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A Population-Based Serosurveillance of Syphilis in Costa Rica

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As part of a case–control study to investigate the high incidence of cervical cancer in Costa Rican women, the seroprevalence of the treponematoses, in particular, syphilis was determined. In each age group, women with a history of two or more sex partners were two to four times more likely to be seroreactive in tests for syphilis than women with zero or one sex partner. The highest percentage of reactive results in the microhemagglutination assay for antibodies to *Treponema pallidum* (MHA–TP) was seen in samples from women aged 50–59 who had had two or more lifetime partners (23.8%). Three observations from our study support reactivity due to syphilis rather than yaws or pinta: (*1*) a similar percent of reactive rapid plasma reagin (RPR) card test results among MHA–TP reactors in the two age groups of women who were surveyed (42 vs. 49%) was observed; (*2*) women who were seroreactive in the MHA–TP had multiple risk factors for STD [low socioeconomic status (9.4%), urban residence (22.8%), first intercourse under 16 years of age (14.1%), and multiple sex partners (26.3%)], and (*3*) only sexually experienced women had reactive results in the MHA–TP test.

IN 1984–1985, the Costa Rican Demographic Association in collaboration with the Centers for Disease Control (CDC), the Costa Rican Social Security Administration, and Family Health International conducted a case–control study of the high incidence of cervical cancer and the low incidence of breast cancer in Costa Rican women. Details of these studies have been published elsewhere.[1–5] Because cervical cancer appears to be associated with sexually transmitted diseases, these studies also investigated the seroprevalence of antibodies to syphilis,[1] genital herpes,[1,6] chlamydia[7] and human immunodeficiency virus.[8] In the report by Irwin et al.,[1] of the 415 patients with carcinoma in situ, 9.1% were reactive in serologic tests for syphilis, whereas 17.1% of the 149 patients with invasive cancer were reactive in these tests.[1] The results that are reported herein are based on the serologic responses of the population-based controls that were selected for the case–control study[1] and describe the total number of both new and old cases in the population (seroprevalence) rather than the total number of new cases in a population at risk over a specific period of time (incidence). Although Shadid–Chaina et al.[9] reported on the seroprevalence of syphilis among applicants for a health card, this study is the first population-based study of seroprevalence of antibodies that are detected in the serologic tests for syphilis. In addition, our study relates reactivity in serologic tests for syphilis with demographic characteristics and the medical and sexual history of Costa Rican women.

Materials and Methods

Population Studied

The sample of women consisted of a nationally representative cross-section of women aged 25–59 years, selected on the basis of the June 1984 census.[1] To match the age distribution of the cancer patients in the original case–control study,[1] the sampling fraction for this population-based control group varied by age group; thus, women in certain age groups were oversampled. Of the 870 women who participated as controls in the case–control study, 767 consented to a venipuncture.[1] The

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social and demographic characteristics of these women did not differ from those of the 103 women who did not give informed consent for venipuncture.[10]

Interviews

Interviews were conducted using a modified version of the questionnaire from the CDC Cancer and Steroid Hormone Study.[11] Questions focused on reproductive, medical, and sexual history, and included age at first intercourse, number of sexual partners, history of sexually transmitted diseases (STD), marital status, and number of pregnancies. Demographic information included socioeconomic level,[12] education, age, and region of residence.

Serum Samples

For each blood sample, serum was separated from other cellular constituents and shipped to the CDC. At the CDC, antibodies to *Treponema pallidum* subspecies *pallidum* and *pertenue,* and *T. carateum,* the causative agents of syphilis, yaws, and pinta, were detected using the microhemagglutination assay (MHA–TP).[13] Currently, all serologic tests for syphilis, including the MHA–TP, cross react with antibodies that are produced in response to these pathogenic treponemes. However, because reactivity in the RPR 18 mm circle card test[14] for the detection of antilipoidal antibodies disappears with time, the RPR card test was used to indicate possible current infection with *T. Pallidum* subspecies *pallidum.*[15] In addition, to indicate possible current primary infection, we used an investigational test that was based on an enzyme-linked immunosorbent assay (ELISA), using antibodies to immunoglobulin M (IgM) to capture IgM (Ortho Syphilis–M, Ortho Diagnostic Systems Inc., Carpinteria, CA).

A portion of each serum was sent to the Division of Pediatric Infectious Diseases, Emory University, Atlanta, Georgia for determination of the presence of antibodies to *Herpes simplex* virus type 2 (HSV–2).[16] Sera were also analyzed by microimmunofluorescence for antibody to *Chlamydia trachomatis*[17] in the chlamydia laboratory of the San Francisco General Hospital, San Francisco, California.

TABLE 1. Percent of Women with Reactive Syphilis Serology by Age

Age Group	Number Reactive	Number Tested	Unweighted Percent	Weighted Percent
25–29	6	123	4.9	4.9
30–39	13	270	4.8	4.8
40–49	17	194	8.8	8.3
50–59	18	180	10	10
Total	54	767	7.1	6.4

TABLE 2. Percentage of Women with Reactive MHA–TP Results by Number of Lifetime Sexual Partners

Number of Sexual Partners*	Total		Age Group 25–39		Age Group 40–59	
	%	N	%	N	%	N
None	0.0	(36)†	0.0	(22)	0.0	(14)
1	4.1	(501)	3.3	(251)	5.4	(250)
2	9.1	(133)	3.6	(65)	17.3	(68)
3+	17.2	(95)	15.4	(54)	20.6	(41)
Total	6.4	(767)	4.8	(393)	9.0	(374)

* Two women with unknown number of sexual partners excluded.
† In this and subsequent tables, numbers in parenthesis refer to the unweighted number of cases in each cell.

Tabulations

The analysis was descriptive using data from cross-tabulations for demographic and medical/sexual history variables. To calculate seroprevalence by demographic characteristics in the general population, the results, except where noted, were age-weighted to compensate for the oversampling of certain age groups (table 1). Since the population survey was based on a complex, multistage, cluster-sample methodology, the unweighted number of cases in each cell is shown in tables 2–4 as an indication of sample sizes rather than standard errors that were based on assumptions of simple random sampling.

Results

In the interviews of the 767 women from whom blood was collected, 17.7% reported a history of STD or pelvic inflammatory disease (PID). Only 8 women (1.9%) reported a history of syphilis. However, 54 of the women (6.4%) had reactive serology by the MHA–TP, indicating either a past or present infection with pathogenic treponemes other than oral treponemes that are associated with peridontal disease. Of these 54 women, 25 (46.3%) also had reactive RPR card test results, suggesting a recent infection.[14] Among MHA–TP reactors, the percent of serum samples that were reactive in the RPR card test was similar in the two age groups; 42% for ages 25–39 compared with 49% for ages 40–59. The distribution of end-point titers of the samples in the RPR card test was also similar between the age groups. Only one serum sample from a woman aged 33 was reactive in the IgM capture ELISA. Only two serum samples had RPR card test titers greater than 1:8.

The percent of women with reactive MHA–TP results increased with age (table 1), most markedly in women with two or more lifetime sex partners (table 2). The highest percentage of seroreactivity (23.8%) was seen

TABLE 3. Percentage of Women with Reactive MHA–TP Results by Marital Status, Reproductive Characteristics, and by Age and Number of Sexual Partners

	Total		Age Group				# Sexual Partners*			
			25–39		40–59		0–1		2+	
	%	N	%	N	%	N	%	N	%	N
Martial status										
In union	4.6	(542)	3.1	(299)	7.6	(243)	3.2	(432)	9.4	(117)
Separated/widowed/divorced	11.1	(106)	11.7	(28)	10.7	(78)	3.1	(54)	19.1	(52)
Single	11.2	(119)	10.1	(66)	13.1	(53)	8.3	(60)	14.1	(59)
Age at first intercourse†										
None	0.0	(36)	0.0	(22)	0.0	(14)	0.0	(36)		N.A.
<16	14.1	(112)	10.2	(66)	23.0	(46)	5.1	(49)	20.8	(61)
16–19	7.2	(281)	5.6	(135)	9.5	(146)	5.9	(178)	9.3	(103)
20+	3.9	(336)	2.8	(169)	5.7	(167)	2.8	(273)	9.3	(63)
Total pregnancies										
0	1.8	(70)	0.0	(42)	6.8	(28)	1.1	(60)	‡	
1–2	6.8	(161)	5.9	(117)	10.9	(44)	3.2	(120)	18.2	(40)
3–4	5.5	(223)	4.6	(153)	8.3	(70)	3.4	(150)	9.5	(72)
5+	8.4	(313)	6.8	(81)	9.2	(232)	5.8	(207)	13.1	(106)
Totals	6.4	(767)	4.8	(393)	9.0	(374)	3.8	(537)	12.6	(228)

* Two women with unknown number of sexual partners excluded.
† Two women with unknown age at first intercourse excluded.
‡ <25 women.

with samples that were collected from women aged 50–59 with two or more partners. In each age group, women with a history of two or more sex partners were two to four times more likely to be seroreactive than women with no or one sex partner. Most notably, all of the 36 women who reported not having coitus were nonreactive in the tests for syphilis (table 2). Women who were currently married were less likely to have reactive results in the tests for syphilis than those women who were not; 4.6 vs. 11% (table 3). The percent of women with an age at first intercourse of <16 years with reactive MHA–TP results was 14.1. A direct association existed between intercourse at <16 years of age and two or more lifetime sexual partners. Reactivity in the MHA–TP test also appeared to be associated with total pregnancies, but this pattern diminished when controlled by age. When ana-

TABLE 4. Percentage of Women with Reactive MHA–TP Results by Selected Demographic Characteristics

	Total		Age				# Sexual Partners†			
			25–39		40–59		0–1		2+	
	%	N	%	N	%	N	%	N	%	N
Region										
San José	5.7	(257)	4.8	(127)	7.1	(130)	4.1	(171)	8.9	(86)
Urban Central Valley	7.1	(130)	8.3	(72)	4.8	(58)	4.8	(108)	*	—
Rural Central Valley	3.3	(127)	1.1	(69)	7.3	(58)	3.5	(98)	3.0	(29)
Urban Outside Valley	10.0	(83)	5.3	(46)	18.7	(37)	5.0	(53)	18.4	(30)
Rural Outside Valley	7.6	(170)	4.8	(79)	11.7	(91)	2.0	(107)	17.7	(61)
Socioeconomic status										
Low	9.4	(270)	7.2	(138)	12.8	(132)	4.7	(151)	15.3	(117)
Medium	5.4	(309)	3.6	(170)	9.0	(139)	4.1	(228)	9.0	(81)
High	4.0	(188)	3.7	(85)	4.4	(103)	2.5	(158)	11.6	(30)
Education										
None	9.3	(79)	*	—	12.1	(56)	4.3	(43)	14.9	(36)
Primary incomplete	7.2	(258)	4.6	(89)	9.2	(169)	3.1	(173)	15.3	(84)
Primary complete	6.8	(188)	4.7	(114)	11.8	(74)	6.0	(129)	8.5	(58)
Secondary complete	6.4	(172)	6.9	(123)	4.3	(49)	3.5	(132)	15.1	(40)
University	0.9	(70)	0.0	(44)	3.3	(26)	1.1	(60)	*	—
Totals	6.4	(767)	4.8	(393)	9.0	(374)	3.8	(537)	12.6	(228)

* <25 women.
‡ Two women with unknown number of sexual partners excluded.

lyzed by geographic region (table 4), the prevalence of antibodies that were detected by the MHA–TP was highest in San José, and in the other urban areas outside the central valley, principally the port cities of Limón and Puntarenas. More women in the lower socioeconomic groups than in higher socioeconomic groups were reactive in the MHA–TP. Of the women in the lowest socioeconomic group and with two or more sexual partners, 15.3% were found to be reactive. When analyzed by education, university-educated women had the lowest prevalence of antibodies that were detected in the tests for syphilis, with the exception of those women without sexual experience.

Discussion

Results of syphilis serosurveillance studies are difficult to interpret in Latin American countries, because the current serologic tests do not distinguish between antibodies that result from infection with *T. pallidum* subspecies *pallidum* or subspecies *pertenue* or *T. carateum*.[18] Pinta and yaws occurred in the Atlantic coastal region of Costa Rica in the 1940s and 1950s. However, documentation of the prevalence of these infections is limited. For example, Costa Rica reported only six cases of yaws between 1951 and 1956.[19] Mass treatment campaigns of the late 1950s and early 1960s eliminated yaws and pinta from Costa Rica, and a resurgence of these treponemal diseases has not been reported. In our study population, ¾ of the women seroreactive by the MHA–TP were old enough to have been exposed to yaws or pinta in childhood. Antibodies to yaws, pinta, and syphilis remain in peripheral blood for many years after the infection has resolved or has been treated. These antibodies may be detected by treponemal tests, such as the MHA–TP, for the lifetime of the individual. Thus, the higher percentage of older women with reactive MHA–TP results may be due to antibodies that remained from childhood infections. In contrast, the antibodies detected in the RPR card test, although not specific for syphilis alone, disappear with time or remain at low levels. Therefore, the equal percentages of reactive RPR card test results in both age groups of women may indicate syphilis rather than yaws or pinta, since the younger women would not have been exposed to these diseases. Unfortunately the IgM capture ELISA, which, at least in our preliminary studies, appears to detect antibodies that were developed in response to a first infection and not in subsequent infections, was only reactive in one patient. However, because reactivity in the tests for syphilis was associated with an increase in the number of sexual partners, many of the reactive women may have had more than one syphilis infection and thus not be reactive for IgM.

Results that were obtained when the serum samples were examined for the presence of antibodies to chlamydia and to HSV-2 also support the reactivity in the MHA–TP as due to syphilis, if one assumes that infection with one STD places a person in a higher risk group for infection with another STD. Forty-four of the 54 (77.5%, weighted) women with reactive MHA–TP results were also reactive by microimmunofluorescence for chlamydia,[6,7] and women whose serum samples were reactive in the MHA–TP were almost twice as likely to have HSV-2 antibody as women whose MHA–TP results were nonreactive (77.5 vs. 36.8%).[6] In addition, the percent of reactive results for HSV-2 antibodies, like that for syphilis, increased as the number of sexual partners increased.

Finally, regardless of age group, all women who did not report coitus were nonreactive in the MHA–TP, indicating that sexual experience was associated with reactivity in the MHA–TP. Thus, syphilis rather than yaws or pinta, which are nonvenereal diseases, appears to be the cause of the reactivity.

Our finding of a confirmed reactive RPR card test prevalence of 3.3% is consistent with the previous seroprevalence study conducted in the late 1970s by Shadid-Chaina et al.,[9] who found a confirmed reactive VDRL test result rate of 3.2% among women who were 20+ years of age and had applied for health cards. More definitive studies of the prevalence of syphilis in Costa Rica await tests that will distinguish among infections with yaws, pinta, and syphilis or the passage of time to eliminate possible exposure of the sexually active population to other pathogenic treponemes.

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