

Detection of Infectious Agents Causing Appendicitis using some Conventional and Special Histochemical Stains, Sudan

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Abstract: Appendicitis is the most common cause of acute abdomen in young adolescents and cause by variety of infectious agent including bacteria, virus, parasite and fungi ,and appendectomy is often the first major procedure performed by a surgeon¹ . This is cross sectional laboratory based study conducted in Omdurman Teaching Hospital during the period from 2018 to 2019. The study aimed to detect the infectious agents causing appendicitis in appendix tissue samples .Fifty samples were randomly collected after appendectomy, their ages ranged between 1-80 years , with mean age (32.6) , 11-20 years was the most frequent age group. section were prepared and stained using four different stains , Gram stain for demonstration of bacteria, Macchiavellos stain for demonstration of viral inclusions ,periodic acid schiffs (PAS) for demonstration of fungi, Hematoxylin and eosin (H&E) for general morphology and demonstration of parasite , Data was analyzed using SPSS computer program. Histopathologically ,43(86%) of the cases were diagnosed as acute appendicitis, 7(14%) as chronic appendicitis .15 (30%) of cases shows presence of bacteria . Intranuclear inclusion (viral infection) were seen in 13 (26%) of cases. and no fungal and parasitic infection were noticed. The study concluded that bacterial and viral infection associated with appendicitis particularly the acute form .The study recommended using some Conventional and Special Histopathological stain for detection of the infectious agent causing appendicitis . and using larger sample size to identify the infectious agent causing appendicitis.

Keyword: Detection, Infectious, Appendicitis Conventional , Histochemical Stains , Sudan

Introduction : The Vermiform appendix present only in human beings, certain arthropod Apes and the wombat (a nocturnal, burrowing Australian marsupial) was probably first noted as early as the Egyptian civilization .It was described as "worm of the intestines" ⁽²⁾ . The vermiform appendix is located in right lower quadrant of abdomen ⁽³⁾ . It is a narrow, worm shaped tube which arising from the posteromedial caecal wall, approximately 2 cm below the end of the ileum⁽⁴⁾ . Its length varies from 2-20 cm , with an average length of 9 cm⁽⁵⁾ . Appendix of the male is, on an average, 1 cm longer than that of the female ,its opening is occasionally guarded by a semi circular fold of mucous membrane known as the valve of garlach. It is the only organ in the body that has no constant anatomical position⁽²⁾ . The attachment of the base of appendix to the caecum remains constant, where as the tip can be found in a retrocaecal , pelvic ,subcaecal , paracolic, preileal and postileal position .It is connected by the short mesoappendix to the lower part of the ileal mesentery .This fold is usually triangular, extending almost to the a ppendicular tip along the whole tube. The mesoappendix has a free border which carries the blood supply to the organ, by the appendicular artery, a branch from the ileocolic artery⁽²⁾. Acute appendicitis is the most common cause of "acute abdomen" in young adolescents and appendectomy is often the first major procedure performed by a Surgeon⁽¹⁾ . Variations in the position of the appendix ,age of the patient and degree of inflammation make the clinical presentation of appendicitis not odiously in consistent. Misdiagnosis in different age groups is from 10 to 33% ⁽³⁾ . Despite extraordinary advances in modern radiographic imaging and diagnostic laboratory investigations, the diagnosis of appendicitis remain essentially clinical requiring a mixture of observations, clinical examination and surgical

sense. Appendicitis may be cause by variety of infectious agent, some with specific histological appearance, and other with non specific feature that may necessitate Abroad diagnostic assessment⁽⁶⁾ .

Yersinia is one of the most common causes of bacterial enteritis in Western and Northern Europe. It has a worldwide distribution, the incidence of infection is rising both Europe and the United States, Although this may be due to better methods of detection and wider recognition of Yersinia as important enteric pathogens. Yersinia infection can be transmitted by both food and water⁽⁶⁾ . Yenterocolitica and pseudo tuberculosis are the species that cause human GI disease, These fastidious gram negative coccobacilli have been implicated in numerous GI illnesses, including appendicitis (particularly granulomatous appendicitis) and mesenteric lymphadenitis⁽⁷⁾ . Fever, pharyngitis, and leukocytosis may be present as well Symptoms often have been present for weeks to months, leading to misdiagnosis as chronic idiopathic inflammatory bowel disease. Reactive poly arthritis and erythema nodosum are also associated with yersinia infection. Infants, children, and young adults are most commonly infected. Patients with granulomatous appendicitis caused by Yersinia often present with signs and symptoms indistinguishable from acute non specific appendicitis. However, some patients with yersiniosis are initially believed to be suffering from appendicitis.

Many parasites can be found in the lumen of the appendix, including pinworms (most commonly), Ascaris (roundworms), Giardia, and Entamoeba histolytic. Clinicians should be alerted when parasites are found in the appendix that could affect other parts of the GI .

Pinworms are one of the most common human parasites. These nematodes have a worldwide distribution, but are more

common in cold or temperate climates and in developed countries. Prevalence is highest among children ages 5 to 10 years, and it has been reported that pinworm infections of the Gastro intestinal tract affect 4% to 28% of children around the globe. These infections are common in the United States and North western Europe. The infective egg resides in dust and soil, and transmission is believed to be via the fecal oral route. In the appendix, the mucosa seems normal, and pinworms are most often found in the appendiceal lumen. Even invasive pinworms incite little, or no, inflammatory reaction, but rarely an inflammatory infiltrate composed of neutrophils and eosinophils may occur⁽⁸⁾, along with hemorrhage and ulceration. Granulomas, sometimes with necrosis, may develop rarely as a reaction to degenerating worms or egg. Adenovirus is one of the more common viruses described in the appendix, is also associated with ileal and ileocecal intussusception, particularly in children⁽⁸⁾. The virus is believed to cause intussusceptions by producing lymphoid hyperplasia, Altering intestinal motility, or a combination of both. Morphologic changes are subtle, including lymphoid hyperplasia and overlying disorderly proliferation and degeneration of surface epithelium.

Fungal infection of the appendix is rare. Mycetomycosis has been reported to cause inflammatory masses of the right lower quadrant involving the appendix, ileum, and cecum in patients undergoing chemotherapy⁽⁹⁾. Histoplasmosis may involve the appendix as part of generalized infection of the GIT usually in immune compromised patients. Patients usually require anti fungal therapy following resection. An obstruction, or blockage, of the appendiceal lumen is the dominant factor of appendicitis. Mucus backs up in the appendiceal lumen, causing bacteria that normally live inside the appendix to multiply. As a result, the appendix swells and becomes infected. Some unusual factors could also be the reason. This may be due to lymphoid hyperplasia, intestinal worms, malignant or benign tumor, foreign material.⁽¹⁰⁾

Materials and methods :

Study design: This is cross sectional laboratory base study in Omdurman Teaching Hospital at Khartoum state during 2019 to 2020, fifty appendix samples of tissue after appendectomy were taken from patients with appendicitis and Questionnaire was designed to collect the data. Data were analyzed by using SPSS.

Ethical considerations : The specimens and information that were collected from patients is not used for any purposes rather than this study. This study were approved by faculty of medical approval permission department of histopathology and cytopathology. Permission this approve research committee

Sample collection : Appendix sample were collected from the surgical theatre immediately after Appendectomy and preserved in 10% formalin⁽¹⁰⁾.

Sample processing : Specimen were collected from Omdurman Teaching Hospital and preserved in 10% formalin solution. They were examined grossly for signs of inflammation, any swelling, hemorrhage or perforation and measurements were recorded. Three cut sections were made at the following sites: base, tip and middle of the appendectomy specimen as well as from any other abnormal region. The lumen of the appendix was examined for obstruction and contents. Selected pieces of tissue have been inserted in a plastic cassette and labeled, then processed in a tissue processor according to the following procedure. Sample were dehydrate through 50% ethanol for half an hour, 70% ethanol for one hour, 90% ethanol for one hour, and two changes of absolute ethanol for two and half hour, then were cleared in two changes of xylene for four hours, then impregnated in paraffin wax for six hours, then embedded in cassette block using moulds. four sections of 3-5 thickness were obtained from each tissue samples using rotary microtome.⁽¹¹⁾

Sample staining: Section were dewaxed in hot plate oven and cleared in 2 changes of xylene for 2 minutes, then rehydrated through ethanol (100%, 90%, 70% and 50%) and water for two minutes, then stained using hematoxylin and eosin. Gram stain, PAS stain, Macchiavello stain. stained sections were examined by light microscopy for histopathological diagnosis.

Hematoxylin and eosin : All section were deparaffinized and rehydrated through graded alcohols to distilled water, after that sections were stained with Mayer hematoxylin for 5-10 min, and washed well in running tap water until section blue for 5 minutes or less, differentiated in 1% acid alcohol (1% HCL in 70% alcohol) for 5-10 sec, and washed well in tap water until sections are again blue (5-10 minutes), or blued by dipping in an alkaline solution (e.g. 1% eosin Y for 10 minutes, washed in running tap water for 1-5 min, dehydrated, cleared and mounted⁽¹¹⁾

Periodic acid shifts for fungi : All section were deparaffinized and rehydrated through graded alcohols to distilled water, then treated with periodic acid for 5 min, and washed well in several changes of distilled water, then covered with shifts solution for 15 min, washed in running tap water for 5-10 min, and stained with Harris Hematoxylin, the differentiation as appropriate in acid alcohol and blued as usual, after that washed in water, rinsed in absolute alcohol, cleared and mounted⁽¹¹⁾

Grams stain for bacteria : All section were deparaffinized through graded alcohols to distilled water, after that stained with crystal violet solution, 2min, rinsed in tap water and drained, then covered with Iodine solution for 2 min, rinsed in tap water, after that blotted and flooded with acetone for 1-2 seconds, and washed in tap water, then Counterstained in neutral red for 3min, blotted, dehydrated, rapidly, cleared and mounted⁽¹¹⁾

Macchiavellos stain for rickettsia and viral inclusions : All section deparaffinized and rehydrated through graded alcohols to distilled water, then stained in 0.25% basic fuchsin for 30 min, and differentiated in 0.5% citric acid for 3 seconds, washed in tap water for 2 min, counterstained in 1% methylene blue for 15-30 seconds, rinsed in tap water, dehydrated, cleared, and mounted⁽¹¹⁾

Result Interpretation : H and E stained tissue sections, PAS, Macchiavellos and gram stained sections were examined using light microscopy and seen to find the infectious agent or marker for their present in the tissues section

Results : A total of 50 tissue samples of appendicitis were examined to detect the infectious agent causing appendicitis using some Conventional and Special Histopathological Stains. All patients were Sudanese. The patients age ranged between 1 to 80 years old with mean age (32.6), 11-20 years was the most frequent age group 15 (30%) out of 50, followed by 21-30 years 10 (20%) see Table (4-2).

The majority of study population was males 33 (66%) while female were 17 (34%). With male to female ratio of (1.5 : 1) that illustrated in figure (4-1). The histopathological diagnosis classified the cases as acute, chronic, distributed as 43 (86%), 7 (14%) respectively, the highest incidence of acute and chronic inflammation in the age group from 11 to 20 years, the lowest incidence is in the age group from 1 to 10 years, and from 61 to 70 years (table 4-2).

In gross examination the length and diameter of each appendix sample was measured and recorded and the mean length were found 7.4, and 0.8 as mean diameter.

Association of bacteria and virus inclusion bodies were present in 3 cases (6%), both are males, more than 20 years old.

Bacteria present in 17 samples (34%). all of them found in acute appendicitis group 8 (47%) out of 17 cases were gram positive bacilli, and 4 (23.6%) out of 17 cases were gram positive cocci, 2 (11.8%) out of 17 cases is gram negative cocci, 3 (17.7%) showed mixed population of gram positive bacilli and gram negative cocci. 16 (94.2%) out of 17 cases showed bacteria were males and 1 (5.9%) were females. Table (4-1) shows the result of gram stain.

Virus inclusion bodies were present in 13 (26%) of cases and absent in 37 (74%). 7 (53.8%) out of 13 cases were males and 6 (46.2%) were females.

Table (4-2) show distribution of viral inclusion bodies in different types of histopathological diagnosis.

Periodic acid shiffs stained sections doesn't reveal any fungal infection.

Hematoxylin and eosin stained sections doesn't reveal any parasitic infection.

Lymphoid hyperplasia were seen in one case with acute appendicitis. the sum of an acute appendicitis cases with lumen obstruction was 2 those represent (4.7%) of acute appendicitis cases.

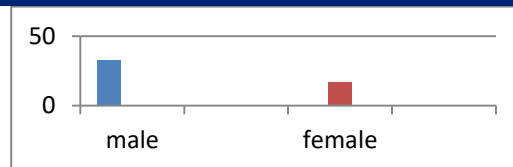


Figure (4-1): Distribution of study population according to sex

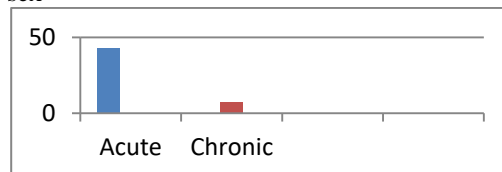


Figure (4-2): frequency of histopathological result among study population

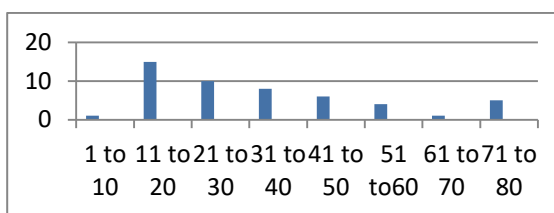


Figure (4-3): frequency of age groups among study population

Table (4-1) :Distribution of bacteria and sex between different types of histopathological results

Presence of bacteria		Histopathological results				Total 1
		Acute		Chronic		
		Male	Female	Male	Female	
Negative	Count	18	8	6	3	35
	Percentage	51.4 %	22.8 %	17.2 %	8.6 %	70%
Positive	Count	9	6	0	0	15
	Percentage	60%	40%	0%	0%	30%
Total	Count	27	14	6	3	50
	Percentage	54%	28%	12%	6%	100 %
Total :males - females	Count	41		9		50
	Percentage	82%		18%		100 %

Table (4- 2): Distribution of viral inclusion bodies and sex between different types of histopathological results.

Presence of virus		Histopathological results				Total 1
		Acute		Chronic		
		Male	Female	Male	Female	
Negative	Count	20	12	3	2	37
	Percentage	54.1 %	32.5 %	8.2 %	5.5 %	74 %
Positive	Count	6	2	4	1	13
	Percentage	46.2 %	15.4 %	30.7 %	7.6 %	26 %
Total	Count	26	14	7	3	50
	Percentage	52 %	28 %	14 %	6 %	100 %
Total: males-females	Count	40		10		50
	Percentage	80 %		20 %		100 %

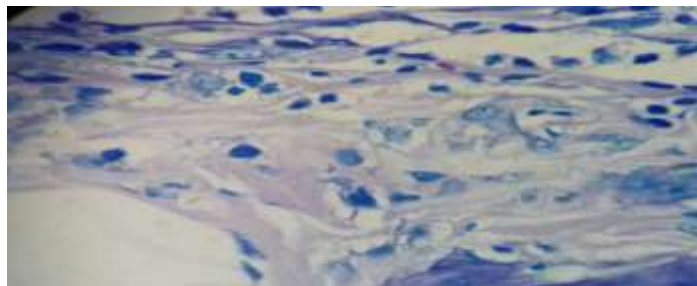


Figure (4-4): A specimen taken from 16 years old boy showing Gram positive cocci (Gram stain 40 x)



Figure(4-5 :A specimen taken from 20 years old boy showing intranuclear inclusion bodies in the nucleus of the epithelial cells with margination of chromatin to nuclear border (Macchiavellos stain 40x).

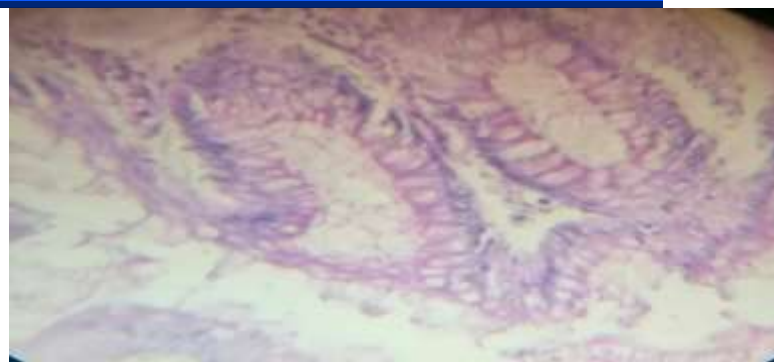


Figure (4-6: A specimen taken from 17 years old girl from appendix tissue Peroidic Acid Shifts show no fungal infection seen (PAS 40x).

Discussion : Appendicitis is predominantly a disease of modern Western culture and communities and is influenced by it . Abdominal pain, loss of appetite, nausea, vomiting weakness and sometimes diarrhea are signs and symptoms. Pain traditionally starts from the epigastrium and descends to the right lower quadrant of the abdomen and settles down there. Rebound tenderness is generally absent when the pain first appears in the epigastrium, but is experienced later when the pain settles in the right lower quadrant of the abdomen. It suggests that the inflammation has reached the parietal peritoneum⁽¹²⁾

Appendicitis is most common between the ages of 10 and 20 years but can occur at any age and is more common in men may be most of them eating out of house , the food habit and less of environmental health in restaurants and moving sellers . In the present study out of 50 specimens 15 cases were between 11-20 years (30%) of age in that 12 were males (80%) and 3 were females (20%). According to⁽¹³⁾ . variation in incidence by age and sex. In this study, the highest incidence of appendicitis was observed in males had a higher incidence of appendicitis than females in all age groups. .The reasons for age, sex and in the occurrence of acute appendicitis are not clear. The study revealed that 43(86%) out of 50 cases show acute appendicitis ,7(14%) chronic appendicitis .this finding agree with⁽¹⁴⁾ whos found (59,8%) cases of acute appendicitis, (22%) cases of chronic appendicitis. In males, the average length was 6.61 cm, where as in female it was 6.06 cm as registered in the work of ⁽¹³⁾ , in this study the mean length was 7,4 cm, and the mean diameter was 0.8cm, with an average length of 7 cm in female and 7.5cm in males.

in the present study the bacterial infection found in 17(34%) , and bacterial infection is commom associated with appendicitis agree with⁽¹³⁾ parasitic infection doesnot reveal in this study disagree with⁽¹⁵⁾ Parasites were present in 5.5% (88 patients), and of those 88 parasitic infestations, 45 (51.1%) were Enterobaisis, 8 (9.1%) were Schistosomiasis, 23 (26.1%) were Ascariasis, 7 (8%) Trichuriasis, and 5 (5.7%) were Teania Saginata. The percentage of patients with suppurative, gangrenous or perforated appendicitis was similar in both groups with no statistical significance, irrespective of the presence or absence of parasitic infestation

. Periodic acid shifts stained in this study doesnot reveal any fungal infection, and fungal infection is rarely associated with appendicitis agree with ⁽⁹⁾

Conclusion: The study concluded that bacterial and viral infections associated with appendicitis particularly the acute form .11-20 years was the most frequent age group , and the males are more affected than female with appendicitis, parasitic infection may associated with normal or inflamed appendix, fungal infection and parasitic infection were not associated with appendicitis in this study.

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