



Original Contribution

Is urinary 5-hydroxyindoleacetic acid helpful for early diagnosis of acute appendicitis?

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Abstract

Objective: Acute appendicitis is the most common abdominal emergency in children and young adults. There are a lot of serotonin-containing cells in the appendix, which release serotonin into the bloodstream in response to inflammation. Consequently, serotonin is converted to 5-hydroxyindoleacetic acid (5-HIAA) and secreted into the urine. On this basis, urinary 5-HIAA could be a marker for acute appendicitis. In this study, we investigated the value of 5-HIAA levels in spot urine in the diagnosis of acute appendicitis.

Methods: The urinary 5-HIAA was measured by an enzyme-linked immunosorbent assay in the spot urine of 70 patients who presented to the emergency department with a clinical picture of acute appendicitis. Urine concentration results were correlated to final histopathologic reports, and the diagnostic value of this factor was measured.

Results: Diagnosis of appendicitis was confirmed by histopathologic reports in 59 of 70 patients with presumptive diagnosis of appendicitis. Considering 5.25 mg/L as the cutoff point for urinary 5-HIAA, 28 patients had high urinary 5-HIAA levels, whereas 42 patients had values within reference range. The sensitivity and specificity of this test was 44% and 81%, respectively.

Conclusions: The measurement of urinary 5-HIAA levels is not an ideal diagnostic tool for ruling out or determination of acute appendicitis.

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1. Introduction

Acute appendicitis is the most common surgical abdominal emergency all over the world [1,2]. This disease is more common in the young population with the highest incidence in the second and third decades of life [3,4]. The diagnosis is mainly based upon history and physical examination, but still many of the patients may not show typical characteristics of this disease, which necessitates more accurate diagnostic imaging studies and laboratory investigations [5-7]. Although, there is an increasing interest in the paraclinical studies for diagnosis of acute appendicitis, but the negative appendectomy rate is still between 20% and 40% [8-10]. On the other hand, the morbidity and mortality of acute appendicitis occur because of delayed diagnosis. Therefore, further studies are needed to find more reliable diagnostic methods for resolution of the dilemmas in diagnosis of acute appendicitis.

One of the laboratory tests recommended by some clinicians for early diagnosis of acute appendicitis is the measurement of urinary level of 5-hydroxyindoleacetic acid (5-HIAA) [11,12].

5-Hydroxytryptamine is a vasospastic mediator, which is created and stored within enterocromaffine cells. These cells, which are present in all parts of the gastrointestinal tract, especially in the appendix, produce more than 95% of the circulatory level of 5-hydroxytryptamine. This mediator that is released into the circulation during inflammatory processes is metabolized to 5-HIAA by mono-oxidase system of the liver and excreted in the urine [13,14]. The measurement of urinary level of this factor may help to the early diagnosis of acute appendicitis. In this study, we investigated the diagnostic value of 5-HIAA levels in the spot urine of patients with acute appendicitis.

2. Methods

This is a double-blind study, which was performed on 70 consecutive patients with initial diagnosis of acute appendicitis who were admitted in the emergency surgery department of Imam Reza Hospital from March 2007 to September 2008. Patients' history, physical examination, white blood cell count, and the percentage of polymorphonuclear leukocytes were used by the surgeons to admit or discharge the patients suspicious for acute appendicitis. All patients who were operated on with the initial diagnosis of acute appendicitis were included in the study. Patients who were candidates for nonoperative management based on the surgeon's decision and patients with a history of ingestion of special foods that might raise the U-5-HIAA were excluded. Special questionnaires were used to record positive findings in history, physical examination, white blood cell counts, and the percentage

of polymorphonuclear leukocytes. Informed consent was obtained from all participants.

The spot U-5-HIAA was measured in 30 healthy people (19 male and 11 female) without history of ingestion of special foods, which might have increased the U-5-HIAA in the last 72 hours, and the mean value was used as the reference range.

Various techniques have been used for determination of urinary 5-HIAA in which high-performance liquid chromatography (HPLC) (11) and enzyme-linked immunosorbent assay (ELISA) (15) are more commonly used for this purpose. To our knowledge, there is no study to compare the accuracy of these tests for U-5-HIAA measurement. However, because acute appendicitis is a common disease, any diagnostic test for this disease should be available and economical. Having these characteristics, ELISA (5-HIAA ELISA Kit; IBL International GmbH, Germany) in comparison with HPLC was, therefore, the preferred method in this study.

To establish a definitive diagnosis, all surgically removed appendices were subjected to microscopic examination. The histopathologic findings reported as normal appendix, acute mucosal appendicitis (mucosal infiltrate of neutrophils), acute suppurative appendicitis (inflammation into submucosa and/or muscle), acute suppurative appendicitis with abscess formation, acute gangrenous appendicitis, and acute suppurative appendicitis with perforation.

In patients with a suspicious intraoperative finding, the surgeon investigated for other possible pathologies. The data were recorded in the questionnaires.

A urine sample was taken from all patients within the first hours of admission. An ELISA was used for measuring urinary level of 5-HIAA. To reduce the technical errors of measurement, an experienced laboratory employee was selected.

Considering 98% sensitivity for 5-HIAA [12], with confidence interval of 95% and accuracy of 0.05%, the sample size was estimated to be 35 patients. To achieve a high accuracy, it was determined to be 70. The data collection was performed during an 18-month period.

Using SPSS 11.5 software (SPSS Chicago, IL), the statistical analysis was performed on all collected data from laboratory tests, histopathologic reports, and intraoperative findings.

t Test, χ^2 test, and 1-way variance analysis were used to detect correlation between different variables. Using receiver operating characteristic (ROC) curve, the test values were analyzed by plotting sensitivity against 1-specificity for various probable decision levels. The SPSS program was used to calculate the area under curve (AUC). The AUC represents the probability that a randomly selected patient of the test group is properly diagnosed [15]. The value of 1 shows the optimal test, whereas 0.5 demonstrates a useless test. The optimal cutoff value for urinary 5-HIAA was obtained by comparing specificities and sensitivities at various levels (Fig. 1).

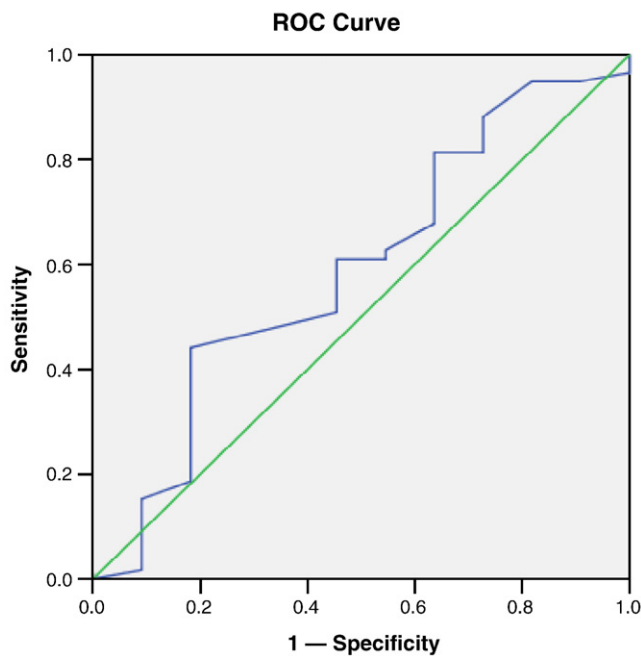


Fig. 1 Receiver operating characteristic plots show the power of 5-HIAA in diagnosis of acute appendicitis.

3. Results

Participants included 38 males (54.4%) and 32 females (45.6%). The mean age of the patients was 25 ± 9.5 years (range, 9–50 years). Pathologic review of the specimen revealed 59 cases (84.2%) of appendicitis, whereas 11 patients (15.7%) had a normal appendix. The acute suppurative appendicitis was the most common pathologic stage (35.5%), whereas the incidence of other stages was as follows: acute appendicitis (27.1%), acute gangrenous appendicitis (10%), acute suppurative appendicitis with perforation (3%), and acute suppurative appendicitis with abscess formation (23.7%).

The mean U-5-HIAA concentration was 5.6 ± 2.49 mg/L in healthy people, whereas it was 6.0 ± 6.52 mg/L in patients with any sort of inflammatory appendiceal disease. The difference was not statistically significant ($P = .20$).

By using a ROC curve, cutoff points were determined for leukocyte count, the percentage of neutrophil count, and U-5-HIAA (Fig. 1), which were 10 500, 71%, and 5.25 mg/L, respectively (Table 1). These data show that although the specificity of U-5-HIAA is higher than 2 other parameters, its sensitivity is very lower than them. In

addition, the relationship between U-5-HIAA and other parameters including age, clinical findings, interval between pain onset, and U-5-HIAA measurement and stage of appendicitis showed that there were statistically significant differences between “shift of pain” and “stage of appendicitis” and urinary 5-HIAA level ($P = .027$ and $P = .015$, respectively) (Table 2).

In comparison with paraclinical assessments, the Alvarado score was used as the clinical scoring system. The mean Alvarado score was 8.54 ± 1.31 (range, 4–10). There was a significant relationship between Alvarado score and final histopathologic diagnosis of appendicitis ($P = .000$), but there was not any correlation between level of U-5-HIAA and the Alvarado score ($P = .16$).

In patients admitted with the initial diagnosis of acute appendicitis, the data were analyzed in 3 ways. In one set, we compared U-5-HIAA level between patients without appendicitis and those with any stage of appendicitis. In the second set, this comparison was made between patients without appendicitis and patients with “acute appendicitis.” Finally, we compared patients without appendicitis with those in any stage of appendicitis except acute appendicitis. The study indicates that there was no significant difference in U-5-HIAA level of patients with pathologically normal appendix and those with appendicitis ($P = .34$). In addition, we did not find any significant difference between patients with normal appendix and acute appendicitis ($P = .98$). However, in the third set when the acute appendicitis group was excluded from the comparison, the difference became statistically significant ($P = .038$).

4. Discussion

Our study demonstrates a low sensitivity for U-5-HIAA in diagnosis of acute appendicitis. It indicates that positive result for this test may suggest a high probability of acute appendicitis, although a negative result does not rule out the diagnosis. This finding is consistent with those of some previous studies [16–18], but others claim that this test is strong enough to rule out the diagnosis [11,12]. The disagreement may result from different techniques of measurement because the former studies used HPLC and the latter used ELISA method for the measurement of urinary 5-HIAA.

In some studies, urinary 5-hydroxyindoleacetic acid to creatinine ratio (U-5-HIAA/Cr) has been used rather than

Table 1 Diagnostic value of different factors in acute appendicitis

Test/cutoff	TN (n)	TP (n)	FN (n)	FP (n)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
U-5-HIAA ≥ 5.25 mg/L	9	26	33	2	44	81	92	21

TN (n) indicates true-negative cases; TP (n), true-positive cases; FN (n), false-negative cases; FP (n), false-positive cases; PPV, positive predictive value; and NPV, negative predictive value.

Table 2 Relationship between urinary 5-HIAA and other variables

		U-5-HIAA level (mg/L)			<i>P</i>
		Mean	SD	Median	
Age (y)	<20	5.6	6.4	3.7	.65
	20-29	6.9	10.2	5.0	
	≥30	5.7	3.9	6.0	
Stage of appendicitis	acute mucosal	4.7	6.9	3.0	.015
	acute suppurative	4.3	3.3	2.8	
	acute suppurative with abscess formation	9	9.2	7.8	
	acute gangrenous	8.2	6.2	6.3	
	acute suppurative with perforation	7.5	0	7.5	
Interval between pain onset and U-5-HIAA measurement					.74

U-5-HIAA alone, although it is not yet verified to be more accurate in discriminating acute appendicitis. Bolandparvaz et al [11] showed that both tests had a significant degree of correlation. However, the value of AUC of U-5-HIAA/Cr in their study was about 10% lower than that of U-5-HIAA. They concluded that correction of U-5-HIAA/Cr did not significantly change the *P* value but decreased the diagnostic value of U-5-HIAA in discrimination of acute appendicitis. We studied the value of U-5-HIAA in diagnosis of acute appendicitis.

According to this study, the mean U-5-HIAA concentration is not significantly higher in the appendicitis group than in the healthy control group and is not of value as the single test for diagnosis of acute appendicitis.

This study showed that U-5-HIAA does not help us to differentiate between the normal appendix and acute simple appendicitis. It may be interpreted that mild inflammatory changes in appendix do not elevate the U-5-HIAA level significantly. On the other hand, U-5-HIAA is helpful for diagnosis of more severe forms of appendicitis and not acute simple appendicitis. The studies by Apak et al [16] and Mendes et al [19] are in conflict with this result. They concluded that the patients' elevated level of U-5-HIAA is observed in early stages of acute appendicitis. So, they recommended it as an appropriate test for early diagnosis of acute appendicitis. We have no evidence to interpret the conflict.

Our study showed that the level of U-5-HIAA in gangrenous appendicitis is higher than that of uncomplicated appendicitis. It is consistent with the study by Hernandez et al [20] but conflicts with that of Bolandparvaz et al who believed that with progression of appendicitis to gangrene, the urinary 5-HIAA level is decreased [11]. This discrepancy seems to be the result of different degrees of gangrene, which have been considered for "gangrenous appendicitis" by the pathologists. Because most of the 5-HIAA secretory cells are located in the submucosal layer, early stages of gangrene, which are limited to the mucosal layer, do not have any significant effect on secretion of this factor. But, with progression of gangrene to deeper layers and destruction of secretory cells, the circulatory and, consequently, urinary levels of this factor are decreased.

In our study, the calculated cutoff point for U-5-HIAA was 5.25 mg/L, whereas in the studies by Bolandparvaz et al [11] and Ilkhanizadeh et al [12], it was 10 and 20 $\mu\text{mol/L}$, respectively. The difference probably comes from different methods of U-5-HIAA measurement.

This study showed that age and interval between pain onset and U-5-HIAA measurement do not have any effect on urinary 5-HIAA level. Furthermore, a significant relationship was established only between shift of pain and U-5-HIAA level, which does not seem to give us a helpful interpretation.

5. Limitations

A research limitation in this study was that we did not measure serum creatinine level to compare the diagnostic value of U-5-HIAA/Cr with U-5-HIAA in discriminating the acute appendicitis.

In conclusion, although U-5-HIAA may be useful to increase the accuracy of diagnosis, especially in more severe stages of appendicitis, it is not reliable enough to be used as a single test for definitive diagnosis or ruling out of acute appendicitis.

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