The Role of Vascular Endothelial Growth Factor as a Predictor of Complicated Appendicitis in Animal Model Oryctolagus cuniculus

Erjan Fikri1*, H. M. Nadjib Dahlan Lubis2, Bachtiar Surya3, Kamal Basri Siregar4

1Department of Surgery, Division of Pediatric Surgery, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia; 2Department of Pathology Anatomy, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia; 3Department of Surgery, Division of Digestive Surgery, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia; 4Department of Surgery, Division of Oncology Surgery, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia

Abstract

BACKGROUND: Appendicitis is the most common abdominal disease that requires surgery in children. The mortality rate in appendicitis is greatly affected by rupture or leakage of the appendix. In establishing the diagnosis of appendicitis, several modalities are needed, namely, with a pediatric appendicitis score and ultrasound. In addition to operative measures, non-operative therapeutic research is also developing, especially for cases of appendicitis without complications. Pathologically, the occurrence of complications in appendicitis is influenced by good vascularization for tissue healing through the process of angiogenesis by vascular endothelial growth factor (VEGF).

AIM: We aimed to see the effect of VEGF and folic acid levels on the occurrence of complicated appendicitis.

METHODS: This research method is laboratory experimental design. A total of 32 rabbits used were male rabbits weighing 2500–3000 g that met the inclusion and exclusion criteria. Samples were examined for VEGF levels and the appendix lumen was obstructed with Silk No.-O thread. Thirteen hours later, blood samples are taken back and inspection difficulties [5].

RESULTS: There was a decrease of basal VEGF levels regarding histopathological results. In complicated appendicitis, the basal VEGF level was lower than acute appendicitis and normal; 8.61 (±4.87), 7.66 (±3.47), and 5.75 (±4.88), respectively. Similar trend was found in basal FA levels. In complicated appendicitis, it has a lower level than both acute appendicitis and normal; 2.00 (±2.08), 1.14 (±1.00), and 0.44 (±0.22) in order. However, there is no statistical significance in both trends (p = 0.775 and p = 0.058).

CONCLUSION: There was a trend showing low value of VEGF and FA that were a predictor of appendicitis and complicated appendicitis. Unfortunately, no statistical significance was shown in this study.

Introduction

Appendicitis is the most common abdominal disease that requires surgery in children. In the 21st century, the incidence (per 100,000 people/year) in the world is quite varied. In the northern part of the Americas, there was an incidence of 100, in North Europe 112, in South Europe 112, in East Europe 105, in West Europe 151, and in the Oceania region 140. Whereas for data in Asia, there was an incidence of 206 in a country of South Korea [1].

The thing that is of concern for cases of appendicitis in children is the occurrence of negative appendectomy, which is surgery performed on patients with normal appendixes. In all children, it is suspected that the negative appendectomy rate is 8.4%, but for children under 6 years, the number increases to 56.7% [2]. In a 2009 study in the United States, this negative appendectomy rate reached 15% [3]. In addition, the problem of cost is also a concern because the negative action appendectomy will cost more than the cost of establishing the diagnosis [4]. However, even with a complete investigation, the diagnosis of appendicitis in children does not have significant development. This difficulty is based on communication and inspection difficulties [5].

Pathologically, the occurrence of complications in appendicitis is influenced by good mucosal resistance and vascularity (microvessel density) in the appendix mucosa. Mucosal resistance is determined by the ability to regenerate, where folic acid (FA) plays a role in this process [6]. Angiogenesis is also an important factor in the success of wound healing. One of the angiogenic growth factors is vascular endothelial growth factor (VEGF), which has a pleiotropic role in tissue healing through the process of neovascularization, reepithelialization, and regulation of extracellular matrix [7]. This VEGF is specific to blood vessel endothelial cells [8].

Based on this statement, FA and VEGF are believed to be related to complicated appendicitis which can be confirmed by histopathological examination in...
the form of microvessel density. Therefore, researchers want to see the effect of VEGF and FA levels on the occurrence of complicated appendicitis. For this reason, animal experiments were conducted to measure VEGF and FA as well as histopathology in the case of appendicitis. The animals used were rabbits that were obstructed by the appendix lumen so that they experienced appendicitis given the similarity in structure and function of the appendix of rabbits with humans [9]. In this study, we aim to find out the role of VEGF and FA as the predictor of complicated appendicitis.

**Materials and Methods**

This study was an observational study with laboratory experimental design for the analysis of VEGF and FA levels as a predictor of the occurrence of complicated appendicitis seen through histopathological examination in treated rabbits.

The study was conducted at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (USU) and examination of VEGF and FA levels was carried out at the Clinical Pathology Laboratory of the Faculty of Medicine, USU. Histopathology examination was carried out in the Anatomy Pathology Department of Faculty of Medicine, USU.

The inclusion criteria in this study were (1) male rabbit. The selection of male sex is intended to avoid the hormonal influence on the results of the study [10] and (2) rabbits weighing around 2500–3000 g, while the exclusion criteria were rabbits that have abnormalities in the digestive tract, especially in the appendix, are enforced during a laparotomy.

The experimental animals used in this study were male rabbits (*Oryctolagus cuniculus*), weighing 2500–3000 g and healthy. Rabbits were chosen because in the previous studies, the success rate of appendix lumen obstruction has a 100% success rate [10].

**Experimental model of acute appendicitis in the rabbit**

Thirty-two male rabbits (*O. cuniculus*), weighing about 2500–3000 g blood samples, were taken for VEGF and FA as initial samples before treatment. Then, the rabbit was given anesthesia intramuscularly with 0.5 mg/kg body weight, which had no effect on spontaneous breathing. Abdominal exploration through a midline incision and appendix is identified. In all rabbits, Silk No.-O thread is inserted through the mesentery at the base of the appendix, but the lumen of the appendix is obstructed by binding with Silk No.-O thread. The abdomen was closed and the rabbit returned to its cage and given food and drink.

Thirteen hours later, rabbits blood samples were taken for VEGF and FA as samples 13 h after the appendix lumen obstruction treatment. Then, the rabbit is again anesthetized and has an appendectomy, and blood is drawn from the heart to check VEGF and FA levels. Then, the termination of the rabbit is carried out, and a histological examination is performed on the appendix (Figures 1 and 2).

**Statistical analysis**

Analysis of research data is presented in mean ± SD for normally distributed data and median (min-max) for data that are not normally distributed. Then, Mann–Whitney U-test was used because the data were not normally distributed, then proceed with bivariate analysis with Kruskal–Wallis test. The significance limit is if p < 0.05, which means that there is a significant relationship between the two variables tested.

Data analysis was performed with the statistical analysis software.
Results

A total of 32 samples were included in this study. Of the 32 samples, we found 17 samples with normal histopathological results (53.1%), 13 samples with acute appendicitis (40.6%), and the last two samples with acute appendicitis + serosal hemorrhage (6.3%) (Table 1).

Table 1: Characteristic of the study sample

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
<th>Mean (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histopathological results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>17 (53.1)</td>
<td></td>
</tr>
<tr>
<td>Acute appendicitis</td>
<td>13 (40.6)</td>
<td></td>
</tr>
<tr>
<td>Acute appendicitis + serosal hemorrhage</td>
<td>2 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Rabbit’s body weight (g)</td>
<td></td>
<td>2679.34 (±131.92)</td>
</tr>
</tbody>
</table>

In the analysis of the enzyme-linked immunosorbent assay examination, the results of VEGF levels were obtained. The median of basal VEGF level was 6.83 (2.04–17.8) pg/mL, while 13 h post-intervention was 7.07 (4.20–55.10) pg/mL. There was a slight increase in VEGF level, however, there was no statistical significance proven (p = 0.172) (Table 2).

Table 2: Basal and 13 h VEGF and folic acid level

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Basal (median [min-max])</th>
<th>13 h (median [min-max])</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF level</td>
<td>6.83 (2.04–17.8)</td>
<td>7.07 (4.20–55.10)</td>
<td>0.172*</td>
</tr>
<tr>
<td>Folic acid level</td>
<td>1.08 (0.28–7.25)</td>
<td>0.78 (0.39–7.97)</td>
<td>0.900*</td>
</tr>
</tbody>
</table>

*Wilcoxon test; p<0.05 means statistically significant. VEGF: Vascular endothelial growth factor.

We also analyzed the basal FA levels with the histopathological results. As a result, basal FA levels in the normal group were 2.00 (±2.08) ng/mL, and the acute appendicitis group was 1.14 (±1.00) ng/mL, and the complicated appendicitis group was 0.44 (±0.22) ng/mL. The difference in basal FA levels between the three anatomic pathology results shows that the lower the basal FA level, the more likely there is a complicated appendicitis. However, based on the Kruskal–Wallis test, there were no statistically significant differences (p = 0.058) (Table 4).

Table 4: Basal folic acid level to histopathological results

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Basal (median [min-max])</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid level</td>
<td>2.00 (±2.08)</td>
<td>1.14 (±1.00)</td>
</tr>
</tbody>
</table>

*Kruskal–Wallis test; p<0.05 means statistically significant.

Discussion

In appendicitis, the process of angiogenesis, the process of forming new blood vessels from existing blood vessels, occurs. The formation of new blood cells is needed to meet the tissue’s need for oxygen and nutrients, as well as to repair damaged blood vessels. The process of angiogenesis is assisted by VEGF, which is a substance formed by cells that stimulate the formation of new blood vessels, a mitogen for the endothelial lining of blood vessels.

FA and VGEF are believed to have an association with complicated appendicitis. It can be found on histopathological examination in microvessel density. In this study, we will look at the effect of VGEF and FA levels on appendicitis.

From a total of 32 rabbits, 17 rabbits (53.1%) had normal anatomic pathology, 13 rabbits (40.6%) had acute appendicitis, and 2 rabbits (6.3%) had appendicitis results acute + serosal hemorrhage. This shows that there are several variations of appendicitis that occurred in this study, namely, acute and complicated appendicitis.

In this study, 32 male rabbits weigh 2500–3000 g. Based on previous study, rabbits were chosen because the success rate of appendix lumen obstruction could achieve a 100% success rate [10].
From the results of this study with a total sample of 32 male rabbits, it was found that the mean rabbit body weight used in this study was 2679.34 (±131.92) g. Rabbits weighing 2500–3000 g were chosen because they have been used in the previous studies [10], and rabbits are in the post-weaning phase, where rabbits can find their own food and move actively [11].

In this study, we compared the basal VEGF levels (median of 6.83 (2.04-17.8) pg/mL) and VEGF levels 13 h post-intervention (median of 7.07 (4.20-55.10) pg/mL). The results of the analysis with the Wilcoxon test obtained p = 0.920, which means that there are no significant differences in each variable.

The results of Eržen's research et al. in “Stable phase post-MI patients have elevated VEGF levels correlated with inflammation markers, but not with atherosclerotic burden,” it was found that plasma VEGF levels increase in a stable phase after myocardial infarction and are associated with inflammatory cytokines, but not with atherosclerotic burden. Thus, this shows that increased levels of VEGF are part of the ongoing inflammatory activity. Because VEGF stimulates neovascularization of inflamed plaque and induces destabilization, VEGF levels can have important negative prognostic values [12].

According to the research of Ramakrishnan et al. in “Vascular Endothelial Growth Factor Signaling in Hypoxia and Inflammation,” hypoxia-inducible factor (HIF) is a key evolutionary conserved transcription factor that regulates vascular biology, angiogenesis, metabolic reprogramming, and inflammation. HIF regulates the recruitment of inflammatory cells, erythropoiesis, tissue remodeling, and pH homeostasis. In addition, metabolic adaptations mediated by HIF create a place for stem cells to develop in a hypoxic micro-environment. One of the main target genes regulated by HIF is VEGF-A. VEGF is needed for the development of angiogenesis, collateral vessel growth in peripheral arterial disease, stroke, and myocardial infarction. Inducing local production of VEGF is therapeutically useful in limiting tissue damage due to hypoxia/ischemia [13].

Wan et al., in “Supplemental Oxygen Reverse Hypoxia-induced Smooth Muscle Cell Proliferation by Modulating HIF-alpha and VEGF Levels in a Rabbit Arteriovenous Fistula Model,” showed that short-term administration of additional oxygen inhibits HIF and VEGF signaling to reduce the proliferation of smooth muscle in local blood vessels. This provides strong support for the use of additional oxygen therapy after arterial surgery to reduce intimal hyperplasia [14].

In this study, we obtained the comparison between basal FA levels (median = 1.08 (0.28-7.25) nmol/L) and FA levels 13 h post-intervention (median = 0.78 (0.39-7.97) ng/mL). The results of the analysis with the Wilcoxon test obtained p = 0.920, which means that there are no significant differences in each variable.

The study of Park et al. trying to find a relationship between VEGF inhibition in heart tissue in experimental animals. The result is, with the blockade of VEGF, it will cause more damage to the heart tissue. Hence, it can be concluded that the decrease in VEGF levels will cause more severe tissue damage [15]. Another study from Quarck et al. tried to look at the relationship of VEGF to chronic thromboembolic pulmonary hypertension (CTEPH). In that study, a healthy group of subjects as controls had higher VEGF values compared to patients suffering from CTEPH, so this study concluded that angiogenesis disorders could be related to disease progression [16].

In line with the two studies above, in this study, the mean VEGF was lower in the acute appendicitis group compared to the normal group (7.66 [±3.47] vs. 8.61 [±4.87]) pg/mL. Then, acute appendicitis + serosal hemorrhage is also lower than acute appendicitis (5.75 [±4.88] vs. 7.66 [±3.47]) pg/mL. However, in this study, there were no statistically significant differences (p = 0.775).

For differences in basal FA levels on the results of anatomic pathology in this study, a lower mean of FA was found in the acute appendicitis group compared with the normal group (1.14 [±1.00] vs. 2.00 [±2.08]) ng/mL. Then, acute appendicitis + serosal hemorrhage is also lower than acute appendicitis (0.44 [±0.22] vs. 1.14 [±1.00]) ng/mL. However, in this study, there were no statistically significant differences (p = 0.058).

In the research of Akbulut et al., they were trying to see the relationship between homocysteine levels and FA on the incidence of ulcerative colitis. Lower levels of FA were obtained in patients with ulcerative colitis when compared with the control group. This difference was considered statistically significant (p < 0.001) [17]. Another study from Ma et al. looked at homocysteine and FA levels in coronary heart disease. The results are differences in FA levels between controls, stable angina pectoris, unstable angina pectoris, and acute myocardial infarction. Sequentially, each FA level was 12.86 (±5.85) mmol/L; 10.33 (±3.95) mmol/L; 9.21 (±4.38) mmol/L; and 7.08 (±3.43) mmol/L. From these results, there is a tendency that the lower the levels of FA, it is associated with the more severe conditions of coronary heart disease that occurs. This difference was shown to be statistically significant (p < 0.001) [18].

Thus, it can be concluded that there is no significant relationship between basal FA levels with the anatomic pathology of the appendix.

The difference in VEGF and FA levels in the appendicitis histopathology is also not significant, so we cannot determine with VEGF and FA the histopathological form. The most accurate examination to determine appendicitis histopathology is, of course, anatomical pathology analysis. Research on VEGF
regarding tumor events has been done, but research on VEGF and FA related to the incidence of appendicitis is still very little.

Conclusion

Although no statistical significance was found, there was a trend showing the low value of VEGF, and FA was a predictor of appendicitis and complicated appendicitis, especially if combined with 5-hydroxyindoleacetic acid can be accurate diagnostic.

References