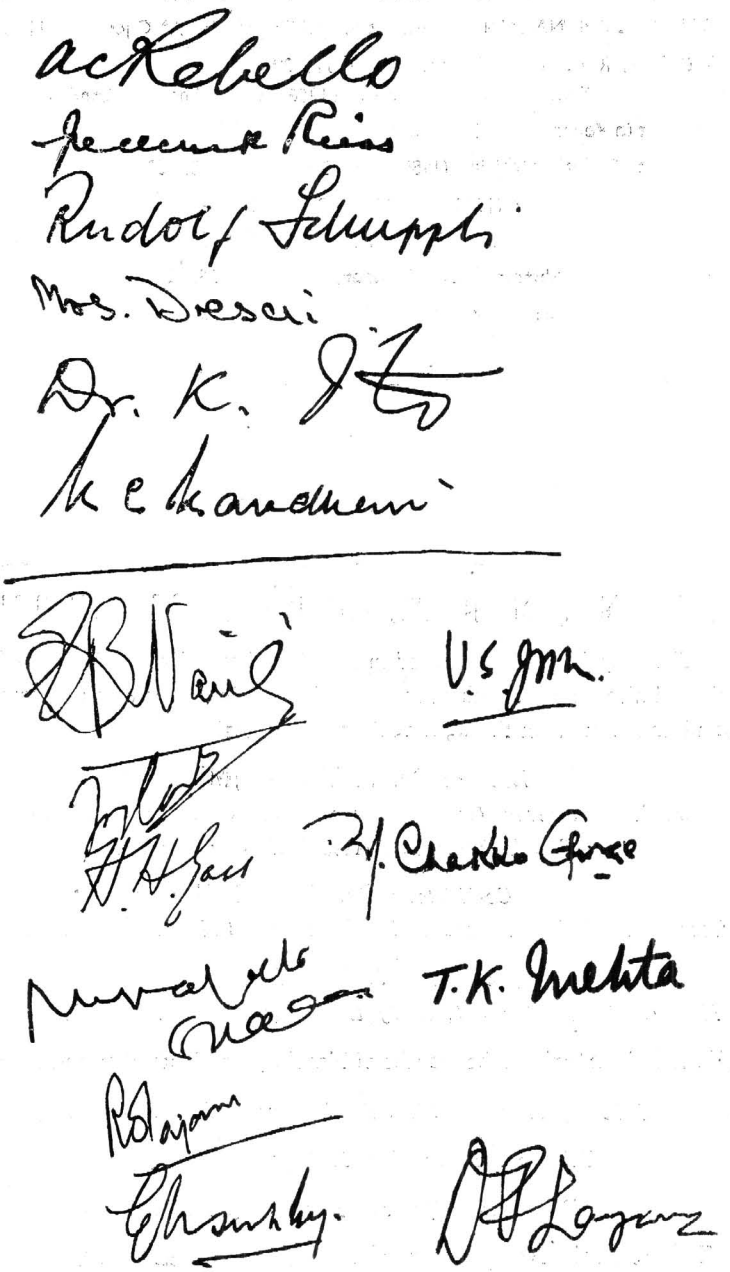
4th All India Conference.

Autographs of Delegates at the Lunch at Radio Club, Bombay, hosted by President, Dr. J. Fernandez.



EVALUATION OF R. M. T. & V. D. R. L. TESTS CARRIED OUT SIMULTANEOUSLY IN 1594 CASES*

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The evoluation of the serology of syphilis is one of the fascinating topics in the field of immunology. In 1906 Wassermann[6] introduced a complement-fixation test for the diagnosis of syphilis. The first flocculation test was described by Michaelis[2] in 1907. This was soon followed by the introduction of many other similar tests. The isolation of cardiolipin from the crude beef heart extract by Pangborn[5] in 1941 was another important milestone in the sero-diagnosis of syphilis. In 1959 Nelson and Mayer[3] introduced the first specific sero-diagnostic test for syphilis using live Treponema pallidum as antigen. The original Treponema pallidum immobilisation test was followed by other tests employing similar antigens such as Treponema pallidum agglutination test. Treponema pallidum immune-adherence test, Treponema pallidum complement-fixation test etc. Though both highly specific and highly sensitive, these tests for certain reasons cannot yet replace the standard serological tests for syphilis. Besides, the cost and technical limitations of these tests have limited their use to a few large laboratories only. The standard serological tests are still the tests of choice.

The standard serological tests are often employed as a matter of routine in medical examinations. It is necessary to assess the relative merits of these tests in the different stages of syphilis. Osmond[4] has rightly pointed out that the value of a test depends as much upon the skill and experience of the tester, as on the specificity and sensitivity of the test. Owing to the non-specific nature of the antigen used, it is sometimes an advantage to use more than one test. All antigen do not possess the same degree of sensitivity and specificity and serological discords are to be expected in certain cases.

MATERIALS AND METHODS

Samples of sera were received from the wards, out-patient departments, Blood Bank and the antenatal clinic of the J. J. Group of Hospitals, Bombay. Grossly contaminated and hemolysed sera were excluded from the present study. Meinicke and V. D. R. L. Slide flocculation tests were carried out. Kahn test was carried out on a few sera only. The standard technique was employed for all three tests and this helped to minimise the element of personal error.

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DISCUSSION

During the year 1960, 1,70,011 new patients attended the various O. P. D.s of the J. J. group of Hospitals, Bombay and 32 478 were treated as indoor patients. During the year, 18,169 V. D. R. L. tests, 16,252 R. M. Tests, 432 Kahn tests and 295 Wasserman tests were carried out. The slightly higher number of V. D. R. L. tests is due to the fact that sera received from the Blood Bank were subjected to V. D. R. L. tests only.

Before evaluating the data in the present study, certain facts need to be borne in mind. The majority of patients attending the J. J. Group of Hospitals, Bombay, are from the lower strata of society, of average intelligence and limited means. Their approach to the hospitals is for the alleviation of symptoms. Not all of them stay on to complete the treatment and very few bother to attend for a systematic follow up. Many patients reported to the clinic after having received injections of penicillin outside. This fact has to be born in mind when interpreting the results of serological tests.

PRIMARY SYPHILIS

Out of the 514 cases diagnosed as primary syphilis comments are offered here on 511. In 73 cases the diagnosis was made on D. G. I. examination alone, patients not reporting for the serological tests. 483 cases were subjected to serological tests. In 417 i.e. 94.97%, both R, M. T. and V. D. R. L. tests were positive. 21 i. e. 5.04% sera presented discord. 19 showing only V. D. R. L. positive and 2 showing R. M. T. positive. Kahn test was performed in seven cases. Wilkinson[7] is of the opinion that such a discord occurs when primary syphilis enters sero–reactive stage and later after treatment when it enters sero–nonreactive stage. As Daruwala[1] has pointed out, the discord is even more apparent in the static or deaclining phase of the sensitivity of these tests than in the rising phase. It is due to the varying sensitivity of the different tests employed and is likely to occur when serum contains less reagin.

SECONDARY SYPHILIS

141 cases were diagnosed as secondary syphilis. In 139 cases both R. M. T. and V. D. R. L. tests were strongly positive. In the remaining two, the diagnosis was based on the D. G. I. results, the patients not reporting for serological tests. In the majority of cases, the V. D. R. L. titre was over 1 : 16, the maximum titre observed being 1 : 512. There was not a single case of serological discord.

LATENT SYPHILIS

Out of the 953 cases diagnosed as latent syphilis, comments are offered here on 723. In 666 i. e. 92.13% cases, both R. M. T. and V. D. R. L. tests were positive. 57 i. e. 7.8% sera showed serological discord. In all these the V. D. R. L. test was positive and R. M. T. negative. In 12 of these cases Kahn test was found to be positive.

PRENATAL SYPHILIS

Sera of 3795 pregnant women attending the Antenatal Department were examined during the year. 208 i. e. 5.4% sera were found to be positive. Both R. M, T. and V. D. R. L. tests were positive in 140 i. e. 67.31% cases. In a large number of cases tests were positive in low titre being under 1:16. Remaining 68 i, e. 32.66% cases showed serological discord. In the majority of these cases V. D. R. L. was positive and R. M. T. negative. Pregnancy is no longer accepted to be a cause of biological false positive or false negative with the more modern standard serological tests. However, in the final analysis, we have offered our own comments.

LATE BENIGN SYPHILIS

Of the 25 cases diagnosed as late benign syphilis, comments are offered on 15. The distribution of these was as follows: Skin 4, Bone 7, Eye 4, In all cases except one, both R. M. T. and V. D. R. L. were positive. In the majority of cases, the titres observed were high. One case presented a serological discord; V. D. R. L. being positive, and R. M. T. negative.

CARDIO-VASCULAR SYPHILIS

Out of 54 cases diagnosed as cardio-vascular syphilis comments are offered on 35. The distribution of these was as follows; Aortic incompetence 24 (one with C. C. F.) and Aneurysm 11, 10 being thoracic confined to the arch and 1 abdominal. Other types of cardio-vascular syphilis were not observed in these series. Post-mortem confirmation of these lesions was not available. In 34 cases R. M T. and V. D. R L. were positive. There was one case of discord, V. D. R. L. being positive and R. M. T. negative.

NEURO SYPHILIS

Out of 35 cases diagnosed as neuro-syphilis, comments are offered here on 30. The distribution of these was as follows: Optic Atrophy 15, G. P. I., Hemiplegia and Transverse myelitis three each, Tabes Dorsalis and VIII Nerve deafness two each, Monoplegia and Meningo-vascular syhpilis one each. The diagnosis was based on clinical findings, serology of blood and C. S. F. and clinical pathology of C. S. F. In 29 cases both R. M. T. and V. D. R. L. on blood were positive. There was a single case of discord, V. D. R. L. being positive and R. M. T. negative.

CONGENITAL SYPHILIS

Out of 10 cases diagnosed as congenital syphilis, comments are offered on 8. All were cases of late congenital syphilis. Two cases reported with interstitial keratitis Stigmata observed were depressed bridge of the nose in 4, Hutchinson's teeth in 1, perforated palate in 1. In 5 cases both R. M. T. and V. D. R. L. were positive. There were 3 cases of serological discord V. D. R. L. being positive and R. M. T. nsgative. The number of cases of congenital syphilis is small as most of them must have reported to the B. J. Hospital for children.

It may be pointed out here that out of 1594 cases in the present series, both R. M. T. & V. D. R. L. tests were found to be positive in 1444 i. e. 90.47% cases. Of

TABLE No. 1

Distribution of Cases in different stages of syphilis and their diagnosis.

Stage of Syphilis.	Total cases diagnosed	Comments offered on.	Diagnosis by D. G. I.	Diagnosis by Serology	Both RMT & VDRL positive.	Serological Discord								Total
M	+	—	+	—	+	—	+	—						
V	—	+	—	+	—	+	—	+						
K	—	—	+	+	D	D	-	-						
Primary	513	511	73	438	417	1	7	0	5	0	4	1	3	21
Secondary	141	141	2	139	139	0	0	0	0	0	0	0	0	0
Latent, Early & Late	953	723	0	723	666	0	23	0	12	0	8	0	14	57
Prenatal	208	208	0	208	140	0	24	9	12	0	2	3	13	68
Late Benign	25	15	0	15	14	0	1	0	0	0	0	0	0	1
Cardio-vascular	54	35	0	35	34	0	1	0	0	0	0	0	0	1
Neuro	35	30	0	30	39	0	0	0	0	0	0	0	1	1
Congenital	10	8	0	8	5	0	0	0	0	0	0	0	3	3

TABLE No. 2.

Distribution of V. D. R. L. Titres in blood in the different stages of syphilis.

Stage of Syphilis.	W. P.	1:1	1 2	1:4	1:8	1:16	1 32	1:64	1:128	1:256	1 512	Total
Primary	8	28	47	50	71	57	66	50	33	7	0	417
Secondary	0	0	0	2	6	5	23	40	46	15	2	139
Latent	9	42	84	101	109	83	90	81	58	9	0	666
Prenatal	18	13	32	21	17	14	11	8	5	1	0	140
Late benign	0	0	0	1	3	2	2	3	3	0	0	14
Cardiovascular	0	0	1	3	5	7	13	3	2	0	0	34
Neuro	0	0	3	4	5	11	2	3	0	1	0	29
Congenital	1	0	0	0	0	0	1	2	0	1	0	5

the remaining 152. only in two cases R. M. T. was positive and V. D. R. L. was negative. In 150 cases R. M. T. was negative and V. D. R L. was positive. Rapid Meinicke Test as its prefix implies, is a screen test and as such must possess a very high degree of sensitivity, even at the sacrifice of specificity. This is not borne out by the results shown here. This test has since been dispensed with being found unsatisfactory, the antigen probably being at fault. However, we have reproduced the results as we found them.

The V. D. R. L. test was carried out with the antigen supplied by the Antigen manufacturing unit, Calcutta.

Certain significant facts have emerged from this series. In primary syphilis the titres varied from weak positive to 1:256. In secondary syphilis, out of 139 cases only 13 cases showed titres of 1:16 or under. The highest titre observed was 1:512. In no case was the blood found to be negative. In the latent stage of the disease titres showed all dilutions from weak positive to 1:256. Almost all the cases of prenatal syphilis were cases of latent syphilis. Only 25 out of 140 showed a titre higher than 1:16. This is not the same as in the latent group. Could it be that pregnancy is responsibe for these low titres? If so, could pregnancy also not be the cause of a few false negatives? In late benign syphilis more than 50% of the cases showed a titre higher than 1:16. Comparatively lower blood titres are observed in cases of neurosyphilis. Ail cases except one, in late congenital syphilis, showed titres higher than 1:16. It may be concluded that V. D. R. L. has proved to be a very sensitive and specific test and is the test of choice praticularly when only one test is to be employed, for the diagnosis of syphilis.

SUMMARY

Simultaneous testing of 1594 sera of patients in different stages of syphilis has shown that in 1444 i. e. 90.4% cases both the tests were positive. The remaining 152 i. e, 9.5% cases which have presented serological discord has shown greater positivity rate with the V. D. R. L. tests. This relationship has been maintained uniformly in all stages of syphils. The unsuitability of R. M. Test is pointed out. Certain signifiicant observations from the present series have been included. V. D. R. L, test has rendered a good account of itself and is recommended as the test of choice, particularly when only one test is employed for the diagnosis of syphilis.

REFERENCES

1. Daruvala B. A. "Sero-diagnosis of Syphilis" indian Year Book of Medical Sciences Vol. 1:428, 1958.
2. Michaelis A. "Serum diagnosis of syphilis by precipitation" Kahn 1st Edition: 7,1928
3. Nelson R. A. & Meyer M. "Modern Diagnosis of sypilis" W. H. O. Bulletin Vol, 14: 263, 1956
4. Osmond T, E. "Value of Serum reactions" British journal of Venereal Diseases, 11:177, 1935
5. Pangborn M. C. "A new serological active phospholipoid from beef heart" Prcc. Soc. Exp. Bibl. & Medicine 48:484, 1941
6. Wasserman A. "Textbook of Bacteriology" Zinsser 11st Edition: 528, 1957
7. Wilkinson A. E. "A Universal Case of Serological Discord" British Journal of Venereal Diseases 30:38, 1954.