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The role of urinary 5- hydroxyindoleacetic acid determination in diagnosis of acute appendicitis

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Abstract

Background. Appendicitis is associated with high rate of wrong diagnosis. Objective. To evaluate the predictive value of 5-HIAA in diagnosis of appendicitis. Methods. Spot urine samples were collected from 147 patients with appendicitis and 45 healthy controls. 5-HIAA was detected in urine samples in all patients using ELISA method. Results. The mean 5-HIAA level was 4.5 $\mu\text{mol}/\text{mmol}$ creatinine in healthy individuals, 5.8 $\mu\text{mol}/\text{mmol}$ creatinine in patients with wrongly diagnosed appendicitis and 15.7 $\mu\text{mol}/\text{mmol}$ creatinine in patients with appendicitis. The difference between patients group with appendicitis and other 2 groups was statistically highly significant ($P < 0.001$). However, the difference between the normal individuals group and wrongly diagnosed group was statistically not significant. The test had 81% sensitivity and 85% specificity. Conclusion. This study indicated that determination of 5-HIAA as non-invasive test was with predictive value in diagnosis of appendicitis. Thus reducing the malpractice in surgical operation in Iraqi community.

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INTRODUCTION

Appendicitis (or epityphlitis) is a condition characterized by inflammation of the inner lining of the vermiform appendix that spreads to its other parts [1]. It is the most common acute abdominal condition, and one of the leading indications for surgery [2]. Peak incidence occurs between the ages of 10 – 20 years but can occur at any age. There is a slight male to female predominance [3].

The overall diagnostic accuracy achieved by traditional history, physical examination, and laboratory tests which has been approximately 80%. The ease and accuracy of diagnosis varies by the patient's sex and age. In atypical cases, ultrasonography and computed tomography (CT) may help lower the rate of false-negative appendicitis diagnoses, reduce morbidity from perforation, and lower hospital expenses [4]. Early diagnosis is a major concern for the prevention of complications and decreasing general costs of the

treatment. There is no diagnostic test to detect acute appendicitis with 100% specificity and sensitivity, for this reason, many clinical studies has been performed in an attempt to decrease false-negative appendicitis [5].

One of these attempts is measurement of the urine 5-hydroxyindoleacetic acid (5-HIAA). Serotonin is released during inflammation and cell damage and theoretically might be released during acute appendicitis. 5-HIAA is a serotonin metabolite excreted in urine and could be a reliable marker of inflammation of the appendix. When the inflammation of the appendix shifts to necrosis, the level of (5-HIAA) will decrease, therefore, such decrease could be an early warning sign of perforation of the appendix [6].

The aim of the study is to investigate the significance of spot urine 5-hydroxyindoleacetic acid (5-HIAA) as a diagnostic approach for acute appendicitis. The study protocol was proved by Tikrit University College of

Medicine Ethical Committee.

PATIENTS & METHODS

Patients

One hundred forty seven patients, who were admitted to Emergency Department of Tikrit Teaching Hospital between August 2009 and August 2011 with the complaints of abdominal pain, were evaluated prospectively. Urine samples were collected from patients in sterile container for 5-HIAA measurement. The patients were also evaluated for other causes of increased 5-HIAA secretion such as fruit ingestion, medications and clinical conditions like gastroenteritis. Additionally urine samples were collected from 45 healthy persons for control. A spot urine samples were collected into test tubes and were acidified with 6 M HCl and were kept at -20°C until time of measurement.

Measurement of 5-HIAA

5-HIAA was measured in patient's urine samples using ELISA method. The ELISA kit was purchased from Immune - Biological Laboratories (IBL). The ELISA used with micro plate detector to 450 nm with reference wavelength between 620-650 nm.

Statistical Analysis

The results were presented as mean \pm SD values. $P < 0.05$ was considered to be statistically significant. The significance of mean differences between groups was determined using student t test.

RESULTS

Among (147) patients of clinically diagnosed acute appendicitis, 79 (53.7%) were females and 68 (46.3%) were males, with a male: female ratio of (1:1.16). Their age ranged from (5- 46) years with a mean age of 22.3 ± 6.9 year. After the clinical and histopathological follow-up, appendicitis was detected in 113 patients and abdominal pain was found to be due to causes other than appendicitis in 34 patients and was considered as non-appendicitis group. Forty five healthy individuals without any complain, came to the emergency department as relatives to the patients and were considered as a control group. The mean 5-HIAA levels were 4.5 ± 1.9 $\mu\text{mol}/\text{mmol}$ creatinine in healthy control, whereas 5.8 ± 2.8 $\mu\text{mol}/\text{mmol}$ creatinine in non-appendicitis group and 15.7 ± 7.6 $\mu\text{mol}/\text{mmol}$ creatinine in appendicitis patients, respectively. The comparison of 5-HIAA levels between non-appendicitis and appendicitis patients was found to be statistically highly significant ($P = 0.001$), also significant difference was found between control group and appendicitis group. The comparison between non-appendicitis and control groups was not significant ($P=0.09$), Table (1). The sensitivity and specificity of 5-HIAA measurement for the diagnosis of acute appendicitis were 81%, 85% respectively, Table (2). In addition, the positive predictive value was 95%, the negative predictive value was 57% and test accuracy of 82.

Table 1. Mean urine level of 5-HIAA in studied groups

Cases	No.	Mean ($\mu\text{mol}/\text{mmol}$ creatinine)	\pm SD	P value
Appendicitis group	113	15.7	7.6	0.001
Control group	45	4.5	1.9	
Appendicitis group	113	15.7	7.6	0.001
Non- appendicitis group	34	5.8	2.8	
Non- appendicitis group	34	5.8	2.8	0.09
Control group	45	4.5	1.9	

Table 2. The specificity and sensitivity of urinary 5-HIAA in the diagnosis of acute appendicitis

Urinary 5HIAA	Patients without acute inflammation	Patients with acute inflammation	Total
High	5 (FP)	91 (TP)	96
Normal	29 (TN)	22(FN)	51
Total	34	113	147
Specificity = 85%			
Sensitivity = 81%			
Positive predictive value = 95%			
Negative predictive value = 57%			
Accuracy = 82%			

*(FP=false positive, TP=true positive, TN=true negative, FN=false negative)

DISCUSSION

Appendicitis is one of the more common surgical emergencies, and it is one of the most common causes of abdominal pain. Persons of any age may be affected, with highest incidence occurring during the second and third decades of life. In this study, acute appendicitis was higher in female (53.7%) compared to male (46.3%) with a male to female ratio of (M: F ratio= 1:1.16). A comparison between the two groups of gender did not reveal any significant differences ($P>0.05$). This finding was in agreement with that reported by Nampet *et al.* [7] and Mowlavi *et al.* [8] and was disagreed with others [9,10]. Nampet *et al.* [7] reported a female preponderance exists in his study, where a female to male ratio of 1.15:1. In addition, the same result was obtained by Mowlavi *et al.* [1] where acute appendicitis was higher in female (52%) than in male (48%). Furthermore, Cerva *et al.* [11] who reported a high frequency rate in female (64.9%) compared to male (35.1%). This result was disagreed with other studies by Ramezani *et al.* [9] who reported male (54.2%) and female (45.8%), AL-Salami [10] reported a high frequency rate of male (62.7%) than in female (37.3%). Also this increments of female than male disagree with international figure that appendicitis occurs more frequently in males than in females, with a male-to-female ratio of 1.3:1 [12]. The present study shows a significant differences upon age factor since the results indicated that the peak of incidences observed in the age group (11-15) years old (37.5%), followed by the age group(16-20)years (23.75%).

In addition, the lowest rate (5%) of patients was observed within the age groups of (>30) years and in age group (5-10) years (7.5%). The present study was in agreement with the international figure where incidence of acute appendicitis occurs in adolescent and young patients [13]. The same results were being reported by AL-Salami in Babylon [10] who reported

that high incidence of acute appendicitis in age group (11-20) years (51.8%). Other study by Ali *et al.* [14] in Sudia Arabia revealed a maximum incidence (38.4 %) was found in the age group (10-19) years. The same results were reported in different geographical areas by Colleran *et al.* [15], Ozgür [16], Cerva *et al.* [11], and Thanikachalam *et al.* [17]. The individuals of (11-20) years old are being more susceptible for appendicitis may ascribed to the nature of physiological and anatomical factors of appendix tissue which is composed mainly of lymphoid tissue [18], since these lymphoid tissue is the most susceptible for infection gradually increase up to the 20 years of age and begins to decrease with advancement of the age up to 60 years old when this tissue is totally disappear [19].

Out of the 147 patients included in the study, 113 (76.9%) cases were found to have acute inflammation on histopathological examination (appendicitis cases), while 34 (23.1%) cases were found to have non acute inflammation on histopathological examination (non-appendicitis cases). Negative appendectomy is defined as one which is performed for a clinical diagnosis of acute appendicitis but in which the appendix is found to be normal (no acute inflammation) on histopathological examination. Thus, the overall negative appendectomy rate in our study was (23.1%). The percent reported in literature by different others indicated a variable figures depending on study population. Gladman [20] reported negative appendectomy in (31%), while Fatemeh [21] reported a percent of (34.2%) and lower than that reported by others, Thanikachalam *et al.* [17] (5.7%), Nampet *et al.* [7] (11.8%), Khanzada *et al.* [22] (12.3%), and Herd *et al.* [23] (17.3%). However, Colleran *et al.* [15] reported about the same percent (24%) of negative appendectomy as this study indicated. Thus, negative appendectomy rates in the literature range between 5.7% and 34.2% of all these patients who are suspected of having acute appendicitis

undergo surgery and several authors consider higher negative appendectomy rates acceptable in order to minimize the incidence of perforation [22]. However, this suggestion may be not accepted due to patient exposure to hazard of surgery and economical burden on patient and hospital. Thus the surgeon and health care providers' main goal is the development of novel approach for accurate and early diagnosis of appendicitis.

Early and correct diagnoses are the main goals in the treatment of acute appendicitis. Many diagnostic modalities, both biochemical and radiological, have been suggested in the literature for this purpose. Recent interest has been focused on the level of spot urine 5-HIAA levels in appendicitis [24]. 5-HIAA is a metabolite of serotonin and is used in the diagnosis of carcinoid tumors. Although the appendix is rich in serotonin, only a few studies have evaluated the relationship between serotonin levels and acute appendicitis. Rordam *et al.* [25] has previously showed the increase of serotonin from the EC cells in the appendix in cases of acute appendicitis of children as first study, reported the sensitivity and specificity of high serotonin levels as 45% and 95% [25] and another study as 93.8% and 95.7% [26]. A total of 147 patients were included for this study: 113 had (acute appendicitis) histologically, whereas 34 had (non-appendices) histologically and 45 cases as healthy control admitted to hospital for another complain. The acute appendicitis group had a mean urinary 5-HIAA level of 15.7 $\mu\text{mol}/\text{mmol}$ creatinine which was much higher than the mean of non-appendicitis group, 5.8 $\mu\text{mol}/\text{mmol}$ creatinine and 4.5 $\mu\text{mol}/\text{mmol}$ creatinine in healthy control group. The calculated sensitivity and specificity of this test for acute appendicitis as 81% and 85%, respectively, with a positive predictive value of 95%. The comparison of 5-HIAA levels between non-appendicitis and appendicitis patients was found to be statistically highly significant ($P = 0.001$) and the comparison between non-appendicitis and control groups was not significant ($P=0.09$). Various other studies have confirmed this observation; Ilkhanizadeh *et al.* [27] measured 5-HIAA levels in spot urine of patients with acute appendicitis and found sensitivity as 98% and specificity as 100%, whereas the sensitivity was 84% and specificity was 88% in the study of Bolandparvaz *et al.* [6]. In our study, the sensitivity of 5-HIAA levels was low 76.9%, while the specificity was high (85.3%). On the other hand, Oruc *et al.* [28] have given sensitivity of 58% and a specificity of 48% in their study, Hernandez *et al.* [29] have a sensitivity of 63% and a specificity of 33% and Mihmanli *et al.* have given a sensitivity of 22% and a specificity of 93% in their study, who considered this test to be unreliable in the diagnosis of acute appendicitis [5]. In the present study, no patient in the control group had

elevated 5-HIAA level. It means that when a patient has a positive test result, this patient is not free of disease. However, nearly 1/3 of the patients with acute appendicitis in the present study displayed negative test result and this is the point largely restricting the usefulness and confidence of the test. When we consider our findings, due to a high relatively specificity in comparison to low sensitivity, this test seems to have a questionable superiority over the conventional methods in the diagnosis of acute appendicitis. Urinary 5-HIAA has been determined with ELISA method in this study, other studies has been determined with high performance liquid chromatography (HPLC) which were studied shortly after were kept at 4 °C that make this method is fast, unlike ELISA method. A 5-HIAA level has been known to have no diurnal change and no variation between males and females. 5-HIAA levels may be changed after ingestion of certain foods and in certain clinical conditions like carcinoid tumors, acute gastroenteritis, inflammatory bowel disease, coeliac disease and patients treated with aminosalicylates. These factors should be excluded before the tests to avoid false positive results [24]. This study idea is not novel and is essentially replicating previous work from other groups, however, we used different estimation procedure for urine 5-HIAA. The test used is the ELISA and to our knowledge, it is the first time to be used for evaluation of determination of urine 5-HIAA in diagnosis of appendicitis.

CONCLUSION

Urinary 5-HIAA significantly rises in patients with acute appendicitis in comparison to control. This study indicated that determination of 5-HIAA as non-invasive test was with predictive value in diagnosis of appendicitis. This is reducing the malpractice in surgical operation in Iraqi community. There is a need for future research that determine the predictive value of combination of determination of 5-HIAA with conventional methods to select the best combination.

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