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Immunological and histopathological study in patients With acute appendicitis in karbala city

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Abstract:-

Acute appendicitis represent a terrible problem for community for many reasons such as there were no exact identification for it and surgery might lead to many difficulties and the diagnostic ways for appendicitis were significantly not altered over in the past few years ;therefor our study designed to identify different cytokines levels (Monocyte chemotactic protein- 1 (MCP-1) and Macrophage inflammatory protein (MIP-1α) as predicator for specific diagnostic tests of appendicitis and study histological change accompanied with the appendix .

Our study is carried out on(100)specimens , which divided into two groups: group I , included (50) patients who were suffered from fever, diarrhea , nausea, vomiting and abdominal pain which migrate to the right iliac Fossa , to explain the presence of acute appendicitis , specimens were collected from patients who were attending to Imam Hussein medical city in Holy Karbala during the period January 2024- June 2024 . Patients specimens include blood and appendix in compere with (50) blood samples collected from healthy control (group II),that were identical to patients group but not suffered from any symptoms of appendicitis.

The result of our study of histopathological changes included macroscopic and microscopic examination of all appendices , some of these appendices are enlarged and tissue surrounded by vesicles, and some have fibrous and ulcerated walls of varying colors. The tissue changes showed changes in the histological structure of appendix, represented by expansion of its lumen and congestion of venous blood vessels in the mucosa and sub mucosa , and increasing amount of lymphatic tissue spread through the layers of appendix, lymphoid follicular hyperplasia , large amount of adipose tissue with fatty necrosis , infiltration of inflammatory cells that spread through the layers of the appendix.

The serological result demonstrated that there were highly significant differences for monocyte chemotactic protein- 1 (MCP-1) and macrophage inflammatory protein (MIP)-1α (p=0.0001 **) in the serum levels of appendicitis group and healthy Control group.

Introduction;-

Appendix is an abdominal organ include parts of the lymphatic tissue related with the intestine and serves as a reservoir for intestinal microbial flora; It also plays a role in the correct development of lymphatic tissue in the stomach (Radenković *et al.,* 2021). Appendix is the abdominal organ that differs the most in location, peritoneal connections, size, and organ connections. The position of the vermiform appendix plays essential role in the clinical presentation of a patients with acute appendicitis (Choudhary & Ghatak, 2018). Histologically, the four typical sub layers that form appendix wall beginning from the inside are mucosa, sub mucosa, muscularis external and serosa (Bandyopadhyay *et al*., 2022) .The presence of lymph nodes in the sub mucosa suggests their role in the immune system. Acute appendicitis is a high prevalence diseases that needs rapid and accurate diagnosis that confirm or exclude perforation which causes abdominal inflammation , with surgical appendectomy is the standard choice of the treatment and is still considered an emergency (Teng *et al*., 2021). diagnosis of acute appendicitis is clinical and combined with laboratory investigations, supplemented by selectively focused imaging , late diagnosis of acute appendicitis lead to complications such us perforated appendix, peritonitis, sepsis, increased morbidity and mortality; nevertheless its diagnosis remains challenging (Li *et al.*, 2021). two essential components are attributed to the pathogenesis of acute appendicitis : obstruction and infection by the pathogen in the most patients with acute appendicitis, the latter thought due to the luminal obstruction which may result from a diversity of reasons, i.e., lymphoid hyperplasia, fecoliths and parasite (Shahmoradi *et al*., 2021). Bacterial infection is believed to be essential for appendix inflammation (Takahashi, *et al.,*2021) it is evident that several bacteria can pass through intact appendix wall before perforation, whereas progressive infection and subsequent tissue damage with necrosis permit the bacteria to enter the abdominal cavity (Sakellaris *et al.,* 2023). Acute appendicitis is associated with production of cytokines, the cytokine profile varies dependent on how long the infection has been present and whether or not it has had any consequences (Wheeler, *et al.,* 2021) . The histological investigation of resected appendices aims to achieve two purposes: the first is to increase confirming the diagnosis of the inflammatory condition , especially if no macroscopic changes are observed during the surgical operation and the second to detect additional causes that may be discovered during the investigations (Jones *et al.,* 2007). Pathological valuation was the gold standard method for diagnoses of appendicitis by doing the routine histopathological valuation which performed to confirm the diagnosis of appendicitis and it might reveal additional important pathological details (Lal *et al*.,2014).This study is aimed to Investigate histological change accompanied with appendix and Investigate and compare the level of several immunological factor (MCP-1 and MIP -1α) in peripheral blood of patients with acute appendicitis and healthy control and identified the significant level to be used as a diagnostic test for acute appendicitis.

Methods:-

The present study was carried out on (100) specimens, which divided in to two groups: group I , included (50) patients who wear suffered from nausea, vomiting and abdominal pain which migrate to the right iliac Fossa and fever . specimens were collected before the operation from patients who were attending to admitted to Imam Hussein medical city in holy Karbala during the period January 2024- June 2024 ,and (50) specimens collected from healthy control group

II that were identical to patients group but not suffered from any symptoms of appendicitis. 5 ml of a sample of intravenous blood was taken from each patient before operation using disposable syringes, control blood samples were taken from healthy people, as well as blood samples were taken and placed in the gel tubes . Data were collected including age, sex, symptoms , and other parameters through using a short questionnaire, each serum samples should be divided into small (200 µl) aliquots keep at deep freeze (-20°C) until used. All the immune markers were measured in the serum samples by using Enzyme Linked Immuno Sorbent Assay (ELISA) technique**.**

A pieces of appendix was placed in 10% formalin for 24 hours and replaced with another fresh formalin 10% to complete 48 hours, specimens were washed (3x 10 minutes) in a fresh phosphate buffer pH 7.4 at the room temperature, followed by washing in the distilled water (3x10 minutes) at room temperature, the specimens were transferred into gradually increased grade ethanol starting from 35% up to 90% for 15 minutes each at the room temperature. Finally they were transferred into 100% absolute ethanol for one hour (2 changes).specimens were transferred in the fresh pure xylene for 1 hour (2 changes), then appendices specimens were embedded in fresh paraffin wax, wax mounted specimens were re-blocked in the fresh paraffin wax and prepared for sectioning via microtome *(LEICA RM2125RT).* Thin pieces of 5 *µ*m were cut from each block and water bath was used to stretch sections before the collection on clean glass slide and dried out at room temperature. The 5 *µ*m sectioned specimens from a water bath was collected on clean glass slide were de-waxed in xylene for 5 minutes, transferred to another xylene followed by hydrating a sections through descending grades of alcohol (100%, 96%, 80%, 70%, 50, 30%) down to distilled water , after that the sections were stained in hematoxylin for five minutes and were blued for six minutes in running tap water followed by staining with eosin for two minutes . Sections were then passed through 3x 96% alcohol for one minute each (dipped sections up and down to remove the extra of stain). Sections were passed through 3x100% alcohol for one minute, followed dipping them in the 2x xylene, lastly, stained sections were permanently mounted by using DPX and cover slips and kept in the safe place at room temperature for examination. The histological sections were photographed using an olympus light microscope, an olympus high-resolution digital camera equipped with a microscope digital camera.

Statistical Analyses:-

The results were analyzed statistically in SPSS to find out Chi-square , ANOVA (One away) at significance level (α) in (0.01and 0.05) .

Results & Discussions:-

Macrophage inflammatory protein (MIP)-1α:-

In table (1) the results were significantly increased (*P* = 0.0001 **) in the mean of MIP-1 in patients of acute appendicitis comparison with control, as the mean of MIP-1 for patients and the control (45.91 and 2.493) % respectively. This results agreement with (Stankovic *et al.,* 2019) who concluded statistically significant differences between patient with a cute appendicitis and healthy for MIP-1α . Our result disagreement with (Peeters *et al.,*2020) who observed no significant differences between patients and control .

Table 1: The mean MIP-1α of appendicitis patients and healthy group

Markers	Healthy (N=50) Mean ± SD	Patients(N=50) Mean ± SD	*P value*
MIP-1	2.493 ± 2.432	45.91 ± 9.38	0.0001 **

Studies suggest that increased levels of MIP-1α in appendicitis may correlate with inflammatory response associated with the condition. For instance elevated MIP-1α levels are thought to facilitate recruitment of monocytes and other immune cells to the site of inflammation, contributing to pathological process. (Davis *et al.,*2021). Macrophage inflammatory protein 1α (MIP-1α) belongs to a family of chemokine's primarily produced by macrophage cells activated by bacterial endotoxin, and has a crucial role in immune response to infection , the pro-inflammatory role of this cytokine reflected in the activation of granulocytes and the induction of synthesis of other pro-inflammatory cytokines .This chemokine plays significant role in attraction and activation of granulocytes, and its serum concentration was expectedly higher in acute appendicitis (Cook,1996).

Monocyte chemotactic protein- 1 (MCP-1) :-

In table (2) the results were significantly increased (P = 0.0001 **) in the mean of MCP-1 in patients of appendicitis comparison with control, as the mean of MCP-1 for patients and the control (151.21 and 34.53) respectively**.** This results agreement with (Shommu , 2017) who founded elevated of MCP-1 in the patients with acute appendicitis, also (Naqvi *et al.,*2019) have observed Plasma levels of MCP-1 were significantly different ($p < 0.001$) in children with appendicitis compared to those with non-appendicitis abdominal pain. while our results disagreement with **(** Zviedr *et al.*,2016) who concluded that there were no significant differences between MCP-1 in both appendicitis and healthy . Also our result disagreement with (Peeters *et al.,*2020) who observed no significant differences between patients and control .

Table 2: The mean of MCP-1 of appendicitis patients and healthy group

Markers	Healthy (N=50) Mean ± SD	Patients(N=50) Mean ± SD	*P value*
MCP-1	34.53 ± 11.98	151.21 ± 39.00	0.0001 **

Monocyte chemo attractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) are members of the C-C chemokine family, which has been shown to play a major role in the migration of monocytes to an inflammatory focus (Koch *et al.*,1994).

Our results are consistent with other studies that have focused on bacteria and their products especially polysaccharides , and endotoxin is one of the most important factors that causes an increase in the secretion of a number of cytokines during infection such as MCP-1to increase the speed of the immune response, both local and systemic (Paajanen *et al*.,2002).Local MCP-1synthesis of endothelial cells in the peritoneal abdominal fluid respond to the activity of microorganisms and stimulating formation of operative septic complications (Riese *et al.,* 2002; Riese *et al.,* 2004).This is the explanation to the statistically significant increase MCP-1

concentrations in cases of acute appendicitis in our study.

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Histopathological features of acute appendicitis:-

All specimens in our study showed changes in the histological structure of appendices . An inflammation of appendices which were noted by hypertrophy increasing in the size of lymphoid follicles noted in (figure 1) which was appeared to be agreed with (Bokhary & Riddell, 2009 & Singhal & Jadhav,2007).

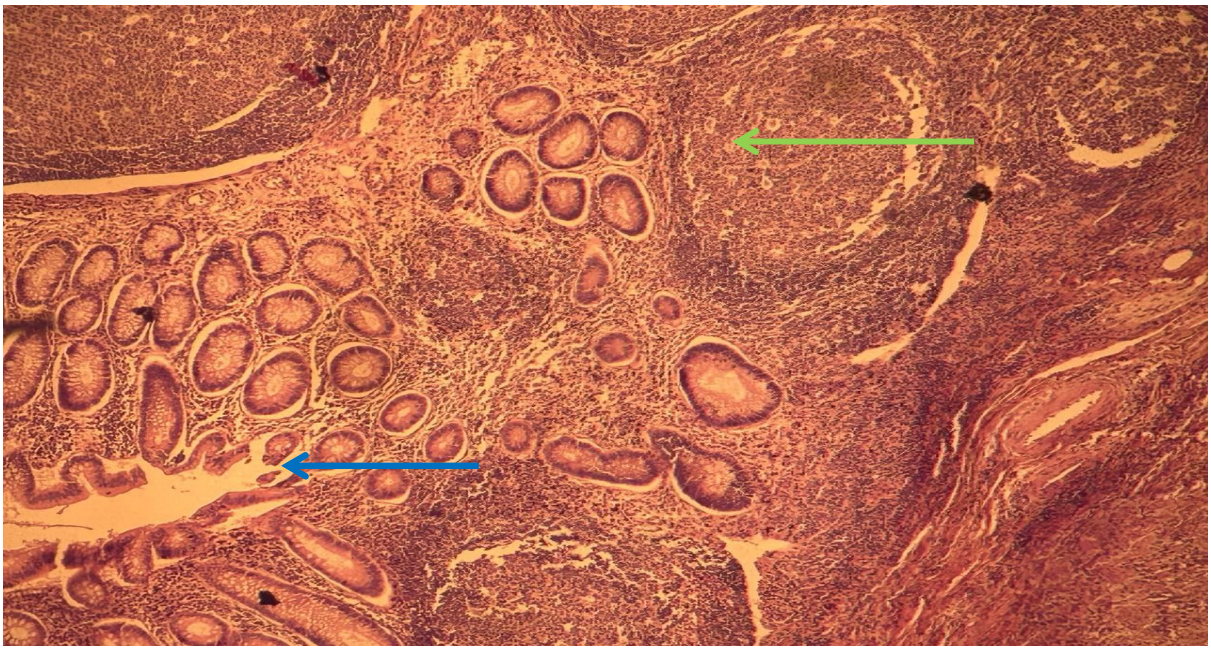
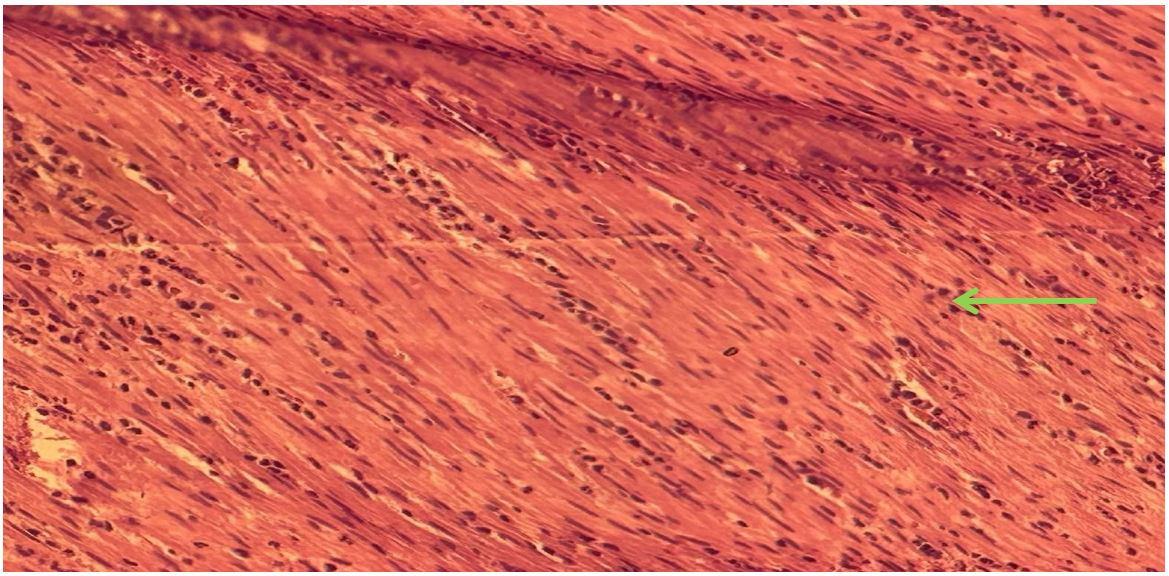


Figure1 acute appendicitis, lymphoid follicles are more active and larger than normal (reactive hyperplasia) (green arrow). Note appendix epithelium (blue arrow) (H&E 40x)

This may be occurred as a result of viral gastroenteritis or mesenteric adenitis (XU *et al*.,2016) while (Torigian *et al* .,2001) demonstrate that lymphoid hyperplasia might occur as a result of bacterial infection. Lymphoid hyperplasia is a physiologic response to inflammation particularly in gastrointestinal infections can regress if the accompanying gastrointestinal inflammation is resolved, it is most commonly identified in pediatric patients (Xu *et al*., 2016).

Appendical lymphoid hyperplasia maybe occur as secondary to stimulation, especially for viral or bacterial infections, giardiasis, cow milk protein allergy, or IgA deficiency (Mansueto *et al*., 2012). Lymphoid hyperplasia of the appendix is typically associated with inflammatory conditions such as viral gastroenteritis and mesenteric adenitis, it is maybe obstructing the appendicular lumen, cause acute appendicitis, which can progress to peritonitis, and resolves spontaneously only in a few cases (Whittle *et al*., 2020).

It was also observed increasing in numbers of different types of leukocyte such as lymphocyte and neutrophil, as in (figure 2).



Figure(2) acute supparative appendicitis note the neutrophil,(green arrow), (H&E 40x)

As a result for an immune response to infection to occur , appendix is one of the lymphatic organs involved in defense against Infection with pathogenic agents , including bacteria , also that would explain increasing in numbers of different types of leukocyte such as lymphocyte and neutrophil.

It was also observed in some cases the appendical cavity expanded significantly, and vascular changes were observed, represented by congestion blood vessels and their cavities expand in the serosa and sub mucosal layers (figure 3). which was appeared to be agreed with (Bokhary and Riddell,2009). and agreement with Carr (2000) who found dilatation in the blood vessels of the serosal layer which might belong to explain our finding of hyperemia and congestion.

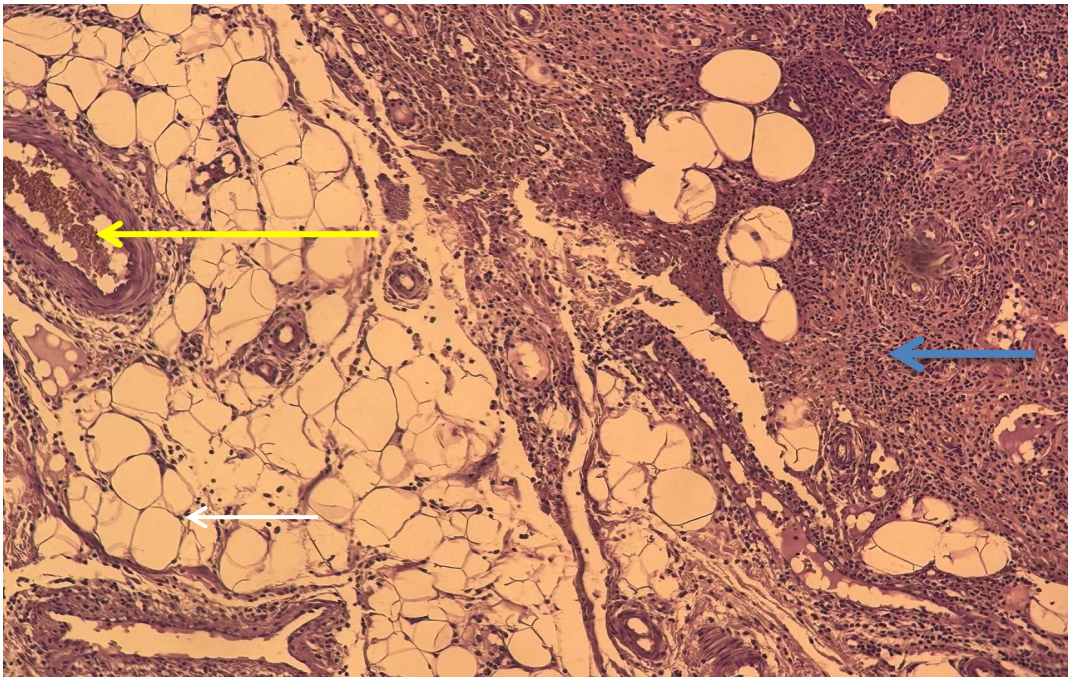


Figure (3) acute suppurative appendicitis and periappendicitis note the congested blood vessels (yellow arrow),neutrophil,(blue arrow), periappendicitis (white arrow) (H&E 40 x)

In most cases, gases produced by bacterial species that cause inflammation as metabolites contribute to increased pressure on the walls of appendix (Robbins *et al.,*1996) The expansion of appendix cavity causes pressure on the arteries and blood vessels supplying appendix which leads to interruption of blood supply, so it appears dark in color and blood clotting is observed in the blood vessels with exudation of red blood cells within the tissues of the appendix wall and its cavity (Burhan et al.,2006). When the luminal pressure exceeds the venous pressure, ischemia or vasospasm occurs, it may cause clotting of the veins and capillaries, and when there is continued arteriolar inflow and impaired lymphatic and venous drainage, mucosal ischemia develops, and inflammation extends to the submucosal, muscular layers and serosa causing congestion and haemorrhage in blood vessels (Alvarado, 2018).

The histological investigation showed that there is destruction of the epithelial wall lining appendix resulting from its obstruction by a fecolith (figure 4).

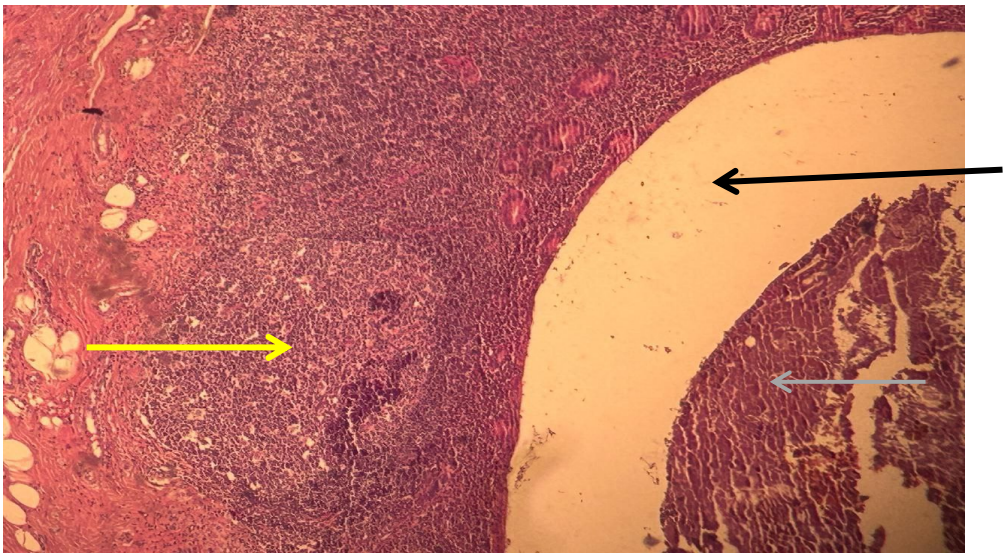


Figure (4) follicular hyperplasia with fecolith note the fecolith (green arrow), lumen (black arrow) , follicular hyperplasia (yellow arrow) (H&E 40 x)

Most of the destruction occurs in the mucosal and sub mucosal layer, and the expansion and swelling of the appendix makes the wall thin, and this result is similar to a number of studies (Truty *et al.,*2008).

It was also observed in some appendices no significant pathology seen (figure 5), which was appeared to be agreed with (Hiatt & Gartner 2000) which indicated that no clear-cut damage occurred in appendix tissue

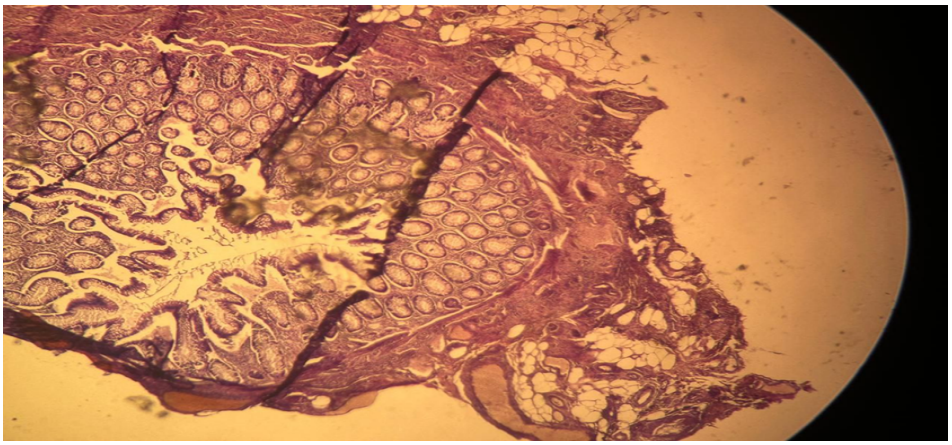


Figure (5) appendix with no significant pathology seen (H&E 40 x)

This may be due to the time of the excision procedure which may be early in the occurrence of inflammation, such that this period is not enough to show clear tissue changes that may appear later.

Histological sections of appendages showed the accumulation of a large amount of adipose tissue, in addition to fatty necrosis of some adipose tissue (figure 6).

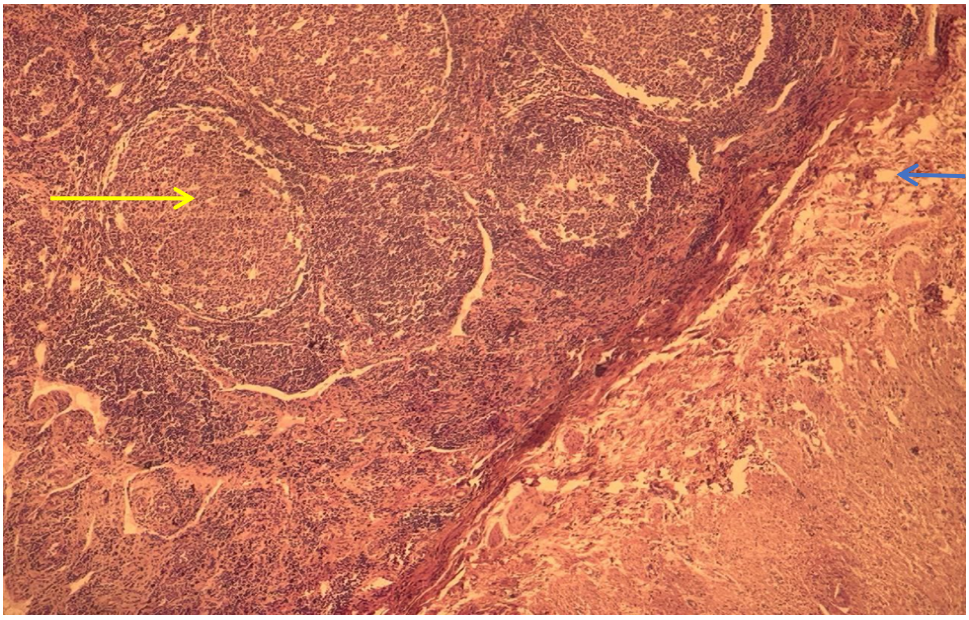


Figure (6) appendix with follicular hyperplasia note the fatty necrosis (blue arrow) , lymphoid follicles are more active and larger than normal (reactive hyperplasia or hyperplastic changes) .(yellow arrow) (H&E 40 x)

The term "necrosis" refers to the uncontrolled death of a cell, which often occurs in response to a severe injury and characterized by the fact that the cell's contents seep out into the tissues that are in close proximity to it so causing harm to those tissues (D'Arcy, 2019).Breach of the plasma membrane, which enables the release of damage related molecular patterns that cause necroinflammation as an immunological reaction (Tonnus *et al.,* 2021). The primary idea postulates that necrosis of tissue and subsequent bacterial invasion are the effect of blockage of appendix discharge , which is frequently caused by a feces. (*Dimberg et al.,* 2020).

The histological changes of the excised appendices in this study varied greatly among them ,

This may be due to the grade of appendicitis in those affected , or it may be due to physiological and anatomical nature for appendiceal tissue , which differs according to age groups , despite the seriousness of the condition, the surgeon's try to perform the surgical operation before it reaches progressive stages to avoid perforation of the appendix, sometimes such cases happen this may be due to the differences in symptoms of appendicitis and its development the inflammatory conditions between one patient and another, especially young or old patients, the development of the conditions in them is faster than in young people due to their weak in immune resistance (Butler,1981).

Conclusion

Our conclusion that the serum concentration (MCP-1), and (MIP-1α) can be used as a predictor for the diagnosis of acute appendicitis and histological changes in inflamed appendices confirm the activity of the immune system with appendicitis ,this is due to the abundance of lymphatic tissue in the examined species where histopathological examination of all appendix specimens showed inflammation in all layers of the tissues characterized by dilatation of blood vessels with increasing amount of lymphatic tissue spread through the layers of appendix , lymphoid follicular hyperplasia , large amount of adipose tissue with fatty necrosis , infiltration of inflammatory cells that spread through the layers of appendix..

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