

Original Research Article

Clinicopathological study of bacterial lesions in a tertiary care hospital

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ABSTRACT

Background: The incidence of bacterial lesions has been on the rise over the decades. In our study, we assessed and studied the spectrum of bacterial lesions retrospectively for one year and their clinicopathological correlation which was diagnosed on histopathological examination in a tertiary health care center.

Methods: The present study (retrospective) has been done to analyse the incidence of bacterial infections obtained in the histopathology laboratory and study its clinical correlation and significance. Clinical details of the cases were accessed from biopsy requisition forms and included tissues from various sites in the body. Special stains were done which included acid fast bacilli (AFB), Fite Farraco (FF) stain and gram stain for the identification of bacteria.

Results: A total of 318 cases including 288 *H. pylori* infections, granulomatous inflammation 18, Hansen disease 9 and 3 actinomycosis infections.

Conclusions: The histopathological diagnosis of bacterial lesions are of utmost importance as it is more reliable than culture in circumstances of reduced tissue availability. Early reporting is extremely necessary to reduce complications related to bacterial lesions.

Keywords: AFB, Bacteria, Culture, Special stains

INTRODUCTION

Bacteria are prokaryotes, they have a cell membrane but lack membrane-bound nuclei and other membrane-enclosed organelles. There are two common forms of cell wall structure: a thick wall that retains crystal violet stain (gram-positive bacteria) and a thin cell wall surrounded by an outer membrane (gram-negative bacteria). Depending upon the staining, shape, and their requirement for oxygen bacteria are classified by gram positive or negative), spherical, called cocci (Figure 1a), or rod-shaped, called bacilli (Figures 1 b-d), and aerobic or anaerobic. Motile bacteria have flagella, long helical filaments extending from the cell surface that rotate and move the bacteria. Some bacteria possess pili, another kind of surface projection that can attach bacteria to host cells or extracellular matrix. Bacteria synthesize their own DNA, RNA, and proteins, but they depend on the

host for favourable growth conditions. Many bacteria remain extracellular when they grow in the host, while others can survive and replicate both outside and inside of host cells (facultative intracellular bacteria such as mycobacteria), and some grow only inside host cells (obligate intracellular bacteria, such as rickettsia). Bacteria cause a range of infections from common pharyngitis and urinary tract infections to rare diseases such as leprosy.

The pathologist plays a crucial role in the diagnosis of both routine and new upcoming infectious diseases. The bacterial infections can be diagnosed by many methods like clinical features, histopathological examination of tissues, microbial culture, and other techniques like direct fluorescence antibody, immunohistology, enzyme immunoassay, in situ hybridization, PCR, etc. Even though culture is a gold standard for identification of

microorganisms, the histopathological examination of tissues is more diagnostic than the culture because of the inadequate tissue samples in formalin, and also delay in the isolation of the organisms by the presence of unwanted material like fibers, dead tissue. For the diagnosis of microbial infections, the pathologist should be knowledgeable about bacterial morphology in tissue and their reactions in response to the infection in various tissues.¹

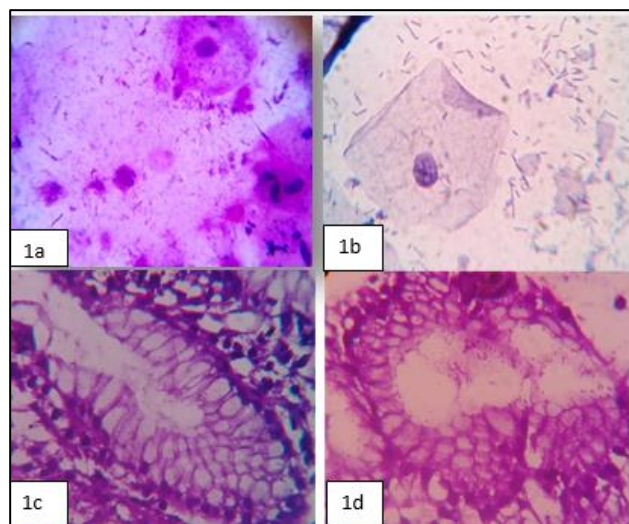


Figure 1: (a) Cocci, H and E 40x; (b) bacilli, pap stain, 40x; (c), (d) *H. pylori*, H and E and giemsa, 40x.

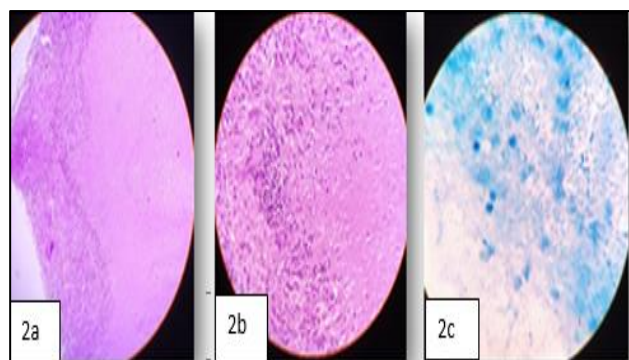


Figure 2: (a), (b) Caseating granulomas, H and E, 10x and 40x; (c) tuberculosis bacilli, Zielh-Nelson stain, 40x.

Most of the time special stains are required to identify the morphology of bacteria in tissues because unable to identify in the hematoxylin and eosin stains. So the incidence of bacterial infections is increasing steadily, hence active search of these organisms in tissue sections in correlation with clinical history is important for early diagnosis, treatment, and complications.

The study aimed to detect the various types of bacterial infections and their distribution according to age, sex, clinical presentation, and site of infection in

histopathological specimens received in the pathology department, Saveetha Medical College.

METHODS

The study (retrospective and observational) was carried out in the department of pathology, Saveetha Medical College, for a period of one year from 2020 January to 2020 December.

Inclusion criteria

In this study, we included all histopathological specimens received during the study period in both clinically suspected cases and incidentally detected bacterial infections.

The data was entered in Excel sheet and analysed descriptively. Along with specimens, the relevant clinical data was obtained from the request form and the tissues were fixed in 10% formalin, processed and the sections were stained with hematoxylin and eosin stain and special stains like Acid fast bacilli (AFB), Fite Farraco (FF) stain and gram stain were used whenever required. The study was approved by the institutional ethics committee (IEC).

RESULTS

This study was done from 2020 January to 2020 December. The total number of bacterial infections were 318. Out of 318 cases, 288 (90.6%) cases were chronic active gastritis with *Helicobacter pylori*, 19 (5.9%) cases were granulomatous inflammation with AFB positive in 13 cases and 6 cases were AFB negative, 8 (2.5%) cases were Hansen disease and 3 (0.9%) cases were actinomycosis in various tissues of different sites, as shown in (Tables 1 and 2). The most common site of *H. pylori* infection was in the antrum followed by the body, antropylic and lesser curvature with the most common clinical presentation was gastritis, dyspepsia, and acid peptic disease etc. The most common sites of tuberculous infection was in the cervical and axillary lymph nodes, spine/vertebra, lung, prostate and bladder etc. The biopsy sites for Hansen disease was skin of forearm or leg. The most common site of actinomycotic infection was in the tonsils and aryepiglottic folds which was an incidental finding.

Infectious diseases were more common in males than females. The bacterial infections occur in the age group of 6 to 75 years. In *H. pylori* infection the males (56%) were affected more than females (44), 2:1 ratio, the incidence was high in the age group ranging from 21 to 60 years (Table 3). In TB infection, out of 19 cases females 10 (53%) and males 9 (47%) were affected. In Hansen disease out of 8 cases, males 7 (87.5%) and females 1 (12.5%) were affected. In actinomycosis infection all females 3 (100%) were affected.

Table 1: Bacterial lesions age, sex, site, clinical and HPE diagnosis.

Age	Sex	Site	Clinical diagnosis	HPE diagnosis
52	F	Rt side of neck	? TB Lymphadenitis	Tuberculous lymphadenitis (AFB+ve)
45	M	Rt lung bx	B/L pleural effusion	TB, AFB +ve
60	M	Skin	Histoid Hansen disease	Histoid Hansen disease FF+ve, BI 6+
32	M	2 nd metatarsal	Osteomyelitis? TB	TB Osteomyelitis, AFB+ve
25	M	Skin	? Hansen disease	Borderline lepromatous leprosy, FF+ve
6	F	tonsils	tonsillitis	Chronic tonsillitis with actinomycosis
46	M	Skin	Cutaneous TB	Lepromatous leprosy FF+ve, 4+
75	M	Prostate	BPH	TB prostatitis, AFB+ve
25	F	Skin	Hansen disease	Hansen disease-BL, FF+ve
53	M	Skin	Hansen disease	Hansen disease-BL, FF+ve
15	F	Rt cervical node	MRTB, retrovirus+	TB lymphadenitis, AFB+ve
46	M	skin	Histoidhansen	Lepromatous leprosy, FF+ve, 4+
34	M	skin	Leprosy-BT	BT-FF+ve, 2
42	F	D12-L1	Epidural mass	Caseating granulomatous inflammation-Kochs, AFB -ve
49	M	Cervical node	TB cervical lymphnode	Caseating granulomatous lymphadenitis, AFB -ve
58	F	D5-D6 disc	Potts spine	Chronic granulomatous inflammation-Kochs
18	F	Rt axillary lymphnode	lymphoma	granulomatous lymphadenitis, AFB -ve
65	F	Rt axillary lymphnode	TB abscess	Chronic granulomatous inflammation
27	M	L3-L4 vertebral body	TB intestine	Necrotizing granulomatous inflammation, probably TB, AFB -ve
35	M	ileum	Ileal TB	Tuberculosis Ileum AFB +ve
50	M	Cervical lymphnode	TB lymphadenitis	TB lymphadenitis, AFB +ve
65	F	bladder	GUTB	TB bladder, AFB +ve
28	F	Cervical LN	Lt cervical lymphadenopathy	Caseating granulomatous lymphadenitis
30	F	tonsills	Chronic tonsillitis	Chronic tonsillitis with actinomycosis
55	F	Ileum and caecum	? ileocaecal TB	Ileocaecal TB, AFB +ve
47	M	D3-D4	Potts spine	Tb osteomyelitis, AFB +ve
27	F	Lt scapula	Cold abscess	TB abscess, AFB +ve
28	F	arytenoids	Abductor palsy	Acute on chronic inflammation with actinomycosis
17	M	Axillary LN	TB lymphadenitis	TB lymphadenitis, AFB +ve
45	M	skin	Lepromatous leprosy	Lepromatous leprosy-BL, FF +ve, 4+

Table 2: The incidence, site and clinical presentation of bacterial infections.

Bacterial infections	Total number of cases	Site	Clinical presentations
H.pylori	288 (90.6%)	i. Antrum ii. Body iii. Pylorus iv. Lesser curvature	a) Gastritis b) Dyspepsia c) Acid peptic disease d) Oesophagitis e) others
Tuberculosis	19 (6%)	i. Lymphnodes ii. Lung iii. Spine iv. Ileum v. Caecum vi. Bladder vii. Metatarsal viii. prostate	a) TB lymphadenitis b) Cold abscess c) Pleural effusion d) Ileal TB e) GUTB f) Potts spine g) BPH h) Osteomyelitis i) ?lymphoma
Hansen disease	8 (2.5%)	i. Skin	a) Hansen disease/Histoid leprosy/Lepromatous leprosy
Actinomycosis	3 (0.9%)	Tonsils arytenoids	a) Tosillitis b) Abductor palsy

Table 3: Total cases of various pathogenic bacterial organisms or lesions-age and sex wise distribution.

Organisms	F (%)	M (%)	Total number (%)
<i>H. pylori</i>	108 (44)	180 (56)	288 (90.6%)
Granulomatous inflammation	10 (53)	9 (47)	19 (6%)
			AFB +ve 13 (68.4%)
			AFB -ve 6 (31.6%)
Hansen disease	7 (87.5)	1 (12.5)	8 (2.5%)
Actinomycosis	3 (100)	-	3 (0.9%)

DISCUSSION

Helicobacter pylori are gram-negative, microaerophilic, spiral organism which inhabits the gastric mucosa. *H. pylori*-related diseases are the most prevalent in the world especially in the subcontinent of India and are an important etiological factor of numerous benign, premalignant and malignant lesions like peptic ulcer, chronic gastritis, intestinal metaplasia, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma (MALT).^{2,3} It was first discovered by Warren and Marshall in 1983. The occurrence of *H. pylori* infection is more common in developing countries when compared with developed countries and the incidence increases with age.⁴ In the present study, male patients were 180 (62.5%) and females were 108 (37.5%) with male to female ratio being 2:1.5 and which was almost equal to the study done by Dandin et al, and Dogar et al.^{5,6} In the study by Abu-Ahmad et al, the percentage of male and female cases were 75%, 62% respectively.⁷ All studies showed males were commonly affected, except Adisa et al showed females (53.2%) were more affected than males (46.8%).

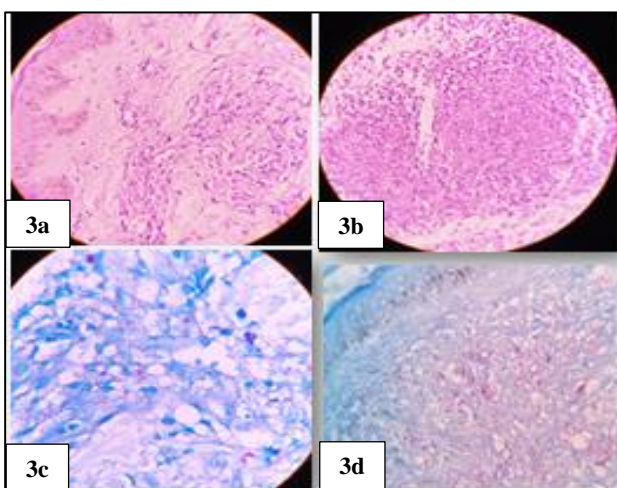


Figure 3: (a), (b) Lepromatous and tuberculoid leprosy, H and E, 40x; (c), (d) Lepra bacilli, Fitefarraco stain, 40x.

The H and E stain is the routinely used stain for interpretation of gastric biopsies, which is cost effective, easy to use. *H. pylori* can be identified easily with a careful examination by using any stain. The *H. pylori* appeared as pink in H and E stain, dark blue against a pink-pale blue background in modified Giemsa stain (Figures 3, 4).

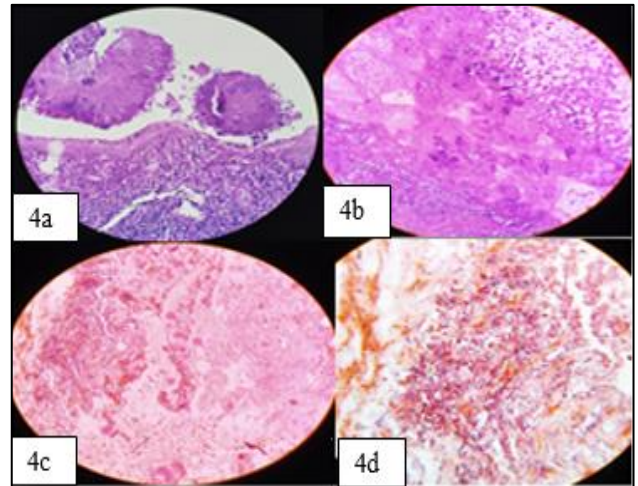


Figure 4: (a) Actinomycosis basophilic filamentous aggregates, H and E, 40x; (b) necrotic debris with bacterial colonies, H and E, 40x; (c), (d) bacteria brown in colour, gram stain, 40x.

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* and still it is a common disease in developing countries. Zheil-Neelsen stained smear and culture on Lowenstein Jensen (LJ) media are conventional methods used for the diagnosis of *Mycobacterium tuberculosis* in most developing countries (Figures 2 a-c). In our study male (9) and females (10) were affected almost equally. The results obtained by previous studies reported TB was more common among men than women. According to Zaman et al, Vijayakumar et al, Amany et al found that men are more commonly affected by pulmonary tuberculosis than women.⁸⁻¹⁰ The most common site of involvement is lymphnodes.

Mycobacterium leprae is a microaerophilic, acid-fast bacillus which causes leprosy and cannot easily be cultured in the lab. The infection is thought to be spread through the skin and nasal mucosa. Ridley and Jopling (1966) proposed a five group histological classification reflecting the immunological spectrum: tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), lepromatous borderline (BL) and lepromatous (LL).¹¹ Diagnosis is typically made upon recognition of acid-fast bacilli in a skin biopsy of a lesion by Fite-Faraco stain (Figure 3 a-d). In our study out of 8 cases 3 cases were lepromatous leprosy, 3 cases were borderline lepromatous and one case of histoid and borderline tuberculoid leprosy cases. The males (7) were affected more than the females (1), comparable to study done by

Nandwani et al showed 35 (70%) were males and 15 (30%) were females.¹²

Actinomyces are gram-positive, pleomorphic, non-spore forming anaerobic, non-acid-fast branched filamentous bacteria.¹³ *Actinomyces* species normally colonize tonsillar crypts and the oral cavity. The main clinicopathological manifestations are cervicofacial, thoracic, and intestinal actinomycosis. The organisms are usually tangled together in a matted colony forming granuleorain and filaments are hematoxyphilic and gram positive. They stain with the special stain as Grocott silver method, often only the peripheral filaments stain with the gram method, because of degeneration of inner bacteria and often the peripheral filaments terminate in a club and in ulcer foot the bacterial colonies appear dark brown (Figures 4 a-d). Aydin et al and van Lierop et al and Toh et al and colleagues found that actinomycosis was more prevalent in adults than children.¹⁴⁻¹⁶ Some authors reported a female predominance of tonsillar actinomycosis, while one study did not show such a predominance.^{14,17,18} In our study 3 cases occurred accidentally in tonsils and arytenoids and all are females, out of 3 cases one case was at the age of 6 years and two cases were seen in adult age group 25-35 years age, correlating with the above studies.

The limitations of study was no availability of culture report.

CONCLUSION

The role of histopathology for the diagnosis of infectious diseases is well established.

The most common bacterial infection is *H. pylori*, followed by tuberculosis, Hansen disease and actinomycosis. The correct diagnosis of bacterial infections were important for early treatment and also to prevent further consequences and complications.

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