Indian Journal of
Dermatology & Venereology

(Incorporating Indian Journal of Venereal Diseases & Dermatology)

Vol. 29; No. 2. April-June 1963

ORIGINAL ARTICLES

SEROLOGIC TESTS FOR SYPHILIS IN LEPROSY.

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The Standard Serologic tests for Syphilis (S. T. S.) using the non-specific tissue lipid antigen is reported in the literature, to produce the so-called "biological false positive" (B. F. P.) or non-treponemal sero-reactions, in leprosy, more frequently than in any other non-treponemal diseases. The frequency of incidence of the B. F. P. reactions for Syphilis reported in leprosy, runs the range of 0 to 100 percent.

This variation in the incidence is apparently due to the differences in the Serologic techniques and the types of antigen used in them. Edmundson etal (1954) reported, in 224 leprosy patients from U. S. A. Sero-reactivity rates of 63.4% for the Kolmer cardiolipin complement fixation test, 52.7% for the Kahn Standard precipitation test and 46.9 per cent for the V. D. R. L. Cardiolipin slide flocculation test for Syphilis. The fact that the specimens of serum have been drawn from patients, in the various clinical stages of leprosy and infected for varying periods may also have caused the variability in the incidence of B. F. P. reactions reported by various investigators in this field at various times. Moore and Mohr (1952) found 40 to 60 percent of B. F. P. reactions in patients with lepromatous leprosy, while in the tuberculoid type, B. F. P. reactions were significantly of much less frequency comparitively. Ruge (1955) examined 820 leprosy patients in Egypt and reported sero-reactivity for Syphilis, on 45% of them with cutaneous type of leprosy, on 35% with mixed leprosy, but only 11% of patients with neural leprosy. SCHMIDT (1959) showed evidence to suggest that the duration of infection with leprosy is of great significance, the group of patients in his series, infected for the shorter periods, giving greater number reactive Sera with S. T. S.

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In India, Kvittingen etal (1952) made a special study of Serologic reaction for Syphilis in leprosy, sponsored by the W. H. O. at Simla during 1949-51. This is apparently one of the very few investigations in this field in India. They have also reported high incidence of B. F. P. reactions for Syphilis, in leper patients in India too, using the V. D. R. L., the Kahn Standard, and the Meinicke slide tests for Syphilis, on 821 cases of leprosy drawn from the northern regions of India.

In this clinio-serological study of 753 cases of leprosy from Madras, with serologic tests for Syphilis using both the non-specific crude and purified tissue extract antigens and the specific antigens of virulent T. Pallidum origin, the incidence of false positive reactions for Syphilis in leprosy in India is confirmed. However, the frequency of incidence of B. F. P. reactions has appeared in Madras leprosy patients to be comparitively less than those reported from abroad or from the North of India. Further, it has appeared that in 288 (309) specimens of leprosy sera in this study, investigated with the specific T P. I. test for Syphilis in parallel tests with other standard Serologic tests for Syphilis both at Madras and Copenhagen State Serum Institute, that a significant percentage of the so-called biological false positive reactors had possibly simultaneous infection with a treponemal disease.

MATERIALS AND METHODS.

Clinical Material.

The clinical cases in this study consisted of adult male leper patients made available through the kind co-operation of Dr. Z. Ahmed from the special leprosy clinic of the Government General Hospital, Madras, and children of both sexes varying from 5 to 12 years of age, provided through the kind courtesy of Dr. Ramanujam of the Government Silver Jubilee children leprosy clinic, Saidapet, Madras. These leprosy patients as they came as outpatients, everyday, during the periods of this study were thoroughly examined physically and historically by the specialist Medical Officers concerned, and the diagnosis of infection with Myco-Leprae was established clinically and in some cases confirmed bacteriologically. They were classified into three types namely 1. Lepromatous, 2. Non-Lepromatous, 3. Borderline cases. They were examined further by Dr. P. N. Rangiah, Professor of Venereology, Madras Medical College for evidence of any other infections, particularly treponemal or any other venereal infection, past or present. Actually 753 patients have been investigated in three separate batches. During May 1955 to December 1956, 142 adults, and during June '56 to January '58, 302 children, were investigated with parallel Wassermann Complement Fixation Reaction, the V. D. R. L. slide and Kahn Standard flocculation tests for Syphilis. During February to August 1959, additional 309 adults were investigated in parallel series with the V. D. R. L., the Kahn, the W. R. and the T. Pallidum Immune Adherence for Syphilis tests at Madras. At the same time duplicate split samples of these 309 sera were also tested in parallel with the V. D. R. L., the Kahn, the Meinicke, the Cardchol W. R. and the Cardiolipin W. R. (Morch-C. W. R. M.

tests for Syphilis at the State Serum Institute at Copenhagen where they had been sent by air particularly for testing with the known specific T. Pallidum Immobiliza tion (T. P. I.) test for Syphilis.

LABORATORY METHODS

5 to 10 ml. of blood had been taken with sterile precautions daily as the patients were clinically examined day by day. The sera were separated, soon after, in the Madras V. D. Laboratory and preserved in the cold at 5°C until they were ready to be tested with the Serological tests for Syphilis performed in parallel series. The tests were performed quantitatively once a week, after inactivation at 56°C for half an hour. The V. D. R. L. Cardiolipin slide flocculation and the kahn Standard precipitation test were performed according to the technique dessribed in Manual of S. T. S. (1955)

The following Cardiolipin antigens namely, Lederle batch No. 2740–21a, Italian Sclavo Batch No. 036, and A. P. U. Calcutta No. V. 758 were used for the V. D. R. L. tests in the first Batch of 142 specimens. The Italian Sclavo, No. 036 Cardiolipin antigen was used for second Batch of 302 specimens of leprosy sera from children. Italian Sclavo No. 036 and A. P. U. Calcutta No V. 758 Cardiolipin antigens were used in the V. D. R. L. test for the 3rd Batch of 309 specimens.

The Kahn Standard test was performed using Difco Kahn antigen No. B–218 for all the specimens at Madras.

The Wassermann Reaction was performed according to PRICE (1950) using A. P. U. Calcutta crude heart extract antigen Batch No. MF/A/54 in the first two Batches of sera and A. P. U. Cardiolipin Lot No. W. 2–56 was for the last Batch of sera.

The T. Pallidum Immune Adherence (TPIA) test was performed according to the technique of DAGUET (1956) using specific antigen of virulent Nichol's strain of T. Pallidum origin from rabbits, prepared locally at Madras.

The specimens of sera from the third batch of 309 patients were collected in adequate quantities and were preserved at –20°C until about 60 specimens had been collected at a time. Then each of the frozen specimen was thawed and split into two duplicate halves, one being sent as unknown to Copenhagen by air parcel, the duplicate being retained for testing at Madras.

The Madras test were performed in parallel, on batches of about 60 specimens at time during a week, after each batch of split samples of sera had been despatched by air to Copenhagen. The despatch of the 309 specimens to Copenhagen was completed during the course of 6 months. Each batch was kept preserved at –20°C at Madras for about a month and it was in transit under conditions of air transport to Copenhagen for 6 to 8 days.

The Copenhagen tests including the T. Pallidum Immobllization (T. P. I.) test for Syphilis, were carried out with the kind co-operation of Dr. H. Schmidt

according to the Standard techniques in routine use for them in the State Serum Institute. (Schmidt 1959).

RESULTS

753 cases of leprosy investigated in this study are classified as shown in the table 1 according to their clinical categories, to their age groups, and to the batches in which their serum specimens were tested with the various Serologic tests for Syphilis at Madras and Copenhagen.

TABLE 1

753 Leprosy cases classified.

LEPROSY (Clinical Category)	Batch 1 (Adults)	Batch 2 (Children)	Batch 3 (Adults)	Total
Lepromatous	57	50	112 (111)	219 (111)
Non-Lepromatous	83	233	192 (184)	508 (184)
Border-line	2	19	5 (5)	26 (5)
	142	302	309 (300)	753 (300)

Batch 1 and Batch 2 sera were tested at Madras only.

Batch 3 sera were tested both at Madras and Copenhagen.

Figures in brackets are the exact number of specimens tested at Copenhagen – a few specimen were lost in transit.

TABLE 2.

Percentage rectivity of the various S. T. S. at Madras compared in 753 leprosy cases classified according to age groups and clinical categories.

LEPROSY		VDRL	Kahn	WR	TPIA
All cases	Total	11.1 (753)	12.2 (753)	25.3 (750)	17.8 (309)
	Adults	17.1 (451)	14.9 (451)	26.4 (450)	17.8 (309)
	Children	1.99 (302)	8.3 (302)	23.7 (300)	Nil ( )
Lepromatous	Total	19.6 (219)	22.8 (219)	34.4 (218)	25 (112)
	Adults	23.1 (169)	23.1 (169)	35.1 (168)	25 (112)
	Children	8 (50)	22 (50)	32 (50)	Nil ( )

LEPROSY		VDRL	Khan	WR	TPIA
Non Lepromatous	Total	7.9 (508)	8.3 (506)	21.5 (506)	14.1 (192)
	Adults	13.8 (275)	10.2 (274)	21.8 (275)	14.1 (192)
	Children	0.9 (233)	6 03 (232)	21.2 (231)	Nil ( )
Borderline	Total	0 (26)	0 (26)	23.1 (26)	0 (5)
	Adults	0 (7)	0 (7)	0 (7)	0 (5)
	Children	0 (19)	0 (19)	31.6 (19)	Nil ( )

(Figures in brackets are the numbers of which the percentages are based.)

In the table 2 is shown the comparitive reactivity rates obtained with the various Serologic tests for Syphilis with which 753 leprosy cases were investigated at Madras. It may be particularly noted that, of all the currently used tests for Syphilis at Madras, the V. D. R. L. test has been the least reactive with 11.1 per cent of all 753 leprosy cases, 19.6 percent of 219 lepromatous and 7.9 percent of 508 non-lepromatous leprosy cases. Further all the tests for Syphilis are comparitively more reactive in the lepromatous than the non-lepromatous cases and more reactive in adults than in the children with leprosy.

TABLE 3.

Sero reactors in 300 leprosy cases, to Serologic tests for Syphilis at Madras and Copenhagen compared.

TEST FO \ SYPHILIS.		All cases	Lepromatous	Nonlepromatcus	Border line
V. D. R. L. (Cardio-lipin)	Madras	19%(300)	25.2%(111)	15.7%(184)	0 (5)
	Copenhagen	16%(300)	20.7%(111)	13.6%(184)	0 (5)
KAHN (Crude lipid antigen	Madras	23.6%(55)	31%(29)	17.4%(23)	0 (3)
	Copenhagen	18 2%(55)	27 6%(29)	8.7%(23)	0 (3)
MEINICKE – (Crude lipid antigen)	Copenhagen	29.9%(244)	37.5%(80)	26.5%(162)	0 (2)
W. R.	Madras (Cardiolipin)	23 3%(120)	35.2%(54)	14.7%(61)	0 (5)
	Copenhagen (Cardio ipin)	21.7%(120)	38.8%(54)	8 2%(61)	0 (5)
	Copenhagen (Cardchol)	29.0%(120)	67%(54)	21%(61)	0 (5)
T. P. I. A. (Treponemal dead Antigen)	Madras	18.1%(288)	24.8%(109)	14 4%(174)	0 (5)
T. P. I. (Treponemal live Antigen)	Copen-hagen	16.7%(288)	17.4%(109)	16.6%(174)	0 (5)

In the table 3 is given the percentage frequency obtained in 300 out of 753 specimens of adult leprosy sera specially investigated in parallel at Madras and Copenhagen, with all the Standard Serologic tests for Syphilis in use at both places particularly the T. P. I. test for Syphilis.

In this smaller group also it is to be noted that the lepromatous cases have been more reactive than the non-lepromatous leprosy cases, with all the Standard tests for Syphilis at both places.

The V. D. R. L. test for Syphilis at Copenhagen has been again the least reactive with 16 percent of 300 cases of leprosy.

It may be particularly noted that there are differences in the frequency of reactivity between any 2 tests of the same name performed at Madras and Copenhagen – for example the V. D. R. L. test at Madras has been reactive in 19 percent while it has been so in only 16 percent of the same 300 cases of leprosy at Copenhagen.

The percentage of reactivity of about 17 percent of 288 leprosy cases, obtained with the T. P. I. test for Syphilis irrespective of the types of leprosy may be specially noted in the table 3.

It has been interesting to note again, that the T. P. I. A. test of Madras also using a specific virulent T. Pallidum antigen like the T. P. I. test for Syphilis has shown the same reactivity tendency as the V. D. R. L. test for Syphilis using the non-specific cardiolipin antigen, in this particular group.

In the Table 4, agreements between the reactivity of any 2 Serologic tests for Syphilis performed on 309 cases of leprosy sera either at Madras or Copenhagen are analysed and compared.

Taking the T. P. I. test for Syphilis as the specific control test, its highest agreement in reactivity has been with the Copenhagen V. D. R. L. and Kahn test for Syphilis with 90.9%, and the lowest agreement has been with the W. R. using cardchol antigen at Copenhagen with 67.5 percent.

The greatest agreement in reactivity between any two tests performed either at Madras or at Copenhagen is between Madras V. D. R. L. and Madras T. P. I. A. tests for Syphilis, and least agreement has been between the Copenhagen T. P. I. test and the Copenhagen Cardchol WR, and between the Copenhagen Cardchol WR and the Madras V. D. R. L. and T. P. I. A. tests for Syphilis.

Further, no two tests of the same laboratory or of the same name or type, have had 100 percent agreement in this study.

TABLE 4.

Percentage Agreement in the results among serologic tests for syphilis for all possible pairs of tests on 309 cases of leprosy investigated at Madras and Copenhagen.

TESTS FOR SYPHILIS	Copenhagen TPI	Copenhagen VDRL	Copenhagen MEINEIKE	Copenhagen KAHN	Copenhagen CWRM	Copenhagen WR	Madras T.P.I.A.	Madras VDRL	Madras KAHN	Madras WR
Copenhagen TPI (Treponemal Antigen)		262/288 90.9%	189/244 77.5%	50/55 90.9%	103/120 85.8%	81/120 67.5%	254/288 88.2%	258/288 89.6%	246/287 85.7%	233/287 80.9%
Copenhagen VDRL (Cardiolipin antigen)			192/242 79.3%	52/55 94.5%	106/120 88.3%	84/120 70%	276/300 92%	275/300 91.7%	268/299 89.5%	255/299 85.3%
Copenhagen MEINEIKE (Crude lipid antigen)				7/8 87.5%	4/63 77.8%	45/63 72.4%	188/244 77.05%	187/244 76.6%	186/243 76.5%	182/243 74.9%
Copenhagen KAHN (Crude lipid antigen)					53/55 96.4%	41/55 74.5%	50/55 90.9%	49/99 89.1%	51/55 92.7%	49/55 89.1%
Copenhagen CWRM (Cardiolipin antigen)						96/120 80%	101/120 84.2%	101/120 84.2%	105/120 88.3%	120/102 85%
Copenhagen WR (CARDCHOL antigen)							81/120 67.5%	81/120 67.5%	86/120 71.7%	88/120 73.3%
Madras TPIA (Tepronemal antigen)								305/309 98.7%	286/30 92.9%	276/308 89.6%
Madras VDRL (Cardiolipin antigen)									263/308 91.9%	272/308 88.3%
Madras KAHN (Crude lipid antigen)										273/307 88.9%
Madras WR (Cardiolipin antigen)										...

TABLE 5.

Agreements and Disagreements between the specific TPI test for Syphilis at Copenhagen and current STS performed at Madras Laboratory on 288 cases of leprosy.

Other TESTS for Syphilis		TPI TEST FOR SYPHILIS. Reactive	Non-Reactive	Total
VDRL	Reactive	35	19 (7.9%)	54
	Nonreactive	13(27%)	221	234
KAHN	Reactive	29	23 (9.6%)	52
	Nonreactive	18(38.3%)	217 1, not done	235
WR	Reactive	31	37 (15.5%)	68
	Nonreactive	17(35.4%)	202 1 anticomplementary.	219
TPIA	Reactive	33	19 (7.9%)	236
	Nonreactive	15(31.3%)	221	236
Total		48	240	288

The table 5 shows the details of the agreements and disagreements between the Reactive and the Non-reactive results obtained in the specific T. P. I. test for Syphilis, and such results obtained in the various serologic tests for Syphilis performed at Madras in parallel, on 288 cases of leprosy. It is to be particularly noted that 240 were nonreactive and 48 were reactive to the T. P. I. test for Syphilis in 288 cases of leprosy. In the 240 T. P. I. test nonreactive group, the V. D. R. L. and the T. P. I. A. tests for Syphilis were the least reactive with 19 or 7.9 percent each, while in the 48 T. P. I. test reactive group, the V. D. R. L. test for Syphilis was again the least non-reactive with 13 or 27 percent.

TABLE 6.

Correlation between the titre of reactions with V. D. R. L. tests in the two T. P. I. test reactive and nonreactive groups of leprosy cases.

V. D. R. L. test titre in dils	T. P. I. Reactive cases Numbers.		T. P. I. Nonreactive Numbers.	
1 dil	6	14 (low titre)	7	14 (low titre)
2 dils	3		5	
4 ,,	5		2	
8 ,,	4	21 (high titre)	–	5 (high titre)
16 ,,	5		3	
32 ,,	4		1	
64 ,,	7		1	
128 ,,	1		–	
Total	35		19	

In the Table 6 is shown the comparitive titres of the reactive V. D. R. L. tests in 2 groups of leprosy cases reactive and Non-reactive to the T. P. I. test for Syphilis. It may be noted that in the T. P. I. reactive group, 21 out of 35 were 8 dils and higher in contrast to 5 out of 19 of such high titre in the V. D. R. L. test in the group of T. P. I. nonreactive cases. Alternatively, the V. D. R. L. test was reactive 4 dils and less in 14 out 35 T. P. I reactive group, in contrast to such low titre in 14 out 19, T. P. I. nonreactive grop.

TABLE 7.

Correlation between the duration of leprosy infection and the age of patients on the one hand, and the percentage incidence of the V. D. R. L. test for Syphilis reaction, in 201 TPI test nonreactive leprosy cases.

DURATION OF INFECTION

V. D. R. L. TEST REACTORS	Below 1 month	1 to 10 months	1 to 5 years	6 to 10 years	11 to 15 years	16 to 20 years	21 to 25 years
170/201	0/7	3/43 = 7%	6/106 = 5.2%	6/29 = 20.7%	1/7 = 14.3%	0/6	1/3 = 33.3%

AGE OF LEPROSY PATIENTS.

VDRL test Reactors 19/240	0–10 years	11to20 years	21to30 years	31to40 years	41to50 years	51to60 years	61to70 years
	0/1	3/38 = 7.9%	10/94 = 10.6%	3/62 = 4.8%	3/31 = 9.7%	0/9	0/5

TABLE 8.

Agreements and disagreements between the T. P. I. test for Syphilis and other tests for Syphilis particularly the F. T. A. test for Syphilis investigated in a special fourth batch on 33 cases of lepromatous leprosy at Madras and Copenhagen on split samples of the sera.

Other tests for Syphilis		T. P. I. test for Syphilis (Copenhagen)			
		REACTIVE	WK. REACTIVE	NONREACTIVE	TOTAL
F. T. A. 200 (Copenhagen)	REACTIVE	2	0	0	2
	WK. REACTIVE	2	0	0	2
	NONREACTIVE	2	2	24	28
F. T. A. 200 (Madras)	REACTIVE	2	0	0	2
	WK. REACTIVE	3	0	0	3
	NONREACTIVE	1	2	24	27
R. P. C. F. (Madras)	REACTIVE	1	0	0	1
	WK. REACTIVE	2	0	0	2
	NONREACTIVE	3	2	24	29
V. D. R. L. (Madras)	REACTIVE	1	0	0	1
	WK. REACTIVE	2	0	0	1
	NONREACTIVE	3	2	24	29
Total		6	2	24	32*(33)

*(1 specimen was inconclusive in the TPI test and nonreactive in all other tests.)

In the Table 8 is analysed the agreements between the rectivity of the T. P. I. test for Syphilis and other Serologic tests for Syphilis particularly the recently described Fluorescent treponemal antibody (F.T.A.) tests and the Reiter Frotein Complement Fixation (R.P.C.F.) test for Syphilis on 33 specimens of lepromatous leprosy sera studied in a special fourth batch in parallel tests at Copenhagen and Madras during 1961.

It may be noted particularly that there were no instances where the T. P. I. test was nonreactive while any of the other test was reactive in 33 cases of lepromatous leprosy sera in this batch. In 24 cases where T. P. I. test was nonreactive, all other tests also were nonreactive. However in cases when the T. P. I. test was reactive, there was major nonreactive disagreement with it in the F. T. A. of Copenhagen in 2 cases, in the F. T. A. of Madras in 1 case, and in both the R. P. C F. and the V. D. R. L. tests of Madras in 3 cases. There were a few minor disagreements too where the T. P. I. test was reactive or weakly reactive, while all other tests were weakly reactive or nonreactive respectively.

It may also be noted that there was complete agreement in the results of the R. P. C. F. and the V. D. R. L. tests of Madras and almost complete agreement between the results of F. T. A. tests of Copenhagen and Madras in 33 lepromatous cases of leprosy.

DISCUSSION.

Infection with Myco leprae does seem to produce false positive Serologic reaction for Syphilis in patients. This phenomenon however has not been adequately explained. An antibody antilipoidal in nature, has been demonstrated by various investigators to varying high frequencies, in the sera of leprosy patients on testing them with all the Standard Serologic tests for Syphilis employing the non-specific lipoidal tissue extract antigen. But it has been difficult to obtain a correct estimate of the frequency of incidence of these reactions because of several factors involved.

First of all, when a Serologic test for Syphilis is rective in leprosy, it is difficult to prove that the reaction is due to leprosy alone and not due to a possible simultaneous treponemal infection. This is because of the fact that leprosy and treponemal infections inspite of their distinct etiological agents, have a number of points of contact. Leprosy and Syphilis have similarities in their epidemiology, both flourishing side by side in the urban and the rural areas, among the same socio-economic class of the population. There may be clinical resemblances particularly between leprosy and yaws in the rural population.

Then, it is not easy to exclude simultaneous past or present latent troponemal infection, in the presence of more obvious leprosy lesions, by the absence of clinical and historical evidence alone. In fact, all the cases investigated in this study, had obvious lesions of leprosy with no presenting lesions of Syphilis or

yaws, and yet these treponematoses in their latent form could not be excluded or established unequivocally in them clinically.

The specific T. Pallidum Immobilization test for Syphilis only, can possibly verify if the positive reactions obtained in leprosy patients, tested with the standard Serologic tests for Syphilis in current use, are true or false biologically. Very few early investigators in this field had the benefit of T. P. I. test for Syphilis to verify their Standard Serologic test results. Therefore their estimations, often of very high incidence of false positive reaction for Syphilis in leprosy with their Standard tests, my be questioned at present in view of the fact that the T. P. I. test is now available as a specific verification test of false reactions.

In an earlier investigation in this field in India too, the T. P. I. test for Syphilis was not available to KVITTINGEN etal (1952). Therefore their figures for sero-rectivity of 25 percent of 818 leprosy cases in the V. D. R. L. test, and 66 percent of 526 cases for the Kahn Standard test, may be considered rather high and may not be accepted as the true incidence of false positive reaction for Syphilis discovered in leprosy in India since the possible simultaneous treponemal infection had not been excluded for certain in them.

In this Serological study of leprosy in the South of India at Madras, it may be seen from the figures shown in table 2, that the corresponding sero-reactivity figures in all 753 cases of leprosy were comparitively less, with 11.1 percent for the V. D. R. L. test and 12.2 percent for the Kahn Standard test for Syphilis. When the the adult 451 cases out of 753 leprosy cases were alone taken, the reactive rates were only 17.1 percent and 14.9 percent for the V. D. R. L. and the Kahn test for Syphilis respectively. Even when the lepromatous leprosy cases alone were taken, the V. D. R. L. were reactive in 19.6 percent and the Kahn test in 22.4 percent of 219 cases.

The T. P. I. test for Syphilis was not available in the investigation of the above group of all 753 leprosy cases at Madras and yet the reactive rates obtained for the V. D. R. L. and Kahn tests were comparitively less than that of Kvittingen's rates for these tests in leprosy from the North of India. In the study of 288 leprosy cases at Madras in the batch 3, in which the T. P. I. test was available as a specific verification test, it will be seen from table 5 that the V. D. R. L. test was reactive in 55 or 18.8 percent, while the T. P. I. test in the same group was reactive in 48 or 16.7 percent of 288 leprosy cases. If this known specific reaction of the T. P. I. test is taken as evidence of simultaneous treponemal infection of these leprosy patients, and the percentage reaction with the T. P. I. test for Syphilis is subtracted from the percentage reaction obtained for the V. D. R. L test performed in parallel in the same group, then the actual incidence of false positive reaction for the V. D. R. L. test in this particular would be as low as 2.1 percent.

But this large reduction in incidence of the possible false positive reactivity of the V. D. R. L. test by this method may be questioned on the possibility that a V. D. R. L. test type of positive lipoidal antigen antibody reaction, in T. P. I. test reactive leprosy patients, may be provoked in independently by the leprosy infection too, simultaneously present with a treponemal infection. Therefore, the rate of the T. P. I. test reactivity incidence may not justifiably be so subtracted to come to a correct estimate of the frequency of the false positive reactivity incidence with the V. D. R. L. test in leprosy.

However, if the specific value of the T. P. I. test for Syphilis is admitted, the 240 T. P. I. non-reactive leprosy caces in this particular batch 3 may be considered not to have a simultaneous treponemal infection. Any incidence of positive reaction given by any other serological tests for Syphilis in these 240 leprosy cases may be considered to be a true incidence of its biologic false positive reaction. As seen in the table 5, then the true incidence of Biologic false reaction for the V. D. R. L. test for Syphilis in leprosy at Madras would be 19 or 7.9 percent of 240 cases.

It may be noted in this connection as seen from the results shown in the table 8 that in 33 lepromatous cases of leprosy, specially investigated in a separate fourth batch again, both at Madras and Copenhagen, there were not any false positive cases at all with any of the other Serologicel tests for Syphilis including the V. D. R. L. test. All the 24 T. P. I. non-reactive leprosy cases in this group were also non-reactive to all other tests and all cases reactive to the other tests for Syphilis were also reactive to the T. P. I. test for Syphilis performed with them in parallel series.

It is interesting in this connection to note that DAGUET (1961) also recently reported that, in his serological investigation of 150 leprosy cases at the Institute Marchon de Bomako Africa, he found only 4 percent false positive reactivity in his standard serological tests using lipoldal antigen, on parallel check with the T. P. I. test for Syphilis. Therefore, it is necessary to reconsider the common view that leprosy produces very high incidence of positive serological reaction for Syphilis. Simultaneous infection with T. Pallidum should be always kept in mind under such circumstances. Leprosy infection might be chronogically an earlier infection having been infected in childhoor and the later Syphilis infection may be latent with the possibility of manifesting itself with late lesions. There for appropriate anti-syphilis treatment too should be given to such patients while under treatment for leprosy.

From the results analysed in the table 5, it would appear that the false positive reaction for Syphilis obtainable in leprosy with the V. D. R. L. test for Syphilis may be comparitively of lower titre quantitatively. 14 put of 19 T. P. I. non-reactive cases were reactive to the V. D. R. L. test to 4 dils and less in contrast to 14 out 35 in T. P. I. reactive leprosy cases, The false positive reaction

for Syphilis in leprosy when they occur have appeared to have certain positive correlation to the duration of the infection and to the age of the patient as seen in table 7.

The results of this study, as seen in the table 2 and 3 confirm the findings of earlier investigators in this field that the false positive reaction for Syphilis, when they occur in leprosy do so significally more frequently in lepromatous than in non-lepromatous cases. This goes for all types of Serologic tests for Syphilis employing the non-specific lipoid tissue extract antigen, irrespective, of the fact that the antigen is crude or purified. In striking contrast to the reactions of the Serologic tests for Syphilis using lipoidal antigen, the reaction of the T. P. I. test in 300 cases of leprosy cases was not significantly affected whether these cases were lepromatous or non-lepromatous as seen table 3. This finding may be considered as indicative of the truly specific type of reaction in which T. P. I. test is concerned in Syphilis.

Kvitringen etal (1952) reported that the Meineike test for Syphilis had given fewer number of positive reactions than all other Serologic tests employed, with 12 percent of their 815 leprosy cases from North India. The presence of "balsam of Tolu" in the Meinicke test antigen in place of "Cholesterol" which is usually included in all other lipodal antigen for the Standard Serologic tests for Syphilis was given the credit by Kvittingen for this comparatively favourable figure for the Meinicke test for Syphilis in leprosy. The high cholesterol content of leprosy sera was considered a probable cause of the hypersensitivity of the serologic reactions in leprosy with tests for Syphilis employing antigen containing cholesterol.

But in this study, the Meinicke test for Syphilis of Copenhagen was reactive in 29.8 percent of 244 leprosy cases and actually it was the most highly reactive of all other tests for Syphilis used in parallel with it (table 3). It may be said that the technique used to perform the Meineike test at Copenhagen was the "original tube clarification" method of Meineike, while Kvittingen had used a slide modification of it in his study of leprosy in North India. But the constitution of antigen with particular regard to "Tolu balsam" about the same so that the comparatively higher specific worth claimed by Kvittingen for the Meineike test for Syphilis in leprosy may not be quite justified.

According to Schmidt (1961) of Copenhagen, "Cardchol antigen" which does not contain lecithin but only cholesterol and Cardiolipin was found by him, to be more reactive or sensitive in leprosy sera than any other antigens containing Cardiolipin, lecithin and cholesterol used together usually in standard Serologic tests for Syphilis. Therefore, he has suggested that in his "Cardchol Wassermann Reaction" this antigen reacted almost specifically with the antilipoidal antibody produced in leprosy sera distinct from the antilipoidal antibody also produced in

Syphilis. However, he did not claim that Cardchol W. R. is a specific Serologic tests for leprosy.

Since the T. P. I. test for Syphilis could not be established in India, alternative tests for Syphilis using specific antigen of T. Pallidum origin were also under trial in this study for their possible specific value, using the T. P. I. test of Copenhagen as the control specific test.

The T. P. I. A. test for Syphilis described as an alternative to the T. P. I. test as a specific verification test has not been proved in this study to have the specific worth reported for it, as seen in the table 3. It seemed to show no particular advantage over the V. D. R. L. test atleast in leprosy sera.

The reported potential specific worth of the more recently described Fluorescent treponemal antibody (F. T. A.) DEACON etal (1957) test for Syphilis was checked on 33 specimens of lepromatous leprosy sera with the T. F. I. test for Syphilis at Copenhagen as shown in the table 8. The F. T. A. test had 26 complete, and 5 partial agreements in its results with T. P. I. test on 32 cases. But in this group of leprosy there were no obvious false positive cases so that it has not been possible to confirm the reported specific value of the F. T. A. test in leprosy in this preliminary study of it.

The Reiter Protein Complement Fixation (R. P. C. F.) test (Cannefax, 1959) also under study as a possible specific verification test for Syphilis has 25 complete and 4 partial agreements with the T, P. I. test results in the same 32 cases of leprosy sera, and it showed no advantage over the V. D. R. L. test in this particular group of leprosy case in which unfortunately there were no false positive cases. However, in a separate investigation of leprosy cases with parallel V. D. R. L. and the R. P. C. F. tests for Syphilis not included in this report, there were 9 instances out of 72 cases where the V. D. R, L. test was reactive while the R. P. C. F. was non-reactive, indicating the possible use of the R. F. C. F. tests as a verification test of B. F. P. reactions in this field.

It was also noticed in this study as seen in the table 5 that most of the other serologic tests for Syphilis have the tendency to be non-reactive in some cases of leprosy where the T. P. I. test has been reactive. The significance of the "false negative reaction for Syphilis " in these tests must be considered with reference to lower sensitivity or reactivity level at which they are functioning if the same type of antibody is involved, or considered with regard to a distinctly different antibody concerned in the T. P, I. test for Syphilis.

From the results seen in the table 3 and 4 it is apparent that there has been differences in the percentage reactivity of any two tests of the same name or type from the two laboratories at Madras and Copenhagen. Again 100 percent agreement in the results was not obtained between any two tests for Syphilis of same name or different type, from the same laboratory or from 2 different labo-

ratories at Madras and Copenhagen. In Explaining this, it may be said that the difference between 19 percent and 16 percent reactivity obtained in the Madras and the Copenhagen laboratory respectively for the V. D. R. L. test on the same, 300 cases of leprosy may not be considered statistically significant. If it is so, the lower percentage reactivity obtained with almost all the S. T. S. at Copenhagen on the same 300 cases (table 3) may be explained on the possibility of the poor stability of the antilipoidal antibodies in leprosy sera while under transport under unfavourable conditions from Madras to Copenhagen. Further the possibilities of the modification or variability of techniques in the tests of the same name in different laboratories and the different levels of reactivity at which the different tests are standardized and set in the same and different laboratories are to be considered.

Schmidt (1961) had observed from the results of his serological investigation of leprosy cases from Egypt that the frequency of the reactions he obtained on them with T. P. I. test for Syphilis more or less corresponded to the frequency of Serological reaction for Syphilis reported in the general population of Egypt. Therefore, it was suggested by him as an anology that 15 percent reaction that he obtained with T. P. I. test for Syphilis in this 300 cases of leprosy from Madras may be considered to represent the incidence of positive Serology for Syphilis or in other words, incidence of treponematoses infection in the general population of Madras. Although the leprosy and Venereal disease patients come from the same socio-economic class in India and in Madras generally, it is stressed that these 300 leprosy cases may be considered a selected group attending a special leprosy clinic in Madras City and therefore the incidence of sero-reaction for Syphilis obtained in them may not be taken strictly to represent the incidence of Syphilis in the general population of Madras or even of Madras City. The frequency of sero-reaction for syphilis in pregnant women from an antenatal clinic would be a more reliable index of incidence of syphilis in the general population. Actually it has been found that only 8 present of 48674 specimens of sera from pregnant women investigated as a routine from a Madras City antinatal clinic during the the period of this investigation were reactiv to the V. D. R. L. test for Syphilis in this laboratory.

SUMMARY

The frequency of incidence of the so called biologic false positive reactions for Syphilis in leprosy, specially investigated with a battery of currently used standard Serologic tests for Syphilis using non-specific tissue extract antigen and controlled by the known specific T. P. I. test for Syphilis using specific treponemal antigen at Madras, is presented. It is confirmed that all the currently used S. T. S. produces B. F. P. reactions for Syphilis in leprosy in the absence of a simultaneous treponemal infection. However, the frequency of incidence of B. F. P. reaction with Standard test for Syphilis obtained in leprosy in this study at Madras has been found to be far less than those reported earlier by other investigators in this field in

North India and abroad. A significant proportion of the leprosy patients at Madras who were found reactive to the current Standard tests were found to have simultaneous latent treponemal infection. The comparitively higher figures reported by others is considered to be due to the fact that a possible simultaneous treponemal infection in leprosy patients could not be easily excluded by them clinically alone, in the lack of the specific T. P. I. test for Syphilis. The various aspects of this problem are discussed.

ACKNOWLEDGEMENTS

The authors gratefully express their thanks to the Government of Madras and the Indian Council of Medical Research for providing facilities for this study. To Dr. R. V. Rajam, Ex-director and Dr. P. N. Rangiah present Director and other staff of the Clinic and Laboratory of the Madras Institute of Venereology, To Dr. Dharmendra, Director General Leprosy Institute, Chingleput, Madras, To Dr. Ramanujam, Medical Officer, Silver Jubilee Children Leprosy Clinic, Saidapet, Madras, To Dr. Z. Ahmed, Medical Officer, Leprosy Clinic, Government General Hospital, Madras and To Dr. H. A. Nielsen and Dr. H. Schmidt of the Copenhagen State Serum Institute, they are obliged for their advice, help and co-operation in this study.

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