



The Determination of Appendicitis from Folate Acid and Vascular Endothelial Growth Factor Level in Animal Model: A Review

Erjan Fikri^{1*}, Putri Chairani Eyanoe²

¹Department of Surgery, Division of Pediatric Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia;

²Department of Community and Preventive Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Abstract

Edited by: Ksenija Bogoeva-Kostovska

Citation: Fikri E, Eyanoe PC. The Determination of Appendicitis from Folate Acid and Vascular Endothelial Growth Factor Level in Animal Model: A Review. Open Access Maced J Med Sci. 2021 Oct 10; 9(F):395-398. <https://doi.org/10.3889/oamjms.2021.7069>

Keywords: Appendicitis; Animal model; Folate acid;

***Correspondence:** Erjan Fikri, Department of Surgery, Division of Pediatric Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: binnazief@gmail.com

Received: 15-Aug-2021

Revised: 26-Sep-2021

Accepted: 30-Sep-2021

Copyright: © 2021 Erjan Fikri, Putri Chairani Eyanoe

Funding: This research did not receive any financial support

Competing Interest: The authors have declared that no competing interest exists

Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

BACKGROUND: Appendicitis is one of the most common abdominal emergencies that require surgery in children. The morbidity and mortality rate in appendicitis is greatly affected by rupture or leakage of the appendix. In establishing the diagnosis of appendicitis, several modalities are acknowledged, namely, pediatric appendicitis score and ultrasound. Pathologically, severity and complications of appendicitis occur related to good vascularization and tissue healing process through process of angiogenesis influenced by folic acid (FA) and vascular endothelial growth factor (VEGF). This is clinically important as currently non-operative and non-invasive therapies were developing in stratification of mild appendicitis.

AIM: This study aimed to review the determination of appendicitis from FA and VEGF levels in animal model.

METHODS: This study was conducted in accordance with the PRISMA guidelines for reporting systematic reviews. Articles were reviewed for relation of FA and VEGF in determining appendicitis in PubMed and Science Direct. Articles on experimental animal model published from 1990 to 2020 were included, while articles in English were excluded from the study.

RESULTS: The articles we reviewed conduct an assessment of appendicitis by FA and VEGF level in animal model. Three articles were reviewed ranging from 1990 to 2020. One article presented a significant association of decreased level of FA in determining appendicitis while the other two mentioned trends of decreased level of FA and VEGF in appendicitis without statistically significance.

CONCLUSION: Reduction of folate acid levels could be a critical prescient factor for the weight of muddled an appendicitis in animal model, however, there was a pattern demonstrating low estimation of VEGF as an indicator of appendicitis and convoluted appendix with no measurable note worthiness appeared.

Introduction

Appendicitis is the most well-known gastrointestinal emergency requires a surgical approach in pediatric. In the 21st century, prevalence of appendicitis fluctuated over the world, ranging from 100 in North America to 140 in Oceania. A report from South Korea stated 206 cases of appendicitis in every 100,000 population [1].

Appendicitis in pediatric has burden of the event of negative appendectomy, which surgical procedure performed on patients with normal appendix. In pediatric, the negative appendectomy rate is 8.4%, however for children under 6 years, the number increases to 56.7% [2]. In a recent report in the United States, this negative appendectomy rate was 15% [3]. Hence, cost issue is an additional to problem to expend in those occasions with no appendicitis occurred [4]. Nonetheless, even with a total examination, the conclusion of an appendicitis in children did not present huge turn of events. This diagnostic challenge depends on correspondence and examination challenges [5].

Obsessively, confusions in appendicitis were related to acceptable mucosal opposition and vascularity (microvessel thickness) in the reference section mucosa. Mucosal opposition was strongly correlated to the capacity to recover, where folic acid (FA) assumes a part in this cycle [6]. Angiogenesis is additionally a significant factor tissue remodeling and healing. One factor affecting angiogenesis was vascular endothelial growth factor (VEGF), which has a pleiotropic feature in tissue remodeling through the cycle of neovascularization, reepithelization, and guide of extracellular framework [7]. This VEGF is explicit to vein endothelial cells [8].

Current development in laboratory diagnosis with biomarkers supports and increases accuracy in diagnosis of appendicitis, namely, severe appendicitis. This may suppress the rate of negative appendectomy. In contrast, there were no current guideline mentioning such approach [9]. These mechanisms were associated to activity of subepithelial macrophages and the surface epithelium in the appendix with luminal antigens which exerts a provocative reaction of inflammation in the appendix [10]. In this term, FA and VEGF may be

identified in the appendix by histopathological approach examining microvessel thickness. Consequently, it is need for researchers to analyze the impact of FA and VEGF levels on the event of a appendicitis. Therefore, we conducted a systematic review among animal research model of VEGF and FA on account of an appendicitis.

Methods

This study was conducted in accordance with the PRISMA guidelines for reporting systematic reviews. This study reviewed experimental studies in animal model reporting the determination of appendicitis with the FA and VEGF levels that were published from 1990 to 2020 using PubMed and Science Direct. The literature search was conducted with keywords and combined by Boolean Logic of (Appendicitis OR Acute Appendicitis) AND (FA OR Folate Acid OR FA) AND (VEGF OR VEGF) AND (Animal Model). Only animal research model was included with publications ranging from 1990 to 2020. Articles instead of English were excluded due to language inability of authors. References from selected articles were inspected to detect additional potential studies. The criteria of this study were as follows: (1) Reported experimental data on the determination of appendicitis, both chronic or acute case; (2) focused on animal model; (3) they described the methods used to assess or diagnose appendicitis, FA and VEGF level; and (4) the full text was available. We excluded studies focusing on other study method (e.g. cross-sectionals, cohort, and case series) and review articles. A pre-designed data extraction form was used to extract information on the following variables: Country, sample size, appendicitis examination, FA and VEGF examination, and the result of each study.

Results

Figure 1 shows the flowchart of the literature search strategy and the study selection process. Initially, 453 potential records were identified, of which 87 were retrieved from PubMed and 366 were retrieved from Science Direct. From title screening, the articles remaining were 17 articles and eight of them were excluded for not meeting inclusion criteria in full-text read. Subsequently, data of the remaining nine articles were read and six articles were removed for not being eligible, not analyzing the determination of appendicitis with FA and VEGF levels and for not being an animal model study. Therefore, we finally included three in our systematic review.

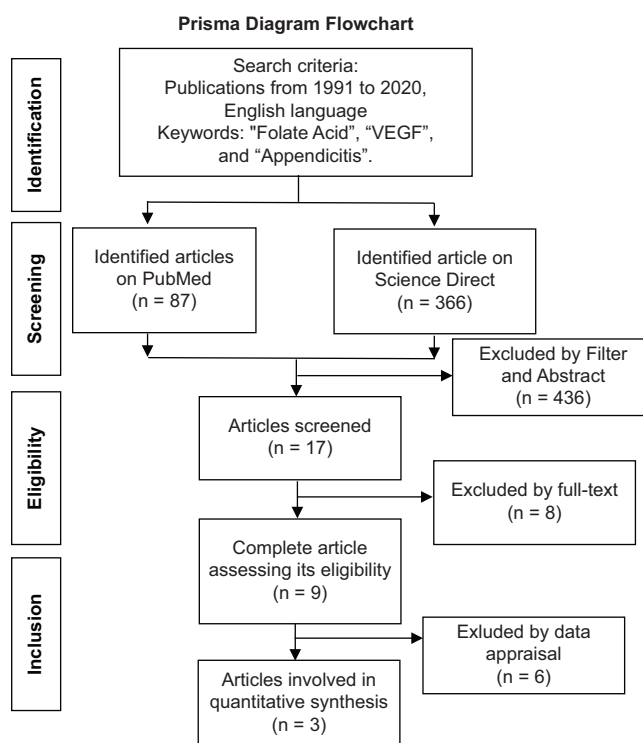


Figure 1: The PRISMA flowchart in identifying the literature included

Table 1 summarizes the characteristics of the included studies, two of which were from Indonesia and one from Israel. The sample size ranged from 20 male rabbits weighing for 2.500 to 3000 g, 32 rabbits used were male rabbits weighing 2500 to 3000 g, and one research used an 18 New Zealand type rabbits, males and females, 2–3 kg in weight. There three studies conducted similar methods, where obstruction of the appendix lumen with silk was no.0 at 18, 24, 36, and 48 hours. Blood samples were taken for serological examination of folate acid and VEGF using ELISA method. Appendectomy was performed for histopathological examination.

Discussion

From three studies evaluated, only one study (Fenny *et al.*, 2020) demonstrated a factually significant contrasts in the appendicitis assurance with FA. This exploration indicated that folate acid levels for all sample sources (18 h, 24 h, 36 h, and 48 h) were typically appropriated ($p > 0.05$), ANOVA test was directed to see distinction of folate acid levels between groups of samples. It additionally demonstrated that there was distinction in folate acid levels between groups ($p < 0.05$). Moreover, *post hoc* test was directed to analyze the distinction of folate acid levels in groups, utilizing least square difference indicated huge contrast of folate acid levels between samples collected in 18 h with 24 h, 24 h with 36 h, and 48 h. This clarifies that the connection between constant incendiary conditions, for

Table 1: Characteristics of studies

Article detail	Sample size	Methods	Treatment	Outcome	p-value
Fenny, <i>et al.</i> (2020) [11]	20 male rabbits (<i>Oryctolagus cuniculus</i>) weighing for 2500–3000 g	Experimental analytic research on animal model	<ul style="list-style-type: none"> The appendix lumen was then obstructed with silk no.0. at 18, 24, 36, and 48 h. Blood samples were taken for serological examination of folate acid using ELISA method. Appendectomy was performed for histopathological examination. 	Decreased levels of folate acid could be a predictive factor for complicated appendicitis in experimental animals	Folate acid (p=0.044)
Fikri, <i>et al.</i> (2020) [12]	32 male rabbits (<i>Oryctolagus cuniculus</i>) weighing 2500–3000 g	Experimental analytic research on animal model	<ul style="list-style-type: none"> Samples were examined for VEGF levels and then the appendix lumen was obstructed with silk no.-O thread. Thirteen hours later, blood samples are taken back to check VEGF and folate acid (FA) levels and an appendectomy is performed for histopathological examination 	There was a trend showing low value of VEGF and FA that were a predictor of appendicitis and complicated appendicitis.	VEGF (p=0.755) Folate acid (p=0.058)
Katz, <i>et al.</i> (1990) [13]	18 New Zealand type rabbits, males and females, 2–3 kg in weight, were used.	Experimental analytic research on animal model	<ul style="list-style-type: none"> An experimental model of acute appendicitis was established using no.-0 silk ties to block the base of the appendix. The clinical and histopathological picture of acute appendicitis was seen after 12 h in all the rabbits in the model group (9/9) and in none of the control group. Blood samples were taken at 0, 6, and 12 h for assay of LAP and Acid-P activities. 	No statistically significant difference between the experimental group and the control group, in the activities of LAP and Acid-P, was found at any time interval (p=0.928 and 0.802).	LAP (p=0.928) Acid-P (p=0.802)

example, systemic inflammatory illness and carcinoma have a solid relationship, and perhaps including folate acid job in directing DNA solidness through DNA methylation, combination, and fix component [11].

Low folate acid serum and raised degrees of homocysteine (Hcy) were generally detailed in patients with constant provocative problems, for example, systemic inflammatory illness and rheumatoid joint pain. Folate acid has been widely concentrated in cardiovascular sickness and malignancy, with an expanded danger of this infection in those with insufficient degrees of folate acid. Folate acid is likewise a contributing element to irritation, different examinations have demonstrated connection between folate acid status with cardiovascular illness and persistent provocative infections, for example, endothelial damage. Endothelial damage was characterized as a diminishing in vasomotor tone guideline through lopsided vasodilator and vasoconstrictor levels, which makes the endothelium move toward an inflammatory state. Folate acid can forestall endothelium damage by keeping up degrees of Hcy, vasodilators, and nitric oxide [14].

Fikri *et al.*, 2020, reported that there was a decrease in basal VEGF levels contrast to histopathological results. In appendicitis, the basal VEGF level was lower than control and typical; 8.61 (± 4.87), 7.66 (± 3.47), and 5.75 (± 4.88), respectively. Comparative pattern was found in basal FA levels. In convoluted an appendicitis, it has a lower level than both intense an appendicitis and typical; 2.00 (± 2.08), 1.14 (± 1.00), and 0.44 (± 0.22) all together. However, there were no statistical significance among both groups ($p = 0.775$ and $p = 0.058$). These outcomes have no significance but provide trend or pattern demonstrating low estimation of VEGF and FA as an indicator in diagnosis of appendicitis [12].

Other previous researches explained that plasma VEGF levels increase in a steady stage after myocardial localized necrosis and are related with

incendiary cytokines, yet not with atherosclerotic weight. The study presented that expanded degrees of VEGF are important for the progressing neovascularization of aroused plaque and initiates destabilization, VEGF levels had significant negative prognostic qualities. Hypoxia-inducible factor (HIF) is a mediator that controls vascular science, angiogenesis, metabolic reinventing, and irritation [5]. HIF manages the enrollment of inflammatory cells, erythropoiesis, tissue remodeling, and pH homeostasis. Furthermore, metabolic variations interceded by HIF for cell maturation and replication in a hypoxic state. Effects of HIF presented by VEGF-A, required for the advancement of angiogenesis, vascular patency development in fringe blood vessel infection, stroke, and myocardial dead tissue. Secretion VEGF is restoratively helpful in restricting tissue harm because of hypoxia/ischemia [15].

In accordance with another case clarified that with the barricade of VEGF, it will make more harm the heart tissue. Thus, it tends to be inferred that the diminishing in VEGF levels will cause more serious tissue harm. In another examination, control group had higher VEGF esteems contrasted with patients experiencing chronic thromboembolic pulmonary hypertension, which represents that angiogenesis problems may occur in disease progress [6], [16].

Katz *et al.* (1990) stated that there were no measurably critical contrasts found between the benchmark group and the trial bunch at any of the time stretches tried of Acid-P exercises [13]. Same as LAP, no measurably huge contrasts were found between the benchmark group and the test bunch at 0, 6, and 12 h. Between the two results no distinctive result shown in the range of 0 and 12 hours. The author clarified that their sever appendicitis causes extensive tissue necrosis and increase of permeability due to recruitment of GALT cells from circulations and vice versa. If so, one would expect changes in these particular blood test during severe acute appendicitis [17]. Author considered to quantify protein test in blood samples, before being mixed into systemic circulation with other protein expressions. This

blood test to diagnosis may provide potency of easier identification of appendicitis [18].

There were limited reports regarding blood tests detecting biomarkers in the unanesthetized animal model. The author did not discover any of those study related to a appendicitis [19]. In this perspective, their work is likely the primary utilizing nonstop catheterization and continued inspecting of blood samples, for the estimation of chemical exercises, in a test model of severe appendicitis, which was led in 1990 [20].

Conclusion

Reduction of folate acid levels provided potency as a critical prescient factor for the severity of appendicitis in animal model, however, there was a pattern demonstrating low estimation of VEGF that was an indicator of an appendicitis and convoluted appendix with no measurable noteworthy appearance.

References

- Ferris M, Quan S, Kaplan BS, Molodecky N, Ball CG, Chernoff GW, et al. The global incidence of appendicitis: A systematic review of population-based studies. *Ann Surg.* 2017;266(2):237-41. <https://doi.org/10.1097/sla.0000000000002188> PMID:28288060
- Smink DS, Finkelstein JA, Kleinman K, Fishman SJ. The effect of hospital volume of pediatric appendectomies on the misdiagnosis of appendicitis in children. *Pediatrics.* 2004;113(1):18-23. <https://doi.org/10.1542/peds.113.1.18> PMID:14702441
- Turhan AN, Kapan S, Kütükçüütu Yığıtbaş H, Hatipoğlu S, Aygün E. Comparison of operative and non-operative management of acute appendicitis. *Ulus Travma Acil Cerrahi Derg.* 2009;15(5):459-62. <https://doi.org/10.5505/tjtes.2013.81889> PMID:19779986
- D'Souza N, Marsden M, Bottomley S, Nagarajah N, Scutt F, Toh S. Cost-effectiveness of routine imaging of suspected appendicitis. *Ann R Coll Surg Engl.* 2018;100(1):47-51. <https://doi.org/10.1308/rcsann.2017.0132> PMID:29046077
- Rothrock SG, Skeoch G, Rush JJ, Johnson NE. Clinical features of misdiagnosed appendicitis in children. *Ann Emerg Med.* 1991;20(1):45-50. [https://doi.org/10.1016/s0196-0644\(05\)81117-5](https://doi.org/10.1016/s0196-0644(05)81117-5) PMID:1984727
- Ponziani FR, Cazzato IA, Danese S, Fagioli S, Gionchetti P, Annicchiarico BE, et al. Folate in gastrointestinal health and disease. *Eur Rev Med Pharmacol Sci.* 2012;16(1):376-85. PMID:22530356
- Inoshima N, Nakanishi Y, Minami T, Izumi M, Takayama K, Yoshino I, et al. The influence of dendritic cell infiltration and vascular endothelial growth factor expression on the prognosis of non-small cell lung cancer. *Clin Cancer Res.* 2002;8(11):3480-6. [https://doi.org/10.1016/s0959-8049\(01\)80925-4](https://doi.org/10.1016/s0959-8049(01)80925-4) PMID:12429638
- Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun.* 1989;161(2):851-8. [https://doi.org/10.1016/0006-291x\(89\)92678-8](https://doi.org/10.1016/0006-291x(89)92678-8) PMID:2735925
- Marchand A, Van-Lente F, Galen RS. The assessment of laboratory tests in the diagnosis of acute appendicitis. *Amer. J. Clin. Pathol.* 1983;80(3):369-74. <https://doi.org/10.1093/ajcp/80.3.369> PMID:6881101
- Giampalmo A, Berardi L, Martucciello G, Musso GL, Rossi F. Enzymatic activation of lymphoid population following inflammatory reactions in the human appendix. *Mikrosk Anat Forsch.* 1983;97:785.
- Fenny N, Fikri E, Eyanor P. Folate Acid Analysis in Predicting Complicated Appendicitis in Animal Model *Oryctolagus Cuniculus*. Utara: Department of Surgery, Universitas Sumatera Utara; 2020.
- Fikri E, Lubis HM, Surya B, Siregar KB. The role of vascular endothelial growth factor as a predictor of complicated appendicitis in animal model *Oryctolagus cuniculus*. *Open Access Maced J Med Sci.* 2020;8(A):261-5. <https://doi.org/10.3889/oamjms.2020.3902>
- Kats E, Hana R, Jurim O, Pisuv G, Gutman A, Durst A. Experimental model of acute appendicitis in the rabbit with determination of leucine amino peptidase (LAP) and acid phosphatase (Acid-P) activities in portal blood samples. *J Surg Res.* 1990;48(3):230-6. PMID:2314096
- Brunicaardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Matthews JB. *Schwartzs Principles of Surgery.* 10th ed. New York: McGraw-Hill Education; 2015.
- Jones P, Lucocka M, Scarletta CJ, Veysey V, Becketta EL. Folate and inflammation links between folate and features of inflammatory conditions. *J Nutr Intermed Metab.* 2019;18:1-6. <https://doi.org/10.1016/j.jnim.2019.100104>
- Hansen LW, Dolgin SE. Trends in the diagnosis and management of pediatric appendicitis. *Pediatr Rev.* 2016;37(2):52-8. <https://doi.org/10.1542/pir.2015-0021> PMID:26834224
- Ramakrishnan S, Anand V, Roy S. Vascular endothelial growth factor signaling in hypoxia and inflammation. *J Neuroimmune Pharmacol.* 2014;9(2):142-60. <https://doi.org/10.1007/s11481-014-9531-7> PMID:24610033
- Wan J, Lata C, Santilli A, Green D, Roy S, Santilli S. Supplemental oxygen reverses hypoxia-induced smooth muscle cell proliferation by modulating HIF-alpha and VEGF levels in a rabbit arteriovenous fistula model. *Ann Vasc Surg.* 2014;28(3):725-36. <https://doi.org/10.1016/j.avsg.2013.10.007> PMID:24345704
- Quarck R, Wynants M, Verbeken E, Meyns B, Delcroix M. Contribution of inflammation and impaired angiogenesis to the pathobiology of chronic thromboembolic pulmonary hypertension. *Eur Respir J.* 2015;46(2):431-43. <https://doi.org/10.1183/09031936.00009914> PMID:26113681
- Marchand A, Van-Lente F, Galen RS. The assessment of laboratory tests in the diagnosis of acute appendicitis. *Am J Clin Pathol.* 1983;80:369-74. <https://doi.org/10.1093/ajcp/80.3.369> PMID:6881101