

Histopathological changes of parasitic infection associated with appendicitis in Thi- Qar province

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Abstract

The research include the study of prevalence of Parasite associated with appendicitis and to know the kind of histopathological changes of parasitic infection appendectomies in from patients of Al-Husseini hospital in Nassriya city in Thi-Qar province.

The study included 70 patients with appendicitis of both sexes, The total rate of parasitic infection 20 %

The results of histopathological study reveal's kinds of histological changes : necrosis of submucosal layer , inflammatory cell , Degeneration of lymphatic nodules, Submucosal oedema and Lymphofollicular hyperpla

التغيرات النسيجية المرضية للإصابة الطفيلية المرافقة لالتهاب الزائدة الدودية في محافظة ذي قار

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الخلاصة

تضمن البحث دراسة انتشار الطفيليات المرافقة لالتهاب الزائدة الدودية ومعرفة أنواع التغيرات النسيجية التي تسببها الإصابة الطفيلية للزوائد الدودية المستأصلة من المرضى الراقدين في مستشفى الحسين التعليمي في مركز قضاء الناصرية في محافظة ذي قار .

تضمنت الدراسة 70 مريضاً بالتهاب الزائدة الدودية من كلا الجنسين. كانت أعلى نسبة إصابة 10% سجلت للطفيلي *Entamoeba histolytica* كما بينت نتائج الدراسة النسيجية وجود أنواع من التغيرات النسيجية منها تنخر الطبقة تحت المخاطية وظهور خلايا التهابية وتنكس العقيدات اللمفاوية وظهور وذمات تحت مخاطية وفرط نسيجي .

Introduction:

Intestinal parasites represent as one of the hygienic problems spread in many poor countries especially in hot humid climate country, and consider a reason of death and malnutrition diseases Protozoa and intestinal helminthes represent a wide group of parasites that normally exist in the intestine and become infected depends on type, duration of infection, climate factors, diet, age and hygienic costumes (WHO, 1981). Appendicitis, is one of the disease that

parasites one of the causes for it, its sudden appendix hypertrophy, it's occurs also as a result of bacterial or viral infection and because of calculi, calcification fecaliths obstruction (Schneider and Szanto, 1993).

Appendix is a closed end elongation of the intestine consists of many layers.

The appendiceal lumen appears in the transactional view small and irregular because of the high existence of lymphatic nodules. and it might be closed because of this. (Itskowitz and Jones, 2004), (Mariano,1976).

This study tries to explain the histological changing that occurs due to parasitic appendicitis

The aim of this study was determine the prevalence of parasitic infection in appendectomy specimens and to study the histopathology changes associated with appendicitis.

Materials and methods:

2-1- A collection samples of infected Appendix: 70 samples have been collected (40 from male, 30 from female), for the period of Dec 2008 – April 2009 From the patient entered in the Al-Hussein Educational hospital, the ages of the patients ranged from 10-50 years old. The samples saved in well locked agars with saline solution to do the microscopic examination and after the saline solution replaced with formalin 10% to be ready for histological examination.

1-b Microscopical examination of samples of vermiform appendix:

A- Direct smear method examination.

This method include prepare the glass slide and putting one drop of saline solution in the right side of the slide and one drop of iodine sol. In the left side of the slide , by a wood sticks a samples(stick head size) have been taken from different areas of container of the appendix and mixed well with the saline solution and with the iodine drop and covered with slide(Davey and Crewe,1973).

b- Floation method examination

it's depends on mixing the faecal materials with a high concentration to get the granules and materials be float and this include the parasites, zinc sulphur used for this because of his high molecular weight 1.8 , and it's good in float sacs , eggs , except the Belharsia eggs (Baker and sliverton,1985).

Step of work:

1- 3grams of the container of the appendix mixed with 10 mml of saline solution in lab glass

2-The mixture has been filtrated by cotton membranes to lab packer.

3- Filtrated materials been put in centrifugation system (2000-3000) Rpm for 2 min.

4- The participates has beenliqefaction with distal water and re put in the centrifugation.

5- The participate taken and mixed with zinc sulphur and put in the centrifugation .

6- The float of the result has been taken with a loop and put on slid to microscopic examination

2-3 Histological Study The waxing of paraffin method used to light microscopically examination (Luna, 1960)

1-Fixation: Neutral buffered formalin solution used to fix the tissue samples (appendix).

This solution prepared by dissolving: formalin 100 ml, NaH₂PO₄ 4grams and Na₂HPO₄ 6.5 grams in distilled water 900ml. The pH has been corrected to be 7, the fixation period lasted for 24 hours

2- Washing: the samples have been washed for many times by tap water.

3 - Dehydration: the samples have been passed through Ascending concentration of Ethanol for 2 hour to each concentration (50, 80, 100)

4- Clearing: the samples treated with mixture of pure ethanol and xylene for 3 hours.

5-infiltration: to immerse the tissue in the paraffin wax it, should be heated to heat for 3 hours 60 cent and transport to the pure paraffin for 24 hours in the same cent degree .

6- Embedding: the samples have been putting in plastic blocks contain a solid wax in 60 cent .

7- Sectioning: The samples been cut by rotary microtome Reichert-Jung in 5 micrometer in a ribbon shape then removed to a sterilizer path water under 45-50 cent degree till it be flattened and then moved to a clean dry test slides painted with sticky layer of Mayer's albumen and then the slides put on a hot plate or fisher slide warmer 50 cent degree for 24 hours.

8- Staining: Harris Haematoxylin and Eosin stain as following:

The wax removed by hot xylene and then passed with gradually concentration of Ethanol starting with 70, 80, 99% for 2 min to each concentration, and washed with distilled water.

The slides put in Haematoxylin to 3-5 min and passed in Alcoholic acid 70% for seconds to remove the extra stain, then washing the slides with clean tap water to 5 min . The slides put in Eosin stain to 2 min and passed with different concentration of Ethanol 70, 75, 80, 99% to 10 sec for each concentration this slides put in xylene to 5-10 min for clearing

9 - Mounting: The slides loaded with Distrene plasticizer xylene DPX and the slides then put on hot plate 45 cent degree to be dry

11- Examination and photography: the examination been made by photo microscope / phase contrast (kind Olympus) .

Results:

Appendix is a closed end elongation of the intestine consists of many layers:

1- Mucosa which consist of:

* Epithelial layer consist of connective tissue saturated with lymphatic nodule with blood vessels.

* Thin smooth muscle layer

2- Sub Mucosa layer consist of blood vessels and nerves. 3- Muscular mucosa.

3- Serosa, consist of lymphatic tissue extended to the in the mucosa and submucosa layer

The appendice lumen appears in the transactional view small and irregular because of the high existence of lymphatic nodules. And it might be closed because of this. (Figure (1,2))

APPENDIX (PANORAMIC VIEW, TRANSVERSE SECTION)

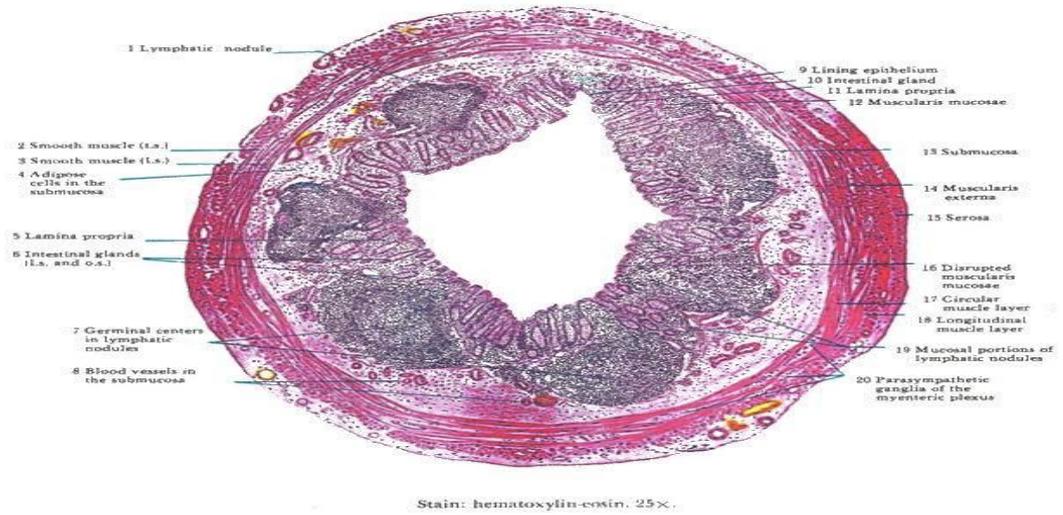
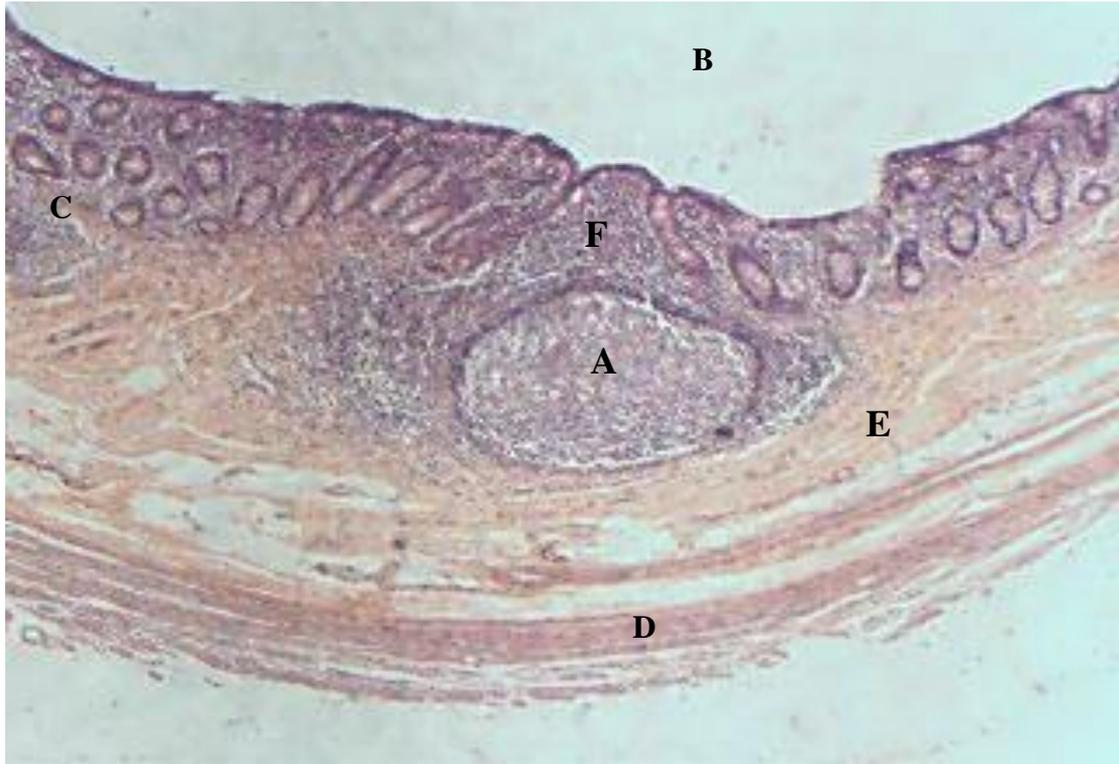


Figure (1) Section of normal appendectomy

Figure (2) Section of normal appendectomy A=lymphatic nodule ,B=lumen ,C=intestinal gland ,D=serosa ,E=submucosa,F=mucosa (E&H),(40X)



After examination 70 appendectomy specimens, the total rate of parasitic infection 20%. The higher percentage of infected was with *Entamoeba histolytica* (10 %) and the lowest percentage was with *Entrobins vermicularis* (1.4%)(table 1).

Table (1): Distribution of Parasite infections in appendectomies

parasite	Infective number	Education Level	
		Educated (%)	Uneducated (%)
<i>Entamoeba histolytica</i>	7	4 (57.1)	3 (42.9)
<i>Entamoeba Coli</i>	4	1 (25)	3 (75)
<i>Giardia lamblia</i>	2	0 0	2 (100)
<i>Entrobins vermicularis</i>	1	1 (100)	0 0
Total	14	6 (42.9)	8 (57.1)

Parasite	Number of infected appendix	%
<i>Entamoeba histolytica</i>	7	10
<i>Entamoeba Coli</i>	4	5.7
<i>Giardia lamblia</i>	2	2.9
<i>Entrobins vermicularis</i>	1	1.4
Total	14	20

The study it was found that the higher percent of infected individuals were uneducated (57.1 %) more than those who were educated (42.9 %)(table 2).

Table (2): Distribution of parasite infection in infected. individuals according to educational level

Regarding the histopathological changes in the 14 appendices that's been subjected in this study which that appendix with infected parasite reveal that 66.7%(Table 3) involved with necrosis(necrosis is refers to a sequence of morphological change that follow cell death in living tissue its most common manifestation is coagulative necrosis) in submucosal layer (figure4,5,7), 55.6% involved with inflammatory cell infiltrations(figure3) ,55.6% involved with degeneration of lymphatic nodules 22.2% involved with submucosal oedema and 77.8% involved with Lymphofollicular hyperplasia (figure3,6,7) with ulceration (figure 4,5).

Table (3): Histopathological changes in the Appendixes

Type of histopathological changes	Positive appendix for parasite (9)		Negative appendix for parasite (6)		Total appendix (14)	
	number	%	number	%	number	%
Necrosis of submucosal layer	6	66.7	6	100	12	80
Inflammatory cell in filtrations	5	55.6	0	0	5	33.3
Fibrosis	0	0	1	16.6	1	6.7
Degeneration of lymphatic nodules	5	55.6	0	0	5	33.3
Submucosal oedema	2	22.2	1	16.6	3	20
Lymphofollicular Hyperplasia	7	77.8	2	33.3	9	60

Figure (3):Section of appendectomy A,B,C,= Lymphofollicular hyperplasia (E&H) (40 X)

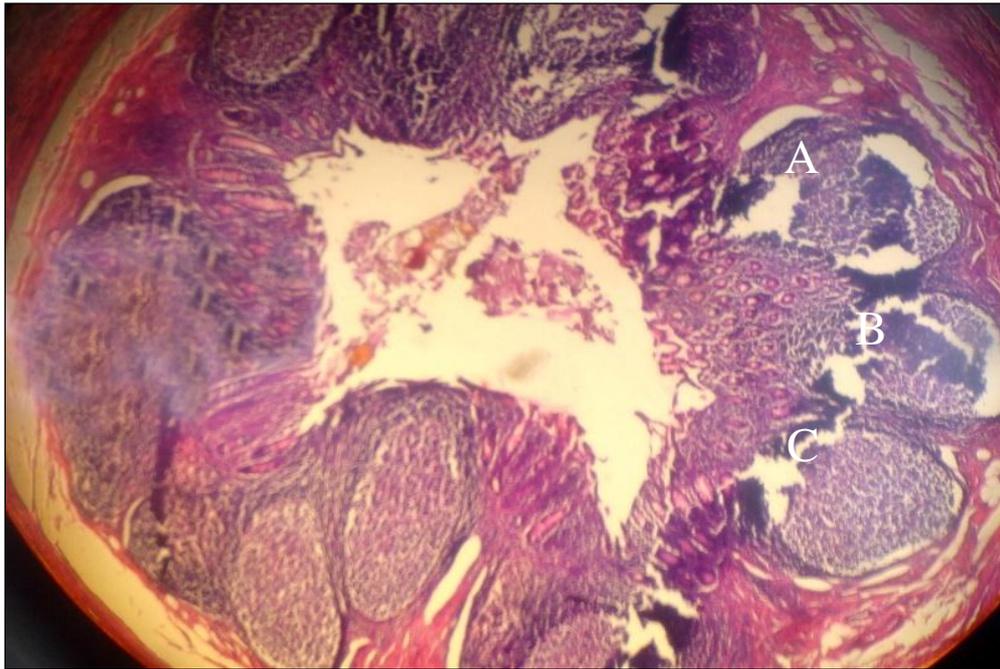


Figure (4)Section of appendectomy A=necrotic area ,B= ulceration(E&H) (100 X).

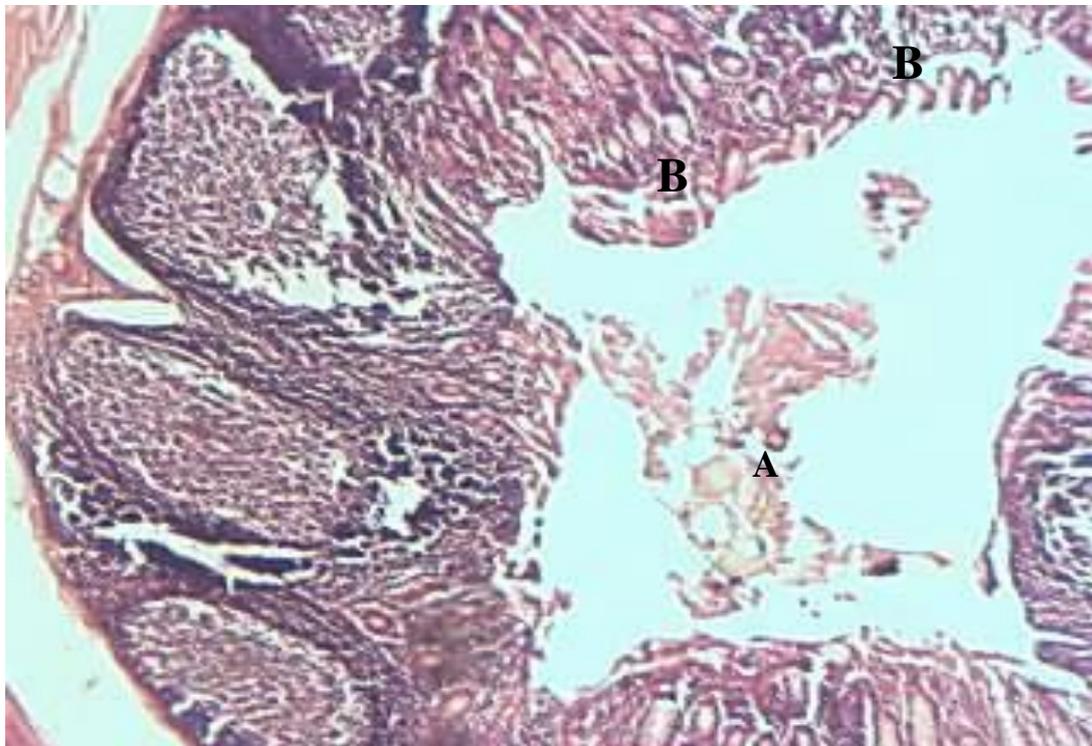


Figure (5)Section of appendectomy A=necrotic area ,B= ulceration ,C=hyperplasia (E&H) (100 X).

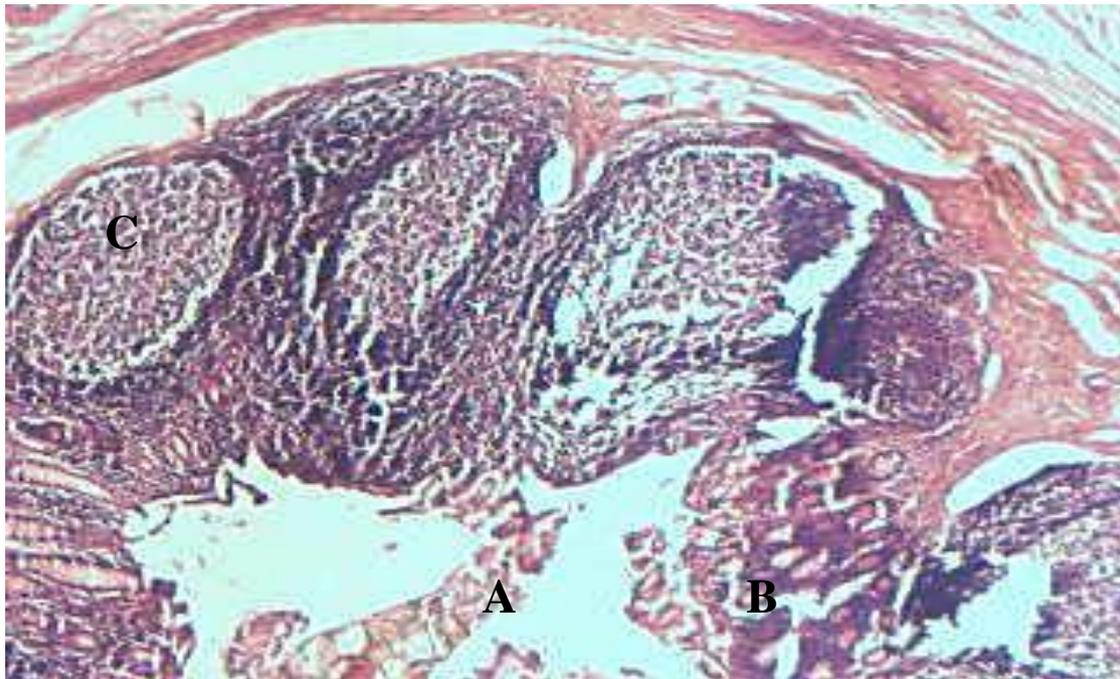


Figure (6)Section of appendectomy A=lymphofollicular nodule with hyperplasia (E&H) (400 X)

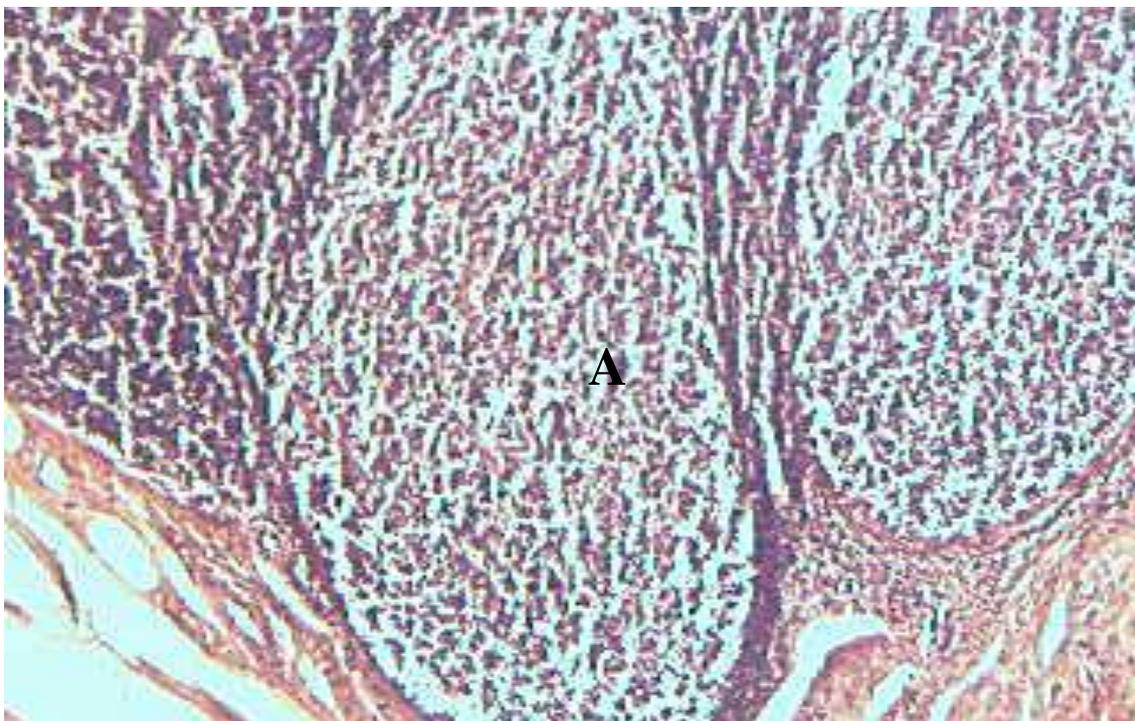
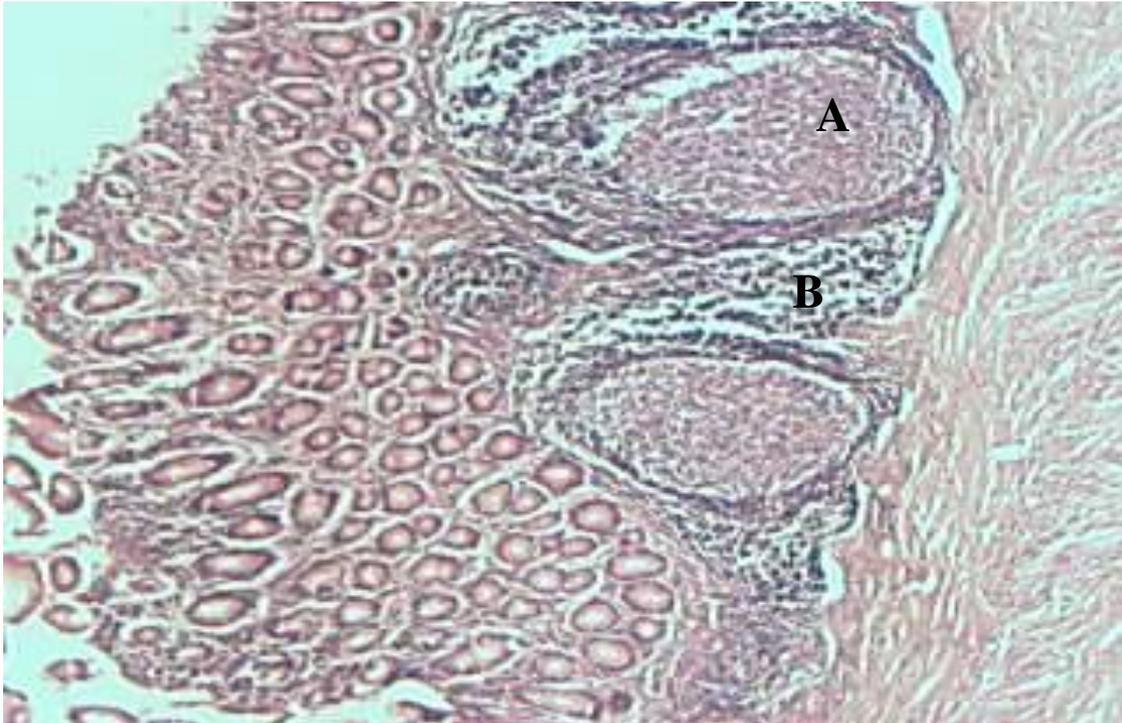


Figure (7)Section of appendectomy A=lymphofollicular nodule with hyperplasia ,B=necrosis area (E&H) (100 X)



Discussion:

The parasitic infections are causing a negative effect in health, nutrition and growth because it lead to pathological complications and with surgical interference as what's happened in appendicitis (Gillesse *et al.*, 1987) in addition in competition with host food.

This study reveals that the percentage of overall intestinal parasitic infestation is 20% which's higher than percentages in similar studies in Iraq in Teaching Mosul Hospital which's 4.9%(Majeed and Al Bakri,1984) , in Al-Mosil also which's 13.6%(Al-Dabbagh *et al.* 1999) and in Al-Najaf which's 16.2%(Al-Shaddod, 2001) .

This difference of percentage can be attributed to the variation in socioeconomic and health status in different sites of Iraq, besides the differences in methods laboratory tests, duration of study and size of sample subjected in different studies leading to different percentages.

Our study reveal that the appendices been infected by 2 groups of parasites: protozoa and intestinal worms ,amoebic dysentery cases were higher than other parasites 10% which's high percentage in comparison which's 2.8% in Al-Najaf (Hussein (1995)) .

Regarding the worldwide percentages, the percentage in our study is higher than Ahmed *et al.* (1994) which's 0.8 % in Karachi (Pakistan), while in Royal Darwin Hospital in Australia which's 100% during testing of six of appendices containing amoebic dysentery Zardawi *et al.*(2003) that's mean it's higher than our study.

This variation attributed to variation in ecological environments and difference in healthy economic status for individual and community and due to using different testing methods.

In this study *Entamoeba coli* infection in 5.7% *E. coli* humen commensal and have no pathogenic effect but its presence indicate contamination of food and water by faeces of other

human beings . *Giardia lamblia* infection in 2.9% and this resemble the percentages of Al-Shaddod 2002 in Najaf which's 2%. Pin worms in 1.4% which less than percentage of Majeed and Al-Bakri (1984) in Al-Mosil Teaching Hospital which 2.9% and Al-Dabbagh *et al.* (1994) .

which's 12.2% and less than Hussein (1995) in Baghdad which's 4.7% and less than international percentages of Boulos and Cowie (1973) in Royal College in London which's 11.2% ,but higher than Sarmaste *et al.* (2005) in Marshes South- west of Iran which's 0.7% and resemble to Williams and Dixon (1995) in two teaching hospitals in Britain where the percentage was 1.5%.

The problem of parasitic diseases was more frequent among individuals of low education illiterate and educated individuals 42.9% statistically there was significant difference. In fact, families' individuals having good lifestyle that enhance their levels of health would be less susceptible to parasite infection more than those of low educational levels who have not wellness principle (WHO, 1989).

Regarding the histopathological changes in the 14 appendices that's been subjected in this study which reveal the following:-

- 1- 80% involved with suppuration in submucosa layer.
- 2- 33.3% involved with inflammatory cells infiltration.
- 3- 33.3% involved with lymphoid follicles formation.
- 4- 6.6% involved with fibrosis in all layers of appendix wall.
- 5- 20% involved with oedema formation in submucosal layer.
- 6- 60% involved with Lymphofollicular Hyperplasia .

When comparison applied between appendices negative for parasitic infestation and appendices positive for parasitic infestation in this study it show that all appendices negative for parasitic infestation reveal suppuration in submucoza , fibrosis and oedema in submucoza while the appendices positive for parasitic infestation reveal all these histopathological changes except fibrosis. These results are resembling the results of Ahmed *et al.* (1994) where that histological study discovered suppuration in the submucoza, also another study (Ramadial *et al.* ,2002) reveal presence of ulceration in wall of appendix with suppuration and presence of vegetative amoebic stages with RBCs and inflammatory cells infiltration.

The explanation of the mechanism of occurrence of appendicitis is agreeing the opinion that attribute the appendicitis occur sometimes because of the role of parasites in causing histological tissue damage in appendix that lead to obstruction and then inflammation.

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