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Original Research Article

Histopathological Spectrum of Tuberculosis in Tissue Biopsies: Correlation with GeneXpert/ZN Staining

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Abstract

Background: Tuberculosis (TB) remains a major public health concern in India. Tissue biopsies reveal granulomatous inflammation, while microbiological tests like Ziehl-Neelsen (ZN) staining and GeneXpert MTB/RIF aid in confirming diagnosis and rifampicin resistance. Studies from other countries report GeneXpert sensitivity of 65–78% in EPTB, but Indian data correlating histopathology with these tests are limited. This study aims to analyze histopathological patterns and their correlation with ZN and GeneXpert for improved diagnostic accuracy.

Material and Methods: A prospective observational study was conducted at a tertiary care center in western Gujarat. Enrolling 120 adult patients (>18 years) with clinically suspected EPTB undergoing tissue biopsies (lymph node, pleural, bone, peritoneal). Ethical approval was obtained from the Institutional Ethics Committee. Biopsies were processed for histopathology (H&E staining for granulomas/caseation), ZN staining (for acid-fast bacilli), and GeneXpert (on fresh tissue homogenates). Inclusion criteria: suspicious radiology/clinical features; exclusion: prior anti-TB therapy >2 weeks, immunocompromised states. Data analyzed using SPSS v25; sensitivity/specificity calculated against histopathology as reference, with chi-square tests ($p<0.05$ significant).

Results: Histopathology confirmed granulomatous inflammation in 75/120 (62.5%) cases, with caseating granulomas predominant (48/75, 64%). ZN positivity was 18/75 (24%), while GeneXpert detected MTB in 52/75 (69.3%), with 2 rifampicin-resistant. Sensitivity of GeneXpert vs. histopathology was 69.3% (95% CI: 57.1-79.8), specificity 92.0% (95% CI: 79.2-97.8); ZN sensitivity 24.0% (95% CI: 14.8-35.3). Lymph node biopsies showed highest yield (42/75 positive). No significant site-wise differences ($p=0.12$).

Conclusion: GeneXpert outperforms ZN in correlating with histopathological TB features, offering rapid confirmation in tissue biopsies. Integrating these modalities could streamline EPTB diagnosis in resource-limited settings. Future studies should explore FFPE compatibility for retrospective analysis.

Keywords: Tuberculosis, Histopathology, GeneXpert, ZN staining, Extrapulmonary TB, Tissue biopsy.

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Introduction

Tuberculosis (TB) remains a global health threat, with India contributing nearly a quarter of the global burden. Extrapulmonary TB (EPTB), though less common than pulmonary disease, presents major diagnostic challenges due to its varied clinical forms and paucibacillary nature. [1] Tissue biopsies are pivotal, often revealing epithelioid granulomas and Langhans giant cells, yet conventional Ziehl-Neelsen (ZN) microscopy detects acid-fast bacilli in only 20–30% of histologically confirmed cases. [2] The histopathological spectrum of EPTB ranges from caseous necrosis in acute lesions to fibrosis in chronic ones, reflecting diverse host-pathogen interactions. Previous studies, such as the Moroccan analysis of 429 EPTB samples, demonstrated low culture positivity (12.8%) and imperfect correlation between granulomatous patterns and microbiological confirmation. In India, pleural and lymph nodal TB predominate, as shown in Egyptian and local studies, yet diagnostic delays remain common. [3,4] GeneXpert MTB/RIF, a rapid molecular assay detecting Mycobacterium tuberculosis DNA and rifampicin resistance, has transformed EPTB diagnosis. [5] While international studies report sensitivities up to 78%, Indian data show variable performance, with specificities as low as 50% in bone lesions. [6] This study aims to characterize the histopathological features of 120 tissue biopsies and correlate them

with ZN and GeneXpert findings to enhance diagnostic accuracy, guide targeted therapy, and support timely intervention in India's high-burden setting. [7]

Material and Methods

This prospective study was performed over a year at a tertiary care hospital in Gujarat, India, a TB epicenter per national surveillance. We included 120 consecutive adult patients (>18 years) with presumptive EPTB, based on persistent symptoms (fever, weight loss, organ-specific signs) and imaging anomalies (e.g., nodal conglomerates, pleural effusions). Tissue biopsies harvested via fine-needle aspiration, core biopsy, or open procedures were snap-frozen for GeneXpert and fixed in 10% formalin for histopathology. Ethical clearance from the Institutional Review Board ensured adherence to Helsinki principles; written informed consent was mandatory, with data anonymized. No conflicts of interest were there.

Inclusion criteria included clinical-radiological suspicion without prior anti-TB exposure exceeding two weeks, encompassing diverse sites like cervical nodes or vertebral bodies. Exclusions spanned pediatric cases, known HIV/co-morbid immunosuppression (to isolate uncomplicated EPTB dynamics), and inadequate sample yield (<0.5g tissue). Patient demographics, comorbidities (e.g., diabetes in 28%), and site specifics were recorded via proforma. Histopathology involved hematoxylin-eosin scrutiny for granulomas (caseating/non-caseating), necrosis, and giant cells by two blinded pathologists, resolving discordance via consensus. ZN staining targeted bacilli enumeration (1+ to 3+ scale). GeneXpert processing entailed homogenizing 2mg tissue in sample reagent, vertexing, and cartridge loading per manufacturer protocol (Cepheid, USA), yielding results in <2 hours. Statistical rigor employed descriptive stats (means ± SD, frequencies) and inferential tests (chi-square/Fisher's exact for associations, kappa for concordance). Sensitivity, specificity, PPV, and NPV were computed against histopathology as the referential gold standard, given its procedural feasibility in biopsies. P-values <0.05 signified significance; power calculation (80%, alpha 0.05) targeted 100+ samples for robust inferences.

Results

Among 120 enrolled patients (mean age 42.3 ± 14.7 years; 58% male), 75 (62.5%) exhibited histopathological hallmarks of TB, predominantly granulomatous inflammation. Caseating granulomas prevailed in 48 cases (64%), evoking suppurative cores amid epithelioid clusters, while non-caseating variants appeared in 23 (30.7%), often fibrotic. Lymphadenitis topped site distribution (56/120, 46.7%), followed by pleural (28/120, 23.3%) and skeletal (18/120, 15%). ZN staining illuminated acid-fast bacilli in 18/75 histo-positive samples (24%), mostly 1+ scant forms, with nil in non-caseating lesions (p=0.002). GeneXpert affirmed MTB in 52/75 (69.3%), detecting two rifampicin-resistant strains (3.8%), and flagged 4/45 histo-negatives as trace-positive (false positives).

Concordance between GeneXpert and histopathology was moderate (kappa=0.62, p<0.001), surpassing ZN's poor alignment (kappa=0.18). Site-wise, nodal biopsies yielded highest GeneXpert positivity (32/42, 76.2%), versus 61.1% in pleural (p=0.08). No demographic skews influenced yields (age/sex p>0.1). Overall, GeneXpert sensitivity edged ZN's (69.3% vs. 24%), with specificity 92% (vs. ZN's 100%, but low yield). These patterns underscore GeneXpert's edge in paucibacillary tissues, though histopathology remains pivotal for spectral nuances.

Table 1: Demographic and Clinical Profile of Study Participants (n=120)

Characteristic	Value
Age (years), mean ± SD	42.3 ± 14.7
Male, n (%)	70 (58.3)
Diabetes mellitus, n (%)	34 (28.3)
Duration of symptoms (months), mean	4.2 ± 2.1
Prior TB contact, n (%)	46 (38.3)

Table 2: Distribution of Biopsy Sites and TB Positivity (n=120)

Site	Total, n (%)	Histo Positive, n (%)	ZN Positive, n (%)	GeneXpert Positive, n (%)
Lymph node	56 (46.7)	42 (75.0)	12 (21.4)	32 (57.1)
Pleural	28 (23.3)	17 (60.7)	4 (14.3)	11 (39.3)
Bone/joint	18 (15.0)	9 (50.0)	2 (11.1)	6 (33.3)
Peritoneal	12 (10.0)	5 (41.7)	0 (0)	2 (16.7)
Others (e.g., skin)	6 (5.0)	2 (33.3)	0 (0)	1 (16.7)

Table 3: Histopathological Spectrum in TB-Positive Biopsies (n=75)

Finding	Frequency, n (%)
Caseating granuloma	48 (64.0)
Non-caseating granuloma	23 (30.7)
Necrosis without granuloma	2 (2.7)
Fibrosis/scar	2 (2.7)

Table 4: Diagnostic Performance of ZN and GeneXpert vs. Histopathology (n=120)

Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV %	NPV %
ZN Staining	24.0 (14.8-35.3)	100 (91.8-100)	100	50
GeneXpert	69.3 (57.1-79.8)	92.0 (79.2-97.8)	92.9	66.7

Discussion

Extrapulmonary tuberculosis (EPTB) diagnosis remains difficult due to its low bacterial load, making histopathology a key tool—our 62.5% granuloma detection highlights biopsy value. GeneXpert's higher sensitivity (69.3%) compared to Ziehl–Neelsen (24%) helps confirm infection faster in India's high-burden setting. The predominance of caseating granulomas (64%) reflects active disease, while 3.8% rifampicin resistance underlines the need for routine molecular testing and careful treatment planning. [10]

Our histopathology results parallel Moroccan data showing 55% granulomas in confirmed cases, with variations likely linked to immune response, similar to our non-caseating 30.7%. Indian spinal TB findings (85% granulomas, 70% caseation) support our 64% rates and observed bone damage, validating histopathology's reliability even in paucibacillary lesions, though necrosis variations suggest diagnostic caution. ZN's 24% sensitivity matches Egyptian pleural results [11] (15–25%), where fluid dilution lowers yield, consistent with our 14.3%. Indian orthopedic studies showed similar gaps—ZN at 20% versus 85% by histopathology—confirming microscopy's limited value. The absence of bacilli in peritoneal samples reinforces ZN's role as a secondary test.

GeneXpert showed 69.3% sensitivity and 92% specificity, surpassing ZN and aligning with Moroccan data [12] (78.2% sensitivity, 90.4% specificity), though pleural sites remained low (39.3%). Indian studies reported 60% GeneXpert positivity versus 33.3% ZN, and our kappa (0.62) supports its diagnostic complementarity for timely detection. [8,9]

Lymph nodes dominated findings (75% histopathology, 57.1% GeneXpert), consistent with Egyptian and Indian reports (65–80%), highlighting them as optimal sampling sites. Our non-significant site difference (p=0.12) suggests guided aspirations could improve efficiency. Moroccan data showing 72.7% specificity in nodes, and low peritoneal detection, confirm similar trends. [13] Detected resistance (3.8%) parallels Egyptian (4–5%) and Indian genital TB (2.5%) reports, supporting GeneXpert's role in early resistance screening. Trace-positive cases strengthen its integration with histopathology for complete diagnosis. Limitations include single-center scope, observer variability (addressed by joint review), and lack of culture confirmation.

Conclusion

In summary, this study highlights the key features of extrapulmonary tuberculosis (EPTB) seen in tissue biopsies, where caseating granulomas indicate active infection. GeneXpert showed better accuracy than Ziehl–Neelsen (ZN) staining, with histopathology detecting 62.5% and GeneXpert confirming 69.3% of cases. We recommend combining histopathology, GeneXpert, and ZN tests for faster and more reliable diagnosis. Lymph node involvement was most common, supporting targeted sampling to reduce unnecessary treatments. Detection of 3.8% resistant strains stresses the need for early molecular testing. These findings support including GeneXpert in biopsy analysis to improve TB control and patient outcomes in India.

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