Chapter

Endothelial Dysfunction in Appendicitis

Erjan Fikri, Ahmad Razi Maulana Alnaz and Fini Meirisa Alnaz

Abstract

In an inflammation, including appendicitis, vascular adequacy is required to supply anti-inflammatory substances and nutrition due to inflamed tissue remodeling. Normal tissue has balanced tissue regeneration and tissue destruction from apoptosis. While in inflammation, inflammatory substances tend to cause tissue destruction and lead to necrosis. This requires the tissue to increase cell regeneration to maintain tissue homeostasis in the appendix, induced mainly by oxygenation, nutrition, growth factors, and mainly anti-inflammatory substances that are obtained with vascular adequacy. This process needs active vascularization that can be achieved with neovascularization to ensure good vascularization to the tissue lacking from vascular damage. The ability of neovascularization is mainly related to growth factors acting in the endothelium and inducing neovascularization process. This mechanism is impaired in the process of inflammation by inflammatory substances causing endothelial dysfunction. As stated that vascular adequacy is related to growth factors such as vascular endothelial growth factors (VEGF) that may differ from one person to another, external and internal factors plays role in affecting individualized difference in adapting to inflammatory process, the expression of the VEGF may be a novel distinction to cut-off requirements of inflammation process in appendicitis would be self-limiting or continue to cause tissue necrosis and perforating appendicitis that urges surgical treatment to encounter the unstoppable inflammatory process in the appendix.

Keywords: appendicitis, endothelial dysfunction, inflammation, neovascularization, VEGF

1. Introduction

Appendicitis is an inflammation of the vermiform appendix, which presents as one of the causes of acute abdomen leading to emergency surgical indication. The acute appendicitis presented as the most common indication of nontraumatic emergency surgeries around the world. Annually, more than 100 cases of appendicitis per 100,000 persons are recorded around the world. About 16.33% of men and 16.34% of women mostly in the second and third decades of life were at risk of experiencing acute appendicitis [1, 2].

More than 108,000 surgical procedures were conducted to treat appendicitis in a year. Acute appendicitis may be treated with surgical treatment and conservative treatment. Treatment choices were considered in acute appendicitis by classification of clinical uncomplicated or complicated appendicitis occurred [3].

Distinguishing indications of surgery or appendectomy as treatment of appendicitis might be challenging. It was more subtle in pediatric patients, as more consideration and careful examination needed to be conducted prior to the surgical procedure. Statistics had recorded more events of negative appendectomy, the fact that vermiform appendix presented to be normal or not inflamed after the process of appendectomy. The incidence was commonly only about 15% in adults but raised up to 56.7% or even more in pediatric patients presenting with related symptoms to appendicitis. These procedures were stated as a burden in medical decision-making as non-indicative surgeries may harm patients or even cause expenditures for non-beneficial procedures [4].

The main aim of diagnostic and decision-making in appendicitis was to accurately distinguish any chance of currently symptomatic appendicitis appearing normal as presented during operation. This means that appendicitis may be reversible and basic inflammation of the appendicitis was not causing permanent damage to the appendix vermiform itself. Currently, there was still no such strong evidence to prove whether an appendicitis process that occurred would end as permanent tissue damage and causes complications that are mandatory for surgical treatment of the appendix vermiform or would heal without risk of complications [5].

We analyzed the possibilities of differentiation of the two conditions in appendicitis. The main key to understand and accurately differentiate risk in appendicitis would be clearly explained in a basic inflammatory mechanism on how appendicitis would occur. The promising approach was glancing at the pathways and mechanism of inflammation would occur specifically, as related with factors lying alongside the pathophysiological process of inflammation and cure of the inflammation itself, by sufficient oxygenation and adequate metabolism of remodeling or tissue repair.

2. The appendix vermiformis

The appendix vermiform is an anatomical structure located at the end of cecum, commonly in posteromedial projection, located about 1.7 cm below the ileocecal valve, at the end of the taenias of the colon converging on the cecum. Its size is about 91.2 mm long in men and 80.3 mm in women, respectively. The appendix is a true diverticulum, as its layer is made up of mucosa, submucosa, longitudinal and circular muscle, and serosa. Anatomically, the position of the appendix just located anterior to the iliopsoas muscle and the lumbar plexus, and posterior to the layers of abdominal wall muscles. The main blood supply to the appendix comes from the appendicular artery, one branch of ileocolic artery, which extends along the mesoappendix to the distal tip of the appendix. Mesoappendix is a mesenterium consists of connective tissue anchoring the appendix into the mesentery of the intestines which size varies to the size of the appendix itself. Somehow, angle and projection of the appendix may differ from one to another: retrocecal, subcecal, preileal, postileal, and pelvic (**Figure 1**) [6, 7].

Nutrition of the vermiform appendix was obtained by special vessels vasculating the appendix running along the mesoappendix. Main nutrition and oxygenation living the appendix are supplied by the appendicular artery, derived and branched from the ileocolic artery alongside the ileum, cecum, and ascending colon. The

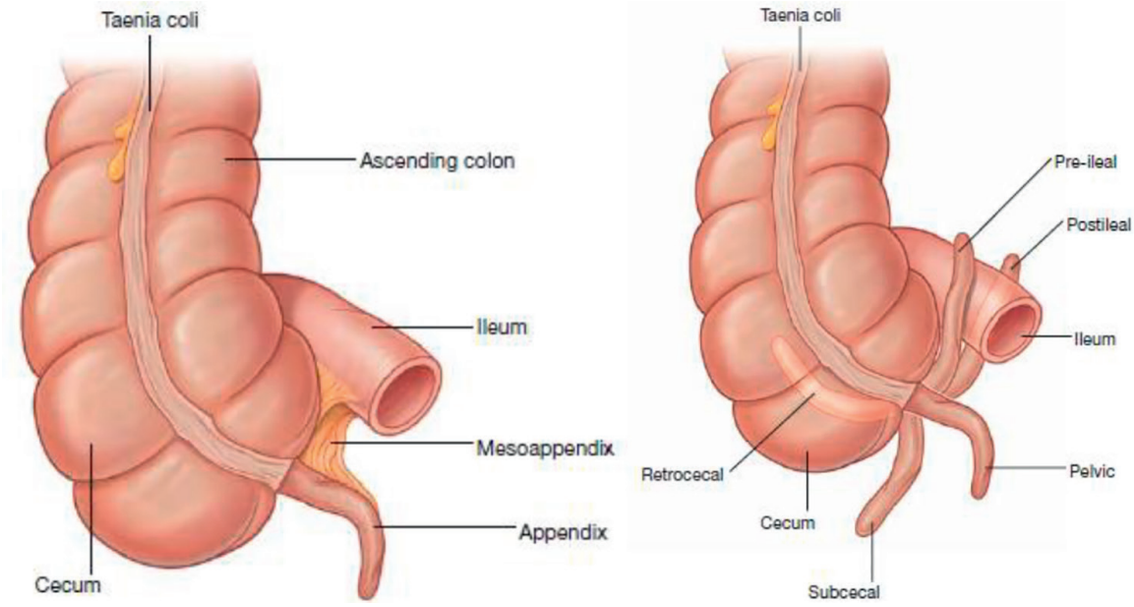

Figure 1.
The anatomy of vermiform appendix and the cecum (left) and its anatomical variations (right) [6].

appendicular artery was 1 single branch from the ileocolic artery vascularize the whole appendix which then performed networks of smaller arteries perforating into the layers of appendix. The vascularization of the appendix originates from mesenteric blood circulation which is also responsible for other parts of the intestinal circulation. The appendicular artery is one of the most distal parts of the branches from the superior mesenteric circulation. Although it is part of the huge mesenteric circulation, as appendiceal circulation took a little part and branch among the circulation, the regulation of blood flow is less and lower than in other parts of the mesenteric circulation such as in the ileum or ascending colon. However, regulation of the blood flow is commonly maintained by local factors with vasodilator effects induced and attributed by hypoxia or cytokines of inflammation to cause an increase in blood flow to the appendix. Certain osmotic mechanisms, autonomic and neurohumoral, also the intestinal wall activity of peristalsis affect the blood flow in the mesenteric circulation primarily the special circulation to the appendix [6, 8].

Metabolic end products from the tissue in the vermiform appendix were drained away by the venous drainage which anatomically will join the venous blood flow in the ileocolic vein and the superior mesenteric vein. Another non-venous drainage of the appendix occurs by the lymphatic drainage of the cecum and the appendix which passes the lymph nodes in the mesoappendix and ileocolic lymph nodes surrounding the ileocolic artery into a group of superior mesenteric lymph nodes (**Figure 2**) [9].

The vermiform appendix is known as a vestigial organ embryologically and by the evolution of mammals. The main functions of vermiform appendicitis itself remain unknown. Theories stated that in humans the vermiform appendix no longer functions but other theories counters. As histologically it is rich in lymphoid tissue and its vascularization and lymphatics, its function is mostly discussed related to the immunological functions, especially to the gastrointestinal system. As its luminal and non-continuous structure, the vermiform appendix was also hypothesized to be a reservoir for gut microbiota. This function and structure were suspected to have strong correlations to inflammatory mediators and microbiological mechanisms of the bacteria.

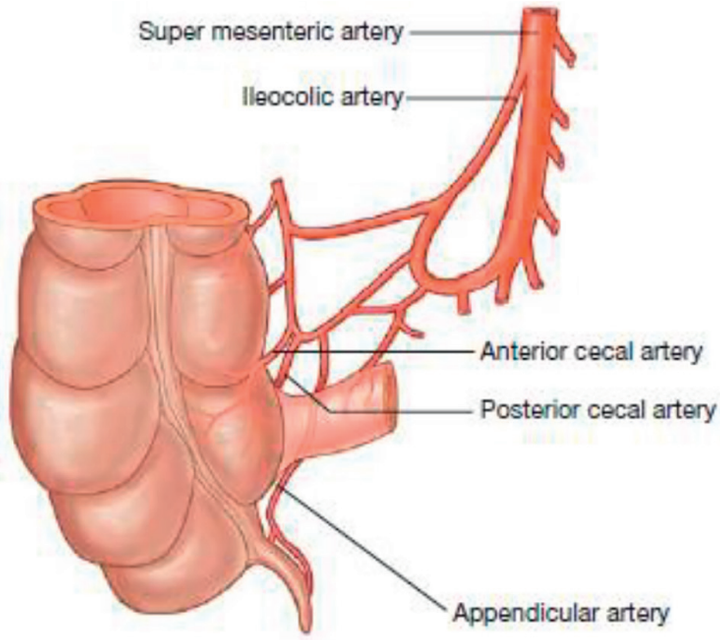

Figure 2.
Arterial supply of the vermiform appendix [6].

This puts the vermiform appendix as an organ with a lot of risks of inflammations and infections its homeostasis was disrupted [10].

3. Appendicitis

Appendicitis is defined as inflammation of the vermiform appendix and represents the most common cause of acute abdomen and emergency surgical indication in the world [1]. As common to all kinds of inflammation and precisely to the gut, appendicitis was commonly related to biochemical, histological, and physiological changes to the vermiform appendix itself. Inflammatory mediators regardless of factors precipitating lead to common manifest of inflammatory signs of a fluid shift, size changes (enlargement), increased blood flow and perfusion, inflammatory cell infiltrations, and also tissue remodeling, especially to the lymphoid tissues of the appendix. The inflammatory process as occur in all tissue may be reversible and some tend to be permanent remodeling or end with tissue damage and causes complication of appendicitis [11].

Presentation of appendicitis occurs by luminal obstruction of the appendix lumen that may be precipitated by a variety of etiologies, whether due to mass, faecolith or appendicolith, mucosal inflammation, lymphoid tissue hyperplasia, parasite infestations, or other mechanism leading to disruption of the passage of fluid and any luminal contents in the appendix to be propelled away to the cecum, and causing maladaptive mechanism that started certain cascade of pathophysiological events of inflammation that would be manifested clinically [12].

Clinical manifestation of appendicitis may be challenging. Most common symptom that occurs and causes patients to seek medical care is abdominal pain, although other symptoms such as fever, constipation, diarrhea, anorexia, and nausea are also reported as the main symptom. Pain in appendicitis starts in periumbilical and epigastric region at the beginning of the onset, and later migrates to the lower right

quadrant where classic McBurney sign of classic lower right quadrant pain occurs. However, the history of migratory pain from one to another abdominal region occurs only in 50–60% of patients with acute appendicitis. Symptoms of nausea and vomiting start as the effect of abdominal pain, and fever starts about 6 hours after the onset of pain where an inflammatory process in the appendix had been established. The history of symptoms may be different from one patient to another, related to the anatomical variation of the appendix. Anteriorly located appendix commonly causes more marked and localized pain in the right lower quadrant, and the variation of retro-cecal one commonly has a dull abdominal pain manifestation or may be interpreted as a lower lumbar region pain. Furthermore, as appendicitis occurs with inflammation not restricted only to the appendix itself but may affect surrounding organs, other symptoms such as urinary urgency, dysuria, or rectal symptoms may appear but some cases [13, 14].

Physical examinations of patients with appendicitis include basic vital sign findings followed by an appendicitis-specific examination. Patients with appendicitis mostly present as febrile with a temperature greater than 38°C, tachycardia, and tachypnea may be found. Most early clinical manifestation of appendicitis are mostly non-specific and mimics other gastrointestinal disturbances. Obvious manifestation would present when inflammation progresses when inflammation had involved the parietal peritoneum in the serosa of the appendix which causes localized right lower quadrant tenderness that further exacerbates by specific physical examination such as McBurney sign, Rovsig sign, or other signs of appendicitis. However, the pain would progress more to be exacerbated by movement or cough causing an increase in intraabdominal pressure. Routine laboratory test usually provides an increase in leukocytes, especially neutrophil as an acute reaction to the inflammatory process presents a shifting to the left in leukocyte differential count. C-reactive protein indicates that systemic inflammation with greater than 1.5 mg/l may be one of the likely diagnostic indicators of appendicitis [15].

Further complicated and severe appendicitis usually has leukocytosis counts more than 20,000/μl and commonly related to perforation dan peritonitis and high level of C-reactive protein or even Procalcitonin. However, perforation and complicated appendicitis were also reported in about 10% of appendicitis with normal to mild increase in leukocyte count and C-reactive protein. This could not exclude the possibility of perforation in normal laboratory values in appendicitis. This because low sensitivity of leukocyte count in the diagnosis of appendicitis with only 65–75% while only 57–87% for C-reactive protein. Therefore, many studies had been conducted on early specific diagnosis; such as procalcitonin, as it is a good biomarker in sepsis and appendicitis may lead to sepsis but is still limited in appendicitis with no sepsis [15].

3.1 Pathophysiology of appendicitis

Exact pathophysiology of appendicitis itself remains a struggle for physicians. The process of appendicitis itself is related and basically similar to other pathophysiology of inflammations. Commonly appendicitis began with a luminal obstruction. Several causes of obstruction may occur such as lymphoid hyperplasia, parasitic infections, fecalith, or intra and extra luminal mass. This causes an increase of intraluminal and intramural pressure which causes small vascular and lymphatic occlusion collapsed by the tension of the lumen and mural. Obstructed appendix tends to cause overgrowth of bacteria, mostly aerobic bacteria dominate in acute appendicitis [16].

The obstruction may also cause mucous plaques and accumulated causing distension. Distension of the appendix may progress vary from one patient to another up to 50–65 mmHg. When the luminal pressure increases, vascularization in the mural may be disrupted. Increase in the pressure may beyond the lymphatic and venous pressure and prevents fluid drain from the two vessels due to a weak wall of vein and lymphatics [17]. First collapsed vessel would be the lymphatic drainage preventing fluid back into circulation to remain in the appendix tissue. Soon as pressure increases in the lumen, the pressure disrupted the lumen of the vein and causes collapse of both lymph and blood flow from the tissue of the appendix. This process causes the edema process which occurred by disrupted fluid drainage [14, 18].

This state of appendicitis consisted of inflammation and edema alone possibly start the clinical symptoms of appendicitis, but this stage is considered as a mild process in which conservative treatment for appendicitis may be available. Surgical treatment may be offered, and still may be beneficial but as it is an invasive procedure and has a number of complications, the surgical procedure which is still not yet urgent to be performed may not be a favorable choice of treatment. Antibiotics and good fiber intake may be one of the choice and helps relieve symptoms and reduce the inflammation of appendicitis [5, 19].

But the mild state of appendicitis also has a risk to develop further. Fluid accumulation and edema also cause more tension to the vascular wall causing further obstruction and disruptions of vessel flows. Soon as the pressure increases more the arterial walls were collapsed due to pressure to its wall. The blood flow containing oxygens and nutrients was decreased due to arterial obstructions. This stated the condition of hypoxia in the distal of the arterial obstruction in the appendix [20].

Hypoxia state of the mucosa and the wall of the appendix begins further tissue damage in the appendix. Tissue damages were due to hypoxic stress of the cells in the appendix which then undergo a cell apoptosis process of even necrosis of the tissue. Tissue damage causes less strength of the appendix wall from distensions of the edema and fluid accumulation that is then related to complications by ulcerations, perforations, and necrotic appendix. This process occurs only if the vascularization were disrupted. Hypoxic environment also tends to be favorable for growth of intestinal flora mostly the Gram-negative bacterias such as *Escherichia coli, Enterococcus, Bacteroides,* and *Pseudomonas*. Bacterial growth also elicits more inflammatory and immunologic processes in the appendix itself. *E. coli* itself as the main flora normal in the large intestine may cause activity changes and be pathogenic as shifted and trapped in the appendix with different microenvironments and releases toxins exacerbates inflammation [13, 20].

The tissue damage and necrosis in appendicitis risk a thin and fragile wall of the appendix. This at one point with more pressure would lead to a tear of the mucosal or even the muscular and serous layer of the wall causing perforation of the appendix. Once a perforation occur, acute appendicitis occurred with complication. As bacterial overgrowth inside the lumen of the appendix came in contact with the sterile peritoneum cavity begins further immune and inflammatory responses cause peritonitis. As peritonitis occurred in complicated appendicitis, operative treatment with laparotomy may be inevitable. Further inflammatory process of the peritoneal wall also risks the spread of bacteria into the bloodstream and may complicate more into sepsis with all its high risk of mortality [21, 22].

Bacterial growth causes inflammation, especially in the mucosa of the appendix. The incompetence of the appendiceal wall risk the development of spreading infections and inflammatory mediators causing inflammation of the serous layer of the

appendix. A close anatomical layer of the serous into the parietal peritoneum and other adjacent organs. The inflammation of the peritoneum led to appendicitis complicated with peritonitis. Stimulation of pain fibers of the afferent visceral pain nerves in the layer of the peritoneum to the level of medulla spinalis. Pain sensation is interpreted as epigastric and periumbilical pain, which quality could not be specifically localized, but the right lower quadrant has the most accumulation of inflammatory mediators that would inflame more and be more painful [12, 20].

Untreated and uncompensated process of inflammation leads the more serious complications of appendicitis. The longer the hypoxia occurs to the tissue, the more risk of tissue damage. Risk of infarction multiplied by time followed by progression into perforated or even gangrenous appendicitis. More severe symptoms as mentioned previously would progress and limits daily activity. More pain and nausea and vomiting would occur. Systemic involvement in sepsis may be part of the risk of prolonged appendicitis. The process and pathophysiology off appendicitis may be illustrated in **Figure 3** [16, 22].

Basically, no major changes occur in mesenteric circulation in appendicitis. However, as a reaction to inflammation, local vascularization specifically targeting the inflamed tissue increased and was hypothesized to activate the neovascularization in the collateral circulations nearby mucosa and muscular layer of the appendix. The tiny new collateral vasculature along the local inflammation we aimed to increase perfusion into the center of inflammation, which is highly fragile and requires

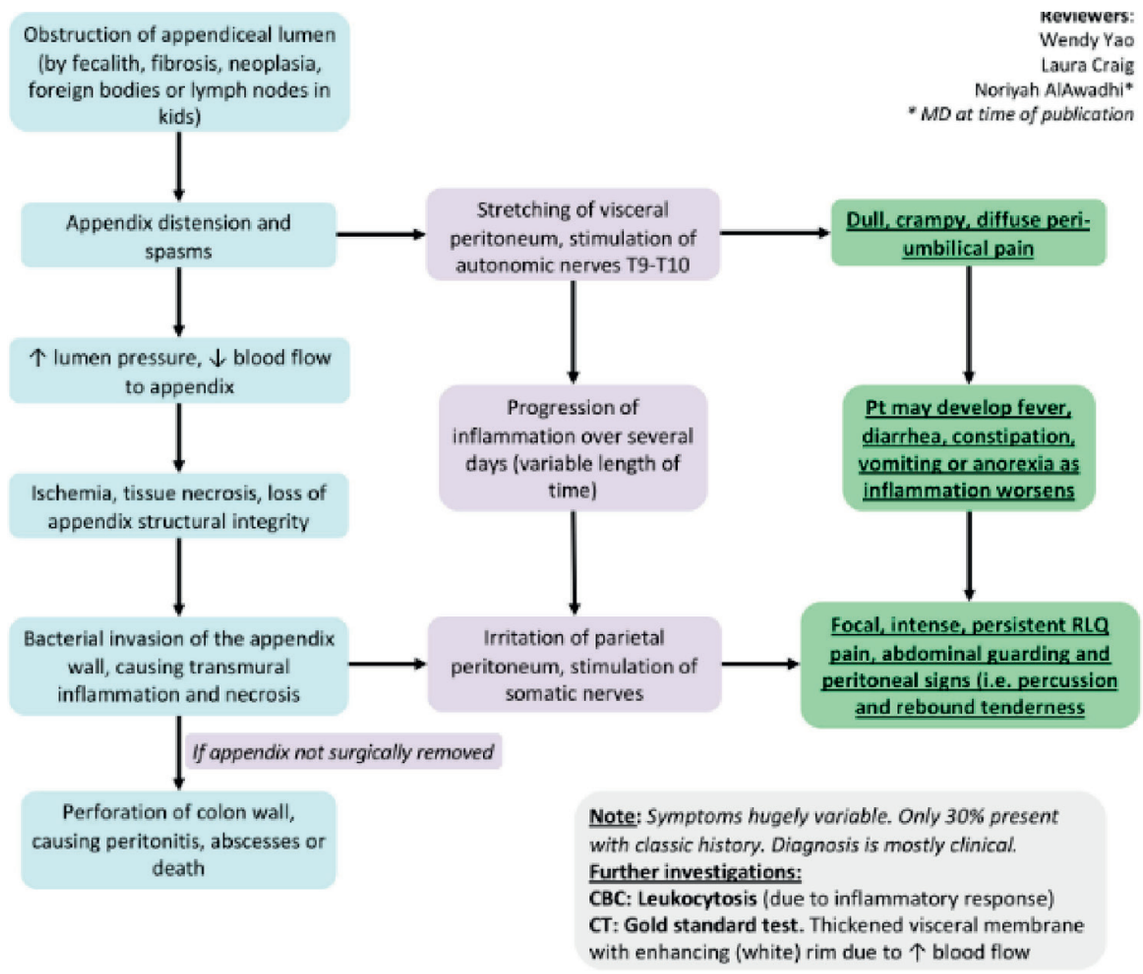

Figure 3.
The pathophysiology of appendicitis [23].

strong endothelial stability which may be supported by certain endothelial factors to maintain perfusion against high intraluminal pressure from the appendix itself and preventing collapsed or burst of the vascularization [23, 24].

Severity and complications of appendicitis are known to be related with necrosis or ischemic tissue in the appendix vermiform. Basic consideration on factors affecting the vascularization patency to tissue damage in appendicitis. Arteries with dysfunctioned endothelium occasionally damaged and unable to adapt and perform potent perfusion to tissues in systemic changes caused by the inflammatory mediators. While arteries with good endothelium tend to be able to keep strong perfusion to maintain oxygenation preventing cell death. This differentiates variations of symptoms in patients with appendicitis who develops complication and whom did not [25].

This means mucosal vascularization of the appendix was considered as a barrier preventing further damage to the mucosa from increased intraluminal pressure in appendicitis. So as the mucosal and muscular layer of the appendix receives adequate vascularization, tissue elasticity and cell regeneration would take place so that the tissue would able to adapt against stretch elicited by the increase of the intraluminal pressure. Furthermore, enough perfusion to the appendiceal muscular layer will be able to initiate appendiceal contractility to drain out fluid or any fecalith obstructing and causing trapped intraluminal contain. This tissue competence may be a key role in preventing the perforation of other complications of appendicitis [26].

3.2 Recovery process in appendicitis

The appendix as a rich in lymphoid tissue part of the intestine has a high reserve of natural killer cells (NK)111 CD31 T cells (NK T lymphocytes). This cell produces cytokines and chemokines early since activated by the local inflammatory process. Cells such as B2201CD31 T cells in the lymphoid of the appendix express CD45R indicates for T cell activations more than any part of the intestine. Certain factors related to a great number of lymphocytes in the appendix came as the presence of CCL21, a chemokine embedded to the lymphatic endothelial cells and luminal surface of endothelial venules around the parafollicular areas in MALT. CCL21 binds to CCR7 to promote recruitment of B and T lymphocytes to the appendiceal lymphoid tissue and migration of dendritic cells (DC) back to appendiceal lymph nodes [15].

Apart from the abundant lymphocytes in the appendix, the molecular expression on the surface of the lymphocytes in the appendix differs from lymphocytes in the intestinal lymphocytes. In the lamina propria, the T cells in the lamina propria of the appendix express more integrin subunit b7 than B cells and also than the lymphocytes in the other parts of the intestine. Integrin a4b7 is expressed on T cells located between lamina propria and epithelium, and on macrophages and dendritic cells located in the mucosa of the appendix [22]. The molecule binds to mucosal addressin cell adhesion molecule 1 (MAdCAM-1), which mediation process of “tethering and rolling” and “homing” attracts lymphocytes into it. The localized expression of these molecules of a4b7 is considered a trafficking signal. Conversely, the aEb7 is responsible for the retention of these lymphocytes, via binding with its ligand E-cadherin. The dendritic cells express aEb7 stimulate the differentiation of forkhead box protein 3 (FoxP3)1 Treg cells soon after the interactions with antigens. Therefore, the suppression of regulatory expression would prevent lymphocyte differentiation and lead to a proinflammatory state [14].

CD51 cells or B1 lymphocytes are expressed more in a healthy appendix than the rest of the gut. When the appendix inflamed, the expression increased even

more. These CD51 B cells produce IgM antibodies specific to certain pathogens. The synthesis of the IgM could take place directly in case of the absence of antigen presentation by other T cells, similar to innate-like immune response expressed by IELs. Despite the ability to synthesize IgM similar to immune response, the IgM antigen has low affinities, it still has major importance in reaction to microorganisms. Increase in the expression would be explained by an alteration of the intestinal microflora that occurs along the pathogenesis of appendicitis. Moreover, the CD51 cells also produce anti-self antibodies and an anti-inflammatory molecule such as IL-10 which means the increase of the expression was process to prevent inflammation currently occurring [27].

Pathologically, the complications of appendicitis were affected by the mucosal resistance to stress and adequate vascularity (microvessel density) in the appendix mucosa. This prevents further tissue damage. The mucosal resistance is determined by its adequacy to regenerate in case of stress or damage, producing new and strong mucosal layer which is influenced by folic acid (FA) metabolism. Adequate vascularity is then determined by ability of angiogenesis which plays as on of the most important factors in wound healing process. The angiogenesis itself is induced by growth factors namely vascular endothelial growth factor (VEGF), which role is fundamental by mediating and inducing the neovascularization, reepithelialization, and regulation of extracellular matrix. However the VEGF expression itself is endothelial cells in the blood vessels [28].

The angiogenesis occurs and induced in appendicitis, forming novel microvasculatures around the inflamed appendix to sustain adequate perfusion. The formation of new vascularization is required undergo the increased tissue's requirement of oxygen and nutrients of parenchymal remodeling, as well as to repair damaged blood vessels induced by pressure of inflammatory cytokines. Angiogenesis itself depends on VEGF, which is produced by damaged endothelial cells that stimulates mitosis in the endothelial lining of blood vessels creating new blood vessels other than currently damages vessel. This mechanism relates the VGEF to be believed associated with complicated appendicitis. Further evidence presented that different expression of VEGF may be found on histopathological examination of microvessel density in appendicitis specimens [28, 29].

Differentiating between risk of having complication may be a cut off on physician to take a concise decision on therapy of the patient. Patient which endothel may be strong enough responding the inflammatory process may not need to undergo operative treatment as tissue repair and remodeling were likely. However in chronic inflammation and weak endothelium possess a risk of further harm and requires surgical procedure [4, 12, 25].

Factors determining endothelial stabilities are regarding on tissue strength itself. Subgroup of individuals who tend to have a strong connective tissue subtypes of collagens has a chance to have a stronger endothelial stability. Factors effecting the endothelial growth and proliferations subsequently backs-up cells of the endothel to proliferate in preventing the endothelial damage. Also neovascularization may occur and possibly perfuse other sites of inflamed tissue to receive strong and supports of the vascularization. This prevents further complications to occur and more invasive treatment procedure may not be required or indicated as if antibiotics are capable [4, 19, 30]. The growth factor such as the vascular endothelial growth factor (VEGF) has a main role determining the strength of the endothelium against inflammation, especially in cases of appendicitis itself in the vascularization of the appendix [31].

4. Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF, now referred to as VEGF-A) is a member of a family of proteins including VEGF-B, VEGF-C, VEGF-D, VEGF-E (virally encoded), and PlGF. VEGF-C and VEGF-D are primarily implicated in regulation of lymph angiogenesis. Given the dominant role that VEGF-A plays in regulating angiogenesis and disease, it will be referred to as VEGF. VEGF undergoes multiple splicing alternative creating several exon leading to multiple isoforms. Common isoforms include VEGF 165, VEGF 206, VEGF121, and VEGF189. VEGF165 (VEGF164 in mice) is the most frequently expressed isoform in majority of tissues. The VEGF165 is also the most physiologic isoform, with characteristics connected to the highly diffusible VEGF121 and the extracellular matrix (ECM)-bound VEGF189 [32, 33].

Less other isoforms of VEGF, such as VEGF145 and VEGF183 currently been described in several studies. Main features differentiates one isoforms than another were differential ability to bind heparin. The lowest affinity to heparin belongs to VEGF121, while strong affinity known for VEGF189 and VEGF206 which consist of two heparin-binding domains (encoded by exons 6 and 7), that may also bind to protein in the cell surfaces or the ECM. The most common VEGF165 has an intermediate binding ability with a single heparin-binding domain, encoded by exon 7, and has ability for ECM bound. In inflammatory process such as appendicitis, several proinflammatory molecules with protease ability such as the MMP3 and plasmin may alter the binding site of VEGF primarily at the COOH terminus and turns VEGF from ECM-bound peptides into non-heparin-binding, diffusible, molecular species which leads to less ability inducing angiogenesis [32].

Several inhibitory isoforms of VEGF have also been recently described, including VEGF165b and VEGFAx, but there is some controversy regarding the mechanisms of inhibition, and VEGF-Ax has now been shown to actually have pro-angiogenic and pro-permeability features. VEGF expression is majorly regulated by the hypoxia state by a transcription factor named hypoxia-inducible factor (HIF). The HIF and other genes related and activated by hypoxia plays role in diverse contexts activating several transcription of other growth factors including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and some oncogenic gene mutations (RAS, VHL, WNT-KRAS signaling pathway genes) which may control the VEGF expression in other side alters the VEGF-driven signaling [31].

The most understood VEGF signaling now is through VEGFR1/R2 regulation which controls the activities of several kinases and activation of its cascades to promote cell proliferation, survival, migration, and even influencing vascular permeability on angiogenesis. The endothelial cell, which consist of both tip and stalk cells are at the main site of vascular proliferation. VEGF gradients induce tip cells and promote the formation of filopodia. The molecular regulation of these events is via activation of notch signaling and by increased expression of notch ligands on endothelial cells, including but not limited to delta-like 4 (DLL4). The increased signaling of the notch in neighboring cells will reduces the expression of VEGFR2, which is causing a negative feedback loop to the signaling process. This main signaling pathway of the VEGF plays a critical role to maintain homeostasis, but as alteration of the pathway lead to hyperactivation by pathologic process leading to pathologic angiogenesis. Another pathway were described in 2014, named as a non-canonical pathway of VEGFR2 that was characterized in neurons. It is known to be expressed more in retinal neurons but are lacking in endothelial cells. Study reveals that a deletion gene responsible in

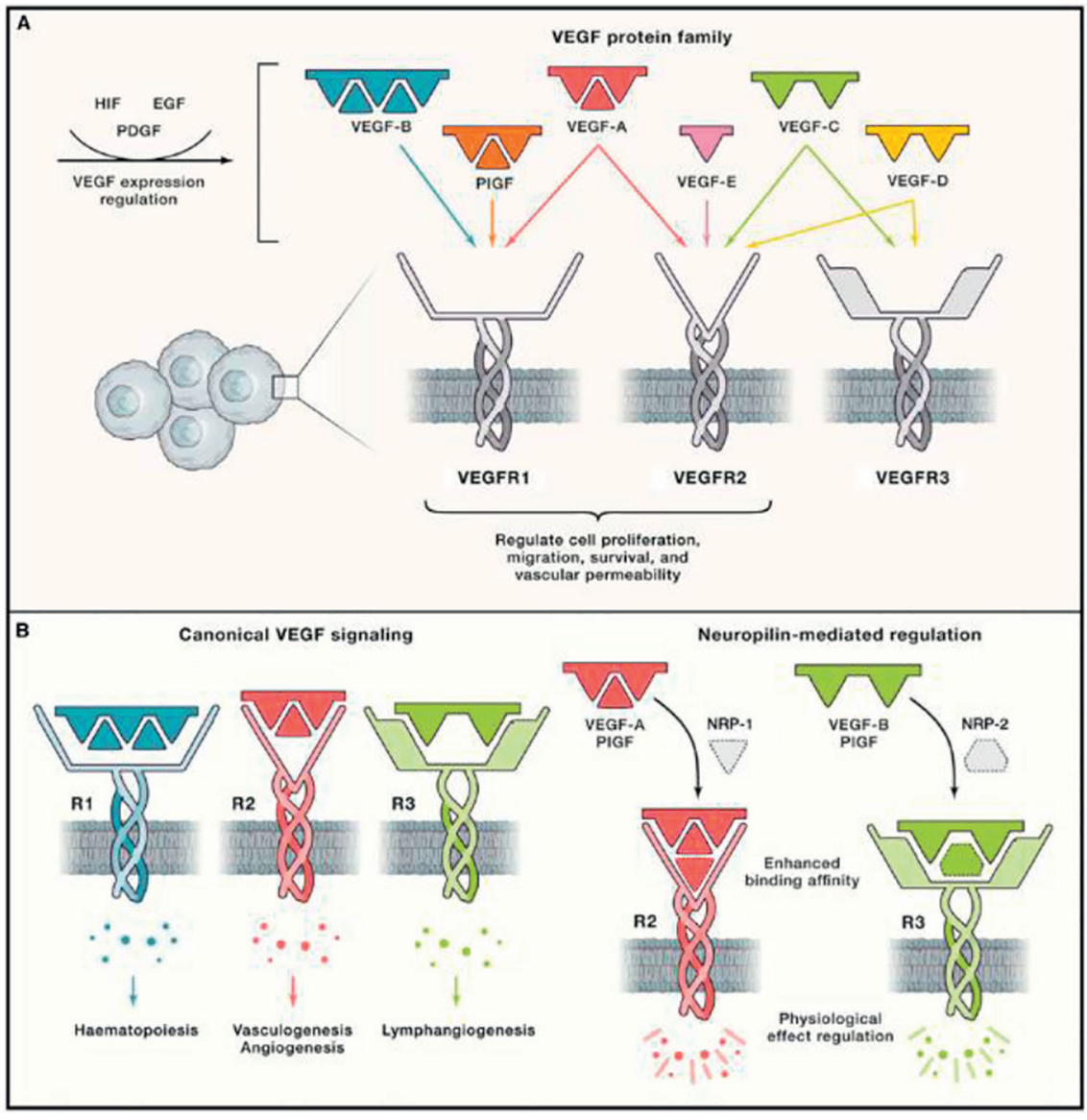

Figure 4.
VEGF activation and signaling pathways [33].

VEGFR2 pathway in neurons causes abnormal angiogenesis process by high VEGF expressions around the neuron tissue in response to deficiency of the VEGFR2. In other hand, the abnormal angiogenesis at the juxta-neural cells were common in response to maintain homeostasis in cases of ischemic retinopathy to ensure regenerative phase. This similar mechanism were a point of interest as number of VEGF expressed would be a critical factor to maintain tissue vascularization in several pathogenesis of tissue damage (**Figure 4**) [31, 33].

The findings that anti-VEGF antibodies decreased the growth of tumor cells implanted in immune-deficient mice opened up translational possibilities for targeting VEGF-VEGFR signaling. In addition, it was also demonstrated that inactivation of a single allele of the VEGF-A gene in mice resulted in defective vascular development and early embryonic lethality, highlighting the importance of VEGF during embryonic development. Inactivation of both copies of vegfr2 largely pheno-copied vegfa single-allele deletion. The ability to delete VEGF in target tissues with the advent of cre-lox systems created the possibility of assessing the role of VEGF in individual

tissues/cells. Numerous studies employing this approach have documented the important role of VEGF in angiogenesis and homeostasis in a variety of pathophysiological circumstances [34].

4.1 Role of VEGF in appendicitis

Appendicitis is the most well-known gastrointestinal emergency and requires surgical approach in the pediatric population. The negative appendectomy rate is 8.4%, however largely higher among children aged <6 years at 56.7%. However, the diagnosis of appendicitis in children is often missed due even in a total examination. This article summarizes the current evidence on the influences of folic acid (FA) and vascular endothelial growth factor (VEGF) in appendicitis. The pathological processes of appendicitis could be approached by histopathological examination of microvessel thickness. Further analysis reveals that folic acid (FA) assumed a role in mucosal opposition and its capacity to recover, and VEGF (explicitly found in vein endothelial cells) was associated in tissue remodeling through a cycle of neovascularization, reepithelization, and guide of extracellular framework and has an important pro-angiogenic activity, having a mitogenic and an anti-apoptotic effect on endothelial cells, increasing the vascular permeability, promoting cell migration, etc. Due to these effects, it actively contributes in regulating the normal and pathological angiogenic processes [29].

Both folate acid and VEGF had a role as mentioned previously by certain cascades in the endothelial cells which may increase endothelial proliferation and induces branching of new collaterals during inflammation. This mechanism ensure enough and adequate blood flow locally around the appendix. The VEGF-induced proliferation among the endothelial cells adapts to race cell damages from stretch, cytokine induced cell death, and increase intraluminal pressures. This means collateral vascularization in the appendix were stabilized and able to receive more blood flow for tissue healing process. This puts VEGF has a special role in preventing tissue damage and the complication of appendicitis such as perforation or necrosis [35].

Folate acids had been widely concentrated in cardiovascular sickness and malignancy and an increased risk of infection among patients with insufficient degrees of folate acid. A low folate acid serum and raised homocysteine was shown to be found among patients with constant provocative infections and conditions such as systemic inflammatory illness and endothelial damage. A higher folate level would prevent endothelial damage as it would help maintain levels of homocysteine, vasodilators, and nitric oxide [28, 29, 36].

Similar patterns of reduction were also observed in basal VEGF levels among patients with appendicitis as reported by Fikri et al. However, the lower level in both FA and VEGF among patients with appendicitis were significant compared to control and a possible indicator in diagnosing complicated appendicitis. Another studies had reported increases in VEGF levels, namely in myocardial localized necrosis and was related to incendiary cytokines. The increasing levels of VEGF was directly associated with the number of hypoxia-inducible factors as it regulated the advancements of angiogenesis, vascular patency in atherogenic vessels. Fikri et al. further explained that VEGF levels in appendicitis has similar pattern as increase of VEGF during the stable phases after myocardial infarction and hence signifying that VEGF as a part of an ongoing inflammatory activity. The lower levels of VEGF often signifies a worsened condition and could be associated with a more complicated case of appendicitis [28, 36].

VEGF in conclusion, a histopathological examination of microvessel thickness is required to investigate the influences of FA and VEGF towards the pathological process of appendicitis among the pediatric population. Both FA and VEGF could be associated with disease progression where lower levels often indicated a more complicated case. A higher FA was associated with less provocative conditions with less inflammation and endothelial damage and a higher VEGF often suggested better prognosis as VEGF was used in angiogenesis etc. However, in both studies FA and VEGF were still limited of evidence as statistically significantly different towards controls in their use as a biomarker in the diagnosis complicated appendicitis were done in animals but human reaches are still conducted. However other factors regarding the endothelial functions in appendix are still limited to VEGF in current studies, other mechanism related to endothelin and the Fas-ligand were also in conduct for further evidence for the current update [26, 35].

5. Conclusions

Appendicitis is known as one of the most cause of emergency surgery. But beyond facts of its surgical emergency, basic pathophysiology of the appendicitis were not completely a surgical process. Manifestations and process of the inflammation of the appendicitis were also related to its vascularization and the stability of perfusion into the inflamed tissue. Factors contributing the quality of vascularization were considered to have a significant role in determining whether an appendicitis is a process of inflammation without or with complication, between non-surgical and surgical case. This fact may be guide further study physicians to differentiate indications of appendectomy and to be selectively careful and to reduce the number of negative appendectomies. Hance, current information and data were still provided by animal research model and laboratories studies, but rationally related to clinical manifests. However researches on the current topic with human sample of appendicitis is currently still conducted.

Conflict of interest

The author declared no conflict of interest in the process of writing this article.

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