

RESEARCH

Open Access



5-Hydroxyindoleacetic acid as a biomarker for revealing perforation in acute appendicitis

Arash Mohammadi Tofigh¹, Majid Samsami¹ , Alireza Haghbin Toutounchi¹ , Shervin Tavakoli² , Zeinab Taabzadeh³, Hojatolah Khoshnoudi¹ and Ehsan Adinevand^{1*}

Abstract

Background The present study investigated the level of 5-hydroxyindoleacetic acid (5-HIAA) in perforated and nonperforated appendicitis patients. This issue is important for timely diagnosis of acute appendicitis complications and making decision about the surgical plan and type of incision.

Methods This prospective study was conducted on patients with acute appendicitis. 5-HIAA levels were measured in urine spot sample of every patient before the surgery. The patients were finally included based on result of pathology for presence of appendicitis or not and sorted by direct surgical detection for exist of perforation or not. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 5-HIAA were investigated. The data were statistically analyzed by SPSS v.27.

Results A total of 150 patients were included in two groups as 40 patients in the perforated appendicitis group and 110 patients in the noncomplicated acute appendicitis group. The average age of patients in the perforated appendicitis group was 28.8 ± 6.07 years, and that of patients in the acute appendicitis group was 29.6 ± 6.96 years. 94 patients (63%) were male, and 56 (37%) were female. No significant difference was observed in terms of age or sex between the two groups. The difference in the 5-HIAA concentration between the acute appendicitis group (0.3 ± 0.04 mg/dl) and the perforated appendicitis group (0.5 ± 0.03 mg/dl) was significant ($P < 0.001$). The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were 82%, 62%, 75%, 77%, and 88% respectively.

Conclusion This study shows that the 5-HIAA concentration in patients with perforated appendicitis can potentially increased significantly more than that in patients in the early stages of acute appendicitis. further studies with larger sample sizes are needed to prove the present results.

Keywords 5-Hydroxy Indole Acetic Acid, Appendicitis, Perforated appendicitis

*Correspondence:

Ehsan Adinevand
Dr.Adine.md@gmail.com

¹Department of General Surgery, Imam Hosein Medical and Educational Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

²Department of General Surgery, Guilan University of Medical Sciences, Rasht, Iran

³Department of Internal Medicine, Imam Hosein Medical and Educational Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Background

Acute appendicitis is one of the most common surgical emergencies, making timely diagnosis and appropriate management critical [1, 2]. Delays in diagnosis can lead to complications such as gangrene or perforation of the appendix [3]. These complications necessitate different treatment approaches and underscore the importance of prompt diagnosis [4]. The distal part of the appendix contains enterochromaffin cells, which secrete large amounts of serotonin [5]. During inflammation and infection, the secretion of serotonin by these cells increases. Serotonin is then metabolized in the liver to 5-hydroxyindoleacetic acid (5-HIAA) and excreted in the urine [6]. Recently, studies have investigated the potential of 5-HIAA in the diagnosis of acute appendicitis [7].

This study aims to compare the levels of the marker 5-HIAA in cases of perforated appendicitis versus non-complicated appendicitis. Our objective is not to use 5-HIAA for diagnosing appendicitis in general, but rather to investigate whether elevated levels of 5-HIAA can be used to identify perforation. This distinction is crucial because the type of surgical incision varies depending on the presence of perforation. For uncomplicated acute appendicitis, an appendectomy is typically performed through a McBurney incision. However, if the appendicitis is perforated, a lower midline incision is necessary for proper management [3, 4]. Identifying perforation before surgery would enable better surgical planning and decision-making.

Materials and methods

Study design

This prospective study was conducted at a tertiary medical center and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. Inclusion criteria consisted of patients aged 18 to 65 years who were candidates for surgery due to acute appendicitis, had no history of abdominal surgery or malignancy, and serotonin-related drug use, had no consume of banana, caffeine, and chocolate in past 24 h, and provided informed consent. Urine spot samples were collected from patients meeting the inclusion criteria before surgery to measure random urine 5-HIAA level. Following appendectomy, the presence of perforation was documented by the surgeon. Only patients with pathologically confirmed appendicitis were included in the final analysis, and their

5-HIAA levels considered for evaluation. The measurement was done using ELISA methods. To increase the stability of 5-HIAA in urine samples, hydrochloric acid (HCl) was added as a preservative directly to the collection container before urine collection to maintain the pH at the desired level. Because the acidification of the urine sample to a pH of around 3 helps to stabilize 5-HIAA, reducing its degradation and ensuring more accurate measurement. Additionally, the samples were stored in dark and cold condition [8].

Sample size

The sample size was calculated based on a sensitivity (Se) of 70%, specificity (Sp) of 90%, a prevalence (P) of 20%, and a desired confidence level of 95% (Z-value=1.96), resulting in a requirement of 150 participants.

Statistical analysis

Descriptive statistics, including frequency, percentage, mean, median, range, and standard deviation, were used to summarize the data. Analytical methods included the chi-square test, Mann-Whitney test, Kruskal-Wallis test, and other nonparametric tests. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 5-HIAA were calculated. Data analysis was conducted using SPSS version 27 software.

Results

In this study, a total of 150 patients were divided into two groups as 40 patients in the perforated appendicitis group and 110 patients in the noncomplicated acute appendicitis group. The mean and standard deviation of the age of the patients were 29.3 ± 6.7 years (Table 1). The results showed that there was no significant difference between the two groups in terms of age ($P=0.50$). Out of 150 participants in the study, 94 (63%) were male and 56 (37%) were female. Additionally, the frequency distributions of men and women were investigated and compared between the two groups (Table 1), and the results showed that the two groups were not significantly different in terms of sex ($P=0.26$).

Based on the objectives of this research, the difference in 5-HIAA levels between patients with acute and perforated appendicitis was investigated (Fig. 1). The overall mean 5-HIAA level was 0.35 ± 0.09 mg/dl. The level of spot urine 5-HIAA was significantly higher in the

Table 1 Demographic information of patients in the studied groups

Variables	Perforated appendicitis (N=40)	Acute appendicitis (N=110)	Total (N=150)	P Value
Age (Year)	28.8 ± 6.07	29.6 ± 6.96	29.3 ± 0.7	0.500
Gender				0.260
Male	28 (70%)	66 (60%)	94 (62.7%)	
Female	12 (30%)	44 (40%)	56 (37.3)	

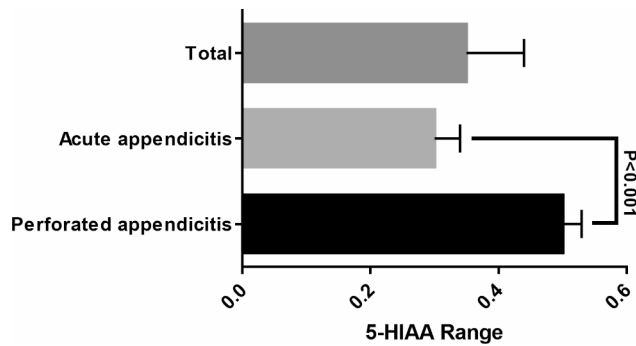


Fig. 1 Comparison of 5-HIAA between acute and perforated appendicitis patients (mg/dl)

perforated appendicitis group (0.5 ± 0.03 mg/dl) compared to the acute appendicitis group (0.3 ± 0.04 mg/dl) ($P < 0.001$). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 5-HIAA for predicting perforation were 82%, 62%, 75%, 77%, and 88%, respectively.

Discussion

In recent years, despite the rapid availability of diagnostic tools in emergency units, there has been little progress in reducing the misdiagnosis rate of appendicitis. Delays in diagnosing acute appendicitis increase the risk of perforation, potentially leading to peritonitis or abscess formation [9]. The symptoms of appendicitis often overlap with those of other conditions, such as gastroenteritis, urinary tract infections, and pelvic inflammatory disease [10]. In cases of suspected perforated appendicitis, further investigation can be done using a contrast-enhanced CT scan to reveal complications [11]. However, in this study, we explored the potential of using a biomarker as an alternative. This approach may reduce costs, minimize radiation exposure, and decrease the reliance on contrast agents for assessing perforation. It is more useful in special populations like pregnant patients. This distinction is crucial because the type of surgical incision varies depending on the presence of perforation [5]. For uncomplicated acute appendicitis, we typically perform an appendectomy through a McBurney incision. However, if the appendicitis is perforated, a lower midline incision is necessary for proper management [3, 4]. Identifying perforation before surgery would allow for better surgical planning and decision-making.

The appendix is rich in enterochromaffin cells, which synthesize and store serotonin (5-hydroxytryptamine or 5-HT). The digestive system contains approximately 95% of the body's serotonin [12, 13]. Recent studies have shown that during an inflammatory process in the appendix, blood serotonin levels increase. In the liver, 5-HT is rapidly metabolized to 5-HIAA by the monoamine oxidase system and subsequently excreted in the urine [14].

Several studies have investigated the use of plasma serotonin concentrations in diagnosing acute appendicitis. Kalra et al. and Singh et al. found that plasma serotonin concentration is a reliable marker for early acute appendicitis, especially when physical symptoms are unclear. However, they noted that serotonin concentration is less useful in diagnosing gangrenous appendicitis. This suggests that 5-HT levels decrease as the inflammatory process progresses toward severe mucosal necrosis and cell destruction [15, 16]. A clinical trial by Khirallah et al. (2021), showed a significant higher level of 5-HIAA in acute appendicitis than control. Their optimal cutoff value was $5\text{-HIAA} > 16.0$ mg/gCreatinine with 91.8% sensitivity, 87.1% specificity in detection of acute appendicitis [17].

In a clinical trial by Jangjoo et al. (2012), no statistical difference was found in level of 5-HIAA between control and acute appendicitis patients. They stated that 5-HIAA is not reliable enough to be used as a single test for definitive diagnosis or ruling out of acute appendicitis, while it may be useful to increase the accuracy of diagnosis, especially in more severe stages of appendicitis [18]. Bolandparvaz et al. (2004), showed a significant higher level of 5-HIAA in acute appendicitis and gangrenous cases than control, while 5-HIAA was significantly lower in gangrenous cases than acute appendicitis ones. They also stated that the performance of spot 5-HIAA was higher than adjusted with creatinine. Finally, they concluded physiological reduction in level of 5-HIAA on severe stages and suggesting perforation [19]. Kamal et al. (2017), showed a significant higher level of 5-HIAA in mild acute appendicitis with specificity of 100%, sensitivity of 94%, and diagnostic accuracy of 97%. But they found many reductions of 5-HIAA in severe cases (perforated or gangrenous) and no statistical difference between severe ones and normal population with specificity of 100%, sensitivity of 37%, and diagnostic accuracy of 68% [20]. In a study by Mendes et al. (2009), 5-HIAA levels were evaluated in patients with acute appendicitis. The levels of 5-HIAA increased in all groups of appendectomy cases within 24 h after the operation, with the greatest increase observed 12 h after cecal ligation. The diagnostic accuracy of 5-HIAA in this study was estimated to be approximately 76%, similar to the findings of our study [21]. In a systematic review and meta-analysis by Montero et al. (2023), the authors concluded that while 5-HIAA is potentially a useful marker for diagnosing acute appendicitis, it cannot differentiate between complicated and noncomplicated cases [22]. This conclusion is based on the fact that most studies reported no significant difference in 5-HIAA levels between these two groups, while the result of our work is supporting the potential of 5-HIAA in detection of complicated cases in against other works. In our study, the difference in 5-HIAA levels between patients with acute

and perforated appendicitis was significant. The sensitivity, specificity, positive predictive value, and negative predictive value were 82%, 62%, 75%, and 77%, respectively, based on statistical analysis.

The differences between these studies and ours may be due to sample size, sampling considerations, and disease severity [20]. The excretion of 5-HIAA can be influenced by various medical (carcinoid tumors, gastroenteritis, IBD, celiac disease, and etc.), pharmacological, and dietary conditions, which limits its utility as a routine diagnostic test for acute appendicitis [22, 23]. To mitigate these factors, we excluded patients with a history of serotonin-related drug use and those who consumed coffee or chocolate which have a high amount of caffeine within 24 h prior to sample collection. However, many nutrients may also affect urine 5-HIAA levels. We preserved the samples using HCl, light protection, and cooling. These considerations were taken to minimize 5-HIAA degradation and ensure more accurate measurements, as darkness, cold temperatures, and acidification enhance the stability of 5-HIAA [8].

The most significant limitation of this study was its small sample size, which was constrained by the COVID-19 pandemic, the limited duration of the study, and a lack of resources. As a pioneering study in our country, no similar research has been conducted thus far. At the time of this study, providing enough sample kits was challenging due to restrictions. Additionally, 5-HIAA measurement methods were referenced for 24-hour urine samples, making the use of kits for random urine samples uncommon. Future studies should be conducted with larger sample sizes and improved resources to obtain more accurate and reliable results and to establish an optimal cutoff for clinical use.

Conclusion

The results of this study indicate that 5-HIAA concentrations can be significantly higher in cases of perforated appendicitis compared to uncomplicated acute appendicitis. Therefore, measuring 5-HIAA levels maybe potentially used in revealing perforation and help surgeons determine the most appropriate incision and surgical approach for appendectomy. However, further research is needed to provide more definitive evidence and conclusions on this subject.

Acknowledgements

N/A.

Author contributions

A.M.T participated in Supervision and validation. M.S participated in Conceptualization and Methodology. A.H.T and S.T participated in Writing - Original and Formal analysis. Z.T and H.K participated in Writing - Review & Editing. H.K and E.A participated in Data curation. E.A participated in Project administration, corresponding author.

Funding

The authors declare that no funds, grants, or other support was received during the preparation of this manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

Ethics approval was obtained from the Research Ethics Committees of School of Medicine - Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1401.094). Informed consent was obtained from all individual participants included in the study.

Consent for publication

N/A.

Competing interests

The authors declare no competing interests.

Received: 9 June 2024 / Accepted: 3 October 2024

Published online: 14 October 2024

References

- Acharya A, Markar SR, Ni M, Hanna GB. Biomarkers of acute appendicitis: systematic review and cost-benefit trade-off analysis. *Surg Endosc*. 2017;31:1022–31.
- Baird DL, Simillis C, Kontovounisios C, Rasheed S, Tekkis PP. Acute appendicitis. *BMJ*. 2017;357.
- Almström M, Svensson JF, Patkova B, Svenningsson A, Wester T. In-hospital surgical delay does not increase the risk for perforated appendicitis in children. *Ann Surg*. 2017;265(3):616–21.
- Nordin AB, Diefenbach K, Sales SP, Christensen J, Besner GE, Kenney BD. Gangrenous appendicitis: no longer complicated. *J Pediatr Surg*. 2019;54(4):718–22.
- Sheikh U, Henderson-Jackson E, Muhammad J, Coppola D, Nasir A. Neuroendocrine Neoplasms of the Appendix. *Neuroendocrine Tumors: Review of Pathology, Molecular and Therapeutic Advances*. 2016:289–308.
- Versic AB, Glavan N, Bukvic N, Tomasic Z, Nikolic H. Does elevated urinary 5-hydroxyindole acetic acid level predict acute appendicitis in children? *Emerg Med J*. 2016;33(12):848–52.
- Apak S, Kazez A, Ozel SK, Ustundag B, Akpolat N, Kizirgil A. Spot urine 5-hydroxyindoleacetic acid levels in the early diagnosis of acute appendicitis. *J Pediatr Surg*. 2005;40(9):1436–9.
- Petersen V, Hermann K, Lund JO, Paulsen J, Larsen ML. Stabilization of 5-hydroxyindoleacetic acid in human urine by acidification. *Clin Chim Acta*. 1990;192(3):201–9.
- Lau W, Fan S, Yip W, Chu K, Yiu T, Yeung C, et al. Acute appendicitis in children. *Aust N Z J Surg*. 1987;57(12):927–31.
- Franz MG, Norman J, Fabri PJ. Increased morbidity of appendicitis with advancing age. *Am Surg*. 1995;61(1):40–4.
- Hernandez R, Jain A, Rosiere L, Henderson SO. A prospective clinical trial evaluating urinary 5-hydroxyindoleacetic acid levels in the diagnosis of acute appendicitis. *Am J Emerg Med*. 2008;26(3):282–6.
- Kim D-Y, Camilleri M. Serotonin: a mediator of the brain-gut connection. *Am J Gastroenterol*. 2000;95(10):2698–709.
- Gershon M. Serotonin: its role and receptors in enteric neurotransmission. *Kynurenine and Serotonin Pathways: Progress in Tryptophan Research*. 1991:221–30.
- Ilkhanizadeh B, Owji AA, Tavangar SM, Vasei M, Tabei S. Spot urine 5-hydroxyindole acetic acid and acute appendicitis. *Hepatogastroenterology*. 2001;48(39):609–13.
- Kalra U, Chitkara N, Dadoo R, Singh G, Gulati P, Narula S. Evaluation of plasma serotonin concentration in acute appendicitis. *Indian J Gastroenterology: Official J Indian Soc Gastroenterol*. 1997;16(1):18–9.
- Singh S, Dean H, de Dombal F, Wilson D, Flowers M. Concentrations of serotonin in plasma—a test for appendicitis? *Clin Chem*. 1988;34(12):2572–4.

17. Khirallah MG, Ghafar MTA. Diagnosis of acute appendicitis in children using urinary 5-hydroxy indol acetic acid and pediatric appendicitis score: a randomized control trial. *Annals Med Surg* 2021;65.
18. Jangjoo A, Varasteh A-R, Bahar MM, Meibodi NT, Esmaili H, Nazeri N, et al. Is urinary 5-hydroxyindoleacetic acid helpful for early diagnosis of acute appendicitis? *Am J Emerg Med*. 2012;30:540–4.
19. Bolandparvaz S, Vasei M, Owji AA, Ata-Ee N, Amin A, Daneshbod Y, et al. Urinary 5-hydroxy indole acetic acid as a test for early diagnosis of acute appendicitis. *Clin Biochem*. 2004;37(11):985–9.
20. Kamal ZB, Naji RE, Eessee RM, Abdulghafoor YA. Assessment of urinary-5-Hydroxyindolacetic acid as a diagnostic parameter in early detection of Acute Appendicitis. *Health Sci*. 2017;6(4):71–4.
21. Mentos O, Eryilmaz M, Harlak A, Yaman H, Yigit T, Ongoru O, et al. The importance of urine 5-hydroxyindoleacetic acid levels in the early diagnosis of acute appendicitis. *Am J Emerg Med*. 2009;27(4):409–12.
22. Montero JA, Asfura OEB, Riveros BPP, Burgos EL, Jiménez MR. Diagnostic performance of urinary 5-Hydroxyindoleacetic acid in acute appendicitis: a systematic review and diagnostic test accuracy meta-analysis. *Int J Colorectal Dis* 2023;38.
23. Maghsoudi LH, Soltanian A, Shirzadi A, Alizadeh-Kashani R, Ahmadinejad M. Biomarker of urinary 5-HIAA as a valuable predictor of acute appendicitis. *Practical Lab Med*. 2021;23:e00198.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.