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Detection of *Treponema pallidum* DNA in oropharyngeal swabs and whole blood for syphilis diagnosis

Lauren C. Tantalo, BS[1], **Haley Mendoza, BS**[1], **David A. Katz, PhD, MPH**[2], **Sharon K. Sahi, BS**[1], **Christina M. Marra, MD**[1]

[1]Department of Neurology, University of Washington School of Medicine, Seattle, WA, USA

[2]Department of Global Health, University of Washington School of Medicine, Seattle, WA, USA

Abstract

Background: Syphilis diagnosis relies on serological tests, which may be falsely nonreactive or may be reactive but not reflect current syphilis.

Methods: Polymerase chain reaction for detection of *T. pallidum* DNA was performed on 123 oropharyngeal swabs, 120 whole bloods and 46 lesion exudate swabs from 123 untreated individuals with syphilis (cases); oropharyngeal swabs from 148 at-risk controls without syphilis; and 73 oropharyngeal swabs and 36 whole bloods from 73 individuals recently treated for syphilis.

Results: Most (90.2%) cases had early syphilis. *T. pallidum* DNA was detected in 33 (26.8%) of 123 oropharyngeal swabs, 32 (26.7%) of 120 bloods, and 30 (65.2%) of 46 lesion exudate swabs. *T. pallidum* DNA was detected in 49 (40.8%) of 120 individuals in whom both oropharyngeal swabs and blood were tested. *T. pallidum* was more likely to be amplified from oropharyngeal swabs when it was amplified from blood than when it was not (15 [46.9%] of 32 vs. 17 [19.3%] of 88, p=0.003). For each 2 fold increase in serum RPR titer, the odds of detection of *T. pallidum* DNA in oropharyngeal swabs increased by 1.44 (95% CI, 1.14-1.82, p=0.003). *T. pallidum* DNA was not detected in oropharyngeal samples from controls, but it was detected in 3 (8.3%) of 36 bloods from individuals recently treated for syphilis: two at 1 day and one at 5 days after initiation of syphilis treatment.

Conclusion: Nucleic amplification tests can identify recent *T. pallidum* infection and may be particularly useful for diagnosis of very early or asymptomatic syphilis.

Summary

Treponema pallidum DNA was detected in 27% of 123 oropharyngeal swabs, and in 27% of 120 bloods from individuals with untreated syphilis. *T. pallidum* DNA was detected in 41% of those in whom both oropharyngeal swabs and blood were tested.

Correspondence: Christina M. Marra, MD, Harborview Medical Center Box 359775, 325 9th Avenue, Telephone: 206-744-3251, Fax: 206-744-8787, cmarra@uw.edu.

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Introduction

Syphilis, caused by the bacterium *Treponema pallidum* subspecies *pallidum* (hereafter *T. pallidum*), is increasing in incidence in the United States and other high income areas, particularly among men who have sex with men (MSM) (1, 2). Syphilis diagnosis mainly relies on serological tests, which may be insensitive (falsely negative) early in the course of disease (3). Moreover, reactive serological tests in individuals who are asymptomatic may reflect previous, and not current, syphilis (3). While nucleic acid amplification tests are in common use for other sexually transmitted infections, such as chlamydia or gonorrhea, and can be applied to oropharyngeal, vaginal or rectal swabs (3), such tests are not routinely available for syphilis diagnosis.

Previous reports have documented amplification of *T. pallidum* nucleic acid from oral or pharyngeal swabs (4, 5), or in one instance, saliva (6), in individuals with syphilis. These studies differed in patient selection, inclusion of individuals without syphilis, and concomitant amplification of samples from blood or lesion exudate. Only one study attempted to amplify *T. pallidum* DNA from samples collected after treatment (6). Herein we report the results of polymerase chain reaction (PCR) testing for *T. pallidum* DNA from oropharyngeal swabs, blood and lesion exudate swabs from individuals with syphilis, from oropharyngeal swabs from two control groups at risk for but without syphilis, and from oropharyngeal swabs and blood from individuals after treatment for recent syphilis.

Methods

Study Participants

Between February 2015 and December 2017, oropharyngeal swabs, blood and lesion exudate (if available) were collected from untreated individuals with clinically- and laboratory-confirmed syphilis who underwent care at a County Sexual Health Clinic. These samples were collected as part of a public health investigation, and, as such, informed consent was not required.

Between September 2017 and June 2018, oropharyngeal swabs were collected from persons living with HIV (PLWH) who did not have syphilis and were attending regular appointments in an HIV clinic, and from individuals attending the Sexual Health Clinic who did and did not receive a syphilis diagnosis; for those with syphilis, blood was collected, and lesion exudate swabs were collected if available; all samples were collected before syphilis treatment. In addition, between March 2016 and March 2018, oropharyngeal swabs and blood were collected before and after treatment for uncomplicated syphilis from participants who were enrolled in a study focusing on cerebrospinal fluid abnormalities in syphilis. Study protocols were reviewed and approved by the University of Washington Institutional Review Board, and written informed consent was obtained from all participants. Individuals with syphilis are designated as cases; control group 1 included PLWH without syphilis, control group 2 included Sexual Health Clinic attendees without syphilis, and group 3 included those recently treated for uncomplicated syphilis.

Procedures

A Dacron swab was rubbed over the entire inside surface of the mouth including buccal mucosa, gums inside and out, posterior pharynx and tonsils. Note was made of any visible oral lesions. The margins of primary chancres or moist secondary lesions were firmly squeezed and exudate collected with a Dacron swab. Swabs were inserted into sterile 1.7 ml tubes and mixed with 1 ml of 1X lysis buffer (10 mM Tris-HCl, 0.1 M EDTA, 0.5% SDS) and stored at −80°C until DNA extraction. DNA was extracted from 200 ul of the swab material using the QIAamp DNA Mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The final elution yielded 200 ul of DNA in molecular grade water and was stored at −80°C.

DNA was extracted from whole blood collected into ethylenediaminetetraacetic acid (EDTA) tubes using the QIAamp DNA Blood Midi kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Extracted DNA was precipitated using 2.5 volumes of 100% ethanol with 0.1 volume of 3M sodium acetate and 20 ug of glycogen overnight at −20°C. The DNA pellets were washed twice with 75% ethanol, resuspended in 60 ul of molecular grade water and stored at −80°C.

Amplification of a portion of the *tp0548* gene from blood, oropharyngeal or lesion swab DNA by conventional PCR and amplification of a portion of the *tp0574* (Tp47) gene from blood (only for individuals in group 3) by real time PCR were performed as previously described (7, 8). Serum rapid plasma reagin (RPR) tests were performed in a CLIA certified clinical laboratory or in a research laboratory using published methods (9).

Data Analysis

Descriptive statistics are expressed as number (percent) or median (interquartile range [IQR]). Proportions were compared by the Chi-square or Fisher's exact test, and odds ratios with 95% confidence intervals (95% CI) were determined by binomial logistical regression using SPSS version 27 (IBM Corporation, Armonk, NY, USA). Differences in proportions were compared using the two sample test of proportions, Stata version 11.2 (StataCorp, College Station, TX, USA). Two sided p-values <0.05 were considered significant.

Results

The characteristics of the study participants are shown in Table 1. Of the 123 cases, oropharyngeal swabs were collected in 123, whole blood in 120 and lesion exudate swabs in 46. Oropharyngeal swabs were collected in 221 other participants: 60 in control group 1 (PLWH without syphilis), 88 in control group 2 (Sexual Health Clinic attendees without syphilis) and 73 in group 3 (recently treated for uncomplicated syphilis). Oropharyngeal swabs were collected a median of 7 (4-17) days (range 1-56 days) after syphilis treatment began for group 3.

Serum RPR was reactive in 119 cases. Of the four cases with nonreactive serum RPR, three were in the primary and one was in the late latent stage. Four individuals, all with secondary syphilis, had oral lesions.

In the cases, *T. pallidum* DNA was detected in 33 (26.8%) of 123 oropharyngeal swabs, 32 (26.7%) of 120 bloods, and 30 (65.2%) of 46 lesion exudate swabs by amplification of the *tp0548* gene. *T. pallidum* DNA was detected in 49 (40.8%) of 120 individuals in whom both oropharyngeal swabs and blood were tested. *T. pallidum* DNA was more commonly amplified from oropharyngeal swabs in cases with secondary and early latent syphilis than in individuals with primary or late latent stages (28 [34.6%] of 81 vs. 5 [12.5%] of 40, p=0.01), and it was more commonly amplified from blood in cases with primary and secondary syphilis compared to individuals with early or late latent stages (27 [33.3%] of 81 vs. 5 [13.5%] of 37, p=0.03), (Table 2). *T. pallidum* was significantly more likely to be amplified from oropharyngeal swabs when it was amplified from blood than when it was not amplified from blood (15 [46.9%] of 32 vs. 17 [19.3%] of 88, p=0.003). There was no relationship between detection of *T. pallidum* in oropharyngeal swabs and in lesion exudate (data not shown). Detection of *T. pallidum* in oropharyngeal swabs, but not in blood or lesion exudate, was more common with increasing serum RPR titers. For each 2 fold increase in serum RPR titer, the odds of detection of *T. pallidum* DNA in oropharyngeal swabs increased by 1.44 (95% CI, 1.14-1.82, p=0.003). *T. pallidum* DNA was more likely to be detected in oropharyngeal swabs when oral lesions were present compared to when they weren't (4 [100.0%] of 4 vs. 21 [26.9%] of 78, p=0.007). Of the 4 cases with nonreactive serum RPR, two had detectable *T. pallidum*, one from an oropharyngeal swab and the other from lesion exudate. Both individuals had primary syphilis.

T. pallidum DNA was not detected by amplification of the *tp0548* gene in any of the oropharyngeal samples from controls. In group 3 (recently treated for syphilis), *T. pallidum tp0574* DNA was detected in 3 (8.3%) of 36 blood samples collected between 1-8 days after treatment: two at day 1 and one at day 5. Two were treated with one dose of intramuscular benzathine penicillin G, and one (detectable at day one) after beginning treatment with an oral course of doxycycline.

Discussion

Syphilis diagnosis relies heavily on serological tests, which may be nonreactive in very early syphilis, and which may indicate active or treated syphilis in asymptomatic individuals (3). While darkfield examination of lesion exudate may identify seronegative syphilis, this diagnostic modality is not available in most clinical settings, and even if it is, not all patients with syphilis have genital or skin lesions. We were able to amplify *T. pallidum* DNA from 27% of oropharyngeal swabs from individuals with all syphilis stages, including two individuals with nonreactive serum RPRs, and we were able to amplify *T. pallidum* DNA from blood from a similar proportion. *T. pallidum* DNA was identified in 41% of individuals with syphilis in whom both an oropharyngeal swab and blood were tested. Although detection from oropharyngeal swabs was more likely in those with oral lesions, we were able to identify *T. pallidum* DNA in 30% of oropharyngeal swabs from individuals without oral lesions. The odds of detection in oropharyngeal swabs increased by 44% for each two-fold increase in serum RPR titer.

Previous studies have demonstrated detection of *T. pallidum* nucleic acid in oral and pharyngeal swabs. Yang and colleagues (5) identified *T. pallidum* DNA in 40% of

oral swabs from 240 individuals with 267 syphilis episodes, and they identified similar associations with oral lesions and serum RPR titer as we did. Golden and colleagues (4) identified *T. pallidum* 23S rRNA in 7 (32%) of 22 pharyngeal specimens from patients with syphilis; 20 of 21 had early syphilis and stage was unknown in one individual. As in our study, *T. pallidum* nucleic acid was detected in samples from two individuals with nonreactive serological tests. Blood samples were not tested. Wang and Hu (6) used a nested PCR to detect *T. pallidum* DNA in saliva and plasma from 234 individuals with syphilis, and they found that detection in saliva (44%) was significantly greater than in plasma (30%). Similar to ours and other studies, detection in saliva was significantly more common in those with detectable DNA in plasma, and in those with higher serum RPR titers.

We were not able to detect oropharyngeal *T. pallidum* in any individual without syphilis nor in any individual who had been treated for syphilis before sample collection, even with an interval as short as one day. Wang and Hu (6) collected serial saliva samples from 9 participants at 4 hour intervals after treatment; all but one individual were treated with intravenous aqueous penicillin G, 4 million units every 4 hours for 14 days. The median clearance time (defined as time to three consecutive negative samples) was 64 hours. In four participants, serial saliva samples were collected after clearance, and *T. pallidum* DNA could be detected as late as 88 hours after treatment. The difference between detection of *T. pallidum* DNA in saliva in that study compared to oropharyngeal swabs in our study is likely due to the larger volume of saliva compared to oropharyngeal swab fluid. We were able to detect *T. pallidum* DNA in blood at 1 and 5 days after treatment, which adds plausibility to the findings in saliva.

Limitations of our study should be acknowledged. We did not test additional sample sources such as urine or vaginal swabs. Few of our cases had late latent syphilis, and two-thirds had symptomatic early syphilis, which usually does not present a diagnostic dilemma. The amount of oropharyngeal fluid collected in a swab is small, and it may limit detection of *T. pallidum* DNA when the oropharyngeal concentration is low; saliva may ultimately be a better oral sample, although it may be more complicated to collect. The characteristics of the cases were most similar to group 3, who were individuals recently treated for uncomplicated syphilis, and cases were less similar demographically to the two control groups. Nonetheless our cases were well characterized, and we included a large number of controls who were representative of a wide spectrum of individuals at-risk for sexually transmitted infections.

Detection of DNA in oropharyngeal swabs or blood is not the same as detection of viable organisms. However, the observation that *T. pallidum* DNA is not detected beyond 5 days after initiation of treatment suggests that detection of *T. pallidum* DNA in these samples represents recent infection, which is the potential advantage of nucleic acid amplification over serological tests. In our study, testing both oropharyngeal swabs and blood increased the proportion of positive individuals from 27 to 41%. From a clinical standpoint, DNA testing would be most useful when positive, particularly when there is no physical examination evidence of syphilis, because it would confirm the diagnosis. Future prospective investigation is required to determine the utility and cost effectiveness of detection of *T. pallidum* DNA in conjunction with serological tests for syphilis diagnosis, particularly in individuals with asymptomatic syphilis.

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Table 1.

Characteristics of study participants

Characteristic	Cases n=123	Other Study Participants n=221		
		Controls		
		Group 1 n=60	Group 2 n=88	Group 3 n=73
Male	119 (96.7)	45 (75.0)	72 (81.8)	71 (97.3)
White	74 (60.2)	24 (40.0)	51 (58.0)	55 (75.3)
Black	12 (9.8)	28 (46.7)	11 (12.5)	6 (8.2)
Other	37 (30.1)	8 (13.3)	26 (29.5)	12 (16.4)
Age (years)	33 (27-41) n=122	50 (46-57)	30 (24-36)	37 (29-48)
Men who have sex with men	105 (92.9) n=113	32 (53.3)	55 (62.5)	67 (91.8)
Stage				
Primary	29 (23.8)	NA	NA	12 (16.4)
Secondary	55 (45.1)			26 (35.6)
Early latent	26 (21.3)			23 (31.5)
Late latent	11 (9.0)			12 (16.4)
Sexual contact with an individual with infectious syphilis in the last 90 days	1 (0.8) n=122			--
Serum rapid plasma reagin titer	64 (16-128) n=119			64 (32-256)
PLWH	32 (26.2) n=122	60 (100.0)	3 (3.4)	39 (53.4)

Cases were individuals with untreated syphilis diagnosed at a Sexual Health Clinic. Control group 1 included persons living with HIV (PLWH) without syphilis, control group 2 included Sexual Health Clinic attendees without syphilis, and group 3 included those recently treated for uncomplicated syphilis. Stage for group 3 refers to the stage for which they were treated. Values are expressed as number (percent) or median (interquartile range). Abbreviations: NA, not applicable.

Table 2.

Detection of *tp0548* DNA in oropharyngeal swabs, whole blood and lesion exudate swabs from cases according to syphilis stage

Stage[a]	**Sample Source**		
	Oropharyngeal swab, n=122	**Whole blood, n=119**	**Lesion exudate swab, n=46**
Primary	4/29 (13.8)	8/28 (28.6)	19/24 (79.2)
Secondary	22/55 (40.0)	19/53 (35.8)	11/22 (50.0)
Early latent	6/26 (23.1)	4/26 (15.4)	--
Late latent	1/11 (9.1)	1/11 (9.1)	--
Sexual contact with an individual with infectious syphilis in the last 90 days	0/1 (0.0)	0/1 (0.0)	--

[a] Stage was missing in one participant.