Study of sensitivity and specificity of five rapid plasma reagin test kits in syphilis

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Background: Syphilis caused by *Treponema pallidum* has been associated with humankind since ancient times.

Materials and Methods: Blood samples from 408 healthy Nepalese male population was collected and tested to find out the sensitivity and specificity of five different (a, b, c, d and e) non-treponemal syphilis serological test rapid plasma reagin (RPR) as well as for the screening of the healthy target group for syphilis.

Results: Six subjects (1.47%) were found positive with the test and by *Treponema pallidum* Haemagglutination (TPHA) test was identified as a syphilitic subject. With the test kits a, c and d 34 false positive cases were detected. The sensitivity was 100%, and the specificity was 92% only. Whereas with test kits b and e only six true positive cases were detected thus making their sensitivity and specificity 100%.

Conclusion: Therefore, it is concluded that test kits a, c and d should only be used for the screening purpose. On the other hand, the test kits b and e can be used for the diagnostic purpose.

Key words: RPR, TPHA, syphilis, sensitivity, specificity

Introduction

Syphilis caused by *Treponema pallidum* has been associated with humankind since ancient times. All the cases of syphilis are not reported as the primary chancre is overlooked and unheeded due to it being painless. They are usually hidden sites and shame associated with it. At the same time, the social behavior of hiding such lesions due to social stigma attached to sexually transmitted diseases make the screening dependable on serological screening tests. Furthermore, 50% of female and 30% of males do not notice the primary lesions[1].

For finding out the infection, the non-treponemal test RPR test is the preferred one than other non-treponemal tests like complement fixation test, venereal disease laboratory (VDRL) test. Biological false positive reaction is the drawback of all non-treponemal tests like RPR test. Therefore, all positive cases by such tests should be confirmed by the confirmative treponemal test like TPHA test. Biological false positivity is possibly due to the formation of antibody against tissue cardiolipin liberated during various infectious diseases[2]. In biological false positive cases the titre is generally less than 8 and the titre decrease without therapy [3-4]. The patients suffering from different diseases like pneumonia, malaria, hepatitis leprosy, autoimmune disease, mononucleosis, drug addiction etc. usually exhibit biological false positive reaction [2,3,4,5,6,7]. The diseases allied to syphilis such as yaws, pinta also give positive reaction. In fact no serological test can differentiate syphilis from other treponemal infections [8].

The incidences of false positive and false negative results are partly due to the poor specificity. The disadvantage of non-treponemal serological test for syphilis is their lack of specificity [2]. One percent of apparently normal people exhibit a positive reaction in non- treponemal serological test for syphilis. Therefore, to study the sensitivity and specificity of five different RPR kits available in the market, this study is conducted.

Materials and Methods

In this study 408 blood samples was collected from healthy Nepalese male population during July 2001 to August 2001.

The healthy subjects were those who had no symptoms of any syphilitic lesion and had not been under treatment for the same. Consent for the test was taken from all the subjects and privacy was assured. The serum samples were separated, numbered and tested exactly as recommended by the manufacturers. Each single sample was tested by five different RPR test kits which were decoded as a, b, c, d and e. All the samples positive by any of the kits were confirmed by using TPHA test kit, Biokit, Spain. The subjects giving false positive reaction (RPR positive and TPHA negative) were retested after two months and were not found positive in TPHA test, hence were identified as false positive cases. The calculation of sensitivity, specificity, positive predictive value, negative predictive value, was calculated as described by Park and Pulse Diagnostics[4-5]. All the subjects confirmed positive by TPHA test were treated by the registered medical practitioner.

Results

Of the 408 samples 6 were positive by all five kits a, b, c, d and e. They were confirmed positive by TPHA test, thus making the prevalence of syphilis 1.47% in the target group. The kits a, c and d gave positive reaction in 40 subjects in total, however only 6 of them were confirmed positive cases of syphilis and 34 were false positive reactions. (table 1). Therefore, the sensitivity of these three kits was obtained 100%, whereas specificity was only 92%.

Table 1: Showing reaction of different RPR kits and TPHA

Positives	Kits used					
	a	b	c	d	e	TPHA
Total positive	40	6	40	40	6	6
True positive	6	6	6	6	6	6
False positive	34	0	34	34	0	x

Calculaiton No 1. Showing sensitivity, specificity, positive and negative predictive value; and false positive and negative percentage of a, c, and d test kits

Testkit	Syphilis		
	+	-	
+	(a) 6	(b) 34	(a+b) 40
-	(c) 0	(d) 368	(c+d) 368
	(a+c) 6	(b+d) 402	

Sensitivity = a x 100/ (a + c) = 6 x100 / 6 = 100%

Specificity = d x 100/ (b + d) = 368 x 100/ 402 = 92%

Positive Predictive value = a x 100/ (a + b) = 6 x 100/ 40 = 15%

Negative Predictive value = d x 100/ (c + d) = 368 x 100/ 368 = 100%

Calculaiton No 2. Showing sensitivity, specificity, positive and negative predictive value; and false positive and negative percentage of b and e test kits

Testkit	Syphilis		
	+	-	
+	(a) 6	(b) 0	(a+b) 6
-	(c) 0	(d) 368	(c+d) 368
	(a+c) 6	(b+d) 368	

Sensitivity = a x 100/ (a + c) = 6 x100 / 6 = 100%

Specificity = d x 100/ (b + d) = 368 x 100/ 368 = 100%

Positive Predictive value = a x 100/ (a + b) = 6 x 100/ 6 = 100%

Negative Predictive value = d x 100/ (c + d) = 368 x 100/ 368 = 100%

On contrary, the test kits b and e gave positive reaction only in 6 cases which were confirmed positive by TPHA test. The sensitivity and specificity of these kits were 100% (calculation 2). No false positive cases were detected. Furthermore, there were no cases of false negative by these kits in comparison to a, c and d, since the subjects positive by a, c and d were TPHA negative.

Discussion

In this study purport to find out the sensitivity and specificity of five different RPR test kits available in market, an indication was obtained that one must not rely on at any particular kit till there is surety that it has high sensitivity and specificity. If it is for the screening purpose, a kit with high sensitivity (giving few false negative) should be used. Whereas, for diagnostic purpose a kit with high specificity (giving few false positive) should be used. In this study, positivity in TPHA test was taken as standard as the subjects were healthy and did not exhibit any clinical symptoms.

This study was also a screening study as healthy population was tested to find out the disease. Such studies are important epidemiologically and are considered these days as preventive care function and a logical extension of health care[6]. In this study 1.47% (6/408) of the asymptomatic subjects were treated and their progression to late stage of syphilis (tertiary stage) was stopped. One third of the infected people in primary, secondary and latent infection may proceed to tertiary stage[7].

The test kits a, c and d did not have high specificity (92%),

so were not good enough for the diagnostic purpose. However these could be used for the screening purpose (sensitivity 100%). In contrary, the test kits b and e were good enough for the diagnostic as well as screening purposes (both sensitivity and specificity 100%). It has been stated that no screening test is perfect, that is, 100% sensitivity and 100% specificity [10]. But this was the finding of this research for the test kit b and e. therefore, it is recommended that such research should be continued with a large number of sample for assuring laboratory personnel of the quality of a particular test kit. Such study will be helpful for the consistency of reports of different laboratories. Whatever specificity a RPR test kit has it should always be confirmed with the confirmative treponemal tests like TPHA, Fluorescent antibody absorption (FTA Abs) test or other tests. When FTA-Abs is not available, RPR and TPHA are complementary tests and provide excellent screening for detection of syphilis at all stages[8].

The test kits a and b were the product of two different batches of the same company. It is astonishing that the test kits were giving totally different results. Hence, it is worth stressing here that one should be careful in purchasing kits keeping in view the attitude/knowledge/service of supplier which encompasses the proper storage and handling of the kits which play very important role in the proper functioning of the kits. The test kit with 100% specificity is not comparable with a kit with 92% specificity

Conclusion

It is solely the duty of laboratory personnel to purchase a kit having high sensitivity and specificity. At the same time the suppliers have as big a responsibility to provide the kits to the user in exactly the same state the manufacturer has shipped. Furthermore, research work of this type with large sample size should be conducted by the government authorities to assure that only high quality kits are available in the country, which also assures the consistency of the reports produced by different laboratories.

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