Significance of Urinary Tartaric Acid

To the Editor:

In 1995, Shaw et al. (1) reported the gas chromatographic–mass spectrometric identification and quantification of various compounds in the urine of two autistic brothers. In that report (1) and a subsequent one (2), the authors concluded that the presence of several compounds, including tartaric acid (excreted as tartarate), may be causally related to the autistic symptoms and that their origin is an overgrowth of intestinal yeast. The practice of analyzing urine for these compounds to detect intestinal yeast continues, although several lines of evidence contradict these conclusions.

Dietary intake is usually the major contributor to urinary tartarate. The US Food and Drug Administration considers it a generally recognized as safe (GRAS) human food ingredient (3). Gram quantities of tartaric acid are commonly consumed in grapes and grape products, and many prepared foods contain large quantities of the acid or its salts (4, 5). Dietary intake of tartaric acid by the two individuals in the study by Shaw et al. (1) was not evaluated.

To demonstrate the magnitude of dietary impact from grape juice, we measured urinary tartarate concentrations in 23 individuals on 2 consecutive days. On day 1, participants restricted their diets by avoiding any products that contained tartaric acid on their labels. Each participant collected an overnight urine. On day 2, participants followed no restrictions, but drank 28 mL (10 oz) of grape juice with a tartaric acid content of 2.0 g/L between 1900 and 2200 and again collected an overnight urine. Urinary tartarate was measured by a liquid chromatographic–electrospray tandem mass spectrometric method similar to that reported by Pitt et al. (6). The results, normalized to creatinine, are presented in Fig. 1. Ingestion of 28 mL of grape juice containing 590 mg of tartarate increased mean urinary tartarate concentrations from 7.4 to 282 μg/mg of creatinine, with the lowest concentration measured on day 2 being 131 μg/mg of creatinine. Shaw et al. (1) reported that the two brothers had a mean urinary tartaric acid of 69.2 mmol/mol of creatinine, equivalent to 91.9 μg/mg of creatinine. Thus, ingestion of moderate amounts of grape juice produces urinary tartarate concentrations above those suggested by Shaw et al. (1) to produce toxic effects.

Regarding the production of tartaric acid by intestinal yeast, the evidence supports the opposite conclusion: that it is destroyed by fungi and bacteria. At least 23 varieties of bacteria are able to degrade tartaric acid (7), and there is no evidence that any type of yeast or fungus can produce tartaric acid as a metabolic end product.

Even when urinary tartaric acid is greatly increased, there is no evidence of physiologic harm. The majority of tartaric acid is destroyed in the intestinal tract by microbial action (8, 9). The presence of tartaric acid in human urine is not associated with any toxic consequences at doses up to 20 g. Long-term studies in rats demonstrated that a diet containing up to 1.2% tartaric acid for 2 years produced no significant toxic effect (10).

We conclude that dietary sources strongly influence the concentration of urinary tartarate and that its production by intestinal yeast or bacteria is insignificant. Ingestion of 28 mL of grape juice frequently produces urinary tartarate concentrations >300 μg/mg of creatinine. Furthermore, tartaric acid in human urine specimens provides no evidence regarding yeast overgrowth or toxic consequences. At this time, there is no justification for routine clinical measurement of tartaric acid in human urine.

Fig. 1. Urinary tartarate excretion (Day 1) and after ingestion of 28 mL of grape juice (Day 2).
Genetic Screening for Hemochromatosis: A Cautionary Tale

To the Editor:

Hemochromatosis is a common autosomal recessive genetic disorder of iron metabolism. In the United Kingdom, more than 90% of patients with hereditary hemochromatosis are homozygous for the C282Y mutation of the HFE gene, but other single-nucleotide polymorphisms (SNPs) within the HFE gene, namely H63D and S65C, have also been associated with the hemochromatosis phenotype. Various PCR-based methods can detect these SNPs, including a multiplex PCR for the two common SNPs, C282Y and H63D (1). This method involves PCR-mediated site-directed mutagenesis for C282Y and H63D to create a BbrPI restriction site in the wild-type PCR products. The presence of polymorphic alleles for both C282Y and H63D abolishes the restriction site so that the mutated allele remains undigested.

Using this method (1), we found two family members who appeared to be homozygous for H63D, but another laboratory had found these individuals to be heterozygous. DNA sequencing confirmed heterozygosity for H63D and, in addition, heterozygosity for S65C. We postulated that the presence of the S65C polymorphism interfered with the BbrPI restriction enzyme site and analysis of the H63D polymorphism by our method (1).

We reanalyzed 250 patients for H63D and S65C, using an alternative PCR method (2) with the same forward primer but a different reverse primer and producing a 174-bp product. The H63D genotype was determined by use of bcl-1 restriction enzyme digestion, which produced two fragments of 104 and 70 bp in the mutated allele and an undigested product in the wild-type allele. The S65C genotype was determined by use of the same PCR for H63D but with a Hinf-1 restriction enzyme digestion producing two products of 113 and 61 bp in the wild-type allele but an undigested product with the mutated allele.

According to the multiplex PCR (1), 4% were homozygous H63D and 19% were heterozygous; 4.8% were heterozygous S65C and none were homozygous. By the alternative PCR method (2), six cases that had been genotyped as homozygous for H63D by the multiplex PCR method (1) were found to be compound heterozygotes for H63D and S65C. This was confirmed by DNA sequencing.

The presence of the S65C mutated allele interferes with detection of the H63D genotype by the multiplex method of Stott et al. (1). This erroneously leads to ~1–2% of tested individuals being labeled as homozygous for H63D when they are in fact heterozygous for H63D. The presence of the S65C allele appears to lead to a failure to produce the expected digestion products. In all six such patients, an undigested PCR product was produced, indicating that it was not a failure of PCR but a failure of digestion. In four other patients who were heterozygous for S65C and did not have H63D complete digestion at the BbrPI site did occur. This failure of digestion occurred only in those S65C-heterozygous patients who were heterozygous for H63D.

The S65C SNP is outside of the restriction site and would not be expected to interfere with digestion by BbrPI according to available information on the restriction enzyme site. One possible explanation is that methylation occurs to prevent restriction enzyme digestion in this region. Such methylation, however, would have to be highly specific for this DNA sequence.

A recent UK NEQAS survey indicated that laboratories in the United Kingdom are using many different PCR methods for detecting the common hemochromatosis polymorphisms, and several use the multiplex PCR method (1). Unfortunately, this multiplex method may lead to error in up to 2% of samples. Our current practice is to test for all three mutations.

References


Jane Starczynski
Laura Hooper
Nageeb Ali
Mark Hill
Chris Fegan
Guy Pratt

1Department of Haematology
Birmingham Heartlands
and Solihull NHS Trust
Bordesley Green East
Birmingham, United Kingdom
2 Institute for Cancer Studies
University of Birmingham
Edgbaston, United Kingdom

*Address correspondence to this author at: Department of Haematology, Birmingham Heartlands and Solihull NHS Trust, Bordesley Green East, Birmingham, B9 5SS, United Kingdom. Fax 44-121-7667530; e-mail guy.pratt@heartsol.wmids.nhs.uk.

DOI: 10.1373/clinchem.2004.04925

Aberrant Thyroid Testing Results in a Clinically Euthyroid Patient Who Had Received a Tumor Vaccine

To the Editor:

We describe here the first case of immunoassay interference from...