Br J Vener Dis 1981;57:95-9

Syphilis in Swaziland

A serological survey

J P URSI,* E VAN DYCK,† C VAN HOUTTE,* P PIOT,† J COLAERT,† M DLAMINI,‡ AND A MEHEUS§

*From the *Department of Microbiology, Antwerp Academic Hospital, Edegem, and the †Department of Bacteriology, Institute for Tropical Medicine, Antwerp, Belgium; the ‡Ministry of Health, Mbabane, Swaziland; and the §Department of Epidemiology and Social Medicine, University of Antwerp, Wilrijk, Belgium*

SUMMARY Sera from 536 adults and children in Swaziland were examined for their reactivity in the rapid plasma reagin (RPR) and *Treponema pallidum* haemagglutination (TPHA) tests. None of 130 sera from children was reactive in either test; 8·6% of sera from 185 healthy adults were reactive in the RPR test and 33% in the TPHA test; 24·5% of 220 sera from patients with genital ulcers were RPR-positive and 45·9% TPHA-positive. The RPR positivity rates were not related to age, but the percentage of RPR-negative, TPHA-positive sera increased with age in both the healthy adults and the patients with genital ulcers. Thus venereal syphilis appears to be responsible for these high positivity rates. Estimates of the yearly incidence of syphilis are identical for both groups—approximately 1·4%, an unusually high figure.

Introduction

In 1978 serological tests for syphilis on patients attending an antenatal care unit in Swaziland showed a very high rate of positive reactions.[1] Other population groups in Swaziland were surveyed on a larger scale in 1979 to obtain a clearer epidemiological picture. The results of serological tests for syphilis in these groups are presented.

Patients and methods

STUDY POPULATION

The characteristics of the groups studied are summarised in table I.

Group 1 consisted of 130 healthy children aged between 6 and 14 years from a primary school in Sipophaneni (rural area).

Group 2 consisted of 90 healthy women aged between 15 and 43 years attending consecutively the antenatal and family planning clinics of the King Sobhuza Clinic in Manzini, the second city of Swaziland, during a four-week period.

Group 3 consisted of 95 men aged between 15 and 45 years with urethral discharge but without any sign of present or past genital ulcerative disease attending consecutively the sexually transmitted diseases (STD) clinic of the State Hospital in the capital city, Mbabane, during a four-week period.

Groups 2 and 3 constituted a "healthy sexually active population"; no difference in RPR positivity rates was found between the two groups.

Group 4 consisted of 161 male and 59 female patients aged between 15 and 50 years with genital ulcers attending the same STD clinic consecutively during two periods each of four weeks. Of 149 dark-field examinations carried out, 30 (20·1%) showed *Treponema pallidum*. The sensitivity of dark-field examinations in this study was estimated to be 55%, suggesting that about one-third of the ulcers were syphilitic in orgin.

SERA

Ten millilitres of blood (5 ml for group 1) were drawn with Vacu-Tainer tubes (Beckton, Dickinson and Co) from all persons examined. The blood was centrifuged within six hours and the serum stored at −20°C. The sera were transported to Belgium in dry ice at −70°C within six weeks and kept at this temperature until thawed for testing.

SEROLOGICAL TESTS

The rapid plasma reagin (RPR) tests were performed with a commercial kit (RPR Macro-Vue card test; Beckton, Dickinson and Co) and the *T pallidum*

Address for reprints: Dr J P Ursi, Department of Microbiology, Akademisch Ziekenhuis Antwerpen, Wilrijkstraat 10, B-2520 Edegem, Belgium

Accepted for publication 10 September 1980

TABLE I *Number of subjects in each group according to age*

Group	Total No of subjects	Age group (years) 5-9	10-14	15-19	20-24	25-29	30-34	⩾35 (mean)
1. Healthy children								
Boys	70	29	41					
Girls	60	28	32					
2. Healthy women	90			13	38	19	15	5 (39·3)
3. Men with urethritis	95			12	41	24	9	9 (37·3)
4. Patients with genital ulcers								
Men	161			22	75	43	11	10 (41·6)
Women	59			24	20	8	3	4 (41·5)

haemagglutination assay (TPHA) with a commercial kit (Gist-Brocades) using sheep erythrocytes. Tests were performed according to the manufacturer's instructions, except that the lowest test dilution in the RPR test was 1/2.

STATISTICAL ANALYSIS

For statistical analysis, the χ^2 test was used to compare frequencies and Student's t test to compare regressions and mean values. In calculating geometric mean inverse titres (GMIT) for the TPHA test, negative sera were assigned a titre of 1/40—that is, the dilution below the lowest dilution tested. The entire sexually active population tested (groups 2, 3, and 4) was taken as the standard population in standardising for age.

Results

RPR TEST

Group 1

None of the 130 children's sera was positive in the RPR or TPHA tests; they were not therefore considered in the analysis of data from groups 2-4.

Groups 2 and 3

Sixteen (8·6%) of 185 sera from the healthy sexually active population (groups 2 and 3) gave a positive RPR test result; there was no difference between men and women (7·4% and 10% respectively). The men, who had a urethral discharge, could have been part of a more highly promiscuous group than the general population. As their RPR and TPHA positivity rates (see below) were not different from those of healthy women, they were included in the "healthy" group. Although the numbers were relatively small, the RPR positivity rates were clearly not related to age.

Group 4

Among the patients with genital ulcers 54 (24·5%) of 220 sera were reactive in the RPR test—35 (21·7%) of 161 sera from men and 19 (32·2%) of 59 sera from women ($\chi^2_1 = 2·00$; $0·10<P<0·20$). The difference in RPR reactivity between groups 4 and 2 and 3 was highly significant ($\chi^2_1 = 18·06$; $P<0·001$). RPR positivity rates were not related to age.

TPHA TEST

Groups 2 and 3

In the healthy sexually active population 31 (32·6%) of 95 men and 30 (33·3%) of 90 women had positive TPHA test results; after standardisation for age these figures were 31·2% and 35·1% respectively. This difference was not statistically significant. The age distribution (fig 1) shows a linear increase in positivity rates in men (group 3) ($t = 2·055$; $0·025<P<0·05$). The slope of the regression line for women (group 2) is not statistically different from 0. When data in both figures are analysed, the slope of the regression line is nearly significant ($t = 1·932$; $0·05<P<0·10$).

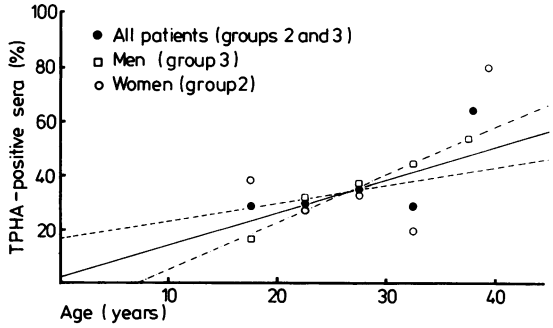

FIG 1 *Regression lines of percentages of TPHA-positive sera in relation to age in men with urethral discharge (group 3) and in healthy women (group 2).*

Group 4

In the patients with genital ulcers (group 4) 68 (42·2%) of 161 sera from men and 33 (55·9%) of 59 sera from women were TPHA-positive; the figures

standardised for age were 41·8% and 60·6% respectively ($\chi^2_1 = 5·47$; $0·01<P<0·25$). The regression lines of TPHA positivity in relation to age (fig 2) have a highly significant slope (table II); they are not significantly different for men and women ($t = 1·476$; $0·10<P<0·20$).

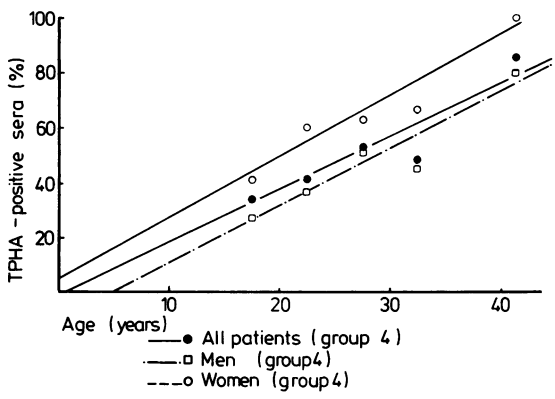

FIG 2 *Regression lines of percentages of TPHA-positive sera in relation to age in men and women with genital ulcers (group 4).*

TABLE III *Percentage of TPHA-positive/RPR-negative sera for "healthy" adults (groups 2 & 3) and patients with genital ulcers (group 4)*

% TPHA-positive/RPR-negative sera:					
Groups 2 and 3			*Group 4*		
Men	*Women*	*Total*	*Men*	*Women*	*Total*
24·4	24·4	23·8	20·4	27·5	22·9

Groups 2 and 3—"healthy" adults; group 4—patients with genital ulcers

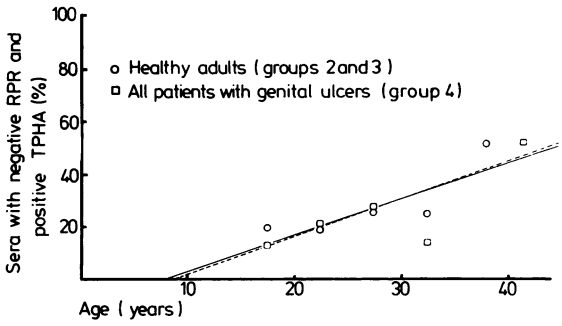

FIG 3 *Regression lines of percentages of TPHA-positive, RPR-negative sera in relation to age in "healthy" men and women (groups 3 and 2) and in patients with genital ulcers (group 4).*

TPHA-POSITIVE/RPR-NEGATIVE SERA

When the percentages of sera with positive TPHA and negative RPR test results (figures standardised for age) are compared there were no significant differences between healthy subjects (groups 2 and 3) and patients with genital ulcers (group 4) or between men and women in each of the groups (table III). In fig 3 the same parameter is regressed towards age. When compared with figs 1 and 2 the age-positivity relationship remained, which was to be expected, as RPR positivity rates were unrelated to age. The regression lines for this relationship show a highly significant slope (table II). As regressions for healthy adults (groups 2 and 3) and patients with genital ulcers (group 4) were very similar ($t = 0·042$; $0·95<P<0·975$), no difference being found between men and women, a common regression was calculated; the slope shows a 1·39% increase of TPHA positivity per year of age (table II).

GEOMETRIC MEAN INVERSE TITRES

The geometric mean inverse titres of the TPHA tests are given in table IV. The mean is 101 in the sexually

TABLE II *Statistical analysis: regression lines of percentage of TPHA-positive sera and TPHA-positive/RPR-negative sera in relation to age*

Regression of	*Groups*	*Slope (% increase per year)*	*95% Confidence interval limits (% per year)*	*Probability that the slope of the line is equal to 0*	
				P value	*t**
% of TPHA-positive sera *vs* age	3	1·76	0·05 – 3·47	0·025<P<0·005	2·055
	2	0·65	0·0 – 1·53	0·40 <P<0·50	0·746
	2 and 3	1·18	0·0 – 2·40	0·05 <P<0·10	1·932
	4 (men)	2·08	0·76 – 3·40	0·001<P<0·005	3·157
	4 (women)	2·23	0·37 – 4·95	0·02 <P<0·025	2·393
	4 (all patients)	1·92	0·84 – 3·00	P<0·001	3·558
% of TPHA-positive, RPR-negative sera *vs* age	2 and 3	1·38	0·27 – 2·49	0·01 <P<0·02	2·495
	4 (all patients)	1·42	0·50 – 2·34	0·001<P<0·005	3·092
	2, 3, and 4	1·39	0·70 – 2·09	P<0·001	4·008

Group 2—healthy women; group 3—men with urethritis; group 4—men and women with genital ulcers
*Student's *t* test

TABLE IV *Geometric mean inverse titre in TPHA test according to subject group and age*

Subject group	Geometric mean inverse titre (TPHA) according to age (years):					
	15-19	*20-24*	*25-29*	*30-34*	*⩾35*	*Total**
Groups 2 and 3	97	103	96	78	125	101
Men (group 3)	71	90	101	160	123	99
Women (group 2)	129	134	89	50	139	107
Group 4	175	160	247	320	580	202
Men	150	122	191	300	394	171
Women	202	437	987	403	1522	517

*Standardised for age
Groups 2 and 3—"healthy" adults; group 4—patients with genital ulcers

active healthy population without any relation to age ($t = 0 \cdot 077$; $0 \cdot 90 < P < 0 \cdot 95$) or sex ($t = 0 \cdot 217$; $0 \cdot 80 < P < 0 \cdot 90$). In the group with genital ulcers, on the contrary, there is a definite relationship to age ($t = 2 \cdot 2128$; $0 \cdot 025 < P < 0 \cdot 05$) and significantly higher titres in women ($t = 2 \cdot 279$; $0 \cdot 2 < P < 0 \cdot 025$).

Discussion

At the start of this investigation, it was thought that non-venereal treponematosis would account for the unusually high positivity rate of tests for syphilis in Swaziland. The local medical staff, however, was unaware of this condition being prevalent in the country. None of 130 sera from schoolchildren was seropositive, making the non-venereal hypothesis very unlikely. These children live in a rural area in contrast to the other subjects tested, who represent an urban population. However, as Burney[2] pointed out, many attendants at Mbabane Hospital outpatient department and probably also at the King Sobhuza Clinic in Manzini are migrant workers from rural areas. Non-venereal treponematosis, if prevalent in Swaziland, should occur more frequently in the region of Sipophaneni, where it is warmer because of the lower altitude. Moreover, non-venereal treponematosis is generally more frequent in rural areas.[3] As only sera from sexually active adults were reactive in both the TPHA and the RPR tests the only possible conclusion is that venereal syphilis was responsible for the high positivity rate.

The RPR test is a sensitive and specific non-treponemal test for syphilis.[4-8] It is positive in late primary syphilis and afterwards.[9] In some cases of apparently untreated latent syphilis it may eventually become negative.[6 10 11] After treatment the RPR test usually soon becomes negative except when treatment is instituted in the late secondary or tertiary stage.[10] A positive RPR test result indicates active treponemal disease if a biological false-positive reaction can be excluded by a positive treponemal test. In our study population only three patients with genital ulcers had both a positive RPR and a negative TPHA test result. Of these, two had confirmed early primary syphilis, which accords with the lack of sensitivity of the TPHA test at this stage of the disease.[12 13] This leaves one possible biological false-positive reactor. This rate (0·2%) is extremely low when compared with the results of other workers,[4 5 7 8] but can be explained by the fact that our lowest test dilution was 1/2 instead of 1/1. We have to admit that approximately 8% of the healthy Swazi population has active syphilis, which is extremely high. This is confirmed by the results of TPHA testing.

The TPHA test is highly specific and highly sensitive,[7 10 12-19] although some workers[20] have doubted this. Except in early primary syphilis its sensitivity is practically equal to that of the FTA-ABS test.[10 12 13] The TPHA test titre rises as the infection progresses; typical values are 1/80, 1/320, and 1/5120 in late primary, early secondary, and late secondary syphilis respectively.[9 13 18] After treatment, the TPHA test titre declines only very slowly, seroreversal being much less frequent than in the FTA-ABS and the *T pallidum* immobilisation (TPI) tests.[10 12 13 18 21] When treatment is started in late syphilis, the TPHA test result usually remains strongly positive.[12] The TPHA remains as a "serologic scar" of past disease, whether treated or not,[11] so one can expect the TPHA positivity rate to increase with age in a population with a high prevalence of syphilis. This is indeed the case in Swaziland, at least in men, as shown in fig 1. In the patients with genital ulcers (group 4) the same increase in TPHA positivity rate can be seen (fig 2).

To eliminate interference from active cases of syphilis, individuals with positive TPHA and negative RPR test results are analysed in fig 3 and table II. This combination of results occurs only in latent syphilis, tabes dorsalis, treated syphilis, and old yaws.[9] Since it occurs in only one-third of cases of latent syphilis and even less often in tabes

dorsalis,[6 10 11] and since yaws can be excluded, figures for this combination can be regressed towards age, and this gives an accurate estimate of the cumulative frequency of cases of treated syphilis. The slope of the regression is the yearly incidence of treated syphilis, which in turn is a minimal estimate of the yearly incidence of new cases of syphilis. Surprisingly enough, the regressions calculated for "healthy" individuals and patients with genital ulcers were almost identical, which might indicate that sexual promiscuity is not notably higher in the groups with genital ulcers. The common regression has a slope of 1·4% per year of age, which means that the yearly incidence of syphilis in Swaziland is at least 1·4%. As far as we know, this is the first time such a high incidence of venereal syphilis can be proved.

The absence of any correlation between age and the GMIT of the TPHA tests in the healthy group indicates that most cases have old treated syphilis. In the group with genital ulcers more have untreated active syphilis, and as titres rise with time the relation between age and GMIT was to be expected. High titres in women may be due to underdiagnosis and subsequent lack of treatment.

Figures from other sources (unpublished) have estimated the yearly incidence of syphilis in Swaziland to be about one-third that of gonorrhoea, which is at least 3·75% in this country.[22] This estimate of 1·25% is comparable with the 1·4% calculated from our serological data. With this high incidence, one would expect a high rate of congenital syphilis; this should be reflected in positive serology in children, which was not the case. Possible explanations are the selection of normal healthy schoolchildren in group 1 or a high mortality rate from congenital syphilis in the country or both. An investigation of the incidence and manifestations of this condition in Swaziland is needed.

The authors wish to thank Dr N Van Meirvenne and Professor S Stadsbaeder, who performed part of the serological testing. The study was supported by grants from the World Health Organisation and from the University of Antwerp (UIA).

References

1. Meheus A, Friedman F, Van Dyck E, Gyver T. Genital infections in prenatal and family planning attendants in Swaziland. *East Afr Med J* 1980;**57**:212-7.
2. Burney P. Some aspects of sexually transmitted disease in Swaziland. *Br J Vener Dis* 1976;**52**:412-4.
3. Guthe T. Clinical, serological and epidemiological features of framboesia tropica (yaws) and its control in rural communities. *Acta Dermatovenereol (Stockholm)* 1969;**49**:343-68.
4. Buck AA, Mayer H. Comparative studies of the rapid plasma reagin card test for syphilis and the VDRL slide test in Ethiopia. *Am J Hyg* 1964;**80**:85-90.
5. Falcone VH, Stout GW, Moore MB jun. Evaluation of rapid plasma reagin (circle) card test. *Publ Hlth Rep* 1964;**79**:491-5.
6. Paris-Hamelin A. Aspect actuel de la syphilis et de son sérodiagnostic. *Feuillets Biol* 1977;**17**:23-30.
7. Peter CR, Thompson MA, Wilson DL. False-positive reactions in the rapid plasma reagin card, fluorescent treponemal antibody-absorbed, and hemagglutination treponemal syphilis serology tests. *J Clin Microbiol* 1979;**9**:369-72.
8. Portnoy J, Brewer HH, Harris A. Rapid plasma reagin card test for syphilis and other treponematoses. *Publ Hlth Rep* 1962;**77**:645-52.
9. O'Neill P. A new look at the serology of treponemal disease. *Br J Vener Dis* 1976;**52**:296-9.
10. Miller JN. Value and limitations of nontreponemal and treponemal tests in the laboratory diagnosis of syphilis. *Clin Obstet Gynecol* 1975;**18**:191-203.
11. Thivolet J, Salussola D, Sepetjian M. Actualités sur le sérodiagnostic de la syphilis en pratique courante. *Revue Praticien* 1976;**26**:4095-109.
12. Lesinski J, Krach J, Kadziewicz E. Specificity, sensitivity, and diagnostic value of the TPHA test. *Br J Vener Dis* 1974;**50**:334-40.
13. O'Neill P, Warner W, Nicol C. *Treponema pallidum* haemagglutination assay in the routine serodiagnosis of treponemal disease. *Br J Vener Dis* 1973;**49**:427-31.
14. Johnston NA. *Treponema pallidum* haemagglutination test for syphilis. Evaluation of a modified micromethod. *Br J Vener Dis* 1972;**48**:474-7.
15. Le Clair RA. Evaluation of a qualitative hemagglutination test for antibodies to *Treponema pallidum*. *J Infect Dis* 1971;**123**:668-70.
16. Logan L, Cox P. Evaluation of a quantitative automated micro-hemagglutination assay for antibodies to *Treponema pallidum*. *Am J Clin Pathol* 1970;**53**:163-6.
17. Robertson D, McMillan A, Young H, Henricksen C. Clinical value of the TPHA test. *Br J Vener Dis* 1975;**51**:79-82.
18. Simonart JM. Intérêt du TPHA *(Treponema* haemagglutination test) dans le sérodiagnostic de la syphilis. *Arch Belg Dermatol* 1974;**30**:15-24.
19. Young H, Henricksen C, Robertson D. TPHA test as a screening procedure for the diagnosis of syphilis. *Br J Vener Dis* 1974;**50**:341-6.
20. Blum G, Ellner PD, McCarthy LR, Papachristos T. Reliability of the treponemal haemagglutination test for the serodiagnosis of syphilis. *J Infect Dis* 1973;**127**:321-4.
21. Lefevre JC, Dabernat H, Galthier M, *et al.* Diagnostic sérologique de la syphilis par le test d'hémagglutination passive. *Nouv Presse Med* 1975;**4**:2511-3.
22. Meheus A, Ballard R, van Dyck E, Piot P, Ursi JP, Dlamini M. Epidemiology and aetiology of urethritis in Swaziland. *Int J Epidemiol* 1980;**9**:239-45.