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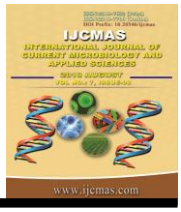

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Evaluation of the Rapid Plasma Reagin (RPR) and *Treponema Pallidum* Hemagglutination Assay (TPHA) Test Results, Which Used in Diagnosis of Syphilis Infections between 2009-2017 in a Tertiary Care Center

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A B S T R A C T

Knowing the profile of the disease in the working hospital is important in terms of differential diagnosis. Unfortunately, no current laboratory test can distinguish one treponematosis from another, and this must be considered in serology.

Introduction

Syphilis is caused by the spirochete bacterium, *Treponema pallidum* and was first recognized as a sexually transmitted disease in the 1400s (1). Syphilis is transmitted sexually via direct contact with mucocutaneous syphilitic lesions (e.g., chancre and condylomalata) or during pregnancy via vertical transmission from them other to the fetus (2). Which affects at least 11 million people worldwide every year. *T. pallidum* cannot be cultured *in vitro* (3).

Syphilis is a disease easily treated when timely diagnosed; however, it continues to be a global public health issue. It can present with a wide range of medical symptoms and signs, and devastating sequelae can develop decades after untreated infection. Syphilis is a reportable disease in every country. Interpretation of serological tests for syphilis requires expertise to distinguish true infection from false-reactive tests (4). For this reason, patients found to have positive syphilis serological tests should be managed by those

with relevant experience (5). In this study, we aimed to evaluate the RPR and TPHA test results retrospectively in relation of these tests with demographic data. Serum samples derived from potential blood donors or patients during pre-operation and antenatal routine testing were examined for syphilis. The examinations took place in the Serology Laboratory, Department of Medical Microbiology, Uşak University Training and Research Hospital.

Materials and Methods

In this study, serology laboratory records of Uşak University Training and Research Hospital between January 2009 and December 2017 were examined retrospectively. The syphilis screening test examined the number of samples studied, the male / female ratios of these patients, and the distribution of positive and negative samples by sex.

The TPHA test was run with the commercial kit of Immutrep TPHA, (Plasmatec Healthcare Ltd, UK). The principle of testing is based on the comparison of patient sera with sensitized and non-sensitized erythrocytes in base micropits. RPR test (Plasmatec, UK; Syphilis Ultra Rapid Test Device, Acon, USA and Syphilis Rapid Test Device Assure Tech (Hangzhou) Co., Ltd China) was also performed.

The RPR set is a non-treponemal flocculation test used for the qualitative and semi-quantitative determination of serum or plasma reactive antibodies from syphilis. Test principle; comparing the patient sample with a reagent containing cardiolipin / lecithin is based on the detection of aggregation in samples containing antibodies against the reagents. If there is a Syphilis antibody, agglutination occurs by clotting of the carbon particles present in the antigen suspension appearing as black clusters.

Results and Discussion

Between 2009 and 2017, a total of 19845 RPR tests were performed and 19725 (99.4%) were negative. Only RPR test was found positive in 120 (0.6%) samples. RPR test was 15907 (80%) of the patients who were working females. Of the 120 positive patients, 43 (35.8%) were females. The distribution of RPR positivity by age is given in table 1. (10406 patients' age information was available through the information processing system, 9439 patients were not). The number of RPR positive specimens according to years is given in table 2. Between 2009 and 2017, a total of 245 TPHA tests were performed and 190 (77.5%) of them were negative. Only TPHA test was found to be positive in 55 (22.5%) samples. 158 patients (64%) of the patients who underwent TPHA test. Of the 55 positive patients, 16 (35.8%) were females. The distribution of TPHA positivity by age is given in table 3. (Although the age information of 109 patients could be reached by the information processing system, the age of 136 patients could not be found in the system). The distribution according to the result of the TPHA samples is given in table 4. The number of TPHA positive samples according to years is given in table 5 distributed by TPHA test worked example of the results is given in Table 4. The number of TPHA positive samples according to years is given in table 5.

In our hospital, the syphilis test was used as a routine screening test in women who were monitored for pregnancy. For this reason, the number of female patients is high. However, the population with the disease is significantly male and is widespread in the group of sexually active age. The test RPR was also found to be positive in all patients with TPHA test positive. The observation of agglutination inpatient of 1/80 dilutions was evaluated as positive.

Table.1 Distribution of RPR positivity by age

	POSITIVE EXAMPLE NUMBER	NEGATIVE EXAMPLE NUMBER	TOTAL EXAMPLE NUMBER
Between 0-1 YEARS	0 (%0)	7 (%0,1)	7
Between 1-18 YEARS	1 (%0)	242 (%2,3)	243
Between 18-45 YEARS	39 (%0,4)	8932 (%85,8)	8971
Between 45-65 YEARS	32 (%0,3)	842 (%8,1)	874
65 YEARS	3 (%0)	308 (%3)	311
TOTAL	**75 (%0,7)**	**10331 (%99,3)**	**10406**

Table.2 Number of RPR positive samples according to years

Table.5 Number of TPHA positive samples according to years

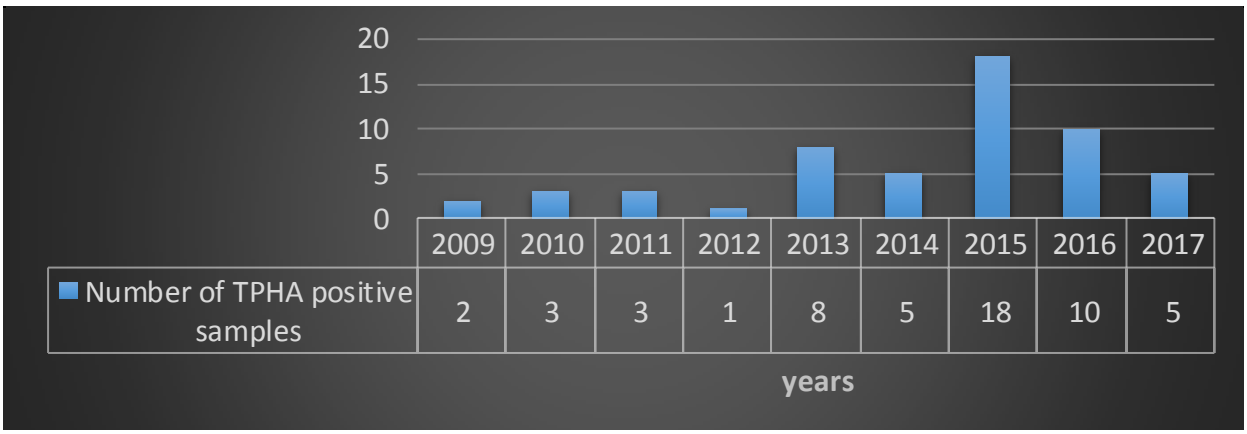


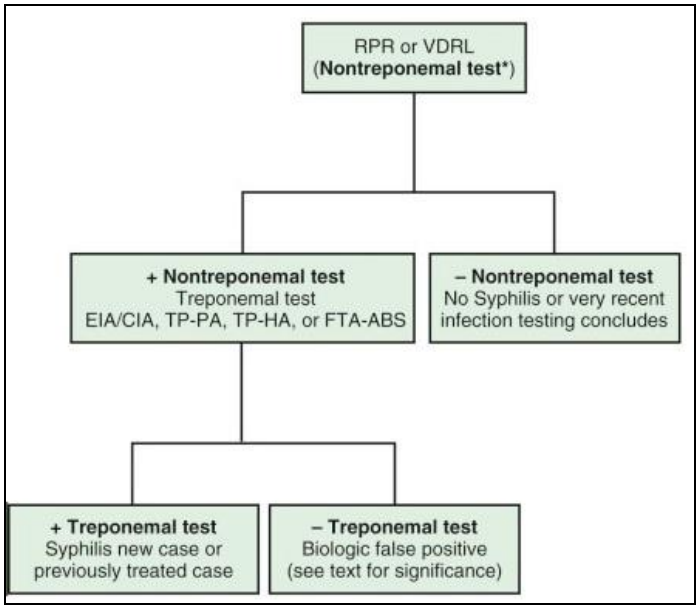
Table.3 Distribution of TPHA positivity by age

	POSITIVE EXAMPLE NUMBER	NEGATIVE EXAMPLE NUMBER	TOTAL EXAMPLE NUMBER
Between 1-18 YEARS	0 (%0)	2 (%1,8)	2
Between 18-45 YEARS	18 (%16,5)	53 (%48,6)	71
Between 45-65 YEARS	14 (%12,8)	19 (%17,4)	33
65 YEARS	1 (%0,9)	2 (%1,8)	3
TOTAL	33 (%30,3)	76 (%69,7)	109

Table.4 Distribution of TPHA-treated samples according to their titers

Result (titration)	Number of Patients
Negatif	190
1/80	17
1/160	15
1/320	8
1/640	13
1/1280	1
1/2560	1
Toplam	**245**

Fig.1 Traditional laboratory testing algorithm for syphilis (Nelson Textbook of Pediatrics. Patterson, Maria Jevitz; Davies, H. Dele. Published January 1, 2016. Pages 1470-14) (Based on data from Workowski KA, Berman S; Centers for Diseases Control and Prevention [CDC]: Sexually transmitted diseases treatment guidelines, 2010. MMWR RecommRep 59[RR-12]:1-110, 26-29, 2010)



The most important performance measure required in a screening test is the ability to detect real patients, that is, their sensitivity is high. In general, when sensitivity is higher than 95% in screening tests, it is usual that the specificity is lower than invalidation tests. (6). False-positive reactions can also occur with treponemal tests, but this is less common than with nontreponemal tests. Rapid Plasma Reagin (RPR) tests often screening purpose. Figure 1 shows the traditional laboratory test algorithm for syphilis. The traditional algorithm using a nontreponemal test followed by a treponemal test remains the standard in many parts of the world.

TPHA is a test that measures specific treponemal antibodies and can result in easy to set up. However, it remains positive for life in treated patients. VDRL are used for initial screening, whereas specific treponemal tests such as the TPHA are used to confirm the diagnosis. RPR is a nonspecific test but it is useful in the following treatment since the antibody titer declines on successful therapy. There was no significant difference between the two tests over the years ($p > 0.05$). It is a qualitative test for screening of syphilis, and currently, all nontreponemal tests are flocculation tests and RPR tests are the modification of original Wasserman reaction. Nontreponemal screening tests have a sensitivity of 70–90% in primary syphilis. It needs to be confirmed by a treponemal test. When a titer of nontreponemal test <1/8, the test should be repeated and a treponemal test should also be performed (7, 8). The causes of false positive results in the treponemal tests are listed as autoimmune diseases, HIV infection, pregnancy and intravenous drug use (9). Nontreponemal tests are recommended for screening purposes only in populations like ours where the incidence of syphilis is high. (10) Positive tests should be verified by a treponemal test such as TPHA. However, these tests may give more false-positive

results in cases such as tuberculosis, red pneumonia, leprosy, leptospirosis, mumps, hepatitis, cirrhosis, pregnancy, Hashimoto thyroiditis, various connective tissue diseases. The combination of TPHA and RPR deviates all treponemal infections except early primer syphilis and, rarely, is difficult to diagnose in low-risk populations (blood donors) in primary syphilis, requiring additional tests such as ELISA (11, 12). Knowing the profile of the disease in the working hospital is important in terms of differential diagnosis. Unfortunately, no current laboratory test can distinguish one treponematosis from another, and this must be considered in serology.

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