Vivek Gupta

Dr. Vivek Gupta is a Pathologist working in division of cytopathology, his area of research interest is Molecular Pathology. His research provided the first direct evidence of tuberculosis in reactive lymph nodes and focused on detection of tuberculosis infection in lymph nodes through molecular techniques.

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ISID Research Grant Report

Assessment of Clinically Suspected and Unsuspected Tubercular Lymphadenopathy by PCR Compared to Non Molecular Methods in Lymph node Aspirates

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Introduction

Peripheral tubercular lymphadenopathy (TBLN) presents a diagnostic challenge especially when it is associated with non-specific symptoms and deceptive clinical signs [1]. The diagnosis, based on Fine Needle Aspiration Cytology (FNAC) or tissue biopsy, which interprets tuberculosis as epithelioid cell granuloma with or without multinucleated giant cell and necrosis [1, 2]. Granuloma formation is an immunological response triggered by Mycobacterium tuberculosis (M.tb) antigens mediating delayed type of hypersensitivity, usually takes 14-100 days to develop [3]. Further, in paediatric patients, the immunity is not developed until the age of 14 years, due to which the cytological features are not evident [4].

The changes that foreruns the granuloma formation are para-cortical hyperplasia (T cell medicated immune response) or accumulation of activated macrophages, which considered as an evidence of reactive lymphoid hyperplasia. Patients with such lymph nodes fail to get anti-tubercular treatment.

Conventional, diagnostic methods like Ziehl Neelsen (ZN) staining for acid-fast bacilli and culture for lymph node aspirates or biopsy can help to provide the evidence of tuberculosis by detecting tuberculosis bacilli, but ZN staining lacks sensitivity and specificity, and culture is time consuming [5,6]. Molecular Pathology technique based on principal of Polymerase chain reaction (PCR) are sensitive, specific and rapid but there role had not yet been utilized in the existing problem scenario.

Objectives

1. To compare the results of real time PCR on fine needle aspirates with laboratory gold standard comprising of culture in clinically suspected and unsuspected tubercular lymphadenopathy.

2. To study the role of PCR in evaluation of lymphadenopathy in paediatric population.

3. To evaluate the role of PCR in diagnosis of Mycobacterium tuberculosis complex in lymph node aspirates compared with culture in evaluation of reactive lymphoid hyperplasia.

Materials and Methods

The study carried out with the guidelines of Central Ethics Committee of Human Research. FNAC performed on patients presenting with lymphadenopathy and aspirate divided into five parts: (a) Papanicolaou staining, (b) ZN stain, (c) May Gürwald Giemsa stain, (d) culture and (e) real time PCR for Mycobacterium tuberculosis complex targeting insertion sequence IS6110. Data was statistically analysed.

Results and Discussion

Total 214 patients including 45 paediatric patients enrolled in this study. The results of real time PCR were as follows: Sensitivity,77.3%; Specificity,69.6%; Positive predictive value,72.8%;

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Negative predictive value,74.4%;Accuracy,73.5%. Pearson Chi-square statistics is significant at P≤0.0005. PCR could additionally detect 82 cases that were missed on ZN staining which is also comparable with other reports [7].The sensitivity of PCR raised up to 84.6% in paediatric age group [8].

The results were analysed further in cases of reactive lymphoid hyperplasia. Out of 112 cases of reactive lymphoid hyperplasia, 35 (31%) cases were positive on both PCR and culture. PCR was positive in 43 cases. Findings are been sent for review in Journal.

We further evaluate the concordance of diagnostic modalities individually and in combination against culture for assessing utility in diagnosis of TBLN. Positive agreement in PCR was 40% and in cytology was 28%, PCR diagnosed additional 12% of positive cases missed by cytology and proved to have better diagnostic power than that of cytology. The concordance rate was high for any one of the diagnostic modality either cytology or PCR being positive at 75%. It was lowest when cytology and PCR combined for making concordance of 60.3%.

Limitations

The target insertion sequence IS6110 adopted and used for TB PCR in the study was for commonly infecting mycobacterium in reference to molecular epidemiology of mycobacterium tuberculosis in India.The atypical mycobacteria were not the part of targeted insertion sequence utilized for PCR. This would have probably limited the detection of atypical mycobacteria commonly associated as a cause of TBLN.

Conclusions

The study concludes that PCR is useful molecular method for detection of for Mycobacterium tuberculosis complex on aspirates of clinically suspected and unsuspected TBLN cases. PCR had a distinct advantage of detecting the TBLN cases that otherwise were missed or diagnosed as reactive lymphoid hyperplasia on cytology/histopathology. Diagnostic method of PCR detected TBLN at an early stage even before its evidence on cytology/histopathology and thus expanded the numerical catchment of cases. PCR for Mycobacterium tuberculosis complex has proved to be highly sensitive, specific and accurate in diagnosis paediatric tuberculosis.

Scope of the Research Work

The pathogenesis of tuberculosis at cellular level passes through various morphological events, providing little evidence of tuberculosis on cytology or histopathology, thus keeping these cases in diagnostic dilemma. PCR in such cases have a wide scope to apply on aspirates in diagnosis of TBLN.

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