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A MORPHOLOGIC STUDY OF CALYMMATOBACTERIUM GRANULOMATIS IN TISSUE BIOPSY

Mithilesh Chandra, D D Ganguly and N C Bhargava

Tissue smears showed *C. granulomatis* in 72.8% of the 151 cases studied. In 15 of the remaining 39 cases, clumps of extracellular bacilli were present, out of which intracellular location could be demonstrated in tissue sections in 5 cases. It is not clear whether the organism in the remaining 10 cases were *C. granulomatis* or secondary contaminants. Intracellular organisms could be demonstrated in the histopathologic sections in 47.6% of the cases. Tissue biopsy was negative for the organisms in 25 tissue smear positive cases. Morphology of *C. granulomatis* in the smears as well as the sections is described.

Key words : Morphology, Donovanosis, *C. granulomatis.*

Calymmatobacterium granulomatis (*C. granulomatis*) is believed to be the etiologic agent of donovanosis (granuloma inguinale).[1,2] The causative organisms are Gram negative bacteria and are antigenically related to Klebsiella.[2] The organisms are of low infectivity and require tropical or subtropical climates for growth.[3] In India, donovanosis is prevalent along the south-east coast though cases have been reported from non-endemic areas also.[4-6] Most of these studies pertain to the clinical characteristics of the disease and there are only a few reports on the pathology of this disease.[7,9]

Though presence of the organisms in the tissue smears is the hall-mark for diagnosis, it is generally thought that donovan organisms are not demonstrable in tissue biopsies. The present study highlights the value of tissue biopsy for the detection of donovan organisms as well as the morphology of *C. granulomatis* in the tissue.

Materials and Methods

The material was obtained from 151 cases of donovanosis. The diagnosis in each case was clinical and other diseases having genital ulcers like primary syphilis, chancroid and herpes progenitalis were excluded by dark field examination, VDRL test and examination of Gram stained and Giemsa stained smears respectively. Tissue smears and tissue biopsies stained with Leishman or Giemsa stains were used to confirm the diagnosis of donovanosis. Presence of intracellular donovan organisms was considered confirmatory of the diagnosis. In the absence of the organisms in the smears, presence of a large number of macrophages in the smears was considered suggestive of donovanosis.

Biopsy was taken from the edge of the lesions, fixed in 10% formalin, routine histopathology processing was done and sections were stained with H and E and Giemsa stains. Thick sections were avoided and care was taken that stain deposits were not produced on the section.

Results

Tissue smears from 112 (72.8%) cases showed non-capsulated and capsulated organisms lying intra as well as extra-cellularly. Non-capsulated organisms were thin and bacillary, and had telephone-handle or safety-pin appearance because of the dark bipolar staining (Fig. 1) as described earlier.[3] The capsulated forms were thicker, oval or round and blue or purplish in colour (Fig.2). Many organisms attained a large size and were arranged in groups. In 39 cases, intracellular organisms could not be demonstra-

From the Institute of Pathology (ICMR), Post Box No : 4909 and the Department of STD, Safdarjang Hospital, New Delhi-110 029, India.

Address correspondence to : Dr Mithilesh Chandra.

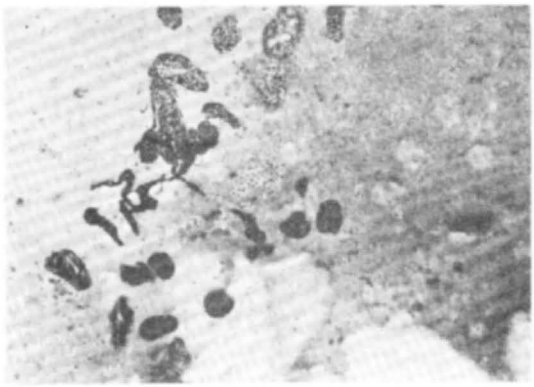

Fig. 1. Young form of thin coccobacillary *C. granulomatis* surrounded by a clear space in the tissue smear (Leishman stain X 800).

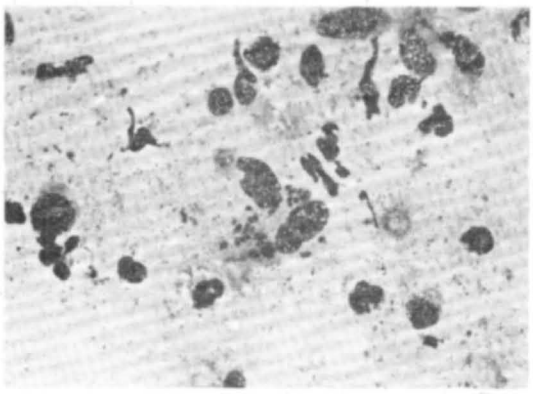

Fig. 2. Thick, oval, capsulated *C. granulomatis* lying intracellularly in the cytoplasm of a macrophage. Some of the organisms show bipolar staining (Leishman stain X 800).

ted in the smears. Of these, 15 cases showed the presence of clumps of bacilli lying extracellularly. Some of the organisms also showed bipolar staining. It was not certain whether these organisms were secondary contaminants or were donovan organisms.

Histopathological examination of biopsies from the ulcers showed dense inflammatory exudate in the upper dermis with predominance of large vacuolated mononuclear cells and plasma cells. In 72 (47.6%) cases *C. granulomatis* were seen lying inside the vacuolated cytoplasm of the macrophages either singly or in clusters though extracellular organisms were also present occasionally.

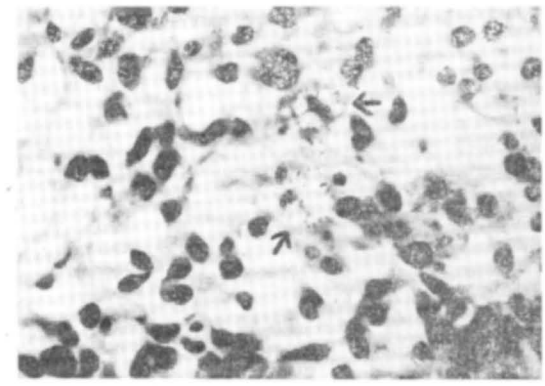

Fig. 3. Thin coccobacillary organisms are present in the vacuolated cytoplasm of the macrophages (←) in the tissue biopsy (Giemsa stain X 800).

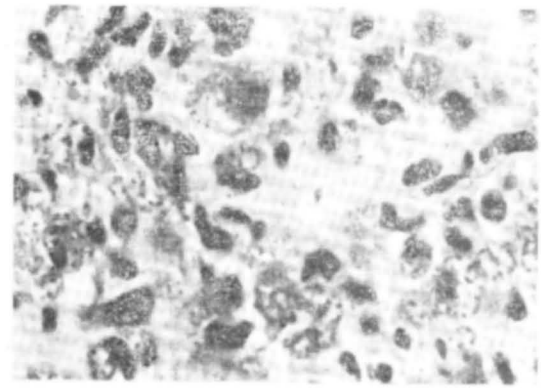

Fig. 4. Vacuolated cytoplasm of a large number of macrophages filled with thick, coccobacillary, *C. granulomatis* (Giemsa stain X 800).

These usually presented as coccobacillary organisms which took blue stain with Giemsa (Figs. 3 and 4). It was difficult to identify non-capsulated and capsulated forms in the tissue sections. Bipolar staining of the organisms could be appreciated if the paraffin sections were thin and were stained well. However, telephone-handle or safety-pin appearances of the bacteri-

were only occasionally (2 cases) seen in the tissue sections. In 79 organism-negative cases, histopathology of the lesion was characterized by the presence of large vacuolated macrophages (Greenblatt cells). In 5 out of 15 cases where only extracellular organisms were present in tissue smears, intracellular presence was detected in the tissue biopsy. However, in 25 smear positive cases, organisms could not be demonstrated in the sections.

In 8 cases, a large number of mast cells filled with red granules were present in the inflammatory exudate and were a source of confusion with *C. granulomatis* (Fig. 5). The features which helped in differentiating these cells from Greenblatt cells were, (a) absence of vacuolated cytoplasm in mast cells, (b) overlapping of the nucleus by the granules in mast cells, (c) coccobacillary shape of the organisms, and (d) the magenta staining of the mast granules. In three cases, precipitation of Giemsa stain caused difficulty in identifying the organisms and the stain had to be repeated for clear-cut demonstration.

Comments

It has been established that donovan bodies are the etiologic agent of donovanosis.[1,2] These belong to the *Calymmatobacterium* species. The diagnosis of donovanosis is made by the demonstration of the organisms in the macrophages in smears stained with Romanoswky dyes.[3] Tissue smears were positive in 72.8% cases. Negative results have been reported in 30-40% cases in the past, despite multiple smear examination.[10] Difficulty was encountered in differentiating secondary contaminants from *C. granulomatis* in 15 cases. Sometimes, contaminants like diphtheroids and bacteroids may cause confusion in the diagnosis. Therefore, due emphasis should be given to the intracellular location of the organism. Histopathology of the lesions is quite characteristic with the presence of Greenblatt cells and has been well described.[7-9] *C. granulomatis* could be demonstrated in 47.6% of the biopsies. Nevertheless, many workers find difficulty in demonstrating the organisms in the sections. Success of demonstration of the organisms in the biopsy lies in preparing thin, well-stained sections where the organisms pick up blue stain with Giemsa.[11] Caution should however be exercised in differentiating the organisms from the mast cells which are generally present among the inflammatory cells. In five smear negative cases, organisms could be demonstrated in the histopathology sections. In other cases, the presence of characteristic Greenblatt cells clinched the diagnosis. Therefore, study of tissue sections can be useful in smear negative cases. Histopathology proves helpful also in cases where secondary contaminants cause confusion in the tissue smears.

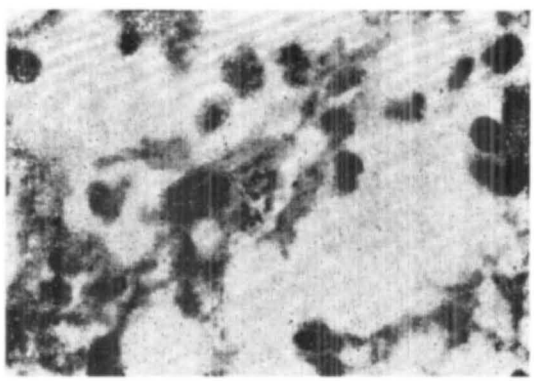

Fig. 5. Granules of mast cells scattered in the cytoplasm masking the nucleus (Giemsa stain X 800).

The limitation of histopathology is evident as negative results were obtained in 25 smear positive cases. Therefore, efforts are necessary to develop more sensitive and simple methods for the diagnosis of donovanosis.

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