

## **Detection of Etiological Agents and Histopathological Changes Associated with Appendicitis**

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**ABSTRACT:** In this descriptive hospital based study, appendix samples were collected from 74 patients after appendectomy. These were fixed in 10% formal saline and prepared in paraffin blocks. Sections were prepared and stained with four different methods: Hematoxylin and eosin (H&E) for general morphology, Gram stain for bacteria, Macchiavello's stain for viral inclusion bodies and periodic acid- Schiff's reaction for fungi. The age of patients ranged from 1-47 years (mean age 19.3) years; 81% of the cases occurred in patients below 30 years of age, with male/female ratio of 1.5:1.0. Histopathologically, 48(64.8%) of the cases were diagnosed as acute appendicitis, 9(12.1%) as chronic appendicitis while 17(22.9%) of cases showed no detectable pathology. Intranuclear inclusions (suggesting viral infection) were seen in 17 (23%) of cases; 10 (13.5%) of cases showed presence of bacteria, one case (1.3%) had parasites (*Ascaris Lumbricoides*), and none had fungal infection. In conclusion the study indicated that acute appendicitis was more frequently encountered than chronic appendicitis and that bacterial and viral infections may be associated with appendicitis, especially the acute form. The age group 11-20 years was the most frequently. No relation was found between chili and lemon consumption and family history of appendicitis.

### **المستخلص :**

في هذه الدراسة الوصفية المستشفوية تم جمع عينات زائده دودية من 74 مريض بعد استئصالها جراحيا. تم تثبيت العينات في 10% فورمالين ملحي وتحضيرها في البرافين. تم تصبغ المقاطع النسيجية باربع طرق مختلفة: صبغة الهيماتوكسيلين والايوسين للشكل النسيجي العام وصبغة جرام للبكتريا وصبغة الميكافيللو للاجسام المشتملة للفيروسات وتفاعل حمض البريودك والشيف للفطريات. تراوحت اعمار المرضى من 1-47 سنة (متوسط الاعمار 19.3) ( 81% من الحالات كانت في المرضى اقل من 30 سنة وكانت نسبة الذكور الى الاناث 1.5:1.0. اظهر

الفحص النسيجي الاتى 48(64.8%) حالة إلتهاب زائدة حاد و 9 ( 12.1%) حالة إلتهاب زائدة مزمن و 17 (22.9%) من العينات لم تظهر أي تغيرات مرضية. لوحظت الأجسام المشتمله للفيروسات في 17 (23%) عينة و البكتيريا في 10 (13.5%) عينات ووجدت الطفيليات (دودة الإسكارس) في عينة واحدة (1.3%) و لم تلاحظ أي إصابة فطرية. خلصت الدراسة الى ان التهاب الزائدة الدودية الحاد هو اكثر حدوثا من النوع المزمن وان الإصابة الفيروسية والبكتيرية تصاحب التهاب الزائدة الدودية خاصة النوع الحاد وان الفئة العمرية من 11-20 سنة هي الاكثر تكرارا من حيث الاصابة ولم تبين الدراسة اى علاقة بين تناول الشطة والليمون اوالتاريخ العائلى لالتهاب الزائدة الدودية.

**KEYWORDS:** *Appendicitis, Infectious agents, Histopathology.*

## INTRODUCTION

The vermiform appendix is a narrow gland appendage arising from the postero-medial wall of cecum <sup>(1)</sup>.The appendix may undergo inflammatory changes associated with variable symptoms and is rarely a site for neoplastic changes <sup>(2)</sup>.

Several factors have been claimed to predispose to appendicitis. Most cases follow luminal obstruction mainly by fecolith and also by other factors such as lymphoid hyperplasia, parasites and tumors and occasionally by fruit seeds like orange, barely, melon, grape, dates and others. <sup>(3)</sup> Relationship between the incidence of appendicitis and the refined carbohydrate content in the diet has been indicated <sup>(4)</sup>. Appendicitis may be caused by infectious agents, including viral,

bacteria, parasitic and fungal infections <sup>(5-8)</sup>. The etiology of appendicitis was first proposed in 1898 by Veillon and Zuber <sup>(9)</sup>.Obstruction of the lumen is the dominant factor for acute appendicitis. The increased intraluminal pressure can cause injury to the mucosa with resultant impaired resistance to microorganisms. Acute appendicitis has been a cause of mortality throughout history and still remains a problem of medical emergency <sup>(10)</sup>. The aim of this study was to determine the etiological agents and to describe the pathological changes of appendicitis.

## MATERIAL and METHODS

### Patients:

Seventy four patients presented to the surgical units at Khartoum State hospitals with signs and symptoms of

appendicitis. Diagnosis was confirmed on the basis of clinical symptoms, physical examination of patients, laboratory investigation and ultra sound imaging. Diagnosed cases were subjected to appendectomy. Before surgery, patient particulars (age, sex, dietary habits and family history of appendicitis) were recorded and each patient was well informed about the study and signed a written ethical consent form approved by the ethical committee, College of Medical Laboratory Research Board; Sudan University of Science and Technology.

**Sample collection:**

The resected appendices were examined grossly for pathological changes and luminal obstruction. Length, width and thickness were also measured. Three tissue samples about 2mm thick were taken from appendix of each patient; one at the tip, the second at the middle and the third at the base. These were fixed in 10% formal saline and processed in paraffin. Four sections, 4-5  $\mu$ m thick, were prepared from each sample; one section was stained with Hematoxylin and eosin (H&E) for general

histopathology, one stained with Gram stain to demonstrate bacteria, the third section stained with Macchiavello's stain for presence of inclusions and the fourth was stained with periodic acid- Schiff's for demonstration of fungi.

**Statistical analysis:** Data was analyzed using SPSS computer program. Frequencies, cross tabulation and chi-square were calculated.

**RESULTS**

**Patient's particulars:**

All patients included in this study were Sudanese except two, one was a Bangladesh national and the other was Yemen national. The patient's ages ranged between 1 to 47 years with a mean of 19.3 years. More than half the patients (52.7%) were within the age group 11-20 years and 28.4% were within 21-30 years old. (Table 1). Forty five 45 (60.8%) of the study population were males with a male to female ratio of 1.5:1. Twenty two (29.7%) of patients had family history of appendicitis.

**Gross lesion:**

The length and diameter of the removed appendices were  $7.4 \pm 1.9$  and  $0.8 \pm 0.1$  cm, respectively. Obstruction of the

lumen with *Ascaris lumbricoides* was detected in one case. Foreign body (pin) and plant seed were seen in one case each. Undigested food was seen in 4 (5.4%) cases, while fecoliths was found in 8 (10.8%) cases. The number of appendicitis with lumen obstruction was 18 representing 24.3% of the total cases. Only two cases showed perforations.

The study revealed no significant association between family history of appendicitis or chili and lemon consumption and occurrence of appendicitis.

#### **Histopathological results:**

Histopathological diagnosis classified the cases as acute (48= 64.9%) or chronic (9 =12.2%) appendicitis and in 17 (23%) of cases there were no significant pathological changes. The frequency of acute and chronic inflammation was highest in the age group 11 to 20 years and lowest in the age group 41 to 50 year (Table 1, Figures 1 and 2).

Gram stained sections revealed the presence of bacteria in tissue sample

taken from six males and four females, all with acute appendicitis (Table 2), gram negative bacteria alone or mixed with gram positive cocci were seen (Figure 4). Hyperemia mural and presence of purulent neutrophils or fibrous exudates were observed in most sections. Lymphoid hyperplasia was seen in 21 cases, 13 (61.9%) of which were acute. Acute appendicitis was diagnosed in 13 (59.1%) of the 22 patients with family history of appendicitis. The acute type was also seen in the 18 appendices with lumen obstruction, in two of the four cases containing undigested food and in three of the cases with fecolithis.

Seventeen (23%) of the sections stained with Macchiavello's stain revealed the presence of magenta intracytoplasmic inclusion in epithelial cells. Eleven of these were from males and 6 were from females (Table 3 and Figure 3).

Nine of the 17 cases were 11-20 years old. Periodic acid Schiff's stained sections showed no evidence of fungal infection.

*Table1: Type of appendicitis in relation to age and sex of patients*

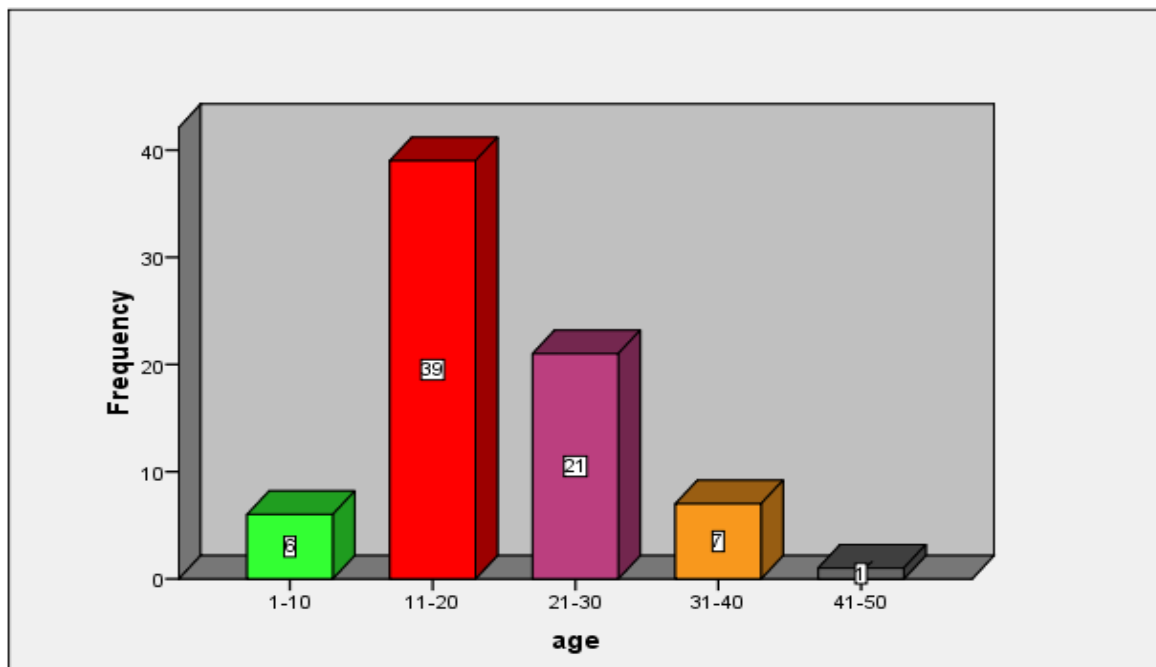
Age groups (years)		Histopathological results						Total
		Acute		Chronic		no change		
		Male	female	Male	female	Male	female	
1-10	N	2	2	0	0	1	1	6
	%	33.3%	33.3%	0%	0%	16.7%	16.7%	8.1%
11-20	N	14	10	1	4	5	5	39
	%	35.9%	25.6%	2.6%	10.3%	12.8%	12.8%	52.7%
21-30	N	14	2	2	0	0	3	21
	%	66.7%	9.5%	9.5%	0%	0%	14.3%	28.3%
31-40	N	3	1	1	0	1	1	7
	%	42.9%	14.3%	14.3%	0%	14.3%	14.3%	9.5%
41-50	N	0	0	1	0	0	0	1
	%	0%	0%	100%	0%	0%	0%	1.4%
total	N	33	15	5	4	7	10	74
	%	44.6%	20.3%	6.8%	5.4%	9.5%	13.5%	100%

*Table2: Detection of bacteria in sections of acute and chronic appendicitis*

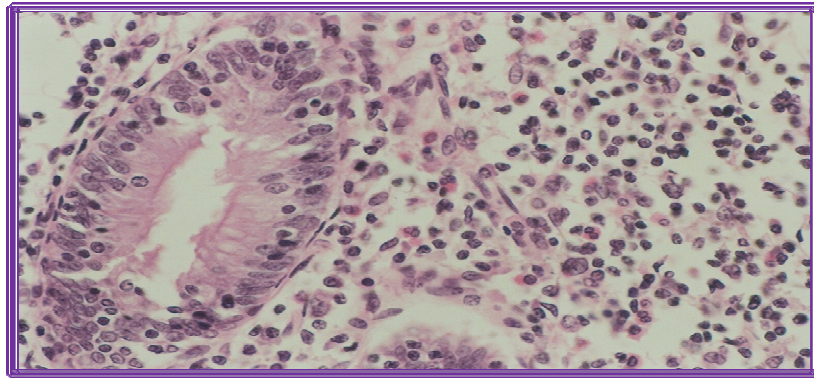
Presence of bacteria		Histopathological results						Total
		Acute		Chronic		no change		
		Male	female	Male	female	Male	female	
negative	N	27	11	5	4	7	10	64
	%	42.2%	17.2%	7.8%	6.2%	10.9%	15.6%	86.5%
positive	N	6	4	0	0	0	0	10
	%	60%	40%	0%	0%	0%	0%	13.5%
total	N	33	15	5	4	7	10	74
	%	44.6%	20.3%	6.8%	5.4%	9.4%	13.5%	100%

*Table3: Detection of cellular inclusions in sections of acute and chronic appendicitis*

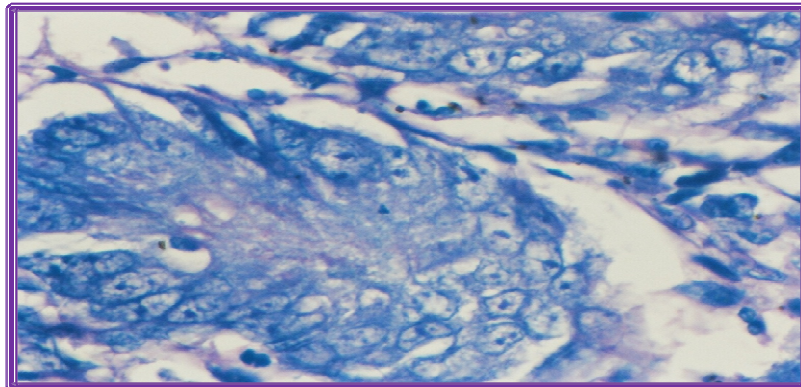
Presence of viral inclusion bodies		Histopathological results						Total
		Acute		Chronic		no change		
		Male	female	Male	female	Male	female	
negative	N	25	10	2	3	7	10	57
	%	55.6%	34.4%	4.4%	10.3%	15.5%	34.4%	77%
positive	N	8	5	3	1	0	0	17
	%	17.7%	17.2%	6.6%	3.4%	0%	0%	23%
total	N	33	15	5	4	7	10	74
	%	72%	51.6%	10.1%	13.7%	15.5%	34.4%	100%



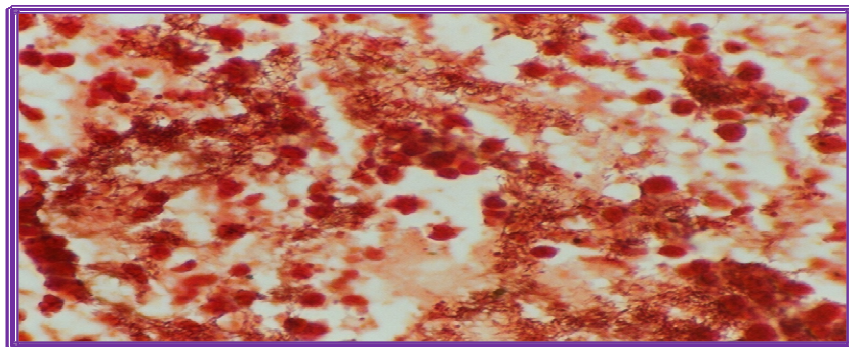
*Figure 1: Frequency of age groups (years) of appendictomized patients.*



*Figure 2: Appendix section (27 years old male) showing transmurular infiltration of lymphocytes and macrophages all over the tissues (H&E 40x).*



*Figure 3: Appendix section (35 years old male) showing intranuclear inclusion bodies in epithelial cells with margination of nuclear chromatin (Macchiavello's stain, 100x).*



*Figure 4: Appendix section (13 years old male) showing Gram negative bacilli (Gram stain ,100x).*

## DISCUSSION

Appendicitis is one of the commonest surgical emergencies<sup>(11)</sup>; it can be treated with antibiotics, which relieve inflammation, but surgery remains to be the first and best option in treatment. Appendectomy samples are usually referred to histopathological evaluation which is the gold standards for diagnosis and classification of appendicitis<sup>(12)</sup>. In the present study 74 appendicitis patients were investigated. Their mean age was 19.3 (range: 1-47) years. Eighty one percent of cases occurred in patients below 30 years, and this seems to agree with Andersson,*et al*<sup>(13)</sup>, and Chua,*et al*,<sup>(14)</sup>. Male to female ratio was (1.5:1) suggesting that males may be more prone to appendicitis than females. Such sex difference has also been indicated by Mellinger,*et al*.<sup>(15)</sup>

Histopathological evaluation of appendix samples showed that 64.9% and 12.2% of patients had acute or chronic appendicitis, respectively, while 23% showed no detectable pathology, indicating that acute appendicitis is more frequently encountered. This may be supported by

the findings of Helmy *et al*, who reported more cases of acute appendicitis (59.8%) versus chronic appendicitis (22%) and also found no detectable changes in 15.7% of samples<sup>(16)</sup>. In similar studies, cases of appendicitis with no detectable histopathological change were reported to range between 10 and 25%<sup>(17)</sup>.

The initial event in the pathogenesis of acute appendicitis is obstruction of the appendix lumen by factors such as appendicolith, foreign bodies, parasites, tumor and lymphoid follicular hyperplasia due to viral infections. Following obstruction, the appendix wall swells with thrombosis of small vessels, lymphatic stasis, necrosis and leakage out of bacteria through the damaged wall causing appendicitis and peri-appendicitis<sup>(18)</sup>. If untreated, appendicitis may be complicated by perforation, gangrene and sepsis. In the present study 18 (37.5%) of the acute appendicitis cases had lumen obstruction. Obstructive elements have also been observed in 30-40% of removed appendices<sup>(19)</sup>.

The mean length of the removed appendices seen here was 7.5 cm for



males and 7.0 cm for females which is longer than the corresponding values, 6.6 cm and 6.0 cm<sup>(20)</sup>. Our findings showed two cases of perforated acutely inflamed appendix, one was in male and the other was in female below 15 years old. Paulson and Kalady reported that perforations occurred in 50%, 10% and 30% of patients under 10 years, between 10 and 50 years and over 50 years old, respectively<sup>(21)</sup>. Khorasani and Pasha reported that the highest frequency of perforation was within the age group 15-34 years, 70.2% of whom were males<sup>(22)</sup>. The presence of Gram negative bacteria in tissue sections observed here, suggest that leakage and growth of bacteria inside the appendix is the cause of acute infection. Many organisms have been reported to be involved with acute appendicitis including: *Yersinia species*, *Actinomyces species*, *Campylobacter species*, *Mycobacterium tuberculosis* and fungi<sup>(23)</sup>. Rautio *et al*, isolated aerobic and anaerobic bacteria from appendectomy samples in children<sup>(24)</sup>. These included: *Bilophie wadsworthia*; *Fusobaclerum nucleatum*; *Eggerthella lenta*; *E. coli* and *Streptococcus*

*anginosus* group. The intranuclear inclusions demonstrated in epithelial cells of acute (76.5%) or chronic (23.5%) appendicitis samples may suggest viral etiology. Viral infections have been associated with appendicitis<sup>(25)</sup>.

Periodic acid Schiff's stained sections were negative for fungi. This may be attributed to the fact that our patients were in good health, none of them was immunocompromised or have received immunosuppressive therapy. Our study showed no significant association between family history and occurrence of appendicitis. This, however, disagrees with Ergul *et al.*, who found that family history is an important parameter while predicting acute appendicitis<sup>(19)</sup>. The results also do not support the common belief that chili and lemon habitually used in or consumed with Sudanese food, cause acute appendicitis; no significant relation between the two was found.

## CONCLUSIONS

The study indicates that acute appendicitis is more frequently encountered than chronic appendicitis and that bacterial and viral infections may be associated with appendicitis especially

the acute form. 11-20 years was the most frequently affected age group. No association was found between chili and lemon consumption and family history with appendicitis.

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