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Line Probe Assay Is Better Than CBNAAT for Diagnosis of Ileocolonic Tuberculosis: A Prospective Study

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ABSTRACT

Introduction:
Diagnosis of Ileocolonic Tuberculosis remains a challenge due to its nonspecific clinical and laboratory characteristics. Role of Line probe assay in Ileocolonic ulcers for the diagnosis of intestinal tuberculosis and Non Tubercular Mycobacterium is not well defined. We report the utility of Line Probe Assay (LPA) testing in patients with Ileocolonic ulcers and its comparison with Cartridge based nucleic acid amplification test (CBNAAT) and histopathology. **Methodology:** A prospective study of 35 patients with Ileocolonic ulcers detected during colonoscopy. Ileocolonic ulcer tissue was subjected to Line probe assay (GenoType Mycobacterium CM kit) for detection of Mycobacterium tuberculosis (MT) and Non tubercular Mycobacterium (NTM), CBNAAT (Gene Xpert), and Histopathology. Patients were followed up for six months and repeat colonoscopy was done for response of therapy and to assess diagnostic accuracy. **Results:** Out of 35 patients, 31 patients were analysed. 16 patients diagnosed to have TB were started on anti-tubercular treatment based on clinical presentation, LPA, CBNAAT and histopathology. 15 patients had other diagnosis. Sensitivity and specificity of LPA for diagnosis of ITB was 28.57% and 50%whereas The sensitivity of CBNAAT was 7.14% and the specificity was 100%.Histopathology was positive in 12 patients (38.7%). Positive predictive value of both LPA and CBNAAT were comparable. **Conclusion:** LPA provides an additional investigation in the armamentarium for the diagnosis of Ileocolonic tuberculosis .Although CBNAAT is generally recommended in the diagnosis of tuberculosis in extra-pulmonary specimens, utility of the same in the setting of Ileocolonic ulcers appears limited as demonstrated in this study.
Keywords: Intestinal tuberculosis, Line probe assay, Polymerase chain reaction, Cartridge based Nucleic acid amplification, Gene Xpert, Crohn's disease, Ileocolonic Ulcers

Introduction:

Intestinal Ulcers (Colonic and Ileal) are common finding during colonoscopy. The aetiology of these ulcers includes Tuberculosis, Crohn's disease, Nonsteroidal anti-inflammatory drugs, infectious causes like Yersinia, Amoebiasis, Ischemic and Idiopathic ulcers. When histopathological examination reveals granulomas in these ulcers, differential diagnosis of Tuberculosis vs. Crohn's disease is usually considered. Although there are few differentiating points to diagnose either of these

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conditions, in reality it is difficult and many patients end up taking empirical anti tubercular treatment. Hence we need better diagnostic tools for diagnosis of intestinal tuberculosis.

Literature review:

Tuberculosis is one of the major infections worldwide. Extrapulmonary forms of tuberculosis are increasingly reported. The aetiology of Crohn's disease is also not clear. Infectious causes including Non tubercular mycobacterium (NTM) has been postulated as etiological factor for Crohn's disease.[1,2,3].There are, however, many diverse opinions[4-5].To date, more than 160 NTM species have been recognized, while many others await classification. Approximately one-third have been associated with diseases in humans[6].The most frequent forms of NTM include *Mycobacterium avium* complex (MAC, 61%), *Mycobacterium fortuitum* (19%) and *Mycobacterium kansasii* (10%), with smaller percentages of *Mycobacterium gordonae* and *Mycobacterium chelonae*[7]. Mycobacterial involvement of the bowel either by tuberculosis or MAC may lead to diarrhoea. Antony et al[8] reported that MAC is the most commonly identified organism in patients with chronic diarrhoea and low CD4 lymphocyte counts.

There is a possibility that some patients thought to have Crohn's disease or tuberculosis are indeed cases of atypical mycobacteriosis of the intestine. Correct species identification of NTM isolates is important clinically since NTM species differ in their potential to cause clinical disease in humans and in their response to specific antibiotics[9]

Over recent years clinical laboratory methods of speciation have moved from biochemical to molecular techniques. Matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry has shown good promise in providing rapid speciation of NTM [10]

Of the molecular techniques available, line probe assay (eg, Hain CM and AS) is widely used allowing reasonable identification of common species of NTM[11,12] These molecular techniques are applied on respiratory samples for detection of NTM.

Clinical presentation of Extra-pulmonary tuberculosis (EPTB) is diverse and it may lead to delayed diagnosis due to paucibacillary nature of the samples. Sensitivity of acid-fast bacillus detection by microscopy is poor (0-40%) and smear positive samples requires 10^4 bacilli/ml of the sample[13,14].BACTEC-MGIT cultures established from colonoscopic biopsy specimens were positive for M. tuberculosis only in 20.29% GITB patients[15] Long incubation period is required to grow Mycobacterium Tuberculosis, limit the usefulness of culture methods for diagnosis of TB. Recently, nucleic acid amplification tests, which can be used for direct detection of TB from samples, have emerged as potentially useful tools for rapid diagnosis of TB[16].

World Health Organization has recommended a cartridge based Nucleic acid amplification test (CBNAAT), Xpert MTB/RIF (by Cepheid, CA, USA) for extra pulmonary samples. But it has limitation of detecting RIF resistance only[17]. Line probe assay (LPA) detects resistance to both Isoniazid (INH) and Rifampicin (RIF). LPA Studies have been performed in EPTB diagnosis, but there is limited evidence of LPA use in Ileocolonic ulcers. This study was designed to evaluate the use of LPA for detection of MTB and NTM directly from Colonic and Ileal ulcers, and to compare with Histopathological examination (HPE) and CBNAAT.

Methods:

Methodology: The study was conducted at a tertiary referral centre in India. Ethical committee clearance was obtained from Institutional Ethics Committee. Thirty five consecutive patients with provisional diagnoses of Ileocolonic ulcers detected during colonoscopy were prospectively recruited

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to the study. Patients with Ileal / colonic ulcers more than 1 x 1 cm in size were included in the study. Malignant ulcers and ulcers less than 1 x 1 cm were excluded from the study. Biopsies were obtained from the colonic and Ileal ulcers and transferred into three separate containers one for Histopathology, two other containers containing saline for CBNAAT and Line probe assay.

The Line probe assay was done using GenoType Mycobacterium CM kit which is a qualitative in vitro test for the identification of the Mycobacterium tuberculosis complex as well as the following nontuberculous mycobacterial species: M. avium, M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. intracellulare,M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. marinum/M. ulcerans, M. peregrinum, and M. xenopi.

Genotype Mycobacterium CM test is based on the DNA Strip technology. The whole procedure is divided into three steps:

1. DNA extraction from tissue material

2. A multiplex amplification with biotinylated primers

3. A reverse hybridization.

The CB-NAAT system detects DNA sequences, specific for Mycobacterium tuberculosis. It purifies and concentrates Mycobacterium tuberculosis bacilli from tissue samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR. The process identifies clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the Mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons.

Patients were monitored until the final definite diagnosis was made. If the symptoms improved, Colonoscopy was performed after six months to look for resolution of the ulcers. If the symptoms persisted for two months after starting treatment or worsened after initiating treatment, repeat colonoscopy was done at two months. The patients' clinical, endoscopic, laboratory and histological features were stored in a database.

Data analysis: Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions. Continuous data was represented as mean and standard deviation. Chi square was used to check for association of qualitative data. Sensitivity , Specificity , Positive and Negative Predictive Value and Diagnostic Accuracy of the test was done in comparison with gold standard test .p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Results:

A total of 16 patients were diagnosed to have tuberculosis based on clinical presentation, lab investigations and histopathology whereas 15 patients had other diagnoses (Crohn's disease, Amoebiasis, nonspecific Ileitis). In patients treated with anti-tubercular therapy (16), Abdominal pain was present in 11 (68.7%) patients, diarrhoea in 9 (56.2%) Weight loss in 6 (37.5%), fever in 2 (12.5%) and bleeding per Rectum in 1(6.2%). Two patients were HIV positive. Ascites was present in 2(6.4%) and 11(35.4%) patients had anaemia. Summary of clinical and laboratory parameters are mentioned in table 1

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Table 1. Clinical parameters of the patients studied.

		Patients started on ATT (n=16)	Others (n=15)	P Value
Clinical features	Pain abdomen	11 (68.7%)	7 (46.6%)	0.213
	Diarrhoea	9 (56.2%)	10 (66.6%)	0.552
	Weight Loss	6 (37.5%)	2 (13.3%)	0.124
	Fever	2 (12.5%)	2 (13.3%)	0.945
	Bleeding per Rectum	1 (6.2%)	3 (20%)	0.254
Investigations	Haemoglobin	11.1 ±2.2	11.42 ± 2.22	0.701
	Total Leucocyte count	6701.7 ±3019.6	6643 ±3051.9	0.957
	ESR	38.89±28.08	37.13± 27.5	0.861
Colonoscopy	Ileum	6 (37.5%)	8(53.3%)	0.376
	Caecum	2 (12.5%)	1 (6.6%)	0.583
	Ileo-caecal	9 (56.2%)	1 (6.6%)	0.003*
	Other Part of Colon	4 (25%)	7 (46.6%)	0.208
Histology	Granuloma	11 (68.7%)	0 (0%)	0.001*
	Caseation necrosis	1 (6.2%)	0 (0%)	0.325
	Inflammation	4 (25%)	4 (26.6%)	0.916
USG Findings	Lymphadenopathy	2(12.5%)	1 (6.6%)	0.583
	Wall Thickening	6 (37.5%)	5 (33.3%)	0.809
	Ascites	3 (18.7%)	1 (6.6%)	0.316
CT Findings	Lymphadenopathy	4 (25%)	3 (20%)	0.739
	Intestinal Wall Thickening	5 (31.2%)	4 (26.6%)	0.779
	Ascites	1 (6.2%)	1 (6.6%)	0.962

USG-Ultrasonography, CT-Computerized tomography

LPA was positive in 9(29.03%). MTB Species was positive in 5(16.12%) and NTM was positive in 4 (12.1%). 12(38.7%) Patients had features of tuberculosis on biopsy. Out of the 31 patients, only one was positive for CBNAAT (3.3%).

Diagnostic Ability of Line probe assay for diagnosis on Intestinal Tuberculosis in mentioned in table two.

Table 2

		Patients treated with Anti Tubercular Therapy(16)			
		Improved (14)		Not improved (02)	
		Frequency	%	Frequency	%
LINE PROBE ASSAY for MTB	Positive	04	28.57%	01	50%
	Negative	10	71.42%	01	50 %

SN- 28.57% (95%CI- 8.39% to	SP- 50.00% (95% CI- 1.26% to 98.74%)	PLR-0.57 (95%CI- 0.11 to 2.87)	NLR- 1.43 (95%CI 0.34 to 5.94)	PPV-80.00% (95% CI 44.32% to 95.26%) .	NPV- 9.09%(95% CI 2.35% to 29.37 %)	Accuracy- 31.25%(95%CI- 11.02% to 58.66%)

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58.10%)						

(SN- Sensitivity, SP- Specificity, PLR- Positive Likelihood Ratio, NLR- Negative Likelihood Ratio, PPV- Positive Predictive Value, NPV -Negative Predictive Value, MTB-Mycobacterium Tuberculosis)

The diagnostic ability of CBNAAT for ITB is mentioned in the Table 3

TABLE 3

		Patients treated with Anti Tubercular Therapy(16)				
		Improved (14)		Not improved (02)		
		Frequency	%	Frequency	%	
CBNAAT	Positive(1)	01	7.14%	0	0%	
	Negative(15)	13	92.85%	02	100%	
SN- 7.14% CI 95% (0.18%-33.87%)	SP- 100% CI 95%(15.81%-100.00%)	PLR- --	NLR- 0.93(95% CI 0.80-1.07)	PPV-100%	NPV-13.33%(95%CI 11.74 to 15.10 %)	Accuracy-18.75% (95%CI 4.05-45.65%)-

(SN- Sensitivity, SP- Specificity, PLR- Positive Likelihood Ratio, NLR- Negative Likelihood Ratio, PPV- Positive Predictive Value, NPV -Negative Predictive Value CBNAAT – Catridge based Nucliec acid amplification test)

Diagnostic Ability of Line robe Assay in Diagnosis ITB when compared to CBNAAT is mentioned in table 4

		CBNAAT			
		Positive (1)		Negative (30)	
		Frequency	%	Frequency	%
LINE PROBE ASSAY	Positive	1	100.0%	8	26.6 %
	Negative	0	0.0%	22	73.4 %

SN- 100.00% (95% CI- 2.50 % to 100.00%)	SP- 73.33%(95%CI 54.11% to 87.72%)	PLR- 3.75 (95% CI- 2.07 to 6.79)	NLR-0.00	PPV-11.11% (95%CI- 6.46% to 18.45%)	NPV-100.00%	Accuracy-74.19% (95% CI- 55.39% to 88.14%)

(SN- Sensitivity, SP- Specificity, PLR- Positive Likelihood Ratio, NLR- Negative Likelihood Ratio, PPV- Positive Predictive Value, NPV -Negative Predictive Value CBNAAT- Catridge based nucleic acid amplification)

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Diagnostic Ability of Line robe Assay in Diagnosis TB when compared to Histo pathology is mentioned in table 5

Table 5

		(Total 31 cases)			
		Histopathology Positive(12cases / 38.7%)		Histopathology Negative(19cases / 61.3%)	
		Frequency	%	Frequency	%
LINE PROBE ASSAY(only MTB)	Positive	4	33.3%	1	5.2%
	Negative	8	66.6%	18	94.7%

SN- 33.33% (95%CI- 9.92% to 65.11%)	SP- 94.74% (95% CI- 73.97% to 99.87%)	PLR-6.33 (95% CI 0.80 to 50.13)	NLR- 0.70 (95% CI - 0.47 to 1.06)	PPV- 80.00% (95% 33.57% to 96.94%)	NPV- 69.23% (95% CI 59.80 to 77.29 %)	Accuracy- 70.97% (95% CI- 51.96% to 85.78%)

(SN- Sensitivity, SP- Specificity, PLR- Positive Likelihood Ratio, NLR- Negative Likelihood Ratio, PPV- Positive Predictive Value, NPV -Negative Predictive Value, MTB-Mycobacterium Tuberculosis)

Discussion

Our study included total of 35 patients with Ileocolonic ulcers. 4 patients were excluded from the final analysis as one developed road traffic accident and died. Three patients stopped the recommended treatment midway and were lost for follow up. 31 patients were considered for final analysis. Of the 31 patients 17 (54.8%) were males. Mean age was 41.58 ± 17.31years. 16 patients were started on ATT after considering their clinical presentation, laboratory investigations, imaging studies, Monteux test, intestinal biopsy histopathology, LPA and CBNAAT.

Among the patients who had been diagnosed with Tuberculosis, abdominal pain was present in 11 (68.7%) patients, Chronic diarrhoea in 9 (56.2%), weight loss in 6 (37.5%), fever in 2 (12.5%) and bleeding per rectum in 1 patients (6.2%). In a study done by Makharia et al[18]on intestinal tuberculosis, chronic diarrhoea was present in 37%, blood in stools in 16%, abdomen pain in 90%, fever in 41% and weight loss in 83% which is almost similar to our study.

On colonoscopy, ulcers were noted most commonly at the ileocaecal area in 9 (56.2%), followed by terminal Ileum in 6 (37.5%), Caecum in 2 (12.5%) and rest of the colon in 4 (25%). These findings were almost similar to the findings Mukewar et al [19]

On Computer tomography findings in 16 of 31 cases started on ATT included bowel thickening in 5 (31.2%) patients, lymphadenopathy in 4 (25%) and ascites in 1 (6.2%) patients which is in contrast to study done by Tariq Sinan et al [20]where ascites was seen in more than half the cases (55.2%). Other findings in this study included lymphadenopathy (46.9%), bowel wall thickening (38%) and solid organ involvement (20.4%).

Histological features suggesting ITB include confluent granulomas, multiple granulomas, large granuloma size, bands of epithelioid histiocytes lining ulcers and disproportionate submucosal inflammation that significantly exceeds mucosal inflammation. In our study on intestinal biopsy, in

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addition to other findings, granuloma was present in 11 (68.7%).In a study done by Makharia et al[18], granulomas were seen in 62.5% of the patients which was similar to our study.

Of total 31 colonic ulcers patients, 12 cases (38.7%) were suggestive of TB on histopathological examination. Out of these 12 cases, 5 were positive on Line Probe Assay (16.1 %). Of 19 cases which were nonspecific on histopathological examination, 4 were positive for Mycobacterial species (NTM) on Line Probe assay (12.9 %).

The sensitivity of LPA compared to HPE for diagnosis of ITB was 33.33% (95% confidence interval: 9.92% -65.11%) while the specificity, negative predictive value, the positive predictive value, and accuracy were 94.74% (73.97- 99.87%), 69.23% (59.80-77.29%), 80.00% (33.57- 96.94%) and 70.97 % (51.96- 85.78%), respectively

Out of the 16 patients started on ATT, only one patient was CBNAAT positive. This patient was positive for MTB by LPA also. When LPA was compared to CBNAAT for diagnosis of ITB, Sensitivity was 100% (95% confidence interval: 2.50 to 100%), specificity 73.33% (54.11 to 87.72%).These were similar to observations of Syed et al [21]

The sensitivity of CBNAAT in our study for diagnosis of ITB was 7.14% (95% confidence interval: 0.18% - 33.87%) while the specificity, negative predictive value, the positive predictive value, and accuracy were 100% (15.81- 100%), 13.33% (11.74%- 15.10%), 100.00% and 18.75 % (4.05%- 45.65%), respectively. However Balaji et al [22] had demonstrated higher sensitivity (32%) of CB NAAT compared to our study.

Multiple studies have been performed on the role of CBNAAT in extra-pulmonary tuberculosis and the sensitivity for detection of tuberculosis has varied from 39 to 82 % and the specificity has varied from 26 to 98%[23, 24,25]

The sensitivity of LPA for diagnosis of in our study for ITB was 28.57% (95% confidence interval: 8.39% - 58.10%) while the specificity, negative predictive value, the positive predictive value, and accuracy were 50.00% (1.26- 98.74%), 9.09% (2.35%- 29.37%), 80.00% (44.32% - 95.26%) and 31.25 % (11.02%- 58.66%), respectively.

Other studies of LPA on extra-pulmonary samples have obtained a sensitivity ranging from 45-71% and specificity ranging from 92-94% [26, 27]. This is first study of LPA on intestinal ulcers. The sensitivity and specificity of LPA appears to be less as compared to respiratory and other extra-pulmonary samples probably due to the paucibacillary nature of the specimen.

Out of remaining 15 patients who were not started on ATT, 3 were diagnosed as Crohn's disease, 2 as amoebic colitis and 10 showed nonspecific ulceration.

Of total 31 cases, 9(29.03%) were LPA positive. Out of the 9 LPA positive, 5 (16.12%) were of mycobacterium TB and 4(12.9%) were mycobacterial species positive (NTM). Species differentiation was not possible probably due to low bacterial load in the biopsy specimen. All these patients 9 patients were started on ATT. Four improved among mycobacterial TB positive group and three improved among mycobacterial species positive group. The patient who did not improve in MTB group improved after starting treatment for multidrug resistant TB and the patient who did not improve in the NTM group improved after addition of macrolide.

Of total 31 cases, after considering the clinical presentation, colonoscopy findings, histopathology, imaging, LPA and CBNAAT, 16 Patients were started on ATT. Among the 16 patients who were

started on ATT, overall response to therapy was positive in 14 patients. Among the improved cases, 7 patients were positive for LPA. Among two cases who did not improve on ATT, one was LPA positive for TB and one positive for NTM..

LPA is a better investigation tool compared to CBNAAT for diagnosis of Ileocolonic TB. It can be added as a routine tool in diagnosis of Ileocolonic TB along with histopathology and CBNAAT till better tests arrive.

Conclusion: LPA provides an additional investigation in the armamentarium for the diagnosis in intestinal ulcers. Although CBNAAT is recommended in the diagnosis of tuberculosis in extra pulmonary specimens, utility of CBNAAT in the setting of Ileocolonic ulcers appear limited as demonstrated in this study.

Treatment of intestinal ulcers involves application of multiple clinical and laboratory imaging parameters to reach to a diagnosis. The sensitivity and specificity of all the available investigative modalities are poor and we need better tools.

Limitation of the study : Sample size was small, more studies need to be done with large samples. Further speciation of NTM was not possible probably due to the paucibacillary nature of the intestinal ulcers

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References:

1. Burnham WR. Lennard-Jones JE, Stanford JL, et al. Mycobacteria as a possible cause of inflammatory bowel disease. *Lancet* 1978; 2: 693-6.

2. Chiodini RJ, Kruiningen HJV, Thayer WR, et al. Possible role of mycobacteria in inflammatory bowel disease. An unclassified mycobacterium species isolated from patients with Crohn's disease. *Dig Dis Sci* 1984; 29: 1073-9.

3.Thayer WR Jr, Coutu JA, Chiodini RJ, et al. Possible role of Mycobacteria in inflammatory bowel disease II. Mycobacterial antibodies in Crohn's disease. *Dig Dis Sci* 1984; 29: 1080-5.

4. Gitnick G. Is Crohn's disease a mycobacterial disease after all? *Dig Dis Sci* 1984; 29: 1086-8.

5. Graham DY, Markesich DC, Yoshimura HH. Mycobacteria and inflammatory bowel disease: result of culture. *Gastroenterology* 1987; 92: 436-42.

6. J. P. Euzeby, "List of Prokaryotic names with Standing in Nomenclature-GenusMycobacterium,"2013, http://www.bacterio.cict.fr/m/mycobacterium.htmlView at: Google Scholar

7. Phillips MS, von Reyn CF. Nosocomial infections due to nontuberculous mycobacteria. *Clin Infect Dis* 2001; 33:1363-1374

8. Antony MA, Brandt LJ, Klein RS, Bernstein LH. Infectious diarrhea in patients with AIDS. *Dig Dis Sci* 1988; 33: 1141-1146

9. Brown-Elliott BA, Wallace RJ. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev* 2002;15:716–46

10. Saleeb PG, Drake SK, Murray PR, et al. Identification of mycobacteria in solidculture media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2011;49:1790–4

11. Suffys PN, da Silva Rocha A, de Oliveira M, et al. Rapid identification of Mycobacteria to the species level using INNO-LiPA Mycobacteria, a reverse hybridization assay. *J Clin Microbiol* 2001;39:4477–82.

12. Lebrun L, Gönüllü N, Boutros N, et al. Use of INNO-LIPA assay for rapid identification of mycobacteria. *Diagn Microbiol Infect Dis* 2003;46:151–3.

13. CLSI. Laboratory detection and identification of mycobacteria. *2nd ed, CLSI guideline* M48-A, Wayne, PA: Clinical and Laboratory standards Institute; 2008.

14. Meenal Bagdia1, Sanjay Bijwe2, Nilma Hirani3 . Lab Diagnosis of Extra PulmonaryTuberculosis: Comparison ofHistopathology, Cytology, ZeihlNeelsenstain and Light Emission DiodeMicroscopy with Culture and Nucleic AcidAmplification Tests.*Int J Cur Res Rev*.2018:10(8);15-19

15. Bhumit Patel, Vipul D Yagnik.Clinical and laboratory features of intestinal tuberculosis. *Clinical and Experimental Gastroenterology* 2018:11; 97–103.

16. Promod K. Mehta, Ankush Raj, Netrapal Singh, Gopal K. Khuller.Diagnosis of extrapulmonary tuberculosis by PCR. FEMS *Immunol Med Microbiol*.2012:66; 20–36

17. World Health Organization. Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children .Policy update. 2013.ISBN: 9789241506335.

18. Makharia, G. K., Srivastava, S., Das, et al. Clinical, Endoscopic and Histological Differentiations Between Crohn's Disease and Intestinal Tuberculosis. The American *Journal of Gastroenterology*. 2010: 105(3); 642–651.

19. Mukewar S, Mukewar S, Ravi R, Prasad A, S Dua K. Colon tuberculosis: endoscopic features and prospective endoscopic follow-up after anti-tuberculosis treatment. *Clin Transl Gastroenterol* 2012; 3(10): e24

20. Tariq Sinan,Mehraj Sheikh,Salwa Ramadan,et al. CT features in abdominal tuberculosis: 20 years' experience. *BMC Med Imaging*. 2002; 2: 3. PMID: 12427257.

21. Syed Beenish Rufai, Parveen Kumar, Amit Singh, Suneel Prajapati, Veena Balooni, Sarman Singh. Comparison of Xpert MTB/RIF with Line Probe Assay for Detection of Rifampin-Monoresistant Mycobacterium tuberculosis. *J Clin Microbiol*. 2014 Jun; 52(6): 1846–1852.

22. Balaji L Bellam , Harshal S, Mandavdhare,et al. Utility of tissue Xpert-Mtb/Rif for the diagnosis of intestinal tuberculosis in patients with ileocolonic ulcers. *Ther Adv Infectious Dis*. 2019, Vol. 6: 1–5.

23. R. Rakotoariveloa, J. Ambrosionic, V. Rasolofoe,et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of smear-negative pulmonary and extrapulmonary tuberculosis in Madagascar. *International Journal of Infectious Diseases* 69 (2018) 20–25

24. K.Suresh, Dr.Y.Vimala, Dr.Pv Ramana, et al. Diagnosis of Extra Pulmonary Tuberculosis By Using Xpert® MTB/RIF Assay (CBNAAT) And MGIT Liquid Culture. *Journal of Dental and Medical Sciences*.2018; 17(9):65-70.

25. Abhay Uppe, Sayli Sawant, Deepak Gupta, Girija Nair. Comparison Study of GENEXPERT versus TB MGIT Culture in Extra Pulmonary Tuberculosis. American *Journal of Infectious Diseases and Microbiology*. 2020;8(1): 1-13

26. Charuta Ghanekar , Divya Patel , Madhuwanti Abhyankar , et al. Role of line probe assay in detection of extra-pulmonary tuberculosis: Experience from a tertiary care hospital in western Maharashtra. *Indian journal of tuberculosis*. 2019; 66: 325 – 330

27. JoveriaFarooqia, Salima Qamara, Imtiaz Alib, et al. Utility of Line Probe Assay for diagnosis of extra pulmonary tuberculosis. *International Journal of Mycobacteriology*. 2015 ;4 (5): 110