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Research Article

Evaluation of Fine-needle Aspiration Cytology (FNAC) Sensitivity Compared to PCR for Diagnosing Tuberculosis Lymphadenitis

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Abstract

Background: Tuberculosis (TB) is a major healthcare burden in Sudan and other developing countries, it is considered the second most common cause of death from infectious diseases after those due to AIDS. In Sudan, TB lymphadenitis (TBLA) remains one of the major health problems. This descriptive cross-sectional study was conducted at the University of Medical Sciences and Technology (UMST) and Total Labcare Diagnostic Center (TDC). The study aims to compare the sensitivity of Fine Needle Aspiration Cytology (FNAC) smears with that of the Polymerase Chain Reaction (PCR) for the diagnosis of TBLA.

Methods: Fifty-five dry smears were obtained using fine-needle aspiration (FNA) from an enlarged lymph node. PCR was applied to detect the target gene (IS*6110*). May-Grunwald-Giemsa (MGG) or Diff quick stains were used.

Results: Two (4%) patients with TBLA were non-necrotic, while fifty-three of them (96%) were necrotic. Moreover, 17 (30%) fine-needle lymph node aspiration specimens were confirmed by PCR to be positive for Mycobacterium tuberculosis complex (MTB complex) while 38 (70%) of them were negative.

Conclusion: There was no significant difference between the sensitivity of PCR and that of FNAC (*P*-value = 0.33).

Keywords: tuberculosis, lymphadenitis, lymph node, FNAC, PCR

1. Introduction

Tuberculosis (TB) is a common infectious agent associated with prominent levels of morbidity and mortality, especially in developing countries [1, 2]. Globally, in 2018, about 10 million TB cases and 1.5 million TB deaths were estimated [3]. In Africa, the prevalence of TB remains as one of the major health problems due to malnutrition, poverty, and poor diagnosis. Based on a previously published report, about 30–40% of HIV patients die from TB in African countries [4]. Sudan is considered one of the endemic areas of TB. According to the WHO, in 2013, 20,181 TB cases were detected, of which 980 (30%) were new sputum smear-positive cases. However, there are many unreported cases due to the low-quality system of data reporting [5, 6]. One of the WHO TB strategies after 2015 is global reduction in TB epidemic death and incidence rate of up to 90% and 95%, respectively [7]. TB is caused by a bacterium called *Mycobacterium tuberculosis*, which is a member of *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. microti, and M. africanum*). There are two forms of clinical TB – pulmonary TB (PTB) which usually attacks the lungs and extrapulmonary TB (EPTB) that attacks other organs such as the kidneys, spine, and brain [8]. However, the major EPTB is TBLA. It causes an enlargement of lymph nodes caused by infection or inflammation [9]. Both the diagnosis and therapy for TBLA represent a challenge because it has physical and laboratory findings feature similar to other pathologic processes [10]. It is difficult to diagnose TBLA by routine methods such as the microscopic Ziehl-Neelsen (ZN) stain and microbiology culture in the Lowenstein-Jensen medium. Among the most practical applications for cytological analysis of lymph node aspirates is fine needle aspiration cytology (FNAC) [11, 12]. Fine needle aspiration (FNA) is accepted by most patients as a noninvasive method and is considered by pathologists for evaluating lymphadenopathy and preserving lymph node structure [13]. Enlarged lymph nodes are a prime target for FNA.

Although, as mentioned in the previously published studies, cytology can provide a definitive morphological prognosis of lymphadenopathy, but combination with confirmatory techniques is recommended. In the current study, the molecular technique used is polymerase chain reaction (PCR) to detect the *M. tuberculosis* complex. PCR is the molecular tool that permits the exponential amplification of target DNA [14]. The use of PCR to diagnose mycobacterial infection is not a novel procedure, however, PCR still represents a gold standard for molecular techniques and adds diagnostic value

for suspicious results. The goal of this study was to assess the sensitivity of FNAC technique compared to PCR in confirming TBLA.

2. Materials and Methods

A descriptive cross-sectional laboratory-based research was carried out at the University of Medical Sciences and Technology and Total Labcare Diagnostic Center. Fifty-five patients were included from both genders of different age groups. Fine needle lymph node aspiration specimens were collected under all aseptic precautions, using standard disposable 27-gauge needles (Figure **1**). High-quality smears were prepared and stained with Diff-Quick (Romanowski stain). In the staining procedure of

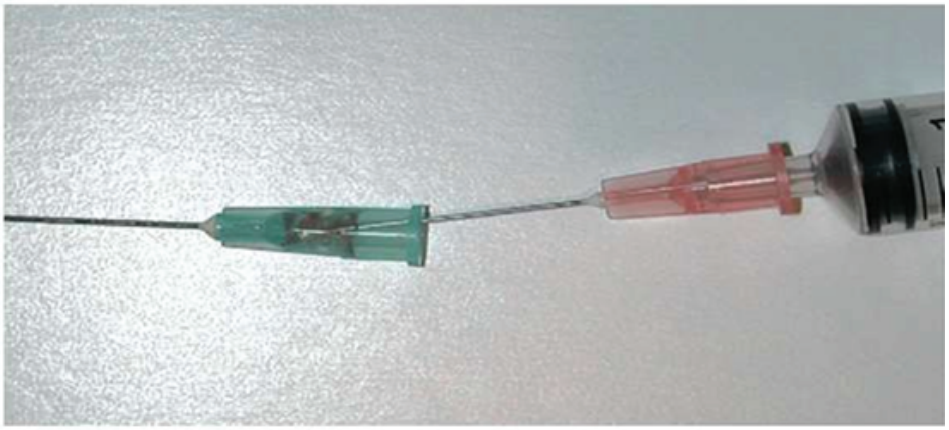

Figure 1: Collection of a sample trapped in the hub of the biopsy needle, the sample was aspirated with another needle mounted on a syringe.

Diff-Quick, air-dried smears are fixed in Diff-Quick fixative (or methanol) for 30 sec followed by stain with Diff-Quick solution I for 30 sec, and then stain with Diff-Quick solution II for 30 sec. Then, they are rinsed in tap water to remove excess stain and rapidly dehydrated in absolute alcohol. After that the slides are cleared and mounted.

2.1. DNA extraction

DNA was extracted by scraping lymph node smears material. Cinnagen, Iran Kit was used, the kit contains lysis buffer to rupture and release the cells' constituents, the precipitation solution was used to precipitate proteins with other substances, but the DNA flooded in the mixture. This method used column tube which contains silica particles plate, positive charge of the silica attached to DNA and passed other substances. The

elution buffer of this kit was warmed at 64°C, which eluded the DNA and the DNA was obtained at the bottom of new 1.5 ml eppendorf tubes.

2.2. PCR reagents

DNA template; PCR master mix contains TaqTMDNA Polymerase (5U/µl), dNTPs 2.5 mM each, reaction buffer (10×); stock primers (forward and reverse primers were 5′-GCCTACGTGGCCTTTGTCAC-3′ and 5′-C3-GTCCAGATGGCTTGCTCGAT-3′ respectively [15]; DNA MTB-positive; DDH_2O (Double Distilled water); and gel loading dye (1×).

2.3. PCR protocol

Extracted DNA was brought at –20°C, thawed, and kept on ice cryo-rack for processing. At the same time, stock primers, dNTPs, and reaction buffer were brought at room temperature (RT) and kept on ice cryo-rack for thawing. Sterile PCR water was brought out from refrigerator and aliquoted on 1.5-ml tubes.

2.3.1. PCR optimization

Small fraction of positive control (brought from tuberculosis unit in the national health laboratory) was first subjected to PCR amplification. After successful amplification, the rest of the samples were analyzed. In the events of the negative results, samples were diluted from the outer product up to one in hundred and the original DNA was diluted up to tenfold to minimize the inhibitors.

2.3.2. PCR procedure

In PCR room at the Biological Safety Cabinet Class II, the maxim PCR premix tubes (20 µl) and the DNA sample both stored at –20°C were taken out and, respectively, placed on ice and bench for thawing. Next, 2-µl TB primer mix (forward and reverse) was added to each maxim tube and 13-µl ddH_2O was added to the sample tube and positive control while 18-µl ddH2O was added to the negative control. Moreover, 5-µl DNA was added to each tube (filter tips were used), vortex for 5 sec and all samples were run in PCR machine.

2.3.3. PCR amplification of MTB gene

The amplification was carried out using 10× PCR buffer (10 mMtris-Hcl, Ph 8.3, 50 mM KCL, 1.5 mMMgcl2); 2.5mM of each dNTPs, DNA template and i-TaqTMDNA Polymerase (5U/µl) in a final volume 20 µL. Reactions were performed in a thermal cycler TC-412 with the following thermal profile: primary denaturing at 94°C for 5 min, denaturing at 94°C for 30 sec, annealing temperature at 62°C for 30 sec, extension at 72°C for 1 min, and a final extension at 72°C for 30 sec for 40 cycles for the outer PCR. Then the PCR products were examined in the agarose gel.

2.4. Electrophoresis protocol

2.4.1. 10X TBE Tris-Borate-EDTA (TBE) buffer preparation

The working solution of 1X TBE was prepared from the stock solution (1 L) which contained the following: 89 mMTris base, 89 mM boric acid, and 2 mM EDTA. It was used for agarose gel preparation and as a running buffer for electrophoresis.

2.4.2. Preparation of DNA loading dye solution, (bromophenol blue)

The dye was prepared as follows: 0.25 gm of bromophenol blue (SIGMA), 50% pure glycerol (10 ml) and 0.4 m EDTA. It was then mixed and stored in a brown bottle at 4°C.

2.4.3. Preparation of 100 base pairs ladder

DNA ladder was prepared for electrophoresis as follows: 5 µl DNA ladder (SIGMA), 5 µl water, and 2 µl gel loading dye, the mixture was stored at –20°C.

2.4.4. Preparation of agarose gel for PCR product (2%)

In clean dry bottle, 50 ml of 1X TBE buffer was added, 1 gram of agarose powder was then added to the bottle. The powder was dissolved by heating the solution in a microwave, and then cooled at room temperature (until 50°C). Next, the solution was poured in a gel tray with two combs row (well-maker) until completely solidified. In the running tank, 500 ml of 1X TBE buffer was added with 1 µl of DNA-safe stain. Agarose gel plate was placed in the running direction and the first well was loaded with DNA marker (100 pb) and the second with negative control. Samples were loaded in the next

wells, and the last well was loaded with positive control. The power supply was adjusted to 100mA for 30 min. Finally, the gel plate was transferred to the gel documentation system to visualize the DNA and photograph the bands.

3. Results

The study involved 55 patients, 24 male and 31 female, with an average age of 37.5 years. All cases showed enlarged cervical lymph nodes. Fine needle aspiration for cytological examination showed that 53 (96%) cases had TBLA with necrosis and granulomatous inflammation with or without detection of epithelioid cells, while 2 cases showed non-necrotizing granulomatous lymphadenitis with only epithelioid and inflammatory cells detected (Figures 1 & 2). Products of PCR on gel electrophoresis are shown in Figure **4**. The correlation between cytological findings and PCR results are set out in Table 1, showing a Chi-square with *p*-value > 0.05 which was considered statistically insignificant (<0.05 was considered significant).

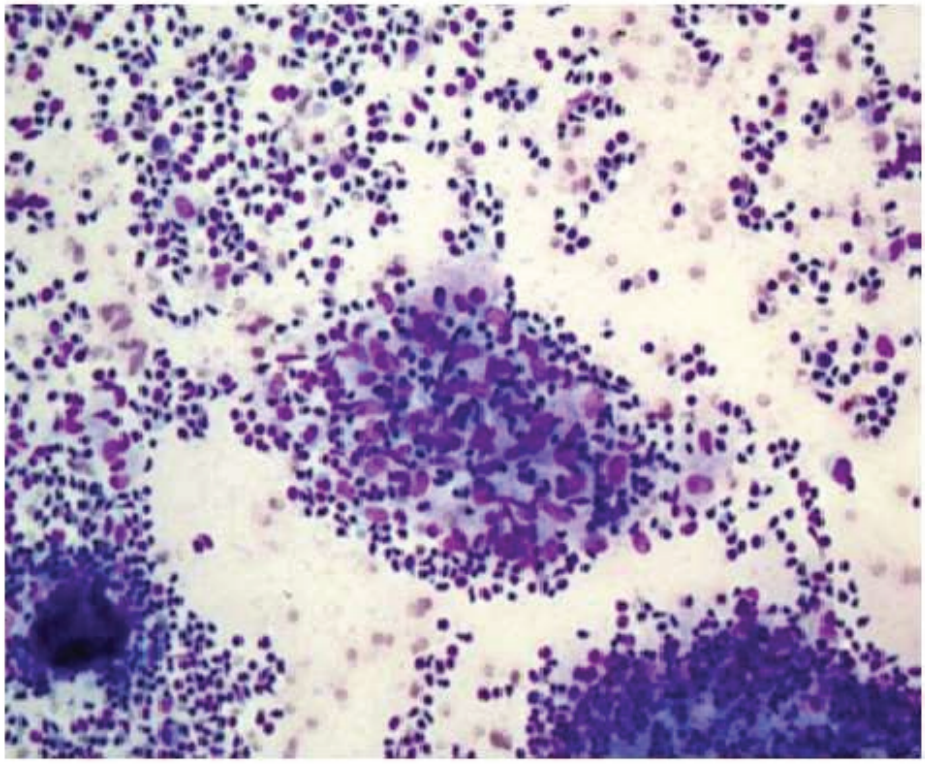

Figure 2: Tuberculosis lymphadenitis with necrotizing shows granulomas variable in size with a mixture of epithelioid macrophages and lymphocytes.

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Chi-square *p*-value was obtained = 0.335*.

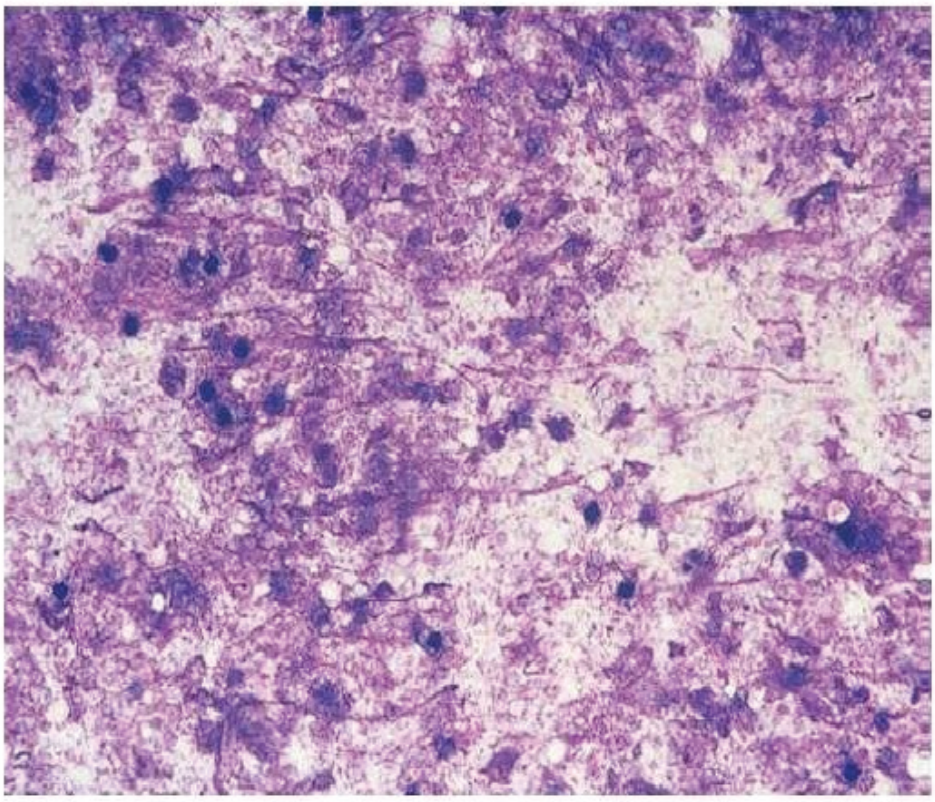

Figure 3: Tuberculosis lymphadenitis with necrotixing material and a few degenerating nuclei (Romanowsky stain).

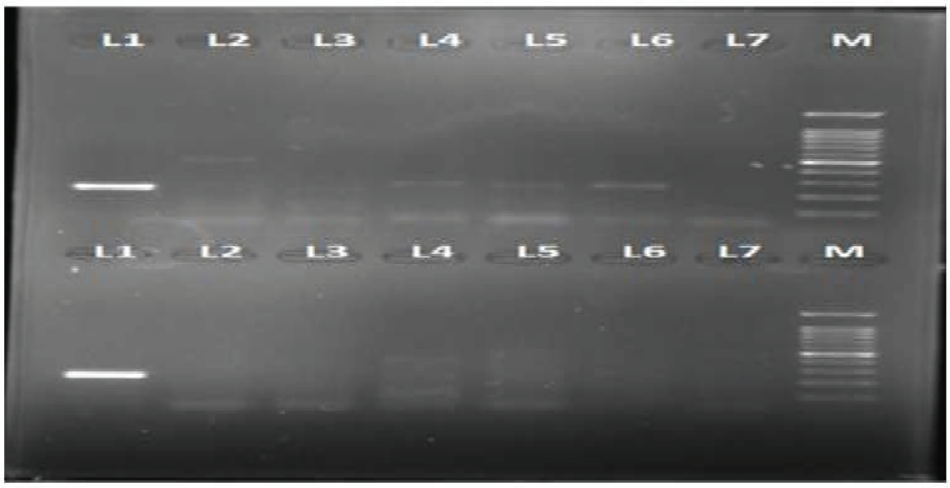

Figure 4: PCR products on gel electrophoresis under UV light. Upper row right of gel (M): DNA marker; L1: positive control; L7: negative control; L4–L6: positive samples for MTB complex; L2 and L3: negative for MTB complex.

4. Discussion

PCR is considered as one of the confirmatory methods for TB. There are other molecular techniques such as Nucleic Acid Amplification Test (NAAT) and GeneXpert (GXP) both

Table 1: Comparison of overall sensitivity of cytology and PCR.

Cross-tabulation		PCR		Total
		Positive	Negative	
Cytology	Necrotizing	17	36	53
		(30%)	(66%)	(96%)
	Non-necrotizing	0	2	2
		0	(4%)	(4%)
Total		17	38	55
		(30%)	(70%)	(100%)

of which depend on the principle of nucleic acid detection of TB. But in the current setting, the PCR technique was used as a confirmatory method for FNAC results. In the present study, 55 patients were enrolled with a suspicion of cervical TBLA. Fine needle lymph node aspirate was collected and examined cytologically. Fifty-three smears showed necrotic material while two were non-necrotic. All these smears were diagnosed as cervical TBLA. PCR was done and 17 (30%) specimens were positive and 38 (70%) were negative for MTB complex. Our specimens have shown lower PCR-positivity numbers which may be attributed to many factors, including the small amount of specimen and subsequently fewer organisms (especially, after splitting the specimen for cytological assays), internal variations in the DNA extraction and concentration. The storage temperature of the specimen could also affect the sensitivity, especially in tropical areas where the temperatures are high [16]. While our cytological findings have shown higher positivity of 53 (96%) smears with necrosis this may be due to their larger size and well-stained smears on the slide. Statistically, there was an insignificant difference between the PCR sensitivity compared to the FNAC with a *P*-value of 0.33. PCR-positive specimens also were positive for cytological smears diagnosed as TBLA. Thus, our results agree with Chantranuwat *et al.* (2006), Tansuphasiri *et al.* (2004) [17, 18].

5. Conclusion

There was no significant difference between the FNAC and PCR sensitivities in the diagnosis of TBLA. All PCR-positive cases also showed cytological positivity for TBLA. PCR could be used as a confirmatory test for MGG or Diff-Quick-stained cytological smears. PCR could be used as a practical and valuable method when no smear specimen is available. However, a molecular technique like PCR is costly and unavailable in a limited resource setting. So, FNAC could be an effective diagnostic technique for

TBLA, because it is easy to perform, cost-effective, and convenient, especially in high prevalence areas in poor economies with limited resources.

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Ethical Considerations

Informed consent was obtained from all patients.

Competing Interests

None declared.

Availability of Data and Material

Data is available with corresponding author upon request.

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