INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Histopathological Changes Associated With Appendicitis And The Influence Of Some Pathogens

Zainab Khairullah Aouda[1], Ahmed Aziz Ahmed*[2], Dmoaa Majid Nasar[3]

[1]*Department of basic medical sciences, College of Nursing, University of Thi-Qar, Nasiriyah/ Iraq*
[2-3]*Department of pharmaceutical sciences, College of pharmacy, University of Thi-Qar, Thi-Qar, 64001, Iraq.*

ARTICLE INFO

Received: 01 May 2024
Accepted: 04 May 2024
Published: 13 May 2024

Keywords:
Influence , Appendicitis, Histopathological, Changes

DOI:
10.5281/zenodo.11185824

ABSTRACT

Appendicectomy for acute appendicitis is one of the most commonly performed surgical procedures. A retrospective study was performed to determine histopathological change accompanying appendicitis and histological diagnosis.

Methods:

Histopathological examination was conducted for 20 appendices. Taken from Nasiriyah General Hospital in Dhi Qar Governorate.Where a tissue fixation was performed to study the histopathological diagnosis and unusual finding on histology

Results:

Out of 20 specimens of appendix. All of them have appendicitis. Histopathological diagnosis include: -mucosal glandular hyperplasia, erosion of mucosal layer 43%. -necrosis of mucosal and submucosal layer 42%.-severe inflammatory cell infiltration 71%.

Conclusion:

The present study showed a high number of appendicitis in adolescents and young adults. The study support routine histological examination of all the appendicectomy specimens to avoid missing any clinically important and treatable.

INTRODUCTION

Appendix is an organ connected to the digestive system and usually referred to as functionless organ in human. It is now well recognized that this organ may play a role in immunity [1].The position of this organ lies in the right lower part of the abdomen. In spite of the minor significance of this organ, it is susceptible for infections as other organs such as hepatitis, pneumonitis, cholecystitis, tonsillitis and encephalitis [2]. The appendix is known as a site of acute and chronic infections, as it is difficult to get an appendix in a normal way, because the appendix has an end. They are clogged and their contents do not regenerate quickly, they are constantly prone to inflammation[3]. Acute appendicitis is one of the

***Corresponding Author:** Ahmed Aziz Ahmed
Address: *Department of pharmaceutical sciences, College of pharmacy, University of Thi-Qar, Thi-Qar, 64001, Iraq.*
Email : ahmed_aziz@utq.edu.iq

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

most common inflammatory diseases of the gastrointestinal tract, [4]. About 7% of the world's population suffers from acute appendicitis during their lifetime. [5] in America, there are about 250,000 cases of appendicitis annually [6]. In the UK, there are approximately 70,000 cases of infection per year [7]. Appendicitis rarely occurs before the age of two years and its symptoms begin during the later stages of [8]. Childhood and adolescence. The incidence of appendicitis increases among the age group 10-30 years [9]. The percentage of passive appendectomy also ranges from 20-40% [10]. Acute appendicitis is not limited to a specific age category and it is possible that parasites can cause acute appendicitis [11] . Most cases of acute appendicitis occur as a result of blockage of the lumen of the appendix due to hardening of the stool or enlargement of the lymphoid-mucous tissue due to viral, bacterial or parasitic infection, such as infection with the pinworm parasite [12]. Appendicitis is a common condition that needs surgical intervention to prevent complications. About 250,000 cases of appendicitis occur annually in the United States. Approximately 80% of cases of appendicitis occur in people under the age of 45, and this inflammation occurs in males 1.4-1.3 times more often than in females [13]. The appendix naturally opens with the intestines but can become clogged with hardened feces, swollen tissue, parasitic worms, or bacteria that can grow and cause appendicitis. Appendicitis is most common between the ages of 30-10 years, although it can occur at any age [14]. It is rare in children under two years of age [15]. Appendicitis has been reported worldwide. In USA, it was estimated as 250,000 case per / year [16]. In Britain the infection rate was estimated at an average of 70,000 case per/ year [17].There are no accurate records about appendicitis in Iraq. The general Cases being registered in Nasiriya General Hospital were 530 case per/ year; while in Al habobi hospital were 450 case per/ year. Appendix can be infected by a variety of microbes (parasite, viral and bacterial). A surgical operation should be done to remove this organ and to protect the body and maintain its health from complications.

MATERIALS AND METHODS:

Study area.

The study was conducted in the year 2022/2023 / Nasiriyah in Thi Qar Governorate, which is in Iraq, which is bordered by the governorates of Basra from the south and Maysan Governorate from the east Muthanna Governorate to the west and Wasit Governorate to the north, conducted a study on appendicitis prevalent in this governorate. The study included collecting samples from patients with this infection.Where the duration of the study took a long period, starting from 10/30/2022 and ending on 4/30/2023, where this study was conducted to find out the main causes of appendicitis. The location and histological structure.Knowing its symptoms and also identifying its didiagnosis.

Sample collection.

Ten samples of appendicitis were collected and fixed in 10% formalin, and the collection period did not exceed 10 days. They were collected from Nasiriyah General Hospital after operations were performed to remove the appendix from patients with appendicitis. They were transferred directly to the laboratory in Al-Hussein Teaching Hospital for bacteriological examination. Where There were several types of appendixes, some thick and some thin, and the process of collecting samples took place after obtaining a letter facilitating a task from the Thi Qar Health Department for the purpose of collecting samples of appendicitis. Several preparatory steps were conducted in Al-Hussein Teaching Hospital in Thi Qar Governorate. As follows:_

1. Appendix 8.5×5 cm two pieces taken.
2. Appendix 5×1 cm two pieces taken.

3. Appendix 8×1 cm around fatty tissue two pieces taken.
4. Appendix 5×1 cm around by fatty tissue two pieces taken.
5. Appendix 7.5×1.5 cm fatty tissue two pieces taken.
6. Appendix 6.5×0.5 cm fatty tissue two pieces taken.
7. Appendix 6.5×1 cm two pieces taken.
8. Appendix 7×1 cm around fatty tissue two pieces taken (thick wall).
9. Appendix 5×0.5 cm two pieces taken.
10. Appendix 10×1 cm around fatty tissue two pieces taken.

Histological cutting. First, the doctor removes the sample from the patient After that we do a macroscopically examined for the sample possibility of ulceration, damage to the outer wall of the sample, inflammation or swelling ، The sample is then passed through the steps below.

1. Fixation:

It is the first step and includes three basic functions: It maintains the shape of the tissue from deterioration by preventing Autolysis and rotting process by small organisms by using fixatives such as 10% formalin [10%formalin it's Consists of 10% formalin and 90% tap water] , gives rigidity to the tissue, and

Facilitates the staining process later.

Tap water is used instead of distilled water in formalin because tap water prevents swelling of the sample due to it containing a percentage of salts. Distilled water is highly osmotic(hypotonic), and this causes its penetration into the tissue and causes swelling in the sample (distilled water free of salts will move to areas of high concentration of salts, and it penetrates the tissue, causing swelling).

2. Washing:

Using tap water for a long (6-24) hours to remove fixative from the tissue ,and It usually depends on the type of fixative.

3. Dehydration:

Common dehydration fluids are Ethanol, commercial industrial methylated spirit, methanol, propanol (isopropyl alcohol), acetone The dehydration process is the removal of water using dehydration liquids and in this samples alcohol was used and alcohol is introduced into the tissue in ascending concentrations are 70%,80%,90%,95% ,100% ,100%, 100% thus, the water was eliminated and the alcohol became inside the tissue.

4. clearing

Substances are used to remove alcohol and are removed by wax these substances are clearing agent and there are common types of clearing agent are Xylene (xylole), toluene chloroform ,benzene ,cedar wood oil . In these samples, xylol was used and it is used with three changes in each change of one hour, so that the xylol became inside the tissue.

5. infiltration (impregnatlon)

Paraffin wax is used where it is melted at a temperature of 60C, and thus the paraffin wax becomes liquid and the fabric is placed inside it using three changes, and thus the paraffin wax becomes inside the fabric and the paraffin wax gives a support to the fabric, and then the fabric and its cavities become hardened and protected with wax.

6. Embedding :

In this step, paraffin wax is also used, but it does not contain residues of xylene, a mold is used, paraffin wax is poured into it continuously, and then the sample is placed inside the mold containing paraffin wax. There is a phenomenon in paraffin wax that is the phenomenon of surface tension and to prevent this phenomenon from happening, the wax is blown into the mold . Another phenomenon that occurs in paraffin wax is the phenomenon of crystallization, and the reason for this phenomenon is that the wax cools slowly, which are bubbles that are inside the wax,

and these bubbles weaken the integrity of the block, and it is possible that during cutting they are crushed by Microtome knives.to prevent this phenomenon, The Block is placed in the refrigerator overnight and as a result, rapid cooling occurs, which prevents the crystallization phenomeno.The purpose of this step Is to support the sample from the outside.

7. Trimming process:

After preparing the wax molds, it is advisable to trim them with a sharp blade so that the sample is in a suitable position For cutting so that its edges become parallel and can apply to the edge of the microtome knife.

8. cutting

After completing the fixation steps, the process of cutting the tissue (sample) using the microtome device: The block that contains the sample is cut with a microtome device with a thickness of (3_5)µm, as soon as a slice containing tissue appears, it is taken directly and placed in the water bath device at a temperature of (40_45)C˚ in order to ensure the expansion of the sample. After that, a new slide is used, and using Mayer's egg albumen [It's consist of egg albumen,glycerin and crystals of thymol to prevent rotting] it works as a glue to stick the sample slic on the slide.

9. Staining

After that, the Staining process begins, but first the wax in the slice (tissue) must be removed by dewaxing process, using the oven at 60 degrees for about half an hour. Immediately after it is extracted from the oven, it is placed in three variables of xylene (xylole), and the sample is left for a quarter of an hour in each variable so the wax was removed. Since the medium that carries dyes inside the tissue is water and because of the removal of paraffin wax by xylene (xylole), the tissue contains xylene (xylole), so it must be expelled from the tissue and replaced with water, but the question is how is this done. The xylene (xylole) is removed by using descending concentrations of alcohol (100_70). After the last concentration, the tissue is taken and placed in water, after which the dyeing process becomes possible.

The Staining process is done by using two dyes:

1. The first is hemotoxylin; It is a basic stain that is attracted to acidic structures, and since the highly acidic structure is the nucleus, it dyes the nucleus in a blue or violet colour. It also stains any acidic structure such as protein, so the dye must be shortened by differentiation, as the dye sticks to high-acid structures only, and an alcoholic acid 1% (consisting of 99 alcohols and 1 HCL compound) is used. The slide was placed four times with alcohol acid 1% and then washed with water, so the dye was confined to the nucleus only.
2. The second dye is eosin: it is an acidic dye that is attracted to the base compounds, where it stains the cytoplasm, because its compounds are alkaline and dye pink. Thus, the cell became dyed in two colors, the nucleus is blue or violet, and the cytoplasm is pink. The water inside the tissue that served as a medium for dye transfer is no longer needed for its presence, so it is expelled from the tissue by dehydration through progressive alcohol concentrations (70_80_90_95_100). It should be noted that the presence of alcohol inside the tissue causes cloudiness, and therefore it must be disposed of, and this is done by using xylene (xylole) , so it will filter the tissue as well. At the end of the process, a cover slip is attached to the sample on the slide with a material called DPX. Thus, it becomes a tissue that can be preserved for decades.

RESULTS:

Histopathological examination was conducted for 20 appendices. There are histopathological changes was recorded in all excised appendices.

These were stained with hematoxylin and eosin and imaged with different magnification power.

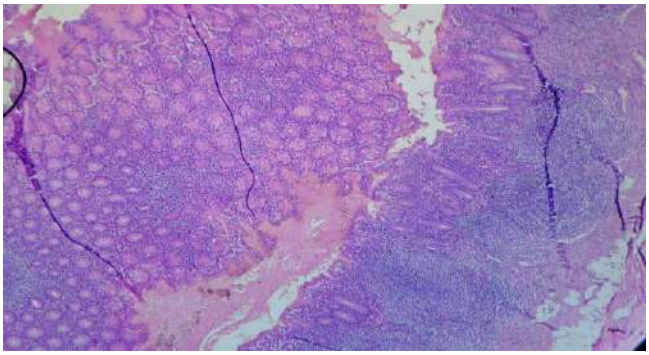

Fig. 1. a. mucosal glandular hyperplasia, b. cavitation of lymphatic follicles, c. mucosal ulcerate and necrosis, d. blood sinusoids (40x) (H&E).

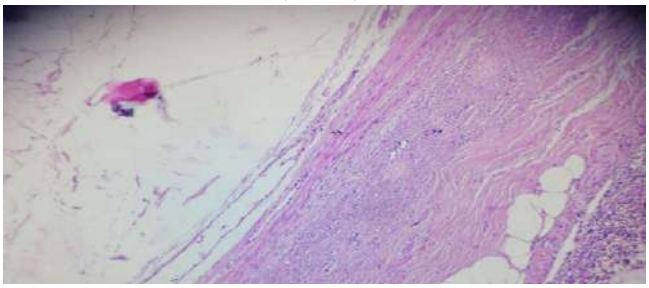

Fig. 2. a. erosion of mucosal layer, b. necrosis of mucosal layer, c. necrosis of submucosal layer, d. oedema, e. mild inflammatory cell infiltrations (10x) (H&E).

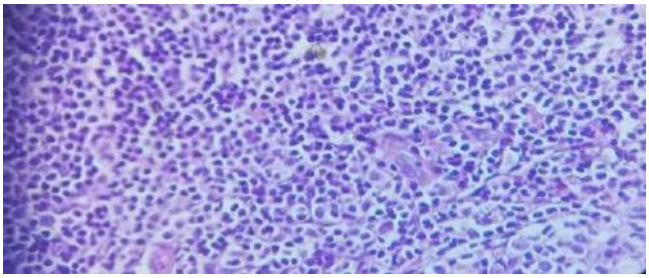

Fig. 3. severe inflammatory cell infiltrations (100x) (H&E)

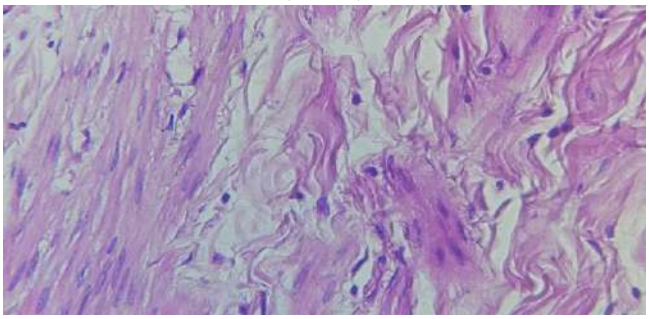

Fig. 4. liquefactive necrosis (400x) (H&E).

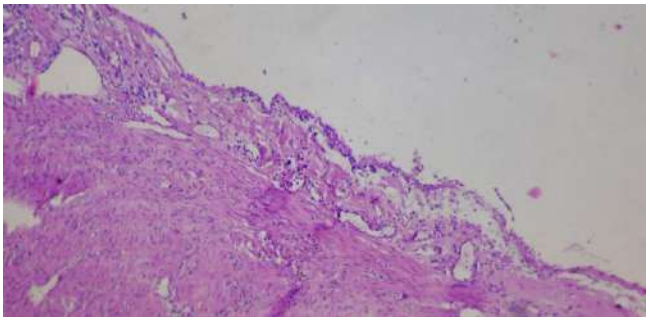

Fig. 5. mucosa atrophy, b. mucosa erosion, c. mild inflammatory cellular infiltrations, d. necrisos of mucosal layer, e. fibroblastic proliferation (100x) (H&E).

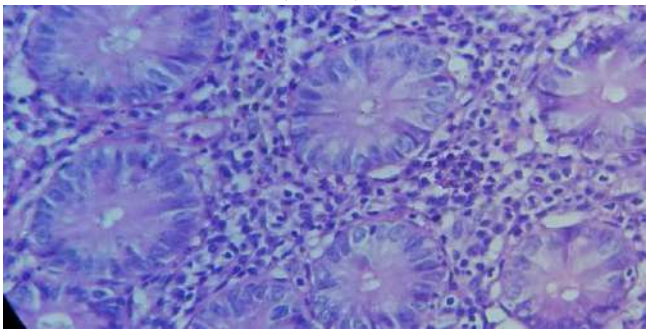

Fig . 6. expansion and hypertrophy of lymphatic ducts and severe inflammatory cell infiltrations (400x) (H&E).

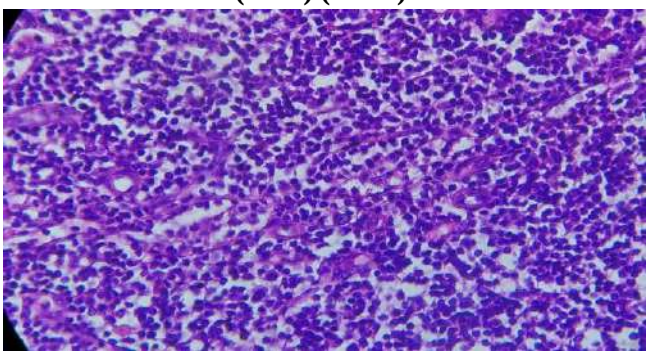

Fig. 7. severe inflammatory cell infiltrations (400x) (H&E).

DISCUSSION:

Appendicitis is of great Importance because of its frequent occurrence and serious complications and because it affects all ages and both sexes, although it is more common between the ages of 10-30 years (18). Many scientific studies and researches have confirmed the role of bacterial, viral and parasitic pathogens in the occurrence of this inflammation. Due to the fact that the appendix is blocked at the end, its contents do not regenerate quickly, and

therefore it constantly becomes a site of acute and chronic inflammations (19), which progress towards reaching the body cavity (20). The rapid reproduction of primary animals and worms makes it a major health problem in developing countries, especially in South and Southeast Asia, where a combination of factors such as heat, monsoons, high population density, lack of education and public health conditions, as well as biological and mechanical resistance to parasitic growth phases in the environment, which together maintain the survival and transmission of primary animals and worms in the environment. Parasitic infections negatively affect health, nutrition and growth, as they lead to serious pathological complications, they block vital organs in his body or invade tissues, which necessitates surgical intervention, as happens in appendicitis(21), as well as host competition in his food(22).As for the role of parasitic factors causing inflammation, it is the subject of controversy and debate.some researchers have proven the presence of some intestinal parasites in the inflamed appendages without determining their pathological role in appendicitis due to the presence of the parasite in a small percentage in the inflamed appendages, as explained (23) when watching the body pieces of tapeworms in the cavity of the appendix. However, these results were documented with some significance and significance by others who confirmed the pathological role of the parasite, especially in histological studies of infected appendages, which counted parasites as pathological organisms closely related to the occurrence of inflammation(24).Confirm (25).The relationship of pinworms and their association with appendicitis, indicating that the percentage of this relationship varies between 0.2-41.8% worldwide.As for the relationship between the total infection of appendages with parasites and the sex of the infected, the current study showed that there is no significant difference in infection between females and males (47% for females versus 61.7% for males), this may be due to the presence of the same opportunity to cause infection in both sexes, such as the same food, presence in the same environment and drinking the same contaminated water…Etc. This is consistent with the findings of (26) (3.3% of females and 4% of males were injured) and the severity (27), where the injury was 41.9% of females and 58% of males. From this, it was found that there is no influence of the sex factor in causing parasitic infection of appendages, which confirms that gender itself is a non-essential factor in determining the susceptibility and predisposition of an individual to infection with such intestinal parasites. In the current study, no infection of the appendix with the bovine tapeworm Taenia saginata and the fish tapeworm Diphyllobothrium latum was recorded, due to the lack of common consumption of raw or poorly cooked meat in Iraq. The chance of infection of the appendix with schistosomiasis worms or their eggs remains low due to the fact that the rate of infection with the blood splinter is low in the city of Baquba, however, the infection of the appendix with these worms was recorded by 44% (28) at Al-Sabah Hospital in Kuwait .The results of the current study showed the presence of common parasitic infections in one appendix by 3.1% in which the dysentery amoeba parasite shared with other intestinal parasites. Co – infection with parasites in the appendix has been recorded by (29) . Between dysentery and pinworm amoeba, and between whipworm and nematode, as well as co-infection was recorded by (30) between pinworm and dysentery amoeba, pinworm and Giardia lamblia, pinworm and whipworm, and between pinworm and nematode. This slight discrepancy is mainly due to the quality of parasites recorded in each study and the infection rates of each.the current study showed the infection of appendages by two groups of parasites, namely primary animals and intestinal

worms. The incidence of dysentery Amoeba was high compared to other parasites, with an infection rate of 53.1%, the highest rate recorded compared to(31) 0.97% recorded at Mosul Teaching Hospital in Nineveh (32). It is 0.21% in Mosul as well and (33) in Baghdad Teaching Hospital which is 0.7% and (34) which is 2.8% in Najaf governorate.

On the one hand, and on the other hand, the presence of parasites in the intestine reduces the efficiency of the immune system and thus reduces the host's resistance to other pathological infections represented by viral and bacterial infections, becoming more susceptible to diseases (35)

REFRENCES:

1. Zuercher, A.W.; Coffin, S. E.; Thurnheer, M. C.; Fundowa, P. & Cebra, J. J. (2002). Nassal associated lymphoid tissue a mucosal inductive site for virus. Specific humoral and cellular immune responses. J. Immuno., 168: 796- 803.
2. Schwartz, S.; shives, G. & Tom, A. (1996). Principle of surgery. 6th ed. Hill book comp., PP: 1307.
3. Junqueira, L.C. & Carneira, J. (2005). Basic histology: text and atlas. 11th ed. McGraw-Hill, USA, PP: 502 .
4. Becker, K. & Höfler, H. (2002). Pathology of appendicitis. Chirurg., 73(8): 777-781.
5. Pasupati, T. M.; Yothasamutr, K.; Wah, M. J.; Sherif, S. E. T. & Palayan, K. (2008). A study of parasitic infections in the luminal contents and tissue sections of appendix specimens. Trop. Biomedicine, 25(2): 166-172.
6. Addiss, D. G.; Shaffer, N.; Fowler, B. S. & Tauxe, R. V. (1990). Epidemiology of appendicitis and appendectomy in the United States. Am. J. Epid.,132 (5): 910-925.
7. Rao, P. M.; Rhea, J. T.; Novel line, R. A.; Mostafavi, A. A. & McCabe, C. J. (1998). Effect of computeriz tomography of the appendix on treatment of patients and use of hospital resources. New. Eng. J. Med., 338(3): 141-146 .
8. Rains, A. J. H. & Capper , W. M. (1988). Baily and Loves short practice of surgery. 20th ed. PP: 1009.
9. Hardin, D. M. (1999). Acute appendicitis: review and update. Am. Fam. Physician, 60: 2027-2034.
10. Dado, G.; Anania, G.; Baccarani, U.; Marcotti, E.; Donini, A. & Risaliti, A. (2000). Application of a clinical score for the diagnosis of acute appendicitis in childhood. J. Pediatr. Surg., 35: 1320-1322.
11. Pasupati, T. M.; Yothasamutr, K.; Wah, M. J.; Sherif, S. E. T. & Palayan, K. (2008). A study of parasitic infections in the luminal contents and tissue sections of appendix specimens. Trop. Biomedicine, 25(2): 166-172.
12. Schneider, A. S. & Szanto, A. A. (1992). Pathology board review Williams & wilkins a waverly comp. Philadelphia, PP: 220-223.
13. Itskowitz, M. S. & Jones, S. M. (2004). Appendicitis. Emerg. Med., 36(10): 10-15.
14. Al-Zwaini, E.J.; Al-Dalla Ali, F.J. & Al-Obaidi, A.F. (2002). Perforated acute appendicitis in a neonate with Down syndrome. Al-Anbar Med. J., 4(1): 73-74.
15. Shamkhi, A.L. & Al-Ameedi, S.H. (2002). Diagnosis of acute appendicitis according to clinical evaluation and operative findings. Kufa Med. J., 5(1): 126-133.
16. Addiss, D. G.; Shaffer, N.; Fowler, B. S. & Tauxe, R. V. (1990). Epidemiology of appendicitis and appendectomy in the United States. Am. J. Epid.,132 (5): 910-925.
17. Rao, P. M.; Rhea, J. T.; Novel line, R. A.; Mostafavi, A. A. & McCabe, C. J. (1998). Effect of computeriz tomography of the appendix on treatment of patients and use of hospital resources. New. Eng. J. Med., 338(3): 141-146 .

18. Shamkhi, A.L. & Al-Ameedi, S.H. (2002). Diagnosis of acute appendicitis according to clinical evaluation and operative findings. Kufa Med. J., 5(1): 126-133.
19. Majeski, J. (2003). Diverticulum of the vermiform appendix is associated with chronic abdominal pain. Amer. J. Surg., 186(2): 129-131.
20. Junqueira, L.C. & Caneiro, J. (2003). Basic histology: Text and atlas, 10th edn. Lange Med. Books, Toronto: 515 pp.
21. Gillesse, E.G.; Mud, H.J. & Prins, M.E. (1987). An unusual cause of acute appendicitis. Neth. J. Surg., 39(5): 153-154. (Medline Abst.).
22. Haque, R.; Huston, C.D.; Hughes, M.; Houpt, E. & Petri, W.A. (2003). Amebiasis. N. Engl. J. Med., 348(16): 1565-1573.
23. Upton, A.C. (1950). Taenial proglottides in the appendix: Possible association with appendicitis. Amer. J. Clin. Pathol., 20: 1117-1120.
24. Al-Dabbagh, N.Y.; Al-Izzi, N.S. & Hayatte, Z.G. (1994). The role of Enterobius vermicularis in human appendicitis. J. Fac. Med. Baghdad, 36(3): 465-473.
25. Arca, M.J.; Gates, R.L.; Groner, J.I.; Hammond, S. & Caniano, D.A. (2004).
26. Clinical manifestations of appendiceal pinworms in children: An institutional experience and a review of the literature. Pediater. Surg. Int., 20(5): 372-375. (Medline Abst.).
27. Husain, M.H. (1995). Microbial causative agents of appendicitis. M. Sc. Thesis, Coll. Med., Univ. Baghdad: 77 pp.
28. Hoda Ali Saleh (2002). Epidemiological study of the parasitic etiology of appendicitis in Najaf governorate. Master thesis, Faculty of Science, University of Kufa: 65 pages.
29. Fataar, S. & Satyanath, S. (1986). The radiographic evaluation of appendiceal calcification due to schistosomiasis. Amer. J. Trop. Med. Hyg., 35(6): 1157-1162.
30. Al-Dabbagh, N.Y.; Al-Izzi, N.S. & Hayatte, Z.G. (1994). The role of Enterobius vermicularis in human appendicitis. J. Fac. Med. Baghdad, 36(3): 465-473.
31. Hoda Ali Saleh (2002). Epidemiological study of the parasitic etiology of appendicitis in Najaf governorate. Master thesis, Faculty of Science, University of Kufa: 65 pages.
32. Majeed, A. & Al-Bakri, H. (1984). Acute appendicitis in Nineva province. J. Fac. Med. Baghdad, 26(1): 71-76.
33. Al-Dabbagh, N.Y.; Al-Izzi, N.S. & Hayatte, Z.G. (1994). The role of Enterobius vermicularis in human appendicitis. J. Fac. Med. Baghdad, 36(3): 465-473.
34. Husain, M.H. (1995). Microbial causative agents of appendicitis. M. Sc. Thesis, Coll. Med., Univ. Baghdad: 77 pp.
35. Hoda Ali Saleh (2002). Epidemiological study of the parasitic etiology of appendicitis in Najaf governorate. Master thesis, Faculty of Science, University of Kufa: 65 pages.
36. Espinosa-Cantellano, M. & Martínez-Palomo, A. (2000). Pathogenesis of intestinal amebiasis: From molecules to disease. Clin. Microbiol. Rev., 13(2)

HOW TO CITE: Zainab Khairullah Aouda, Ahmed Aziz Ahmed, Dmoaa Majid Nasar, Histopathological Changes Associated With Appendicitis And The Influence Of Some Pathogens, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 5, 593-600. https://doi.org/10.5281/zenodo.11185824