

# THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN PREDICTING COMPLICATED APPENDICITIS IN PEDIATRIC APPENDICITIS PATIENTIN 2024

Yolanda Rahayu M Simamora<sup>1</sup>, Erjan Fikri<sup>2</sup>, Kamal Basri Siregar<sup>2</sup>

<sup>1</sup>Resident of General Surgery, Faculty of Medicine, Sumatera Utara University, Haji Adam Malik General Central Hospital, Indonesia

<sup>2</sup>General Department of Surgery, , Faculty of Medicine, Sumatera Utara University, Haji Adam Malik General Central Hospital, Indonesia

## ABSTRACT

**Background:** Appendicitis is one of the most common pediatric emergency surgeries, with complication risks influenced by various factors, including Vascular Endothelial Growth Factor (VEGF). VEGF plays a crucial role in angiogenesis, affecting wound healing and the development of complications.

**Methods:** This prospective analytic study analyzed VEGF levels as a predictive factor for complicated appendicitis in 20 pediatric patients. Blood samples were taken and examined serially at intervals of  $\leq 24$  hours, 25-48 hours, 49-72 hours, 73-96 hours, and more than 96 hours from symptom onset.

**Results:** The average VEGF levels were within the normal range (31.25–2000 pg/ml) in 80% of patients. There was no statistically significant difference between VEGF levels and macroscopic findings of appendicitis ( $p > 0.05$ ). However, a trend of decreasing VEGF levels was observed with the increasing duration of symptoms.

**Discussion:** VEGF plays a role in angiogenesis and mucosal regeneration in appendicitis. Lower VEGF levels indicate an increased risk of complicated appendicitis. These findings align with previous studies showing VEGF reduction is associated with complications.

**Conclusion:** VEGF levels tend to be lower in complicated appendicitis, though not statistically significant ( $p > 0.05$ ). Further research with a larger sample size is needed to determine the VEGF cutoff for prediction.

**Keywords:** Appendicitis, VEGF, Children, Complicated, Angiogenesis

## Introduction

Appendicitis is one of the most commonly encountered conditions in surgical practice, particularly among children. It is the most common emergency surgery in the United States, with over 300,000 inpatient cases each year. The incidence of appendicitis is estimated to be 86 cases per 100,000 children annually, with numbers continuing to rise. In various regions such as Europe, America, and Australia, the incidence of appendicitis has significantly increased during the first half of this century, with approximately 16% of the population undergoing an appendectomy. The lifetime risk of experiencing appendicitis is estimated to be 6-7%, especially during the second decade of life. After reaching middle age, the risk of appendicitis decreases. Before puberty, the incidence of appendicitis is balanced between males and females, but in adolescence and young adulthood, the male-to-female ratio increases to 3:2 by age 25 (Richmond et al, 2017; Peter et al, 2020; Mulsow et al, 2018).

Appendicitis can be classified into non-complicated and complicated types. The pathogenesis of acute appendicitis involves inflammation of the appendiceal wall, which can progress to ischemia, necrosis, and eventually perforation. If perforation occurs, it can lead to local abscess formation or the development of phlegmon, which are characteristic of complicated appendicitis. The risk of perforation in non-complicated appendicitis is relatively low within the first 24 hours after symptom onset, but it increases to 6% after 36 hours and remains at 5% for every subsequent 12-hour

period. Understanding these types of appendicitis is crucial for determining preoperative, intraoperative, and postoperative management approaches (Mulsow et al, 2018; Mariage et al, 2019; Nimmagadda et al, 2019).

Perforation has a significant impact on patient morbidity. Factors such as race, socioeconomic status, and health insurance status can influence the risk of perforation. In perforated appendicitis, the risk of complications such as intra-abdominal abscesses, wound infections, or postoperative ileus increases up to 39%, compared to only 8% in non-perforated appendicitis (Singh et al, 2014; Howell et al, 2018). Further research indicates that morbidity in perforated appendicitis is often difficult to diagnose quickly, leading to delays in treatment.

From a pathological perspective, the resilience of the appendiceal mucosa and good vascularization, including microvessel density, play a crucial role in preventing complications of appendicitis. The process of angiogenesis, triggered by growth factors such as VEGF, is essential for wound healing through neovascularization, reepithelialization, and extracellular matrix regulation (Ponziani et al, 2012). In this context, VEGF has a pleiotropic role in repairing damaged tissue and in the regeneration of the appendiceal mucosa involved in the inflammatory process.

Based on the literature, this study selected the measurement time during the acute phase at 24 hours, as previous studies, such as those conducted by Erjan Fikri (2019), indicated that measurements taken at 13 hours after intervention yield significant results. Additionally, literature described by Jones, MW et al (2021) and Howell et al (2018) suggests that acute appendicitis can develop within 24 hours. For complicated appendicitis, the measurement time was extended to periods beyond 24 hours, up to 48 hours or more, based on the available data (Fikri et al, 2019).

Other research shows that lower VEGF levels are associated with a higher risk of developing complicated appendicitis. In studies involving animal models, significant differences in VEGF levels were observed across different time groups (18 hours, 24 hours, 36 hours, and 48 hours), with  $p < 0.05$ , indicating that VEGF is a significant predictive factor for the occurrence of complicated appendicitis (Simamora, Yolanda et al, 2021).

Based on the theories and research, this study divided the samples into five groups with serial examinations at  $\leq 24$  hours, 25-48 hours, 49-72 hours, 73-96 hours, and more than 96 hours after the onset of symptoms. Blood samples were collected when the patient was diagnosed with appendicitis and before the appendectomy was performed.

## Methods

This study used a prospective analytical research design aimed at analyzing Vascular Endothelial Growth Factor (VEGF) levels as a predictive factor for the occurrence of complicated appendicitis in pediatric patients with appendicitis. The research samples were taken from pediatric patients hospitalized at RSUP Haji Adam Malik Medan and its affiliated hospitals, according to inclusion and exclusion criteria. VEGF measurements were conducted at various time intervals based on the duration of symptoms before the surgical procedure: within the first 24 hours, 25-48 hours, 49-72 hours, 73-96 hours, and beyond 96 hours. VEGF data were obtained through serological blood tests, while histopathological results were examined from appendiceal specimens after appendectomy.

## Result

This study was conducted at RSUP H. Adam Malik and several affiliated hospitals. It was a prospective analytical study aimed at analyzing the serology of Vascular Endothelial Growth Factor (VEGF) in pediatric patients. There were 20 appendicitis patients who met the inclusion criteria.

### 1. Characteristics of The Sample

Table 1 Basic characteristics of The Patient

characteristics	N	Percentase (%)
Age		

Infants and Toddler (< 2 Years)	0	0%
Children (2-11 Years)	6	30%
Adolescents (12-18 Years)	14	70%
<i>Jenis Kelamin</i>		
Man	12	60%
Women	8	40%
<i>Macroscopic findings Apendiks</i>		
Acute	5	25%
Perforation	13	65%
Chronic	2	10%
<i>Time of onset of disease complaints (Hours)</i>		
≤24	6	30%
25-48	6	30%
49-72	4	20%
73-96	0	0%
>96	4	20%

In this study, the age range with the highest number of samples was patients aged 12-18 years, with 14 patients (70%), while no samples were found in those under 2 years of age. The data also show that there were 12 male patients (60%) and 8 female patients (40%). The type of appendicitis was determined after the patients underwent surgery, based on macroscopic evaluation. The study found that 13 samples (65%) had perforated appendicitis, 5 samples (25%) had acute appendicitis, and 2 samples (10%) had chronic appendicitis. Regarding the onset of symptoms, the most common time intervals were ≤24 hours and 25-48 hours, with each interval having 6 patients (30%).

Table 2 VEGF Levels In Pediatric Appendicitis Patients

Variabel		Amount
VEGF	<31.25 pg/ml	4(20)

31.25-2000 pg/ml	16 (80)
> 2000 pg/ml	0 (0)

The average VEGF levels found in this study were normal, within the range of 31.25-2000 pg/ml, in 16 patients (80%).

## 2. Differences in VEGF Levels Based on Macroscopic Findings

Based on this study, VEGF levels in pediatric appendicitis patients were assessed according to macroscopic findings.

Table 3 VEGF Level in Pediatric Appendicitis Patient

Characteristic	VEGF (pg/ml)		P-Value	PR
	Mean ( $\pm$ SD)	Median (Min-Max)		
Macroscopic				
Acute	209.71( $\pm$ 165.4)	170.28(11.96-385.5)	0.298*	1.1
Perforation	130.04( $\pm$ 112.8)	109.68 (10.18-365.0)		(95%CI)
Chronic	53.02( $\pm$ 53.64)	53.02(15.09-90.96)		

\*ANOVA;  $p < 0.05$  considered statistically significant

The results showed that VEGF levels in acute appendicitis were 209.71 ( $\pm$ 165.4) pg/mL, in perforated appendicitis were 130.04 ( $\pm$ 112.8) pg/mL, and in chronic appendicitis were 53.02 ( $\pm$ 53.64) pg/mL. The analysis of VEGF levels relative to macroscopic findings of the appendix yielded a prevalence ratio of 1.1 (95% CI). However, based on ANOVA testing, there was no statistically significant difference ( $p = 0.298$ ).

## 3. Differences in VEGF Levels Based on Symptom Onset Time

Results from the Spearman test showed a correlation ( $r = -0.069$ ) between the time of symptom onset and VEGF levels in pediatric appendicitis patients, although this correlation was not statistically significant ( $p > 0.05$ ). From chart 1, it is observed that VEGF levels tend to decrease as the duration of symptoms increases. Based on the  $R^2$  value, it can be determined that VEGF levels can predict the onset time of symptoms by 6.9%.

VEGF	R2 Value	P Value*
Time	.069	.292*

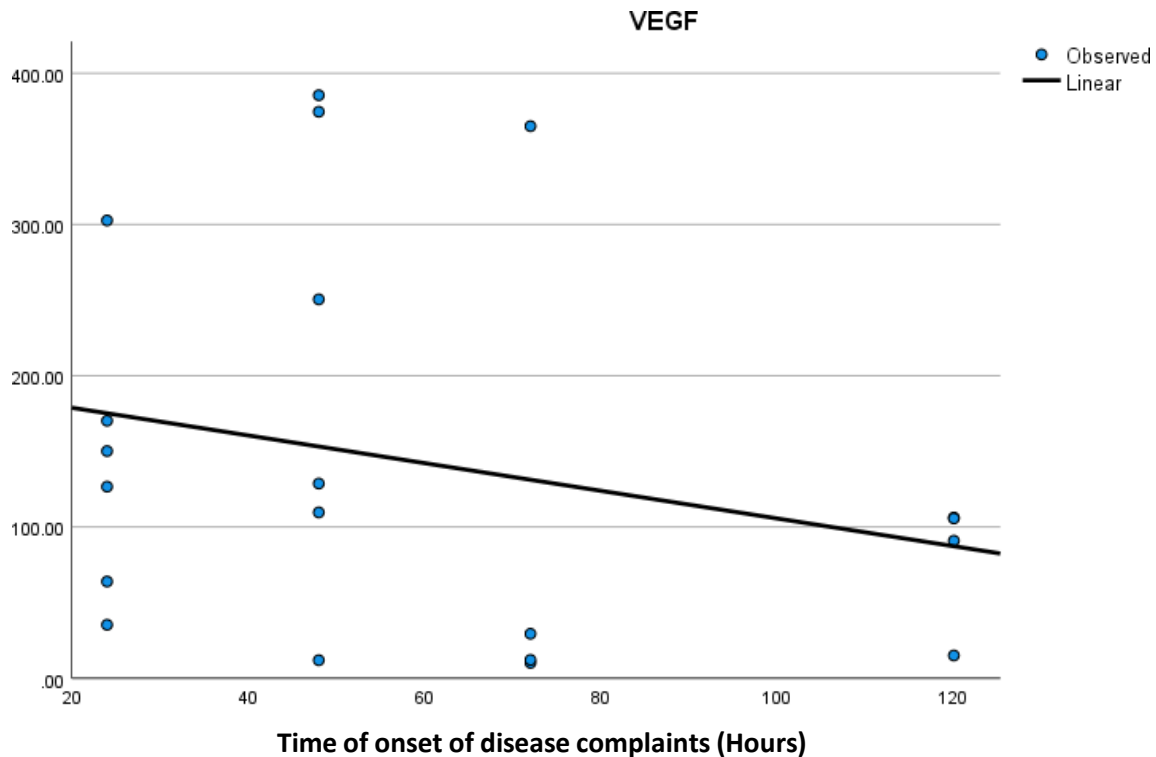


Chart 1 Correlation of VEGF Values with Symptom Onset Time

## Discussion

Appendicitis is one of the most common surgical emergencies encountered in children. Its prevalence increases, peaking during the second decade of life. The incidence of appendicitis in children ranges from 11 to 14 cases per 10,000, but less than 0.4% of cases occur in the first year of life. The classic presentation of appendicitis is the onset of periumbilical pain that migrates to the lower right quadrant, accompanied by mild fever and nausea or vomiting. In children, these symptoms are rarely classic and occur in less than 50% of cases. Due to its atypical clinical presentation, there is a potential for diagnostic delay, which can increase the risk of complications (Bence, 2020; Ericki, 2017).

This study was conducted on patients diagnosed with appendicitis at RSUP H. Adam Malik and its affiliated hospitals, with 20 samples meeting the inclusion criteria. The average age of patients in this study was in the 12-18 year age group (adolescents). Although appendicitis can occur across a range of ages, it is most common between the ages of 10 and 19 years (Gadiparthi, 2024). Another study by Withers in 2019 found that acute appendicitis occurred at an average age of 8.76 years in their study population. It has been demonstrated that inflammation of the appendix occurs more rapidly in younger patients. Variations in the development of appendiceal structures according to patient age may explain differences in disease progression. Thin-walled appendices and omental function in younger children can lead to rapid infection spread (Ericki, 2017). This study did not find any patients diagnosed with appendicitis under the age of 2 years. Research by Bence et al. in 2020 reported that the prevalence of appendicitis in children under 2 years of age is only about 0.38%. The low incidence of appendicitis in this age group is thought to be due to anatomical differences in the first year of life. Initially, the appendix is more funnel-shaped with fewer lymphoid tissues, resulting in a lower risk of obstruction and appendiceal inflammation. The appendix develops into a cone shape similar to adults after the age of one to two years, with lymphoid follicle hyperplasia continuing until late adolescence. Experts have also studied that the soft diet of infants reduces the frequency of appendicitis in children under 1 year of age (Bence, 2020; Turco, 2023).

Based on gender variations, this study found that males suffered from appendicitis more frequently than females, with a ratio of 3:2. Males and females have differences in hormone levels, particularly estrogen, which plays a role in the immune system through pro-inflammatory and anti-inflammatory cytokines. Consistent with this, other studies have reported variations in appendicitis incidence between male and female pediatric patients. Alloo et al. and Ngim et al. reported a male-to-female ratio of 60:40 in their studies. Research by Salim et al. at an educational center in Banten found that 53.3% of all males and 33.3% of all females experienced appendicitis, resulting in a gender ratio of 4:1. A 2020 study from the United States showed similar prevalence data, with 59.4% in males and 40.6% in females. This is comparable to Bansal et al.'s 2012 study, which documented a nearly identical ratio of 51% males and 49% females. Another study reported a contradiction, showing a 12% risk of appendicitis in males and 25% in females (Gadiparthi, 2024). Gender differences potentially affect susceptibility to inflammatory conditions, including appendicitis. Hormonal disparities between males and females may predispose individuals to this condition. Estrogen, through its receptors ER $\alpha$  and ER $\beta$ , can trigger secondary signaling to dysregulate leukocytes (neutrophils, macrophages, dendritic cells, T-helper cells, and B cells) and specific cytokines (interleukin-1, interleukin-6, tumor necrosis factor  $\alpha$ , and immunoglobulins), leading to a pro-inflammatory state in the host and thereby increasing susceptibility to appendicitis (Salim, 2021; Taneja, 2018).

This study also assessed the onset time of symptoms in relation to the diagnosis of appendicitis. Symptoms evaluated included lower right abdominal pain, referred pain, and diffuse abdominal pain, confirmed through clinical examination. On average, patients presented with symptoms between 24-48 hours after onset. The classic presentation of appendicitis includes periumbilical abdominal pain that gradually migrates to the lower right quadrant, usually within 24 hours. Pediatric patients may also experience fever, anorexia, nausea, vomiting, and diarrhea. However, infants and young children may not exhibit this pattern at all. Experienced clinicians can diagnose acute appendicitis with over 90% accuracy. One factor affecting delays is the difficulty of adequately visualizing the appendix in suspected appendicitis patients. Even with step-by-step examinations, visualization failure can occur, especially in patients with severe pain or obesity. Anxiety and uncooperative young patients also contribute to unclear visualization, particularly in younger children (Mallick, 2008).

The duration of symptom onset may be a key factor in the high rate of appendiceal perforation in children, as the signs and symptoms may be less specific (Mallick, 2008). Research by Narsule et al. clearly found that longer delays between symptom onset and surgical intervention are associated with increased perforation rates in children. No children with symptoms for less than 12 hours experienced perforated appendicitis. The prevalence of perforation increased linearly from 10% at 18 hours to 44% at 36 hours. If symptoms persist for more than two days, the risk of perforation exceeds 40%. Literature studying appendicitis in children indicates that the longer appendicitis symptoms are experienced, the higher the risk of perforation (Howell et al., 2018). Regression analysis reports that the relative risk of perforation can increase by 9% (RR 1.09,  $p < 0.001$ ) for each 24-hour delay in the management of acute appendicitis. Increasing age (RR 1.03), male sex (RR 1.50), body temperature upon hospital admission (RR 1.32), and the presence of fecalith (RR 1.89) are statistically significant for increasing the risk of perforation. The risk of postoperative abscess also increased by 8% for each day of delay (RR 1.08,  $p = 0.027$ ) (Westfall, 2019). Research by Jiang in 2020 on adult patients confirmed this, finding that one of the independent risk factors for appendiceal perforation was a symptom duration of  $>48$  hours (OR = 4.64, 95% CI: 1.76-12.27) (Jiang, 2021).

Vascular endothelial growth factor (VEGF) is a crucial mediator in the angiogenesis process. VEGF is secreted by most parenchymal and endothelial cells and by some activated immune cells. This protein plays a major role in both physiological and pathological angiogenesis. However, little is known about the potential use of serum VEGF levels as a biomarker for inflammatory diseases, especially in appendicitis cases. In this study, the serum VEGF levels were found to be normal, with 16 patients (80%) in the range of 31.25-2000 pg/ml, while only 4 patients (20%) had VEGF levels below normal. A study by Kanzawa et al. in 2001 found that in patients diagnosed with inflammatory bowel disease (IBD), VEGF levels increased in serum and colon tissue, suggesting it might contribute to the pathogenesis of the disease. Vascular endothelial growth factor (VEGF) A regulates microvascular development through angiogenic, mitogenic, and strong vascular permeability activities and may play an essential role in neonatal gut (Sabnis, 2015). Research on rodents showed decreased VEGF protein levels in the small intestine specimens of premature neonates with NEC. These findings suggest that reduced VEGF regulation may play a role in small bowel inflammation.

In another study by Eržen et al., VEGF levels in plasma increased during the stable phase after myocardial infarction and were associated with inflammatory cytokines but not with atherosclerotic burden. This suggests that elevated VEGF levels are part of the ongoing inflammatory activity. Since VEGF stimulates neovascularization of inflamed plaques and induces destabilization, VEGF levels may have significant negative prognostic value (Eržen, 2014).

This study also assessed the comparison of VEGF levels with the macroscopic findings of the appendix after surgery. It was found that there was a tendency for lower VEGF levels in perforated appendicitis compared to acute appendicitis, although the results of the ANOVA test were not statistically significant ( $p = 0.298$ ). However, the analysis of VEGF levels with macroscopic findings of the appendix showed a prevalence ratio of 1.1 (95% CI), indicating a 1.1-fold chance of appendicitis developing into perforation with decreased VEGF levels. Previous research on animals by Simamora in 2021 found that VEGF levels in complicated appendicitis were generally lower compared to acute appendicitis. Research by Fikri in 2020 also supports this, showing a correlation between decreased VEGF levels and complications of appendicitis (Fikri, 2020).

Pathologically, complications in appendicitis are influenced by mucosal resilience and good vascularization (microvessel density) in the appendix mucosa. This resilience is determined by the ability to regenerate, where VEGF plays a pleiotropic role in tissue healing through neovascularization, re-epithelialization, and extracellular matrix regulation (Ponziani et al., 2012). VEGF induces the formation of penetrations between endothelial cells in capillaries and venules by altering proteins at cell junctions (occludin, VE-cadherin/ $\beta$ -catenin), thus increasing vascular permeability and facilitating endothelial cell development. Angiopoietins (Ang-1 and Ang-2) are pro-angiogenic and vascular remodeling growth factors related to VEGF levels. In normal adult tissues, Ang-1 maintains vessel integrity, enhances endothelial cell survival (anti-apoptotic effects), and inhibits Ang-2 expression, while Ang-2 has pro-angiogenic or anti-angiogenic effects that are inversely related to VEGF presence. Low or absent VEGF levels lead to Ang-2 promoting endothelial cell apoptosis, vessel regression, and inhibiting angiogenesis (Melincovici, 2018).

VEGF-A is a dominant factor in regulating angiogenesis and endothelial cell growth. Vasculogenesis and angiogenesis are triggered by increased local and systemic VEGF levels. Angiogenesis is a crucial component of tissue growth and repair, relying on the adequate formation of new capillaries from existing vessels. One stimulus for high VEGF expression is hypoxia. Decreased oxygen pressure stimulates vascular endothelial cells to produce VEGF, leading to angiogenesis, an essential process also occurring in inflammation (Suyasa, 2020).

In pathological angiogenesis, VEGF promotes the mobilization of inflammatory cells (macrophages, granulocytes, etc.) to the injury site, sustains local inflammation, and induces the synthesis of pro-angiogenic factors by endothelial cells, cytokines, platelets, fibroblasts, and tumor cells. Hypoxia is a primary trigger for angiogenesis, but other factors may also be responsible: hypoglycemia, hypertension, low pH, mechanical stress, and chronic inflammation. Hypoxic tissue releases hypoxia-inducible factor-1 (HIF-1), which activates the transcription of pro-angiogenic factors such as VEGF. In hypoxic conditions, injured tissue and endothelial cells release NO, promoting vasodilation. Increased VEGF secretion is also induced by other growth factors through paracrine mechanisms (Melincovici, 2018).

In this study, there was also a trend of decreasing VEGF levels after passing through the acute phase. The Spearman test results showed a correlation ( $r = -0.069$ ) between the onset time of symptoms and VEGF levels in pediatric appendicitis patients, but this was not statistically significant ( $p > 0.05$ ). Based on the  $R^2$  value, VEGF levels can predict the onset time of symptoms by 6.9%. Research by Fikri in 2020 observed a trend of increased VEGF levels after 13 hours of intervention, although some samples showed a decrease in VEGF levels after 13 hours of intervention. Wilcoxon test analysis showed a p-value of 0.172, indicating no significant difference between variables. Other research by Simamora in 2021 on *Oryctolagus cuniculus* rodents found VEGF levels at 18 hours were  $6.71 \pm 2.96$ , at 24 hours were  $6.75 \pm 3.15$ , at 36 hours were  $7.57 \pm 2.57$ , and at 48 hours were  $6.58 \pm 2.37$ . However, these differences in VEGF levels based on measurement times were not statistically significant ( $p > 0.05$ ). Research on stroke conducted in 2014 confirmed this, showing an increase in VEGF expression from 2-6 hours after ischemic conditions due to cerebral artery occlusion, normalizing within 12 hours before rising again after 3-7 days and returning to baseline levels after 2 weeks (Zan, 2014).

## Conclusion



The conclusion of this study shows that the majority of the study sample consists of children aged 12-18 years (70%), with the majority being male (60%), and the most common type of appendicitis is perforated appendicitis (65%). ANOVA testing did not find a significant difference in VEGF levels based on macroscopic findings of the appendix ( $p > 0.05$ ), but there is a tendency that as the duration of symptoms increases, VEGF levels decrease, which raises the risk of complicated appendicitis with a prevalence ratio of 1.1. Spearman's test results show a negative correlation between the onset time of symptoms and VEGF levels, although this is not statistically significant ( $p > 0.05$ ). Recommendations for future research include increasing the sample size, standardizing sample groups, adding control variables, and determining a VEGF cut-off level for predicting complicated appendicitis.

## References

- Bence CM, Densmore JC: Neonatal and infant appendicitis . Clin Perinatol. 2020, 47:183-96. 10.1016/j.clp.2019.10.004
- Ericks, V. S. 2017. Pediatric Appendicitis and Its Management: A Review Article. *Remedy Publications LLC*, pp. 1-5.
- Eržen, B., Šilar, M., & Šabovič, M. (2014). *Stable phase post-MI patients have elevated VEGF levels correlated with inflammation markers, but not with atherosclerotic burden.* *BMC Cardiovascular Disorders*, 14(1). doi:10.1186/1471-2261-14-166
- Fikri, E. *et al.* The Role of Vascular Endothelial Growth Factor as A Predictor of Complicated Appendicitis in Animal Model *Oryctolagus cuniculus*. Medan: Open Access Macedonian Journal of Medical Sciences, 2020. 261-265.
- Gadiparthi R, Waseem M. Pediatric Appendicitis. [Updated 2023 Jul 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK441864/>
- Howell, Erin C., Dubina, Emily D., Lee, Steven L. 2018. Perforation Risk in Pediatric Appendicitis: Assessment and Management. *Dovepress*, pp. 135- 142.
- Jiang, L., Liu, Z., Tong, X., Deng, Y., Liu, J., Yang, X., Chan, F.S. and Fan, J.K., 2021. Does the time from symptom onset to surgery affect the outcomes of patients with acute appendicitis? A prospective cohort study of 255 patients. *Asian Journal of Endoscopic Surgery*, 14(3), pp.361-367.
- Jones, MW., Lopez, RA., dan Deppen, JG. 2021. Appendicitis. StatPearls Publishing LLC.
- Mallick MS. Appendicitis in pre-school children: a continuing clinical challenge. A retrospective study. *Int J Surg* 2008;6:371-3
- Mariage M et al, Surgeon's definition of complicated appendicitis: a prospective video survey study. *European Journal of Hepato-Gastroenterology*. 9(1); 2019, pp: 1-4
- Melincovici, C.S., Boşca, A.B., Şuşman, S., Mărginean, M., Mişu, C., Istrate, M., Moldovan, I.M., Roman, A.L. and Mişu, C.M., 2018. Vascular endothelial growth factor (VEGF)-key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol*, 59(2), pp.455-467.
- Mulsow J. *The vermiform appendix*. In: Short Practice of Surgery, 27<sup>th</sup> edition. Williams NS et al, eds. Boca Raton: *CRC Press Taylor & Francis Group*. 2018. pp: 1299-1317
- Nimmagadda N et al. Complicated appendicitis: immediate operation or trial of nonoperative management. *America Journal of Surgery*. 217 (4); 2019, pp: 713-717.
- Peter S.D. & Wester T. *Appendicitis*. In: Holcomb and Ashcraft's Pediatric Surgery, 7<sup>th</sup> edition. Holcomb JW et al, eds. China: Elsevier. 2020. pp: 664- 678.
- Ponziani, F.R.; Cazzato, I.A.; Danese, S.; Fagioli, S.; Gionchetti, P.; Annicchiarico, B.E.; et al. (2012) 'Folate in gastrointestinal health and disease', *European Review for Medical and Pharmacological Sciences*, 16(3), pp. 376-385.
- Richmond, B. *The appendix*. In: Sabiston Textbook of Surgery, 20<sup>th</sup> edition. Townsend CM et al, eds. China: Elsevier. 2017. pp: 1296-1311.
- Salim, J., Agustina F., Maker, J. J.R., 2021. Pre-Coronavirus Disease 2019 Pediatric Acute Appendicitis: Risk Factors Model and Diagnosis Modality in a Developing Low-Income Country. *Pediatr Gastroenterol Hepatol Nutr*. 2022 Jan;25(1):30-40
- Simamora, YRM. 2021. *Analisis Vascular Endothelial Growth Factor (VEGF) dalam Memprediksi Apendisitis Komplikata pada Hewan Coba Oryctolagus Cuniculus Tahun 2021*. USU Repository
- Singh M et al, Complicated appendicitis: Analysis of risk factors in children. *Afr J Paediatr Surg*. Apr-Jun 2014;11(2). pp. 109-13.
- Suyasa IK, Wiradewi Lestari AA. Low expression of vascular endothelial growth factor and high serum level of cyclic guanine monophosphate as the risk factors of femoral head osteonecrosis in alcohol-exposed Wistar rat. *Chin J Traumatol*. 2020 Apr;23(2):107-112.
- Taneja V. Sex hormones determine immune response. *Front Immunol* 2018;9:1931.



Turco R, Angela Mauro, Paolo Quitadamo, Giovanni Gaglione, Francesco Esposito, Giovanni Di Nardo, Vincenzo Tipo; et al. 2023. Acute appendicitis in infants. *Journal of Pediatric Surgery Case Reports*, Volume 88.

Zan, L., Zhang, X., Xi, Y., Wu, H., Song, Y., Teng, G., Li, H., Qi, J. and Wang, J., 2014. Src regulates angiogenic factors and vascular permeability after focal cerebral ischemia–reperfusion. *Neuroscience*, 262, pp.118-128.