National Board of Examination - Journal of Medical Sciences
Volume 1, Issue 12, Pages 751–759, December 2023
DOI 10.61770/NBEJMS.2023.v01.i12.005

ORIGINAL ARTICLE

The histopathology of culture proven Melioidosis

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Accepted: 22-November-2023 / Published Online: 17-December-2023

Abstract

Context: Melioidosis is a bacterial infection with high morbidity and mortality. Published literature on histopatholgical findings of melioidosis is sparse. **Aims:** To describe the histopathological findings in melioidosis. **Settings and design:** This is a retrospective observational study conducted at a tertiary care center in south India. **Materials and methods**: Histopathological findings of tissue samples from which *Burkholderia pseudomallei* was isolated in culture were analysed. **Statistical analysis**: The quantitative variables were expressed as frequency. **Results:** Histopathological findings in melioidosis were either acute (12 out of 18 samples), acute on chronic (5 out of 18 samples) and chronic (1 out of 18 samples). Granuloma was noted in 5 samples. **Conclusion:** Any acute or acute on chronic inflammation with or without granuloma in tissues from patients in endemic regions should raise the suspicion of melioidosis.

Keywords: Melioidosis, Histopathology, Burkholderia pseudomallei, Inflammation, Granuloma

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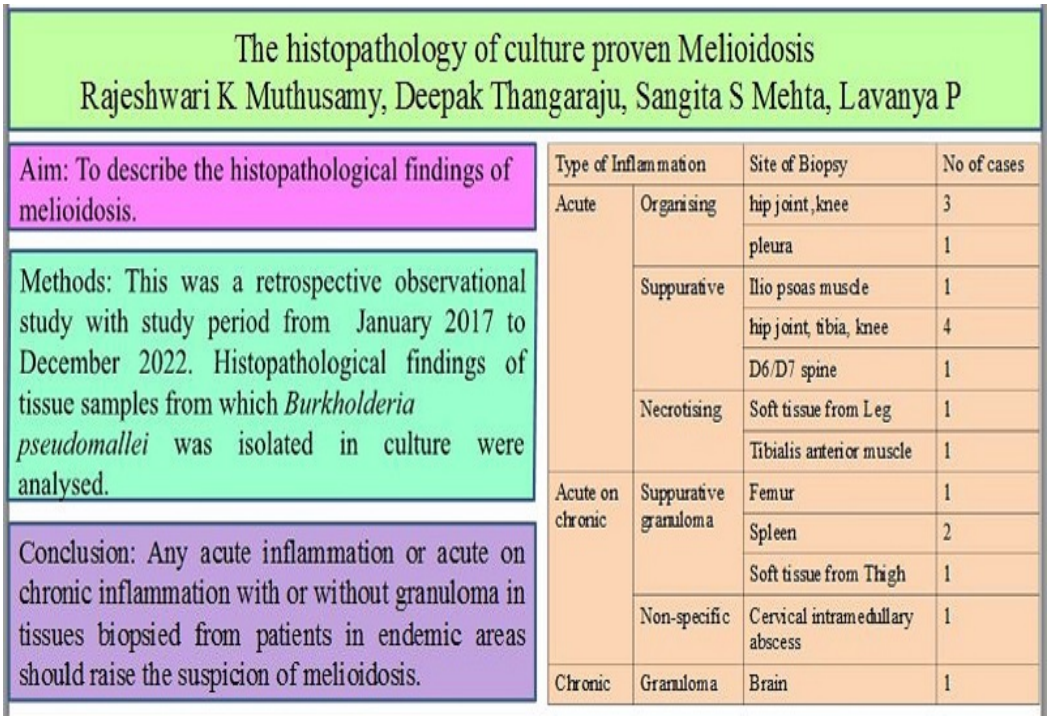
Graphical Abstract



Introduction

Melioidosis, caused by environmental organism *Burkholderia pseudomallei* (B. pseudomallei) is endemic in southeast Asia and northern Australia [1]. The disease has a predicted global burden of 1,65,000 cases per year [1]. It has varied clinical presentation with high morbidity and mortality, as high as 50 percent [1]. The organism must be isolated in culture to confirm the diagnosis. In many instances the diagnosis is delayed even in countries like India where it is being increasingly reported in recent years [2]. It is not suspected in many situations therefore appropriate samples are not sent for culture on time. Even if cultures are done there are problems in identifying the organism due to the lack of experience and inaccuracy of the automated bacterial identification systems widely used in microbiology laboratories [3]. The pathology findings of melioidosis are not well studied. There are only few published literature on this topic. Describing the pathology findings will help in early diagnosis and timely patient management. Therefore, this study was conducted to describe the histopathological findings (HPE) of melioidosis.

Methods

This was a retrospective observational study. The study was conducted in microbiology and pathology laboratory in a tertiary care center in south India. The study period was from Jan. 2017-Dec. 2022. Patients for whom B. pseudomallei was reported in culture were collected from microbiology laboratory records. Samples, collected from melioidosis patients, that were submitted for pathology and microbiology laboratory were identified from hospital information

system. Tissue samples from which B. pseudomallei was isolated in culture were included in the study. Aspirates sent for cytology and tissue samples from melioidosis patients that were not subjected to culture were excluded. Tissue samples were fixed in 10% buffered formalin and processed in automated tissue processor. The processed tissues were embedded in paraffin wax and 5-6-micrometer thick sections were cut and stained with haematoxylin and eosin. The slides were studied, and they were subjected to Gram stain, Ziehl-Neelsen stain, periodic acid-schiff and Grocott's methanamine silver stain wherever necessary. The HPE findings included in the study were verified by the authors and corrections were made after examining the slides retrieved from pathology archives. Clinical details of the patients were retrieved from the hospital records. Qualitative variables were summarised as frequency. This is a retrospective study with neither any contact between the researchers and patients nor any intervention. Hence consent was waived. The study was approved by the institute ethics committee (EC/AP/1032/04/2023).

Results

In the study period, B. pseudomallei was isolated in culture from 58 patients. Samples from which B. pseudomallei was isolated include blood, sputum, endotracheal aspirate, BAL, urine, CSF, tissues, aspirates and abscesses. Eighteen tissue samples from 15 patients were sent for histopathology examination. These were reviewed and included in the study.

Out of 18, 11 samples were from musculoskeletal system (4 from hip joint, 2 from tibia, 2 from knee joint, 1 from femur, 1 from tibialis anterior muscle and 1 from D6/D7 segment of spine), 2 from spleen, 2 from skin and soft tissue (1 from leg and 1 from thigh), 1 from pleura, 1 from brain and 1 from cervical vertebral intramedullary region (Table 1). The mean age group of patients whose HPE reports were included in the study is 52.6 years (13 years to 76 years). Except for one, all the patients were male. Among 15 patients, a sample from hip joint was given for histopathology twice for one patient and 3 samples were given for one patient (synovial tissue from knee, biopsy from tibialis anterior muscle and from medullary cavity of proximal tibia). One sample was given for histopathology from the other 13 patients.

HPE findings are either acute, acute on chronic or chronic inflammation. Twelve out of 18 samples had acute inflammation. Suppurative type was found in 5 samples, organising in 4 and necrotising in 3 samples (Figure 1). In one patient, the first sample from the hip joint showed acute organising inflammation (Figure 2) but the second sample from hip joint obtained twenty days later showed acute suppurative inflammation. In another patient, a sample from the left knee showed acute organising inflammation but samples from tibialis anterior muscle and tibia obtained twenty days later showed acute necrotising inflammation and acute suppurative inflammation respectively. Acute on chronic inflammation was found in 5 samples out of which Suppurative granuloma (Figure 3) was seen in 4 samples, and nonspecific inflammation in one sample. Chronic granulomatous inflammation (Figure 4) was found in one sample.

Table 1. Inflammatory response pattern of melioidosis in various biopsy samples.

Type of Inflammation		Site of Biopsy	Number of cases
Acute	Organising	hip joint	2
		knee	1
		pleura	1
	Suppurative	Ilio psoas muscle	1
		hip joint	1
		Tibia	2
		D6/D7 spine	1
		Knee	1
	Necrotising	Soft tissue from Leg	1
		Tibialis anterior muscle	1
Acute on chronic	Suppurative granuloma	Femur	1
		Spleen	2
		Soft tissue from Thigh	1
	Non-specific	Cervical vertebral intramedullary abscess	1
Chronic	Granuloma	Brain	1

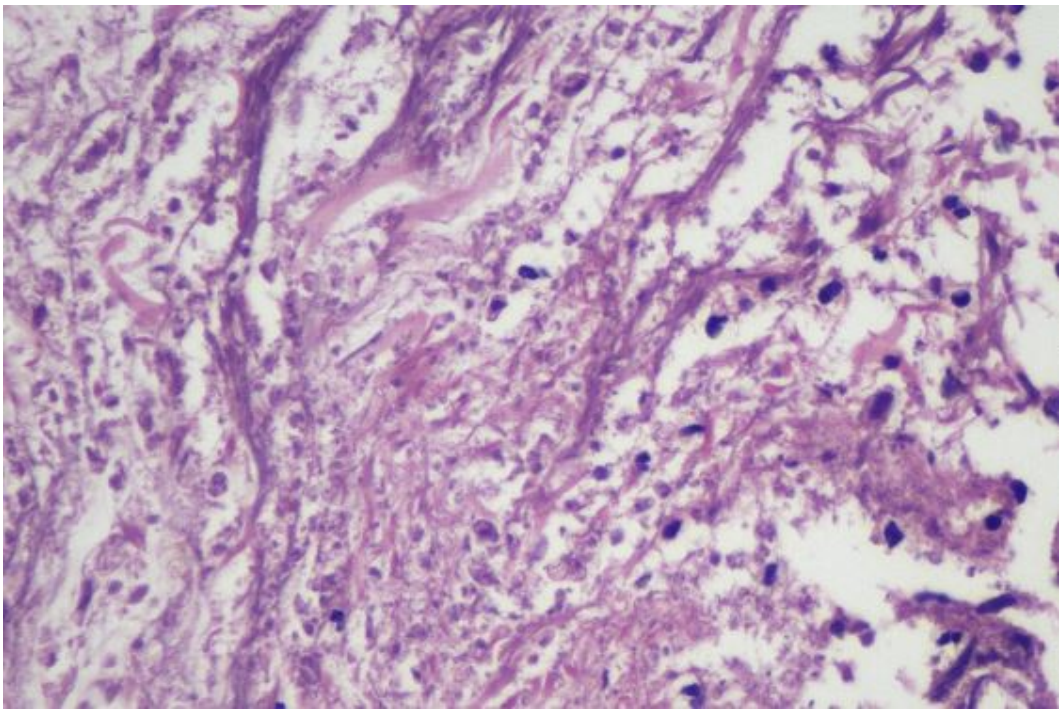


Figure 1: Muscle tissue with necrotising inflammation (H&E 40X).

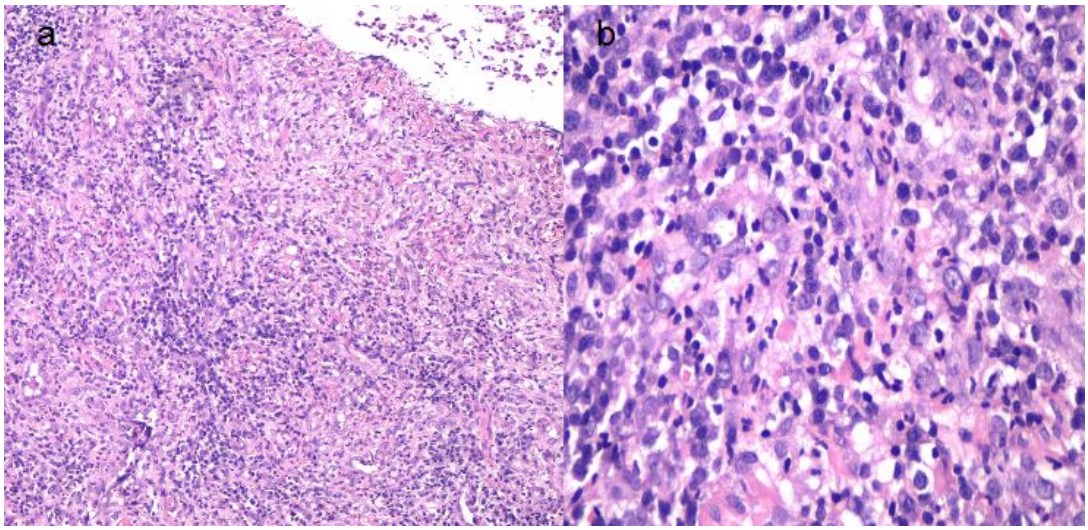


Figure 2a: Cervical vertebral intramedullary tissue with organising inflammation (H&E 10X).
2b granulation tissue with mixed inflammatory cells (H&E 40X).

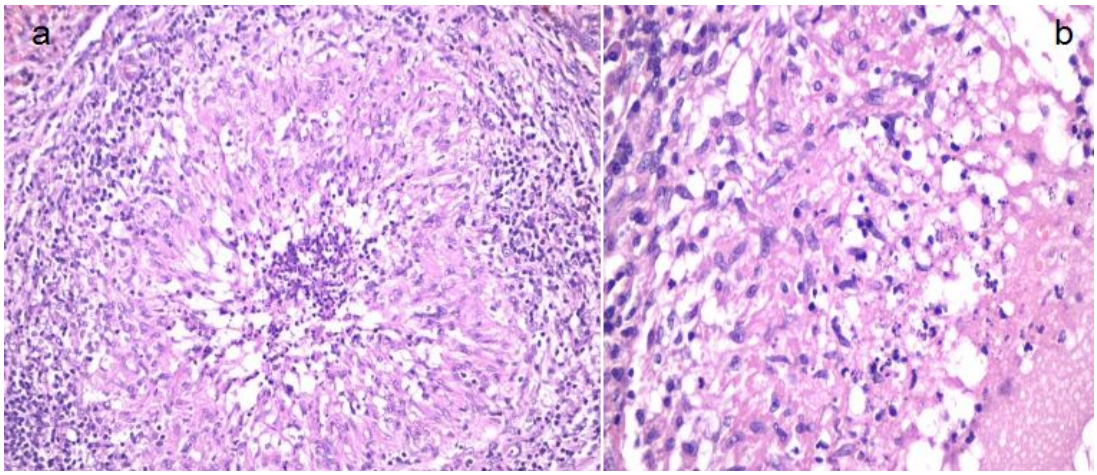


Figure 3a: Spleen with suppurative granuloma (H&E 10X). 3b Granuloma with pallisade of histiocytes and central neutrophilic aggregates (H&E 40X).

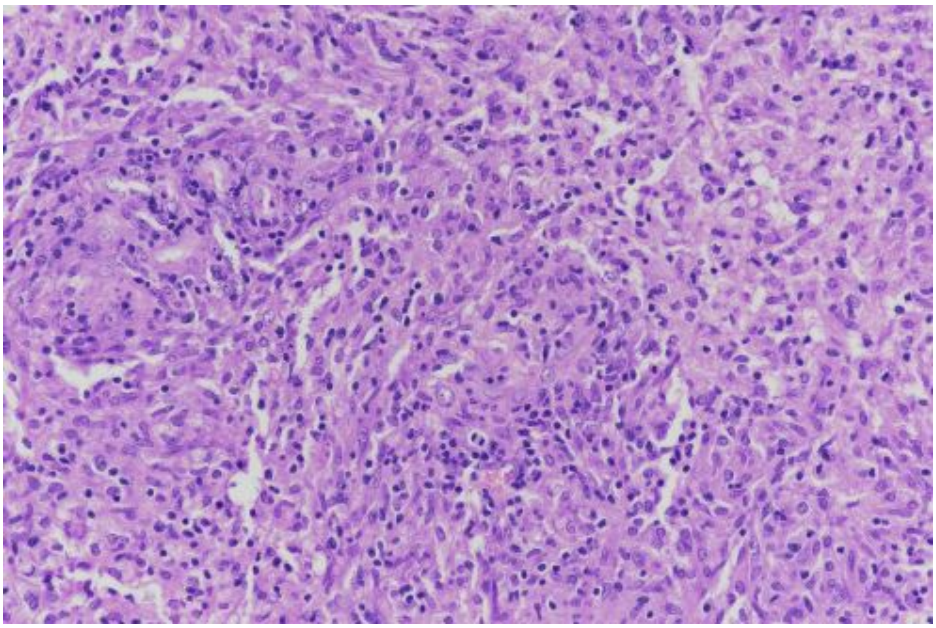


Figure 4: Glial tissue with non-necrotising granuloma (H&E 10X).

Samples with acute inflammation showed sheets of neutrophils forming abscess surrounded by foamy macrophages and mononuclear cells. Granulation tissue with activated fibroblasts were noted in organised foci of inflammation. In addition to mixed inflammatory cells, aggregates of foamy macrophages and epithelioid histiocytes were seen forming granuloma indicating chronicity in 4 of the cases with acute on chronic inflammation. Histiocytic aggregates were predominantly indistinct with central neutrophilic collections

forming suppurative granulomas. Multinucleated giant cells were seen in 3 cases and were occasional in number. Two of the cases had Langhan type giant cell and the other had an irregular outline of the giant cell. A case with brain biopsy showing chronic inflammation had sheets of epithelioid histiocytes and lymphocytic cuffing around vessels. Giant cells and necrosis were not seen.

Gram stain was available for 9 of the cases, of which 6 highlighted gram-negative bacilli, 2 in cases with acute inflammation, one in chronic inflammation and 3 in suppurative granuloma. These bacilli were seen extracellularly and were singly dispersed. Stains for acid fast bacilli and fungi were negative in cases with granuloma.

Majority of the patients had multifocal disease (8/15) and comorbid conditions like Diabetes (9/15). Seven patients needed intensive care during the hospital stay. Three of the 9 patients with acute suppurative/ necrotising/ organising inflammation succumbed to the disease.

Discussion

HPE findings in melioidosis in our study are acute inflammatory response predominantly (12 out of 18) which is either organising, suppurative or necrotising, Acute on chronic in few (5 out of 18) and rarely chronic (1 out of 18). Clinical details of the patients including duration of illness and antibiotic treatment which might influence the type of inflammatory response in the tissue is not analysed in the study. Gram stain is not available for some of the samples. The number of samples included in the analysis is only 18 in spite of many culture proven melioidosis cases during the study period. This is because either samples were not subjected to histopathology, or the samples sent to histopathology were not cultured.

There is scarcity of literature on HPE findings in human melioidosis. Wong *et al* had described HPE findings in autopsy and surgical biopsy specimens from human melioidosis cases [4]. Like our study, the findings noted by them ranged from acute inflammation to acute on chronic and chronic inflammation with focal to diffuse granulomas. The findings are not specific for any organ or tissue. Histopathology presentation of melioidosis is no different from other infectious aetiology.

Most of the samples in our study are from musculoskeletal system (11 out of 18 samples). But in Wong *et al* study out of 14 surgical biopsy specimens only one is from synovium [4]. Among various organ systems affected in melioidosis, musculoskeletal system is one of the common systems affected in our country [5]. In our study, 91% of the inflammatory response in musculoskeletal system are acute in nature. In a study done by Perumal et al, like our study, 80% of the samples from musculoskeletal system in patients with melioidosis showed acute inflammation [6].

Granuloma is noticed in 28% of our samples. But Wong *et al* noticed granuloma in about 50% of surgical biopsy samples in melioidosis [4]. In our study only one sample from musculoskeletal system (9%) had granuloma whereas Perumal *et al* noticed granuloma in 3 out of 15 (20%) musculoskeletal samples from melioidosis patients [6]. Type of host response could depend on the virulence of the organism and immune status of the individual [4]. Three of our samples had a

few giant cells. It is similar to the findings noticed by Wong *et al* in surgical biopsy specimens [4]. Brain biopsy of the patient presenting as acute demyelinating encephalomyelitis included in our study showed chronic inflammation with well-defined granuloma as evidenced by Shimee Ekka *et al.* [7]. Twenty-eight percentage of samples in our study had acute on chronic inflammation. Wong *et al* and Brundage *et al* had described similar findings in their study [4,8].

We could demonstrate gram negative bacilli in 6 out of 9 cases. Three out of four cases with suppurate granuloma shows gram negative bacilli. We could not identify the bacilli in giant cells as described by Wong et al in his study, in which numerous gram-negative bacilli was noted to resemble globi from autopsy samples[4]. In contrast, occasional bacilli seen in our study may be due the fact that all our samples are surgical biopsy samples and not from autopsy. Since Gram stain to demonstrate the bacilli is difficult in biopsy samples, immunohistochemical staining using antibodies targeting the capsular polysaccharide of B. pseudomallei will be useful in cases of diagnostic dilemma [9]. In our study, patients with acute inflammation on histopathology, multifocal disease, comorbidities and need for intensive care had poorer outcome.

Conclusion

Any acute inflammation or acute on chronic inflammation with or without granuloma in tissues biopsied from patients in endemic areas should raise the suspicion of melioidosis. An attempt to demonstrate the organism by gram stain is suggested. Specialised staining techniques with better sensitivity like fluorescent acridine orange can be studied to demonstrate the bacilli. Immunohistochemistry using specific monoclonal antibodies may help in diagnosis[9,10]. This will enhance timely patient management.

Conflict of Interest

The authors declares that the authors do not have conflict of interest

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