

## Pathological Assessments of Ovine Liver Abscesses in Basra Abattoir

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### Abstract

This study was conducted to find out the macroscopical and microscopical lesion as well as the pathological lesions, the hematological study, biochemical analysis, the histopathological study and histochemical study of liver abscesses in sheep. A regular visits were done to Al-Basrah abattoir during the period from October 2019 to January 2020 in which routine postmortem examination to slaughtered sheep was carried out. The livers from sheep showing pathological lesions or abnormalities were collected and thoroughly examined. The liver from 70 sheep were collected and thoroughly examined, sixty of them showed gross lesions (from left and right liver equally), other ten were normal in texture and morphology, used as a control. Our hematological results appear a significant ( $P \leq 0.05$ ) increment in the total white blood cells counts ( $18.4 \pm 0.76$ ), lymphocytes counts ( $7.96 \pm 0.31$ ) and neutrophils counts ( $8.11 \pm 0.16$ ) of ovine liver abscess group when compared to the control group which appear the total white blood cells counts, lymphocytes counts, and neutrophils counts were ( $11.16 \pm 0.47$ ), ( $6.04 \pm 0.2$ ) and ( $3.61 \pm 0.12$ ) respectively. In addition, the results of the biochemical analysis of the study showed significant ( $P \leq 0.05$ ) increase of Alanine aminotransferase (ALT) ( $48.5 \pm 5.4$ ), Aspartate aminotransferase (AST) ( $32 \pm 1.9$ ), C-Reactive Protein (CRP) ( $4.37 \pm 0.3$ ) and lactate dehydrogenase (LDH) ( $195.3 \pm 1.19$ ) of ovine liver abscess (OLA) group when compared to the control group were showed the ALT ( $19.0 \pm 0.5$ ), AST ( $21.3 \pm 1.8$ ), CRP ( $1.63 \pm 0.1$ ) and LDH ( $169.3 \pm 14.8$ ). The macroscopical results of our study of ovine liver abscess showed the plurality of these affected livers had a solo to multiple abscess formation; therefore, the grossly manifest on their surfaces which purulent material and a tender capsule with necrotic tissues in the central area. Besides, the gross examination revealed significant findings of necrotic cavity with a perceptible fibrous wall were observed in liver and multiple abscesses especially surrounded the biliary canal. The histopathological result of ovine liver abscess revealed a dilation of central vein filled with infiltration of inflammatory cells in the per-portal region, in addition, there are a present of a thick fibrous capsule formation surrounded abscess formation of the liver with inflammatory zone consist mainly polymorphnuclear cells surrounded the abscess capsule of the liver, also a present of large area of abscess formation in the liver parenchyma, which stained also positively in

Mallory trichrome stain. The current study concluded that the ovine liver abscesses associated with dangerous increases in the cardinal hematological and biochemical biomarkers as an indicator to the harmful affection of the liver diseased with pyogenic infection.

**Key words:** Ovine liver abscess (OLA) , Biochemical, Hematology, Pathology.

### **Introduction:**

A researchers from Basra, Iraq who had investigate the pathological lesions in liver abscess showed the multiple nodules change in size which scattered on the diaphragmatic and visceral surfaces either a large abscess or small abscess; Besides, there is a remarkable area of congestion and the texture is firm; Also, the surface of the liver in the affected group appear friable areas and multiple grayish /white abscesses that containing whitish cheesy material and enclosed by reddish hyperemic zone or pale yellowish area on the diaphragmatic surface of the right lobe (Alshammari and Ahmed, 2020).

The liver is a signal of the almost necessary biological structure in the animals bodywork. It function a key say in the metabolism of many exogenous and endogenous material and as a result, is one of the repeatedly influenced body part in a sickly body (Alawa *et al.*, 2011). The liver support almost every organ in the body and is vital for survival; therefore, any disorder in the liver will be inverted on the overall validate lead to major economic havoc in animal mass production (Sonawane *et al.*, 2016). The hepatic abscess can be known as an enclosed gathering of suppurative matter during the liver parenchyma, whose may be infected by parasitic, fungal or bacterial microorganisms (Lardièrè *et al.*, 2015). Where the majority of hepatic abscess in the Westerner scholar is infected with bacteria (Sachdev *et al.*, 2013). Hepatic abscesses bring about by a bacterial infection imparted by blood from various provenance fundamentally the portal (Tehrani *et al.*, 2012). (*Fusobacterium necrophorum*) is take into one of the most common reasons for hepatic abscesses in ruminants (Zaki *et al.*, 2000). Another study showed that *Corynebacterium pseudotuberculosis* caused 24.5% of abscesses in sheep and goats examined in Saudi Arabia (Musa *et al.*, 2012). Liver abscesses usually happens because of chronic laminitis in livestock; but they seldom in ovine. Abscesses can happens in animals fed portion high in grain also occur in feedlot lambs (Navarre and Pugh, 2002). The abscesses generally enclosed with thick fibrotic walls. Besides, generally obtain in the liver at the period of necropsy or slaughter (Nagaraja *et al.*, 1996). The use of rise focus diets; fundamentally grains; this form of nourishment push pack carbohydrates that are in a hurry fermentable in the rumen and rise the hazard of metabolic trouble like liver abscesses and ruminal acidosis. Also, they is an progressively common pursuit in ruminant nourishment to get better body weight and milk production (Huo *et al.*, 2013).

Liver abscess promote fundamentally due to that one reasons a falling in ruminal pH (<5.8), also an exaggerated bulk of organic acids in the rumen and lesions in the gastrointestinal partition (Chang *et al.*, 2015).

Amebic hepatic abscess is the utmost popular form of hepatic abscess widespread, while pyogenic hepatic abscess is the generality popular form in North America (Wong *et al.*, 2002), also is commonly the outcome of poly-microbial infection ( Stroup *et al.*, 2007).

### **Aim of the Study:**

The liver abscess is a potentially life minatory disease which had high mortality and morbidity rates; therefore, the current study aimed to:

- Estimate the propagation of hepatic abscesses in sheep and to take out a clear imagine of the pathological lineaments linked escorted by their manifestation, as well as to other related hematological and biochemical changes .
- Giving an ideal screening of the liver abscesses comperes great economic problems and there prevail so little information in synchronism with hepatic abscesses in sheep in Iraq.

### Material and Methods:

Livers from sheep showing pathological lesions or abnormalities were collected and thoroughly examined. Liver from 70 sheep were collected and thoroughly examined. Sixty of them showed gross lesions (from left and right liver equally), other Ten were normal in texture and morphology, used as a control. All animals given for slaughter were physically observed a day before or shortly prior to slaughter. Checking of the animals was made while at comfort or in activity for any clear sign of disease. All lesions were recorded. Then tissue pieces measured as (1cm<sup>3</sup>) were sampled and transferred in a plastic container which contains neutral buffered formalin 10% for 72 hours, to the tissue processing unite in faculty of veterinary medicine, university of Basrah. Post slaughter palpation and incision of doubtful organs visional examination of cadaver and organs with terrible notice being steer to liver. Examination interested gross pathological lesion of each diseased organ was confirmed and on record as described by Gracey (1985). The blood specimens were collected according to (Rahimian *et al.*, 2004) from the external jugular vein by employ a disposable syringe (10 ml) to do complete blood cell (CBC) count MAXM method, were used according to (Blood, 2007), and the serum separation for evaluation of AST, ALT, CRP, and LDH . Histological liver preparation was conducted as follows (Bancroft and Gamble, 2008). After 10% formalin fixation of samples.

The data was analyzed and the significant difference between the liver abscess group and the healthy group was statistically analyzed using (SPSS) program (One way ANOVA).

### Results:

The results of the biochemical analysis of the current study appeared significant ( $P \leq 0.05$ ) increase of ALT, AST, CRP and LDH of ovine liver abscess (OLA) group when compared to control, these values showed (48.5±5.4), (32±1.9), (4.37±0.3) and (195.3±1.19) of ALT, AST, CRP and LDH of OLA group respectively when compared to the control group (19.0±0.5), (21.3±1.8), (1.63±0.1) and (169.3±14.8) respectively as in (table 1). Our hematological outcome appear a significant ( $P \leq 0.05$ ) elevated in the total White blood cells counts, lymphocytes counts and neutrophils counts of OLA group which showed (18.4±0.76), (7.96±0.31) and (8.11± 0.16) respectively when compared to the values of the control group were showed the total WBCs counts, lymphocytes counts, and neutrophils counts were (11.16±0.47), (6.04±0.2) and (3.61±0.12) respectively as in (Table 2).

**(Table: 1): Biochemical result of liver function test among OLA group and control.**

Group	ALT (U/L)	AST (U/L)	CRP (U/L)	LDH (U/L)
OLA	48.5±5.4 a	32±1.9 a	4.37±0.3 a	195.3±1.19 a
Control	19.0±0.5 b	21.3±1.8 b	1.63±0.1 b	169.3±14.8 b

- Different letters vertically refer to present a significant ( $P \leq 0.05$ ) difference between groups, Values are mean± standard deviation of the mean ( $P \leq 0.05$ ).

Group	Total WBCs count (103/ $\mu$ l)	Lymphocytes counts (103/ $\mu$ l)	Neutrophils counts (103/ $\mu$ l)
OLA	18.4±0.76 a	7.96±0.31 a	8.11±0.16 a
Control	11.6±0.47 b	6.04±0.2 b	3.61±0.12 b

- Different letters vertically refer to present a significant ( $P \leq 0.05$ ) difference between groups, Values are mean± standard deviation of the mean ( $P \leq 0.05$ ).

The macroscopical results of the current study of OLA was represented in figures (1 to 3), grossly evident on their surfaces; a tender capsule with necrotic tissues and purulent materials in the central area; therefore, which the preponderance of these affected livers had a single to multiple abscess formation. On other hand, the central areas having creamy to purulent materials and the rest of the examined abscesses had thick fibrous capsule; comparable focal abscesses were noticed in all livers with multiple abscesses, particularly in (figure 1). Besides, the gross examination revealed significant findings of necrotic cavity with a perceptible fibrous wall were observed in liver and multiple abscesses especially surrounded the biliary canals as in (figure 2). In addition, the surface of the liver showed surrounded area of reddish hyperemic zone as in (figure 3).

The histopathological result of OLA revealed the sub-capsular region of abscess formation of the liver appeared severe infiltration of mononuclear cells; as well as to existent of giant cells infiltration; also, there are a present of a thick fibrous capsule surrounded the abscess formation of the liver as in (figure 4). Therefore, the abscess capsule showed a severe infiltration of polymorphonuclear cells in the inflammatory zone of the ovine liver abscess as in (figure 5).

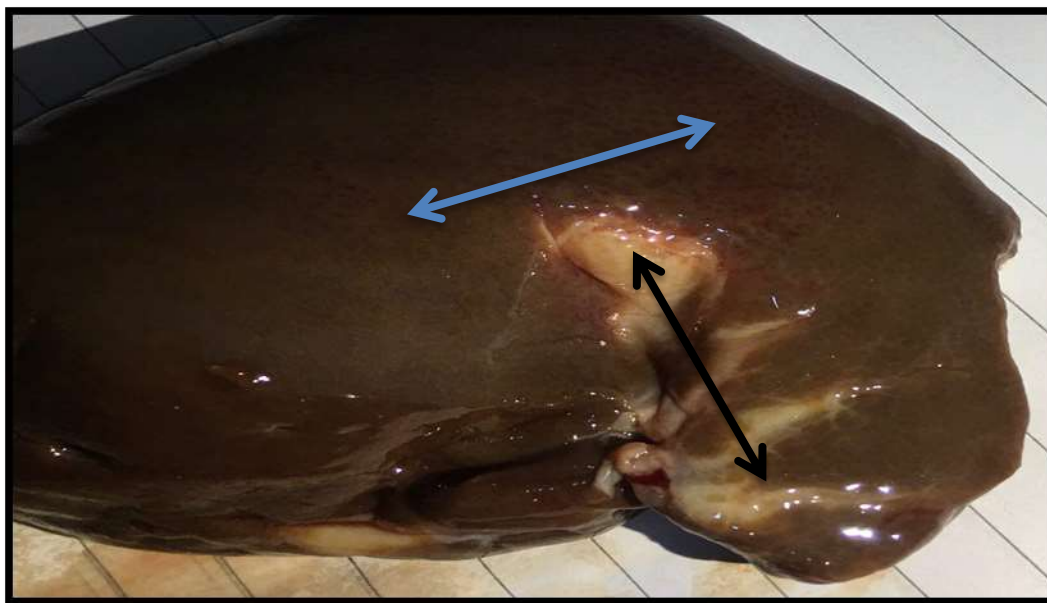
Product for the preparation of histological samples for optical microscopy. Standard procedure for connective tissue; it shows collagen (Sheehan and Hrapchak, 1980). The histochemical section of liver of OLA group showed formation a thick fibrous capsule surrounded the abscess formation (black arrows), which appeared the collagen fibers were stained blue, and their nuclei were stained black as in (figure. 6).



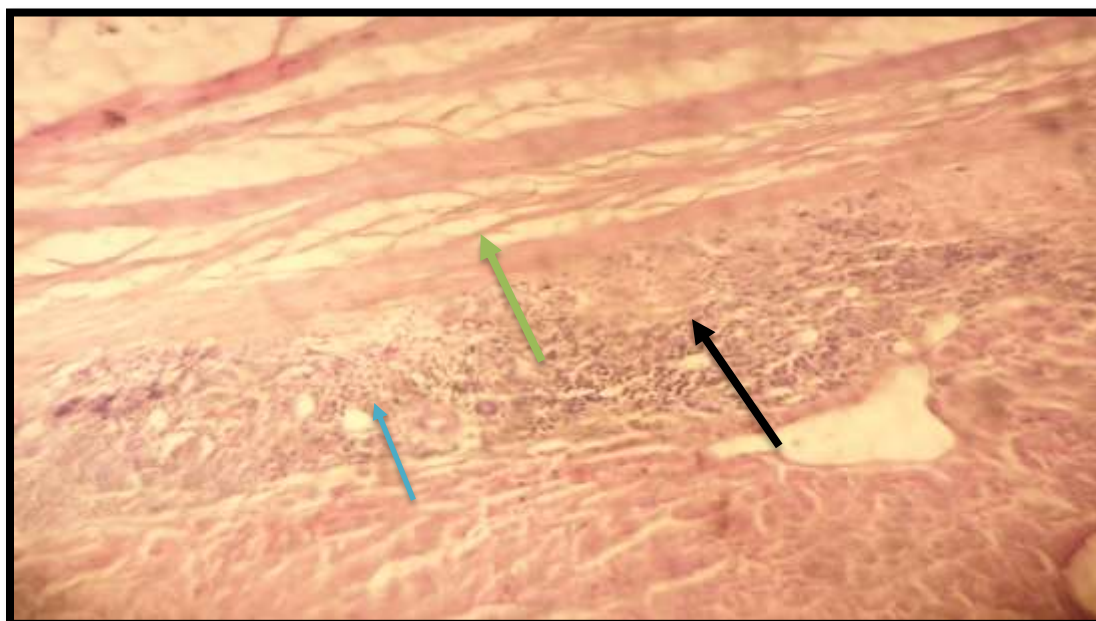
**Figure. (1):** Macroscopical section of OLA group showed encapsulated White-yellowish abscess with a thick capsule in anterior border of the hepatic lobe (Black arrows).



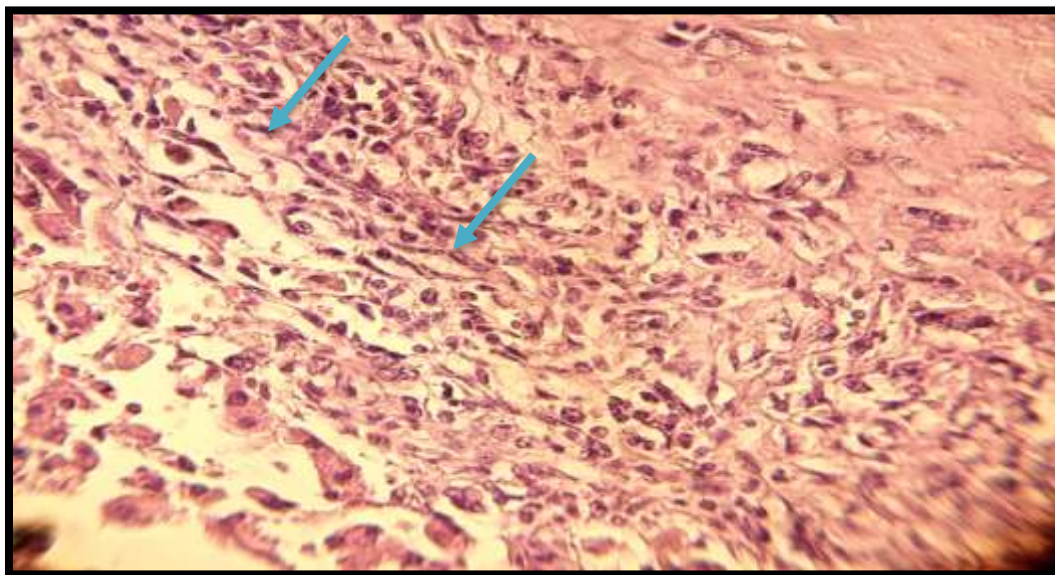
**Figure, (2):** Macroscopical section of liver of OLA group showed multiple abscesses formation as well to necrotic cavity in the biliary canals with a perceptible fibrous wall in visceral surface of the liver (Blue arrows).



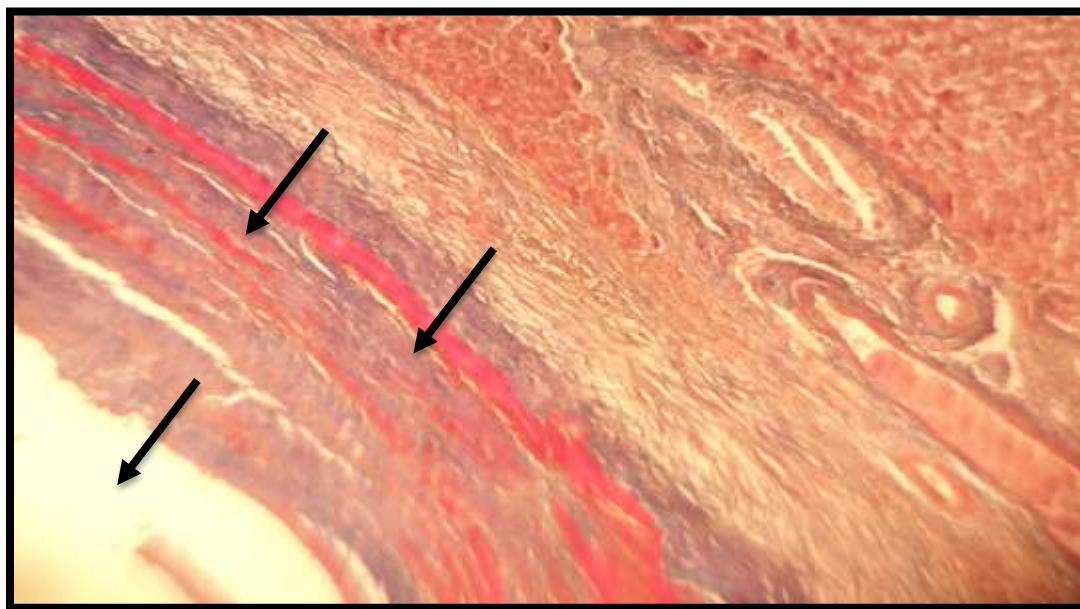
**Figure. (3):** Macroscopical section of liver of OLA group showed a swollen area with multifocal and circular areas of hepatic necrosis in the left anterior segment (Black arrows). Also, the dorsal surface of liver showed a reddish hyperemic zone (Blue arrows).



**Figure. (4):** Histopathological section of liver of OLA group showed severe infiltration of mononuclear cells in the sub-capsular region surrounded the abscess formation (black arrow); also present of giant cells infiltration (blue arrow) ; a thick fibrous capsule surrounded the abscess formation of the liver (green arrow) (H&E stain, 10X).



**Figure. (5):** Histopathological section of liver of OLA group showed severe infiltration of polymorphonuclear cells in the inflammatory zone surrounded the abscess capsule (blue arrows) (H&E stain, 40X).



**Figure: (6):** Histochemical section of liver of OLA group showed formation a thick fibrous capsule surrounded the abscess formation (black arrows), which appeared the collagen fibers were stained blue, and their nuclei were stained black. (Mallory trichrome stain, 40X).

## Discussion:

Due to being liver is an important meat by-product, it must be protect; as possible, free from all pathogens inclusive bacteria and parasites that so affect the hepatic tissue preforms it incompetent for human consumption through partial or total condemnation in slaughterhouse.

The biochemical study analysis is a group of blood tests used to appraise the functional capacity of several critical organs and systems, such as the liver and kidneys. The serum values of these enzymes are often routinely used for assessing liver function. In this completed study, the activities of AST, ALT, and LDH were investigated to establish an extensive biochemical perspective for the investigation and thus health status was viewed in detail. The main purpose of liver tests in sheep is to clarify the liver functions that effect the total health of the organism. The serum values of these enzymes are often routinely used for assessing liver function (Braun *et al.*, 2010).

Therefore, Our biochemical analysis appeared a significant ( $P \leq 0.05$ ) elevated of (ALT) of OLA group when compared to control group. The serum level of ALT in group a was significantly elevated than the group b, similar observations were made by previous workers like (Kitila and Megersa, 2014) and (Pandya *et al.*, 2015), they observed a significant increase in the mean values of serum enzymes in various hepatic diseases. The increase in serum enzyme in the present study might be due to hepatocellular necrosis, degenerative changes and other pathological conditions encountered. Moreover, according to (Hodžić *et al.*, 2013), the plasma concentration of ALT increases with hepatocellular spoiling/ necrosis or degeneration and liver cell proliferation.

Furthermore, our biochemical analysis appeared a significant ( $P \leq 0.05$ ) elevated of (AST) of OLA group when compared to control group. Specific data was also found in other liver enzymes elevation (AST); As the results showed observed by (Pang *et al.*, 2011; Mudroň *et al.*, 1999; and Bionaz *et al.*, 2007), who they reported animals in liver disease, also have elevated concentrations of AST, which mention that the formation of abscesses compromises liver health and indices to liver damage.

Moreover, with the attack of inflammation the (CRP) C- Reactive Protein synthesis rise during 4-6 h; doubling every 8 hour. The CRP level reaches to the peak rate (around 150-350 mg/L) during 36-50 hours after infection. The high levels persist through the inflammation period. Therefore, when the infection is controlled; the CRP levels decrease quickly, and the decrease is strongly correlated with the relief from symptoms and with the duration of the treatment. However, the CRP level is not affected by factors such as gender; age; anemia; hyperglobulinemia; and pregnancy. These finding agree with (Jaye *et al.*, 1997). To date, the results of the biochemical analysis of the current study appeared significant ( $P \leq 0.05$ ) increase of CRP of OLA group when compared to control group. Elevated of the serum level of C-Reactive Protein (CRP), these results may agree with (Vanderschueren *et al.*, 2006), who reported the utmost infections were bacterial and were easily diagnosed. A wide assortment of infections accounted for almost 90% of the inflammatory conditions. Pneumonia and intra-abdominal infections were the predominant focal point. A few fungal infections were meet, confirming that fungal disease may induce high CRP values. therefore, laboratory analysis showed leukocytosis with an expressive increase in CRP, suggesting inflammatory response. Mensuration of CRP levels can help differentiate inflammatory from non-inflammatory conditions and are particularly useful in managing disease since they often reflect the need for, and the response to, therapeutic intervention. For this latter purpose, CRP has largely replaced erythrocyte sedimentation rate (ESR), an indirect, less reproducible and less sensitive acute phase reactant (Morley *et al.*, 1982).



Indeed, the current results of lactate dehydrogenase (LDH) of the OLA group appeared significant ( $P \leq 0.05$ ) increase in the liver abscess group compared to control. These data were comparable with this study (Brogly *et al.*, 2007), who revealed a LDH is a cytoplasmic enzyme expressed in roughly all types of cells of the body. Because it is expressed in various organs/tissues by high concentration, the seepage of LDH from even a small scale of injured tissue can result in a significantly elevated serum level. It is broad into blood when the cells experience injury or death caused by dehydration, ischemia, bacterial toxins, drugs, and chemical poisonings. It has been used as an signal of cellular injury promote by various etiologies.

The current hematological results of liver abscess animals showed result to increases in the total WBCs counts of OLA group when compared to the values of the control group. Leukocytosis, take place most often due to certain infections or inflammatory processes, these idea with agreement with (Wanahita *et al.*, 2002). White blood cells (WBCs) and hepatic function tests are usually increased. Our observations are also consistent with prior reports in several other ways. We noted similar pause of elevated WBC counts (Branum *et al.*, 1990) and (Chang *et al.*, 2000). Moreover, the result of lymphocyte count showed a significant ( $P \leq 0.05$ ) increases of OLA group when compared to the values of the control group. These finding agreement with (Radostits *et al.*, 2006 ; Gameel and Tartour 1974), who demonstrated that in chronic cases lymphocytes and monocytes may increase altogether with development of moderate normochromic anemia and mild proteinuria. Microscopically abscess appeared by necrotic areas infiltrated with dead neutrophils and surrounded by connective tissue capsule. The hepatic infection were also detect in all investigation of farm animals in the form of multiple abscesses. Noted observed by (Borai *et al.*, 2013).

Indeed, the hepatic infection were also observed multiple abscesses formation as well to necrotic cavity in the biliary canals with a perceptible fibrous wall in visceral surface of the liver (Mohamed *et al.*, 2013). In addition, the liver of OLA group showed a large noticed area of the yellowish pus content surrounded by a pale zone. As the results with observed by (Conter *et al.*, 1986).

In this study, the outcome of histopathological changes of liver abscess was dilation of sinusoid that the abscess surrounded by the pyogenic membrane, showed neutrophils infiltration standing by sinusoids surrounded by pyogenic membrane consisting from proliferation of fibrous connective tissue, looked dilation of central vein filled with inflammatory cells. In addition, these changes similar described by (Raji *et al.*, 2012), who reported the histopathological examination for 268 abscessed livers appeared well-circumscribed area of liquefactive necrosis enclosed by leucocyte infiltration and fibrosis. As well as, the histopathological section of liver of OLA group showed infiltration of inflammatory cells in the periportal region, this finding is likely similar to (Hanazaki *et al.*, 2001), who they Suggested that a hepatic abscess may command to infectious damage of the portal vein; which might result in thrombosis. Our microscopical results which agreed with the current biochemical results, which showed a significant increase in total WBC, neutrophils and lymphocytes which it recruited to the site of infection in order to scavenger and phagocytized the invaders.

Besides, the histochemical section of liver of OLA group showed a thick fibrous capsule surrounded the abscess formation, which stained positively in Mallory trichrome stain; also a present of large area of abscess formation in the liver parenchyma, which stained also positively in Mallory trichrome stain. Our observations are in agreement with (Suriawinata and Thung, 2011), who reported the special stain for connective tissue, commonly trichrome stain; for the guess of the degree of fibrosis in chronic hepatic disease and recognize normal structures, such as portal triads and terminal hepatic venules, substantial for the valuation of the lobular architecture. Immature fibrous tissue is pale blue, while mature fibrous tissue are dark blue. The current results of histochemical of Mallory trichrome stain were agreed with the microscopical results which showed severe area of fibrosis particularly in the peri-central vein region as well to the portal area of the affected liver.

### Conclusions:

The current study concluded many of critical points that associated with the OLA endpoints as in the followings:

- OLA associated with significant elevation in the ALT, AST, LDH and CRP when compared to control.
- OLA associated with significant increases in the total WBCs, Neutrophils, Lymphocytes counts when compared to control.
- The current study showed a thick capsule that composed of a thick fibrous tissue surrounded mainly the abscess formation as well to inflammatory cells that appeared positively in PAS and Mallory trichrome stain.

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## التقييمات الباثولوجية لخراجات كبد الغنم في مجزرة البصرة

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### الملخص

أجريت هذه الدراسة لمعرفة الآفات العيانية وكذلك الآفات المرضية، ودراسة الدم، والتحليل الكيميائي الحيوي، ودراسة الأنسجة المرضية، والدراسة النسيجية الكيميائية لخراجات الكبد في الأغنام. تم القيام بزيارات دورية إلى مجزرة محافظة البصرة خلال الفترة من أكتوبر 2019 إلى يناير 2020 حيث تم إجراء الفحص الروتيني بعد الذبح للأغنام. حيث تم جمع الكبد من 70 نعجة وفحصها بدقة، أظهرت ستون منهم آفات جسيمة (من الكبد الأيمن والأيسر بالتساوي)، وعشرة أخرى كانت طبيعية في التركيب والتشكل حيث استخدمت كمجموعة سيطرة. تظهر نتائج أمراض الدم لدينا زيادة كبيرة ( $P \leq 0.05$ ) في إجمالي تعداد خلايا الدم البيضاء ( $18.4 \pm 0.76$ )، وتعداد الخلايا الليمفاوية ( $7.96 \pm 0.31$ ) وعدد العدلات ( $8.11 \pm 0.16$ ) من مجموعة خراج كبد الأغنام عند مقارنتها بالقيم من مجموعة السيطرة وكان مجموع عدد خلايا الدم البيضاء وعدد الخلايا الليمفاوية وعدد العدلات: ( $11.16 \pm 0.47$ ) و ( $0.2 \pm 0.2$ ) و ( $6.04 \pm 0.12$ ) على التوالي. بالإضافة إلى ذلك، أظهرت نتائج التحليل البيوكيميائي للدراسة الحالية زيادة معنوية ( $P \leq 0.05$ ) في ALT ( $48.5 \pm 5.4$ )، AST ( $32 \pm 1.9$ )، CRP ( $4.37 \pm 0.3$ ) و LDH ( $195.3 \pm 1.19$ ). أظهرت مجموعة OLA عند مقارنتها بقيم مجموعة السيطرة ( $19.0 \pm 0.5$  ALT و  $21.3 \pm 1.8$  AST و  $1.63 \pm 0.1$  CRP و  $169.3 \pm 14.8$  LDH).

أظهرت النتائج الماكروسكوبية لدراستنا لخراج كبد الأغنام أن تعدد هذه الاكباد المصابة كان له تكوين خراج فردي إلى خراج متعدد؛ لذلك، تظهر بشكل صارخ على أسطحها مادة قيحية وكبسولة رقيقة مع أنسجة نخرية في المنطقة الوسطى. إلى جانب ذلك، أظهر الفحص الإجمالي وجود نتائج مهمة للتجويف النخري بجدار ليفي محسوس في الكبد وخراجات متعددة تحيط بالقناة الصفراوية بشكل خاص. أظهرت النتيجة النسيجية المرضية لخراج كبد الأغنام اتساعاً في الوريد المركزي مملوءاً بتسلل الخلايا الالتهابية

في منطقة كل بوابة، بالإضافة إلى وجود تكوين كبسولة ليفية سميكة تحيط بتكوين خراج للكبد مع منطقة النهائية تتكون بشكل رئيسي تحيط الخلايا النوى متعددة العقارب بكبسولة الخراج في الكبد، وهي أيضاً موجودة في مساحة كبيرة من تكوين الخراج في حمة الكبد، والتي تلتخ أيضاً بشكل إيجابي في صبغة مالوري ثلاثية الكروم. خلصت الدراسة الحالية إلى أن خراجات كبد الأغنام مرتبطة بزيادات خطيرة في المؤشرات الدموية والكيميائية الحيوية الأساسية كمؤشر على التأثير الضار للكبد المصاب بالعدوى القحبية.

**الكلمات المفتاحية:** خراج كبد الأغنام، الكيمياء الحيوية، أمراض الدم، علم الأمراض.

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