

Diagnostic dilemma of the single screening test used in the diagnosis of syphilis in Nepal

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ABSTRACT

Syphilis screening by the nontreponemal rapid plasma reagin (RPR) test is not usually followed up by specific treponemal tests in most of the resource poor healthcare settings of Nepal. We analyzed serum specimens of 504 suspected syphilis cases at the immunology department of the national reference laboratory in Nepal during 2007-2009 using RPR test and *Treponema pallidum* hemagglutination assay (TPHA). In overall, 35.7% were positive by both methods (combination) while 13.1% were RPR positive and TPHA negative, 8.7% were positive by TPHA only and 42.5% were negative by both methods. Among the RPR reactive (n =246), 73.2% were positive by TPHA. Non-specific agglutination in RPR testing was relatively higher (26.8%) compared to TPHA (19.6%). Although TPHA was found more specific than RPR test, either of the single tests produced inaccurate diagnosis. Since the single RPR testing for syphilis may yield false positive results, specific treponemal test should be routinely used as confirmatory test to rule out false RPR positive cases. More attention needs to be paid on formulation of strict policy on the implementation of the existing guidelines throughout the country to prevent misdiagnosis in syphilis with the use of single RPR test.

Keywords: Syphilis; rapid plasma reagin test; *Treponema pallidum* hemagglutination assay; Nepal.

INTRODUCTION

The appropriate diagnosis of syphilis is of particular importance because of its considerable but preventable obstetric and gynecological morbidity and mortality.¹ Moreover, syphilis being an epidemiological synergy and cofactor in transmission of HIV and other sexually transmitted infections (STIs), its screening and management among HIV infected population has gained a new insight in HIV prevention and control strategies.¹⁻³ Since the cross reacting serological response of non-venereal treponema⁴ and the biological false positivity⁵ compromise the appropriate diagnosis of syphilis, the conformation of cases with positive nontreponemal test is recommended.⁶ Screening by venereal disease research laboratory (VDRL) and/or the rapid plasma reagin (RPR) followed by conformation using fluorescent treponemal antibody (FTA) and/or *Treponema pallidum* hemagglutination (TPHA) test is the recommended guidelines and mainstay in syphilis diagnosis.⁴

Currently, syphilis diagnosis in Nepal is entirely based on the serological methods and most healthcare providers prefer single nontreponemal tests without further confirmation.^{7,8} We conducted this study keeping in view of the following situations prevailing in Nepal. Firstly, burden of HIV is increasing with the government estimate of more than 17000 infected people⁹ though the non-governmental organizations' estimate is far

beyond this figure. Secondly, a high prevalence rate of syphilis (4.7% in asymptomatic and up to 18.0% in symptomatic individuals) reported from the country¹⁰ and a considerable proportion (14.2%) among HIV infected population (unpublished data, NPHL). Thirdly, despite the provision for confirmation of RPR screening by TPHA in the national guidelines,¹⁰ majority of the health care providers/polyclinics use only single RPR test for syphilis diagnosis.

Irrespective of its use in screening syphilis, how helpful is the RPR result to clinical staff in developing countries is of particular interest. Data are merely available among Nepalese populations concerning the misdiagnosis of syphilis as a consequence of single RPR testing. Here, we report on the diagnostic inaccuracy of single syphilis screening test in the clinical settings so that appropriate actions can be taken by the authority towards implementation of proper testing algorithm.

METHODS

Specimens of the adult patients (15-60 years) requesting for the syphilis screening at National Public Health Laboratory (NPHL) were included during June 2007 and May 2009. NPHL is the government's national reference centre for infectious diseases diagnosis and surveillance. These specimens were previously tested for either RPR or TPHA as per the patient's request form. All the patients

Table-1: RPR and TPHA test results

Test results		TPHA		Total
		Positive	Negative	
RPR	Positive	180	66	246
	Negative	44	214	258
Total		224	280	504

were from the urban and semi-urban areas of the Kathmandu valley (Kathmandu, Lalitpur and Bhaktapur districts). The identity of individuals and the primary screen results were blinded before these specimens were made available for this study. Specimens from 504 individuals were investigated for syphilis by the combination of RPR and TPHA tests at the immunology department of NPHL using commercial kits (HUMAN Diagnostics Worldwide, Wiesbaden, Germany) following manufacturer’s guidelines. Quantitative tests were performed on all RPR reactive specimens. Positive and negative controls were used in each batch of testing and the discordant results were repeated twice before being recorded as negative or positive.

Syphilis status of each participant was defined by a gold standard composed of the TPHA and RPR results. Possible combination were: "no syphilis" for negative in both tests, "active syphilis" for TPHA positive and RPR positive; "old or treated syphilis" for TPHA positive and RPR negative, and biological false–positive for TPHA negative and RPR positive results. Specificity, sensitivity, positive predictive value, negative predictive value and likelihood ratios for single RPR and TPHA test were determined against their combination which was considered as gold standard. Data analysis was performed using SPSS Statistics Student Version 17.0. This study was conducted as a part of government’s program for which ethical approval was not required by the Ministry of Health and Population.

RESULTS

The mean age of the individuals under study was 30.3 years (95% confidence interval [CI], 29.2-31.3) with the range of 17-59 years while the mean age of those positive for syphilis was 31.8 years (95% CI, 30.2-33.4) with the range of 18-57 years. Majority of the patients in the study were males (77.8% males). Out of the 504 specimens, 246 (48.8%) were reactive by the RPR test and among these RPR reactive, 180 (73.2%) were positive by TPHA (Table 1). Males showed relatively higher proportion (57.6%) of false positives by RPR. In overall, 35.7% were positive by both methods (combination) while 13.1% were RPR positive and TPHA negative, 8.7% were positive only by TPHA and 42.5% were negative by both methods (Table 1). Based on the results of combination tests, 180 (35.7%), 44

(8.7%), 66 (13.1%) and 214 (42.5%) were grouped into active syphilis cases, old/treated syphilis cases, biological false–positive cases and patients with no syphilis respectively.

None of the positive results obtained by RPR and TPHA testing was invalid. Non-specific agglutination in RPR testing was relatively high (26.8%) compared to TPHA (19.6%). Performance characteristics of the single RPR and TPHA tests against their combination have been presented in table 2. TPHA test was found to be more specific than RPR test.

DISCUSSION

Despite tremendous intervention against syphilis, chiefly through penicillin therapy and several public health initiatives, the world continues to be burdened by this disease urging for more appropriate diagnostic facilities in healthcare setting.⁶ In Nepal, syphilis is commonly reported in various population sub-groups including HIV infected individuals and sex workers,^{8,10} immigrants,^{7,11} and blood donors.⁷ We also found syphilis among 14.2% of the HIV infected population (unpublished data, NPHL). In addition to the high risk group, syphilis has been frequently reported from family planning attendees, pregnant women and other asymptomatic populations in the country.¹⁰ Although it is equally important in general population, screening of syphilis in these specific sub-populations with appropriate testing algorithm should be emphasized to minimize syphilis related health consequences in pregnant women, unborn babies and HIV infected individuals. Since the rate of syphilis is very high among the commercial sex workers,¹⁰ appropriate and regular screening should be in place to tackle role of syphilis in HIV transmission rates.

In this study, only 73.2% of RPR reactive results were confirmed by TPHA test. RPR being easy to perform and more economic, is the commonly used syphilis screening test in most of the healthcare settings in Nepal. Commercial interest has largely influenced the testing algorithms especially in the mushrooming private poly-clinics in the country. RPR positive sera when not confirmed by specific treponemal test may also lead to miscalculation of the disease burden in the country. Although RPR screening detects the symptomatic primary, secondary and late syphilis,^{12,13} positive test results is wrongly interpreted in some patient with symptoms suggestive of syphilis (e.g. genital ulcers) because such condition may be caused by

Table-2: Performance characteristics of single RPR and TPHA tests against their combination

Tests	Sensitivity	Specificity	PPV	NPV	PLR	NLR
RPR	100	79.6	73.2	100	4.9	1.2
TPHA	100	86.4	80.4	100	7.3	1.1

PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio

Haemophilus ducreyi, herpes simplex virus 2, *Chlamydia trachomatis*, and many other genital ulcers disease.¹⁴ Attention has to be paid to the bad consequences of misdiagnosis produced by the financial constrains and low cost testing strategies. Confirmation of RPR results should be done by TPHA or other specific treponemal tests in all populations as mentioned in the national STI management guidelines.¹⁰ It is the responsibility of each health care provider in the country to follow the national guideline's algorithm to ensure that appropriate syphilis diagnosis has been made.

Only 80.4% of the TPHA positive cases were positive by RPR test. Although some false-positive RPR screens may be confirmed by TPHA merely because the patient has a history of syphilis that had been treated successfully,^{4,13} TPHA demonstrated relatively less false positive results compared to RPR in our study. TPHA test was positive in 17.1% of our RPR negative specimens. Such TPHA positive/ RPR negative result is generally common in old or treated syphilis arguing lower utility of TPHA for detecting false positive RPR screening in a population with high incidence of syphilis.¹³ Furthermore, false-positive TPHA results can be obtained due to the presence of other pathogenic treponemes not associated with syphilis.^{13,15} Our findings suggest on the use of combination test to avoid any ambiguous results in syphilis diagnosis.

Another unseen truth is the socio-economical aspect of syphilis misdiagnosis due to single RPR test which should not be undermined. A huge Nepalese population is attracted to abroad jobs and this remittance has been a good source of national income. Since syphilis screening is mandatory for medical certification both in the country and abroad, false negative or false positive results may end up with their embarkation back to the country or the loss of abroad job opportunity or the social stigma producing huge societal and economical impact. Unfortunately, most of the medical centers and poly-clinics in the country use single RPR test for syphilis screening.

In light of the observations we made, it seems unreasonable to subject patients unnecessarily to penicillin injections, anxiety and other psychosocial consequences when single RPR or TPHA test is positive. If RPR test is all that laboratories can afford, then they should not be used as a solo screening tool for clinical decision making. Test limitations must be well notified to treating physicians, and the laboratory reports must state clearly that the RPR test alone cannot confirm or exclude syphilis infection. As syphilis screening has now embarked into new dimension,^{16,17} additional studies are warranted to provide a better move towards the serologic syphilis testing practices that are helpful in developing countries like Nepal. More attention needs to be paid on formulation of strict policy on the implementation of the existing guidelines in all the

health care centers throughout the country to prevent possible misdiagnosis of syphilis with the use of single RPR test.

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