

Serological tests for syphilis in Saudi Arabia

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SUMMARY A total of 6684 sera were initially screened for syphilis by the Venereal Disease Research Laboratory (VDRL) test and the *Treponema pallidum* haemagglutination assay (TPHA). Reactive sera from either or both these tests were tested for confirmation by the fluorescent treponemal antibody-absorbed (FTA-ABS) test. VDRL biological false positive reactors were detected in 0.5% of the total sera examined, with 0.4% and 0.8%, respectively, obtained in pregnant women and blood donors. Eight sera (0.1%) were found to be positive in the TPHA test alone.

An overall positivity of 2.7% for syphilis was detected, with a 0.85% positivity in antenatal patients. Infection with *T pallidum* seemed to be more common in men than in women (1.6:1) and predominated in the age group 20-39 years. Serological testing of sera from 26 mother and infant pairs allowed one case of congenital syphilis to be detected by FTA-ABS (IgM) and identified VDRL biological false positivity in seven infants.

Introduction

Two categories of serological tests for the diagnosis of syphilis are currently available: cardiolipin tests, which detect antibodies to lipoidal antigens; and specific tests, which detect antitreponemal antibody.¹

The most widely used cardiolipin (non-treponemal) and specific tests are, respectively, the Venereal Disease Research Laboratory (VDRL) test² and the *Treponema pallidum* haemagglutination assay (TPHA).³ The fluorescent treponemal antibody-absorbed (FTA-ABS) test is used mainly for confirmation.⁴ The proper use of these tests in combination with clinical information makes the serodiagnosis of syphilis in both adults and children relatively straightforward and useful.

The present study is the first broad based investigation of syphilis in Saudi Arabia; and it attempts to obtain a profile of serological testing for syphilis in various groups of patients, to relate testing of sera to ultimate diagnosis, and to evaluate the effectiveness of the tests used. The FTA-ABS (IgM), which is still a test for the specialist laboratory, has been used to good effect here for the diagnosis of congenital infections.

Patients, materials, and methods

PATIENTS

The patients in this study included all those attending the dermatology and venereology clinic, as well as those with suspected syphilis attending the ophthalmology, otorhinolaryngology, and medicine clinics at this hospital. Serological testing for syphilis was also carried out on blood donors and pregnant women as routine screening.

SEROLOGICAL TESTS

An FTA-ABS test was carried out if the results of either or both tests were positive. Tests with doubtful results were repeated sequentially with a second sample obtained from the patient. The infants' cord blood sera were tested by VDRL, TPHA, FTA-ABS (IgG), and FTA-ABS (IgM).

VDRL

The VDRL slide flocculation test was performed manually using the Oxoid VDRL carbon antigen (Oxoid Diagnostic Ltd, United Kingdom) according to the manufacturer's instructions.

TPHA

The Fujizoki preparation was used according to the manufacturer's instruction. Sensitised cells were added to alternate rows of the quantitative tests performed in microtitre plates, with the results read as twofold dilutions. The screening and initial dilution

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was 1/80, and the last serum dilution of the quantitative test was 1/20 480. Specimens showing doubtful readings were retested.

FTA-ABS

The reagents used in the FTA-ABS test were obtained from Wellcome Reagents Ltd, United Kingdom; the laboratory used its own syphilitic sera as controls. The test was carried out using multipost slides with two rows of six spots. Positive (4+), weakly reactive (2+), and negative control sera were included on each slide. Tests were read on a Leitz microscope with a 100 watt halogen-quartz light source and an oil immersion dark field condenser $\times 40$ objective $\times 63$ eyepiece.

FTA-ABS (IgM)

The test was carried out with reagents obtained from Wellcome Reagents Ltd, United Kingdom. Monospecific antihuman IgM conjugates were used at optimal titres derived from a chessboard titration obtained against known IgM positive serum. IgM positive and IgM negative sera were included as controls in every test.

Any serum sample reacting positively in the rheumatoid factor latex tests (Wellco rheumatest, Wellcome Reagents, United Kingdom) was retested by the FTA-ABS (IgM) test after absorption with an equal volume of insolubilised human globulin to remove anti-IgG antibody.

Results

Table I shows the distribution of syphilis by sex and the total number of patients and positive cases in the

TABLE I *Prevalence of syphilis by sex in different clinics*

Clinic	Total No examined			No (%) positive		
	Men	Women	Total	Men	Women	Total
Obstetrics and gynaecology		4361	4361		37 (0.9)	37 (0.9)
Nursery	11	15	26	9 (34.6)	10 (38.5)	19 (73.1)
Blood donors	1263		1263	38 (3.0)		38 (3.0)
Ophthalmology	23	7	30	3 (10.0)		3 (10.0)
Otorhinolaryngology	113	98	211	6 (2.8)	11 (5.2)	17 (8.1)
Medicine	185	70	255	14 (5.5)	6 (2.4)	20 (7.8)
Dermatology and venereology	246	55	301	28 (9.3)	7 (2.3)	35 (11.6)
Miscellaneous	148	89	237	4 (1.7)	9 (3.8)	13 (5.5)
Total	1989	4695	6684	102 (1.5)	80 (1.2)	182 (2.7)

different clinics at this hospital. An overall positivity of 2.7%, with 1.2% in women and 1.5% in men was detected. The prevalence of syphilis among men, however, was much higher at 5.1% (102 positive cases of 1989 investigated) compared with 1.7% (80 positive cases of 4695 examined) among women.

Table II shows the prevalence of cases of syphilis by age and sex. Most (60%) of the patients with reactive serology were in the age group 20-39 years in both sexes.

TABLE II *Incidence of syphilis by age and sex*

Age (years)	Men	Women	Total
0-14	10	13	23
15-19	1	4	5
20-24	13	13	26
25-29	26	10	36
30-34	16	12	28
35-39	13	7	20
40-44	7	5	12
45-49	8	6	14
50 and older	8	10	18
Total	102	80	182

Table III shows the results of testing 6684 sera from various patient groups. Of 4361 serum samples obtained from presumably healthy adult pregnant women attending the obstetrics and gynaecology clinic, a total of 27 cases positive by VDRL, TPHA, and FTA-ABS tests were obtained; a further 10 cases, representing treated cases of syphilis (TPHA and FTA-ABS positive), were detected; overall prevalence was 0.9%. A total of 17 biological false positive reactors representing an incidence of 0.4% were detected. Six sera that were positive only in the TPHA test, probably representing false positive results, were detected in the sera obtained from pregnant women. Of the group representative of presumably healthy normal adults – that is, the blood

donors – 22 cases were found to be positive in VDRL, TPHA, and FTA-ABS. The overall incidence of syphilis in this group was 3%. A rather low incidence of biologically false positive reactions (0.1%) among blood donors was found. The highest number of positive cases were from the dermatology and venereology

TABLE III Serological patterns obtained from testing 6684 sera from various patient groups

Type of patient	No of sera	No (%) of sera that were positive by:						
		VDRL	TPHA	VDRL and TPHA	TPHA and FTA-ABS	VDRL, TPHA, and FTA-ABS	No (%) VDRL biological false positive reactions	No (%) TPHA false positive reactions
Pregnancy	4361	44 (1.01)	39 (0.9)	27 (0.62)	10 (0.23)	27 (0.62)	17 (0.4)	6 (0.14)
Blood donors	1263	23 (1.82)	38 (3)	22 (1.74)	16 (1.3)	22 (1.74)	1 (10.1)	
Nursery	26	26 (100)	19 (73)	6 (23.1)	13 (50)	6 (23.1)	7 (14.3)	
Ophthalmology	30	0	3 (10)		3 (10)			
Otorhinolaryngology	211	15 (7.1)	18 (8.53)	14 (6.64)	3 (1.42)	14 (6.64)	1 (0.48)	1 (0.48)
Medicine	255	9 (3.53)	20 (7.84)	9 (3.53)	11 (4.3)	9 (3.53)	1 (0.47)	
Dermatology and venereology	301	32 (10.6)	36 (11.96)	31 (10.3)	4 (1.33)	31 (10.3)	1 (0.33)	1 (0.33)
Miscellaneous	237	15 (6.33)	13 (5.49)	13 (5.49)		13 (5.49)	2 (0.84)	
Total	6684	168 (2.5)	188 (2.8)	122 (1.83)	60 (0.9)	122 (1.83)	30 (0.45)*	8 (0.12)†

VDRL = Venereal Disease Research Laboratory test;
 TPHA = *Treponema pallidum* haemagglutination assay;
 FTA-ABS = fluorescent treponemal antibody absorbed test.

*VDRL titres ranged from neat to 1/8.

†TPHA titres were 1/80 and 1/160.

clinics. Of the 6684 sera examined, 164 (2.5%) were positive by VDRL; 188 (2.8%) by TPHA alone, 122 (1.8%) by VDRL and TPHA; and 60 (0.1%) by TPHA and FTA-ABS. A total of 122 (1.8%) sera were positive in all three tests. The overall incidence of biological false positive reactions was 0.5%. Eight of the 6684 sera tested (0.1%) were found to be positive by the TPHA test alone; these most probably represented false positive results. The VDRL titres in the biological false positive reactions ranged from neat to 1:8; for TPHA false positive results the titres were 1:80 or 1:160.

Table IV shows the serological results on sera obtained from 26 newborn infants and their mothers

TABLE IV Serological results on sera from newborn infants and their mothers during pregnancy

No of cases	Mother	Infant
7	VDRL positive only	VDRL* positive, FTA-ABS (IgM) negative
13	TPHA and FTA-ABS positive	TPHA and FTA-ABS positive, FTA-ABS (IgM) negative
4	VDRL, TPHA and FTA-ABS positive	VDRL*, TPHA and FTA-ABS positive, FTA-ABS (IgM) negative
1	VDRL, TPHA and FTA-ABS positive	TPHA and FTA-ABS positive, FTA-ABS (IgM) negative
1	VDRL, TPHA and FTA-ABS positive	VDRL, TPHA and FTA-ABS positive, FTA-ABS (IgM) positive †
Total 26		

VDRL = Venereal Diseases Research Laboratory test.

FTA-ABS = Fluorescent treponemal antibody absorbed test.

TPHA = *Treponema pallidum* haemagglutination assay.

*VDRL titres in these infants were \leq than the VDRL titre on the mothers' serum sample obtained during pregnancy.

† This was a cause of intrauterine growth retardation leading to death.

during pregnancy. The FTA-ABS (IgM) test was performed on all of the infants' sera. The infants born to VDRL positive mothers were positive. Of five infants born to mothers whose sera were reactive in all the three tests during pregnancy, one was born with TPHA and FTA-ABS positivity only, whereas the remaining four retained reactivity to all three tests. Only one case of congenital syphilis was detected by a positive FTA-ABS (IgM).

Discussion

The serology of syphilis has become much more simple and rational in recent years because of the widespread use of specific antibody tests and mono-

specific fluorescent antibody procedures, which provide an insight into the immunoglobulin class of antibodies.⁵ In a comprehensive investigation of syphilis in Saudi Arabia we used a set of tests (quantitative TPHA, VDRL, and FTA-ABS, and monospecific (IgM) FTA-ABS tests) and these have proved particularly useful for the routine serodiagnosis of syphilis. Screening for *T pallidum* infection by VDRL and TPHA and final confirmation of syphilis positivity by FTA-ABS indicated an overall prevalence of 2.7% for syphilis, which is comparable with that in Nigeria.⁶ Most of the cases with reactive serological tests for syphilis seemed to be in the sexually active age group (20-39 years); and this seems to be the trend elsewhere.⁷ In antenatal patients the prevalence of syphilis of 0.9% is close to that of 0.8% detected in pregnant Saudi women by Hossain *et al*, but extremely low compared with the number of women seen in Africa.⁹

In the serum of a patient with no history or clinical evidence of syphilis, or other treponematoses,¹⁰ a biological false positive reaction is ordinarily noted as a positive reaction to reagin tests and a negative reaction to treponemal tests. VDRL biological false positive reactions occurred in 0.5% of the total sera tested, a figure that is low compared with that reported in Indonesia.¹¹ Biological false positive reactions were detected primarily in antenatal patients and in blood donors, which is consistent with the findings of others,¹² but the incidence of 0.4% and 0.8% seems to be lower than that found in pregnant women in Malaysia¹³ and that found in blood donors in Australia.¹⁴ Titres of the VDRL biological false positive results ranged from neat to 1/8; such a trend has been noticed by another worker elsewhere.¹⁵

Positive TPHA reactions could be the result of the failure of Reiter treponemes to remove all antibody from test serum, but this has rarely been reported.¹⁶ In this study we found eight sera that were positive in the TPHA test but negative in the FTA-ABS. Further, titres of these false positive reactions were either 1/80 or 1/160, which are considered to be low.

Diagnosing congenital syphilis is problematic: it depends mainly on the results of serological tests; and most syphilitic neonates are asymptomatic at birth. Interpretation in neonates is extremely difficult as IgG antibody found in the serum of neonates is largely passively acquired through the placenta and is not representative of the infant's own response.¹⁷ We have effectively used the monospecific FTA-ABS (IgM) test for the serodiagnosis of congenital syphilis. This has been used by others.¹⁸ We carefully standardised the FTA-ABS (IgM) test to avoid false negative and positive findings. Thus its use obviates any need for protracted follow up of patients: this is often the case when serodiagnosis of syphilis is based entirely on the FTA-ABS (IgG) test. A case of congenital syphilis

was unequivocally shown by a positive FTA-ABS (IgM) reaction, which was consistent with clinical findings. During the course of this study a few cases with the clinical signs of late congenital syphilis¹⁹ were found; among them was one such case in a six year old girl with bilateral tinnitus and a persistently positive FTA-ABS (IgM) reaction.

Non-venereally transmitted endemic syphilis (bejel), indistinguishable from venereally transmitted syphilis by serological tests, has been reported in a Bedouin community in the western region of Saudi Arabia.²⁰ Positive FTA results were more common in nomadic and "semi-settled" communities than in urban communities in the age groups 0-4 and 5-19 years. These differences were not apparent in the over 20 age group. Pinta and yaws, with distinguishable clinical symptoms, have so far not been reported as occurring in Saudi Arabia. The clinical presentations, age, and history of patients indicate that the positive treponemal serology in our study resulted from venereal syphilis, and that bejel was an unlikely cause of such reactivity.

The excellent technical help of Jahangir Shere, Jeremias Adrias, Luzminda Ocsio, Syed Nageeb Ahmed, Azhar Hossain, Jim Nograles, and Mila Tenefrancia is highly appreciated. Thanks are due to Maria Eleanor T Padua for invaluable secretarial help.

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