

Laboratory testing of women in reproductive age for infection sifilis, through detection of IgG and IgM antibodies

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Abstract

Syphilis is a sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum*. It is widespread throughout the world, especially in underdeveloped countries, affecting the health of the population of these countries. For this reason, this study aims to test a population group consisting of women of reproductive age (18-46 years old) for syphilis, by investigating the presence of IgM and IgG antibodies against *T. pallidum* in the serum, allowing us to evaluate prevalence for this infection in the population. Since in most cases the presence of STIs is asymptomatic, it is important to detect them as quickly as possible not only for the health of the affected person but also for the consequences in the development of babies during pregnancy. Immunological methods allow rapid and accurate identification of IgM and IgG antibodies in patient serum.

1287 sera were analyzed (divided into 7 years) for the presence of specific IgM and IgG antibodies against *T. pallidum* (anti-TPA) in women of reproductive age, with the ECL method in the Cobbas apparatus. All samples were analyzed in the Intermedica laboratory during the years 2011-2017. The prevalence of Syphilis in the study group is 5.28%. Anti-TPA IgM antibodies were detected in 1.71% of cases, while anti-TPA IgG antibodies were detected in 4.12% of cases included in our study group. These numbers show that *Treponema Palladium* (Syphilis) infection, which is associated with fetal damage when the infection occurs during pregnancy, or congenital Syphilis exists at low levels, to be assessed in Albanian women of reproductive age. The infection represents a slight increase over the years, which also shows the tendency for this infection to increase. The age group of 26-35 years is the age group that shows a higher level of infection. It is also noted that Syphilis shows a slight tendency to increase in prevalence with increasing age.

Keywords: *Treponema pallidum*, Syphilis, anti-TPA IgG antibody, anti-TPA IgM antibody, bridging system.

Introduction

T. pallidum is a member of the Spirochetaceae family that is characterized by its distinctive spiral shape. The most interesting thing about the structure of *T. pallidum* is the endoflagella that gives special motility (La Fond & Lukehart, 2006). It has one of the smallest bacterial genomes, so it has limited metabolic capacities (Fraser et al., 1998).

Syphilis was very common in Europe during the XVIII-XIX centuries. In the early 20th century, infections declined rapidly due to the widespread use of antibiotics, until the

1980s and 1990s (Franzen, 2008). Since 2000, rates of syphilis have increased in the US, Canada, the UK, Australia and Europe, mainly among gay men. In China and Russia since 1990 the prevalence rate among heterosexual men has increased (Stamm, 2010). This has been attributed to unsafe sexual practices, such as prostitution, and the declining use of condoms and condoms (Stamm, 2010; Kent & Romanelli, 2008; Ficarra & Carlos, 2009). Cases are proportionally higher among intravenous drug users, those infected with HIV, and homosexual men (Coffin et al., 2010; Gao et al., 2009; Karp et al., 2009). In 2012, about 0.5% of adults worldwide were infected with syphilis, with 6 million new cases (Newman et al., 2015). Syphilis affects 700,000-1.6 million pregnancies annually, causing miscarriages, stillbirths, and congenital syphilis. (Woods., 2009). As of 2014, syphilis infections continue to increase in the United States (Clement et al., 2014; Cantor, 2016). During 2015, syphilis caused an estimated 107,000 deaths, up from 202,000 in 1990 (Global Burden of Disease, 2015). In many Eastern European countries the incidence of syphilis is significantly higher than in Western Europe. The incidence of syphilis per 100,000 inhabitants for the countries of the region has been reported at the following levels: Bulgaria (2009) 5.5 cases, Romania (2007) 25.71 cases, Slovenia (2009) 2.3 cases, Turkey (2000) 4.95 cases. (Herbert & Middleton, 2012).

In Albania, from 1973 to 1995, no case of syphilis was reported, but the number of infections increased about 20 times after 1995 as a result of the country's opening to the West, high emigration of the population and the liberalization of life in place. From a study conducted for the period April 1, 2011 to May 1, 2012, 59 new cases of syphilis were found out of 336 sera examined (16.22%). (Torba. et al., 2013).

Syphilis is mainly transmitted by sexual contact (oral, vaginal and anal sex), from mother to her baby during pregnancy or by uncontrolled blood products and by sharing needles among drug users (Kent & Romanelli, 2008; Bhatti, 2007).

Syphilis infection affects the genitals, skin, mucous membranes, but can also affect other parts of the body, including the heart and brain

The disease develops in 4 stages: primary, secondary, latent and tertiary infection. Congenital syphilis also exists when the disease passes from the mother to the fetus, giving serious complications (Gjestland T, 1955).

Regional lymphadenopathy is characteristically associated with the primary stage. (La Fond & Lukehart, 2006). Sore throat, muscle pain, fatigue, weight loss, widespread skin rash are some of the systemically variable symptoms during secondary syphilis. General lymphadenopathy is encountered in up to 85% of cases (Chapel, 1980). Syphilis is considered latent when it is asymptomatic for a period of one year or more from the time of infection. Up to 25% of individuals with early latent syphilis manifest periodic symptoms of secondary syphilis (Gjestland, 1955; Eccleston et al., 2008). Serological testing during the late latent phase is positive, but sexual transmission of the disease is very rare. The microorganism can occasionally appear in the blood and can infect the fetus during pregnancy. (Bhatti, 2007). Tertiary syphilis can appear after 3-15 years of infection with primary syphilis in three different forms: gumma, nervous syphilis, cardiovascular syphilis. If left untreated, syphilis leads to the death of the affected individual several years after infection (Kent & Romanelli, 2008; Bhatti, 2007).

T. pallidum can be transmitted through the bloodstream from an infected mother to her developing fetus at any time during pregnancy, but the risk of fetal infection is much higher when the mother has primary syphilis (Woods, 2009). . Antibiotic treatment of the mother during the first two trimesters of pregnancy is usually sufficient to prevent adverse outcomes, but late treatment or lack of treatment may result in fetal death, fetal harm, or the birth of an infected child. Effects of the disease include: spontaneous abortion, stillbirth and premature birth. Affected children are underweight. Pulmonary hemorrhage, secondary bacterial infection, and severe hepatitis cause the death of approximately 4% of infected newborns shortly after birth (Cunningham, 2013). Early manifestations are infectious and resemble the severe symptoms of secondary syphilis in adults. Other sequelae of infection are anemia, jaundice, hepatosplenomegaly, and renal involvement. Affecting osteochondritis of the long bones can lead to pain and lack of movement of the upper and lower limbs (Woods, 2009; Nissanka-Jayasuriya et al., 2016). Late manifestations of congenital syphilis occur after the first two years of life. Between the ages of 5 and 25, keratitis can cause damage to the cornea and iris. Nerve deafness may be evident. Asymptomatic and symptomatic neurosyphilis, arthropathy, damage to the knees and elbows (Clutton's joints) and damage to the palate and nasal septum can also occur. Many of these manifestations progress regardless of treatment. Deformities of the teeth and nasal bridge may also occur (Woods, 2009; Nissanka-Jayasuriya et al., 2016).

Material and methods

A total of 1287 sera were analyzed (divided into seven years) for IgG and IgM-specific anti-CMV antibodies in females of reproductive age by the ECL test on the Cobas 6000 instrument. All samples were analyzed in the Intermedica Laboratory. Samples were taken from two state maternity hospitals, as well as from several private gynecological clinics in Tirana Collaborating laboratories and clinics include all 12 counties of the country..The testing was performed on healthy women, who performed the above analyzes before the start of a pregnancy, on women who had problems conceiving a child, or who were tested during pregnancy. There are no cases included in the study with suspect diagnosis: infectious disease. For each patient, after filling in a form with personal data, 5cc of blood was taken in a gel tube from which, after centrifugation for 10 min at 25,000 rpm, serum was extracted. Testing on the Cobas 6000 was performed within two hours of blood sampling. The bridging principle is used to detect anti-TPA IgM and IgG. The bridging principle is programmed to measure antibodies in biological fluids (IgG or IgM).

Results and discussions

In accordance with the aim of the study, a total of 1287 sera were analyzed (during the seven years of the study) for specific anti-TPA IgG and IgM antibodies in women of reproductive age, with the ECL test on the Cobas 6000 instrument, and these results were obtained:

For anti-TPA IgG: in the study group, in the period 2011-2017, the presence of IgG antibodies to *Treponema pallidum* was observed in 4.12% of cases.

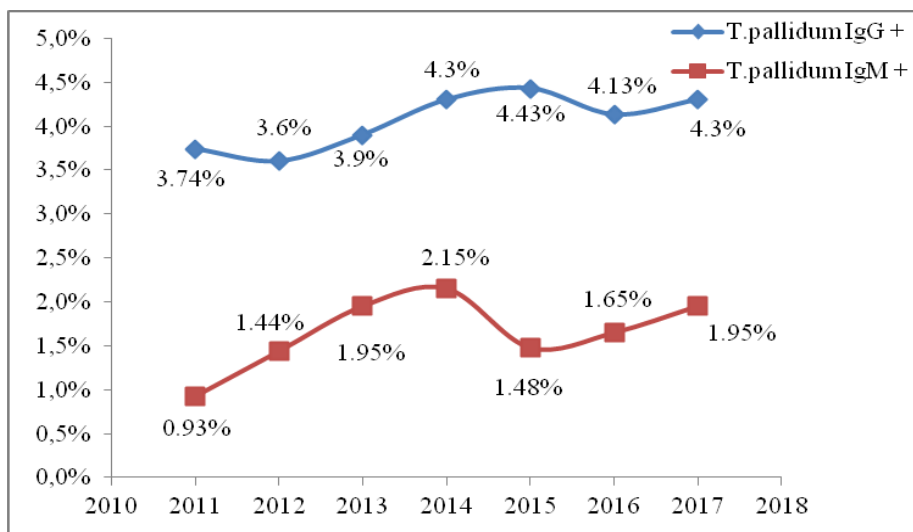
For anti-TPA IgM: the presence of IgM antibodies against *Treponema pallidum* is

observed in 1.71% of cases in the study population, in the period 2011-2017. IgM-positive cases are fewer than IgG-positive cases. Percentage in years of anti-TPA IgG and anti-TPA IgM antibodies in the study population is presented in **Table 1**.

Year	Tested	Positive for IgG (in no)	Positive for IgG (in %)	Positive for IgM (in no)	Positive for IgM (in n%)
2011	107	4	3.74%	1	0.93%
2012	139	5	3.6%	2	1.44%
2013	154	6	3.9%	3	1.95%
2014	186	8	4.3%	4	2.15%
2015	203	9	4.43%	3	1.48%
2016	242	10	4.13%	4	1.65%
2017	256	11	4.3%	5	1.95%
Total	1287	53	4.12%	22	1.71%

Table1: Seropositive females to anti-TPA IgG and to anti-TPA IgM

From the graphic representation of the data in the table above, the variation over the years of the immunological situation of IgG and IgM for *T. palladium* can be seen (**Graphic 1**)

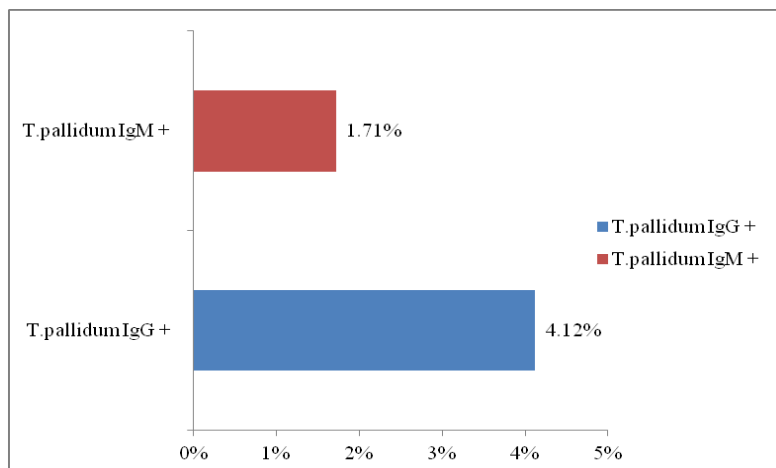


Graphic 1: Immunological situation for *T. pallidum* IgG and IgM

From the graph, it can be seen that we have a slight increase in IgG infection over the years, which also shows the tendency of this infection to increase.

And primary infection has a small upward trend with small fluctuations over the years. Thus, it shows a small increase in the first four years, decreases in 2015 and then increases again.

By comparing the values of anti-TPA IgG and IgM antibodies in total, a similar tendency of the two types of antibodies is observed, explained by the fact that, in contrast to other STIs, anti-TPA IgM antibodies remain positive longer during the course of infection.

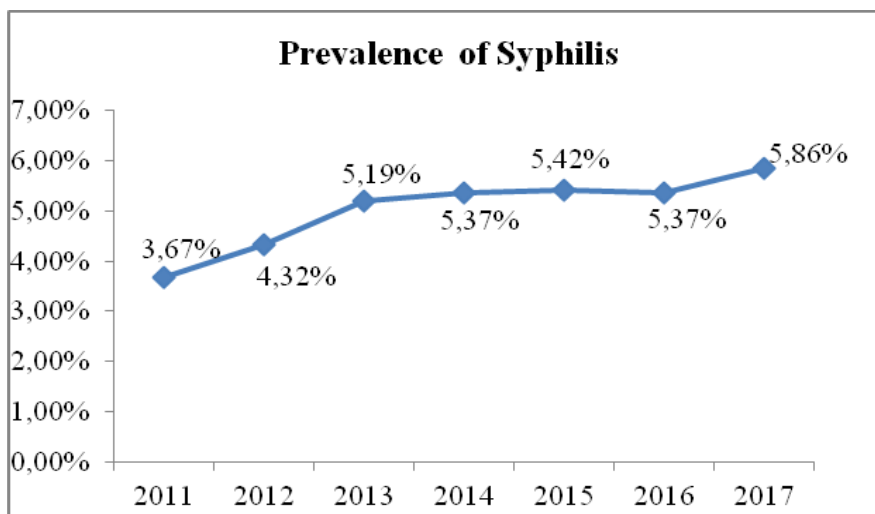


Graphic 2: Prevalence of *T.pallidum* IgG and IgM infection

Year	Tested for anti-TPA	Positive (in no)	Positive (in %)
2011	107	5	3.67%
2012	139	6	4.32%
2013	154	8	5.19%
2014	186	10	5.37%
2015	203	11	5.42%
2016	242	13	5.37%
2017	256	15	5.86%
Total	1287	68	5.28%

Table 2: Seropositive females to anti-TPA

The prevalence of Syphilis in the study group is 5.28% (**Table 2**). From the analysis of the results of the total number of antibodies (IgG and IgM), the tendency to increase the prevalence of Syphilis in our study group is clearly observed (**Graphic 3**).



Graphic 3: Prevalence of Syphilis

In Albania, from 1973 to 1995, not a single case of syphilis was reported. The number of syphilis cases has increased about 20 times after 1995 as a result of the liberalization of life in the country, the opening of the country to the West and the high emigration of the population. From a study conducted for the period April 1, 2011 to May 1, 2012, 59 new cases of syphilis were found (17.6%). The age group of 25-34 years has been the most exposed to syphilis. Positive cases have prevailed in the district of Tirana, 64.22% (Torba et al., 2013).

The lower percentage in our study can be argued with:

- the change of the study group, since in our study only women of reproductive age (16-45 years old) are included, while in the study conducted by Torba et al., 2013, volunteers, patients from the Infectious Diseases Service at QSUT "Mother Tereza" were included and the cases for confirmation by the National Center Blood Donation Tirana as men and women
- in our study for the evidence of acute and chronic infection, evidence was used through the antibody test with the ECL method, which has quite good sensitivity and specificity, while in the study conducted by Torba et al., 2013, the examination of serums was carried out with the tests : Rapid plasma reagin (RPR) and Treponema pallidum haemagglutination assay (TPHA)
- our sample includes a large number of 1287 tested samples which affect the accuracy of the conclusions drawn. while in the study conducted by Torba et al., 2013, 336 samples were examined
- our sample spanned a period of 7 years, while the study conducted by Torba et al., 2013 was conducted over a period of one year

- our group includes a subgroup of women who have difficulty conceiving a child, and one of the reasons may be the presence of an STI

Prevalence of infection and age

From the analysis of the data related to the age of the people who participated in the study, the average age of the tested was 28 years old.

Year	2011	2012	2013	2014	2015	2016	2017	Total
Tested	107	139	154	186	203	242	256	1287

Table 3:Number of tested in the years 2011-2017

In **Table 3** shows the ever-increasing number of women tested from one year to the next, indicating that over the years, Albanian women are more sensitized to be tested for STIs.

To study the influence of age on the spread of Syphilis, for each year of the study, the people who participated in the study were divided into three groups:

- Age group I, which includes people from 16-25 years old
- Age group II, which includes people from 26-35 years old
- Age group III, which includes people from 36-45 years old

The age group with the most tests was age group II. Almost half of the people who participated in the study for each year belong to this age group. (**Table 4**).

After dividing by age groups, the number of positive persons in each age group was determined.

The frequency of infection for each age group in total as well as the frequency within the group was then determined.

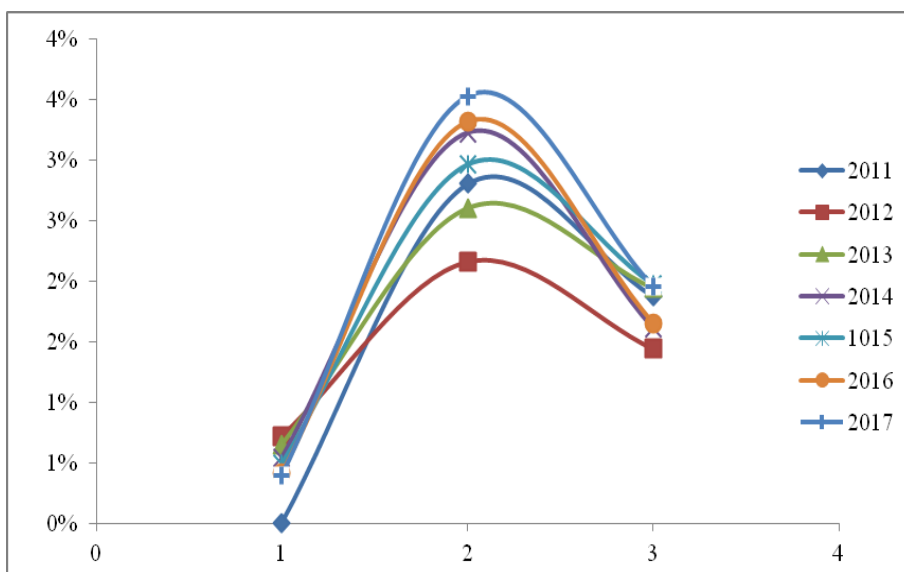
All the data collected for seven years were arranged in a summary table to study the variation of infection for each age group during the time span of the study.

Year		No of testees	Syphilis IgM+ (in no)	Syphilis IgG+ (in no)	Syphilis + (in no) total	% within group	% of the group
2011	Age group I	22	0	0	0	0.00%	0.00%
	Age group II	58	1	2	3	5.17%	2.80%
	Age group III	27	0	2	2	7.41%	1.87%
	total	107	1	4	5		3.67%
2012	Age group I	29	1	0	1	3.45%	0.72%
	Age group II	76	1	3	3	3.95%	2.16%
	Age group III	34	0	2	2	5.88%	1.44%
	total	139	2	5	6		4.32%
2013	Age group I	34	1	0	1	2.94%	0.65%
	Age group II	81	2	3	4	4.94%	2.60%
	Age group III	39	0	3	3	7.69%	1.94%

	total	154	3	6	8		5.19%
2014	Age group I	39	1	1	1	2.56%	0.54%
	Age group II	104	3	4	6	5.77%	3.22%
	Age group III	43	0	3	3	6.98%	1.61%
	total	186	4	8	10		5.37%
2015	Age group I	38	1	1	1	2.63%	0.49%
	Age group II	122	2	4	6	4.92%	2.96%
	Age group III	43	0	4	4	9.30%	1.97%
	total	203	3	9	11		5.42%
2016	Age group I	36	0	1	1	2.78%	0.41%
	Age group II	148	3	6	8	5.41%	3.31%
	Age group III	58	1	3	4	6.90%	1.65%
	total	242	4	10	13		5.37%
2017	Age group I	41	1	1	1	2.43%	0.39%
	Age group II	153	3	6	9	5.88%	3.52%
	Age group III	62	1	4	5	8.06%	1.95%
	total	256	5	11	15		5.86%

Table 4: Immunological situation according to age groups for syphilis

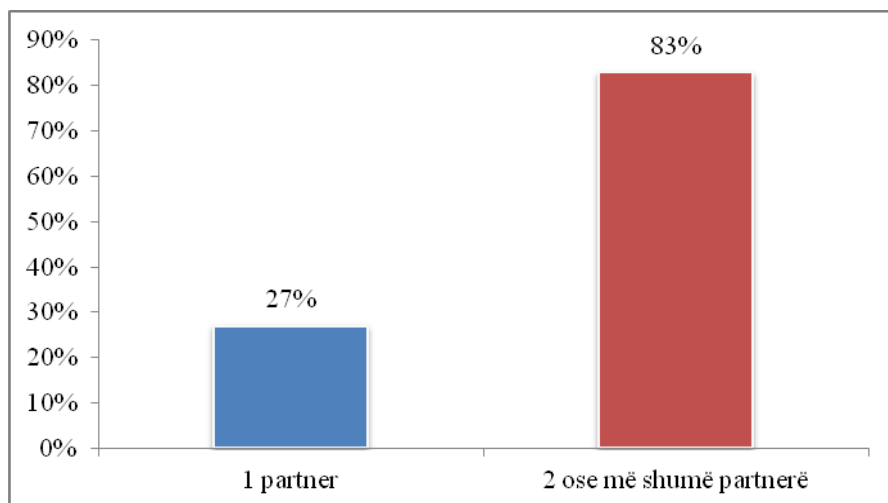
From the graph, it can be seen that the distribution of syphilis is normal, but there is a greater difference between the prevalence of the first and third age groups in favor of the third age group, due to the characteristics of the development of this disease.



Graphic 4: Immunological situation for syphilis according to age groups in the years 2011-2017

Prevalence of infections and number of partners

The question regarding the number of partners in the survey of our study sample was not answered by all respondents due to the nature of the question.



Graphic 5: Number of partners for syphilis positive female

And the results obtained from women who chose to answer the question are not 100% reliable as experience has shown that not everyone answers such questions honestly. 83% of women positive for syphilis infection, who answered the question about the number of partners, declared that they had more than one partner. (**Graphic 5**).

Conclusions

From the above study we reached the following conclusions:

Over the years, Albanian women are more sensitized to be tested for STIs, which is shown by the ever-increasing number of women tested.

The prevalence of Syphilis in the study group is 5.28%.

Anti-TPA IgM antibodies were detected in 1.71% of cases, while anti-TPA IgG antibodies were detected in 4.12% of cases included in our study group. These numbers show that *Treponema Palladium* (Syphilis) infection, which is associated with fetal damage when the infection occurs during pregnancy, or congenital Syphilis exists at low levels, to be assessed in Albanian women of reproductive age.

Syphilis infection shows a slight increase over the years, which also shows the tendency of this infection to increase.

The age group of 26-35 years is the age group that shows a higher level of infections. It is also noted that Syphilis shows a slight tendency to increase in prevalence with increasing age.

The spread of infection increases with the increase in the number of partners.

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