

Folic acid levels in predicting complicated appendicitis in pediatric patients

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Abstract

Background: Appendicitis is the most common surgical emergency in children. Nearly 30% of children present with complicated appendicitis. A study conducted with animal models indicates that lower folate levels were associated with a higher likelihood of complicated appendicitis. Another study suggested that folate levels could be a significant predictive factor for the occurrence of complicated appendicitis in animal models. **Objective:** The aim of this study is to analyze the level of folic acid in pediatric patient with appendicitis as a predictor of complicated appendicitis. **Method:** This study is a prospective cohort study with observational analytical approach involving serological blood tests and histopathological examination of pediatric patients with appendicitis from October 2022 to March 2023. Subject involved nine pediatric patients who were then grouped and upon admission, underwent folic acid measurement, and later intervened surgically with either appendectomy or laparotomy. Kruskal-Willis test and Spearman test would be performed. **Result:** Kruskal-Wallis test showed no significant difference in folic acid levels among the three groups based on the onset of symptoms ($p > 0.05$). Spearman correlation test showed a moderate negative correlation ($r = -0.609$) between the onset of symptoms and folate levels in pediatric appendicitis patients, but it was not statistically significant ($p > 0.05$). From the R^2 value, it can be inferred that folate levels can predict 53% of the onset of symptoms. **Conclusion:** Future research should assess relationship with other risk factors with increased number of subjects and cut-off value for folic acid as a predictor should also be established

Keywords: folic acids; complicated appendicitis; pediatric

1. Introduction

Appendicitis is the most common surgical emergency in children. The risk of developing appendicitis is approximately 9% in males and 7% in females. Nearly 30% of children present with complicated appendicitis, which includes abscess or phlegmon, while another common type is acute appendicitis. In cases of appendiceal perforation, it can lead to generalized peritonitis. The perforation occurs when the appendix wall ruptures due to the inflammatory process in the appendix.¹

Consistently, in many other literature, it has been suggested that in patients with appendicitis who experience prolonged duration of symptoms, there is often an association with appendiceal perforation in both children and adults. In pediatric literature, it is mentioned that there is a tendency for an increased risk of perforation with longer duration of appendicitis symptoms experienced.² In another study of 102 cases of appendicitis that underwent surgery, four (44.4%) out of nine patients aged < 5 years experienced perforation,

while five (12.5%) out of 40 patients aged between 5 and 10 years experienced perforation. Therefore, perforation was more common in patients younger than 5 years ($p=0.06$). The average duration of pain symptoms among all patients was 38.6 hours. And more than 60% of patients presented with complicated appendicitis with a duration of pain symptoms >72 hours.³

Vitamin B, including folic acid, riboflavin, pyridoxine, and cobalamin, is important for methylation reactions, nucleotide synthesis, and the stability and repair of Deoxyribonucleic Acid (DNA). In a randomized clinical trial examining individuals exposed to arsenic, supplementation of folic acid at a dose of 0.4-0.8 mg/day for 8 weeks resulted in a significant reduction in DNA damage. In vitro and in vivo studies also show that folate deficiency exacerbates DNA damage by reducing thymine, causing uracil to fail to incorporate into DNA, and inhibiting DNA repair responses.⁴

A study conducted with animal models showed that there was a difference in folate levels based on pathological anatomical examination results, indicating that lower folate levels were associated with a higher likelihood of complicated appendicitis, although the statistical analysis using Kruskal-Wallis test did not show a significant difference with a p-value of 0.058.⁵ In another study, there was a significant difference in folate levels among different time groups (18 hours, 24 hours, 36 hours, and 48 hours) in relation to the occurrence of appendicitis in animal models, with a p-value of less than 0.05. It was further concluded that folate levels could be a significant predictive factor for the occurrence of complicated appendicitis in animal models.⁶

Based on the theories and previous studies, the aim of this study is to analyze the level of folic acid in pediatric patient with appendicitis as a predictor of complicated appendicitis. This study consists of five sample groups, and blood samples are collected serially at 18-24 hours, 25-36 hours, 37-48 hours, 49-72 hours, and more than 72 hours from the onset of symptoms when pediatric patients come to the hospital. Serial blood samples are taken according to the clinical development of the pediatric patients, and appendectomy is performed, followed by histological examination.

2. Method

This study is a prospective cohort study with observational analytical approach involving serological blood tests and histopathological examination of pediatric patients with appendicitis. The study was conducted at Haji Adam Malik General Hospital and affiliated hospitals for a period of 6 months from October 2022 to March 2023. Blood sample was sent to Integrated Laboratory of the Faculty of Medicine, Universitas Sumatera Utara, where examination of Folic Acid would be carried out. This study has been approved by the Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara.

Subject in this study involved twenty five pediatric patient with appendicitis who were admitted for inpatient care. Exclusion criteria include patient who presented with coexisting gastrointestinal disease or other concomitant diseases. The patients were categorized into groups based on the duration of symptoms until they arrived at the hospital: Group 1 (≤ 24 hours), Group 2 ($>24-48$ hours), Group 3 ($>48-72$ hours), Group 4 ($>72-96$ hours), and Group 5 (more than 96 hours). Upon admission, patients underwent folic acid examination. If the decision for surgery was not yet made, patients were re-evaluated at the predetermined time. In case of surgery, appendectomy was performed and the appendix tissue was examined macroscopically. Independent variable in the study was folic acid level and dependent variable was type of appendicitis in pediatric patient.

Data will be analyzed descriptively to examine the frequency distribution based on characteristics and blood test results. The research data analysis will be presented in the form of percentages for categorical data. For numeric data, mean $\pm$ SD will be displayed for normally distributed data, and median (min-max) for non-normally distributed data. To analyze differences between groups, an ANOVA test will be conducted for normally distributed data, while the Kruskal-Wallis test will be used for non-normally distributed data. The

significance level will be set at $p < 0.05$, indicating a significant relationship between the variables being tested. The data analysis will be performed using statistical software.

3. Result

Nine pediatric patients with appendicitis met the inclusion criteria for the study. In this study, most of the subjects was in the age range of 6-10 years with a frequency of 4 (44.4%). The least number of subjects was found in the age group of 16-18 years with a frequency of 2 (22.2%), and no samples were found in the age group below 5 years. Based on macroscopic examination, perforated appendicitis was found in 7 cases (77.8%), and laparotomy was performed more frequently, totaling 7 cases (77.8%). Demographic characteristic is shown below in Table 1.

Tabel 1. Demographic characteristic of the patients

Characteristic	Frequency, n (%)
Age	
<5	0 (00.0)
6-10	4 (44.4)
11-15	3 (33.3)
16-18	2 (22.2)
Macroscopic	
Acute	2 (22.2)
Perforated	7 (77.8)
Types of Intervention	
Appendectomy	2 (22.2)
Laparotomy	7 (77.8)

The mean value of folic acid in the appendectomy group was higher (34313.48 ± 11620.96), followed by the laparotomy group with a mean value of (22530.13 ± 9521.73) as shown in Table 2.

Table 2. Mean Values of Folic Acid Based on Types of Surgical Procedures Performed in Pediatric Appendicitis Patients.

Types of intervention	Mean $\pm$ SD	Md (min-max)
Appendectomy	34313.48 ± 11620.96	34313.48 (26096.22 - 42530.74)
Laparotomy	22530.13 ± 9521.73	20050.23 (14793.05 - 42530.74)

The results of the Spearman correlation test showed a moderate negative correlation ($r = -0.609$) between the onset of symptoms and folic acid levels in pediatric appendicitis patients, but it was not statistically significant ($p > 0.05$). The R^2 value indicates that folic acid levels can predict the onset of symptoms by 53 as shown in Table 3 and Figure 1.

Table 3. Correlation of Folic Acid with Time of Symptom Onset in Pediatric Appendicitis Patients

Folic acid	r value	p value*
Time	-0.609	0.082

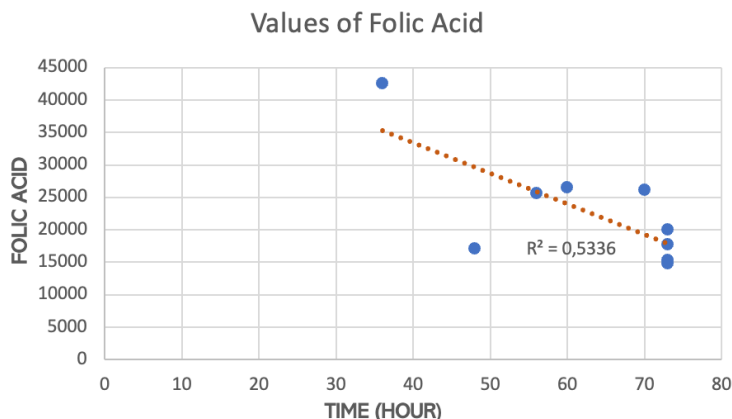


Figure 1. Correlation of Folic Acid with Time of Symptom Onset in Pediatric Appendicitis Patients

4. Discussion

Early diagnosis of appendicitis is crucial in preventing perforation, abscess formation, and postoperative complications. However, diagnosing this condition can be challenging. Patients with gastroenteritis may experience abdominal pain accompanied by nausea, vomiting, diarrhea, or fever. Additionally, pediatric patients may be uncooperative during physical examinations, further complicating the diagnosis. Similarly, a case report conducted by Wang et al. (2019) found that 3 patients presented with nonspecific symptoms, and all patients had no abnormal findings in the right lower quadrant of the abdomen. Furthermore, 2 patients had perforated appendicitis. This further demonstrates that appendicitis in children can present atypically, and perforation can be a possible complication.⁷

All patients diagnosed with appendicitis will receive appropriate medication and treatment chosen based on the type of procedure, effectiveness of the medication, potential side effects, and drug interactions. Approximately 77.8% of newly diagnosed appendicitis patients underwent laparotomy, with an average appendectomy procedure yielding a mean value of 34313.48 ± 11620.96 (range: 26096.22 - 42530.74). On the other hand, laparotomy resulted in a mean value of 22530.13 ± 9521.73 (range: 14793.05 - 42530.74).⁷

In another study involving 102 cases of acute appendicitis diagnosed clinically, histopathological examination revealed that 93 cases showed evidence of appendicitis, while the remaining 9 cases showed no signs of appendiceal inflammation. From the histopathological examination, it was found that 9 patients (8.9%) had a normal appendix, 71 patients (69.6%) had acute appendicitis, and 22 patients (21.5%) had complicated appendicitis with perforation and gangrene. Therefore, a quarter of the patients experienced complicated appendicitis. Perforation occurred more frequently in female patients (30.4%) compared to male patients (13.9%). Based on age, four out of nine patients (44.4%) below the age of 5 experienced perforation, while five out of 40 patients (12.5%) aged 5 to 10 years experienced perforation. Thus, perforation was more common in patients younger than 5 years old ($p=0.06$). More than half of the patients (64%) underwent surgery with a duration of pain symptoms less than 24 hours, while approximately a quarter underwent surgery with a duration of pain symptoms between 24 and 72 hours. The average duration of pain symptoms for all patients was 38.6 hours. More than 60% of the patients presented with complicated appendicitis and a duration of pain symptoms exceeding 72 hours.³

In the study by Fenny et al. (2020) titled "Usage of Folic Acid as a Predictor of Complicated Appendicitis in *Oryctolagus Cuniculus* Animal Experiment in 2020", the research revealed the folate levels in acute appendicitis to be 101.73 ± 11.64 (Mean $\pm$ SD) and 104.93 (86.19-116.14) (Median (Min-Max)). Meanwhile, in complicated appendicitis, the folate levels were found to be 60 ± 20.56 (Mean $\pm$ SD) and 102.06 (54.31-122.24) (Median (Min-Max)). There was a significant difference in folate levels ($p < 0.001$) between the 18-hour group and the 24-hour group, the 24-hour group and the 36-hour group, and the 24-hour group and the 48-hour group after the animal intervention.⁶

In this study, the results of the Spearman correlation test showed a moderate negative correlation ($r = -0.609$) between the onset of symptoms and folate levels in pediatric appendicitis patients, but it was not statistically significant ($p > 0.05$). From the R^2 value, it can be inferred that folate levels can predict 53% of the onset of symptoms.

These findings differ from another study where significant differences in folate levels were found between the groups (18 hours, 24 hours, 36 hours, and 48 hours) with a p-value less than 0.05 regarding the occurrence of appendicitis in animal experiments. It was concluded that folate levels could be a significant predictive factor for the occurrence of complicated appendicitis in animal experiments.⁶

Another study showed that men require more folate than women to achieve the same folate status. Men require higher folate intake than women to reach the same erythrocyte folate concentration, primarily due to men having a larger lean body mass. The findings of this research suggest that the Recommended Dietary Allowance (RDA) for folate should be higher for men than for women.⁸

Folate deficiency can arise from various causes, including inadequate dietary intake. Heating during cooking destroys folate. Folate is absorbed in the jejunum through active and passive transport mechanisms across the intestinal wall. Therefore, conditions such as tropical sprue, short bowel syndrome, amyloidosis, gastric bypass, or mesenteric vascular insufficiency can hinder folate absorption, leading to deficiency. Increased pH, as seen in achlorhydria, can also result in poor folate absorption. Medications such as methotrexate, phenytoin, sulfasalazine, and trimethoprim can antagonize folate utilization, inhibit absorption, or impair its conversion to its active form, thus causing folate deficiency.⁹

Severe Acute Malnutrition (SAM) is often associated with deficiencies of iron, Vitamin B12, and Folic Acid during infancy and early childhood. Inadequate non-vegetarian food intake poses a risk of vitamin B12 and zinc deficiency. Subsequent malnutrition increases the risk of micronutrient deficiencies in low socioeconomic status, inadequate intake, and poor absorption. Deficiencies in vitamin B12 and folic acid lead to megaloblastic anemia, poor growth, and increased infections. The prevalence of folate deficiency was found to be 3 (3.8%). Deficiency is more common in young children, low socioeconomic status, those receiving exclusive breastfeeding, and/or late initiation of complementary feeding.¹

5. Conclusion

This study concludes with Kruskal-Wallis test showed no significant difference in folic acid levels among the three groups based on the onset of symptoms ($p > 0.05$), Spearman correlation test showed a moderate negative correlation ($r = -0.609$) between the onset of symptoms and folic acid levels in pediatric appendicitis patients, which was not statistically significant ($p > 0.05$) with the R^2 value indicates that folic acid can predict 53% of the variation in the onset of symptoms, and observed trend indicating that as folic acid levels decrease with increasing duration of symptoms, the likelihood of experiencing complicated appendicitis increases. Further research using a cohort study design is needed to assess a more accurate relationship with other risk factors and number of subjects should be increased. Cut-off value for folic acid in order to act as a predictor should also be established in future study.

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