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The Incidence of Elevations in Urine 5-Hydroxyindoleacetic Acid*

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ABSTRACT

A 24-hour urine collection for 5-hydroxyindoleacetic acid (HIAA) is commonly performed to evaluate patients with suspected carcinoid syndrome. However, carcinoids are rare, and elevated results are common even when using an analytically specific method. To characterize this problem, the incidence of elevated results was examined in a population of 947 patient specimens received in a clinical reference laboratory setting. Using a reference limit of 15 mg/d identified 7.9 percent of the results as elevated, with 3 percent >100 mg/d, and about 1 percent >350 mg/d. Males showed 14 percent >15 mg/d compared to 5.2 percent for females. Characterization of incomplete and excess 24-hr urine collections is facilitated by use of a creatinine ratio, with a reference limit of 14 mg/g creatinine equivalent to 15 mg/d. Given the frequency of elevated results, HIAA should be used to support the diagnoses of carcinoid only when consistent with other objective findings.

Introduction

5-Hydroxyindoleacetic acid (HIAA) is a metabolite of serotonin (5-hydroxytryptamine), and determination of urine HIAA is often performed in patients when carcinoid syndrome is in the differential diagnosis. There are excellent reviews on HIAA testing,[1,2] but one aspect that is less commonly discussed is the incidence of elevated results in the clinical laboratory setting. To characterize this problem, results of HIAA analysis were examined in a reference laboratory which receives specimens from a wide geographic area. While a laboratory of this type typically knows little of the specific circumstances surrounding a given patient, it is well-situated to provide information about the frequency of elevated results.

The term carcinoid is a generic name for neoplasia which arise from endocrine cells dispersed principally along the gastrointestinal tract, but also in a variety of other sites including lung, thymus, biliary tract, ovary, and elsewhere. Carcinoid tumors are rare, with an estimated incidence between 1.3 per 100,000[2] and 8.5 per 100,000.[3] Carcinoid syndrome occurs in about 5 percent of carcinoid tumors. With GI carcinoids, the syndrome occurs only in association with liver metastases.[2]

Classically, carcinoid syndrome is composed of secretory diarrhea and episodic flushing, sometimes in combination with valvular heart disease or bronchospasm.[4] However, the clinical picture varies widely. As a result, patients who complain of nonspecific symptoms such as

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flushing will often be evaluated for possible carcinoid syndrome, although the vast majority will not have a carcinoid tumor.[5] The test most frequently used for this purpose is HIAA.

Analysis of HIAA was originally based on relatively nonspecific colorimetric-type reactions, which generated frequent false positive and false negative results. The development of high performance liquid chromatography (HPLC) assays was a considerable improvement in terms of analytic specificity.[1] However, serious preanalytic limitations continue to persist, including the problems of serotonin-containing foods, physiologic interferences from medications and alcohol, other non-carcinoid disorders which increase HIAA excretion, and the difficulties associated with 24-hour urine collections. Even with an analytically specific method such as HPLC, the incidence of elevated HIAA results remains common.

Methods

5-Hydroxyindoleacetic acid was determined using a gradient HPLC method employing coulometric array detection.[6,7] Instrumentation consisted of an ESA Model 5500 HPLC system* equipped with a 15 cm × 4.6 mm NBS column.* The mobile phases consisted of 0.05 mol/L sodium phosphate (pH 3.5) mixed with methanol (v/v): Mobile phase A with 1 percent methanol, and mobile phase B with 50 percent methanol. The flow rate was 1 mL/min, and the flow of phase B varied from 6–55 percent over a 20 min run. Eight serial coulometric electrodes, set at increasing potentials from 0 to 560 mV in 80 mV steps, were used to detect the characteristic oxidation profile of HIAA. Additional details of the analysis are described elsewhere.[6]

Specimen preparation consisted of adding 200 μL urine and 3.8 mL HPLC-grade water to a centrifuge filtration device (Centrex 0.2 μL Nylon Membrane Filters† and centrifuging for 5 min at 1500 *g*. Filtrate was then transferred to instrument vials, capped, and placed in a refrigerated autosampler tray. Injection volume was 10 μL. Daily urine-based control material during the study period showed a CV of 16 percent at 3.2 mg/L, and 6.5 percent at 24.7 mg/L. The limit of detection was 0.1 mg/L.

Selection of study specimens was based on the indirect, random sampling strategy described by Solberg.[8] All urine HIAA test results were collected from May 22 to August 15, 1997, for a total of 1083 specimens. Test results were excluded based on the following criteria: (1) No age given; (2) <12 years old; (3) random collection; (4) dilute specimen <25 mg/dL creatinine; (5) 24-hour collection volume <400 mL; and (6) interference with creatinine measurement. Specimens received without specification of sex were placed in male and female categories based on first name gender. Those not excluded were defined as the study population (*ie*, 947 specimens). The study population was ranked in ascending order of HIAA excretion (mg/d), and the rank order and cumulative frequency calculated. The 97.5 percent upper reference limit of the study population was taken as the rank number equal to 0.975 (n + 1), where n was the number of specimens; the 90 percent confidence limits were taken from tables.[8]

An Hitachi model 717 automated analyzer‡ was used to measure creatinine with a kinetic Jaffe method specifically scaled for urine creatinine. In an analogous manner to that described previously, the study population was ranked in ascending order of HIAA mg/g creatinine, and the cumulative frequency calculated. To select a reference limit equivalent to 15 mg/d, the reference limit for mg/g creatinine was taken as that number which identified 7.9 percent of the results as elevated (*ie*, 14 mg/g creatinine).

For those wisely preferring SI units, the following conversion factors apply: HIAA mg/d × 5.23 = HIAA μmol/d,[9] and HIAA mg/g creatinine × 5.92 = HIAA mmol/mol creatinine. The molecular weights (masses) of HIAA and creatinine are 191.2 and 113.1 g/mol, respectively.

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Results

The number of urine HIAA results collected from May 22 to August 15, 1997 (approximately 3 months) totaled 1,083. The following specimens were excluded: 44 with no age given; 3 <12 years-old, 25 random collections; 55 dilute specimens <25 mg/dL creatinine; 8 low 24-hour collection volumes <400 mL/d; and 1 specimen showing an analytic interference with the creatinine measurement. A total of 136 specimens were excluded, and the remaining 947 were defined as the study population. Characteristics of the population included: 338 males and 609 females with an age range of 14 to 109 years old. The 24 hr urine volume range was 440 to 6300 mL (excluding 8 specimens below 400 mL); 26 were between 400 to 600 mL, and 186 were >2500 mL (that is, were probably collected in more than 1 container). The concentration range of HIAA detected was 0.2 to 788 mg/L. The range of HIAA excreted was 0 to 659 mg/day, and 0 to 511 mg/g creatinine. The creatinine range was 25 to 405 mg/dL (excluding 55 specimens (<25 mg/dL), with 23 >200 mg/dL and 2 >300 mg/dL.

A reference limit of >15 mg/day identified 75 specimens (7.9 percent) as elevated; 60 (6.3 percent) were >25 mg/d; 28 (3.0 percent) were >100 mg/d; and 9 (0.95 percent) were >350 mg/d. A reference limit of >7 mg/d would identify 162 specimens (17.1 percent) as elevated.

No significant associations were seen when the results were examined by age (data not shown). In figure 1 is shown the cumulative frequency distribution of the study population categorized by sex. Using the nonparametric rank-order method described by Solberg,[8] the upper 97.5 percent reference limit (90 percent confidence interval) of this patient (*ie*, not normal) population would be: Males 338 (152 to 558) mg/d, and females 39 (25 to 69) mg/d. Of the 75 results > the reference limit of 15 mg/d, 45 (60 percent) were male. Of the 60 results >25 mg/d, 37 (62 percent) were male. Of the 28 results >100 mg/d, 20 (71 percent) were male. Of the 9 results >350 mg/d, 8 (89 percent) were male.

Recalculating the population distribution in terms of mg/g creatinine produced the cumulative frequency distribution seen in figure 2. The reference cutoff of 14 mg/g creatinine is comparable to 15 mg/d in terms of identifying 7.9 percent of the results as elevated. Likewise, 23 mg/g is equivalent to 25 mg/d (6.3 percent of the population distributions), and 300 mg/g is approximately equivalent to 350 mg/d (1 percent).

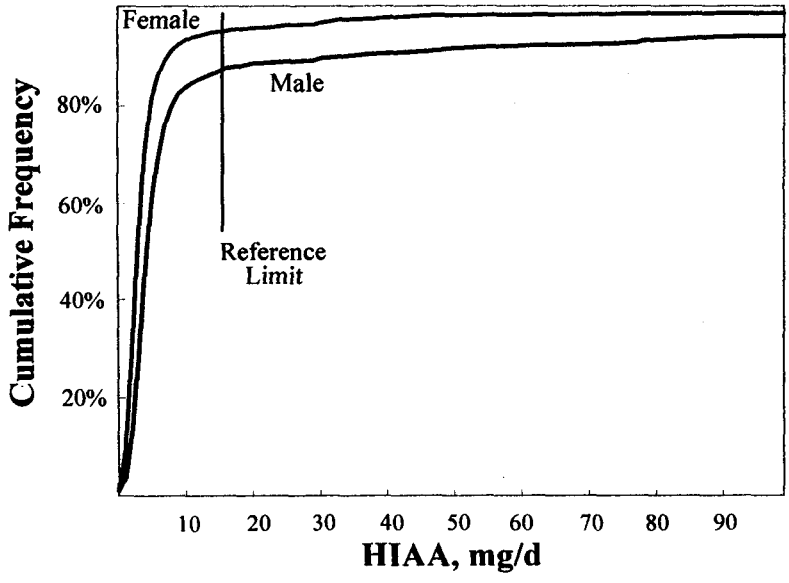

FIGURE 1. The cumulative frequency distribution of the patient study population in terms of mg/d.

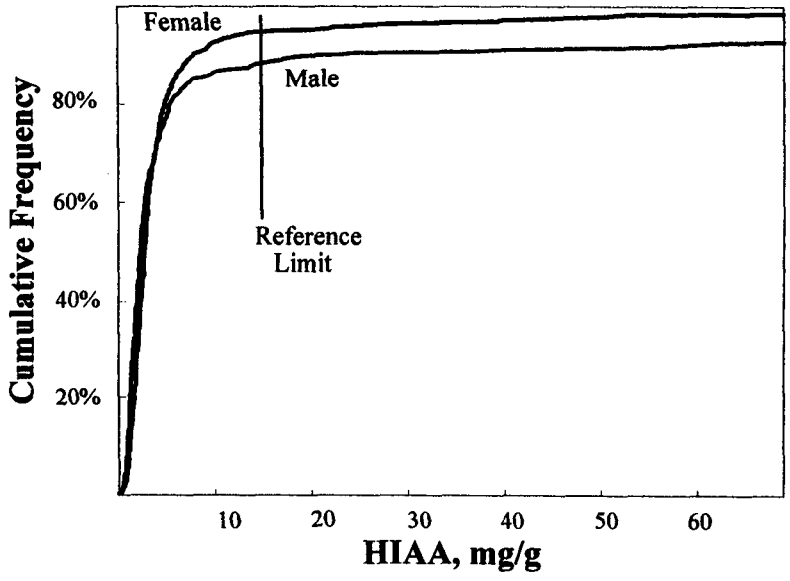

FIGURE 2. The cumulative frequency distribution of the patient study population in terms of mg/g creatinine.

Specimens were compared in terms of mg/d and mg/g creatinine in figure 3; the correlation coefficient of this comparison was 0.92. Specimens in figure 3 can be placed into one of four categories: (1) Those specimens which are within both reference limits (n = 864 or 91.2 percent); (2) those specimens which are elevated by both reference limits (33 in Figure 3 plus 37 which are offscale, n = 70 or 7.4 percent); (3) those within the limit for mg/day but elevated in terms of mg/g (n = 5 or 0.53 percent); and (4) those elevated for mg/day but within the limit for mg/g (n = 8 or 0.84 percent).

Three numbered specimens are shown in Figure 3. Number 17 was a urine specimen from a 59-year-old male; the 24-hr urine volume was 2525 mL, the creatinine concentration was 76 mg/dL, and the HIAA excretion was 20 mg/d or 10.4 mg/g. Number 508 was

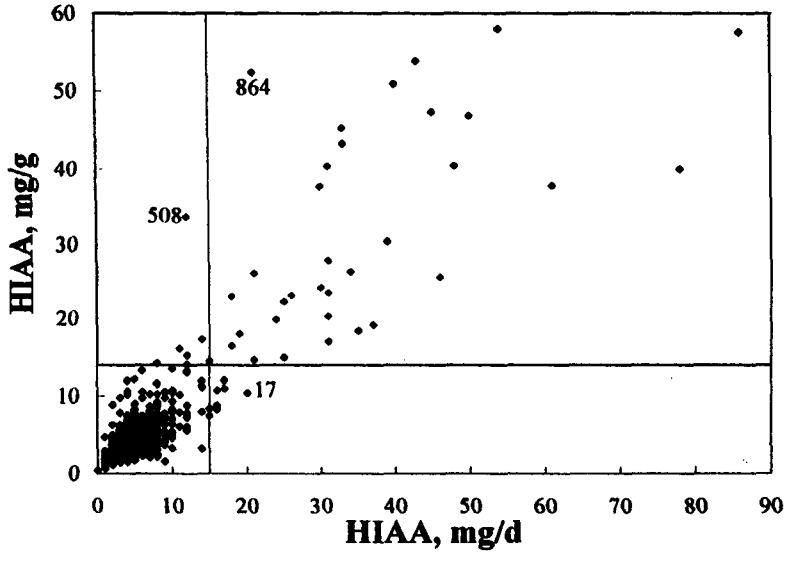

FIGURE 3. Comparison of specimens in terms of mg/d and mg/g creatinine. The reference limits are shown by the vertical line at 15 mg/day and by the horizontal line at 14 mg/g. Specimens numbers 17, 508, and 864 are discussed in the text.

from a 69-year-old female; the volume was 700 mL, the creatinine was 52 mg/dL, and the HIAA was 12 mg/d or 34 mg/g. Number 864 was from a 76 year-old female; the volume was 520 mL, the creatinine was 76 mg/dL, and the HIAA was 21 mg/d or 52 mg/g.

Discussion

Published reference intervals for HIAA vary slightly. Typical values are found in Tietz,[9] which lists a reference interval of 2 to 7 mg/d. Additional interpretive information states that >25 mg/d is diagnostic of carcinoid syndrome "when dietary restrictions are observed," and that functioning metastatic carcinoid tumors often exceed 350 mg/d. Depending on the reference, the upper limit for HIAA may be defined as low as 6 mg/d or as high as 15 mg/d.[1] The reference limit used in the authors' laboratory is 15 mg/d, which classifies 75 of 947 of the patients in the present study population as elevated (7.9 percent). It should be emphasized that the study population was not a "normal" population in good health, but consisted of patients presenting to physicians for the evaluation of complaints suggestive of carcinoid syndrome. Using a lower reference limit of 7 mg/day would identify an additional 87 results as elevated, for a total of 162 (17.1 percent).

Sex Distribution

Reference interval studies in normal populations have reported no distinction based on sex.[1,4] However, the HIAA distribution in figure 1 shows that elevated results are more frequent in males (13 percent >15 mg/d) compared to females (4.9 percent >15 mg/d). This is most likely due to the difference between normal and patient-based populations. Since the distinction between males and females becomes more pronounced at higher HIAA concentrations, it is reasonable that little difference would be seen in normal populations where the concentrations are lower.

If the present patient-based study population was used to calculate "normal" reference limits, a nonparametric method would be preferred since the population is highly skewed, and different limits would be needed for males and females. Using the method described by Solberg,[8] the upper 97.5 percent reference limit (90 percent confidence interval) would be: Males 338 (152–558) mg/d, and females 39 (25–69) mg/d. It is worth emphasizing that the confidence intervals are broad, suggesting considerable uncertainty in the limits. In addition, values in excess of these inflated reference limits still represent 2.5 percent of patients undergoing a relatively common test for a condition which is rare.

Creatinine Ratio

As an alternative to mg/d, specimens may also be examined in terms of a creatinine ratio. In figure 2 is shown the cumulative distribution of the study population when defined in terms of mg/g creatinine. A reference limit of 14 mg/g identifies an equivalent number of elevated results (*ie,* 7.9 percent) compared to 15 mg/g. In figure 3 the study population is compared in terms of both mg/d and mg/g. The correlation coefficient of 0.92 indicates that the majority of specimens produce similar results when defined in either unit, although there are several interesting discrepancies.

Each specimen in figure 3 can be placed into one of four categories: (1) Those which are within both reference limits (90.8 percent); (2) those which are elevated by both (7.4 percent); (3) those within the limit for mg/d but elevated in terms of mg/g (n = 5, 0.53 percent); and (4) those elevated for mg/d but within the limit for mg/g (n = 8, 0.84 percent). Where categories 1 and 2 represent the majority of specimens (98.2 percent), categories 3 and 4 are the most discrepant. When the 24-hr volume is underestimated, an elevated specimen tends to fall into category 3. This is seen in specimen 508, in which the 24-hour collection volume was reported as 700 mL; this almost certainly represents a significant underestimation, and the specimen is more accurately classified in terms of a creatinine ratio. Likewise, specimen 864 had a volume of 520 mL.

Although the HIAA concentration was sufficiently high to fall outside both reference limits, the creatinine ratio places the degree of elevation in a more accurate context.

In contrast to incomplete collections, excess collections tend to fall into category 4. For example, specimen 17 represented a 2,525 mL urine collection in a 59-year-old male. The creatinine concentration was measured at 76 mg/dL, which gives an estimated creatinine excretion of 19.1 g/d, significantly higher than the expected range of 0.8 to 2.0 g/d.[9] Although more definitive analysis would require information which included the patient's weight and diet, this does suggest the volume was overestimated or that the specimen was collected for longer than 24 hr.

As would be anticipated, the creatinine ratio used here has its own set of limitations. Two specimens in the study population had creatinine concentrations >300 mg/dL (*ie*, 307 and 405 mg/dL), values which could potentially mask HIAA elevations. Likewise, dilute specimens (defined as creatinine <25 mg/dL) were excluded from the present study because of the increased analytic uncertainties in measuring low concentrations of both HIAA and creatinine. Where the creatinine ratio is useful for incomplete and excess collections, specimens with high creatinine concentrations may be better evaluated in terms of mg/d. Results at the extremes of both high and low urine creatinine should be scrutinized carefully.

Drugs and Diet

A number of drugs are known to cause physiologic changes which can both decrease and increase the excretion of HIAA.[1] Decreases can be seen in patients using medications such as L-dopa, methyldopa, isoniazid, imipramine, and monoamine oxidase inhibitors, among others. Increases can be caused by guainfenesin (present in many over-the-counter antitussives), cyclobenzaprine (Flexeril), pheonothiazines, and reserpine. Increases can also be seen after the administration of cytotoxic drugs, presumably owing to release by tumor cells. Quantitative information about the degree to which medications may actually induce changes is difficult to find.

Among possible interferences, it is worth considering folk remedies and health foods, such as the recent popular craze in melatonin supplements.[10] Melatonin (N-acetyl-5-methoxytryptamine) is closely related to serotonin (5-hydroxytryptamine), which is the precursor to HIAA. Avoiding melatonin would seem prudent when collecting specimens for carcinoid evaluation, although no interferences have been specifically reported in the literature. Likewise, health food supplements of 5-hydroxytryptophan are sometimes advocated for weight loss. Since 5-hydroxytryptophan is a precursor to serotonin and HIAA, this supplement would also be prudent to avoid. Tryptophan supplements can also raise HIAA in certain conditions.[11] The ingredients in many folk remedies and supplements are often not well defined, and may be a potential source of interfering substances.

It is well known that serotonin-containing foods should be avoided prior to and during collection of a 24-hour urine specimen for HIAA. Most of the serotonin ingested will be metabolized to HIAA by the high levels of monoamine oxidase present in the gastric mucosa. Felman and Lee[12] estimated that the following foods would raise HIAA approximately 1 mg/day: One walnut, eight pecans, one-sixth of a plantain, one-half of a banana, one-twentieth of a pineapple, one tomato, two kiwi fruits, or two red plums.

Elevated Results

Elevations in HIAA are likely to be due to one of the following reasons: (1) patients not following dietary restrictions; (2) patients on medications which induce physiologic increases in HIAA; (3) substances which interfere analytically by coeluting with HIAA; (4) patients with elevations due to diseases other than carcinoids; and (5) patients with carcinoid tumors. Given the low incidence of carcinoids, the last category is probably the least frequent.

Analytic interferences are reduced with the use of HPLC, although they cannot be considered entirely eliminated. Drugs such as sulfasalazine are documented to coelute with HIAA under some HPLC assay conditions.[13] While the coulometric array detection system used in the present study reduces the likelihood that coelution would go unrecognized, analytic-type interferences should always be considered as possible.

A variety of diseases in addition to carcinoids are known to induce elevations of HIAA.[1,2] Symptomatic celiac sprue,[14] tropical sprue, bacterial overgrowth syndromes, Whipple's disease, and other disorders associated with malabsorption syndromes often produce modest elevations. Other neuroendocrine tumors such as pheochromocytoma should also be considered, although the incidence of other tumors is as infrequent as that of carcinoid.

Interpretation

While it is stated in a widely used and highly regarded reference[9] that specimens >25 mg/d are diagnostic of the carcinoid syndrome when dietary restrictions are observed, this obscures how frequently elevated results are actually found. In the present study of 947 patient results, 6.3 percent were >25 mg/d, and 3.0 percent were >100 mg/d. It is worth emphasizing that carcinoid tumors are rare, and that even a finding of 100 mg/d in the present study is unlikely to be diagnostic of that condition. Alternatively, significant elevations clearly warrant further evaluation. Repeat testing with careful attention to preanalytic variables may often be the easiest method for re-evaluation. Testing directly for serotonin can also be considered, as can challenge tests, and imaging studies.[4] Without supporting evidence, increased HIAA should not be considered diagnostic of a carcinoid tumor.

Using a reference limit of 15 mg/d classifies about 92 percent of the study patients as within the normal range. Although not addressed in the present study, it should be emphasized that normal results do not absolutely exclude carcinoid tumors. Confounding factors include: (1) Alcohol use[15] and drugs which can suppress HIAA secretion; (2) physiologic conditions such as bowel resection which can lower HIAA secretion; (3) carcinoid tumors which do not secrete HIAA, but which might secrete other vasoactive substances; and (4) tumors which secrete HIAA episodically. When clinical suspicion is high, a provocative challenge with pentagastrin can be considered, although the possibility of inducing a carcinoid crisis should also be considered.[4]

Conclusion

Elevated urine HIAA concentrations are a common finding even when using an analytically specific method such as HPLC coupled with coulometric array detection. Because elevated results are frequent, they should be used to support the diagnosis of carcinoid syndrome only when consistent with other objective findings. Confounding variables include numerous medications, alcohol, serotonin-containing foods, and malabsorption syndromes. Over-the-counter drugs and herbal remedies should also be considered as sources of physiologic and analytic interferences. Some specimens may be more accurately assessed in terms of a creatinine ratio, particularly those associated with both incomplete and excess collection of 24-hour urine specimens. The reference limit of 14 mg/g creatinine is equivalent to 15 mg/d in terms of identifying a similar number of elevated results. Alternatively, specimens with high creatinine concentrations can potentially mask HIAA elevations, and these may be better evaluated in terms of mg/d. Dilute specimens, defined here as <25 mg/dL creatinine, show a high degree of analytic uncertainty and may give unreliable results. By itself, determination of urine HIAA is not sufficient to diagnose or exclude a carcinoid tumor.

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