, cholesterol and triglycerides utilise peroxidase-redox reactions, with well

Table 2 Mean plasma urate concentrations using the Abbott and Roche assays

	PU, mmol/L (SE) Abbott (non-ascorbate oxidase assay)	PU, mmol/L (SE) Roche (ascorbate oxidase assay)	*p* value
Vitamin C	0.411	0.411	
No vitamin C	0.510 (0.033)	0.525 (0.034)	0.079

PU, plasma urate; SE, standard error.

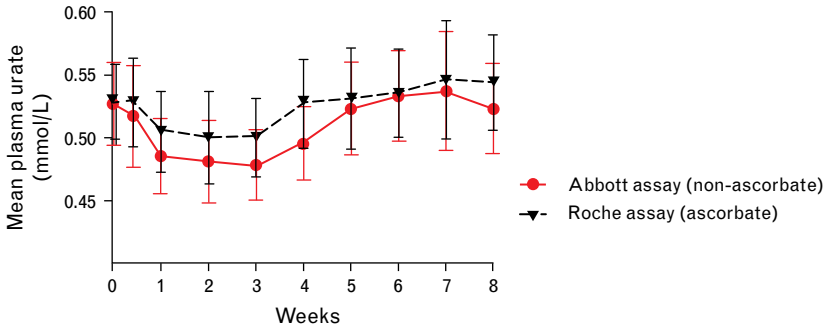

Fig. 1 Mean ± SE plasma urate in patients receiving vitamin C as determined by either the Abbott assay or the Roche Assay.

documented interferences in measured analytes due to ascorbate interferences.[11]

Researchers investigating the use of vitamin C supplementation in the treatment of gout need to be aware of assay differences affecting measured urate levels. It appears that the hypouricaemic effect of vitamin C is clinically modest at best and laboratory assay differences have the potential to influence its perceived effect.

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