Histopathological study on liver of mice during aspergillosis infection

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 Abstract

   In this study ,the   *Aspergillus fumigatus* histopathological activity on the mice livers during aspergillosis became more obvious. The total number of 40 male Albino swiss mice were randomly divided into 8 groups (Five mice/group). The 1st group were immunosuppressed , while the 2nd group are not immunosuppressed , and control mice were instilled nasally with Phosphate buffer saline and Tween 20 (five mice / control). The mice were sacrificed after 7th , 14th and 21st day post infection. It was found   that immunosuppressive treatments increase substantially the susceptibility of animals to infection by invasive aspergillosis, with higher progression of disease and earlier expression of inflammatory cells comparing with the non immunosuppresed mice,as the hepatic fungal colony has increased earlier and become more rapid than in the non- immunosuppressed mice, despite the fact that the initial doses which have been administered were identical in above models.

Keywords: *Aspergillus fumigatus ,*   hydrocortisone acetate , immunosuppressed, aspergillosis.

دراسه نسيجيه مرضيه لكبد الفئران خلال الاصابه بمرض الاسبرجلس

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الخلاصه

   في هذه الدراسه ، الفعاليه النسجيه المرضيه   لفطر   *Aspergillus fumigatus*   بدت واضحه خلال اصابة كبد الفئران بمرض الاسبرجلس ،   اجري البحث على 40 فأر من ذكور الفئران البيض قسمت عشوائياً الى   8 مجاميع (5 فئران /مجموعه) . المجموعه الاولى   تم تثبيطها مناعياً بينما المجموعه الثانيه لم تثبط مناعياً .   مجموعة السيطره تم تقطير انوفها بمحلول الفوسفيت الملحي المتعادل توين 20(5 فئران /سيطره) . تم قتل الفئران بعد اليوم السابع والرابع عشر والواحد والعشرين بعد الاصابه حيث وجد ان الفئران المثبطه مناعياً ازداد تقبلها للاصابه بمرض الاسبرجلس بشكل واضح وكبير بالاضافه الى انتشار المرض وتميز واضح ومبكر   للخلايا الالتهابيه في بداية المرض . مقارنةً بالفئران التي لم تثبط مناعياً بالرغم من ان الجرعه التي تم اعطاءها لكلا المجموعتين كانت متماثله .

Introduction

   Aspergillus fumigatus is a fungus belongs to saprophytics, filamentous a n d Ascomycetes fungi

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which is living in soil as natural habitat. It can colonize and grow on decomposing substrates, making the soil and places like hay or compost, it plays an important role in recycling soil nitrogen and carbon ,during growing on these substrates, it digest them into smaller units by secreting degrading enzymes to facilitate introducing them into the fungal cell [1]. Nowdays it can be considered as one of the most dangerous opportunistic pathogen for humans, especially for immunocompromised individuals with the risk of progressive Aspergillosis which usually affect the respiratory tract, skin, liver , etc., in the human [2- 5].

Once the individuals suffering from impaired immune system that are usually compained by leukemia, neutropenia prolonged treatment with steroids etc inhalate the airborne spores of A. *fumigatus*, that reach immediately to the alveoli of their respiratory system . These airborne spores cause mortality ranges from 30- 90% in this kind of patients due to development of infection with Aspergillosis [6]. In non impaired immune system individuals , the inhaled sporse that reach alveoli may be removed by mucociliary clearance or killed by alveolar macrophages, which are responsible for phagocytosis besides the initiation of a proinflammatory response that recruits neutrophils to the infection site [7].

The corticosteroids drugs interfere with the killing ability the *A. fumigatus* conidia and hyphae by phagocytes [8] . However the effects of steroids on innate immune cell function, neutrophils are recruited to the lung and prevent hyphal invasion, as a result of this , it provid an inflammatory environment . This exacerbated inflammatory response maybe fatal. The drastic differences between fungal growth and host responses in any regimen of immunosuppression show the importance of studying *Aspergillus fumigatus* pathogenesis [9].The major goal of this study is to investigate the effect of immunosuppressive treatment by hydrocortisone acetate on susceptibility to Aspergillosis in infected mice.

Materials & Methods
Laboratory animals
A total number of 40 male Albino swiss mice of male with ages ranged between (6–8) weeks were obtained from the (National Center of Researches and Drugs Monitor in Baghdad) were adapted for two weeks in Biotechnology Division/ University of Technology before start experiment by rearing in separated, cleaned and disinfected cages, they were fed on commercial assorted pellets and tap water.

Microbes used
One fungus (*Aspergillus fumigatus*) was collected from the Biotechnology Division/ University of Technology. Iraq , were used in the present study. The fungus sample was maintained in PDA medium plates until use.

Experimental design
Forty Albino mice were randomly divided into 8 groups and treated as follows :

Groups 1,2,3,4 include 20 mice , were immunosuppressed subcutaneously with 2 mg hydrocortisone acetate and instilled intranasally with $5x10^6$ spore suspension at three periods post infections , each 5 mice sacrified after 7 ,14 ,21 day of infection .The 4 group represent the control .

Groups 5,6,7,8 include 20 mice , were instilled intranasally with spore suspention $5x10^6$ at three periods post infections, each 5 mice sacrified after 7 ,14 , 21 day of infection.The 8th group represent the control .

The 1st group (n=20) were immunosuppressed subcutaneously with two milligram hydrocortisone acetate in 100 microlitre phosphate buffer saline، 0.1% Tween 20 before 4th and 2nd day pre-infection and 2nd ,4th day after infection where the immunosuppression is completed within 8th day .At the day of infection , the mice were instilled nasally with $5x10^6$ conidia in 20 microlitre of phosphate buffer saline ، 0.01% Tween 20 [7,10]. Five mice in each group were sacrificed after 7th, 14th and 21st day post infection. Control group mice were immunosuppressed with hydrocortisone and instilled nasally with 20 microlitre phosphate buffer saline، 0.01% Tween 20.

The 2nd group (n=20) the mice were intranasally instilled with $5x10^6$ conidia in 20 microlitre ، 0.01% Tween 20. (Five mice in each group) of the BALB/c strain Control mice were intranasally instilled with 20 microlitre phosphate buffer saline ، 0.01% Tween 20. Mice were sacrificed after 7th, 14th and 21st day post infection.

Preparation of histopathological sections

Histopathological sections of the liver were prepared from each mice treated and from control and used for comparisons among the groups. Pieces from liver were fixed in 10% formalin for 1 day cut at 5 μm thickness [11]. For histopathological studies this experiment were done in the Pathology Department/ veterinary collage/ Baghdad University, Baghdad, Iraq. Histopathological section were prepared according to the procedure [12]. Hematoxylin-Eosin Staining steps according to the procedure [12]. Periodic Acid-Schiff (PAS) Staining Procedure [13].

Results and Discussion

Histopathological effects of *A. fumigatus* in BALB mice.

The gross-examination of the examined (liver) of immunosuppressed mice   that inoculated subcutaneously with hydrocortisone acetate and are instilled intranasaly with $5*10^6$ conidia on the7 [th] 14th, and 21th day after immunosupresion has showed cleared   pathological changes,   and less pathological changes had showen in examined organ of non immunosuppressed infected animal during 7-21 day post-infection and no gross lesions were seen in examined organ, this observation coincide with [14].

While   histological examination of the control liver treated with only PBS expressed normal structure of the hepatocyte , the sinusoids wall and liver tissue, as shown in Figure-1
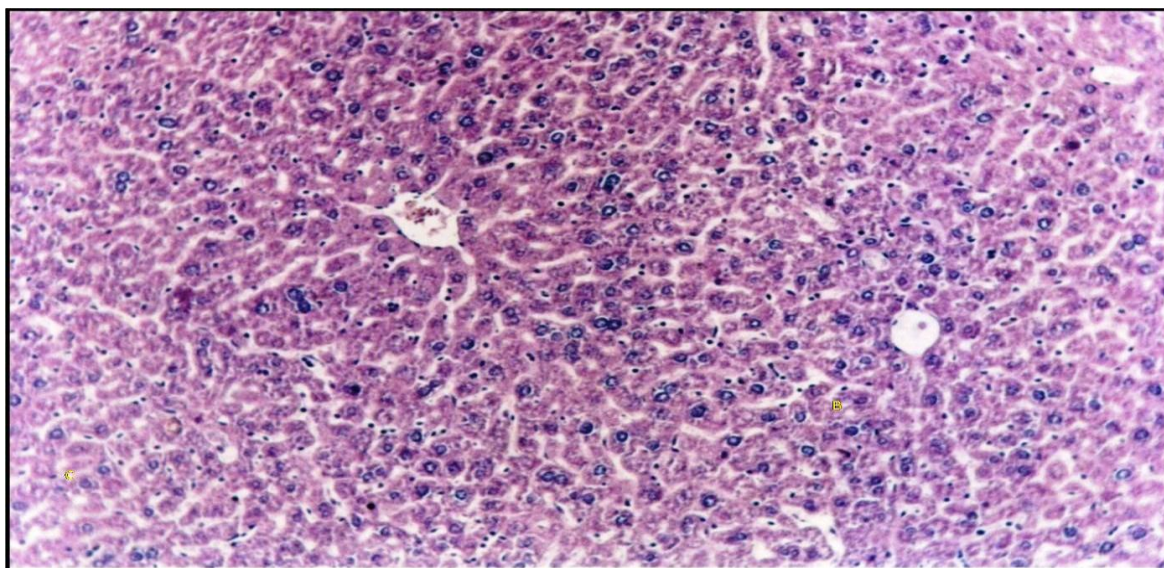

Figure 1- Control liver treated with PBS expressed normal structure of the hepatocyte (H&E stain, 40X) .

In the study of virulence and aggressiveness of the isolate of *A. fumigatus* which are instilled nasally in separated groups of animals and the pathological changes have been monitored periodically . Lastly , pathogenicity of the isolate was shown in two studies of this type of fungus that the virulence varied according to the source of isolation [15].

Histopathological change of liver during 7-21 day post-infection immunosuppressed by hydrocortisone acetate.

Histopathological examination of   liver after 7 days revealed severe neutrophil and macrophage infiltration in the hepatocyte space with increase thickness of intra celluler septa, and hypertrophy of artery muscular layer and congestion of blood vessel, aggregation of neutrophil in the liver lumen Figure-2 .

At 14th day post-infection, the liver showed granulamotus lesion in the wall of sinusoids and severe and defuse aggregation of mononuclear cells and inflammatory cell in the portal area and around the bile duct Figure- 3.

The liver tissue at 21st day post of infection expressed severe died and alive neutrophil filled in the liver lumen, monocyte type 2 proliferation, suppurtive exudates in the lumen of the liver sinusoids also errosion of the epithelial of the bile duct as well as epithelial layer of the sinusoids , larg necrotic areas and MNC infiltaration in the portal area and around the bile duct. Granulamatous lesion in the

liver baranchyma results from aggregation of macrophage and vascular degeneration of the hepatocyte and necrosis of the hepatocyte Figure- 4.
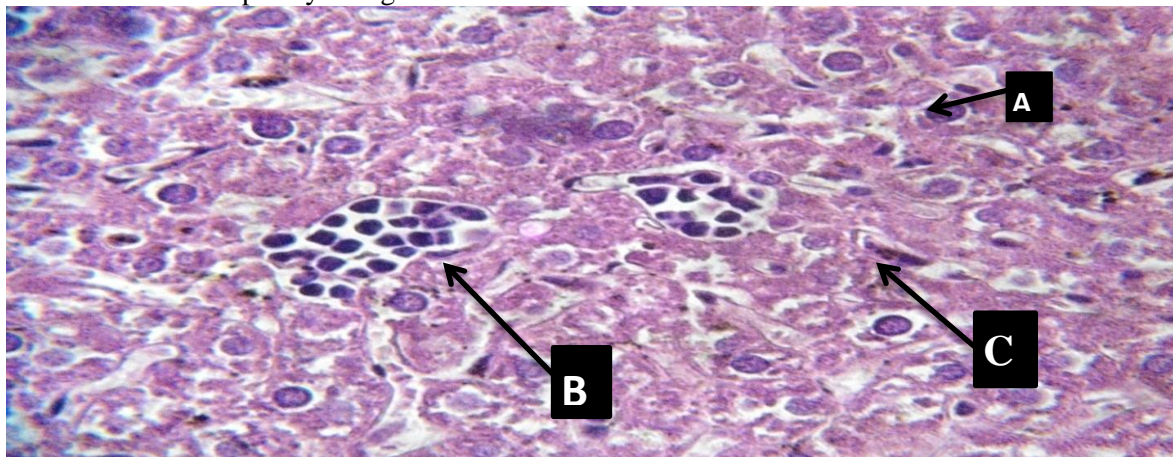

Figure 2- Histopathological section in the liver at day 7 post infection shows A- proliferation of kupfer cell , B- Necrosis of hepatocyte , C- congestion (H&E stain, 40X) .
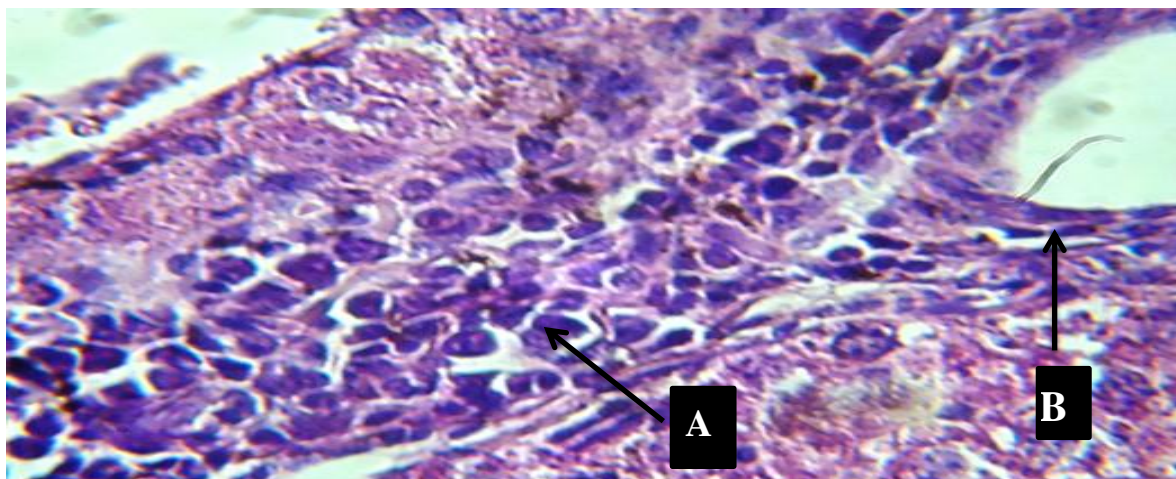

Figure 3 -Histopathological section in the liver at day   14 post infection shows A- larg necrotic area and B- large MNC infeltaration in the portal area and around the bile duct (H&E stain, 40X) .
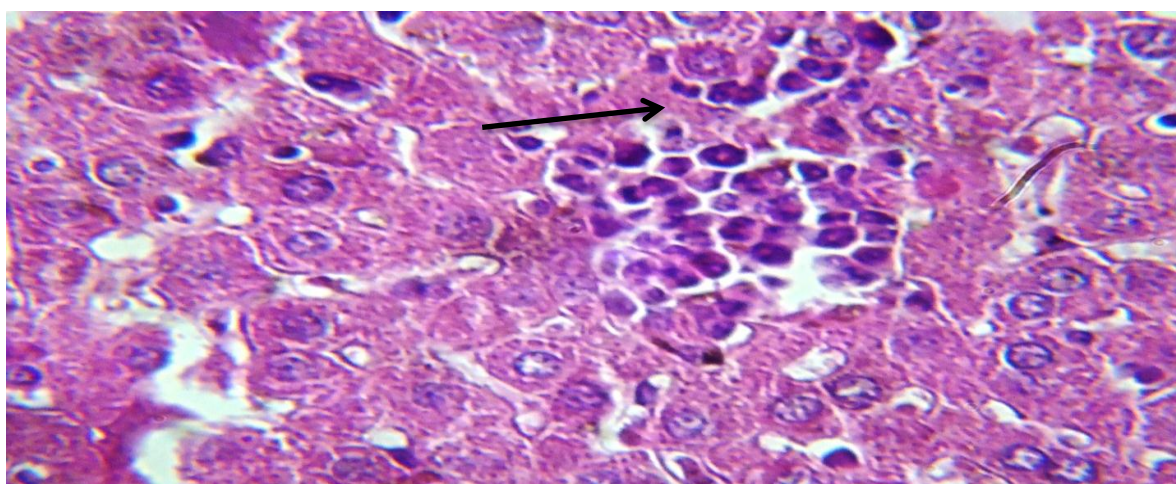

Figure 4- Histopathological section in the liver at day 21 post infection shows sever dead   and alive neutrophil filled in the lumen (H&E stain, 40X).

The liver sections images that are stained with periodic acid Schiff stain at 21st day of infection are

showed fungal lesions and large number of neutrophil that invade the infected liver with *A. fumigatus* with narrowed areas of sinusoids, the nuclei of neutrophils that adjacent to hyphae get stained pinkish color of the *A. fumigatus* isolate are largely intact, whereas the nuclei of neutrophils that are unadjacent to hyphae are not completely intact.

The hyphal invasion of mice hepatic blood vessels (angioinvasion) increase the probability of invasive hepatic aspergillosis . During this invasion , *A. fumigatus* interacts,with the endothelial cells of blood vessels *in vitro* as well as *in vivo* stimulating the endothelial cells to synthesize vascular cell adhesion molecules [16].

Histopathological change of liver during 7-21 days post infection non immunosuppressed by hydrocortisone acetate

Histopathological changes of infected liver during 7 days post infection showed mononuclear cell aggregate in the liver tissue, as well as in the sinusoids. Aggregate of mononuclear cell indicate good immune response [17] as in Figure-5.
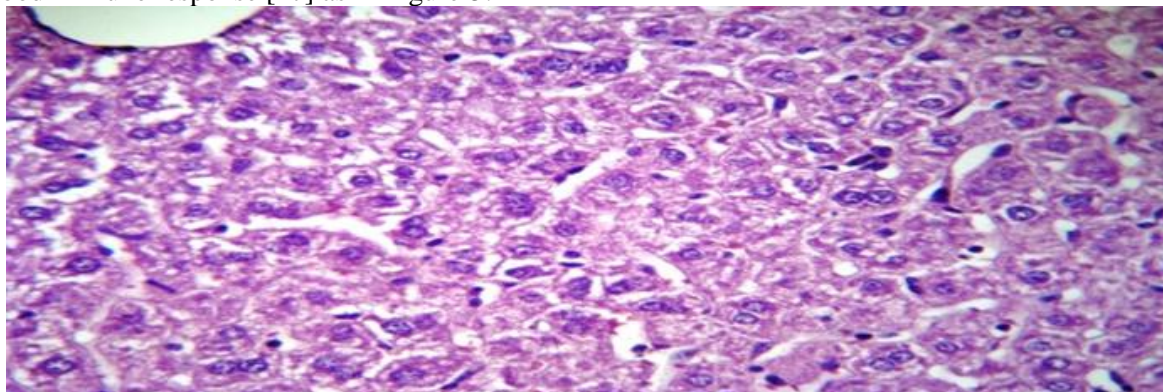

Figure 5- immunosuppressed by Histopathological section in the liver at day 7 post infection non hydrocortisone acetate (H&E stain, 40X) .
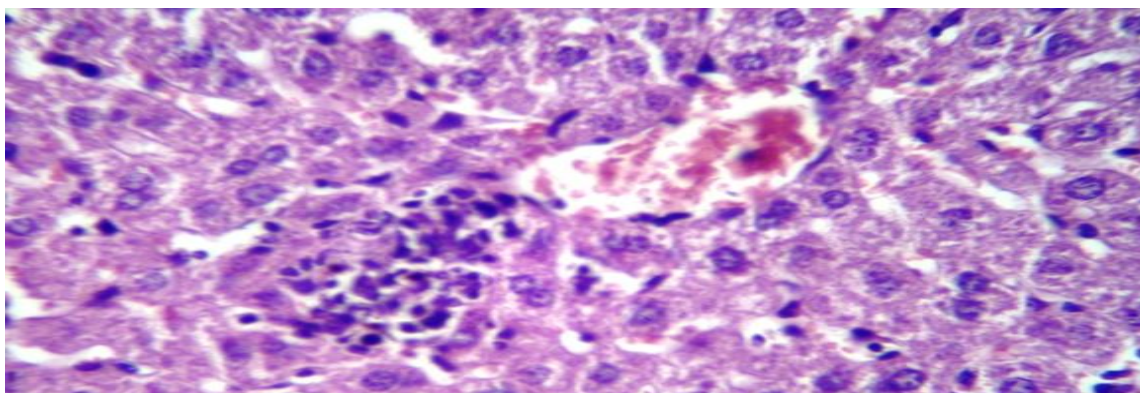

Figure 6- immunosuppressed by Histopathological section in the liver at day 14 post infection non hydrocortisone acetate (H&E stain, 40X) .
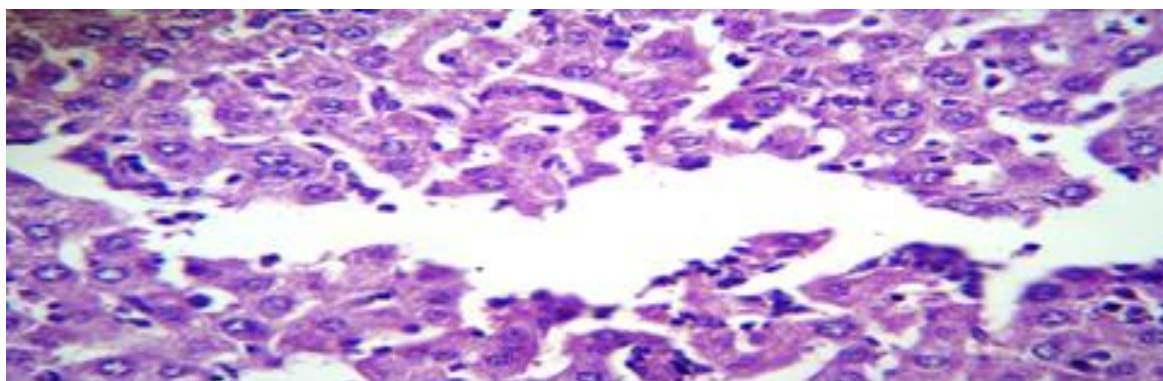

Figure 7- immunosuppressed by Histopathological section in the liver at day 21 post infection non hydrocortisone acetate(H&E stain, 40X) **.**

At 14th day post infection, the liver showed non clear lesions except primary congestion in blood vessels of liver . Figure-6. Histopathological examination of the liver at 21days post infection expressed mononuclear cell aggregation in the wall of the sinusoids with blood vessel dilatation and few inflammatory cell in the same , Figure-7.

The most common animals used in the identification of a virulence factor of *A. fumigatus* are(outbreed Swiss mice) irrespective to their genetic background, while Albino mice are more susceptible to the *A. fumigatus* [18]. This particular difference in host sensitivity against *A. fumigatus* may be useful in the study of the fungal pathogenesis[16].

Invasive aspergillosis has been established in mice in this study, where the animal model were developed to study the efficacy of *A. fumigatus* has invaded the hepatic tissues and induced aspergillosis . The type of immunosuppression using cortison acetate is related to the patterns of infection and inflammation in invasive aspergillosis ,as the immunosuppression treatments increase the susceptibility of mice to infection, and produced progression aspergillosis with earlier expression of inflammatory cells when compared with non immunosuppresed mice and this agree with our result obtained by this study [19, 20].

In human as well as in mice the treatment with cortisone acetate reagent alone is somewhat sufficient to make the host susceptible to invasive aspergillosis depending upon the dose of cortison acetate given even in the absence of neutropenia [21].

Conclusion

In this study , one can concluded that the immunosuppressive treatment of mice using cortisone acetate enhances the infection with invasive Aspergillosis in comparsion with control .

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