Histological Changes Of Mice Skin Infected by *Nocardia asteroides* (Bacteria Isolated From The Soil)

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Abstract

Experimental infection in laboratory Mice of (18-20) gm weight and (4-8) weeks age was made by injection bacterial solution containing *Nocardia asteroides* isolated from the soil in concentration of (1×10^7) CFU/ml, in the foot pads of these animals.

The results proved the ability of these bacteria to cause infection after about 30 days of inoculation and resulted in many histological changes in the sole of the foot of these mice represented by inflammatory cells infiltration and abscess formation containing many granules can be seen by the naked eye represent the *Nocardia asteroides* bacteria whichare thread like, positive for gram stain and can be stain by Modified Ziehl-Neelsen.

This study proved that those mice inoculated by *Nocardia asteroides* suspension by injection in their sole lead to infection and many histological changes in the foot skin were obvious. Bacterial clearance or disease progression is related to pathogenic or virulence factors of the bacteria in the one hand and the immune response killing ability of host in the other hand .

Key words: Nocardia asteroides, Nocardia asteroides in soil, cutaneous Nocardiosis, superficial skin infection.

Introduction

Nocardiae are gram- positive, partially acid-fast, branching filamentous , soil -inhabiting , aerobic Actinomycetes and have the ability to produce Substrate Mycelium and Aerial Mycelium (1).

This species is not well known in the past by doctors, but now and because of the progress in all sciences and due to the presence of laboratory investigation, it is proved to be medically important and to cause many fatal infections in human and animals (2).

This bacteria causes Nocardiosis which could be Pulmonary Nocardiosis or Cutaneous Nocardiosis or systemic Nocardiosis in addition to Neural Nocardiosis (3). The effect of these bacteria is primarily due to its ability to spread through blood vessels or lymphatic vessels leading to wide spread infection (4). Recent studies are directed toward these bacteria which are important opportunistic pathogens especially in Immunocompromised Patients. *Nocardia asteroides* is responsible for about 85%

infection of the immunocompromised patients (5), while in the healthy people the infection occurs in about 15% (6).

The Pulmonary Nocardiosis is a wide spread infection and occurs as a result of inhalation dust particles infected with this bacteria (7), the most common site, (80-90) % of the infection is in lung and is due to *Nocardia asteroides* bacteria (8).

The third most common site for the infection with these bacteria is the skin and it occurs in 15% (9, 10).

Cutaneous nocardiosis is classified into two major categories, primary and secondary. Primary cutaneous nocardiosis includes nocardial mycetoma, localized cutaneous nocardiosis and Lymphocutaneous nocardiosis which occurs due to direct penetration of the skin and the subcutaneous tissues by the bacteria present in water, soil, and plants. The bacteria enter the body through the skin wounds, surgical wound, bullet injury wounds and insect bite (11). The superficial skin infection occurs in the form of Ulcers or Pustules or Pyodermitis or in the form of Papules or chronic local abscess. If the infection is not treated it may extend to involve the lymphatic vessels and reach the lymph nodes.

The abscess formation leads to Sporotrichosis . The Lymphocutaneous Nocardiosis is similar to the Sporotrichosis (12). The Madura foot is swelling characterized by chronic Pustular granules not painful, affect the tissue under the skin. The disease is characterized by the appearance of swelling leading to abscess formation at the site of wound with seropurulent exudate containing granules which appear in different size and colour and can be seen by the naked eye which represent the bacteria (13). This infection could lead to abnormality in the skin and subcutaneous tissues, with time it may extend to involve the bones, muscles and connective tissue and causes fibrosis with malformation in the bones. The exposed parts of the body such as foot, leg and hand are the most common sites to this infection and to less extent the head and shoulders. On the other hand , secondary cutaneous nocardiosis includes (disseminated) subcutaneous nocardial abscess, which is caused by hematogenous dissemination from visceral nocardiosis, particularly from pulmonary lesions (5).

Materials and Methods

1- Laboratory Animals

Ten BALB/c mice were used in this experiment their weights were (18-20) gm and their age (4-8)weeks and were inoculated in the foot pads.

2- Bacterial suspension

For the preparation of bacterial suspension , *Nocardia asteroides* bacteria isolated from the soil, were grown on the Brain Heart infusion broth (BHI) prepared from Oxoid company inoculated at $(37)^{\circ}$ C and for (72) hours calculated by plate available count method.

3- Inoculation of the laboratory animals

To study the ability of the bacteria to cause the cutaneous infection, the mice were inoculated in the foot pads sole by (0.1) ml of bacteria suspension containing (1×10^7) bacterial cells/ml isolated by plate available count method, while in the control group the mice were inoculated by (0.1) ml of normal saline and the animals were left under daily observation. The two groups were sacrificed after (30) days of inoculation using chloroform ,and the skin of the foot was taken to study the histological changes caused by the bacteria.

4-Preperation of the slides from the skin of the foot pads

The skin of the mice feet were kept for few days in (10) % neutral buffered formalin solution. Then the foot skin was cut into pieces of 1cm dimension ,dehydrated by alcohol , cleared with xylol and finally embedded in paraffin blocks. Using Reichert Rotary Microtome ,serial paraffin sections of 5 μ m thickness were made and then were stained with Harris Hematoxylin and Eosin (14) . Seropurulent exudates from the abscess were taken and stained with gram stain to identify this bacteria (1). Modified Ziehl-Neelsen stain (Kinyoun carbol fuchsion) was used to identify the bacteria from other Actinomyces (15).

Results

The skin of the foot sole inoculated by these bacteria produced congested edematous and nodular swelling with abscess formation after (30) days of infection. Fig. (1).



Fig (1) mouse foot inoculated with *Nocardia asteroids* isolated from soil clarify the sever congestion and abscess formation after (30) days.

Oozing of the seropurulent exudates from the infected area contained granules which could be seen by naked eye. These represent bacteria accumulated in the tissue .The microscopical examination of these bacteria, stained by gram stain , showed the presence of the red branched bacteria with different forms and size, fig (2).



Fig. (2) *Nocardia asteroides* species taken from seropurulent exudates from the abscess, stained by gram stain (1000X).

Using Modified Ziehl-Neelsen (Kinyoun carbol fuchsion), the bacteria appeared as long red threads in colour because it contains mycolic acid which can resist the stain using 1 % concentration of acidic alcohol and this makes it different from other Actinomyces fig. (3).



Fig. (3) *Nocardia asteroides* bacteria taken from seropurulent fluid of the abscess stain by Modified Ziehl-Neelsen stain (1000X).

Histological results:-

The important changes appeared in the skin included the epidermis and dermis, and characterized by the appearance of many pustular nodules and granules differ in size and irregular in shape. which appeared as necrotic centers surrounded by inflammatory cells including lymphocytes and phagocytic cells in addition to the hemorrhage and edema, fig.(4,5,6 and 7).



Fig (4): Destruction of the stratum spinosum (arrows) with loss of the continuity of thick skin of mouse skin infected by *Nocardia asteroides* (H&E 40X).



Fig (5): Thickening of the stratum spinosum (arrows) with loss of dermal papillae of mouse skin infected by *Nocardia asteroides* (H&E 40x).



Fig (6): Hemorrhage with the presence of inflammatory cell infiltration of mouse skin infected by *Nocardia asteroides* (arrows) (H&E 40x).



Fig (7): Destruction of the stratum spinosum of the skin with inflammatory cell infiltration of mouse skin infected by *Nocardia asteroides* (arrows) (H&E 40x).

Discussion

Nocardia asteroides recorded as pathogenic bacteria in the animals (16), and in many studies, it was founded that, three species of the genus *Nocardia* was the cause of animals disease, which includes *Nocardia asteroides* , *Nocardia caviae* and *Nocardia brasiliensis* (17, 18, 19) The sensitivity of the animals to infection depends on many factors, the most important is animal species, the way of exposure, the rote bacteria entrance to the body of the animals, and the type of the species, because various types of these bacteria cause acute or chronic infection differ in its intensity in mice (20, 21). There are many types of *Nocardia asteroides* species cause brain infection when injected Intravenously (IV) in the mice.

Previous studies proved that when healthy mice injected (IV) by (1×10^{5}) bacterial cell / cm³ of *Nocardia* asteroides it caused pulmonary infection which extend to the kidney, liver and spleen after 3 months from the injection , while if the mice injected (IV) by (1×10^8) bacteria cells /cm3 of Nocardia otitidscaviarum it produce no clinical signs only after 8 months followed the appearance of chronic infection extend to the abdominal wall followed by the death of the animals, and the histological study of these tissues showed the presence of granules surrounded by inflamantory cells and the Nocardia otitidscaviarum bacteria were isolated from them (21). Other studies showed that when the mice of 4 month age injected intraperitonealy (IP) with (1×10^6) bacterial cell / cm³ of Nocardia brasiliensis isolated from madura foot led to chronic infection in the legs, tail and nose after about (3-6) months (22).

The infection by *Nocardia asteroides* bacteria depends on the defense mechanism and immunity of the person in addition to many enzymes like protease, acid phosphatase, lipase, which are secreted by the bacteria inside human cells. The present results agreed with the results of Komaid and their students, 1987 who showed that injection of *Nocardia asteroides* in the sole of the mice foot resulted in the dermatological infection and occurrence of the disease, (23).

The study also proved the ability of these bacteria to stimulate the inflammatory response by production of chemotactic factors which attract the Neutrophil to come to the inflammatory site, and failure of inflammatory cells to affect the bacteria because the bacteria resist phagocytosis by inhibition of the phagosome-lysosome fusion to produce the phagolysosome. Also the bacteria resist the phagocytosis by secreting chemical substances to decrease the activity of these enzymes, this is due to the presence of mycolic acid in the wall of the bacterial cells. Also the bacteria have cord factor ,which decrease the activity of phagocytic cells and lead to the dissemination of these bacteria in other organs via circulation of these bacteria in the blood or lymphatic vessels. The wall of these bacterial cells have mucolipid

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substances to protect themselves from the phagocytic cells in addition to the ability of these bacteria to form nodular swellings in the organs in which they are present (24). The bacteria have the gatalase and Super Oxide Dismutase (SOD) enzymes which increase their ability to protect themselves leading to other complications, like accumulation of Lipid Peroxidation and increase in the very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the wall of the blood vessels leading to many problems in the heart (25).

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الملخص

تم إحداث إصابة تجريبية في فئران مختبرية بوزن (١٨-٢٠) غم وبعمر (٤-٨) أسابيع عن طريق حقنها في القدم بالمعلق الجرثومي لأفراد النوع Nocardia asteroides المعزولة من التربة ذي التركيز (١× ٢٠) خلية جرثومية / سم^٢ .

بينت نتائج الدراسة المرضية النسيجية للفئران المصابة تجريبيا قدرة الجرثومة على إحداث الخمج الجلدي بعد ٣٠ يوم من الحقن من خلال قدرتها على الاستيطان وإحداث الآفات المرضية والتغيرات العيانية والنسجية في القدم والمتمثلة بحدوث الالتهاب وظهور خراجات موضعية في منطقة لإصابةحاوية على سائل قيحي يحتوي السائل على حبيبات يمكن ملاحظتها بالعين المجردة تمثل جرائيم Nocardia asteroides التي تكون عصوية وتنتظم بشكل خيوط موجبة لصبغة كرام ويمكن صبغهابصبغة , زيل-نلس المحورة Modified Ziehl-Neelsen (صبغة كيزمن).

أثبتت الدراسة الحالية إن معاملة الفئران بمعلق جرثومة Nocardia asteroides عن طريق الحقن في وسادة القدم ،قدرة الجرثومة على تحفيز الخمج الجلدي من خلال الأفات المرضية والتغيرات النسجية في جلد وسادة القدم