Histopathological and enzymatic study on the effect of Aspergillus fumigatus in mice

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Summary:-

Background: - Aspergillus fumigatus is a ubiquitous and opportunistic fungal pathogen causing severe invasive aspergillosis, an important source of morbidity and mortality in immunocompromised hosts (human and animals). The present study was conducted to obtain more clarification about the toxicity and histopathological effects of this pathogen in albino mice.

Material and Methods: - fourteen male mice (6-10) weeks old, (20 – 28) gm weight, divided into two groups, test group (n=8), intranasally infected with 25µl (710 conidia / ml normal saline) of A. fumigatus for 7 successive days, compared with uninfected group (n=6). The weight of all animals were measured at the beginning and at the end of the experiment, in addition to histological study for lung, liver and kidney tissues were performed, with estimation of enzyme activity of GOT (Glutamic Oxaloacetic Transaminase), GPT (Glutamic Pyruvic Transaminase), ALP (Alkaline Phosphatase) and Creatinine Kinase in homogenized liver and kidney tissues were studied by colorimetric method.

Results: - increased (p< 0.05) in body weight, and organs weight (lung, liver, kidney) in infected mice with different histological changes were found in organs tissues of infected mice especially in lung and liver, these changes were supported by variation in enzyme activities of GOT, GPT, ALP and Creatinin kinase in homogenized organs tissues.

Conclusion: - normal host can suffer from systematic diseases after inhalation of infective forms of A. Fumigatus (conidia), and further work is needed to study the active components, which are responsible for pathogenesis of this opportunistic fungi.

Keywords: - Aspergillosis, Histopathology, GOT, GPT, ALP, Creatinine kinase.

Introduction: -

Aspergillus fumigatus is the dominating species causing fungal lung diseases in human and animal, usually acquired through the inhalation of small enough conidia reaching the small airways and alveolar spaces (1). Air, water, soil, plant debris, manure, animal feed are the natural ecological rich of A. fumigatus from which aerosols of conidia are released. Thus, the pathogenesis of invasive aspergillosis (IA) starts with the inhalation of the conidia by the patients, any increase in the concentration of airborne conidia increase the risk of contracting aspergillosis in susceptible individuals with no host specific strains were observed (2). Mice, especially outbred Swiss mice, have been the most common animals used. Most mouse strains, regardless of genetic background are equally susceptible to A. fumigatus, particular difference in host sensitivity may be used in the study of fungal pathogenesis (3). Several studies have been accepted histopathological changes in host with Aspergillosis (4, 5, 6, 7). Moreover, the role of enzymes is well during various diseases among which are liver, kidney and bone diseases, among the isoenzyme of clinical importance are GOT, GPT, ALP (liver function) and Creatinine kinase as kidney function enzyme (8). This study was designed to obtain more clarification about the toxicity and histopathological effects of A. fumigatus in animal model.

Material and Methods: - A. fumigatus clinical isolate from external ear of patients attending Central Health Laboratories – Baghdad. Conidia suspension was prepared according to the method reported by Faraj, 1990(9), then the number of conidia was calculated under high dry power 40X of compound light microscope, using the following equation.

Concentration of conidia = Z × 4 × 10^6 / n.

Where Z= total number of conidia, n= total number of small squares.

Animals: fourteen male white swiss mice (Mus musculus) age ranged from (6-10) weeks, (20-28) gm weight, divided into two groups, test group (n=8) and control group (n=6), The animals were fed with suitable quantity of water and complete diet. The
mouse model of acute aspergillosis was established by intranasal administration of Aspergillus fumigatus conidid (25) µl (10^7 conidia/ml normal saline.) by using micropipette for 7 successive days (3), which represent the test group, while control group was administrated normal saline only. Then all animals were killed by spinal dislocation, lung, liver and kidney were removed for both histological and enzymes activity studies.

Tissues were fixed in 4% neutral buffered formalin, embedded in paraffin and cut into sections which stained with hematoxylin and eosin (H & E) and examined microscopically 40 x (10).

On the other hand, homogenized organs tissues ( liver and kidney) were used to estimate the specific activity of liver enzymes ( glutamic oxaloacetic transaminase (GOT) , Glutamic pyruvic transaminase (GPT) , Alkaline phosphatase (ALP) and kidney enzyme ( Creatinine kinase ) by using colorimetric method (11, 12).

**Statistical Analysis**

Statistical significant was assessed by using least significant differences – LSD (T-test) P-value <0.05 was considered significant. Results were expressed as mean ± S.D. all the statistical analysis were done by using Pentium – 4 computer through the (SPSS) program (version – 10) and excel application (13).

**Results:**

Results showed significant increased (P > 0.05), in the mean of body weight of test group before and after infection with A. Fumigatus (22.41 / 23.07 gm) as compared with control group (20.86 / 22.43). Moreover, significant elevation (P <0.05) in organs weight (mg) in test group in comparison to control group as clearly shown in table (1).

### Table (1).

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Organs weight (mg)</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung</td>
<td>liver</td>
</tr>
<tr>
<td>Control group (n=6)</td>
<td>8.90 ± 0.66</td>
<td>70.63 ± 1.54</td>
</tr>
<tr>
<td>Test group (n= 8)</td>
<td>11.64 ± 1.63*</td>
<td>75.75 ± 1.52*</td>
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</table>

* Significant (P < 0.05)
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**Fig. -1:** histological features of lung, liver in mice intranasally infected with *A. fumigatus* conidia and control group. H. & E. staining sections 40 X.

b- liver tissue in control group
b1- liver tissue in test group

The results of enzyme activity are denoted in table -2 , significant decrease ( P < 0.05) in enzyme activities of GOT and GPT in animals infected intranasally with *A. fumigatus* as compared with control group , concerning enzymatic activity of ALP and Creatinine kinase , results shows non significant decrease ( P >0.05 ) in both enzymes as clearly notice in table (2).

<table>
<thead>
<tr>
<th>Enzymes activities Study Groups</th>
<th>GPT</th>
<th>GOT</th>
<th>ALP</th>
<th>Creatinine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n=6)</td>
<td>143.33 ± 1.20</td>
<td>114.00 ± 0.93</td>
<td>14.00 ± 0.0</td>
<td>0.45 ± 0.69</td>
</tr>
<tr>
<td>Test group (n=8)</td>
<td>106.00 ± 0.66*</td>
<td>80.33 ± 1.10*</td>
<td>13.67 ± 1.22</td>
<td>0.38 ± 0.78</td>
</tr>
</tbody>
</table>

* Significant (P < 0.05)

**Discussion:-**

Infection with *Aspergillus* has been well documented in human and animals (1, 3) Although these species are not considered to be environmental pathogens , they do act as opportunistic pathogens in immuno compromised or debilitated animals. However the circumstances and host conditions allowing such infection are not fully understood (3). Intranasal inoculation mimics the natural route of infection and seen to be a more appropriate route. Following inhalation of conidia , the fungus perferentially localizes to the lungs , and it most often presents as necrotizing pneumonia , also *Aspergillus* species have a propensity to invade blood vessels , and thus systematic dissemination , especially to the brain is often a fatal complication (14) . In this study in addition to histological changes in lung and liver, abnormalities on chemistry panel included significant ( P<0.05) decline in liver enzymes activities (GOT and GPT) with non significant (P >0.05) decrease in both ALP and kidney creatinine kinase activities . These results are related with the degree of organs damages , the more damage and alteration in organ tissues the more decline in homogenized tissues enzymes activities , This is due to release of enzymes to the serum and increament in serum enzymes activities that clearly found in animal with nasal aspergillosis (15) . On the other hand *A. fumigatus* has been reported to produce a large of extrolites including acids and protiens, at least 22.6 potentially bioactive secondary metabolites that can be ordered into 24 biosynthetic families (16). Many extrolites are associated with conidiospores, including gliotoxin, trypacidin, verruculogen and fumigaclavine A and are thus likely to have effects in the initial lung infection process (17, 18). While liver abscesses result from fungus are common, the organisms reach the liver through one of the following pathways: 1- Ascending infection in biliary tract. 2- Vascular seeding, either portal or
arterial, 3- Direct invasion of the liver from a nearby source, or 4- apenetrating injury.(14).

Based on the present findings, it can be concluded that host can suffer from systematic diseases after inhalation of infective forms of A. fumigatus (conidia ) and further work is needed to isolate the active components , which are responsible for pathogenesis of this opportunistic fungi .

References:
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