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Agreement Test of Histopathology in the Diagnosis of Extrapulmonary Tuberculosis with Gold Standard Polymerase Chain Reaction Technique: A Step to Overcome False Diagnosis

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Abstract

BACKGROUND: Tuberculosis (TB) is global health problem which is caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) bacteria. One-quarter of the world's populations is infected by *M. tuberculosis* and only 10–15% of those develop the disease, while the remaining 85–95% of the population are carrying the bacteria and cannot transmit the disease to the others. *M. tuberculosis* bacteria affects the lungs, but any organ in the body can be affected by the bacteria. About 15% of *M. tuberculosis* infections are of in the extrapulmonary type. The diagnosis of extrapulmonary TB (EPTB) is very challenging because most sites are inaccessible and paucibacillary nature of the bacteria in these sites. The need for rapid and more sensitive and specific tests for the diagnosis of EPTB in comparison to culture and histopathology is increasing. The molecular methods for the detection of *M. tuberculosis* gene(s) in the provided sample are now promising.

PATIENT AND METHODS: A cross-sectional descriptive study at AL-Kindy Teaching Hospital at Al-Resaffa part of Baghdad city, Iraq. Data collection has been done in three months duration (July, August, and September) 2015. A total of 74 formalin-fixed paraffin-embedded samples from suspected EPTB cases was collected, both Polymerase Chain reaction test for *M. tuberculosis* and histopathological examination was done for each sample.

RESULTS: A total of 74 patients (18 males, 56 females), mean age 29.72 suspected to had extrapulmonary TB underwent biopsies from different tissue types. The biopsies from the 74 patients were taken from different tissues according to the site of lesion, 49 (66.2%) biopsies were taken from lymph node, 12 biopsies (16.2%) was taken from mass in the axilla, 6 (8.1%) from abscess, 4 (5.4%) from the intestine, 3 (4.1%) from fistula. Of the 74 studied patients 57 (77%) showed positive polymerase chain reaction (PCR) and 17 (23%) showed negative PCR results. Regarding to the histopathological reports of the biopsies, there were 54 (73%) patients had positive histopathological (granuloma) result and 20 (27%) patients had negative results (nongranuloma). The sensitivity of histopathological examination of the biopsies was 91.02%, the specificity 88.2%, and the kappa was 0.748 (p = 0.00) which is mean good agreement between histopathological examination of the biopsies and the polymerase chain reaction test.

CONCLUSIONS: The sensitivity, specificity, and the positive predictive value of histopathology examination of biopsies were 91.02%, 88.2%, and 96%, respectively. The kappa was 0.748 (p = 0.00) which is mean good agreement between histopathological examination of the biopsies and the polymerase chain reaction test.

Introduction

Tuberculosis (TB) is a global health problem which is caused by *Mycobacterium tuberculosis* bacteria. It has been stated that one-quarter of the world's populations is infected by TB and only 10–15% of those develop the disease, while the remaining 85–95% of the population are carrying the bacteria and can't transmit the disease to the others. Ten millions will be ill every year most of them from low and middle-income countries and 1.5 millions die every year according to the WHO report [1]. *M. tuberculosis* bacteria affects the lungs, but any organ in the body can be affected by the bacteria. About 15% of TB infections are of in extrapulmonary type [1]. The most common sites for extrapulmonary tuberculosis (EBTB) are lymph nodes and pleura although other sites such as meninges, bones, intestines, and any other organs may be affected [2], [3]. The diagnosis of EPTB is very challenging because most sites are inaccessible and paucibacillary nature of the bacteria in these sites. Negative smear form acid-fast bacilli and absence of granuloma in histopathology and negative results from TB culture do not exclude the diagnosis [4]. The need for rapid and more sensitive and specific tests for the diagnosis of EPTB in comparison to culture and histopathology is increasing. Several attempts were tried such as the detection of adenosine deaminase detection in serum or effusion fluids but showed variable results [5], [6]. The molecular methods for the detection of TB gene(s) in the provided sample are now promising. In addition, many researches are published for this aspect with variable results [7], [8]. Some manufacturers tried to innovate a method for simultaneous detection of TB genes and the resistance

gene, if present, in the same polymerase chain reaction (PCR) reaction, and some of manufactures success to get Food and Drug Administration approval for this innovation [9].

This study was designed to study the sensitivity of real-time PCR test in the diagnosis of EPTB in comparison to histopathology results.

Patient and Methods

This is a cross-sectional descriptive study carried out by collecting formalin-fixed paraffin-embedded (FFPE) blocks from laboratory for patients suspected to have EPTB from July, 01, 2015 to October 01, 2015.

Inclusion criteria necessitate that all patients should have suspected EPTB affecting any organ except the lung.

Exclusion criteria include any patient with EPTB affecting meningies, pleura, and any organ that necessities a body fluid sample for the diagnosis.

Sample size constituted from 74 patients both males and females are included in the study. Sample size was determined using Epi-Tools Epidemiological calculator with 0.95 confidence interval.

The study was done in accordance with Helsinki Declaration and it received a scientific and ethical approval.

Histopathology examination

Tissue samples were processed by standard wax techniques. The FFPE tissue blocks were cut in 4 μm serial sections. The sections were stained with H and E method [10]. Then slides were evaluated microscopically at increasing magnifications (×100 ×400). Histopathologic Classifications as following:

- **Caseating granulomas**: TB granuloma displaying central necrosis with or without mineralization surrounded by macrophages, lymphocytes, plasma cell, neutrophils, epithelioid cells, and langhan's giant cells and enclosed partly or completely by a thin capsule. Were considered as strong evidence for TB [11]
- **Non-caseating granulomas:** Lesion characterized by irregular unencapsulated clusters of epithelioid macrophages but not langhan's type multinucleated giant cells and necrosis, were considered as weaker evidence for TB [11]
- **Without granulomas:** Features not consistent with TB granuloma, including significant eosinophilic infiltrates, lymphoid hyperplasia, and presence of bacterial colonies within necrotic area or tumors [11].

DNA extraction from FFPE

- Five serial sections (5 μm thick) were cut from each paraffin-embedded tissue block by microtome blade and put in 1.5 mL eppendorf tube for DNA extraction (Leica 2135). For prevention of other contamination, the microtome blade was cleaned with xylene and absolute ethanol after each sample sectioning
- DNA was extracted from FFPE using promega extraction kit according to manufacturer instructions.

MTB gene amplification

DNA was amplified using exicycler 96 real-time PCR machine (Korea) and using Accupower MTB real-time PCR kit which amplifies the target MTB gene IS6110. The kit includes vacuum dried premix, positive control (PC), no template control,and internal PC in all wells to confirm correct PCR amplification. Final results were analyzed by Exidiagnosis software which automatically analyzes the test results based upon the threshold cycle (Ct) value.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-20 (Statstical Packages for Social Sciences- version 20). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different percentages (qualitative data) were tested using chi-square test (c2-test) with application of Yet's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the $p < 0.05$.

Results

A total of 74 different patients (18 males, 56 females) suspected to have extrapulmonary TB underwent biopsies from different tissue types with age between 2 and 58 years (mean age 29.72 years ± 14.16 SD). The biopsies were taken from different tissues according to the site of lesion as shown in Table 1.

For the 74 samples tested, 52 were positive by both PCR and histopathology (caseating and noncaseating granuloma favor TB) and five are positive for PCR with Negative histopathological result (Non specific granuloma not favoring TB) as shown in table 2.

Table 1: Tissue type from whom tissue biopsy taken

Type of tissue	No. of biopsy (%)
Lymph node	49 (66.2)
Axillary mass	12 (16.2)
Abscess	6 (8.1)
Intestine	4 (5.4)
Fistula	3 (4.1)
Total	74 (100)

Sensitivity, specificity, positive predictive value, and agreement

In this study the sensitivity of histopathological examination of the biopsies was 91.02%, the specificity 88.2%, positive predictive value 96% and the kappa was 0.748 (p = 0.00) which is mean good agreement between histopathological examination of the biopsies and the polymerase chain reaction test.

Table 2: Relationship between histopathological result and polymerase chain reaction

Histopathological examination	Polymerase chain reaction		p-value	Total
	Positive	Negative		
Positive	52 (91.2)	2 (11.8)	<0.000001	54 (100)
Negative	5 (8.8)	15 (88.2)		20 (100)
Total	57 (73)	17 (27)		74 (100)

From Tables 3 and 4 below, PCR gives high positive results in L.N biopsies as compared to other tissue biopsies and histopathology evidence (strong or weak evidence).

Table 3: Distribution of tissue biopsies with positive PCR test

Tissue type	Number of positive PCR (%)
L.N	42 (73.7)
Axillary mass	8 (14.1)
Abscess	3 (5.2)
Intestine	2 (3.5)
Fistula	2 (3.5)
Total	57 (100)

PCR: Polymerase chain reaction

Discussion

The diagnosis of EPTB is challenging issue with the need for rapid and highly sensitive and specific techniques. The diagnosis is mostly depending on histopathological examination of the sample. Although histopathology is considered a gold standard method for the diagnosis, this carries some limitations as the reports to be ready this require about 15–20 days with the need of experience of a professional pathologist. In addition to that, the need for the presence of caseating tubercle granuloma is a must for strong evidence of the diagnosis although some cases do not show this evidence [12], [13]. In certain cases of EBTB, rapid diagnosis and initiation of early anti-tuberculous therapy is mandatory to save patients life and this urges the need for rapid and sensitive methods for the early diagnosis. Molecular detection of TB bacilli arises before many years ago to satisfy this demand. However, the detection of TB bacilli in FFPE sample is a challenging technique for acquiring good results, this depends on many factors such as the fixation protocol, the age of the paraffin block, and the presence of endogenous or exogenous inhibitors of the reaction [10], [14].

Table 4: Distribution of tissue biopsies with positive and negative PCR test in relation to histopathology evidence (strong or weak evidence)

Type of biopsy	Positive PCR with Histopathology weak evidence for TB	Positive PCR with Histopathology strong evidence for TB	Negative PCR with Histopathology strong evidence for TB	Negative PCR with Histopathology weak evidence for TB	Total
L.N	0	42 (85.7%)	0	7 (13.3)	49
Axillary mass	0	8 (66.7)	0	4 (33.3)	12
Abscess	1 (16.6)	2 (33.4)	1 (16.6)	2 (33.4)	6
Intestine	2 (50)	0	1 (25)	1 (25)	4
Fistula	2 (75)	0	0	1 (25)	3
Total	5	52	2	15	74

PCR: Polymerase chain reaction, TB: Tuberculosis.

Among the 74 patients selected in this study, 66 % of them are presented with cervical lymph node enlargement from those with LAP, this is in consistent with what was reported previously [15]. Tuberculous lymphadenitis is one of the most common presentations of extrapulmonary TB with rapid and accurate diagnosis is very essential as delaying in the diagnosis results in high risk of complications such as suppuration and fistula formation while the confirmed early diagnosis is required to initiate systemic anti-tuberculous therapy. Furthermore, there is a challenging issue in the diagnosis since there is a possibility of nontuberculous lymphadenitis in the differential diagnosis of neck lymph node mass. In this study, 85% of cervical lymphadenitis is positive for both histopathology and PCR, while 13% are negative for PCR only with histopathology of less evidence for TB. This is can be explained by the fact that there are many diseases can give histopathological features of noncaseating granuloma such as sarcoidosis, then there is a possibility that the 13% of cases that gave negative PCR may be included in this group of disease [16], [17].

For the axillary mass, it represents 16% from samples enrolled in this study; however, there are 66% of axillary mass samples were positive for both PCR and histopathology. This can be explained by the fact that there are many diseases (both benign and malignant) may presented with axillary mass. For the malignant one, lymphoma and metastatic breast cancer, lung, and thyroid cancers may presented with axillary mass. However, benign granulomatous mass may be infectious (TB, toxoplasmosis) and noninfectious (sarcoidosis and berylliosis) [18].

For the intestinal samples, they represent 5.4% of the total samples with 50% showed positive PCR with low evidence of TB in histopathology while the other 50% showed negative PCR. One explanation for this is that inflammatory bowel disease especially Crohn's disease represents an important dilemma in differentiation from TB [19]. PCR will resolve this problem as one protocol for the differentiation is to give anti-tuberculous drugs as a therapeutic trial.

The limitations of this study are: low samples number, the patients whose their FFPE samples give positive or negative PCR results should be followed up to prove the diagnosis, other tests such as Interferon Gamma Release Assay is needed although it is not confirmatory it may give an idea for the patient status, and the PCR tests required to be done both on fresh and FFPE samples to overcome the factors that may affect the results.

Conclusion

In conclusion, this study showed that there is an agreements between histopathology and PCR in regards to sensitivity and specificity (91.02% and 88.2% respectively).

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