Does elevated urinary 5-hydroxyindole acetic acid level predict acute appendicitis in children?

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ABSTRACT

Background Acute appendicitis is the most common abdominal surgical emergency in children, and appendectomy is the most frequent acute abdominal operation. Prompt diagnosis and surgical treatment are required to reduce the risk of perforation and prevent complications, especially in small children. Enterochromaffin cells that contain large amounts of serotonin are mostly located in the distal appendix. Serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) could therefore be a marker for acute appendicitis.

Objective We tested urinary 5-HIAA concentrations in spot urine samples from children with acute appendicitis.

Methods We enrolled 93 patients who underwent surgery for suspicion of acute appendicitis. The diagnosis was made intraoperatively and confirmed histopathologically. Additionally, urine samples from 102 healthy children were collected as controls. Their 5-HIAA was measured using high-performance liquid chromatography.

Results Acute appendicitis was diagnosed in 81 patients, whereas there were other explanations for abdominal pain in the remaining 12 patients in the non-appendicitis group. The control group comprised 102 healthy children. Considering the median of all measured 5-HIAA values as the cut-off, we analysed the proportions of patients with elevated values in all the groups. Our analysis showed that statistically there was no significant difference in the distribution of percentages among the groups. The area under the curve for 5-HIAA was 0.55 (95% CI 0.47 to 0.62) with sensitivity and specificity 60.4% and 48.9%, respectively.

Conclusions Urine 5-HIAA concentration measured in spot samples is not a reliable method for diagnosing acute appendicitis in children.

INTRODUCTION

Acute appendicitis is the most common abdominal surgical emergency in children, with appendectomy being the most frequent acute abdominal operation. Prompt diagnosis and surgical treatment may reduce the risk of perforation and prevent complications, especially in small children. However, the diagnosis is incorrect in as many as 20% of patients, who undergo surgery that reveals no acute appendicitis.

Enterochromaffin cells, which are part of the intestinal nervous system and are mostly located in the distal appendix, contain large amounts of serotonin. Upon inflammation of the appendix, serotonin is released into the blood and metabolised enzymatically in the liver by monoamine oxidase and alcohol dehydrogenase to 5-hydroxyindole-3-acetic acid (5-HIAA), which is subsequently excreted in urine. The 5-HIAA concentration, therefore, could be a marker for acute appendicitis.

What is already known on this subject?

- Upon inflammation, enterochromaffin cells located in the distal appendix secrete serotonin, and 5-hydroxyindole acetic acid (5-HIAA, serotonin metabolite) is excreted in urine.
- During the last decade, researchers have been looking at urinary 5-HIAA levels in patients with acute appendicitis.
- However, the results of the published studies are inconsistent.

What might this study add?

- This study is the second of its kind that was conducted in paediatric population and comprises so far the largest number of participants.
- Our results suggest that elevated urinary 5-HIAA is not a reliable method for diagnosing acute appendicitis in children.

METHODS

Patients

In total, 93 patients who were admitted to the Pediatric Surgery Clinic of the University Hospital Center Rijeka during a 16-month period and who were operated on because of suspicion of acute appendicitis were enrolled in the study. The patients were enrolled in the study after establishing the need for surgery. The patients’ parents or custodian gave consent. The diagnosis was confirmed with histopathological results.

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regular check-up were collected, preserved and analysed using the
same procedure. They served as the controls. Exclusion criteria
included ingestion of some special foods that could increase the
urinary 5-HIAA concentration or a previous appendectomy.\textsuperscript{13}

The sample size was determined according to sample sizes
from previously published studies.\textsuperscript{7, 14} Moreover, the sample
size calculated using MedCalc for an area under the receiver
operating characteristic (ROC) curve (AUC) of 0.903\textsuperscript{7, 15}
\((\alpha=0.005, \beta=0.2)\) and null hypothesis value of 0.8 was lower
(61 participants per test and control group) than our initially
defined sample. Therefore having in mind the incidence of the
disease, we had set the time period to 16 months making sure
to collect at least the minimum of necessary samples.

Spot urine samples (5 mL) were randomly collected in sterile
containers from patients upon admission. These samples were
acidified with 6 M HCl to pH 4–6 and preserved in special
dark containers away from the light at \(-70^\circ\text{C}\) until measure-
ment.\textsuperscript{16} Urine creatine was measured prior to acidification.

The ethics committees of the School of Medicine and
University Hospital Rijeka approved the study.

**Measurement of 5-HIAA**

5-HIAA was measured using high-performance liquid chroma-
tography (HPLC) with the Shimadzu HPLC machine series 10
(Shimadzu Corporation, Kyoto, Japan) with an electrochemical
detector (CLC 100; Chromsystems Instruments & Chemicals
GmbH, Munich, Germany).\textsuperscript{7, 16} The HPLC kit was purchased
from Chromsystems Instruments & Chemicals GmbH, Munich,
Germany. The working potential of the electrochemical detector
was set at 660 mV, and the background current was as high as
5 nA. Flow rate was 1 mL/min at room temperature.

The values were displayed as the urinary 5-HIAA concentration
and the urinary 5-HIAA/creatinine ratio because the actual values
may vary depending on the 5-HIAA concentration in urine.

**Statistical analysis**
The results were analysed with the Kruskal-Wallis test (to test the
difference between the groups), \(\chi^2\) test (to compare distributions),
the \(z\)-test (to compare proportions between the two groups) and
ROC analysis with STATA software (StataCorp, Texas, USA) and
MedCalc for Windows V.14.8 (MedCalc Software, Ostend,
Belgium). The results were evaluated as percentages. A value of
\(p<0.05\) was considered to indicate statistical significance.

**RESULTS**
In all, 93 patients (33 female, 60 male) who were operated on
because they were suspected to have acute appendicitis were
enrolled in the study. The age of the children was 4–18 years.
Acute appendicitis was diagnosed in 81 patients, whereas 12
had other explanations for their abdominal pain and were con-
sidered part of the non-appendicitis group. Our negative
appendectomy rate was thus 12.9%. Patients with acute appen-
dicitis were divided into three groups: phlegmonous, gangren-
ous and perforated appendicitis. The 102 healthy children
comprised the control group (figure 1). The participants’ base-
line characteristics are shown in table 1.

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**Figure 1** Patient flow diagram. Non-AA, non-acute appendicitis; AA, acute appendicitis.
The mean 5-HIAA levels and its associated 95% CIs of the groups are shown in table 2.

Figure 2 shows the distribution of 5-HIAA values per groups. Results of the analysis showed no statistically significant difference between the groups (p=0.48).

The mean 5-HIAA/creatine ratios were 2.09±1.02, 1.87±1.46 and 1.64±0.85 (nmol/μmol) in the control, non-appendicitis and acute appendicitis groups, respectively.

Considering the median of all measured values as the cut-off value (median for 5-HIAA was 21.18 μmol/L and for the 5-HIAA/creatine ratio 1.68 nmol/μmol), we analysed the proportions of patients with elevated values in all of the groups. The distribution of patients with elevated values is shown in figures 3 and 4. First, we analysed the difference between the acute appendicitis group and the healthy children group. There was a 44.4% increase in the urinary 5-HIAA values in the acute appendicitis group and 53.9% increase in the healthy children group. Although there was a difference, it did not reach the level of statistical significance (p=0.20). Furthermore, in the acute appendicitis group, there was a 44.4% increase in the ratio, whereas in the healthy children group the increase was 54.9%. Despite the descriptive difference, it did not reach statistical significance (p=0.16).

Then we analysed the differences between the acute appendicitis group and the non-appendicitis group. There was again a 44.4% increase in urinary 5-HIAA values in the acute appendicitis group, whereas in the non-appendicitis group the increase was less (41.7%), with no statistical significance (p=0.86). Ratios showed identical values: a 44.4% elevation of the ratios in the acute appendicitis group and 41.7% in the non-appendicitis group, with no statistically significant difference (p=0.86).

The χ² test showed that there was no statistically significant difference in distribution per diagnosis of patients with high urinary 5-HIAA levels compared with patients with normal values (p=0.31). We obtained the same result for the 5-HIAA/creatinine ratio (p=0.62).

Furthermore, the positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio for 5-HIAA were calculated as 53.9%, 60.4%, 1.18 and 0.81, respectively. The corresponding values for the 5-HIAA/creatine ratio were calculated as 53.9%, 60.4%, 1.18 and 0.81, respectively.

We also conducted an ROC analysis for elevated 5-HIAA and an elevated 5-HIAA/creatinine ratio as tools for diagnosing appendicitis in children. The ROC analysis of 5-HIAA values with a median cut-off showed that the AUC was 0.55 (95% CI 0.47 to 0.62) (figure 5). The sensitivity and specificity were 60.8% and 49.5%, respectively.

DISCUSSION
We did not identify 5-HIAA concentration in spot urine samples as a predictive indicator of acute appendicitis in children. Children with acute appendicitis do not necessarily present with classic right lower abdominal quadrant pain, nausea, vomiting and fever, making it difficult to diagnose and thus treat them.
Every surgeon should be aware that a delay in establishing the right diagnosis and surgical treatment could result in a high risk of perforation and increased morbidity.\textsuperscript{11} Our negative appendectomy rate in this study was 12.9%, which correlates with rates described in the literature.\textsuperscript{61,7} Various methods can enhance the diagnostic procedure. Ultrasonographic parameters could suggest a diagnosis of acute appendicitis, but they are not 100% reliable.\textsuperscript{18} Also, in many hospitals, ultrasonography is still not available around the clock, and the strength of any ultrasonography report depends largely on the operator’s experience.\textsuperscript{7} CT could be of additional use, even though it has not been clearly shown to be superior to ultrasonography,\textsuperscript{19} and there is sometimes limited access. Additionally, the lifetime cancer mortality risk has been reported to be significantly higher in children who have undergone a CT examination.\textsuperscript{20} There is no unique laboratory marker for acute appendicitis. Many have been suggested, but none has exhibited satisfactory sensitivity and specificity.\textsuperscript{5,6}

During the last decade, researchers have been looking at urinary 5-HIAA levels in patients with acute appendicitis. In vitro studies have shown that there is a correlation between high 5-HIAA levels and appendiceal inflammation.\textsuperscript{31,4} However, the results of studies on adults, are inconsistent. Ilkhanizadeh \textit{et al}.\textsuperscript{11} concluded that measurement of 5-HIAA in spot urine samples is a highly reliable test for supporting the clinical diagnosis of appendicitis (sensitivity 98%, specificity 100%). Bolandparvaz \textit{et al}.\textsuperscript{17} came to the same conclusion but with slightly lower sensitivity (84%) and specificity (88%). Oruc \textit{et al}.\textsuperscript{21} stated that their research showed low sensitivity and specificity for urine 5-HIAA measurement. The research data of Mihmanli \textit{et al}.\textsuperscript{22} showed that measurement of 5-HIAA levels in spot urine samples is not helpful for diagnosing acute appendicitis, which was corroborated by Hernandez \textit{et al}.\textsuperscript{23} Jangjoo \textit{et al}.\textsuperscript{24} concluded that sensitivity (44%) and specificity (81%) do not confirm urinary 5-HIAA measurement as an ideal diagnostic tool for ruling out, or determining the presence of, acute appendicitis. In the most recent study in an adult population, Iraqi authors\textsuperscript{8} described the urinary 5-HIAA assay as a non-invasive test with a high predictive value for diagnosing acute appendicitis. The results of the only study conducted in children showed 70% sensitivity and 67% specificity. Because of the large SD, however, it had limited diagnostic value as a unique parameter.\textsuperscript{25}

According to the available literature, our study is the second study of its kind to be conducted in a paediatric population. To date, it includes the largest number of participants. However, our study did not confirm that urinary 5-HIAA is a good diagnostic method in children with acute appendicitis. Our analysis showed no significant difference of mean 5-HIAA levels among analysed groups. Furthermore, we have not demonstrated statistically significant differences in the percentages of patients with elevated 5-HIAA levels or an elevated 5-HIAA/creatinine ratio between healthy children and children with various stages of appendicitis or with other causes of abdominal pain. Moreover, the specificity and sensitivity were extremely low in our study, as were the positive and negative predictive values and likelihood ratios.

Our relatively small test group size might be a limitation of our study because statistical analyses could be unreliable in small groups. As the purpose of the test would be to differentiate children with abdominal pain of other causes from those who have acute appendicitis, in a larger sample the non-appendicitis group would likely be larger and therefore make the statistical
results more relevant. However, having in mind low incidence of the disease, our time limitation, and the testing cost, the decision was to determine the sample size to at least 80 per test and control group. Additionally, the dependence of variations in concentration on the duration of the symptoms has been described.7

CONCLUSION
Our results suggest that elevated 5-HIAA concentrations in spot urine samples do not offer a reliable method for diagnosing acute appendicitis or for resolving the various stages of appendiceal inflammation.

Contributors
ABV and HN designed the study. All of the authors took part in collecting the samples from the participants and performing the surgeries, depending on the work schedule. ABV performed the sample analysis. ABV, NG and HN wrote the first draft of the paper. ABV, NG, NB, ZT and HN contributed to the revision of the paper and approved the final version.

Competing interests
None declared.

Data sharing statement
Additional data or analyses can be obtained from the corresponding author upon request.

REFERENCES