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SECONDARY AMYLOIDOSIS ASSOCITED WITH *Salmonella typhi* EXPERIMENTAL INFECTION IN WHITE MICE

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ABSTRACT

The present paper reported induction of amyloidosis by intraperitoneal injection of *Salmonella typhi* in white mice. The study was on young mice of 5–6 weeks old, after two weeks acclimatization period, mice were injected intrapritoneally with 0.25 ml of *S. typhi* suspension containing 2.5 x 10^8 bacterial cells and sacrificed daily for 28 days.

The amyloid was systemic, secondary in character and organs mostly affected were spleen, kidney and liver, respectively.

It appeared that this is the first report remarks the induction of systemic secondary amyloidosis by intra peritoneal injection of *Salmonella typhi* in young white mice.

داء النشوانية الثانوي المرافق لحالات الأصابة التجريبية بعصيات التايفوئيد في الفئران البيضاء

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الخلاصــة

يشير البحث الى حدوث داء النشوانية الثانوي المرافق لحالات الأصابة التجريبية بعصيات التايفونيد في الفئران البيضاء وبعمر ٥–٦ أسابيع، حيث ظهر داء النشوانية بمدة أسبوعين من الأصابة وكان من النوع الجهازي حيث أصيبت الأعضاء التالية: الطحال، الكلية والكبد وهذا البحث الأول من نوعه في احداث النشوانية الجهازي المرافق للإصابة التجريبية بعصيات التايفونيد في الفئران البيضاء.

INTRODUCTION

The term amyloid refers to a pathological proteinaceous substance deposited extracellularly (1) and intracellularly (2) in tissues and most commonly identified by light microscopy as a homogeneous eosinophilic material, when stained, with alkaline Congo red (1). Amyloidosis can complicate widely diverse diseases, causes considerable morbidity and mortality and is of trace medical importance in many communities (3). The property of separating amyloid into primary, secondary and experimental types had been debated, since it was maintained that no difference can be found in the composition of amyloid from different sources (4). Secondary amyloidosis is a term applied to the type of

amyloidosis found in mammals with obvious chronic inflammation or in laboratory animals subjected to some procedures not directly aimed at the production of experimental type amyloidosis (3). Secondary and experimental forms of amyloidosis had a different distribution pattern in the organs of mice from the sites of deposition of primary from (4). Many reports of referring to secondary amyloidosis in mammals after parasitic infestations, and some bacterial infections such as tuberculosis and leprosy. After dermatomycosis, it was also noticed in association with the growth of a transplantable tumor, after repeated parentral injection, after irradiation, after hormonal alterations and chronic stress during exposure to chemical carcinogen and in parabiotic mice (4,5).

The purpose of this report is to present the distribution and morphology of secondary amyloidosis in association with *Salmonella typhi* experimentally infected white mice.

MATERIALS AND METHODS

White mice, weighing 15–20 gm, were obtained from Al- Kindi Company for drugs and vaccines production, Baghdad, Iraq. They were healthy reared on the concentrated food for 2 weeks (period of acclimatization) before inoculation.

A local strain of *Salmonella typhi* isolated from febrile patients in Ibn-Al-Khatib Hospital Baghdad, Iraq. The organism was identified previously (6). A logarithmic phase growth of *Salmonella typhi* in trypticase soy broth at 37 C^{o} were taken, washed in phosphate buffer saline and a suspension of viable count of 10^9 bacterial cell/ml was obtained.

One hundred mice were injected intraperitoneally each with 0.25 ml of *Salmonella typhi* suspension containing 2.5 x 10^8 bacterial cell (10 LD50). Three inoculated mice are sacrificed every day for a period of 28 days.

On the other hand control group (40 mice) were injected intraperitoneally with 0.25 ml sections from spleen, kidneys, liver and other visceral organs were taken, fixed in 10% neutral buffered formalin. After fixation, sections were cut at 5 μm thickness, stained with hematoxyline and eosin. From all mice selected samples were also stained with Congo red as differential stain for amyloid.

RESULTS

Both local and diffuse amyloidosis were demonstrated in the spleen, kidneys and liver and in association with the different pathological findings seen in the different organs of the mice infected with *Salmonella typh*i. Histological sections from the infected organs stained with hematoxyline and eosin revealed hyaline like amorphous deposits within the infected tissue, which the Congo red staining of these sections demonstrated a rosy–pink color to these amyloid deposits in the following organs:

1- The spleen:

Amyloid appeared first in the spleen at 14th day post inoculation of *Salmonella typhi* characteristically appeared in the perifollicular (sago spleen type amyloidosis, Fig-1). In advance cases of splenic amyloidosis (18th day), there was marked replacement of the red pulp and sub capsular region.

Following the 24th day post inoculation, all the red and white pulps, wall of arteries, capsule and trabeculae were completely replaced by amyloid giving a feature of Bacon spleen type amyloidosis (Fig- 2 and 3).

2- The kidneys:

Renal amyloidosis started mainly in the glomeruli, by the 16th day, there was complete replacement of glomerular tuft by amyloid (Fig-3, 4 and 5). In advance cases, amyloid deposits were also seen both in cortex and medulla.

3- The liver:

Amyloidosis appeared in liver tissue, soon after its appearance in spleen, at 16th day post inoculation and it was first seen in subendothelia of blood vessels and sinusoid of portal and periportal regions. In sever cases, sinusoidal deposits of hepatic amyloid started to be prominent and progress toward the mid zone of hepatic lobules, also in advanced amyloid, it was associated with signs of pressure atrophy of the adjacent hepatocyte (Fig-6 and 7).

Fig. 1: Spleen: sago spleen type of amyloidosis. Note amyloid deposits in the perifollicular and in red pulp. (H & E) X125.

Fig. 2: Spleen: Bacon spleen type of amyloidosis. Note diffuse type of amyloidosis, replacing most of the white and red pulp. (H&E) X250.

Fig. 3: Spleen: special stain for amyloid (Congo red), note the light pink deposits of amyloid diffusely deposited in white and red pulp. (Congo red) X250.

Fig. 4: Kidney; note glomerular deposits of amyloid, replacing most of the glomerular tuft. (H&E) X125.

Fig. 5: Kidney; special stain for amyloid (Congo red), note glomerular deposits of amyloid light pink in character. (Congo red) X500.

Fig. 6: Liver, periportal and midzonal sinusoidal deposits of amyloid. (H&E) X125.

Fig. 7: Liver, special stain (Congo red), note light pink deposits of amyloid. (Congo red) X250.

DISCUSSION

The term amyloidosis refers to the deposition of pathological pertinacious substances extracellularly (1) and intracellularly (2), tended to replace and destroy the vital tissue. Amyloid deposits can be localized or systemic (7). Amyloidosis in this study was systemic involving many organs such as spleen, kidneys, and liver causing destruction of these affected organs. The association of amyloid deposits with inflammatory process due to *Salmonella typhi* in the present study was not reported previously, except that two reports revealed occurrence of amyloidosis with Salmonellosis in ducklings (8), and in gerbils (9). But most of the reports revealed an association of secondary amyloidosis with the different inflammatory processes (4, 5) due to tuberculin, leprosy, dermatomycosis, parasitic infestations in addition to the non inflammatory pathological processes such as transplantable tumors (4, 5) after repeated parentral injections (4, 5), after irradiation (4, 5), hormonal alteration (4, 5) and during exposure to chemical carcinogens (4,5). The relationship of the inflammatory conditions to generalized amyloidosis has known for decades (4, 5). Further supporting the dependent nature of amyloidosis on these inflammatory conditions is the fact that removal of the inflammatory condition can reverse the development of amyloidosis and be associated with resorption of deposits (7). It therefore appears that any continual immunological or other stimulation of the reticuloendothelial system may result in amyloidosis (5). A characteristic feature of all cases of the secondary amyloidosis in that the amyloid deposits display a typical distribution pattern and a distinct relation to the reticuloendothelial system (5). This study reveals that the spleen was one of the most severely affected organs, and followed by the kidney and then liver. Similar

findings have been reported previously in mice affected with secondary amyloidosis (4). The differences in amyloid picture may be related to the strain of mice. The time as factor may also be important, since primary amyloidosis develops slowly over many months and is often in old mice (10) while secondary and experimentally induced amyloidosis may be induced in young mice in a few weeks time (11), similarly seen in the present study. Experimental models with short introduction time than the spontaneous one was reported in mice and ducks (11-13).

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