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Original Contribution

Spot urinary 5-hydroxyindoleacetic acid is not an ideal diagnostic test for acute appendicitis☆,☆☆

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ABSTRACT

Background and purpose of the study: There is growing evidence to suggest the use of urinary 5-hydroxyindoleacetic acid (5-HIAA) test to help with the diagnosis of appendicitis. The aim of our study was to establish whether urinary 5-HIAA could be used as an effective diagnostic test for acute appendicitis.
Design and methods: A prospective double-blinded study was carried out from December 2014 to October 2015. Patients admitted to the emergency surgical ward of a teaching hospital with suspected appendicitis were included in the study. The diagnostic accuracy of the test was measured by receiver operating characteristic curve.
Results: Ninety-seven patients were divided into 2 groups: acute appendicitis (n = 38) and other diagnosis (n = 59). The median value of urinary 5-HIAA was 24.19 μmol/L (range, 5.39-138.27) for acute appendicitis vs 18.87 μmol/L (range, 2.27-120.59) for other diagnosis group ($P = .038$). The sensitivity and specificity of urinary 5-HIAA at a cutoff value of 19 μmol/L were 71% and 50%, respectively. Receiver operating characteristic analysis showed that the area under curve was 0.64 (confidence interval [CI], 0.513-0.737) for urinary 5-HIAA, which was lower than white blood cell count (0.69; CI, 0.574-0.797), neutrophil count (0.68; CI, 0.565-0.792), and C-reactive protein (0.76; CI, 0.657-0.857). There was no significant difference in the median values of 5-HIAA between different grades of severity of appendicitis ($P = .704$).
Conclusion: Urinary 5-HIAA is not an ideal test for the diagnosis of acute appendicitis.

1. Introduction

Acute appendicitis is one of the most common surgical emergencies, especially in children and young adults [1,2]. Seven percent of the population will develop appendicitis at some point during their lifetime [1]. This condition is treated urgently because progression of the disease leads to life-threatening complications such as sepsis, perforation, and peritonitis [3,4]. The standard treatment of appendicitis is appendectomy.

The diagnosis of appendicitis is difficult, and only half of the cases are correctly identified [5]. The Alvarado scoring system, based on clinical observations and biochemistry measurements, has been used to aid in diagnosis of appendicitis [6]. However, the presenting signs and symptoms vary according to the position of appendix, and nonclassical symptoms are common [7]. Many patients undergo unnecessary appendectomy and are found to have a normal appendix [8]. Computed tomographic (CT) imaging has high sensitivity, but it exposes children and women of childbearing age to extensive ionizing radiation [9]. Ultrasound is associated with low sensitivity and specificity [9,10].

Previous studies have indicated that appendix is enriched with enterochromaffin cells [11]. These cells are densely concentrated with serotonin. Ninety-five percent of serotonin is secreted from enterochromaffin cells in the gut [11]. In addition, lamina propria of appendix also contains enterochromaffin cells secreting serotonin. Once serotonin is secreted in the system, 90% is metabolized in the liver; and the remaining, in lung and kidney. 5-Hydroxyindoleacetic acid (5-HIAA) is the main metabolite of serotonin and mainly discarded in the urine [12].

High levels of serotonin and 5-HIAA are associated with appendicular pathology [12]. Induced appendicitis in rabbits causes a significant rise in 5-HIAA compared to controls [12]. There is growing evidence to suggest the use of spot urinary 5-HIAA test to diagnose appendicitis [13]. The aim of our study was to establish whether urinary 5-HIAA could be used as an effective diagnostic test for appendicitis. Our second objective was to determine if there was an association between urinary 5-HIAA and the degree of inflammation of appendicitis based on histopathologic grading and Alvarado score.

2. Methods

2.1. Participants

This was a double-blinded prospective study conducted from December 2014 to October 2015 at the emergency surgical unit in a

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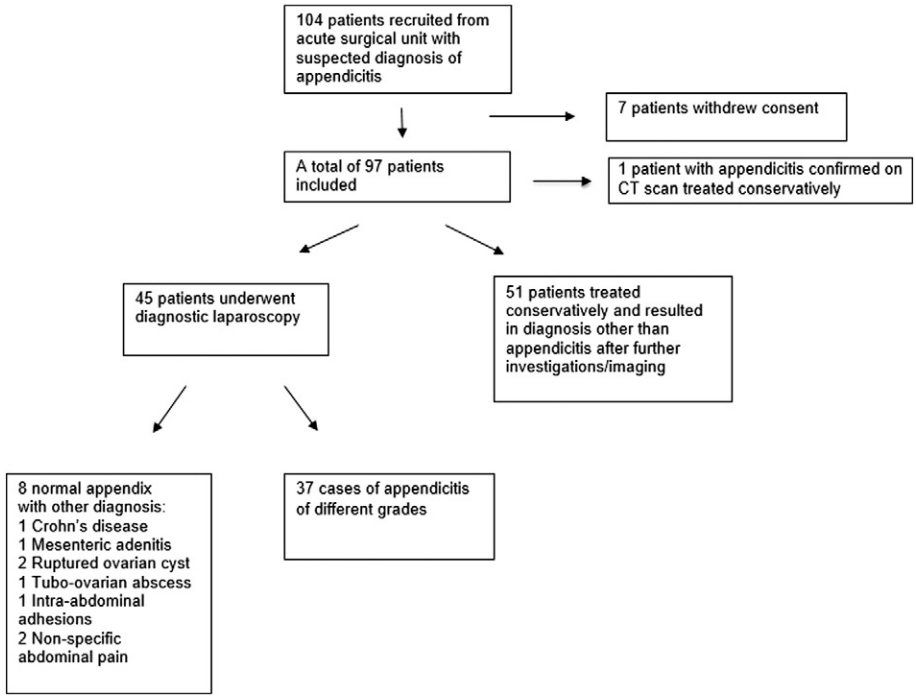

Fig. 1. Patient recruitment to study groups and their final diagnosis.

large teaching hospital. All patients admitted with right iliac fossa pain and presumptive diagnosis of acute appendicitis were asked to participate in the study. Patients were excluded if they were taking drugs interfering with serotonin levels, such as, monoamine oxidase inhibitors, serotonin and norepinephrine reuptake inhibitors, and Lithium. Medical and drug history was noted. Patient consent to participate was obtained once the patient had received initial treatment, and necessary blood and urine tests were taken. The urine samples were collected within 24 hours of the time of admission and before any surgery was performed. The ethical approval to conduct the study was obtained from National Ethics Committee, Health Research Authority UK.

2.2. Samples

Once collected, the urine samples were acidified by 12 N HCl and stored at −70°C. Samples were analyzed using enzyme-linked immunosorbent assay technique. The sample analysis was conducted on ALPCO 5-HIAA enzyme-linked immunosorbent assay. When compared to high-performance liquid chromatography, its methodology has shown strong correlation ($r = 0.99$; n = 47). The sample size of 35 patients was estimated [11] to provide sensitivity of 98% with confidence interval (CI) of 95% and accuracy of 0.05% for urinary 5-HIAA.

Other diagnostic tests used to aid in diagnosis of acute appendicitis were also recorded: white cell count (WCC), neutrophil count, C-reactive protein (CRP), and Alvarado score. Alvarado score is based on clinical observations and biochemistry measurements and ranges from 1 to 10; a score of 5 or 6 is suggestive of appendicitis, whereas score of more than 7 indicates high probability of acute appendicitis.

2.3. Data analysis

Medical information was retrieved from the admission records. This included patient's presenting complaint, duration of symptoms, age, medical history, current medications, and final diagnosis. Results from hematological and biochemical tests along with histopathology reports were acquired from electronic medical records.

Grading of acute appendicitis was based on the same staging system used in earlier studies [11,14]. The categories of acute appendicitis were mild acute appendicitis, acute appendicitis with peritonitis/perforation, acute necrotising appendicitis, and acute gangrenous appendicitis [11,14].

SPSS version 22 program was used to perform statistical analysis. A descriptive analysis was obtained for patients included in the study. Shapiro-Wilk test was conducted to check for normal distribution of dependent variables, such as urinary 5-HIAA, WCC, neutrophil count, CRP, and Alvarado score ($P < .001$). It showed that the data were nonparametric. Mann-Whitney *U* test and Kruskal-Wallis test were used for comparison of 2 and more than 2 groups, respectively. Receiver operating characteristic (ROC) curve was used to plot graph for sensitivity and specificity of urinary 5-HIAA test and other diagnostic tests. For patients who underwent laparoscopy and appendectomy, intraoperative findings and histopathology of specimen were used as criterion standard tests to check diagnostic accuracy of urinary 5-HIAA and other biochemistry tests; however, for those who did not have an operation, CT scan was used. To measure sensitivity and specificity of combination of more than 2 diagnostic tests, binary logistic regression analysis was performed, and the combined predictive score derived from it was used to generate ROC curve [15]. The graph of ROC was plotted for sensitivity against specificity, and the area under curve (AUC) was calculated. It measured the probability of correctly diagnosing a patient in a test group. The value of AUC ranged from 0.5 to 1.0. The ability of diagnostic test to identify patients with appendicitis was considered optimal as AUC value reached closer to 1.0.

Table 1
Comparison of mean values of different diagnostic tests in 2 groups

	Final diagnosis				
	Other diagnosis		Acute appendicitis		
	Median	Range	Median	Range	*P*
5-HIAA (μmol/L)	18.87	2.27-120.59	24.19.	5.29-138.27	.038
WCC ($\times 10^9$/L)	9.50	5.20-20.20	12.50	3.80-23.90	.002
Neutrophil count ($\times 10^9$/L)	6.25	3.40-17.10	10.55	1.80-20.90	.002
CRP (mg/L)	4.00	4.00-334.00	38.00	4.00-434.00	<.001

Table 2
Sensitivity and specificity of different diagnostic test for acute appendicitis

Diagnostic test	Sensitivity	Specificity	PPV	NPV	AUC
5-HIAA (μmol/L)	71%	50%	49%	76%	0.64
WCC ($\times 10^9$/L)	68%	52%	47%	58%	0.69
CRP (mg/L)	84%	59%	56%	85%	0.76
Neutrophil count ($\times 10^9$/L)	55%	84%	69%	74%	0.69
Alvarado score	68%	64%	52%	76%	0.64

Abbreviations: PPV, positive predictive value, NPV, negative predictive value.

Table 3
Median value of urinary 5-HIAA for different grades of acute appendicitis ($P = .328$)

Grade of appendicitis	Median	n	Range
Mild	31.11	12	5.29-89.35
Peritonitis	20.85	21	5.66-138.27
Necrotizing	27.36	4	19.07-44.47
Gangrenous	9.58	1	–

3. Results

A total of 104 patients were initially recruited; 7 subsequently withdrew consent. The outcome of 97 patients is presented in Fig. 1. The study population included 33 male and 64 female participants. The average age of patient population was 35.86.

There were 38 cases of acute appendicitis based on intraoperative, CT, or histologic findings. Of those, 37 cases were treated with appendectomy, and 1 patient was treated with intravenous antibiotics. Based on clinical findings, these patients required diagnostic laparoscopy; however, intraoperatively, 12 cases were found to have mild appendicitis and underwent appendectomy. The final diagnoses in the comparison group of 59 patients included nonspecific abdominal pain (n = 33), ruptured ovarian cyst (n = 5), constipation (n = 4), renal colic (n = 3), urinary tract infection (n = 3), mittelschmerz (n = 2), gastroenteritis (n = 2), chronic abdominal pain (n = 1), intraabdominal adhesions (n = 1), irritable bowel syndrome (n = 1), mesenteric adenitis (n = 1), gastritis (n = 1), colitis (n = 1), and tuboovarian abscess (n = 1). Most of these patients (n = 51) were treated conservatively, which involved supportive treatment with fluid resuscitation, analgesia, and antiemetic. Patients with urinary tract infection were also given antibiotics, and those with constipation were administered laxatives. The remaining 8 patients who underwent diagnostic laparoscopy were found to have a normal appendix. Half of them had other intraabdominal pathology that required surgical intervention. None of these patients developed intraoperative complication; however, one of these patients developed postoperative chest infection. The patient was successfully treated with antibiotics.

The comparison was made between 2 groups: acute appendicitis and other diagnosis (Table 1). The median value of urinary 5-HIAA was 24.19 μmol/L (range, 5.39-138.27) for acute appendicitis vs 18.87 μmol/L (range, 2.27-120.59) for other diagnosis group ($P = .038$).

The sensitivity and specificity of urinary 5-HIAA at cutoff value of 19 μmol/L were 71% and 50%, respectively. The cutoff value was chosen to provide optimum combination of sensitivity and specificity. The sensitivity and specificity of other blood tests are also shown in Table 2. The ROC analysis showed that AUC was 0.64 (CI, 0.513-0.737) for urinary 5-HIAA test, which was lower than WCC (0.69; CI, 0.574-0.797), neutrophil count (0.68; CI, 0.565-0.792), and CRP (0.76; CI, 0.657-0.857) (Fig. 2).

The ROC analysis showed that AUC was 0.76 (CI, 0.663-0.868) for combination of other diagnostic tests (WCC, neutrophil count, and CRP). The AUC value was 0.77 (CI, 0.654-0.858) when 5-HIAA was combined with other diagnostic tests (WCC, neutrophil count, and CRP). Sensitivity and specificity for the combination of diagnostic test were 71% and 74%, respectively.

There was no significant difference in the median values of 5-HIAA between different grades of severity of appendicitis ($P = .704$) (Table 3). Similarly, there was no significant difference among grades of appendicitis for median values of Alvarado score ($P = .771$), WCC ($P = .144$), neutrophil count ($P = .053$), and CRP ($P = .148$).

4. Discussion

There was no significant difference in median values of urinary 5-HIAA for patients with acute appendicitis and those with other diagnosis. The diagnostic test had low sensitivity and specificity, and its combination with other diagnostic tests did not improve its diagnostic ability. Similarly, there was no significant correlation between the levels of 5-HIAA and degree of severity of acute appendicitis.

Previous studies have reported various sensitivity and specificity values for urinary 5-HIAA. Most of the earliest evidence supporting the use of urinary 5-HIAA was obtained from animal studies [12,16]. Studies conducted on human participants compared patients with acute appendicitis to normal healthy individuals [13]. This was not an ideal comparative group, as the test should distinguish true acute appendicitis from other diagnoses with a similar clinical presentation. Most previous studies did not mention the use of statistical tests to evaluate the predictive ability of the test [17]. They had merely calculated differences of mean of 5-HIAA between different groups [17]. Moreover, they chose different cutoff values for 5-HIAA to predict sensitivity and specificity to provide optimum results [17].

The results from our study were similar to a recent study that has evaluated diagnostic ability of urinary 5-HIAA for acute appendicitis [11]. The diagnostic test was found to have low sensitivity and

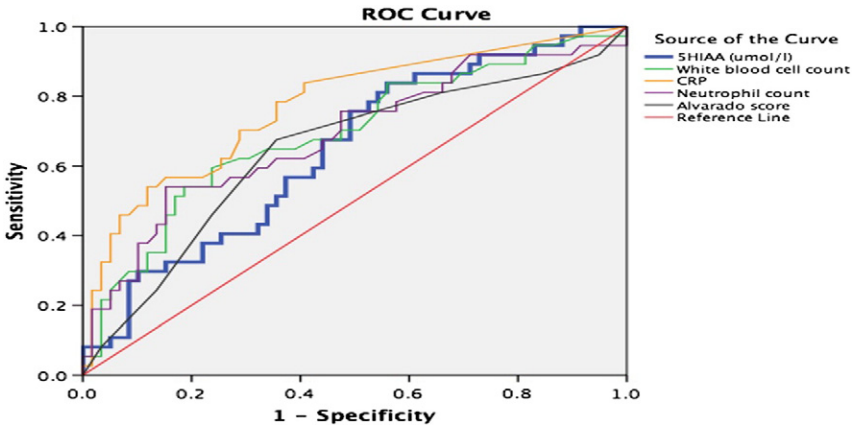

Fig. 2. Receiver operating characteristic curve for urinary 5-HIAA compared to other diagnostic test. The curve more toward top left corner shows increased AUC and, hence, more predictive accuracy of diagnosis.

specificity. Jangjoo et al [11] compared urinary 5-HIAA levels of patients with acute appendicitis and those with similar clinical presentation. It showed sensitivity and specificity of 44% and 81%, respectively, at cutoff value of 27.56 μmol/L [11].

However, the study was based on only 70 participants and assumed sample distribution to be parametric without mentioning outcome of appropriate statistical test.

Area under curve value used in ROC curve was an important statistical test to suggest whether a test had strong diagnostic ability [13]. Area under curve value of 0.60 to 0.70 and 0.70 to 0.80 for a test indicates poor and fair diagnostic ability, respectively. On the other hand, AUC value of 0.80 and higher suggests good diagnostic ability. The AUC value for urinary 5-HIAA test was only 0.64. When combined with other tests, the diagnostic ability improved with the AUC value of 0.77. However, 5-HIAA test was not a significant contributor to improvement in AUC value as the combination of WCC, neutrophil count, and CRP test without 5-HIAA has the AUC value of 0.76. Hence, 5-HIAA only caused the increase in AUC value from 0.76 to 0.77 when combined with other tests. In our study, CRP had higher sensitivity (84%) than 5-HIAA test, and the specificity of 5-HIAA test was lowest of all tests.

Evidence for the use of urinary 5-HIAA was based on cytopathology evidence that the appendix contains numerous serotonin secreting cells [18]. 5-Hydroxyindoleacetic acid levels should rise with severity of inflammation but then fall once the appendix becomes gangrenous and serotonin-secreting cells die. In our study, there was no significant difference between median values of different stages of acute appendicitis. There was only 1 case of gangrenous appendix, which showed low levels of urinary 5-HIAA. Similarly, there was no association between inflammatory markers (WCC and CRP) and levels of urinary 5-HIAA in patients with acute appendicitis.

Our study had certain limitations. We did not perform analysis on healthy individuals; however, our intention was to evaluate the diagnostic role of urinary 5-HIAA to distinguish acute appendicitis from its differential diagnosis with similar clinical presentation. Because the study was not randomized, it was prone to selection bias. The cutoff for diagnostic test of 5-HIAA differed from previous studies. In a previous study, the cutoff value for the diagnostic test was 20 μmol/L; however, the comparison was made between patients with acute appendicitis and healthy individuals [19]. The cutoff value of 5-HIAA in the study was obtained from ROC curve to provide optimum sensitivity and specificity.

In conclusion, urinary 5-HIAA test does not aid in the diagnosis of acute appendicitis. Even when used in combination with other routine diagnostic tests, it clinical usefulness is of no benefit.

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