Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

Percutaneous Transpedicular Biopsy in Patients with Tuberculosis of Spine – How to Increase the Diagnostic Yield

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Abstract

Background: Tuberculosis (TB) is one of the oldest diseases known to humankind, with evidence of its existing dating to over 4000 years ago. Spinal TB is the most common site of skeletal TB, amounting to half of skeletal TB. It is usually diagnosed clinicoradiologically magnetic resonance imaging (MRI) which has been the imaging modality of choice as it has been able to detect the earliest changes. Laboratory diagnostic tests such as histopathology, TB Gene X pert, culture, and sensitivity (TB Bactec) are used to assess bacteriology and sensitivity pattern, especially with the rise in multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), with the growth of *Mycobacterium* in culture specimens obtained from the infected tissue considered to be the single most confirmatory test of spinal TB, and hence considered the gold standard method. However, a negative culture or delay in the setting of resistant strains may lead to neurological deficits or deformity. Overall positive culture yields from abscess sites using core needle biopsies in previous studies, which is 50–83%. Our study aims to assess the efficacy of percutaneous transpedicular biopsy and Gene X pert for the diagnosis of TB of the Spine. Furthermore, we propose systematic area-specific biopsy and laboratory sampling of the affected vertebra (Abscess area), paradiscal region, and a relatively non affected part of the involved vertebra, which will help us understand the area-wise yield.

Materials and Methods: A total of 15 cases with clinical and/or radiological features suggestive of TB of spine were selected for the study. After obtaining an informed consent, the cases were subjected to area-specific percutaneous transpedicular biopsy. Multiple systematic area-wise transpedicular biopsy (abscess area – Area 1, paradiscal partially affected area – Area 2, and area of hyperintensity on MRI – Area 3) were taken. Three samples from obtained from each patient, which were subsequently sent for Gene Xpert and TB culture analysis. Statistical analysis used was IBM SPSS STATISTICS VERSION 20.

Results: The patient age ranged from 14 to 72. The mean age was seen to be 41.4. Nine out of the 15 (60%) subjects were male and six female (40%). Sensitivity of Gene Xpert for Area 1, 2, and 3 was found to be 100% each, specificity of gene Xpert for Area 1, 2, and 3 was found to be 90%, 100%, and 87.5%, respectively. Positive yield for Area 1 was 38.46%, Area 2 was 69.25%, and Area 3 was 53.89% *P* value for comparison of yield between Areas 1 and 2 which was found to be 0.122 (statistically not significant). *P* value for comparison of yield between Areas 1 and 3 was found to be 0.440 (statistically not significant). Erythrocyte sedimentation rate values ranged from 34 to 66, with a mean of 49.47 and standard deviation of 9.015. C-reactive protein values of all patients universally were seen to be positive.

Conclusion: Area specific percutaneous transpedicular biopsy for TB of spine is a good promising modality of investigation in TB spine since it can give a quick definitive diagnosis, by means of Gene Xpert. The accuracy of Gene Xpert can be high since it has shown to have a high sensitivity and specificity. Furthermore, MDR-TB can be diagnosed at an early stage, and appropriate chemotherapy can be initiated.

Key words: Gene Xpert percutaneous Transpedicular biopsy, Tuberculosis of spine

Access this article online

www.ijss-sn.com

Month of Submission : 09-2020
Month of Peer Review : 10-2020
Month of Acceptance : 11-2020
Month of Publishing : 11-2020

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases known to humankind, with evidence of its existing dating to over 4000 years ago.[1-3] Extrapulmonary TB incidence is around 3%,[4] of which 10% contribution is by Skeletal TB. Spinal TB is the most common site of skeletal TB, amounting to

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half of skeletal TB.[5,6] Clinical picture is very variable, and it depends on the severity and duration of the disease, site of the disease, and the presence of complications such as abscess, sinuses, deformity, and neurological deficit.[7] It is usually diagnosed clinicoradiologically. Magnetic resonance imaging (MRI) has been the imaging modality of choice as it has been able to detect the earliest changes. Gadolinium-enhanced MRI further helps in differentiating TB from other causes of infective spondylodiscitis.[8,9] Laboratory diagnostic tests such as histopathology, TB gene Xpert, culture, and sensitivity (TB Bactec) are used to assess bacteriology and sensitivity pattern, especially with the rise in multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), with growth of Mycobacterium in culture specimens obtained from the infected tissue considered to be the single most confirmatory test of spinal TB, and hence considered the gold standard method.[10] However, a negative culture or delay in the setting of resistant strains may lead to neurological deficits or deformity. Overall positive culture yields from abscess sites using core needle biopsies in previous studies, which is 50–83%.[11]

Our study aims to assess the efficacy of percutaneous transpedicular biopsy and gene Xpert for the diagnosis of TB of the spine. Furthermore, we propose systematic area-specific biopsy and laboratory sampling of the affected vertebra (Abscess area), paradiscal region, and a relatively non-affected part of the involved vertebra, which will help us understand the area wise yield.

MATERIALS AND METHODS

Our study was prospective cross-sectional study conducted at orthopedic ward and outpatient department of a tertiary care hospital. Informed consent was taken from all the subjects enrolled. The following was the inclusion criteria – all patients clinicoradiologically (clinical examination, X-Ray, and MRI) diagnosed TB spine cases (thoracic and lumbar). The exclusion criteria were as follows patients with biopsy-proven malignancy and fracture spine. Three areas of vertebrae were biopsied – abscess/area of granulation tissue [Area 1]. Area of osteomyelitis, partially [Area 2], area showing hyperintensity on MRI [Area 3] [Figure 1]. Each of the sample (from Area 1, 2, and 3) was subjected to histopathology, ZN staining, TB Gene Xpert, and Culture and Sensitivity (TB Bactec). Each of this sample was blinded for the pathologist and laboratory technician. The positive outcome was – presence of acid-fast bacilli and/or granulomas (non-caseating or caseating) and/or positive culture growth.

Statistical Analysis

Primary data were collected in paper-based pro forma, and the data were then entered in Microsoft Excel spreadsheets. Statistical analysis was done on IBM SPSS STATISTICS VERSION 20. Means were compared using an independent sample *t* test. Categorical data were plotted using column charts and pie diagrams. Mean values represented by column charts are added with error columns representing actual standard deviation in plus and minus values. $P < 0.05$ was taken significant. $P < 0.01$ was considered highly significant. Receiver operating characteristics (ROCs) curves were plotted to assess the performance of a diagnostic test over the range of possible values of a predictor variable. The area under an ROC curve provided a measure of discrimination to compare the performance of two or more diagnostic tests.

Percutaneous transpedicular biopsy procedure was as follows – After thoroughly explaining the whole procedure to the patient and after obtaining informed consent, the patient was admitted for a single day for the procedure.

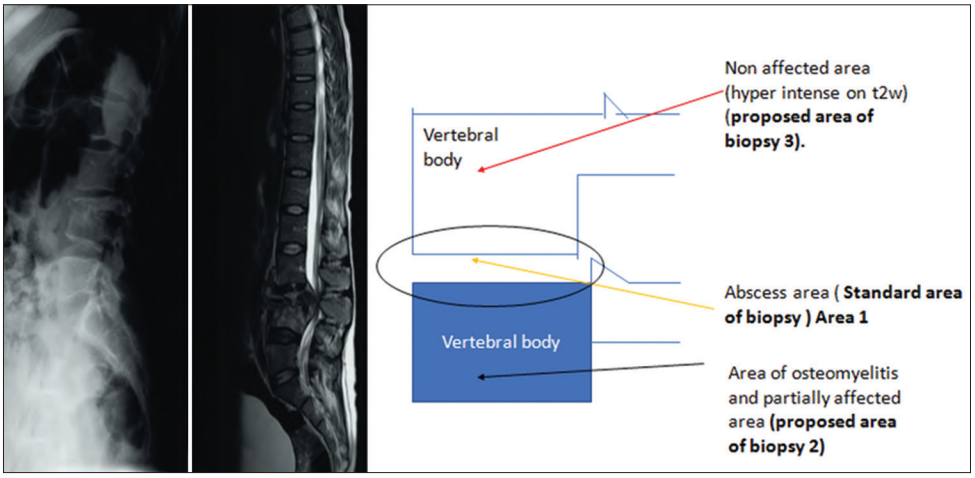


Figure 1: Area specific biopsy

Lignocaine sensitivity test was at least done 30 min before undertaking the procedure. The patient was made to lie prone on a radioluscent operating table, and their vitals were monitored with an anesthetist stand-by.

Planning was done beforehand so as to determine Areas 1, 2, and 3 of biopsy, based on presence of abscess, adjacent area with partial osteomyeltic involvement, and area of hyperintensity, respectively. Scrubbing of the back was done with iodine scrub solutions, followed by painting and draping the biopsy area under all aseptic precautions.

With the use of an image intensifier, the landmarks for the pedicles of the required vertebrae were marked with a sterile skin marker. Local infiltration with 2% lignocaine was given at the biopsy sites. After adequate anesthesia was attained, a small stab incision was taken on the skin, corresponding to the level, slightly lateral to the lateral border of the pedicle. A 10- gauge Jamshedi Needle (J-Needle), with the stylet *in situ*, was introduced through the stab incision to the surface of the pedicle, just medial to its lateral border. Once the position of the J-Needle was confirmed on both lateral and anteroposterior (AP) views such that it was at the center of the pedicle on the lateral view, and just medial to the lateral border on AP view, the J-Needle was advanced very carefully by providing controlled axial and rotational force to the needle. Advancement of the needle was duly monitored with periodic image intensifier images. Once the needle appeared to be reaching the medial wall of the pedicle on AP view, the lateral view confirmed that the J-Needle had traversed the pedicle and was now entering the vertebral body.

After just entering the vertebral body, the stylet was removed, and only the trocar was advanced. Once the needle covered at least 3/4th of the vertebral body, a 20 cc syringe was connected to the back end of the J-Needle and aspirated to collect any liquid content.

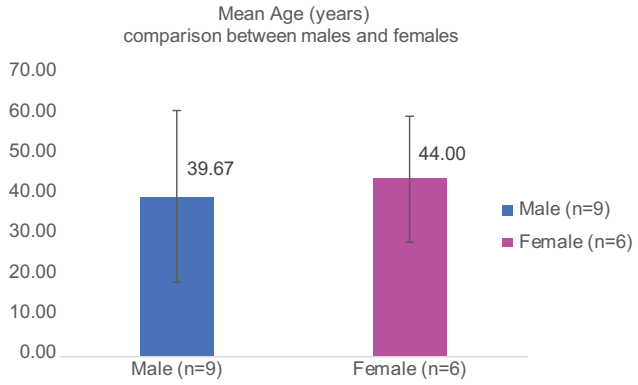
The needle was then rotated at this position a few times to ensure sample gets completely entrapped within the needle before slowly withdrawing it out. The stylet was reintroduced into the trocar, and the sample obtained was collected in a Gene Xpert container under aseptic precautions. These steps were then repeated two more times to obtain samples from the other 2 areas to be biopsied. All collected samples were then immediately sent to the Revised National TB Control Program (RNTCP) approved TB Laboratory, after filling out the appropriate forms.

Blinding was ensured at 3 levels – the personnel carrying the samples to the laboratory for analysis, the technician receiving the sample at the laboratory, and the technician undertaking Gene Xpert, culture, and histopathology.

RESULTS

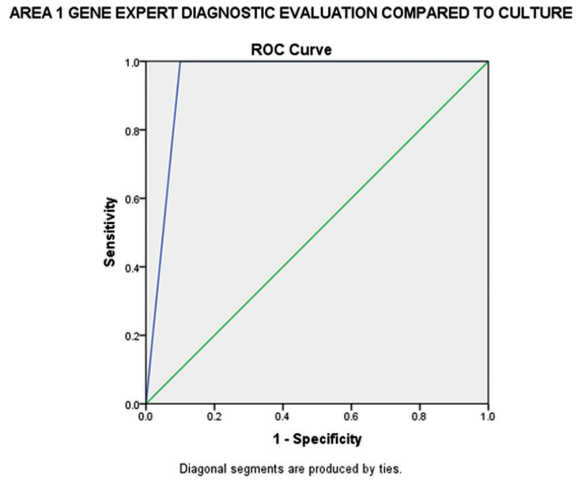
A total of 15 subjects were taken for the study. Their ages ranging from 14 to 72. Mean age was seen to be 41.4, with a standard deviation of 18.681.

Nine of the subjects were male and six were female. The mean age of males was found to be 39.67 with a standard deviation of 21.172. The mean age of female was 44.00 with a standard deviation of 15.697. The standard error mean was 7.057 and 6.408, respectively.

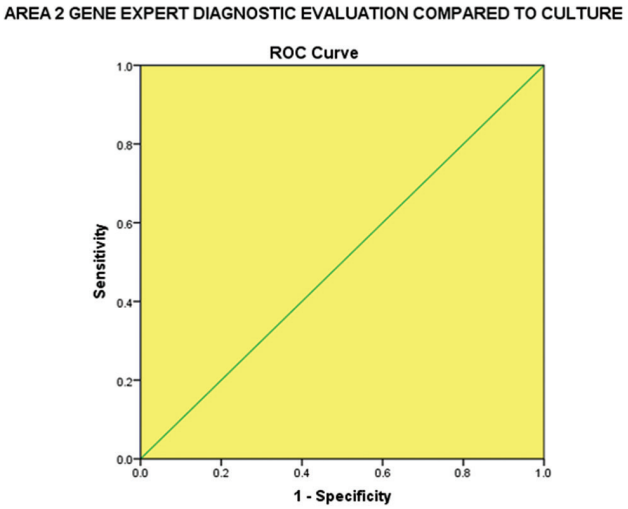


Considering TB culture as the gold standard test,[10] the sensitivity and specificity of Gene Xpert was calculated for each of the areas. These were evaluated using an ROC curve.

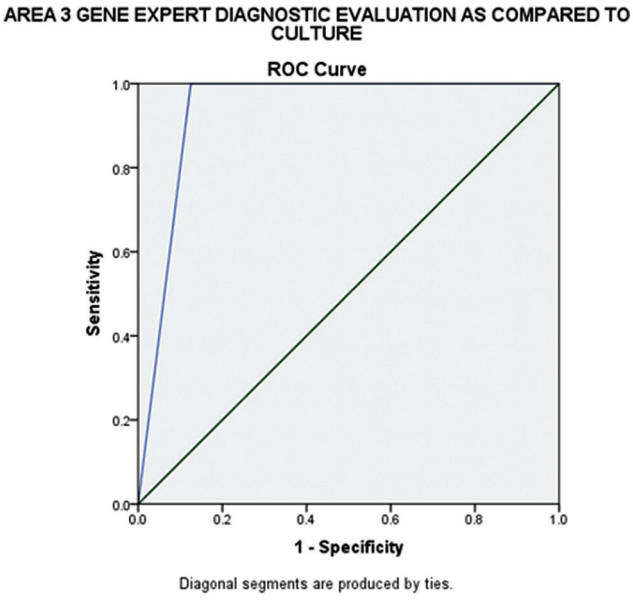
For Area 1, it as seen that it had a 100.00% sensitivity with a 95% CI of 47.82% to 100.00%. Specificity was seen to be 90.00%, with a 95% CI of 55.50% to 99.75%. A positive likelihood ratio of 10 was with a 95% CI of 1.56–64.20. The negative likelihood ratio was 0.



For Area 2, it as seen that it had a 100.00% sensitivity with a 95% CI of 66.37–100.00%. Specificity was seen to be 100.00% as well with a 95% CI of 54.07–100.00%.



For Area 3, it as seen that it had a 100.00% sensitivity with a 95% CI of 59.04–100.00%. Specificity was seen to be 87.50%, with a 95% CI of 47.35–99.68%.



Area under the Curve

The positive yields of Gene Xpert between Area 1 with that of Areas 2 and 3 were compared. It was seen that Area 1 had a yield of 38.46%, Area 2 had a yield of 69.25%, and Area 3 had a yield of 53.89%. Comparison of yields between Areas 1 and 2 gave a $P = 0.122$ (not significant), and that of Areas 1 and 3 gave $P = 0.440$ (not significant).

Erythrocyte sedimentation rate (ESR) values of all patient were compared. A minimum value of 34 was obtained and a maximum of 66. Mean was seen to be 49.47 with a standard deviation of 9.015.

Qualitative CRP analysis was done in all 15 patients. It was seen that all 15 patients had a positive CRP, giving a valid percent and cumulative percent of 100%

Total white blood cell counts were compared in both men and women. In men, a minimum value of 4200 and a maximum of 7800 was seen. Mean was seen to be 6177.78, with a standard deviation of 1068.78.

In women, a minimum value of 5600 and a maximum of 7600 was seen. Mean was seen to be 6425, with a standard deviation of 744.84 (Tables 1-14).

DISCUSSION

In our study, which was conducted over the course of 2 years, a total of 15 patients were enrolled and biopsied. As mentioned, nine of which were men and six women. This correlates with previous studies showing higher incidence and prevalence of TB in men as compared to women, as seen in a study done by Horton *et al.*[12] In this study, which was a systematic review and a meta-analysis, this generalized trend was seen all over the world, including India. This was accounted for due to an increased risk of exposure in men, in general, as well as neglecting to present at an early stage due to socio-economic responsibilities.

There was no specific age group that was found to be affected more than the others. Subjects ranged from ages as young as 14 to as old as 72. This further emphases on the importance of need of better, faster, and more effective modalities for early and accurate diagnosis and subsequent treatment since no age in particular seems to be affected preferentially, and spine seems to cause significant burden on all age groups, in an endemic country such as India.

A generalized consensus of the inflammatory biochemical parameters, namely, ESR and CRP, in our study showed correlation with existing literature[13,14] to the fact that these parameters are raised in the case of TB spine, as all 15 of our study patients showed abnormally high values for both. However, since both parameters are relatively non-specific for the disease itself, their role in diagnosis by itself is close to nil. Also, what needs to be kept in mind is the role of serial monitoring of patients on medical management on a long-term basis. A serial decrease in these parameters would indicate success of therapy. On the contrary, no change, or even increase of these parameters would suggest the clinician look for a possibly more sinister cause, such as MDR/XDR TB, or even perhaps a non-tuberculous or non-infectious pathology.

MDR-TB is that which is resistant to INH and rifampicin. Primarily, it occurs due to improper treatment, but resistant strains can also be communicable. Extensively drug-resistant TB (XDR-TB) is when there is resistance to INH and rifampicin, along with resistance to any fluoroquinolone and at least one injectable second-line anti-TB drugs.[15,16]

Pawar *et al.* suggested the following five predictors for successful outcome in MDR-TB: (1) Progressive clinical improvement at 6 months following chemotherapy, (2) radiographic improvement during treatment, (3) disease with strains that are resistant to < 3 anti-tubercular treatment drugs, (4) use of <4 second-line drugs in treatment, and (5) no changes of regimen during treatment.[17] In 2009, the term "totally drug-resistant tuberculosis" for TB strains that showed *in vitro* resistance to all first and second-line drugs tested was proposed by Velayati *et al.*[18]

Transpedicular biopsy was done as a daycare procedure in all of our subjects. Samples were obtained using stainless steel J-Needles. We found that size 10 and 11 gauge J-Needles gave the best yield in an adult, as the needle bore was sufficiently wide, both for aspirating contents and obtaining a biopsy. All samples were sent for Gene Xpert.

In our study, we primarily assessed the efficacy of Gene Xpert as compared to the TB culture, which is the Gold Standard for diagnosis.[10] We also assessed which areas, with respect to the MRI findings, were giving the best yield of bacilli for diagnosis.

On analyzing our data, we found a sensitivity and specificity of more than 85% in all three areas of biopsy across all the subjects. This is in line with existing literature,[19-21] which gives further credibility to the test. Since Gene Xpert is endorsed by the WHO[19]] and is recommended by RNTCP,[22] and the test requires 2 h to give a result,[23] a quick diagnosis can be achieved with minimal intervention by means of percutaneous transpedicular biopsy. TB culture methods, even newer ones such as BACTEC culture, on the other hand, require at least 2 weeks to give a definitive result.[24] Added of advantage of Gene Xpert over other modalities of diagnosis is that it can determine the presence of rifampicin resistance by detecting the presence of rpoB gene, which confers rifampicin resistance to the bacilli.[25] This can prove to be a huge factor in decreasing the morbidity and mortality associated with MDR TB since in most cases in India, MDR TB is usually suspected only when first-line antituberculous treatment (AKT) seems to show no improvement or there happens to be clinical worsening. By this time, the patient could have already had a significant effect on their disability-adjusted life year. An early diagnosis of MDR TB could mean earlier initiation of second-line drugs. This could be vital in minimizing the complications that may arise due to a late diagnosis.

A total of three biopsies were taken from each subject. First sample was taken from the abscess/diseased area, as is the usual protocol.[26] Second sample was taken from the adjacent vertebrae and a third sample from vertebra showing hyperintense signal on MRI. The abscess area has usually shown to give a yield of around 50–83%.[11]

In a study done by Watt and Davis[27] to assess yield in spinal TB, it was found that the overall positive TB culture rate was 59%, when the sample was taken from the abscess site (Area 1). In our study, we found that the abscess site gave a positive yield of only 38.46% of the samples. However, the adjacent vertebrae (Area 2) gave a positive yield of 69.25% and the area of hyperintensity gave a positive yield of 53.89% (Area 3). On assessing the statistical significance of these findings, Area 1 versus Area 2 gave $P = 0.122$ and that of Area 1 versus Area 3 gave $P = 0.440$, which would suggest statistical insignificance. However, since the sample size was small for this study, and the fact that numerically Areas 2 and 3, in general, showed a higher yield, it would be safe to suggest that a study with a possibly bigger sample size, over a longer duration of time, conducted perhaps

Table 1: Age descriptive

	N	Minimum	Maximum	Mean	Standard deviation
Age (years)	15	14	72	41.40	18.681

Table 2: Frequency distribution of age

Age (years)	Frequency	Percent	Cumulative percent
14	1	6.7	6.7
18	1	6.7	13.3
21	1	6.7	20.0
29	1	6.7	26.7
30	1	6.7	33.3
31	1	6.7	40.0
32	1	6.7	46.7
34	1	6.7	53.3
44	1	6.7	60.0
54	2	13.3	73.3
60	1	6.7	80.0
62	1	6.7	86.7
66	1	6.7	93.3
72	1	6.7	100.0
Total	15	100.0	

Table 3: Comparison of mean age

Gender	N	Mean	Standard deviation	Standard error mean	*P*
Age (years)					
Male	9	39.67	21.172	7.057	0.676
Female	6	44.00	15.697	6.408	

Table 4: Diagnostic evaluation of AREA 1 gene expert compared to culture

Statistic	Value	95% CI
Sensitivity	100.00%	47.82–100.00%
Specificity	90.00%	55.50–99.75%
Positive likelihood ratio	10	1.56–64.20
Negative likelihood ratio	0	

Table 5: Test result variable(s): AREA 1 Gene Expert

Area	Standard error[a]	Asymptotic significance[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
0.950	0.060	0.006	0.833	1.000

Table 6: Diagnostic evaluation of AREA 2 gene expert compared to culture

Statistic	Value	95% CI
Sensitivity	100.00%	66.37–100.00%
Specificity	100.00%	54.07–100.00%

Table 7: Test result variable(s): AREA 2 Gene Expert

Area	Standard error[a]	Asymptotic significance[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
1.000	0.000	0.001	1.000	1.000

Table 8: Diagnostic evaluation of AREA 3 gene expert compared to culture

Statistic	Value	95% CI
Sensitivity	100.00%	59.04–100.00%
Specificity	87.50%	47.35–99.68%

Table 9: Test result variable(s): AREA 3 Gene Expert

Area	Standard error[a]	Asymptotic significance[b]	Asymptotic 95% confidence interval	
			Lower bound	Upper bound
0.938	0.072	0.005	0.795	1.000

Table 10: Comparison of Gene Xpert Yield between areas 1 and 2

	Area 1	Area 2	*P* value
Positive yield	38.46%	69.25%	0.122

over multiple tertiary care centers at once, could give more convincing evidence to prove that Areas 2 and 3 would give a better yield.

Table 11: Comparison of Gene Xpert Yield between areas 1 and 3

	Area 1	Area 3	*P* value
Positive yield	38.46%	53.89%	0.440

Table 12: ESR values

Test	N	Minimum	Maximum	Mean	Standard deviation
ESR (MM/HR)	15	34	66	49.47	9.015
Valid N (list-wise)	15				

ESR: Erythrocyte sedimentation rate

Table 13: C-reactive protein (CRP) values

	CRP values				
		Frequency	Percent	Valid percent	Cumulative percent
Valid	positive	15	100.0	100.0	100.0

Table 14: Total white blood cell count (WBC)

		N	Minimum	Maximum	Mean	Standard deviation
WBC (no/microliter)	Men	9	4200	7800	6177.78	1068.78
	Women	6	5600	7600	6425	744.84

What can be safely assumed, though, is that percutaneous transpedicular biopsy can serve as a good diagnostic investigation. It confers multiple advantages over other modalities of diagnosis. Since each sample is sent for histological confirmation, over-diagnosing and unnecessary treatment with AKT for TB spine could be avoided. The use of Gene Xpert helps give a quick result and also helps determine if there is resistance to first-line drugs, and hence, second-line therapy can be initiated earlier. The procedure by itself is low risk, as it is done under local anesthesia.

In addition, a percutaneous transpedicular biopsy would be superior to a CT-guided biopsy since CT imaging would not show any involvement in adjacent vertebrae. These areas might prove to be hyperintense under an MRI scan. A pre-operative planning using an MRI scan, followed by transpedicular biopsy from multiple areas, as mapped under the MRI, could possibly give a better yield, as opposed to that from a CT-guided biopsy.

CONCLUSION

In summary, percutaneous transpedicular biopsy is a good modality of investigation in TB spine since it can give a quick definitive diagnosis by means of Gene Xpert. The accuracy of Gene Xpert can be considered to be high since

it has shown to have a high sensitivity and specificity when considering TB culture as the gold standard. Furthermore, MDR TB can be diagnosed at an early stage and appropriate chemotherapy can be initiated.

With respect to determining which area of biopsy would give a better yield for diagnosis, probably more studies with a larger sample size and a larger demographic will have to be conducted to give a definitive and a statistically significant answer. However, results found in this study which would suggest that biopsy of adjacent areas as opposed to the diseased area could have promising results.

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How to cite this article: Dhopade V, Bobade SM, Mathesul A, Mohan A, Chandanwale A. Percutaneous Transpedicular Biopsy in Patients with Tuberculosis of Spine – How to Increase the Diagnostic Yield. Int J Sci Stud 2020;8(8):129-135.

Source of Support: Nil, **Conflicts of Interest:** None declared.