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Original Research Paper

Pathology

TO STUDY THE DETECTION OF LATENT TUBERCULOSIS IN HIV POSITIVE PATIENTS FROM CHHATTISGARH STATE

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ABSTRACT

Background- The early diagnosis of latent TB infection helpful to patients for better management and treatment of disease. A new approaches to the development of latent TB diagnostics remains a priority of our goals in developing a more sensitive, specific and affordable test that can be applied to the diagnosis of latent TB. Objective- The estimation of CD4, CD8 count of patients, collection of sputum sample of HIV patients, isolation of DNA from sputum sample and PCR based investigation of latent TB infection. **Methodology-** The study was carried out on 11 subjects with known HIV infection after getting inform consent from the patient. **Result-** In our study HIV positive patients without TB culture positive, for the detection of latent TB by employing PCR method.

KEYWORDS :

INTRODUCTION

HIV positive patients whose immunity is get suppressed, prone to suffer from many opportunistic infections; most common of them is Tuberculosis.[1] Tuberculosis commonly known as TB caused by Mycobacterium tuberculosis (MTB). From Close contact of infectious TB patients, 30% of people get infected with MTB. [2] More than two billion people, equal to one third of world's total population, are infected with MTB. Nearly 9 million people develop TB disease each year and an estimated 1.6 million die from disease. [3]About 30-40 % deaths of HIV positive patients is due to TB. By detecting TB in latent stage, HIV positive patients life can be prolonged for several years.[4]Out of latently infected people, 10% develops active TB disease in their life time.[5]At the most fundamental level, latent TB can be viewed as equilibrium between host and bacillus.[6]Most people, who breathe in TB bacteria, become infected, the body is able to fight the bacteria to stop them from growing.[7] In most cases, the host response is sufficient to forestall active disease for a lifetime. However, occasionally the immune response fails in some way and infection reactivates to cause active disease.[8]TB typically affect the lungs, but it may affect any other organ of the body, even if bones. Pulmonary tuberculosis (PTB) is the consequence, when MTB infect the lungs, extra pulmonary tuberculosis results when, MTB infection occurs in any other part of the body.[9]XDR-TB is less common form of MDR-TB, in which MTB can circumvent two best antibiotics, isoniazid and rifampin as well as most of the alternative drug against MDR TB e.g. fluoroquinolone.[10]The early diagnosis and adequate treatment of infectious patient with TB are considered necessary to reduce transmission of MTB and to achieve disease elimination. IF not treated, each person with active TB can infect 10-15 people a year on an average. [11] One third of the world's population infected with MTB represents more than a quarter of the world's preventable deaths .[12]

MATERIALS AND METHODS

Present study was carried out on 11 subjects with known HIV infection from Chhattisgarh State, after getting inform consent from the patient. Information regarding educational qualification, marital status, and socio-economic status, family history of any bronchial disease, addiction, present illness, ART treatment and physical examination were collected from each subject. About 5 ml of peripheral blood & early morning sputum sample was collected from each subject. Blood was used for estimated CD4 count, TLC and hemoglobin concentration. Sputum sample was used for DNA isolation and PCR studies.

CD4, CD8 Estimation

CD4, CD8 count was estimated by using flow cytometer, Munster, Germany.

Genomic DNA Isolation

For the isolation of genomic DNA standard chloroform-phenol protocol was applied with slight modification. Sputum sample was put in 1.5 ml of micro centrifuge tube.Append off AG, Hamburg Germany. In the sample 0.5 ml of lysis buffer containing, Tris buffer, SDS, EDTA, N.P.40- detergent & 0.01 % of Protease K is added, mixed gently & incubated at 56° C. for 60 minutes. After incubation 400 μl of chloroform& 200 μl of sodium Acetate, were added and mixed gently, & and centrifuged at 10,000 rpm for 5 minutes. The supernatant was transformed to a new micro centrifuge tube and repeat chloroform sodium acetate step. The DNA was precipitated from supernatant with 400μl of isopropyl alcohol. (Ice cold). The DNA pellet was washed with70% of ethanol. Dried dissolved in Tris EDTA buffer containing 10 mmolTris HCL, 1 mmol of EDTA & pH 7.6. & stored at - 20° C. The final conc. of extracted DNA was adjusted to 2μg/ml.

PCR Amplification

PCR reaction was carried out in 25μl reaction volume containing 2μg/μl of genomic DNA, 2.5 μl of 10x Taq polymerase buffer with 1.5 m. mol of Magnesium Chloride, 200 μmol of each DNTP, 15 μg of each primer and 1 unit of Taq DNA polymerase enzyme. The oligonucleotide primer with the SIS 6110 insertion sequence as reverse primer 5'GTGCG GATGGTGGCAGAG and forward primer as 5'CTCGATGCCCTCACGGTT.The DNA amplification was performed in PTC-200 thermal cycler MJ research. U.S.A. The PCR conditions were 95°C for 5 minutes followed by 40 cycles at 94° C for 30 seconds, 65° C for 30 seconds and 72° C for 30 seconds. The thermal cycler was started with initial denaturation of 96° C for 5 minutes and final 72° C extension for 10 minutes, for polishing the ends of PCR product.

Agarose Gel Electrophoresis

The resulting PCR product was resolved. PCR product were mixed with 2μl gel loading dye on 1.5% agarose gel using submerge gel electrophoresis in 1x TBE buffer containing Tris HCL, boric acid & EDTA, pH 8., Subsequently gel was stained with ethidium bromide 10 mg/l and photographed on UV transillumintor using a gel documentation system, Alpha Digi DOC.1201 documentation system U.S.A. A known DNA size marker was ran with every gel.(100 base pair DNA ladder) The reproducibility of PCR was tested by performing duplicate reaction at different time and only reproducible bands were selected.

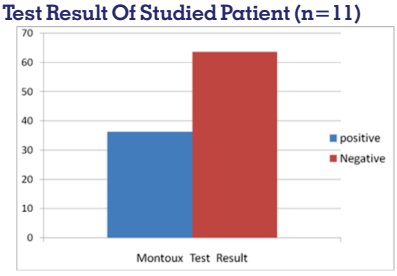
Statistical Analysis

Statistical significance was calculated by using GraphPad Prism software (version 7.0)

OBSERVATIONS AND RESULTS

In the present study we have chosen 11 HIV positive patients without TB culture positive, for the detection of latent TB by employing PCR method.In the present study we selected 05males and 06 females with mean age of patient were 39.88 ± 2.76 years and their age ranges from 32 to 50 years. The mean age of male patient was of 42.20 ± 2.33 and female patient as 36.17 ± 1.42 years ($p<0.0474$). A significant illiterate to primary schooling education was noted in the patient's father. 36.36% patient's father was illiterate followed by 36.36% primary schooling and 18.18% patient's father up to high school. Table no.3 also notes that 54.54% patient's mother having education up to primary schooling, followed by 36.36 % mother education up to high school. None of the patient's mother has education above high school. The family history of any bronchial disease of patients, we observed that 27.27% patient's family with the history of positive bronchial disease. Addiction wise distributions of patients, we observed that maximum patients (n=5) with addiction of tobacco chewing were noted followed by cigarette smoking (n=4) and beetle nut & beetle leaves addiction (n=1). The chosen HIV positive patients were undergone montoux test and their data was summaries in table no.6, 36.36% patients with positive montoux test and 63.63 % patients with negative montoux test resulted. In the clinic-pathological investigation data of patients, BMI of male patients were 19.89 ± 0.41 and female were 21.05 ± 0.44 was shown. A significant decrease in CD_4 counts, that is for Male: 174 ± 4.88 and female: 186 ± 4.06. TLC was significantly decreased in patients, that is for Male: 7.89×10^9, female: 7.793×10^9. A hemolytic anemia was shown in patients as 12.56 ± 0.56 mg% in males & 11.02 ± 0.25 mg% in females. The demonstrated presence of latent TB in patient by employing PCR methodology. 36.36% studied patients were detected positive for TB infection & 63.63% patient's detected negative for TB infection.40% of studied male patients were positive for TB infection while 33.33% females were positive for TB infection.

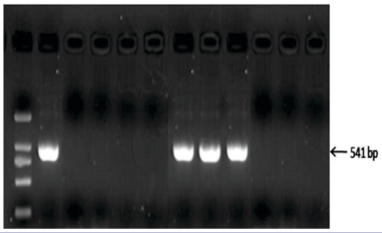
Montoux Test Result Of Studied Patient (n=11)



Clinical Profile Of Studied Patient (n=11)

Gender	B.M.I.	CD4 Count	TLC Count	Hb%
Male (n=5)	19.80±0.41	174.2±4.88	7.89×10^9	12.56±0.56
Female (n=6)	21.05±0.44	186±4.06	7.793×10^9	11.02±0.25

PCR Finding Of Latent TB In Studied Patients And Photograph Of Gel Electrophoresis Of Studied Patients.



DISCUSSION

The course of an M. tuberculosisinfection is very complex, with only 5 to 10% of infections thought to progress to active TB in a short time. In most infected individuals, some bacteria are believed to survive and enter a quiescent phase, resulting in latent infection, which can reactivate later in life. So, infection with M. tuberculosis can give rise to disease at any time, from months to decades, after infection. The estimated one-third of the world's population that harbors latent TB therefore represents an immense infection reservoir and future source of disease transmission.

PCR is the method of choice for the diagnosis of mycobacterium tuberculosis in cases where suspicious is high but ZN staining is negative. When this sample is positive for ZN staining, PCR permits distinction of mycobacterium tuberculosis complex and other mycobacterial infections. DNA PCR method is advantages for reducing false positive reaction and false negative reaction. Resulting in increasing specificity to diagnose clinical samples for latent TB. Diagnosing of HIV positive patients is cost effective, sensitive, specific and required less time. DNA PCR performance for detection of latent TB infection is replacing routine culture for diagnosis and shows superiority and helpful for early diagnosis of latent TB.

CONCLUSION

New approaches to the development of latent TB diagnostics remains a priority of our goals in developing a more sensitive, specific and affordable test that can be applied to the diagnosis of latent TB. The rapid technological evolution in the laboratory diagnosis of TB, especially in the application of molecular biology has diminished the time required for identification and susceptibility testing and may eliminate the need for lengthy culture and protracted biochemical methods. From the study we conclude that on the basis of DNA PCR technique we can diagnose and prognosis latent TB infection in HIV positive patients. This early diagnosis of latent TB infection helpful to patients for better management and treatment of disease. With continuous efforts at increasing their reproducibility, ease of performance and cost containment PCR technique will soon evolve from reference laboratories into clinical diagnostic laboratories.

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