CONCORDANCE OF CYTOMORPHOLOGICAL FEATURES OF CERVICAL LYMPHADENITIS SUSPECTED FOR MYCOBACTERIUM TUBERCULOSIS ON FINE NEEDLE ASPIRATION BIOPSY WITH GENEXPERT FOR MYCOBACTERIUM TUBERCULOSIS ON ASPIRATED MATERIAL

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ABSTRACT

Objective: To correlate different cytomorphological presentations of cervical lymphadenitis suspected for Mycobacterium Tuberculosis (MTB) on Fine Needle Aspiration Cytology (FNAC) with Mycobacterium Tuberculosis detection by geneXpert on aspirated material.

Study Design: Comparative, cross sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology Rawalpindi, Jan 2017 to Nov 2018.

Methodology: Fine needle aspirates of total 100 patients with cervical lymphadenitis suspected for Mycobacterium Tuberculosis were included and the cytomorphology was compared with geneXpert. Data was analyzed using SPSS version 23.

Results: A total of 100 cases were included. A total of 78 cases were positive for Mycobacterium Tuberculosis while 22 cases were negative when compared with molecular analysis on aspirated material. Positivity for Mycobacterium Tuberculosis by geneXpert was seen in 93% of cases with both caseous necrosis and granulomas, 80% of cases with caseous necrosis only, 80% of cases having granulomas along with neutrophilic abscess, 70% of cases showing granulomas and 14% of cases with neutrophilic abscess (p-value<0.05). Sensitivity of Fine Needle Aspiration Cytology was 97% and specificity was 54%. Positive Predictive Value was 88% and Negative Predictive Value was 85%.

Conclusion: Mycobacterium Tuberculosis was detected by GeneXpert in a significant percentage of the Fine Needle Aspiration Cytology samples included in our study. Fine Needle Aspiration Cytology is a rapid, safe, easily available, minimally invasive, outpatient procedure and GeneXpert analysis can be performed on the aspirated material.

Keywords: Cervical lymphadenitis, Cytomorphological patterns, Fine needle aspiration, GeneXpert.

INTRODUCTION

Mycobacterium Tuberculosis (MTB) is a major health problem in developing countries including Pakistan[1]. According to World Health Organization estimate, approximately a third of world's population remains infected with MTB with an incidence of 9 million cases per year[2]. Most of the cases of MTB (81%) account for in twenty-two high burden countries of the world[3]. The major reasons for this high MTB burden are scarce diagnostic and insufficient treatment facilities[3]. Asia (58%) and Africa (27%) share the major MTB burden with approximately 8.6 million new MTB cases reported during the year 2012[3]. The problem is further compounded by emergence of multi drug resistant (MDR) and extremely drug resistant (XDR) strains of MTB, which not only poses a treatment dilemma but also hampering the MTB control measures in these developing countries[4]. Worldwide, 450,000 new cases of MDR TB were estimated in 2012 with high mortality rates; 9.6% of MDR strains were XDR[4]. MTB

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Received: 09 Feb 2019; revised received: 23 Jun 2019; accepted: 07 Aug 2019

is currently said to be the leading cause of death among the curable infectious diseases[5].

Currently, Pakistan is at fifth position among the twenty two countries carrying a high burden of MTB in the world[3]. According to World Health Organizations (WHO) survey, the incidence of MTB cases in Pakistan in 2012 was 231/100,000 population with a prevalence of 376/100,000 population[3]. Unfortunately, majority of MTB cases remain undiagnosed due to poverty, lack of disease awareness, inappropriate diagnostic facilities which further adds to MTB burden in Pakistan[6]. Therefore, a combined approach is required for accurate and timely diagnosis of MTB using precise clinical judgment supported well by diagnostic facilities[6].

Extrapulmonary tuberculosis (EPTB) is an important manifestation of MTB which remains undiagnosed due to diagnostic challenges in developing countries. EPTB can involve any organ of the body including appendix, small and large intestine, skin, soft tissues, genitourinary tract and brain, and most frequently, it manifests as peripheral lymphadenopathy[7]. The prevalence of EPTB is not known yet but local studies done in Pakistan report its frequency to be 25.2% and 33% respectively[8,9]. The frequency of EPTB is on a rise in developing countries due to co-infection with Human Immunodeficiency virus (HIV)[10]. In a study carried out in Pakistan, the frequency of EPTB was found to be 58% with lymph nodes as major extra pulmonary organ involved. WHO recommends the use of molecular techniques for detection of extra pulmonary tuberculosis instead of conventional microscopy smear for AFB[3].

Isolation of the organism in EPTB by culture remains a gold standard, but it requires specialized facility of Biosafety level 3 which is not available widely and takes a lot of time which may delay commencement of ATT[8]. The most commonly used techniques include direct demonstration of organisms by Ziehl Nielsen (ZN) smears which can also be used for monitoring the treatment but it lacks specificity and sensitivity due to sparse number of MTB bacilli in FNAC aspirates[9]. Detection of Mycobacterial deoxyribo-nucleic acid (DNA) using polymerase chain reaction (PCR) is very accurate but expensive which can be another challenge in poor resource settings. It can be readily performed on the aspirated samples for detection of MTB and ruling out other cytomorphological mimics. FNA cytomorphology has recently over-taken other diagnostic modalities in diagnosing EPTB as it is rapid, safe and minimally invasive tool for evaluating peripheral lymphadenopathy.

The rationale of the study was to correlate different cytomorphological presentations of cervical lymphadenitis suspected for MTB on FNAC with MTB detection by geneXpert on aspirated material.

METHODOLOGY

This comparative, cross-sectional study was carried out in the department of Histopathology in collaboration with the department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi and National institute of Health, Islamabad from January 2017 to November 2018 after taking approval of Institutional Review Board and Institutional Ethical Committee, AFIP Rawalpindi.

A total of 100 patients were included in the study using non-probability, consecutive sampling. Written informed consent was taken from the patients with the permission to publish the study later in any journal. Consent of parent/ guardian was taken in patients less than 18 years of age.

Patients of both genders and all ages having clinical suspicion of tuberculosis presenting with cervical lymphadenopathy were included in the study. While, patients declining to give informed consent, previously diagnosed cases of MTB, patients on ATT, patients suspected to have cervical lymphadenopathy other than MTB (reactive lymphoid hyperplasia/lymphoma/ metastatic carcinoma), acellular smears and cases with discrepancy in cytological opinion were excluded from the study.

FNA was performed by on cervical lymph nodes and material was aspirated. The smears were prepared, fixed by air drying and immersion in 95% ethyl alcohol. The smears were stained with Diffquick, Hematoxylin and Eosin, Papanicolaou stains for cytological analysis. Fine cytological details like epithelioid cell granulomata, Langhan's type giant cells, caseous necrosis and abscess (alone or in combination), were noted.

Simultaneously, sample from the aspirate was sent in normal saline for microbiological analysis keeping geneXpert as gold standard for diagnosis. The results were interpreted by the GeneXpert system from measured fluorescent signals and embedded calculation algorithms. Results were displayed on the results window. Lower Cycle threshold (Ct) values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template. Where MTB target DNA is detected the MTB result were displayed at High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample.

Table-I: Gender distribution.

Gender	+ve (%)	-ve (%)	*p*-value
Male	37	16	0.8
Female	35	12	

Table-II: Cytomorphological parameters with GeneXpert results.

Cytomorphological Pattern	Cases	GeneXpert		*p*-value
		+ve	-ve	
Granulomas	10	7	3	<0.05
Caseous necrosis	5	4	1	
Abscess	14	2	12	
Granuloma with caseous necrosis	61	57	4	
Granuloma with abscess	10	8	2	

High standards of biosafety measures were adopted during the procedure. It was ensured that aseptic procedures were employed during sample collection. To avoid cross contamination, personal protective clothing and sterile equipment was used. Sample processing was done in microbiology and cytology laboratory under the supervision of a qualified pathologist. All procedures were performed according to cytopathology and microbiology laboratory biosafety guidelines, standard operating procedures (SOPs) and biohazard waste disposal.

Quality assurance was ensured during the whole procedure. The FNAC slides were interpreted and reviewed by two pathologists to remove bias. Cases having discrepancy in opinion were not included further in the study to ensure uniformity. GeneXpert samples were processed as per SOPs, no expired reagent was used and samples were kept at the required temperature. Positive and negative controls were applied with each batch. Aspirates from lymph nodes showing reactive lymphoid hyperplasia with background polymorphous population of lymphoid cells was taken as negative control.

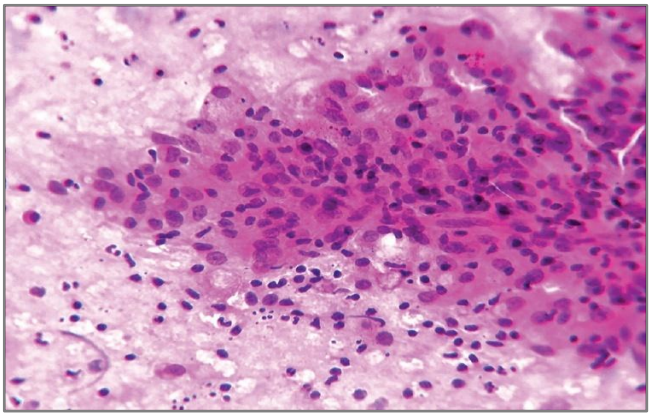

Figure-1: Epithelioid cell granuloma with background caseous necrosis (H& E).

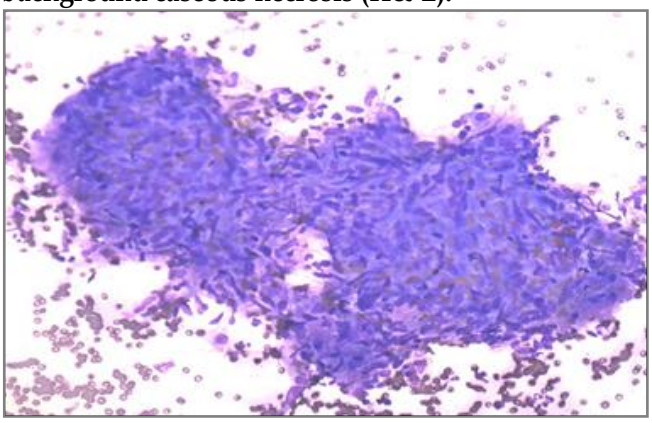

Figure-2: Collection of epithelioid cell granuloma (Diffquick).

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 23.0. Mean and SD was calculated for quantitative variables. Frequencies and percentages were calculated for qualitative variables. Chi square test was used for associations.

RESULTS

A total of 100 samples were evaluated for cervical lymphadenitis out of which 53 were males and 47 were females. Regarding gender no significant gender association was seen in our study (table-I) (*p*-value 0.8). Mean age of population in study was 31 ± 15 years. Minimum age at presentation was 4 years and maximum age was 72 years. Maximum frequency of disease was seen in young patients between 15 and 40 years. On FNAC smears, a total of 61% cases showed granulomas with caseous necrosis, 14% cases showed neutrophilic abscess only, 10% cases showed granulomas only, 10% cases showed granulomas with neutrophilic abscess while 5% cases showed caseous necrosis only. A total of 78% cases out of 100 were positive for MTB by geneXpert on aspirated material, while 22% cases were negative. Smears showing granulomas along with caseous necrosis were 93% positive for MTB by geneXpert while 7% cases were negative. Smears showing caseous necrosis only had 80% cases positive for MTB by geneXpert while 20% were negative. Smears having granulomas with neutrophilic abscess had 80% cases positive for MTB by geneXpert and 20% cases were negative, smears showing only granulomas were 70% positive for MTB by geneXpert while 30% were negative. However, smears showing only neutro-philic abscess had 14% cases positive by gene Xpert for MTB while 12 (86%) were negative (table-II) (*p*-value<0.05). The sensitivity of FNAC was 97% and specificity was 54. PPV was 88% and NPV was 85%.

DISCUSSION

Tuberculosis is a chronic granulomatous disease caused by MTB involving lungs and extra pulmonary sites; of which lymph node is the most common[7]. In spite of good progress made intreatment and prophylaxis, it still is a major global healthproblem[11-14]. Most important cytomorphological features of Extra Pulmonary Tuberculosis (EPTB) lymphadenitis include epithelioid cell granulomas, Langhan's type giant cells, caseation necrosis while few cases show super added neutrophilic abscess as well. However, gold standard for diagnosis remain microbiological culture techniques which is time consuming and molecular analysis which is not widely available[3,15].

In our study mean age of our patients was 31 ± 15 years with a wide range of 68 years. Most of the patients belonged to low and middle socioeconomic class. Maximum prevalence of disease was seen in young patients in second, third and fourth decades of life. Our findings were in line with a local study which showed mean age at diagnosis at 30 years with a range of 2-75 years with maximum incidence of disease in the fourth decade[6]. A similar study in India showed mean age at diagnosis 29 ± 14 years and maximum number of cases were from third decade[14].

No gender predilection was seen in our study with 53 subjects being males and 47 being females. Hence, no significant association was observed between gender and EPTB of cervical lymph nodes. Similarly, no gender association was seen in a local study conducted by Ikram A *et al* in Pakistan[6]. However, a number of studies from the United States, Nepal, Germany and India have reported that females were more likely to develop EPTB[15]. A recent study in Africa showed female majority with male to female ratio of 0.75:1[16]. Likewise, a study in India showed female predominance in EPTB of lymph nodes[17]. Another study in Pakistan conducted by Majeed *et al* also showed female preponderance with 68% of cases being females[7].

In our study, the predominant cytomorphological pattern was granulomas with caseating necrosis 61% followed by abscess 14% and granulomas without necrosis (10%) respectively which is consistent with literature data available based on studies done previously. An African study also showed granuloma with caseous nec-

rosis as predominant cytomorphological pattern 68% followed by granulomas only 22%[16]. Similarly a study conducted in India demonstrated granuloma with caseous necrosis as predominant pattern 49% followed by caseous necrosis 31%[18]. In a separate study, about two third cases were showing granulomas with caseous necrosis and approximately one third of the cases showed either granuloma or caseous necrosis[19]. Another study demonstrated caseous necrosis with granulomas as predominant cytomorphological finding 43% followed by caseous necrosis only 32% and granulomas only 25%[15].

Microbiological confirmation for MTB was done in all aspirates and 93% of smears showing granulomas alongwith caseous necrosis were positive for MTB. A total of 70% smears having only granulomas were positive for MTB while cases showing only abscess had low yield for MTB; 14%. A similar regional study also concluded cases with caseous necrosis with granulomas were 88% positive for MTB, cases with only granuloma were 83% positive and cases with necrosis alone were 84% positive when compared with microbiological results[17]. A study in Pakistan by Majeed *et al* showed significant association between caseous necrosis and AFB detection by microbiological techniques (93%)[7]. These all findings are in agreement with the results of our study.

However, arecent study done in India showed discordant results. It concluded that caseous necrosis with granulomas on FNAC had limited value 53% in predicting MTB lymphadenitis when compared with microbiological results whereas caseous necrosis had greater diagnostic role when tuberculous lymphadenitis is suspected with 78% positive results[18].

Sensitivity of FNAC in our study was 97% while specificity was 54%. A study conducted on fine needle aspirates of lymph nodes suspicious for MTB found similar results and described sensitivity and specificity of FNA showing classical cytomorphology for MTB to be around 97% and 100% respectively when compared with microbiological analysis[16,20]. An Indian study on pediatric Tuberculous lymphadenitis reported sensitivity and specificity of 98% and 100% respectively[21].

The advent of molecular techniques in last few decades has vastly improved diagnostic accuracy of infectious communicable diseases like MTB. However, these techniques are expensive and not widely available in our country where excellent diagnostic healthcare facilities are limited to tertiary care centers only. Our study advocates that FNAC is a reliable diagnostic tool for tuberculous lymphadenitis. Its usefulness has been widely reported by many authors as well[22,24]. Parameters such as caseation necrosis with granulomas show high association with MTB lymphadenitis. However, aspirates showing only abscess or granulomas alone may be evaluated accordingly for other causes like acute bacterial lymphadenitis, sarcoidosis or fungal infection based on clinical suspicion.

CONCLUSION

FNAC was found as a rapid, safe, inexpensive, easily available, minimally invasive and an outpatient procedure for the diagnosis of cervical lymphadenitis suspected for MTB. The results of FNAC are available within few days which can aid clinician in timely initiation of Anti Tuberculous Treatment in country like ours where advanced diagnostic facilities are not easily accessible. Where ever possible, a multi disciplinary diagnostic approach involving clinical suspicion, radiological findings, histopathology and microbiological analysis must be adopted for best possible results and patient management.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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