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Diagnosis of Childhood Leprosy – Changing Trends

Rajeshwar Dayal
Professor and Head, Dept. of Pediatrics
S. N. Medical College, Agra.

SUMMARY

Leprosy, a chronic infectious disease caused by mycobacterium leprae, mainly involves the skin, respiratory mucosa and the peripheral nervous system. Leprosy continues to remain a public health problem. In 2011, the global new case detection was 219075 and in India it was 127295. Thus, India accounts for > 58% of total cases of leprosy worldwide. Pediatric leprosy accounts for around 10% of the total disease burden.

The main source of transmission of leprosy is from the untreated lepromatous patients and the most common route is through the nasal secretions. From the nasal mucosa, the bacteria spreads by hematogenous route to skin and the peripheral nerves. The disease has a long incubation period of 3-5 yrs (can be upto 20 yrs).

After infection, the child first develops indeterminate leprosy which can either get cured spontaneously or on treatment or it can progress to one of the several clinical forms (tuberculoid, borderline or lepromatous). The clinical spectrum varies from tuberculoid, where there are a few, large, anesthetic skin patches with thickened peripheral nerves and no detectable bacilli to lepromatous type where there are multiple, small skin lesions with intact sensation and high bacillary load. In our study spanning over 20 years, we have observed no significant change in the clinical profile.

Early diagnosis of leprosy requires a high index of suspicion on the part of the clinician. It is based on detection of 2 of the following features, namely, characteristic skin lesion, loss of sensation and thickened peripheral nerves or the detection of AFB in skin or nasal smear.

Correspondence : Dr. Rajeshwar Dayal, Professor and Head, Dept. of Pediatrics, S. N. Medical College, Agra – 282002.

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We conducted a number of studies, evaluating various newer techniques for early detection of the disease. In one study, we found the FLA-ABS and Lepromin tests, to be of immense value for identification of "at risk" population in the community and for detecting subclinical infection. We also studied antibody response against 35k Da antigen by SACT and found that nearly 50% smear negative, 42% lepromin +ve and 70% lepromin -ve cases showed positive antibody response with no false positive response.

Gene probes developed at our institute were tried on 100 patients. AII smear +ve cases, lepromin +ve cases and majority of smear- ve cases were detected by this method. 9 cases (4 indeterminate & 5 nonspecific) with inconclusive histopathology were also detected.

In another study on 22 children, in-situ hybridization technique helped in diagnosing the children with negative skin smear and non specific histopathology. It also permitted the concomitant study of tissue pathology.

Again, in our pioneer study, evaluation of the in-situ PCR technique revealed that histopathology detected 45% of total cases, in-situ PCR detected as much as 60% of the total cases. Thus, In-situ PCR offered excellent structural correlation permitting concomitant study of tissue pathology. As contamination by foreign DNA/RNA does not exist, it is a valuable tool for diagnosis of childhood leprosy.

RLEP based PCR is yet another useful tool to detect cases where skin smears are -ve and skin biopsy is not feasible. In our study involving 73 patients, Z-N staining for AFB was positive in 17/73 (23.28%) cases and RLEP PCR in 56/73 (76.71%) cases. All 30 controls showed negative results. RLEP PCR technique had a significantly greater positivity (especially in early stages of leprosy) than ZN staining ($p < 0.001$).

Suggested algorithm for diagnosis, whenever there is clinical suspicion, we can either go for smear for AFB or histology to confirm the diagnosis. A positive smear for AFB is confirmatory. If it is negative then, we can subject the specimen for gene probes or PCR/In-Situ PCR/RLEP PCR. If the result is positive, it is diagnostic of leprosy. On the other hand, if histology shows characteristic features then it is confirmatory; if it is not characteristic, we can go for in-situ hybridization. A positive in-situ hybridization is diagnostic of leprosy; if it is negative then we can opt for in-situ PCR.

To conclude, leprosy often poses a diagnostic dilemma. It is important that after a good clinical assessment, new diagnostic tests be used to diagnose the condition at an early stage & prevent complications/ deformities.

INTRODUCTION

Leprosy is an ancient disease, earliest described in Asia (India & China) around 6th century B.C. It was described as Kustha-Roga (in Sanskrit it means eating away) by *Susruth Samhita* (600 B.C) (1). In 1873 Dr. Gerhard Henrik Armaeur Hansen of Norway discovered *M. leprae* as the causative agent of leprosy (2).

Etiology :

M. leprae is an obligate intracellular bacillus (0.3–1 m wide and 1–8 m long) that is confined to humans and armadillos. The organism is acid-fast, indistinguishable microscopically from other mycobacteria, and ideally detected in tissue sections by a modified Fite stain. The bacilli has an extremely slow dividing time (once every 2 weeks); the incubation period ranges from 6 months to more than 40 years and averages from2 to 5 years (3).

Epidemiology :

Leprosy is almost exclusively a disease of the developing world, affecting areas of Asia, Africa, Latin America, and the Pacific. While Africa has the highest disease prevalence, Asia has the most cases. More than 80% of the world's cases occur in a few countries: India, China, Myanmar, Indonesia, Brazil, Nigeria, Madagascar, and Nepal. Countrywise, maximum number of cases of leprosy reside in India (4) (Table 1).

Table 1 : Trends in the detection of new cases of leprosy, by WHO Region, 2008–2011

WHO Region	No. of new cases detected			
	2008	2009	2010	2011
Global	249007	244796	228474	219 075
India	134184	133717	126800	127 295

Table 2 : Leprosy situation in India first quarter 2012 (WHO)

Registered Prevalence	No. of new cases detected (2011)	No. of new cases of MB leprosy	No. of females among new cases	No. of new cases among children–	No. of new cases with grade-2 disabilities	No. of relapses (2011)	Cure rate (%)	
							PB	MB
83 187	127 295	63 562	47 111	12 305	3 834	690	95.28	90.56

Clinical Manifestations :

The cardinal features of leprosy are a skin patch with sensory loss, nerve enlargement, and acid-fast bacilli in the skin (5). The clinical features are summarized in Table 3.

Table 3 : Clinical Classification of Leprosy (1)

Features	TT	BT	BB	BL	LL
Number of lesions	Single usually	Single or few	Several	Many	Very many
Site of lesions	Variable	Variable	Variable	Variable	Small
Surface of lesions	Very dry, sometimes scaly	Dry	Slightly	Shiny	Shiny
Sensations in lesions	Absent	Markedly diminished	Moderately diminished	slightly diminished	Not affected
Hair growth	Absent	Markedly diminished	Moderately diminished	Moderately diminished	Not affected
AFB in lesions	Nil	Nil or scanty	Moderate number	Many	Very many(plus globi)
AFB in nasal scrapping /in nose blows	Nil	Nil	Nil	Usually nil	Very many(plus globi)
Lepromin test	Strongly positive(+++)	Weakly positive(+ or ++)	Negative	Negative	Negative

After inoculation of M.Leprae, there will be either no disease with complete resistance or there will be clinical disease developing through indeterminate leprosy. Indeterminate leprosy, is an early form of the disease that features only a small number of skin lesions and no nerve involvement. It is a very early form of leprosy and may either be cured or progress to one of the other forms of leprosy depending on their immune status (6, 7). Within each type of leprosy, patient may remain in that stage, improve to a less debilitating form or worsen to a more debilitating form depending on their immune state. The polar forms of leprosy, tuberculoid leprosy and lepromatous leprosy, are immunologically stable, whereas the intermediate forms, including borderline tuberculoid leprosy, borderline leprosy, and borderline lepromatous leprosy, are immunologically unstable and lead to either a gradual decline toward the

lepromatous pole or upgrading 'reversal reactions' toward the tuberculoid pole (8).

Tuberculoid leprosy is characterized by a vigorous cellular immune response and limited humoral immune responses to M. leprae, usually involving the skin and nerves and resulting in few skin lesions. Lepromatous leprosy, on the other hand, is characterized by a minimal cellular immune response and a vigorous humoral immune response and, consequently, extensive skin involvement.

Patients with overt disease form just the tip of iceberg. It is extremely important to identify the at risk population in the community to bring down the disease burden.

Diagnosis of Leprosy :

Clinical diagnosis :

It is diagnosed by the presence of at least two of the following three cardinal signs or the last one independently (5):

(i) Loss/impairment of cutaneous sensation,
(ii) Thickened nerves,
(iii) Presence of AFB.

Slit skin Smear for AFB procedure:

Demonstration of M.Leprae in slit skin smears by Z-N staining method is considered as confirmatory.

Histopathology:

The histopathological examination of skin biopsy can help in confirming the diagnosis, classification of the disease and assessment of bacterial load. In early stage of the disease the diagnostic value of histopathology is limited. The earliest histological response appears in the form of a lymphocytic infiltrate, a non- specific feature, which heralds the onset of almost all chronic dermatoses. A definitive histological diagnosis of leprosy at this early stage requires:

(a) The presence of infiltration within dermal nerves, and
(b) The presence of Acid Fast Bacilli (AFB).

These defining features are, however, not always seen early in the evolution of the disease, and confirmation of diagnosis is often not possible. To add to this, in the pediatric age group it is difficult to elicit impairment of cutaneous sensation and finding of thickened nerves. Majority of cases in children are paucibacillary and demonstration of AFB is very low, making the diagnosis of leprosy even more difficult (9).

With this background, we conducted many studies in our department to find out tests for early and quick identification of leprosy in children. A brief overview of those studies is as follows:-

1. Fluorescent leprosy antibody absorption test (FLA-ABS):

This technique is highly sensitive in detecting the antibodies against M. Leprae antigen by immune fluorescent technique and is useful in identifying healthy contacts of patients who are at risk of developing disease (10) (Table 4).

We conducted a study of healthy children who were close contacts of leprosy patients and followed them for 5 years (from1986-1990) (11) in order to:

1. Detect subclinical infection and observe the development of overt disease by using the Fluorescent Leprosy Antibody Absorption Test (FLA-ABS) and the lepromin test which assesses the humoral and cell-mediated immunity (CM1), respectively;

2. Evaluate the efficacy of dapsone as a chemoprophylactic agent in the 'at risk' contacts.

455 healthy contacts were studied. Majority of the contacts of multibacillary patients (303) were FLA-ABS positive (75 per cent) and lepromin negative (55 per cent) showing that although most of them had been infected, the lepromin status was negative ($P < 0.01$). On the other hand, the majority of the contacts of paucibacillary patients (152) were lepromin positive (57 per cent) ($P<0.05$) indicating a good cell mediated immune response. Furthermore, only 61 per cent of contacts of paucibacillary patients were FLA-ABS positive as compared to 75percent of contacts of multibacillary patients demonstrating that the former had been exposed to a lesser quantum of infection ($P<0.05$).On the basis of results of FLA-ABS and lepromin tests, these 455 contacts were classified into four groups, viz. Group I comprising children who were FLA-ABS positive and lepromin positive; Group II, who were FLA-ASS positive and lepromin negative; Group III, who were FLA-ABS negative and lepromin positive; and Group IV who were FLA-ABS negative and lepromin negative. During the follow-up period of 5 years, only two out of 155 children in Group I developed the disease showing that their good CMI had been able to contain the disease. Out of 166 contacts in Group II, 18 developed the disease mainly of the tuberculoid type. Most of these children were contacts of multibacillary patients. None of the children in Groups III and IV developed the disease. These findings were statistically significant ($P <0.01$) (Table 4, 5).

Out of the 166 children in Group II (the 'at risk' group), 70 were treated as controls while 96 were put on prophylaxis with dapsone which was continued for 3 years after the contact with the source patient had ceased, or for 3 years after the

source patient became non-infective. The incidence of disease was significantly lower among children who received chemoprophylaxis (P<0.05).

Our study demonstrates the value of the FLA-ABS and lepromin tests in detecting sub-clinical infection and for identifying the 'at risk' contacts of leprosy patients in the community. It clearly establishes the chemoprophylactic value of dapsone for the 'at risk' contacts, particularly for those in the 'high risk' category. In pursuance of our Government's policies under the National Leprosy Eradication programme, this study suggests the need to carry out surveillance surveys in the endemic population to identify, follow, and offer chemoprophylaxis to those at risk (12).

Table 4 : Development of Disease in contacts during 5 years follow-up

GROUPS	STATUS		NO. OF CONTACTS			INCID - ENCE (%)	RELA - TIVE RISK
	FLA - ABS	LEPR - OMIN	TOTAL		DEVELOP DISEASE		
I	+	+	155		2	1.29	1:77.51
II (AT RISK)	+	-	166	70 **(CONT.)**	12	17.14	1:9.2
				96 **(DDS.P.)**	6	6.25	
III			68				
IV	-	+	66		-	-	-
	-	-			-	-	-
TOTAL			455		20	4.4	1:22.75

STATISTICAL SIGNIFICANCE
GROUP II Vs I : z=3.705, p<0.01
GROUP II Vs III: z=4.493, p<0.01
GROUP II Vs IV: z=4.493, p<0.01

AT RISK CONTACTS

HIGH RISK GROUP	**LOW RISK GROUP**
< 5 YEARS AGE	>5 YEARS AGE
MALES	FEMALE
BACT. +VE PATIENTS	BACT –VE PATIENTS

Table 5 : Effect of Chemoprophylaxis in 'At Risk' Contacts

GROUPS	CONTROL GROUP		D.D.S. GROUP		EFFICACY RATE (%)	P VALUE
	CONT - ACTS	INCID - ENCE (%)	CONT - ACTS	INCID - ENCE (%)		
HIGH RISK						
< 5 YRS.	25	8 (32.00)	60	1 (1.67)	94.78	<0.01
MALES	30	8 (26.67)	45	2 (4.44)	83.35	<0.05
BACT. +VE	32	9 (28.13)	49	2 (4.08)	85.50	<0.05
LOW RISK						
> 5 YRS.	45	4 (8.89)	36	5 (13.88)	NIL	>0.05
FEMALES	40	4 (10.00)	51	4 (7.84)	21.60	>0.05
BACT. – VE	38	3 (7.89)	47	4 (8.51)	NI L	>0.05

2. Gene Probes :

DNA targeting probes :

Synthetic oligonucleotides are used as probes. A number of such probes are now available for the detection and identification of M. leprae gene sequences.

DNA targeting probes :

They have not been found to be useful for identifying active disease both because of their poor sensitivity in PB cases and also because of persistence of signals for quite some time after bacterial death.

RNA targeting probes:

Targeting of RNA has special importance. RNA is a much more unstable molecule than DNA. As RNA degrades faster after death, their demonstration or quantification is likely to correlate better with the presence of live bacteria in the lesions. rRNA has become a popular target choice for probe development, because of evolutionary conserved as well as variable stretches in rRNA gene region, presence of large copy number and better correlation with viability. A number of rRNA targeting probe for detection of M.leprae have been developed. These probes have been observed to be sensitive enough to detect up to 100 - 1000 live cells directly without amplification. Further assays for quantitative measurement of these signals have been developed. These probes have been found to be useful for confirming active disease, monitoring the course of treatment and also diagnosing a relapse (13). This strategy of targeting rRNA has been observed to 10-100 folds more sensitive than DNA detection in biopsy specimens from leprosy cases. These probes appear to have potential role in diagnosis of MB relapse and also some of the PB leprosy relapses. These rRNA probes has been used by in-situ hybridization to demonstrate M. leprae specific RNA for this signifies the presence of active infection.

We conducted this study from 1992-94 (14) on 651 patients & 40 controls .Children less than 16 yrs age group were selected. History, clinical & smear exam was done in all cases. Majority of cases were of borderline tuberculoid type 291/651. As the age increased the skin lesions also increased. Majority of patients had macular hypopigmented lesions/impaired sensation, with nerve thickening. Nerve thickening was seen in 301 cases, BT = 44.7% cases; BB= 23.5% cases. Lepromin test, skin biopsy, gene probe studies were done in 100 patients & all controls.75/100 cases were positive in probe 1 & 61/100 cases were positive in probe 2.We also studied a correlation of histopathology with probe test (Table 6 & 7).

Results of gene probes :

A total of 87 cases were positive by gene probes ($P < 0.05$).57 cases were detected by both the probes, 22 additional cases were detected by P1 only and 8 additional cases were detected by p2 only. 13 cases were left undetected which may be due to decreased bacterial load or inadequate sample. From the above results it is clear that p1 was better than p2. All smear +ve cases, lepromin +ve cases and majority of smear –ve cases were detected by this method. 9 cases (4 indeterminate & 5 nonspecific) with inconclusive histopathology were also detected by this method (Table 8).

Table 6 : Age & Sex Distribution

Age (Yrs.)	Type of Disease						Total
	I	TT	BT	BB	BL	LL	
0-5	9	9	-	9	-	-	27
6-10	-	9	99	27	9	-	144
11-16	54	9	192	117	81	27	480
Total	63	27	291	153	90	27	651

X^2 = 6.308, df = 4, P < 0.05, Male = 522, Female = 129

Table 7 : Correlation : Smear status with Probe Tests

SMEAR STATUS	NO. OF CASES	PROBE 1		PROBE 2	
		+	-	+	-
POSITIVE	38	35	3	30	8
NEGATIVE	62	40	22	31	31
TOTAL	100	75	25	61	39

P_1:X^2 = 9.564, df = 1, p < 0.05, P_2:X^2 = 8.299, df = 1, p<0.05

Table 8 : Correlation : Histopathology with Probe Tests

TYPE OF DISEASE	NO. OF CASES	PROBE 1			PROBE 2		
		+	±	-	+	±	-
I	4	3	-	1	1	-	3
TT	2	2	-	-	2	-	-
BT	6	3	3	-	3	-	3
BB	4	2	-	2	2	-	2
BL	6	6	-	-	-	2	4
LL	3	3	-	-	3	-	-
NON SPEC	5	5	-	-	5	-	
Total	30	24	4	3	16	2	12

P_1 :X^2=34.667, df=12, p<0.01; P_2:X^2=33.497, df=12, p<0.01

3. Enzyme -linked immunosorbent assay (ELISA) :

We assessed the antibody response against 35 kDa antigen by Serum Antibody Competition Test (SACT). Nearly 50% smear negative cases, 42% Lepromin positive cases & 70% Lepromin negative cases showed positive antibody response. No control had a positive response.

4. *In-situ* Hybridization :

In-situ hybridization uses a labeled complementary DNA/RNA strand to localize specific DNA/RNA in a portion of section of tissue. *In-situ* hybridization significantly enhances the diagnosis in early cases (15).

We evaluated a correlation of clinical, histopathological, in-situ hybridization and PCR features on 22 patients in 2007. Skin smears for AFB were positive in 2/22 cases, histopathology confirmed diagnosis in 6/22 cases and non-specific histopathology was observed in 16/22 cases. In-situ hybridization was positive in 10/ 22 cases (Table 9).

PCR was done in 15 cases and was positive in 10/15 cases. Out of these 15 cases, 1 child had a positive smear; histopathology was positive in only 4 cases (Table 10).

Thus, PCR and IN SITU HYBRIDISATION significantly improved the diagnostic yield in early cases and in those with non specific histopathology.

In-situ hybridization offers excellent structural correlation and permits the concomitant study of tissue pathology. However, this method needs further evaluation on a larger sample size. Therefore, we can conclude that till the potential of *in-situ* hybridization to diagnose leprosy is fully explored, PCR can be used as a diagnostic method in cases of early leprosy where clinical diagnosis is doubtful and histopathology is nonspecific.

Table 9 : Correlation of Clinical Histopathological, In-situ Hybridization and PCR features of cases

Clinical type	No. tested	Skin Smear for AFB		Histopath. Dx confirmed	Histopath. Dx nonspecific	*In-situ* hybridization		PCR	
		+ve	-ve			+ve	-ve	+ve	-ve
I	1	0	1	1	0	1	0	0/1	1/1
BT	6	0	6	2	4	2	4	3/4	1/4
BB	10	0	10	1	9	3	7	6/9	3/9
BL	5	2	3	2	3	4	1	1/1	0/9
Total	22	2	20	6	16	10	12	10/15	5/15

Comparison : Skin smear & in-situ hybridization - p value < 0.05, z value = 3.269
Comparison : Skin smear & PCR – p value < 0.05, z value = 2.967
Comparison : PCR & Histopathology - p value < 0.05, z value = 4.236
Comparison : PCR & in-situ hybridization - p value > 0.05, z value = 1.960

Table 10 : Correlation of Clinical, Histopathological and PCR Features

Clinical types	No tested	Positive skin smear for AFB	Confirmatory histopathological diagnosis	Positive PCR signals
I	1	0	1	0
BT	4	0	1	3
BB	9	0	1	6
BL	1	1	1	1
TOTAL	15	1	4	10

Comparison of skin smear with PCR (1/15 vs 10/15) p value <0.05,z = 3.410.
Comparison of histopathology with PCR (4/15 vs 10/15) p value <0.05, z = 2.196.

5. *In-situ* PCR :

In-situ PCR, also called slide PCR, is a method to run PCR directly on small tissue samples, tissue microarrays (TMA), or other small cell samples, rather than extracting DNA or RNA first and then performing PCR, RTPCR, or q PCR from the extracted material.

In this pioneer work, conducted in our department in 2004, we evaluated a correlation of clinical findings, histopathological features and in-situ PCR on 20 patients. Skin smear for AFB was positive in 2/20 cases (10%), histopathology for AFB was positive in 9/20 (45%) cases. Nonspecific histopathology was observed 11/20 (77.7%) cases. In situ PCR was positive in 12/20 (60%) (Table 11).

Results of in situ PCR with nonspecific histopathology :

Histopathology diagnosed 9/20(45%) cases. In situ PCR done in patients with non specific histopathology was positive in 4/11 (36.3%). Cases confirmed by in-situ PCR and histopathology was 13/20(65%). Histopathology confirmed the clinical diagnosis in 45% of total cases. In situ PCR confirmed the diagnosis in 60% of total cases, thus enhancing the diagnostic yield (16) (Table 12).

Keeping in mind that *In-situ* PCR offer excellent structural correlation permitting concomitant study of tissue pathology & contamination by foreign DNA/RNA does not exist, it is a valuable tool for diagnosis of childhood leprosy.

Table 11 : Correlation of Clinical, Histopathological and in-situ PCR features of Cases

Clini - cal Type	No.	Skin Smear for AFB +	_	Histopath. Dx +ve AFB+		Histopath Dx non -sp	*In -situ* PCR +	_
I	3	0	3	1	0	2	2	1
BT	4	0	4	2	1	1	2	2
BB	9	0	9	2	1	6	4	5
BL	4	2	2	2	0	2	4	0
Total	20	2 (10%)	18 (90%)	9(45%)	2 (10%)	11 (77.7%)	12 (60%)	8(40%)

Comparison : Skin smear with histopathology : Z = 2.694, $p<0.05$
Comparison : Skin smear with *in-situ* PCR : Z = 3.91, $p<0.05$

Table 12 : Results of *in-situ* PCR in cases with nonspecific histopathology

Clini - cal Type	No.	Histopath. Dx +ve +	_	*In -situ* PCR in cases with nonspecific histopathology +	_	Cases confirmed by histopath. + *in-situ* PCR
I	3	1	2	1	1	2
BT	4	3	1	0	1	3
BB	9	3	6	1	5	4
BL	4	2	2	2	0	4
Total	20	9/20	11/20	4/11	7/11	13/20
Percen - tage	(100%)	(45%)	(55%)	(36.3%)	(63.6%)	(65%)

Controls

All 20 samples were negative

X^2 :20.810, P < 0.001, df: 1

6. RLEP PCR :

RLEP PCR detects nucleic acid sequence specific to the pathogen & can be used for definitive diagnosis of leprosy. Due to large size, amplicons of most of the PCR based methods like 65kDa, 18kDa, 36kDa undergo damage/fragmentation during the procedure. This does not occur with RLEP PCR. Donoghue et al found RLEP primer to be 1000 fold more sensitive than 36kDa primers. Higher sensitivity of RLEP PCR than slit skin smear for AFB is due to repetition of RLEP sequence 28 times in M. leprae chromosome. RLEP based PCR on skin smears can be a useful tool to confirm early cases of leprosy, where skin smears are negative and skin biopsy is not feasible. Very few studies are available in the world literature on the diagnostic value of RLEP PCR in childhood leprosy.

With this background we conducted this study from 2007 to 2008 & 2010- 2012. 73 cases of either sex, < 18 years of age, with hypopigmented /erythematous lesions showing partial/total loss of sensation and/or presence of thickened nerves were studied. 30 healthy children were taken as controls. After clinical examination & categorization (TT, BT, BB, BL, LL) two skin smears were taken, one for Z-N staining for AFB & another for RLEP PCR. After DNA extraction & amplification, electrophoresis was done & presence of 129bp fragments was considered as positive result. Z-N staining for AFB was positive in 17/73 (23.28%) cases and RLEP PCR in 56/73 (76.71%) cases. All controls showed negative results. RLEP PCR technique had a significantly greater positivity (especially early stages) than ZN staining ($p< 0.001$) (Table 13).

Thus, RLEP PCR on skin smears can be a useful tool to confirm early cases of leprosy, where skin smears are negative and skin biopsy is not feasible.

Table 13 : Correlation: skin smear for AFB vs. RLEP based PCR

Clinical Type	No. of cases	Smear positive	RLEP PCR positive
T	3	NIL	1
BT	27	NIL	21
BB	31	10	25
BL	11	6	8
LL	1	1	1
Total	73	17	56
%	100	23.28%	76.71%

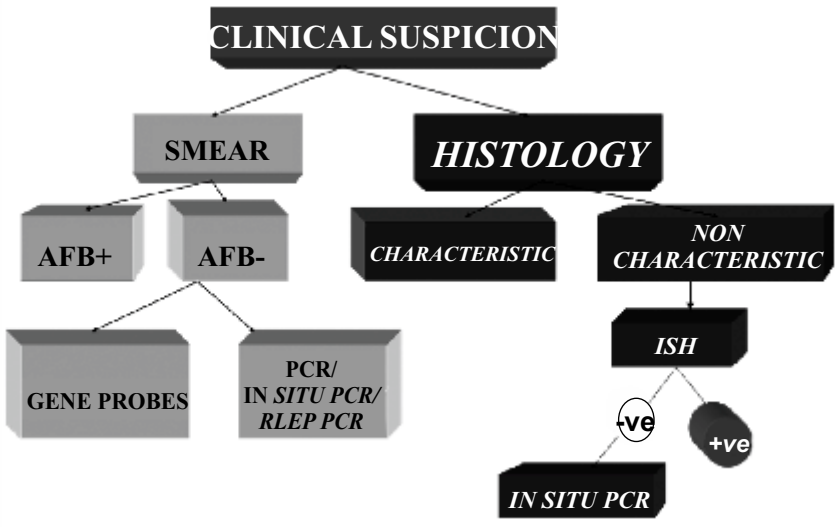
X^2-39.570 ; P < 0.001 D.F. =1

Suggested Algorithm :

On the basis of our 20 years experience with leprosy patients, we propose the following algorithm for diagnosis.

Whenever there is clinical suspicion, we can either go for smear for AFB or histology to confirm the diagnosis. A positive smear for AFB is confirmatory. If it is negative then, we can subject the specimen for gene probes or PCR/In-Situ PCR/RLEP PCR. If the result is positive, it is diagnostic of leprosy. On the other hand, if histology shows characteristic features then it is confirmatory; if it is not characteristic, we can go for in-situ hybridization. A positive in-situ hybridization is diagnostic of leprosy; if it is negative then we can opt for *in-situ* PCR.

SUGGESTED ALGORITHM



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