Induction of brain lesions experimentally by inoculation of Staphylococcus aureus subcutaneously in rabbits (A histological study)

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Abstract

Six adult rabbits of both sexes were used to study the effect of Staphylococcus aureus on the brain tissue. Animals were classified into two groups. Group (A) was the control and Group (B) was the treated. Group (A) was inoculated with 0.8ml saline solution subcutaneously, and group (B) was inoculated subcutaneously with 0.8ml suspension of Staph. aureus. The inoculation process was repeated again with group (B) after 15 days. After (15) days from the second inoculation, all animals of both groups were sacrificed. A brain specimen from the frontal, parietal and temporal lobes of both groups were taken, processed for histological technique, embedded in the paraffin wax, sectioned at (5) pm thickness and stained by the haematoxylin & eosin for examination by the light microscopy.

The brain tissues of group B, demonstrated the histopathological changes of the whole sites of the brain lobes, included focal necrosis of the nerve cells, demyelination of the nerve fibers and gliosis. This effect may reflect the efficacy of the bacteria or its toxin or many both on the brain tissues.

Aحدث أفاته في الدماغ تجريبياً بواسطة حقن المكورات العنقودية الذهبية تحت الجلد في الأرانب (دراسة نسيجية)<br>
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المستخلص

استخدمت ستة أرانب بالغة من كلا الجنسين لدراسة تأثير المكورات العنقودية الذهبية على نسيج الدماغ.
قسمت الحيوانات إلى مجموعتين (A) مجموعة السيطرة ومجموعة (B) مجموعة المعالجة. حققت مجموعة السيطرة بـ 0.8مل من الحلول الملحية السليفي تحت الجلد وحققت مجموعة (B) بمثل المكورات العنقودية الذهبية بـ 0.8مل تحت الجلد أيضاً. وأعيد الحقن بـ (15) يوم تحت الجلد أيضاً لمجموعة (B).
بعد فترة (15) يوم من الحقن الثاني، تم قتل كل الحيوانات وأخذت مقاطع من فصوص الدماغ الجداريةيبة، ودشغية وعامة لكل المجموعتين. عولمت هذه القطع بطرق التقنية النسيجية وطورت بالشم. وقعتت بـ (5) مايكروميتر، وتم صبغها بالبليوتوكسين والأوبيدين لعرض الفصوص تحت المجهر الضوئي.
بين الفصوص النسيجية لفصوص الدماغ في مجموعة (B) حصلت تغيرات نسيجية مرتبطة فيها تمثل في تضرر تجريبي للخلايا العصبية. قدرت النتائج للإعشاب العصبية مع اضطلاع الخلايا العصبية والساندة، هذا التأثير قد يعكس فعالية البكتيريا أو ديفانها أو كلاهما على نسيج الدماغ.
Introduction
Staphylococcal meningitis is an inflammation or infection of the membrane covering the brain and spinal cord caused by the Staphylococcus bacteria (1). Meningitis caused by the Staph aureus or Staph epidermis usually develops as a complication of a surgical procedure or as an infection spread by the blood from another site (2). Borrie (3) revealed that skin infections can arise from the immigration of certain organisms from the external environment through skin breaks or from organism that reach the skin through the blood as part of asystemic disease. Some infections such as brain abscess or skin lesions are caused by toxins produced by the bacteria (4). The present study investigated the efficacy of Staph aureus on the brain tissue using an experimental model for induction of brain lesion in the rabbits.

Literature Review
Ryan (5) revealed that the patient may develop a brain abscess , flumiant meningitis or infection at the other body sites, excessive bleeding and shock can develop from the bacterial invasion.

Bacteria such as Staph aureus may be introduced into the cerebral substance through the penetrating injury. direct spread from sinuses or the middle ear or through embolism to brain tissue (6). Initial infection leads to local suppuration followed by localization of pus within the surrounding wall of gliosis which is chronic abscess may from a tough capsule,multiple abscess occur partuculary with metastatic spread (7). Betty (8) demonstrated that bacterial meningitis is usually secondary to bacteremic illness although the infection may result from direct spread from an adjacent focus.

Materials and Methods
Bacterial suspension was prepared for inoculation the experimental rabbits by obtaining the colonies of Staph aureus from the blood agar and placed in the nutrient broth. 0.8ml of the bacterial suspension has been aspirated and inoculated subcutaneously in the experimental rabbit (9). Six adult rabbits of both sexes, were prepared, weighting 500-750gm, food and water were available. The rabbits were classified into two groups as indicated in the table.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Rabbits</th>
<th>Group Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>Control group</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>Treated group</td>
</tr>
</tbody>
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Group A: inoculated with 0.8ml of physiological saline. Group B: inoculated with 0.8ml of dilevent bacterial suspension.

The animals of the group B were re inoculated with the bacterial suspension after 15 days. All animals were survived for (30) day post 1st inoculation. All animals were deeply, Inhalated with ether and decapitated immediately. Brain specimens from the frontal, parietal and temporal lobes of both groups were taken after breaking down the skull gently. The specimens were rinsed with running water, then placed in 10% formalin for 48 hours and processed through graded alcohol series 70,90 and absolute, xylene. Then infiltrated in paraffine wax, blocked and prepared for sectioning. Sections of (5μm) thickness were prepared, stained by H&E (10), and examined by light microscope.

Results
The animals of group (B) showed decrease in appetite. The site of inoculation become tender and swollen.

- The clinical observations showed violent spasm, lasting for a few minutes and convulsive movement of the feet, these observations occurred (7 days) after second inoculation with Staph aureus and lasting until the time of sacrificing.

- Histological examination of the group A.

The cerebral cortex is thrown into gyri and sulci like elsewhere in the human and other animals. The gray matter was a mixture of nerve cells and nerve fibers, neuroglia and blood vessels. The following types of the nerve cells are present in the cerebral cortex, (1) pyramidal cells (2)stellate cells (3) fusiform cells (4) horizontal cells of cajal.
cells of material. (Fig 1,2). Group B (treated Group) widespread of demyelination with swelling of axons in the white matter (Fig 3), focal necrosis of the nerve cells, atrophy of the nerve and glial cells associated, with few leucocytic infiltration, vaculations or pocket like appearance of most sites of the nervous system.

Discussion

Infection of the skin by the Staph. aureus was well established (3). It forms abscess and boils of the skin, endocarditis of the heart and meningitis of the brain membranes(1). A rabbit model for induction of the brain lesion or meningitis was applied to confirm the concept of the effect of the Staph. aureus on the brain tissue by inoculation of the bacteria subcutaneously.

The structural changes demonstrated in the brain tissue provided a confirmation for the relationship between the skin infection and brain lesion. The hypothesis for the effect of the bacillus or its toxin or both is occur in the form of toxic shock syndrome (11). In the present study, the neuronal damage of the brain tissue was not associated with any inflammation of the meninges, which may reflect the transmission of the bacteria or its toxin or both via blood circulation to the brain, there is another route for infection of meninges and brain tissue via cerebrospinal fluid (6). Blood brain barrier is well protection for the brain tissue (12). We inoculation. Two doses of the bacterial suspension (15) days interval in order to assure the breaking down of this barrier for arriving of the pathogen to the brain tissue. The presence of the extensive vaculation in the brain may consider as a result for degenerative condition of the neurons and glial cells (gliosis), followed by pus or oedematous formation init, and this concept agree with (13).

References


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Fig. 1: Demonstrating the brain of the rabbit
A- meninges
B- blood vessel
C- pyramidal cells
D- horizontal cells. (H&E.40X) Group A.

Fig. 2: Demonstrate the brain of the brain tissue.
A- pyramidal cells
B- fusiform cells. (H&E.40X) Group A
**Fig. 3:** Demonstrates the brain tissue of the rabbit Group B.
A-Demyelination of the nerve fiber of the white matter.
B-Atrophy of the nervous of the gray matter.
C-Gliosis. (H&E.40X).

**Fig. 4:** Demonstrating the brain tissue of group B.
A-Atrophy of the nerve.
B-Nuclation aggregation of the neuron.
C-Vaculation of the neuron. (H&E.40X).