Invited Editorial

How oral sex behaviors can lead to cancer

The sexual revolution in the Western world during much of the 1960s drastically changed behaviors and attitudes towards sexuality. The advent of the birth control pill, the acceptance of non-marital sex, a younger age at first intercourse, and having multiple sexual partners dramatically transformed sexual practices and norms in the last decades[1]. One of the consequences was an upwards trend in the incidence of sexually transmitted diseases (STD), as human immunodeficiency virus (HIV), chlamydia, gonorrhea, and syphilis are efficiently transmitted during sexual contacts. The consequences of the sexual revolution are felt today as more and more individuals engage in vaginal, anal and oral sex, often without protection. More than one million people worldwide acquire a sexually transmitted infection every day[2].

The aforementioned changes in sexual mores also redefined sexual intercourse to include oral sex as a common variation of the act. Long considered safer than genital intercourse, because it avoids unwanted pregnancies, oral sex practices have become more common among adults and adolescents, in both heterosexual and homosexual relationships[3]. According to the U.S. Centers for Disease Control and Prevention, 85% of sexually active adults, and 33% of adolescents reported having oral sex at least once with a partner of the opposite sex[4]. Oral sex is not without danger, since many STDs are caught through unprotected oral sex behaviors. However, of all STDs that can infect mouth and throat, one virus has received major attention by the public and healthcare providers recently, the human papillomavirus (HPV).

Although awareness about risky oral sexual behaviors such as cunnilingus, fellatio and anilingus and their consequences have increased, the population and healthcare providers are still insufficiently educated about oral HPV infection and head-and-neck cancer (HNC) risk. In the general population, between 0.8 and 44.7% of individuals are aware that HPV is a risk factor for oral cancer[5]. For medical and dental professionals, the awareness is between 26 and 88%[5]. Indeed, many health professionals reported that it is difficult to respond to questions and concerns from patients because of their limited knowledge about the role of HPV in oral cancers[6]. There is a need for educational interventions for healthcare providers on how HPV can cause HNCs, because of the compounded stigma that comes from being diagnosed with a dreadful disease and then learning that it was the person's own sexual behavior that led to it. It is not simple for the general population to seek information from credible sources[5].

Historically, HPV was first known for its role in cervical carcinogenesis, and consequently, it has long been perceived as a viral infection of concern mainly to women. HPV is causally associated with several anogenital cancers such as vaginal, vulvar and anal cancers, but nowadays it is clear that HPV infection can also cause a subset of HNCs[7,8]. Oral HPV infection is relatively uncommon in the general population, with a prevalence of 4.5%[9]. In analogy with genital infections, a high proportion of oral HPV infections clear within 6 to 18 months[10], but persistent infections can progress to neoplastic lesions of the head and neck[11,12]. We can differentiate two types of HNCs according to their respective risk factors: those related to smoking and drinking habits, not related to HPV[13], accounting for the majority of HNCs, and the HPV-positive HNCs, mainly oropharyngeal cancers (OPC). The etiological role of HPV in these cancers varies by HNC subsite, but also differs in different parts of the world[14]. Overall, nearly 30% of HNCs are caused by oral HPV infection, mainly with the HPV16 genotype[15,16]. In the last decades, an increase in HPV-positive HNC incidence has been observed in both sexes, with a five-fold higher incidence rate in men[17-20]. Indeed, HPV-positive HNC is particularly common among young white men with high socioeconomic status[21,22]. By 2020, it is expected that HPV-positive OPC will become the most common HPV-related cancer, surpassing cervical cancer[7].

The question of how oral sex can transmit HPV infection and cause mouth or throat cancers has great relevance. The most plausible explanation for HPV transmission to the oral cavity is through orogenital or oroanal contact during oral sex[3]. An increased incidence of oropharyngeal cancer has been reported in many countries, and it is associated with having multiple oral sex partners or oral sex at an earlier age[23]. Oral HPV infection is also associated with higher number of genital sexual partners, younger age at first intercourse, and history of genital warts[24]. Fortunately, oral HPV acquisition rates are low, and infections can clear within a year[25]. Interestingly, differences seem to exist in the gender directionality of transmission. Men acquire oral HPV infection more easily via orogenital contact than women do when practicing oral sex on male genitals[10,26].

Knowing that oral sex behaviors lead to oral HPV infection — which then increases risk of HNCs — is relevant for HNC prevention, since a significant proportion of HPV-positive oral cancers could be prevented by reducing oral infection. The use of condom and dental dam to avoid the direct contact of the mouth with the partner's anogenital sites can lower the chances of getting oral HPV infection. A related question is: does HPV vaccination confer the same protection? Chaturvedi et al.[27] provide new evidence on HPV vaccination and prevention of oral infections. Their study included 2,627 American men and women aged 18 to 33 years enrolled in the National Health and Nutrition Examination Survey. The authors estimated the effects of HPV vaccination on the burden of oral infections. The protective effect of vaccination was striking; the prevalence of oral HPV infection was reduced by 88.2% in vaccinated adults who reported the receipt of one or more dose(s) of the quadrivalent HPV vaccine, targeting HPV 6, 11, 16 and 18, compared to those ones not vaccinated. On the other hand, an important concern raised was the low uptake of the vaccine in the general population (29.2% in women vs. 6.9% in men). The limited knowledge of healthcare professionals and the general population

on the relationship between oral sex practices, oral HPV infection, and risk of OPC are possible explanations for that. These results are the first line of evidence in support of a future reduction on the burden of HPV-positive HNCs. In light of this evidence, HPV vaccination for oral HPV prevention should be seen and promoted as an additional benefit, especially in men[21,22]. Men are generally not targeted for vaccination except for Australia, the United States, and Canada, which adopted this policy in 2012. Unfortunately, even in these countries coverage in men is still low[28].

There are numerous research efforts on the way to learn how we can prevent oral HPV infection, and longitudinal studies are warranted to better understand the natural history of oral HPV infection, the risk factors for oral HPV acquisition and persistence, and the development of HNCs. But one thing is certain: there are fewer options to prevent oral HPV infection and ultimately HPV-positive HNCs than there are for cervical HPV infection and cervical cancer. The latter can also be prevented via screening and treatment, as an established public health practice, an option that unfortunately does not exist for HNCs. Definitely, prevention starts by informing both the general population and healthcare providers about the relationship between oral sex behaviors, oral HPV infection acquisition and transmission, and cancer risks. Meanwhile, promoting safer oral sex practices and HPV vaccination of both sexes could be the only options to prevent oral HPV infections, as well as HPV-related HNC burden in future generations. While healthcare providers deliver these health promotion messages, it also helps to counsel patients about the benefits of smoking cessation and alcohol drinking in moderation.

Conflict of interests

The authors have no conflicts of interest on the topic and contents of this editorial. Dr. Franco has served as occasional consultant or advisory board member to companies involved with HPV diagnostics (Qiagen, Roche, Gen-Probe, BD, Abbott), HPV vaccination (GSK, Merck), and cervical cancer screening or control (3M, Ikonisys, Cytyc). His institution has received unconditional grants from Merck and Roche to supplement publicly funded investigator-initiated studies in his unit. His unit's entire research program has been funded by the Canadian Institutes of Health Research (CIHR), U.S. National Institutes of Health, National Cancer Institute of Canada, Cancer Research Society, Canadian Cancer Society Research Institute, and Fonds de Recherche Quebec-Santé (FRQS). No funding was received by the authors for the writing of this editorial.

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The status of syphilis in pregnant women in Brazil: do we need a national (or world?) day of syphilis combat? Is syphilis a public health problem only in Brazil?

AN OLD PROBLEM

In his verses in the book *Syplilis Sive Morbus Gallicus* (Verona, 1530), in which the word *syphilis* appears for the first time, Hieronymi Fracastorii anticipated that this disease would persist:

> From the purple belly of the night, a slave/ The strangest plague came back to ravage the world/ Infecting the heart of Europe, the plague was thrown/ From Lebanon to the waves of the Black Sea/ When, in war, France marched through Italy/ The disease took its name. I'll dedicate my rhymes to this intruder of twenty pests / That, even if it is not welcome, is eternal since it intends to remain here[1].

Nowadays syphilis continues to plague many countries in the world despite the discovery of penicillin.

In 1905, *Treponema pallidum* subsp. *pallidum* (*T. pallidum*) was identified by Schaudinn and Hoffmann as an etiological agent of syphilis. This motile bacteria, similar to Gram-negative, is environmentally fragile, microaerophilic, with a spiral-shaped structure in a length of 6–20 μm[2].

Syphilis is a chronic and systemic infectious disease that is transmitted sexually or via other types of intimate contact. It may be transmitted from mother to fetus (intrauterine) or through contact between mother and child during birth[3]. Global prevalence for this disease in pregnancy is about 0.5% (0.2 to 1.8%)[4].

The infection can be categorized as recent (primary and secondary), latent (early or late) or late. All stages can occur during pregnancy. Gestational syphilis is estimated in about two million a year, mostly in developing coutries. Less than 10% of these cases are diagnosed[5].

Specifically in Brazil, the Ministry of Health indicates that, in the last five years, the number of syphilis cases has risen in pregnant women, congenital and acquired, partially attributed by the increase of test coverage, the expansion of tests' use , less use of condom, resistance of health professionals to administrate penicillin in primary care and lack of penicillin supply[6]. The underreporting of acquired syphilis cases and inadequate treatment of partners are of particular concern, because they are closely associated with a large occurrence of this disease during pregnancy.

In 2016, 87,593 cases of acquired syphilis, 37,436 cases of syphilis in pregnant women and 20,474 cases of congenital syphilis — among them, 185 deaths — were reported in Brazil. The rate of detection was of 12.4 cases of syphilis in pregnant women per 1,000 live births. Southern and Southeastern states registered the highest rates (16.3 and 14.7 cases, respectively)[6].

The higher rates on Congenital syphilis' rates than on syphilis during pregnancy's show that there is still much to be done for proper diagnosis and treatment during pregnancy. Even worse is that, in 2016, 81.0% of mothers of children with congenital syphilis reported to have prenatal care. Cities like Teresina, Fortaleza, Natal, João Pessoa, Recife, Maceió, Aracaju (Northeast) and Porto Alegre (South) registered more cases of congenital syphilis than syphilis-during pregnancy[6]. This information clearly indicates inadequate prenatal monitoring and lack of diagnosis in pregnant women.

The lack of penicillin in 2016 has caused a major disorder and is partly responsible for the increase in the number of cases. The opening of the Brazilian market and the early signature of trade agreements involving active pharmaceutical inputs in the 1990s showed that Brazilian drug productionwas not competitive at global level. With lower taxes, buying products abroad led to higher profits. Multinational companies operating in Brazil abandoned their production or transferred this stage to branches elsewhere, where the cost of production, including labor ones, was much cheaper. This change devastated Brazilian pharmochemicals' manufacture[7].

To make matters worse, both private and public companies, as well as public health managers, haven't made an adequate plan to maintain strategic stocks of penicillin and other drugswidely used in Brazil.

Similar facts about the lack of penicillin have occurred in other countries. Even the United States, world's largest economy and place of extremely high industrial and scientific development, faces difficulties with the normal use of penicilin. On its website, the Centers for Disease Control and Prevention (CDC) present a roadmap for the rational use of penicillin[8].

However, the high numbers of syphilis cases in Brazil and the lack of planning have made penicillin scarce, especially for its use in pregnant women. It is a true national embarrassment.

Without any clarification for a better understanding, injectable veterinary products with high doses of crystalline penicillin, procaine and benzathine in a single bottle, were never missing in Brazil and did not present any contingencies in 2018.

CDC considers that pregnant women must have access to early prenatal care and be serologically tested for syphilis at their first prenatal visit. In high risk areas, it should be doneonce more at 28–32 weeks pregnancyand at childbirth[9].

The Brazilian Ministry of Health uses three flowcharts aiming at assisting and standardizing immunological diagnosis of syphilis. Two or more combined tests form a flowchart. This combination of sequential testing aims to increase the positive predictive value (PPV) of a reagent result in the initial test. The flowchart in series is logical and cost-effective[10]. Flowchart 1 consists in the conventional approach for syphilis diagnosis by immunological tests, in which a nontreponemal test is used as the first one, followed by a treponemal test to confirm diagnosis. Flowchart 2 embraces unconventional approach for syphilis diagnosis by immunological

tests, in which a treponemal test (Enzyme-Linked Immunosorbent Assay — Elisa, chemiluminescence or other equivalents) is used as the first test, followed by a nontreponemal test to confirm diagnosis. Flowchart 3 encompass unconventional approach for syphilis diagnosis by immunological tests, in which a rapid treponemal test is firstly used, followed by a nontreponemal test for diagnosis confirmation. However, if the nontreponemal test is non-reactive, Flowchart 3 recommends the use of a third treponemal laboratory test.

A therapeutic alternative for primary syphilis, secondary and early latent syphilis (up to one year) is: Penicillin G benzathine, 2.4 million internacional units (IU), intramuscular (IM), single dose (1.2 million IU in each gluteus). As another option, there is: Ceftriaxone 1g, IV or IM, once a day, for eight to ten days for pregnant and non-pregnant women. In later latent syphilis (more than one year of duration) or latent with unknown duration and tertiary: Penicillin G benzathine, 2.4 million IU, IM (1.2 million IU in each gluteus), weekly, for three weeks. Total dose of 7.2 million IU. As alternative: Ceftriaxone 1 g, IV or IM, once a day, for eight to ten days for pregnant and non-pregnant women[11].

Treatment during pregnancy

Adverse pregnancy outcomes are common in women with syphilis[12,13], so the treatment should be provided as soon as possible.

A study carried out in Tanzania found that 25% of women with latent syphilis who had rapid plasm reagin (RPR) titres ≥1:8 gave birth to a stillborn child, and 33% had a preterm birth[14]. A second study (still in Tanzania written by the same researchers) showed that adverse pregnancy outcomes due to syphilis can be prevented with a single dose of benzathine penicillin G before 28 weeks of pregnancy[15]. Treatment with penicillin G benzathine before the 28th week of gestation was one of the most cost-effective interventions available for saved lifetime[16].

However, World Health Organization (WHO) recommends that children with suspected congenital syphilis, including the oneswhose mothers are seropositive for syphilis and not treated with penicillin >30 days before childbirth, should be treated with aqueous benzyl penicillin or procaine penicillin[17]. Beyond that, every child exposed to syphilis, even the ones with no signs or symptoms at birth, should be closely monitored, ideally with non-treponemal test (NTT) titres. Titres should decline by 3 months of life and be nonreactive by 6 months[18]. Treponemal tests (TTs) are not useful in children due to persistent maternal antibodies[18].

Aware of the seriousness of the situation, the Brazilian Ministry of Health created the Strategic Actions Agenda to Reduce Syphilis in Brazil, a collective work with associations of class. Besides, with the objective of emphasizing the importance of adequate diagnosis and treatment for syphilis as a sexually transmitted disease,especially in pregnant women during prenatal care, Brazilian government sanctioned the Federal Law No. 13,430 of March 31, 2017, creating through Law 13,430 / 2017 the National Day for Fighting Against Syphilis[19]. It was a response to a lawsuit commenced by the Brazilian Society of Sexually Transmitted Disease (STD) and the STD Sector of Federal Fluminense University, which in 2004 started a national movement to combat syphilis.

This law establishes that every year, on the third Saturday of October, there must be multiple activities (scientific, educational, dissemination) throughout the country, aiming at acquired and congenital syphilis combat[20].

Considering that syphilis represents an old and continuous problem for public health all over the world, we propose that this Brazilian attitude should be extended to a Global Day for Syphilis Combat. Thus, it is possible to give greater visibility to the serious problems and deaths (abortions, stillbirths and newborn death) caused by this disease.

In the sense that many complications involving syphilis may still appear, we refer to Rekart et al,[21] to the increase in syphilis cases in men who keep sexual relations with other men (MSM) and are submitted to highly active antiretroviral therapy (HAART) for human immunodeficiency virus 1 (HIV-1) infection.

The authours speculate

The prevailing hypothesis is that HAART availability and effectiveness have led to the perception among both individuals who are HIV-1 infected and those who are uninfected that HIV-1 transmission has become much less likely, and the effects of HIV-1 infection less deadly. This is expected to result in increased sexual risk-taking, especially unprotected anal intercourse, leading to more non-HIV-1 STDs, including gonorrhoea, chlamydia and syphilis. However, syphilis incidence has increased more rapidly than other STDs[21]. These authors consider that HAART regulates innate and acquired immune responses to *T. pallidum* and that this biological explanation has an important role in syphilis epidemic.

HIV has evolved with a significant repertoire of mechanisms that target autophagy[22], and who knows if these mechanisms may be connected with the growth of syphilis cases in MSM HIV-1 positive using HAART?

Considering that antiretroviral drugs are increasingly used worldwide in HIV treatment and in pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP) actions, side effects may interfere in epidemics of other STDs.

Syphilis in pregnancy time, although known and well defined, is still a global challenge, especially for developing countries. Intense and well-planned actions must be taken by all agencies involved to reduce the incidence of the disease, otherwise we will continue observing serious complications and death of concepts.

In attention to pregnant women with STDs, it is necessary to get rid of some old habits, like the gynecologist / obstetrician who only attends the woman but doesn't administer treatment during consultation. When prescribing penicillin injections to be taken at another time and not calling patient's sexual partner for first and immediate care, the doctor is deferring the best attention to interrupt transmission chain and prescribe the appropriate treatment.

More than attending a pregnant woman, the doctor and the whole team of health professionals should welcome the pregnant woman, her sexual partner and her family. For only then, it will be possible to break the prejudices (and contagions) that involve STDs, especially syphilis.

To complicate syphilis combat in Brazil, many basic health care services do not apply penicillin in patients. For years, we've had

a standard set by Brazil's Federal Nursing Council that indicated that penicillin should only be applied to medical services that had cardiopulmonary restraint stalls in order to revert a possible anaphylactic shock.

This standard was discared by the Ministry of Health, but, unfortunately, in our everyday life, we have not yet achieved the objective of applying penicillin in all Brazilian basic health units.

Another serious problem is the delay with serological tests results. Often, Venereal Disease Research Laboratory (VDRL) result takes more than 30/40 days.

As many women arrive at their first consultation in the second trimester of pregnancy, the delay to perform exams and result's awareness cause the treatment to be performed near childbirth. The later syphilis treatment is to birth, the higher the chances of congenital syphilisor another serious outcomes.

In 2012, data from WHO estimated the existence of 17.7 million people with syphilis, with 5,590,000 cases of syphilis every year. The numbers by region are: 440,000 in Europe; 496,000 in Eastern Mediterranean; 886,000 in South-Eastern Asia; 937,000 in America; 993,000 in Western Pacificand 1,843,000 in Africa[4].

In many countries, especially in low-income ones, the mother-to-child transmission (MTCT) remains very high and it is a common cause of death[23,23].

Syphilis, after malaria, is the most frequent etiology of avoidable stillbirth in the world[25].

Working with data from the Brazilian Information System for Notifiable Diseases (SINAN), available at the Health Portal of Brazilian Ministry of Health (http://portalsinan.saude.gov.br/ sifilis-em-gestante and http: // portalsinan. saude.gov.br/sifilis-congenita), Saraceni et al.[26] performed a detailed analysis of reports of syphilis in pregnant women and congenital syphilis in six federative units (states) in Brazil. The authors summarize the main results:

> The rate of detection of syphilis in pregnant women grew between 21% (Amazonas) and 75% (Rio de Janeiro). The incidence of congenital syphilis followed the same increase profile, varying from 35.6% in the Federal District to 639.9% in Rio Grande do Sul, with a 0.7% reduction in Amazonas. The performance of prenatal care in women with congenital syphilis outcome ranged from 67.3% in Amazonas to 83.3% in the Federal District. Of the pregnant women with syphilis, 43% had a reported outcome of congenital syphilis. In pregnant women with syphilis and outcome of congenital syphilis, maternal diagnosis occurred during prenatal care in 74% and delivery in 18%. In 8% of the women the diagnosis was ignored.

They also say that these rates may have been led by increased report of cases[26].

Milanez[27], reflecting on the Brazilian capacity to face this problem, says that recent data on syphilis during pregnancy and congenital syphilis in Brazil are alarming. A significant proportion of women and newborns are infected. There was an increase of 1,047% between 2005 and 2013 among pregnant women. During the same period, increase of 135% in congenital syphilis notifications was observed. These numbers might indicate an improvement on notification, which has happened in fact. Nevertheless, it is not enough to explain the number of cases in pregnant women and newborns[27].

The same situation happens in other countries of Latin America and the Caribbean, and it is a must to improve the capacity of countries to collect high-quality data about interventions and inequalities, and to use this data as a basis for a set of decisions to improve pregnant women and children's care[28].

As most of Brazilian mentioned publications work with information from the database of compulsory notification sheets, we believe that it is necessary to cite authors who have worked with syphilis problems in pregnant women and congenital syphilis in their local medical services. The data of these surveys reveal that reality can be much more serious and worrying than the official statistics show. We will present some recent studies from several Brazilian regions.

In a retrospective, prospective, comparative, cross-sectional and observational study of congenital syphilis (CS), the occurrence of cases was observed in two different periods with 512 puerperal women in each one (2006 and 2011), with a total of 1,024 puerperal women from four maternity hospitals in the city of Campo Grande (Mato Grosso do Sul — center-west of Brazil). Figueiró-Filho et al.[29] reported that the prevalence of CS in the first period (2006) was of 2.3% and in the second (2011) 0.58%. On the other hand, the authors observed a significant association between the periods studied and an increase in the frequency of infectious and STDs, from 3.5% (2006) to 10.1% (2011). CS coefficient found for 2006 was 23.4 cases per thousand live births and 5.85 for 2011, which indicates a decrease in the second period. However, it is still ten times higher than the 0.5 rate required for CS elimination.

Cerqueira et al.[30], in a cross-sectional study, pointed out that the prevalence of syphilis in pregnant women was 4.1% in 2012, 3.1% in 2013 and 5% in 2014, with official reporting of 15.6, 25.0 and 48.1%, respectively. This data was collected from 2,041 parturients who had undergone treatment between 2012 and 2014 in the maternity of Pedro Ernesto Hospital of the State University of Rio de Janeiro, in the metropolitan area of Rio de Janeiro. CS incidence was 22/1,000 live births in 2012; 17 in 2013 and 44.8 in 2014. CS underreporting during this period was 6.7%. Vertical transmission occurred in 65.8% of infected mothers' children. It was concluded that in 34.6% of CS cases maternal VDRL titers were of 1/4. Vertical transmission happened in two consecutive pregnancies, one of which being within the low titers group (=1/4). The results also demonstrate the magnitude of the disease, the fragility of the reporting system in assessing its actual prevalence and itsimpact on perinatal outcomes. Moreover, it is a warning about the real situation of syphilis, still underestimated in the state[30].

Holzmann et al.[31] reported, based on data from 107 cases of congenital syphilis in a municipality of the state of Minas Gerais, Brazil, in the period of 2014 and 2015, that 93.5% of these mothers underwent prenatal care, 13.2% of newborns had a diagnosis of neurosyphilis, 23.6% of newborns used drugs other than penicillin, 11.7% of newborns were not referred to outpatient support, only 24.3% were notified, and in 52,3% of all cases there was an inadequate management of newborns, according to the standards recommended by the Brazilian Ministry of Health[31].

Regarding serologies coverage for HIV and syphilis in pregnant womenfrom the private medical service in the state of Rio Grande

do Sul, the percentage of HIV tests performed in childbirth in 2014 was 83.4%, and in 2016 95.8%. In 2014, syphilis exams' coverage was 29%, reaching 82% in 2016. In abortion cases, coverage in 2014 was 55.3% for HIV tests and 86,5% in 2016. In reference to syphilis' examination in cases of abortions, in 2014 24.2% of the cases were examined; and in 2016 this number grew to 73.8%

It is clear that even in private institutions the attention to syphilis is far from ideal. What's more, serology coverage for HIV is always higher than it is for syphilis[32].

Campos et al.[33] analyzed 69 compulsory notification sheets (CNS) in Foz do Iguaçu. From them, nine (13.04%) pregnant women did not perform prenatal care and two (2.90%) were ignored.

Regarding diagnosis of syphilis in pregnant woman, 49 (71.01%) were during prenatal care, nine (13.04%) at the time of childbirth, five (7.25%) after childbirthand three (4.35%) were ignored.Only 10 (14.49%) of all pregnant women were adequate to the treatment.

In reference to newborns with CS, 21 (31.34%) did not undergo any treatment, 21 (31.34%) were ignored, 16 (23.18%) were treated with benzathine penicillin, remaining nine (13, 04%) with another scheme, and two (2.90%) without registration[33].

A descriptive study was carried out using data from confirmed cases of gestational syphilis and CS in Bahia state, Brazil, from 2007 to 2015. This information was obtained from the national database of Brazilian Public Health System (Departamento de Informática do Sistema Único de Saúde — DATASUS). With respect to children with CS, only 6.9% (204/2,948) presented register of an appropriate maternal treatment[34].

With the objective of identifying if Brazilian municipalities (5,300) administered penicillin in 100% of pregnant women with prenatal syphilis, Gonçalves et al.[35] verified that only 52% of the basic health units matched this criteria. This data was obtained from the Program for Improvement and Quality of Life (PMAQ), in order to elaborate a document of good practices concerning these practices, as well as improving the quality of CS' treatment. This result indicates an enormous failure in all prenatal care. About this experience, Brazilian Ministry of Health prepared a document entitled *Good practices booklet: the use of penicillin in basic care to the prevention of congenital syphilis in Brazil*, with the purpose of improving the effectiveness of patients' care , especially pregnant women, with syphilis[35].

This effort makes clear the size of the problem in order to face the effective treatment of syphilis in our country. Detecting who does not apply penicillin in pregnant women at basic health units is essential. Preparing a document for good practices is a great step forward. But to rightly deal with the situation, there are no simple or fast measures. However, it is good that we have already started the job.

For many people, there is still a myth that STDs affect only those from high-risk behavioral groups and / or people of low socio-economic and cultural backgrounds. STDs actually affectthose who have sexual activity and at some point maintain unprotected relationships with an infected person.

In most of the time we use public services' data.

This year we started a project to study the seasonality of anti-HIV and syphilis tests in one of the most traditional supplementary / private clinical laboratory in Rio de Janeiro, which attends, in total, people of high socioeconomic and financial level. Class A, even.

Initial data has high rates of serological reactant results and are surprising, especially for syphilis.

Data from the Richet Laboratory show that in the last years the percentages of positive anti-HIV tests are decreasing: 2.36% (186/7,885) in 2013; 2.04% (199/9,763) in 2014; 1.64% (203/12,397) in 2015; 1.84% (230/12,490) in 2016; and 1.82% (320/17,621) in 2017.

However, syphilis rates show stability: 1.71% (273/15,967) in 2013; 1.56% (310/19,860) in 2014; 1.67% (378/22,679) in 2015; 1.24% (278/24,449) in 2016; and 1.48% (364/42,562) in 2017.

The question is: how many countries in the world have these high syphilis rates in their public and private services?

In Brazil, about 30% of the population does not seek public services in their routine because they have assistance to supplementary medicine (private health plans). In Rio de Janeiro, this share is 40% higher. It is possible to say that the absolute majority of cases of syphilis treated in this segment of health care is not notified to surveillance services of their municipalities. Thus, the numbers of syphilis in Brazil are way different from those ones presented in official data. While we see that, every day we are improving our data on compulsory notifications of infectious diseases.

VACCINES AGAINST SYPHILIS

Genomic sequencing of *T. pallidum* collected directly from samples of recent clinical lesions of syphilis (primary and secondary) is already possible and may overcome the limitation of *T. pallidum* culture and determinants of its virulence[36,37].

Such action can help in developing possible vaccines. Recently, Lithgow et al.[38] showed that immunization of rabbits with lipoprotein TP0751 prevented the spread of *T. pallidum* and thus became a promising vaccine candidate[38].

REFLECTING

Our intention with this article was not to just talk about traditional scientific issues.

What illness has a five-century book that describes it[1]? Have you had an etiologic and serological diagnosis for over 100 years[2]? Have you had a safe and highly effective treatment for more than 70 years[39,40]? Have you studied the natural history of the disease in human experiments for more than 70 years and then a president apologized for these studies on behalf of the nation[40]? Do you make reservations for the lay public based on real facts transformed into a movie, watched until today and serving as material for medical ethics classes around the world[41,42]?

We need to get out of the simplistic lines of safe sex, condom distribution during Carnival, rapid tests in public squares, but we should encourage of open and unprejudiced dialogue, seeking reflection in high school and university institutions several times over the years. It is necessary to have frequency and a sequence of educative actions. Simple tactics have not been working for decades, at least in our country.

Simply say that: every pregnant woman should be tested for syphilis at her first visit at a doctor; her sexual partners should be examined, tested and treated (when necessary) in conjunction with the woman; the administered drug must be penicillin;

if there is a history of allergy to penicillin, the hypersensitivity test should be performed and, if confirmed, desensitization should be done immediately, and, if there are no conditions for these actions to occur, a penicillin dose should be applied in a safe environment. These discourses should get into the daily routine of medical attention.

The Brazilian example of the HPV-vaccine application of the with 100% coverage on adolescents in schools is a milestone.

The coverage of the second and third HPV doses fell by half when schools decline to participate — to the point of the expressive quantity of expired vaccines on refrigerators' shelves in vaccine rooms throughout Brazil, without being applied in Brazilian adolescents.

We hope that more public health managers, educators, representatives of civil organizations, people from the general population, directors from different media sectors, research groups, as well as more companies, invest especially in the awareness of responsibilities with human relationships so that the words of Fracastorii, in 1530, at the beginning of this article, are considered as only an outdated historical verse.

As for Brazil and many other countries in the world to be aware that health education process is based on schools andnot in isolated advertising. To the title of this article we respond yes.

When we were finishing the review of this editorial, we found the article by Takahashi et al reporting the rapid increase in cases of syphilis, including congenital syphilis, in Japan: "Among women, 20- to 24-year-olds consistently had the highest reporting rate, reaching 9.0 per 100,000 in 2016. Although Tokyo prefecture had the highest reporting rate (3.98 per 100,000 person-years)"[43].

And they end up saying that, currently, syphilis is a major public health problem in Japan[43].

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Follow-up of a cohort exposed to vertical transmission of syphilis in Campos dos Goytacazes (RJ), 2016

Seguimento de uma coorte exposta à transmissão vertical da sífilis em Campos dos Goytacazes, Rio de Janeiro, 2016

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ABSTRACT

Introduction: Congenital syphilis (CS) occurs at any time during gestation if the pregnant women were not treated or were incorrectly treated during pregnancy. CS can be avoided with adequate prenatal diagnosis and treatment. Benzathine penicillin is the preferred drug. Objective: To evaluate diagnosis and management of congenital syphilis during pregnancy and the correct management of children exposed in a public hospital at Campos dos Goytacazes, RJ. **Methods:** Retrospective cross-sectional study. Data were obtained from medical records of infants born to mothers with syphilis diagnosed during pregnancy or delivery and followed at Pediatric Infectious Diseases Ambulatory during 2016. **Results:** 84 mother-child binomials were followed-up. The prevalence of syphilis diagnosis was higher in the third trimester of pregnancy 21.40% (18/84) and at delivery, 31% (26/84). About 77.40% of women do not receive adequate therapy during pregnancy. Moreover in 13 cases, investigation was concluded with 2 of them confirmed as CS. **Conclusion:** Late beginning or not attendance to prenatal consults affected management of syphilis at pregnancy; investigation of newborns was inadequate in many cases and treatment demanded alternative drugs in absence of penicillin G.

Keywords: congenital syphilis; newborn; drug therapy; pregnant woman; syphilis serodiagnosis.

RESUMO

Introdução: A sífilis congênita consiste na infecção do feto pelo *Treponema pallidum*, em gestantes não tratadas ou inadequadamente tratadas. A sífilis congênita pode ser evitada por meio do diagnóstico e do tratamento adequado no pré-natal. A penicilina benzatina é a droga de eleição. **Objetivo:** Avaliar o diagnóstico e o tratamento para sífilis realizado na gestação, bem como a abordagem dos lactentes expostos à sífilis congênita em um hospital público de Campos dos Goytacazes, Rio de Janeiro. **Métodos:** Estudo transversal, retrospectivo. Dados oriundos dos prontuários dos lactentes, cujas mães foram diagnosticadas com sífilis durante a gestação ou parto e acompanhados em ambulatório de infectologia pediátrica em 2016. **Resultados:** Foram acompanhados no ambulatório 84 binômios. O diagnóstico de sífilis nas gestantes foi mais prevalente no terceiro trimestre de gestação, 21,40% (18/84), e no momento do parto, 31% (26/84). Em 77,60% (65/84) dos casos não foi realizado o tratamento adequado durante a gestação. Por fim, em 13 casos a investigação foi concluída, sendo dois confirmados como sífilis congênita. **Conclusão:** O início tardio ou a não realização do pré-natal afetaram a abordagem da sífilis na gestação; a investigação dos recém-natos foi incorreta em muitos casos e o tratamento exigiu o uso de drogas alternativas, na falta da penicilina cristalina.

Palavras-chave: sífilis congênita; recém-nascido; tratamento farmacológico; gestante; sorodiagnóstico da sífilis.

INTRODUCTION

Syphilis is a sexually transmitted disease caused by *Treponema pallidum*. Congenital syphilis (CS) is a fetus infection during any gestational time and occurs when the pregnant woman does not receive treatment or has received an inadequate treatment[1]. Its transmission rate is about 100% in primary or secondary phases and decays to 30% in later phase[2].

In Brazil, notified cases of CS have increased in the latest years. Between 2005–2010, 39,789 cases of syphilis in pregnancy and 36,000 cases of CS were notified predominantly in capitals of the Northeast region and São Paulo and Rio de Janeiro States[3]. In 2015, 33,381 cases of CS were notified in the country, with an incidence rate of 11.2 cases per 1,000 newborns[4]. In the period from 2011 to 2016, 129,757 cases of syphilis in pregnancy and 79,670 cases of CS were related, suggesting improvement in epidemiological vigilance, increase in diagnosis and/or more cases[5].

The notifications turn on compulsory for SC since the document 542/1986 of the Health Ministry and of gestational syphilis starting in 2005. The notifications are yet bellow the estimate rates for the year, reflecting the difficulties of case diagnosis and notification[6].

The consequences for the fetus depend on maternal treponemal load at the moment of infection. Beyond the risk of mortality, prematurity, low weight at birth and acute complications, CS also is the cause of deformities, neurologic lesions, and other typical sequelae of this disease[7].

The diagnosis of gestational and congenital syphilis uses non-treponemal testing, once that it is widely available at Brazilian labs, inexpensive and with height of titers tending to correlate with disease activity. This test can get a false positive error and for this reason, treponemal tests are recommended for confirmation by a different format with a specific anti-treponemal antibody[8].

The CS is an infectious disease preventable by gestational diagnosis and suitable treatment. The Brazilian Health Ministry required screening of Venereal Disease Research Laboratory (VDRL) in all pregnant women as early as possible and a second screening in the

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third trimester of pregnancy[9]. In cases of the fault of serologic vigilance or high risk of transmission, maternal serology must be performed during delivery[1]. It is important to assure the quality of public basic and women health service[2].

After maternal syphilis diagnosis, all newborn has to be clinically investigated to CS. In patients with no documented treatment in pregnancy, inadequate maternal treatment or in children with positive VDRL are indicated: long bone radiographical examination, blood count and lumbar puncture for cell count, protein and VDRL. Hematological tests are necessary to detect anemia, thrombocytopenia, leukocytosis, and leukopenia[9].

Penicillin is the only agent that is appropriate for use during pregnancy because of its excellent placental transfer and higher efficiency of reducing perinatal morbidity and mortality. Azithromycin is an alternative, but treatment failures and absence of effect in fetus have been reported[6].

The correct follow-up of this problem during pregnancy has the potential to reduce the incidence of CS to less than 0.5/1,000 of live chilbirth[9].

OBJECTIVE

This study aims to evaluate diagnosis and management of maternal syphilis during pregnancy and the adequate management of children exposed in a public hospital at Campos dos Goytacazes, Rio de Janeiro State.

METHODS

A retrospective cross-sectional study was conducted from January to December, 2016. Data on neonates and infants exposed to syphilis were obtained from medical records and prenatal cards at Plantadores de Cana Hospital, Campos dos Goytacazes, Rio de Janeiro. The analyzed variables included were: gestational data and clinical and laboratorial findings from infants, their treatment and diagnostic of CS. Researchers used the Algorithm of Brazilian Ministry of Health to follow-up CS as the main reference document[5].

This study was carried out by the recommendations of the Brazilian National Ethics Committee (CONEP). The protocol was approved by CONEP (national approval registry CAAE no. 64905917.0.0000.5244).

Statistical analyses were performed using Epidada 3.1 software[10].

RESULTS

In 2016, 84 binomials were followed-up at the Pediatric and Infectious Diseases Ambulatory of a public hospital (**Table 1**). All mothers were diagnosed with syphilis in pregnancy or at delivery. CS cases were more prevalent in later diagnosis of the maternal disease (52.40%; 44/84), in the third trimester of pregnancy (21.40%; 18/84) and at delivery (31%; 26/84). There were no fetus disease at the time of maternal diagnostic in the first and second trimester of pregnancy.

Inadequate maternal treatment until delivery time was present in two-thirds of cases (77.4%; 65/84). No treatment of partner (34.53%; 29/84), it was a current recommendation, and maternal treatment only at delivery (33.34%; 28/84) were the most prevalent situations.

Diagnosis and management of the exposed infants were inadequate in 83.3% (70/84) of cases. The lumbar puncture was performed in 58.35% (49/84) patients whereas the blood count test was performed in only 26.22% (22/84).

When we evaluate the neonate treatment, only 59.5% (50/84) of the cases were correctly treated. Of them, 34 newborns do not receive indicated treatment by the Brazilian Health Ministry. Among the cases of this inadequacy, we found the use of others antibiotics, like a combination of gentamicin and ampicillin. It is important to mention that aqueous penicillin G was not disposable in our country during the period of study.

All children were guided to follow-up at Infectious Diseases Ambulatory. In the second medical appointment day, two-thirds (76.2%; 64/84) of cases were present. Only 13 cases had a complete diagnostic evaluation, with a treponemal test after 18 months of age. Of them, two were diagnosed with CS (15,3%). These cases were from 2015 and they illustrate the difficulty in getting return at 18 months for treponemal testing and conclusion of investigation.

DISCUSSION

Brazil is engaged with CS elimination, although the actual statistic data is highly elevated and far from this scenery. The Panamerican Health Organization aims CS rates below 0.5 case to 1,000 live childbirth, the same objective of the Brazilian Ministry of Health[11].

In 2015, 33,365 cases of gestational syphilis were notified in Brazil (11.2 cases of syphilis in pregnant women/1,000 live childbirth), with 14,959 (44,8%) were living in the southeast region. Other dangerous data is 32.8% of diagnosed pregnant women with positive VDRL in the third trimester of pregnancy[3]. Our study showed the most of diagnosis at delivery time 31% (26/84), followed by diagnostics at the last trimester of pregnancy (21.4%; 18/84) and the first trimester (20.2%; 17/84). These numbers confirm the precariousness of prenatal public service, the difficulty of women in accessing this service and poor adhesion of pregnant women to prenatal follow-up.

Our study showed complete diagnostic evaluation in 13 CS cases with two confirmed infants with CS diagnosis in context of later access to prenatal public service. Early beginning of prenatal care constitutes a protector factor for CS.

In Brazil, CS demonstrated decreasing rates, when we compare 2015 with 6.5 cases per 1,000 live childbirth to 2016, with an incidence rate of 2.0 cases per 1,000 live childbirth. In the state of Rio the Janeiro, the incidence rate in 2015 was higher than the national rate (12.4 cases per 1,000 live childbirth)[3]. This data can be a result of the increase of cases in the city of Rio de Janeiro related to local problems in Public Health or to more effective epidemiological vigilance.

A study at Rondônia, in the city of Porto Velho, showed prevalence of syphilis diagnosis during the pregnancy of 60.1% individuals (119 cases), followed for 28.28% (56 cases) at the delivery[12]. These results are similar to our study's with 69% prevalence of syphilis diagnosis during pregnancy and 31% at delivery. They still invest in the awareness of these pregnant women with specific programs dedicated to them. In Brazil, the most prevalent states with syphilis diagnosis at the time of delivery are Amazonas (59.7%) and Ceará (53.5%)[13], because of their precarious prenatal and basic public service.

In the same study in Porto Velho City, the prevalence of absence of VDRL testing in cerebrospinal fluid (CSF) was 79.29% (157 cases), and the long bone radiographical exam was not performed in 28.79% (57 cases)[12]. The prevalence of testing is heterogeneous in the states of our country. CSF VDRL testing was performed in 5.4% of cases in Amazonas State and in 62.6% in Rio Grande do Sul State. The long bone radiographical testing fluctuated from 19.8% in Amazonas State to 64.5% in Distrito Federal[13]. In this study, the main fault at the approach of neonates was a blood count test, with 73.8% of cases not tested. This comparison confirms that the lack of resources is not the main reason that makes the adequately approach impossible. The absence of information and clarification on the pediatrics team also significantly interferes with its success[12]. Our work is based on the documented case of the newborn and it can be biased due to the possibility of absence of some data in medical records.

The CS has diverse incidence along the countries. The incidence of this disease can be increased in cases of absence or inadequate treatment of pregnant women and their partners (21.4 cases per 100,000 live childbirth)[14]. Our study showed that 77.6% of pregnant women diagnosed with syphilis are inadequately treated. Of them, 34.53% of the partners were not treated, with possibility of reinfection of pregnant women at later stage of pregnancy. Partners must recognize the importance of their treatment and actions have to take place in order to reinforce them. Unfortunately, in 2017 for following recommendations of the Pan-American Organization of Health and World Health Organization, the treatment of sexual partner of the mother was excluded from the definition of CS without considering the relevance of this fact in the reinfection of mothers and in cases of CS[5].

Maternal treatment is important to ensure the prevention of CS. When we analyzed the inappropriate maternal treatment in pregnancy, the highest rates are from the states of Rio Grande do Sul and Amazonas, with 44.7 and 79.8% respectively[13]. Our data showed 77.4% of inappropriate maternal treatment in pregnancy in Campos

Table 1 – Characterization of binomials with congenital syphilis.

Variables	The frequency of variable		The frequency of congenital syphilis confirmed		The frequency of congenital syphilis excluded	
	n	%	n	%	n	%
Gestational age at diagnosis						
The first trimester	17	20.20	0	0	5	38.46
The second trimester	23	27.40	0	0	2	15.48
The third trimester	18	21.40	1	7.69	1	7.69
In delivery time	26	31.00	1	7.69	3	23.07
Treatment of a pregnant woman						
Adequately	19	22.60	1	7.69	2	15.38
Inadequately	65	77.60	1	7.69	9	69.23
Untreated partner	29	34.53	1	7.69	6	46.15
Treatment a month before delivery	1	1.19	0	0	0	0
Antibiotic non-Penicilin	2	2.38	0	0	0	0
inadequately dosage	10	11.90	0	0	0	0
Maternal treatment at delivery	28	33.34	1	7.69	3	23.07
The approach of the neonate						
Adequately	14	16.70	0	0	1	7.69
Inadequately	70	83.30	2	15.38	10	76.92
Do not performed VDRL test	12	14.28	0	0	1	7.69
Do not performed long bone radiographical	45	53.55	2	15.38	7	53.84
Do not performed lumbar puncture test	35	41.65	0	0	5	38.46
Do not performed blood count exam	62	73.78	2	15.38	9	69.23
Treatment of the newborn						
Adequately	50	59.50	1	7.69	6	46.15
Inadequately	34	40.50	1	7.69	5	38.46
Follow-up of the newborn						
Yes	64	76.20	2	15.38	11	84.61
No	20	23.80	0	0	0	0
Complete diagnostic evaluation						
Yes	13	15.47	2	15.38	11	84.61
No	71	84.53	0	0	0	0

VDRL: Venereal Disease Research Laboratory.

dos Goytacazes City, with 33.34% treated only at delivery. The latter may result from the absence of search for treatment or misinformation.

The increased CS incidence in Brazil can also result from the improvement of investigation and notification of cases, in addition to the major quality in the routine screening of pregnant women. However, the universal screening in pregnant women in Brazil is still not a reality. Non-attendance of penicillin feedstock in 2014 can explain the double incidence rate of CS in the years later. Penicillin stock drastically decreased, and many Brazilian states do not have the antibiotics for Syphilis treatment. Moreover, the professionals in health services need continuous updating on the diagnosis and treatment of syphilis[12].

In this study, 76.2% of neonates were followed-up in the medical care in 2016. The pediatric ambulatory's professionals obeyed recommendations of guidelines from the Brazilian Ministry of Health for follow-up of these infants and, during consultations, they reinforced the importance of following-up mothers and partners in specialized services.

In a Bogotá study, in 2016, 29 children diagnosed with CS were followed-up. Only children born of VDRL and Fluorescent Treponemal Antibody Absorption Test (FTA-ABS) positive pregnant women were included in this study. On the other hand of our study, 46.4% of Colombian babies were completely asymptomatic at birth and the persistence of treponemal antibody rates after 18 months of age will probably be higher than the findings in our report whose mothers were only tested by VDRL preventing exclusion of false positive results[1,6].

In 2016, we had 13 complete diagnostic evaluations, with 2 cases of confirmed congenital infection (born in 2015). The Brazilian protocol advocates, after two negative VDRL in treated infants, that they have to return to treponemal test (FTA-ABS) after 18 months and conclusion of investigation. Loss of infants during follow-up was high with implications for vigilance of CS.

We believe that the multidisciplinary team support for the rescue of defaulters would be fundamental. However, the Brazilian Unified Health System has limitations and epidemiological surveillance still prevents the conclusion of these cases.

CONCLUSION

Our data from Campos dos Goytacazes City ilustrate gaps in the diagnosis and treatment of syphilis during pregnancy and in the investigation and treatment of newborns that must be considered to reversion of alarming numbers of congenital syphilis in our state.

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Conflict of interests

The authors declare no conflict of interests.

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Prevalence of positivity in rapid test for syphilis in campaign in Northeastern Brazil

Prevalência de positividade em teste rápido para sífilis em campanha no Nordeste do Brasil

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ABSTRACT

Introduction: Syphilis is a systemic infectious disease caused by the *Treponema pallidum* bacterium of sexual transmission predominance. The cases diagnosed in Brazil increase each year, and the prevalence of syphilis during pregnancy is approximately 1%, but the reported number is lower than the expected, indicating difficulties in the diagnosis. Campaigns for the detection of syphilis are one of the strategies to enable early diagnosis and timely treatment. **Objective:** To survey the prevalence of rapid test reagent for syphilis and infection risk factors. **Methods:** Epidemiological, descriptive, transversal research. Collection was made in 2016's Carnival in the Piauí State capital, Teresina. Population was composed of individuals who agreed to voluntarily participate through express consent (n=532), 57% women and 43% men. The testing was carried out by trained professionals, initiated by the pre-test counseling through individual interview and guarantee of confidentiality of the information. Then, the rapid test was performed by blood puncture from the digital pulp. Finally, the result was passed through post-test counseling, guidance on prevention and referral of positive cases for further investigation of the infection. **Results:** The prevalence of rapid test reagents for syphilis was 5.4%. A predominance of males (65.5%), browns (45%), age group 29–39 years (38%), and singles (65.5%) was observed, 8 to 11 years of school education (34.4%), 52% were tested for the first time. Regarding sexual practice, 76% reported having one to five sexual partners in the last year, and only 24.1% reported using condoms with casual partners, and 55.2% affirmed no use of condoms with fixed partners. The use of alcohol and/or other drugs prior to intercourse was reported by 86.2%. **Conclusion:** The prevalence found justifies the need to intensify actions that increase access to diagnosis and timely treatment for the infection control. The study shows the importance of educational campaigns and cases detection due to the good adherence of the participants, and points out that strategies should be adopted to make the population aware of the increase in cases and the ways to prevent the disease.

Keywords: syphilis; prevalence; epidemiology.

RESUMO

Introdução: A sífilis é uma doença infecciosa sistêmica causada pela bactéria *Treponema pallidum*, de transmissão predominantemente sexual. Os casos diagnosticados no Brasil aumentam a cada ano, e a prevalência da sífilis na gestação é de aproximadamente 1%, sendo o número notificado inferior ao esperado, indicando dificuldades no diagnóstico. Campanhas para detecção da sífilis é uma das estratégias para possibilitar o diagnóstico precoce e o tratamento oportuno. **Objetivo:** Levantar a prevalência de teste rápido reagente para sífilis e os fatores de risco à infecção. **Métodos:** Pesquisa epidemiológica, descritiva, transversal. A coleta foi realizada no carnaval de 2016, na capital piauiense, Teresina. A população foi composta dos que aceitaram participar voluntariamente com consentimento expresso (n=532), sendo 57% mulheres e 43% homens. A testagem foi realizada por profissionais capacitados e iniciou-se pelo aconselhamento pré-teste por meio de entrevista individual e garantia de sigilo das informações. Em seguida, fez-se o teste rápido mediante punção de sangue da polpa digital. Ao final, o resultado foi repassado por aconselhamento pós-teste, com orientações sobre prevenção e encaminhamento dos casos positivos para continuação da investigação da infecção. **Resultados:** A prevalência de testes rápidos reagentes para sífilis foi de 5,4%. Observou-se predominância no sexo masculino (65,5%), de pardos (45%), idade entre 29 e 39 anos (38%), solteiros (65,5%), com escolaridade entre 8 e 11 anos de estudo (34,4%). Do total, 52% realizava o teste pela primeira vez. Quanto à prática sexual, 76% afirmaram ter tido de um a cinco parceiros sexuais no último ano, apenas 24,1% relataram usar preservativos com parceiros eventuais, e 55,2% contaram não usar camisinha com parceiros fixos. O uso de álcool e/ou outras drogas previamente às relações sexuais foi relatado por 86,2%. **Conclusão:** A prevalência encontrada justifica a necessidade de se intensificar ações que possibilitem a ampliação do acesso ao diagnóstico e o tratamento oportuno para controle da infecção. O estudo permite deduzir a importância de campanhas educativas e de detecção de casos em virtude da boa adesão dos participantes, além de apontar que devem ser adotadas estratégias para tornar mais evidente à população o aumento dos casos e formas de prevenção da doença.

Palavras-chave: sífilis; prevalência; epidemiologia.

INTRODUCTION

Syphilis is a systemic infectious disease caused by the *Treponema pallidum* bacterium of sexual transmission predominance. Relevant factors of syphilis transmission may be related to social, biological, behavioral and cultural aspects that influence the occurrence of the disease in the population[1].

The World Health Organization (WHO) estimates that syphilis in pregnancy leads to more than 300,000 fetal and neonatal deaths per year worldwide, and adds 215,000 children to increased risk of premature death. The incidence of congenital syphilis estimated for Brazil was 3.51 per thousand live births, ranging from 1.35 per thousand in the Midwest to 4.03 per 1,000 in the Northeastern region. The number of diagnosed acquired syphilis cases in the country grows every year. In 2015, 65,878 acquired syphilis cases were reported, with the detection rate of 42.7 cases/100,000 inhabitants. It was observed that 32.8% of pregnant women with syphilis were diagnosed in the third quarter of pregnancy. The prevalence of syphilis in pregnancy is approximately 1%, but the notified number is lower than the expected, indicating difficulties of diagnosis[2].

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Campaigns for the detection of syphilis are one of the strategies to enable early diagnosis and timely treatment. Testing for syphilis is recognized as a cost-effective measure[3,4]. The use of tests with immediate result has been indicated in places where access to laboratory tests is infrequent. The rapid treponemal tests (RT) show sensitivity and specificity similar to the treponemal tests performed in the laboratory. Its sensitivity and specificity are comparable to those of non-treponemal tests[5].

OBJECTIVES

Estimate the prevalence of positive serology for syphilis in treponemal test on active search campaign titled Fique Sabendo, in the city of Teresina, capital of Piauí State, Brazil; know the risk behavior of acquiring sexually transmitted diseases (STD); and verify the feasibility of RT for syphilis in campaigns in the population of the mentioned capital of Piauí State.

METHODS

This is a transverse study in non-probability sample of the population above 18 years of age, both sexes, from the syphilis active search campaign titled Fique Sabendo through RT in the city of Teresina. Pregnant women were not included, since all of them are tested during prenatal at the Unified Health System (Sistema Único de Saúde — SUS).

The collection was performed in 2016 at the Carnival's day and night festivities in neighborhoods of the capital, Teresina. The approach of the participants was made in locations with the greatest concentration of revelers. During four days, 532 Rapid Check Sífilis RT were conducted.

The participants agreed to voluntarily take part in the research, by signing a free and informed consent form, with the guarantee of confidentiality of the reported information, and individualized care. The sample was outlined with the free population demand at the site of action in the period set for the campaign.

In the first stage, participants underwent pre-test counseling and responded to structured form containing sociodemographic information, sexual practices, use of alcohol/drugs, level of STD knowledge and antecedents, and participation in prevention activities. In the second step, treponemal RT was conducted, through the collection of blood in digital pulp. Following, in the final stage, the post-test counseling was carried out, and participants received a report describing the test result and information about syphilis, importance of diagnosis, symptomatology, possibility of asymptomatic infection, risk factors for acquisition and transmission. During the counseling, male and female condoms and lubricant gel sachets were available according to individual demand.

The positive results were given guidelines for performing the Venereal Disease Research Laboratory (VDRL) test to confirm the diagnosis, since the RT does not define the active disease, which might be a serological scar. Therefore, participants were referred to the public primary health care network closest to their home for clinical follow-up of the disease investigation. At that time, explanations were also given about understanding the outcome and the importance of timely and appropriate treatment.

The negative results were also discussed on immunological window, prevention importance through safe behavior, as well as information on syphilis testing locations in the city.

Simple descriptive statistics were used to achieve the objectives of determining the prevalence of positivity in the RT for syphilis. The research followed all ethical aspects, guaranteeing confidentiality, privacy, non-stigmatization and non-use of information to the detriment of participants, in accordance with the guiding principles set forth in Resolution no. 466/12 of the National Health Council, which approves rules and regulations of researches involving human beings.

RESULTS

The sample was composed of 532 participants. The positivity of the RT in the sampled participants was 5.4%: 19 (65.5%) males and 10 (34.5%) females. It was observed a predominance among self-referred browns (45%), aged 29 to 39 (38%), singles (65.5%), with education between 8 and 11 years of study (34.4%) (**Table 1**).

Table 2 shows the participants' distribution related to syphilis risk behavior; 52% performed the test for the first time. Regarding sexual practice, 76% reported having one to five sexual partners in the last year, only 24.1% reported using condoms with casual partners, and 55.2% said they did not use condoms with fixed partners. The use of alcohol and/or other drugs prior to intercourse was reported by 86.2%.

Table 1 – Sociodemographic characteristics of the study population, related to the rapid test results for syphilis, Teresina, Piauí, 2016 (n=532).

Variables	Testing	
	Positive cases	Negative cases
	n (%)	n (%)
Sex		
Male	19 (3.6)	513 (96.4)
Female	10 (2.0)	522 (98.0)
Race/Color		
White	6 (1.1)	526 (98.9)
Brown	13 (2.5)	519 (97.5)
Black	7 (1.7)	525 (98.6)
Other	3 (0.6)	529 (99.4)
Marital Status		
Single/Divorced/Widow	19 (3.6)	513 (96.4)
Married/Concubine/Common-law	10 (1.9)	522 (98.1)
Age (years)		
18–28	7 (1.4)	525 (98.6)
29–39	11 (2.0)	521 (98.0)
40–50	4 (0.8)	528 (99.2)
51–61	4 (0.8)	528 (99.2)
≥62	3 (0.6)	529 (99.4)
Education (years at school)		
None	1 (0.2)	531 (99.8)
<4	5 (1.0)	527 (99.0%)
4–7	8 (1.6)	524 (98.4)
8–11	10 (1.9)	522 (98.1)
>11	5 (1.0)	527 (99.0)

DISCUSSION

This study's proposal was to describe the epidemiological profile, risk behavior and frequency of positivity for syphilis through treponemal test available to general population gathered in public space during Carnival festivities in the city of Teresina. The research was carried out through the campaign Fique Sabendo, considered as a prevention of STD, as it offers diagnosis screening through RT. Prevalence of syphilis (5.4%) was observed. National studies conducted with specific population living in the streets found prevalence between 5.7 and 7.0% in São Paulo[6,7]. Prevalence found in this study is considered high, as it was performed with the general population, in which the risks are diluted, and yet are similar to those in specific population, such as the homeless, who usually experience greater vulnerability situations every day.

Syphilis remains an important public health problem, despite having simple diagnostic and treatment methods. The RT for syphilis has been pointed out by the WHO as a tool for rapid diagnosis to be used in specific situations, such as the difficult access to services or laboratory supplies[8]. In this research, it was verified that more than half of those diagnosed with syphilis were tested for syphilis screening for the first time, which shows the insufficiency of the access to diagnosis, as well as the non-perception of health professionals to implement a routine testing request for people with active sex life. It was observed the feasibility of the RT in the population due to the good adherence of the participants through the research for the test even at the time of Carnival street festivity.

The availability of rapid testing to the population is a strategic action that proposes to improve access to diagnosis. Although it constitutes itself as triage, it indicates the chance of infection of those with active disease, because, according to the protocol, all reagent screening cases are guided to the disease investigation and clinical management regarding treatment, notification and follow-up. Thus, it became one of the main goals established by the Brazilian Ministry of Health: in only 2016 more than six million RT were distributed in the country's health facilities[9].

Table 2 – Behavioral characteristics of the study's participants regarding the risk exposure to syphilis, Teresina, Piauí, 2016 (n=532).

Variable	Rapid test results	
	Positive	Negative
	n (%)	N (%)
Tested for syphilis previously		
Yes	14 (2.7)	518 (97.3)
No	15 (2.8)	517 (97.2)
Number of sexual partners in the last 12 months		
None	1 (0.2)	531 (99.8)
1–5	22 (4.2)	510 (95.8)
6–10	2 (99.6)	530 (99.6)
>11	1 (0.2)	531 (99.8)
Alcohol/drugs use before intercourse		
No alcohol or other drugs use	4 (0.8)	528 (99.2)
Alcohol	18 (3.4)	514 (96.6)
Drugs	7 (1.4)	525 (98.6)
Condom use with fixed partner		
Yes	5 (1.0)	527 (99.0)
No	16 (3.0)	516 (97.0)
Sometimes	8 (1.6)	524 (98.4)
Condom use with casual partner		
Yes	7 (1.4)	525 (98.6)
No	5 (1.0)	527 (99.0)
Sometimes	17 (3.2)	515 (96.8)

Despite being a health problem of easy diagnosis and available treatment, there is still a resurgence of this infection. These infection cases grow worldwide exponentially. In 2016, approximately 12 million people were diagnosed all over the world with the active disease, almost two-thirds in sub-Saharan Africa. In the Brazilian context, the growth of syphilis cases also calls attention. WHO estimates that 937,000 new cases occur every year[10,11].

It should be emphasized that, in addition to early diagnosis, it is necessary to provide timely and adequate treatment to break the chain of transmission and, therefore, control the disease. benzathine penicillin was recognized by the 69th World Health Assembly, in May 2016, as an essential drug for the control of syphilis transmission, and has been globally experiencing shortages for some years. In Brazil, as in other countries, since 2014 there has been a shortage of benzathine penicillin due to the lack of raw material for its production worldwide. In addition to the shortage, data from the second cycle of the National Program for Improving Access and Quality of Primary Care (Programa Nacional de Melhoria do Acesso e da Qualidade da Atenção Básica — PMAQ-AB), in 2013/2014, indicate that only 55% of the family health groups administer benzathine penicillin in primary care, which may contribute to the increase of syphilis cases[2].

Other important factors, including the fact that the infection is present in the female population of fertile age, are diagnosis and treatment of pregnant women, in order to prevent the serious consequences of congenital syphilis, and the best results are obtained when the treatment is received from the 24th to the 28th gestational week. In Brazil, there was a considerable increase in syphilis cases, mainly from 2014 to 2015, with 21% increase in congenital syphilis, reaching 19,228 cases. The adequate treatment with penicillin is able to prevent 97% of vertical transmission cases[9,12,13].

Considering the public health point of view evidenced in this research, it is important to emphasize the issues related to vulnerabilities that increase the risk of infection. It was observed, for example, the high proportion of people who reported using alcohol and/or other drugs prior to intercourses, revealing the concern about the power these substances exert, as they lower the level of consciousness, directly influencing safe behavior and risk exposure. Taking into account the negative impact of this fact on the health of the population, actions associated with prevention interventions and integral health care should be taken.

It is also noticeable the low adherence to the use of condoms with both fixed and casual partners. It is known that syphilis is a marker of unprotected sex[7]. Thus, it is necessary to seek strategies to improve access and adherence to male and female condoms, through a facilitated availability in public places with large population movements, such as nightclubs, bars, universities, besides health education mechanisms to make the condom more attractive to the young adult audience.

It is necessary to overcome the failures in the implementation of syphilis control measures, and it is fundamental to look for differentiated strategies to reach the most vulnerable social groups, taking into account the socioeconomic and cultural contexts[14].

Study limitation

This research has some limitations mainly caused by the methodology design (transverse) used, which does not allow the knowledge about the patients follow-up, such as: attendance at health facility for completion of treponemal test, treatment adherence in confirmed cases, notification and research of the scar tissue cases, as well as serological reception in primary health care.

CONCLUSION

The prevalence found justifies the need to intensify actions that can increase access to diagnosis and timely treatment for the infection control. The study shows the importance of educational and case detection campaigns due to the good adherence of the participants. It also points out that strategies should be adopted to make information more evident to the population about the increase of cases and ways of preventing the disease.

Conflict of interests

The authors declare no conflict of interests.

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Conventional and liquid-based cytology (Liqui-PREP™) anal findings in male HIV anoreceptive patients in a referral University hospital in Rio de Janeiro, Brazil

Achados de citologia convencional e de citologia em base líquida (Liqui-PREP®) anal em pacientes masculinos HIV soropositivos anorreceptivos, em um hospital universitário de referência do Rio de Janeiro, Brasil

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ABSTRACT

Introduction: The anal lesions seem to have a natural history that closely resembles cervical lesions, with signs that precede the invasion. Cytological changes of anal epithelium induced by HPV can be detected through cytology, as it is considered an effective screening method. **Objective:** To identify the frequency of atypical epithelial conventional cytology results by comparing anal samples through Liqui-PREP™ technology in HIV-positive men. **Methods:** Cross-sectional descriptive and analytical study of 33 men who have sex with men (MSM), HIV-positive and anoreceptive attended at the Gaffrèe and Guinle University Hospital (HUGG), Rio de Janeiro, from June to July, 2016. Collection of anal samples for the conventional cytology and Liqui-PREP™ cytology was carried out. For significance of findings, Fisher exact test with 95% confidence interval was used and cytological Kappa index was employed for concordance between the two cytological methods. **Results:** The age ranged from 23 to 60 years (mean=39.06). The CD4 cell count was between 200 to 500/mm^3 on 16 (48.5%) and 13 (39.4%), and 50% was diagnosed with HIV for more than 6 years. In conventional cytology one case was considered unsatisfactory (3%). Among the cases considered satisfactory, 9 (28.1%) were diagnosed with ASC-US; 4 (12.5%) LSIL; 2 (6.3%) ASC-H, and 2 (6.3%) HSIL. Through Liqui-PREP™ method, 7 cases were considered unsatisfactory (21.2%). Among the satisfactory cases, 7 showed ASC-US (26.9%); 4 (15.4%) ASC-H; 2 (7.7%) LSIL; and 2 (7.7%) HSIL. The difference of unsatisfactory cases between both methods, although higher for Liqui-PREP™, was not statistically significant (p=0.054). The correlation was moderate (0503; p<0.006 [0.1765–0.8298]). **Conclusion:** The cytologic atypia is common among MSM HIV (+), and the anal conventional cytology and liquid by Liqui-PREP™ cytology are equivalent, although they are more unsatisfactory in the latter technique.

Keywords: anal neoplasm; cytological techniques; HIV; male homosexuality.

RESUMO

Introdução: As lesões anais parecem ter uma história natural, que se assemelha às de lesões de colo uterino, com sinais que precedem a invasão. As alterações citológicas do epitélio anal induzidas pelo HPV podem ser detectadas por citologia, um método de rastreio considerado efetivo. **Objetivo:** Identificar a frequência de atipias epiteliais nos resultados da citologia convencional comparando amostras anais pela tecnologia Liqui-PREP® em homens HIV positivos. **Métodos:** Estudo transversal, descritivo e analítico de 33 homens que fazem sexo com homens (HSH), HIV positivos e anorreceptivos atendidos no Hospital Universitário Gaffrèe e Guinle (HUGG), Rio de Janeiro, no período de junho a julho de 2016. Os pacientes foram submetidos à coleta de amostras anais para citologia convencional e citologia Liqui-PREP®. Para significância de achados, foi usado o teste exato de Fisher com intervalo de confiança de 95%, e para concordância entre os dois métodos citológicos, foi utilizado o índice de Kappa. **Resultados:** A idade variou de 23 a 60 anos (média=39,06). A contagem de células CD4 foi entre 200 e 500/mm^3 para 16 (48,5%) e 13 (39,4%) dos casos analisados, e 50% tinham o diagnóstico de HIV há mais de seis anos. Na citologia convencional, um caso foi considerado insatisfatório (3%). Entre os casos considerados satisfatórios, 9 (28,1%) foram diagnosticados como células escamosas atípicas de significado indeterminado possivelmente não neoplásicas (ASC-US); 4 (12,5%) como lesão intraepitelial de baixo grau (LSIL); 2 (6,3%) como células escamosas atípicas não sendo possível excluir lesão intraepitelial de alto grau (ASC-H) e 2 (6,3%) como lesão intraepitelial de alto grau (HSIL). Pelo método Liqui-PREP®, 7 casos foram considerados insatisfatórios (21,2%). Entre os casos satisfatórios, 7 como ASC-US (26,9%); 4 (15,4%) como ASC-H; 2 (7,7%) como LSIL e 2 (7,7%) como HSIL. A diferença de insatisfatório entre os métodos, embora maior para Liqui-PREP®, não foi estatisticamente significativa (p=0,054). A concordância foi moderada (0,503; p<0,006 [0,1765–0,8298]). **Conclusão:** É frequente a atipia citológica entre HSH HIV (+), e as citologias anal convencional e em meio líquido pela técnica Liqui-PREP™ se equivalem, embora sejam mais insatisfatórias na técnica citológica Liqui-PREP®.

Palavras-chave: neoplasias do ânus; técnicas citológicas; HIV; homossexualidade masculina.

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INTRODUCTION

Anal cancer represents 1 to 2% of all colon tumors, and 2 to 4% of all cancers affecting the large intestine[1]. It is considered the fourth most common type of cancer in the USA, and its incidence is steadily increasing over the last decades[2,3]. It is specially worrying among some population groups at risk, such as: transplanted, non-HIV chronic

immunodeficients, those with autoimmune diseases using medication, anoreceptive individuals of both sexes with sexually transmitted diseases, mainly Human Immunodeficiency Virus infection (HIV) and Human Papillomavirus (HPV), and chronic anal lesions carriers[4-6].

Anal lesions seem to have a natural history that resembles those of the cervix, with signs preceding the invasion. HPV-induced cytologic alterations of the anal epithelium can be detected by cytology, which is considered an effective screening method[7]. There are currently two cytological screening methods: the conventional cytology (CC) and the liquid-based cytology (LBC). Disadvantages with the conventional method include inadequate sample by desiccation of the material, cells obscured by multiple layers of cellular material, by blood, by inflammatory cells or by fecal contamination. LBC would have as characteristic the collected samples stored in a collector with preservative liquid allowing better preservation of the cytomorphological properties, less agglomeration and less obscuration of the cells. An additional advantage is the possible use of the residual material for molecular biology tests[8].

Specifically, the liquid-based cytology can be carried out by automated and non-automated methods, and in Brazil, ThinPrep (Hologic, Inc., Marlborough, MA) and SurePath (Becton, Dickinson and Company, Franklin Lakes, NJ) are available. Liqui-Prep™ is among the non-automated techniques[9].

The liquid-based cytology has been reported as a preferred method for the evaluation of anal swabs as it eliminates air-drying artifacts, fecal material, and bacteria commonly found in conventional anal smears, as well as inflammatory debris that may obscure cellular details, and offers cell performance increase and better preservation of the sample[10,11].

OBJECTIVE

To identify the frequency of epithelial atypia in the results of conventional cytology comparing with the Liqui-PREP™ cytology technology of anal samples in HIV-positive men who have sex with men (MSM) at a referral hospital in Rio de Janeiro, Brazil.

METHODS

A cross-sectional, descriptive and analytical study was carried out with 33 HIV-positive and anoreceptive men who have sex with men (MSM), aged 23–60 years, attended at the Immunology Outpatient Clinic of the Gaffrèe and Guinle University Hospital (HUGG) of UNIRIO in Rio de Janeiro, Brazil, from June to July, 2016.

The study was submitted and approved by the Research Ethics Committee of HUGG / UNIRIO.

Data collection

The medical records were analyzed for the collection of information, such as: age, CD4 lymphocyte count and time of HIV infection diagnosis.

Sample collection

After signing the informed consent form (ICF), the patients were placed in the left lateral decubitus position (Sims position) for the collection of material for conventional oncotic cytology (CC) and liquid base cytology (LBC). Endocervical brush was used.

1. The brush was introduced about 2 to 4 cm from the anal border, 3 to 4 clockwise rotating movements, and removed in spiral motion[12];
2. Shortly thereafter, for CC, the bristles tip of the brush was scrubbed over the entire surface of the previously identified clean opaque edge glass sheet. The slides were put down in 70% plastic bottles;
3. Then the brush bristles tip with the residual material was placed into the bottle (previously identified with patient name and number) with Liqui-PREP™ preservative liquid for the preparation of LBC following the instructions of the product.

The collected material was sent to the Cytopathology Laboratory of the HUGG / UNIRIO for preparation and analysis.

Reading the smears

The reading of the material was performed by four analyzers, under a conventional optical microscope, in increments of 100x and 400x. The Bethesda System (TBS)[13] was used to analyze the sample (anal transformation zone and squamous epithelium), adequacy (satisfactory and unsatisfactory) and interpretation of the material. The results were classified as follows: unsatisfactory (INS) or satisfactory (SAT) in the initial analysis. Following as a negative for intraepithelial lesion or malignancy (NLM), atypical squamous cells of undetermined significance, possibly non-neoplastic (ASC-US), atypical squamous cells of undetermined significance, it is not possible to rule out high-grade lesions (ASC-H), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (CCE), adenocarcinoma *in situ* (ADCIS) and invasive adenocarcinoma (ADCI)[13].

Statistics

For significance of findings, Fisher exact test with 95% confidence interval was used and cytological Kappa index was employed for concordance between the two cytological methods.

RESULTS

Age varied from 23 to 60 years (mean=39.1). Concerning CD4 cell counts, 4 (12.1%) cases had a count lower than 200/mm^3, 16 (48.5%) had a count between 200 and 500/mm^3 and 13 (39.4%) patients had a count greater than 500 cells/μL. Regarding the time of HIV infection among the patients studied, 50% had been diagnosed with HIV for more than 6 years (**Table 1**).

Through the conventional cytology method, 1 case was considered unsatisfactory (3%). Among the 32 (97%) cases considered satisfactory, 15 (46.9%) were NML; 9 (28.1%) were diagnosed as ASC-US; 4 (12.5%) as LSIL; 2 (6.3%) as ASC-H and 2 (6.3%) as HSIL. Through the Liqui-PREP™ method, 7 cases were considered unsatisfactory (21.2%). Among the 26 (78.8%) satisfactory cases, 11 (42.3%) were diagnosed as NML; 7 as

ASC-US (26.9%); 4 (15.4%) as ASC-H; 2 (7.7%) as LSIL, and 2 (7.7%) as HSIL (**Table 2**). The difference of unsatisfactory diagnosis among methods, although higher in Liqui-PREP™, was not statistically significant (p=0.054). However, by evaluating the agreement between the two methods using the Kappa test, it was possible to observe only a moderate concordance (0.503; p<0.006 [0.1765–0.8298]).

Evaluation of the results according to the age shows that patients over 35 years presented around 60% atypia in the anal cytology independently of the technique. Regarding CD4 lymphocyte counts, there was no significant difference between the diagnoses and time of HIV; those with more than 6 years of infection had a discrete and non-significant higher frequency of cytologic atypia (**Table 3**).

Table 1 – Profile of HIV (Human Immunodeficiency Virus) seropositive men who have sex with men (MSM), who underwent conventional anal cytology, and Liqui-PREP™ liquid medium at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

Data	n (%)
Age (years)	
23–35	11 (33.3)
>35	22 (66.7)
CD4 T cell count	
<200	4 (12.1)
200–500	16 (48.5)
>500	13 (39.4)
Time of infection with HIV* (years)	
<1	12 (37.5)
1–6	4 (12.5)
>6	16 (50)

*No information on HIV infection time was found in one patient.

DISCUSSION

Regarding the suitability of the sample, the liquid-based cytology presented 21.2% of unsatisfactory material, while the conventional cytology presented only 3%, and in both methods the cellular shortage was the reason. One possible justification is the fact that conventional cytology was the first to be made, and the remaining material stayed in the brush for cytology in liquid medium. A previous study[12] used the same methodology and the same liquid medium and also found greater unsatisfactory material in the liquid-based cytology compared to the conventional cytology. In another study using only the automated liquid-base technique, the material

Table 2 – Conventional anal cytology and Liqui-PREP™ liquid medium findings in seropositive for Human Immunodeficiency Virus (HIV) men, who have sex with men (MSM), who underwent anal cytology at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

Diagnosis	Conventional cytology n (%)	Liqui-PREP™ n (%)	p-value
Unsatisfactory	1 (3)	7 (21.2)	0.0539
Satisfactory	32 (97)	26 (78.8)	
NML	15 (46.9)*	11 (42.3)*	0.7945
Atypical	17 (53.1)*	15 (57.7)*	
ASC-US	9 (28.1)*	7 (26.9)*	
ASC-H	2 (6.3)*	4 (15.4)*	
LSIL	4 (12.5)*	2 (7.7)*	
HSIL	2 (6.3)*	2 (7.7)*	

*Percentage among the satisfactory cases considered for analysis; NML: Negative for malignancy and squamous intraepithelial lesion; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells, and cannot exclude high-grade intraepithelial lesion; LSIL: low-grade intraepithelial lesion; HSIL: high-grade intraepithelial lesion.

Table 3 – Conventional anal cytology and Liqui-PREP™ liquid medium findings according to age, CD4 lymphocyte count and time of infection among HIV-positive men, who have sex with men, who underwent anal cytology at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

	NML		Atypical		Total		p-value (IC95%)
	CC n (%)	LBC n (%)	CC n (%)	LBC n (%)	CC* n (%)	LBC# n (%)	
Age							
≤35	7 (63.6)	3 (37.5)	4 (36.3)	5 (62.5)	11 (100)	8 (100)	
>35	8 (38)	8 (44.4)	13 (62)	10 (56.6)	21 (100)	18 (100)	ns
Total	15 (46.9)	11 (42.3)	27 (53.1)	15 (57.7)	32 (100)	26 (100)	
CD4 cell count							
<200	0	0	4 (100)	4 (100)	4 (100)	4 (100)	
200–500	9 (60)	7 (58.3)	6 (40)	5 (41.7)	15 (100)	12 (100)	ns
>500	6 (46.1)	4 (40)	7 (53.9)	6 (60)	13 (100)	10 (100)	
Total	15 (46.9)	11 (42.3)	17 (53.1)	15 (57.7)	32 (100)	26 (100)	
Time of infection with HIV (years)							
<1	5 (41.7)	2 (25)	7 (58.3)	6 (75)	12 (100)	8 (100)	
1–6	3 (75)	2 (66.7)	1 (25)	1 (33.3)	4 (100)	3 (100)	ns
>6	7 (43.7)	8 (53.3)	9 (56.3)	7 (46.7)	16 (100)	15 (100)	
Total	15 (46.9)	12 (46.1)	17 (53.1)	14 (53.9)	32 (100)	26 (100)	

*One case was considered unsatisfactory; #seven cases were considered unsatisfactory; CC: Conventional Cytology; LBC: Liquid-Based Cytology; ns: not significant.
The following cytologic results were considered atypical: ASC-US, ASC-H, LSIL and HSIL.

was considered unsatisfactory in 10.7% of cases due to a shortage of cells[14]. Eleutério et al.[11], studying immunocompetent women, did not report unsatisfactory cases using the SurePath™ technique.

About 60% of patients of this study have shown abnormal cytology (ASC-US or more) considering both methods. Several studies have shown that infected with HIV men who have sex with men presented a high prevalence of abnormal cytology. Salit et al.[15] have investigated 401 HIV-seropositive MSM, 67% of which showed abnormal cytology. In a Brazilian study, Silva et al.[16] observed that the highest prevalence (49.5%) was in HIV-seropositive MSM. Selvaggi[14] made a 10-year follow-up on studies that used anal cytology to track anal lesions in HIV-seropositive MSM, and cytology revealed 41 to 93% abnormality, most of which histologically confirmed[14-16].

Concerning the association between clinical data and positive results, patients over 35 years of age often presented more cytological atypia than those younger than 35 years. Studies have shown that anal cancer is much rarer at younger age, and that in the progression of high-grade anal lesions to carcinoma, the median age at diagnosis of cancer is around 51 years[17,18].

In the association with CD4 lymphocyte count, no significant difference between CC and LBC was found. The highest prevalence of altered samples was in patients with more than 500 cells/μL, 41.1 and 40%, with a little difference between the negative ones, 40 and 36.3%. In patients between 200–500 cells/μL count, the percentage of altered cytology was of 35.2 (CC) and 33.3% (LBC), the percentage of altered cytology was of 35.2 (CC) and 33,3% (LBC), and negative cytology was twice high, 60 (CC) and 63.6% (LBC). In patients below 200 cells/μL count, all cytologies were altered in both CC and LBC. Several studies relate the incidence of anal cancer with low CD4 count when associated with HPV infection[19]. Sendagorta et al. [20] reports that the risk of HIV-seropositive presenting anal lesions increases as CD4 decreases. Leeds and Fang[21] consider that the risk of progression of anal lesions is directly correlated with the degree of immunosuppression and with CD4 T cell counts.

Patients HIV infected for more than 6 years showed the highest positivity rates, 52.9 (CC) and 50% (LBC), and with one year or less: 41.1 (CC) and 42.8% (LBC) were nearly ten times more than the ones infected between 1 and 6 years: 5.8 (CC) and 7.8% (LBC). Crum-Cianflone et al.[22], in a cohort with 4,901 HIV-seropositive patients, noted that people infected for more than 15 years had 12 times more chances to develop anal cancer compared to those infected for less than 5 years, concluding that there is an association of high rates of anal cancer with the time of HIV infection, and emphasizing the urgent need to establish screening and prevention strategies for cancer.

CONCLUSION

The cytologic atypia is frequent among HIV (+) MSM, and the anal conventional cytology and liquid-based by Liqui-PREP™ cytology technique are equivalent, although with a small and not significant superiority of conventional cytology.

Conflict of interests

The authors declare no conflict of interests.

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MIXED INFECTION AND TWO DIFFERENT RESISTANCE PROFILES OF *TREPONEMA PALLIDUM* TO MACROLIDES IDENTIFIED IN SOME CLINICAL SPECIMENS OF THE SAME PATIENT

INFECÇÃO MISTA E RESISTÊNCIA DIFERENTE A MACROLÍDEOS EM TREPONEMA PALLIDUM IDENTIFICADAS POR VARIADAS ESPÉCIMES CLÍNICOS EM UM MESMO PACIENTE

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ABSTRACT
Syphilis represents a global public health problem. The resistance of *Treponema pallidum* to macrolides is related to the mutation in the *23S rRNA* gene (A2058G). We reported a case of secondary syphilis in a 52-year-old man presenting two profiles: the first one of susceptibility, and the other one of resistance, when we analyzed the *23S rRNA* gene sequence from two different clinical specimens of the same infectious episode. DNA from *T. pallidum* from skin biopsy presented resistance profile, whereas *T. pallidum* DNA from blood presented a profile of susceptibility to macrolides. These results suggest it was mixed infection or reinfection.
Keywords: syphilis; drug resistance; DNA sequencing.

RESUMO
A sífilis representa um problema de saúde pública mundial. A resistência de *Treponema pallidum* aos macrolídeos está relacionada à mutação no gene *23S rRNA* (A2058G). Relatamos um caso de sífilis secundária, em um homem de 52 anos, com um perfil de suscetibilidade e outro de resistência, ao analisarmos a sequência do gene *23S rRNA* de dois espécimes clínicos diferentes, do mesmo episódio infeccioso. A amostra de DNA de *T. pallidum* proveniente de raspado dérmico da lesão apresentou um perfil de resistência, enquanto aquele que derivou de sangue apresentou perfil de suscetibilidade aos macrolídeos. Esses resultados sugerem tratar-se de infecção mista ou de reinfecção.
Palavras-chave: sífilis; resistência; análise de sequência de DNA.

INTRODUCTION

The reemergence of syphilis in recent decades represents an important public health problem in developed and developing countries[1-5]. Syphilis can facilitate the Human Immunodeficiency Virus (HIV) transmission and increase the risk of adverse pregnancy outcome, besides the substantial social impact. The global syphilis incidence estimation for 2012 was 5.6 million cases. In Brazil, the primary and secondary syphilis in the population between the age group 15–49 years in 2010 was of 843,300 cases[4,6-9]. From 2005 to 2012, 57,700 prenatal syphilis cases were reported, most of which in the Southeast region[3,8-9].

A dose of 2.4 million units of penicillin G benzathine administered intramuscularly is the drug of choice for the syphilis treatment[10-12]. However, the intermittent lack of penicillin in the Brazilian market and the number of patients with reported allergies to penicillin[13] require other processes of treatment. The relatively low toxicity and strong macrolides bacteriostatic effect are the reasons for the use of erythromycin and azithromycin in the treatment of syphilis[11-14].

Unlike penicillin, the treatment of syphilis with macrolides represents risk of failure due to the resistance of *T. pallidum* subspecies *pallidum* to these antibiotics. The primary mutation in *T. pallidum* macrolide resistance is related to A2058G, as it occurs at the coding gene for 23S ribosomal RNA subunit (*23S rRNA*). Macrolide-resistant *T. pallidum* isolates represent a major challenge to public health in both developed and developing countries, making monitoring of *T. pallidum* resistant to macrolides an integral part of the syphilis control programs[5,9,10].

CASE REPORT

A 52-year-old man was treated at the Souza Araújo Clinic, of Oswaldo Cruz Institute (IOC) of Oswaldo Cruz Foundation (Fiocruz), in Rio de Janeiro, Brazil — a reference outpatient clinic of the Ministry of Health for the treatment of Hansen's disease —, with persistent skin and mucosal efflorescence for more than three months. The patient also complained of generalized symptoms such as fever, malaise, adenomegaly and intense headache. His background included infection with *Neisseria gonorrhoeae* two years before and genital lesion one year before. The patient reported having had sexual intercourse with several partners in the last six months.

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The patient was afebrile and revealed a 5 mm genital exulceration and macular rash on the torso and upper limbs on physical examination (**Figure 1**). Serological tests showed the following results: Venereal Disease Research Laboratory (VDRL) 1:256, haemagglutination for *T. pallidum* (TPHA) reagent, fluorescent treponemal antibody absorption test (FTA-ABS) reagent and HIV non-reagent. The genital lesion was not tested on a dark field test.

The molecular diagnosis was performed by polymerase chain reaction (PCR) specific for the amplification of gene *tpp15*, and for *T. pallidum* subspecies *pallidum*. The four clinical samples collected confirmed the diagnosis, and two of them (blood and *swab*) amplified for the *23S rRNA* gene, which were sequenced. The triplicate confirmation was analyzed in the SeqScape Software, that generated the chromatograms shown in **Figures 2 and 3**.

MATERIALS AND METHODS

Clinical samples, total blood with anticoagulant, Vacuplast (Beicheng, Zhejiang, China), serum and lesion scraping were collected prior to treatment initiation. For *T. pallidum* resistance diagnosis and molecular detection, DNA was extracted using *QIAamp DNA mini Kit* (Qiagen, Hilden, Germany), according to the manufacturer's instructions. PCR for the molecular diagnosis of *T. pallidum* amplifies a 120 bp fragment of the *tpp15* gene[15]. This reaction was followed by agarose gel electrophoresis analysis. The molecular detection of resistance to macrolide was carried out from PCR, that amplifies a 628 bp sequence of the *23S rRNA* gene, followed by a direct PCR product sequencing. Isolates of *T. pallidum* resistant to macrolides exhibited the A2058G mutation, as described by Lukehart *et al.* [8].

ETHICAL CONSIDERATIONS

This study was approved by the Ethics Research Committee of Fiocruz, Brazil (Certificate of Presentation for Ethical Consideration — CAAE — no. 58752716.5.0000.5248).

Figure 1 – Case patient presenting disseminated skin rash (syphilitic roseola).

RESULTS

Based on the clinical and serological findings, and evolution time, the patient was diagnosed as having secondary syphilis. Treatment with penicillin was administered intramuscularly in three doses of 2.4 million units. A monthly follow-up was carried out, and the patient reported improvement of symptoms, and at a physical examination four weeks after the last penicillin dose VDRL decreased (1:6).

Molecular detection of treponemal DNA may have identified the *T. pallidum* infection, as well as the macrolide resistance in clinical isolates. All clinical samples (biopsy, serum and total blood) collected from this patient were used for this detection. The PCR result, targeting the *tpp15* gene, was positive for all samples, thus confirming *T. pallidum* infection. Molecular detection of the *T. pallidum 23S rRNA* gene from the biopsy showed a transition from A to G at position 2058 (A2058G) related to the *T. pallidum* resistance to macrolides. This same DNA analysis obtained from total blood and serum showed a macrolide susceptibility profile for both samples, *i.e.*, without A2058G transition in the *23S rRNA* gene.

DISCUSSION

Although the patient was treated with penicillin, the discovery of at least one isolate of *T. pallidum* with a genotypic profile of macrolide resistance showed the importance of treating syphilis with the first antibiotic of choice[9,12]. Other drugs, such as azithromycin[8,11,16] and erythromycin, are recognized effective in the treatment of early syphilis in human beings. However, the increasing incidence of isolates of macrolide-resistant *T. pallidum* was reported in recent years[8,16]. It is believed that resistance to macrolides is due to the frequent use of these antibiotics for the treatment and prevention of a number of non-syphilitic infections[16].

Syphilis testing for macrolide-resistant treponemal are limited by the fact that *T. pallidum* cannot be grown *in vitro*, thus the routine test is restricted to the PCR amplification and sequencing of the *23S rRNA* gene from DNA of clinical samples of patients with syphilis[8]. The data on the occurrence of isolates resistant to macrolides are scarce, and we believe it could be explained because the syphilis molecular detection is not routinely performed.

This case proved to be even more interesting as it presents two resistance profiles, wild and mutant, in different samples, probably coming from the same episode of secondary syphilis. The *T. pallidum* isolate from genital lesion (*swab*) showed a genotypic profile in the *23S rRNA* gene with A2058G mutation, corresponding to an isolate macrolide-resistant, while *T. pallidum* DNA from total blood and serum did not show this transition. There are two possible explanations for this fact:

- the patient might have been infected with one sensible *T. pallidum* isolate and other macrolide-resistant isolate from the simultaneous intercourse with different partners, or at different moments, that formerly hosted the wild or mutant type in relation to drug sensitivity;
- the subject might have had sexual intercourse with an individual who also carried hetero-drug resistant isolates.

CONCLUSION

These data support the PCR testing utilization, since in this case it is possible to use different types of clinical samples for syphilis diagnosis and molecular identification. PCR can probably be used to diagnose syphilis in its different stages. Surveillance for resistance of *T. pallidum* to macrolides is also an important strategy for guiding the syphilis treatment recommendation, especially in periods of epidemic outbreaks and/or absence of the drug of first choice.

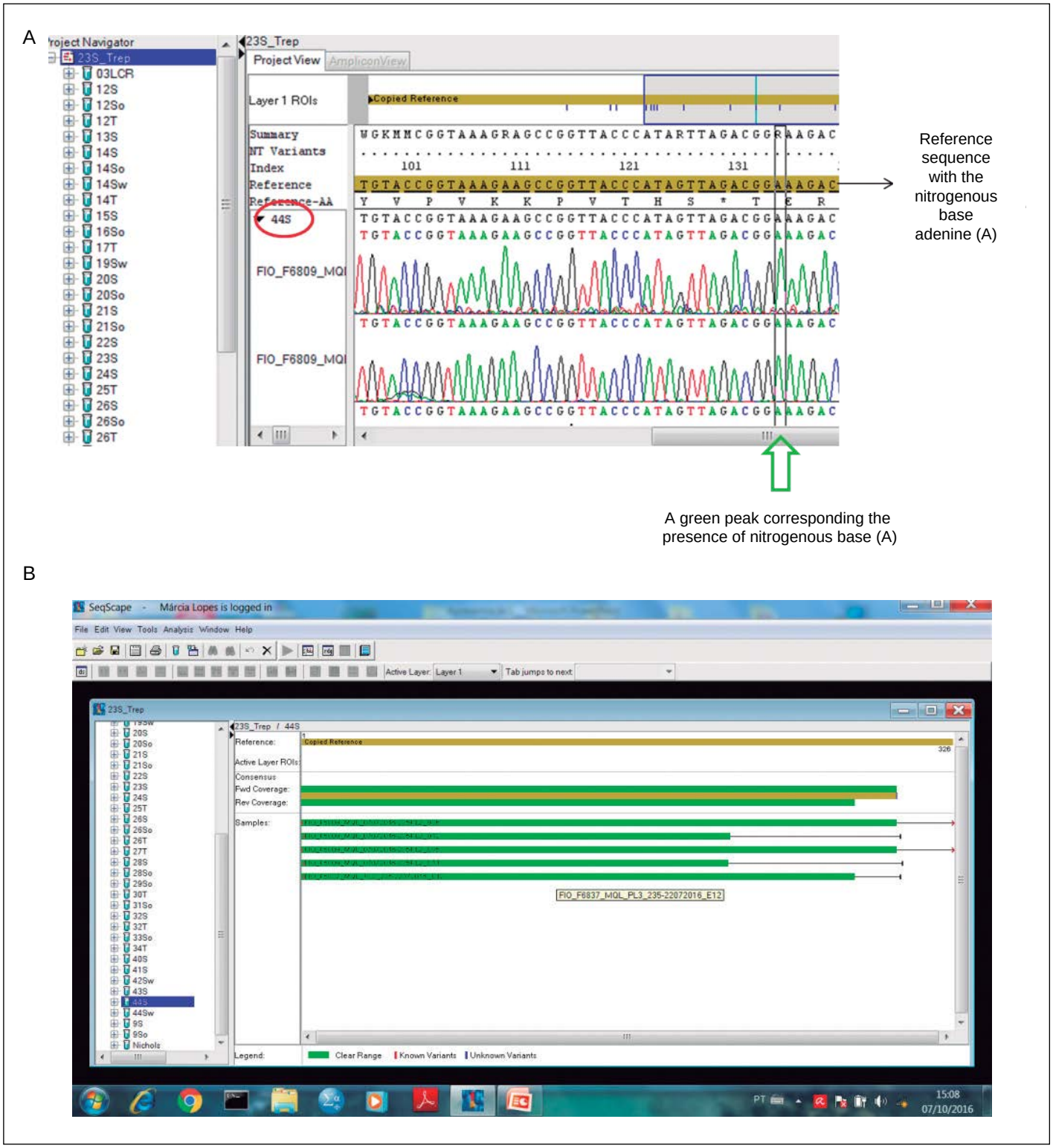

Figure 2 – Sample without mutation A2058G. (A) Visualization of the chromatogram within the SeqScape® program in which no nucleotide other than the reference sequence was found. (B) Image showing the sequences obtained from each initiator, as well as the region of exploitation of each of them (green). No base other than the reference sequence was identified in the sample.

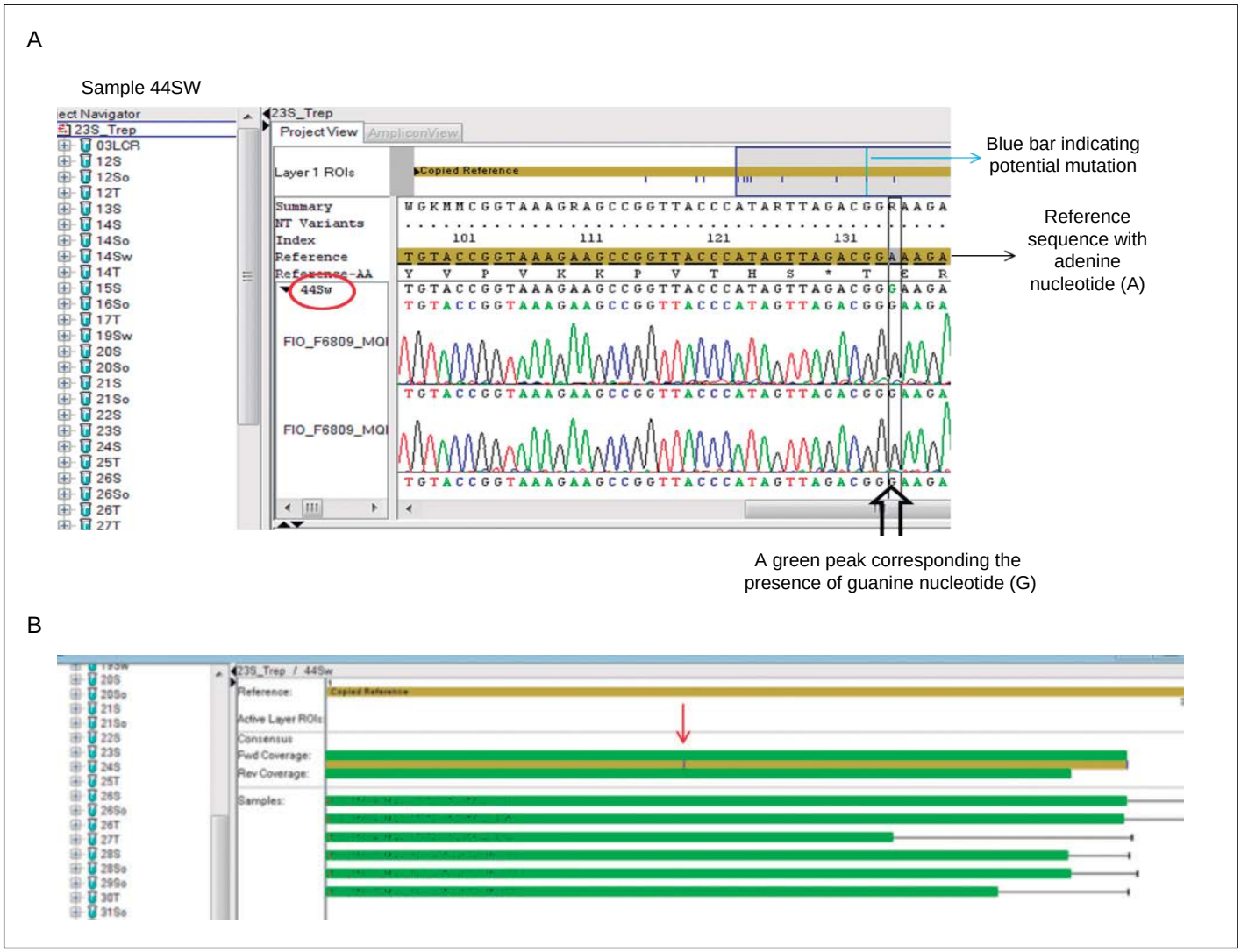

Figure 3 – Samples of the patient with mutation A2058G. (A) Visualization of the chromatogram within the SeqScape® program in which a nucleotide other than the reference sequence was found. (B) Image showing the sequences obtained from each initiator, as well as the region of exploitation of each of them (green). In this sample, a nucleotide (red arrow) distinct from the reference sequence was identified.

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Conflict of interests

The authors declare no conflict of interests.

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Neisseria gonorrhoeae conjunctivitis in a prepuberal girl - a dilemma: sexual violence or non-sexual transmission?

Conjuntivite por Neisseria Gonorrhoea em menina prepubere – um dilema: violência sexual ou transmissão não sexual?

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ABSTRACT

Introduction: Sexually transmitted infections (STIs) are a public health issue of global concern and frequently lead to important sequelae if not diagnosed and properly treated. *Neisseria gonorrhoeae* (NG) infection is one of the most prevalent STIs worldwide and recently presents increasing incidence and antimicrobial resistance rates. Apart from the neonatal period, NG infection during childhood is considered evidence of sexual violence (SV). However, defining perpetration of violence can be challenging in clinical practice. **Objective**: To report a case of conjunctivitis due to NG in a prepuberal girl and discuss possible means of infection acquisition and medical forensic implications. **Case report:** A 7-year-old female Caucasian student from São Paulo was referred to the Rape Care Center (*Núcleo de Atendimento a Vítimas de Violência Sexual* – NAVIS) outpatient clinic to investigate sexual violence in September 2013. At admission, she reported right ocular hyperemia for 10 days with no response to tobramycin eye drops. Personal history: nothing noteworthy. She lived with her mother and grandmother and visited her father every two weeks. Physical and gynecological examinations were normal. Eye examination: Left eye — nothing noteworthy. Right eye — palpebral edema, conjunctival hyperemia with purulent exudate and upper corneal perforation. Bacterioscopy of conjunctival secretion was positive for Gram-negative diplococci and NG was isolated in culture. The patient was submitted to suturing of right eye perforation and received 1g intravenous ceftriaxone per day for 10 days. During investigation at the NAVIS outpatient clinic, the mother denied any SV episode or school behaviour change. Multidisciplinary psychosocial care was provided to the child and her mother for over 6 months, but SV could not be characterized. STIs investigation for HIV, hepatitis B and C infections and syphilis resulted negative. Based on the literature, a hypothesis of accidental intra-familial non-sexual transmission of NG was then considered. Endocervical, vaginal and urethral secretions were collected from the mother and yielded isolation of endocervical beta-lactamase producing NG. Hygiene measures and contact isolation were recommended and the mother underwent treatment with ceftriaxone single dose 1G. During follow-up the child developed corneal opacity in her right eye. **Conclusion:** In prepuberal children presenting with unusual but compatible clinical manifestations, STIs should always be considered and investigated to enable prompt treatment and avoid sequelae. If gonococcal infection is diagnosed, the possibility of sexual violence should be thoroughly investigated, preferably in a comprehensive multidisciplinary approach to rule out non-sexual contamination and avoid emotional damage to the child and family. Clearly defining SV and proposing proper interventions in these circumstances is, however, challenging for healthcare providers.

Keywords: Neisseria gonorrhoeae; conjunctivitis; disease transmission, infectious; sex offenses; rape; child.

RESUMO

Introdução: As infecções sexualmente transmissíveis (ISTs) são um problema de saúde pública global e com frequência deixam sequelas se não diagnosticadas e tratadas adequadamente. A infecção por *Neisseria gonorrhoeae* (NG) é uma das ISTs mais prevalentes em todo o mundo e, recentemente, tem apresentado crescentes taxas de incidência, além de resistência a antimicrobianos. Após o período neonatal, a infecção por NG na infância pode ser uma evidência de violência sexual (VS), no entanto a comprovação da violência é um desafio na prática clínica. **Objetivo:** Apresentar um caso de conjuntivite por NG em uma menina pré-púbere e discutir as possíveis vias de contaminação e implicações médicas forenses. **Relato de caso:** Trata-se de uma criança caucasiana de 7 anos de idade do sexo feminino, estudante, procedente de São Paulo, que, após uma internação, foi encaminhada ao Núcleo de Atendimento a Vítimas de Violência Sexual (NAVIS) para investigação de violência sexual, em setembro de 2013. Na admissão intra-hospitalar, houve relato de hiperemia ocular direita, iniciada havia 10 dias, sem resposta ao tratamento com colírio de tobramicina. Antecedentes pessoais: nada digno de nota. Ela morava com a mãe e a avó e visitava o pai a cada duas semanas. Os exames físico e ginecológico foram normais. Exame oftalmológico: olho esquerdo — nada digno de nota. Olho direito — edema palpebral, hiperemia conjuntival com exsudato purulento e perfuração da córnea superior. A bacterioscopia de secreção conjuntival foi positiva para diplococos gram-negativos e a NG foi isolada em cultura. A paciente foi submetida a sutura cirúrgica de perfuração do olho direito e, enquanto internada, recebeu 1 g de ceftriaxona endovenoso por dia, por um período de 10 dias. Durante a investigação no ambulatório de NAVIS, a mãe negou qualquer episódio de VS ou mudança de comportamento escolar. Foi oferecida assistência psicológica e social à criança e à mãe por mais de seis meses, mas a VS não pôde ser caracterizada. A investigação de IST para o HIV, infecções por hepatite B e C e sífilis resultou negativa. Com base na literatura, a hipótese de transmissão não sexual acidental de NG intrafamiliar foi então considerada. As secreções genitais da mãe (endocervical, vaginal e uretral) foram coletadas e o isolamento endocervical da NG produtora por betalactamase foi positivo. Medidas de higiene e isolamento de contato foram recomendados, além ser prescrito o tratamento com ceftriaxona em dose única de 1 g para a mãe. Durante o acompanhamento, a criança desenvolveu opacidade corneana em seu olho direito. **Conclusão:** Em crianças pré-púberes que apresentam manifestações clínicas incomuns, as ISTs devem sempre ser consideradas e investigadas para permitir o tratamento imediato e assim evitar sequelas. Se uma infecção gonocócica for diagnosticada, a possibilidade de (VS) deve ser minuciosamente investigada, de preferência com uma abordagem multidisciplinar abrangente para descartar a contaminação não sexual e evitar danos emocionais à criança e à sua família. Definir com precisão se houve VS e propor intervenções adequadas nessas circunstâncias mostra-se um desafio para os profissionais de saúde.

Palavras-chave: Neisseria gonorrhoeae; conjuntivite; transmissão de doença infecciosa; delitos sexuais; estupro; criança.

INTRODUCTION

A laboratory confirmed sexually transmitted infection (STI) in a prepuberal child should be considered evidence of sexual violence (SV), according to international clinical guidelines and forensic

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medicine regulations[1,2]. According to the United Nations (UN) definition, childhood is considered as the lifetime from birth to 10 years of age, whereas in Brazilian legislation since 1990 — *Estatuto da Criança e do Adolescente* (ECA) —, childhood includes age groups from 10 to 12 years, the average age when menarche occurs[3,4]. However, it is not always easy to prove the perpetration of violence when physical evidence is lacking or when a statement by the victim cannot be obtained due to immaturity of poor understanding of the child. Under these circumstances, the decision-making process on how to intervene can be very challenging for healthcare providers. Not protecting children as suspected victims of SV may lead to severe deleterious consequences with effects that may last throughout their lifetimes[5,6]. On the other hand, removing suspected victims from their parents' company can also be harmful for the child's good development in case SV is eventually ruled out. We thus report a case of a child who presented conjunctivitis due to Neisseria gonorrhoeae (NG) infection and discuss the possible means of infection acquisition and the corresponding forensic medicine implications, so as to support healthcare providers in the proposal of proper interventions.

CASE REPORT

A 7-year-old female Caucasian student from São Paulo was referred to the Rape Care Center (*Núcleo de Atendimento a Vítimas de Violência Sexual* — NAVIS) outpatient clinic at Hospital das Clínicas in São Paulo, Brazil, in September 2013, to investigate SV. According to the mother, the child sought a pediatric emergency service with complaint of right eye hyperemia for 10 days and was prescribed tobramycin eye drops, without any improvement. No noteworthy information was detected in assessment of the child's personal history. She lived with her mother and grandmother and visited her father every two weeks. Physical and gynecological examinations were normal. Eye examination: Left eye — nothing noteworthy. Right eye — palpebral edema, conjunctival hyperemia with purulent exudate and upper corneal perforation. Bacterioscopy of conjunctival secretion was positive for Gram-negative diplococci and NG was isolated in culture (automated Vitek method; beta-lactamase producing NG). In addition, the secretion yielded no evidence of fungal infection. Vitreous and aqueous humor cultures resulted negative after first antibiotic dose, for aerobic and anaerobic bacteria and fungi. With diagnosis of corneal perforation secondary to gonococcal conjunctivitis, the patient was submitted to suturing of the perforation and received 1g of intravenous ceftriaxone per day for 10 days.

As part of the routine management of cases at the NAVIS outpatient clinic, the judicial system was notified of suspected SV. During investigation, the mother denied any intrafamilial SV episode or school behavior change. Multidisciplinary psychosocial care, consisting of psychotherapy and social counseling, was provided to the child and her mother for over 6 months. Adherence to follow-up consultations with an attending physician, a social worker and a psychologist was good. Although intensively investigated, the multidisciplinary team of care providers could not characterize SV.

Complementary clinical investigation for other STI, including HIV, hepatitis B and C infections and syphilis resulted negative. Based on the literature, a hypothesis of accidental intrafamilial non-sexual gonococcal transmission was then considered. Endocervical, vaginal and urethral secretions were collected from the mother and yielded isolation of endocervical beta-lactamase producing NG. The mother was appropriately treated with a single dose of 1g ceftriaxone. Hygiene measures and contact isolation were also recommended. Despite receiving systemic antibiotic therapy, the child developed unilateral visual deficit in her right eye during follow-up, probably due to the delay in establishing the etiological diagnosis of conjunctivitis and its proper therapy.

DISCUSSION

At first, this clinical case led us to hypothesize sexually transmitted gonococcal conjunctivitis. Bearing this in mind and taking the patient's age into account, based on international medical and forensic guidelines a suspicion of SV was raised, and the case accordingly reported to judicial authorities in charge of children's rights protection. However, SV could not be characterized in this case, despite a thorough investigation carried out by a skilled multidisciplinary care team. Vulnerability issues and alternative modes of acquisition of gonococcal infection should therefore be considered in this situation.

In this regard, it is important to point out that the incidence of gonococcal infection, which remained stable in previous decades, has been rising in both sexes, especially among men who have sex with men (MSM) since 2010 in several countries[7,8]. In addition, recent evidence has shown that asymptomatic individuals may carry NG in the oropharynx, resulting in increased risk of transmission through oral and genital contact, more often reported among MSM[9]. Increased gonococcal antimicrobial resistance may have contributed to increased transmission[7,8], once that single-dose antibiotic therapy regimens have been shown ineffective in eradicating oropharyngeal NG. Oral antiseptic use is therefore being studied for oropharyngeal decontamination[9]. In a context of increased NG infection prevalence, unusual clinical presentations and alternative modes of transmission may occur.

Particularly among prepuberal children, gonococcal infection is described more often in girls, and is usually presented clinically as vulvovaginitis, with no evidence of endocervical or internal genitalia involvement, in contrast to what is described among female adolescents or adult women. At older age, NG infections, due to its protracted course and frequent asymptomatic presentation, may lead to pelvic inflammatory disease and, subsequently, to infertility[1,7]. It is important to highlight, among prepuberal girls, that gonococcus may also be isolated from oropharynx and anorectal swabs[7]. According to the literature, the lack of genital estrogen renders prepuberal girls significantly more vulnerable to NG acquisition. Immature development of the outer lips in younger girls provides more intense vaginal exposure in the absence of growth of protective bacterial flora. Genital infection may thus occur in these girls without any physical lesion, i.e., even in the absence of hymnal rupture[10,11].

Regarding the clinical presentation of the present case, gonococcal conjunctivitis has been previously described in prepuberal children of both sexes[10-13], alone or associated with genital infection. Its occurrence is of major clinical and epidemiological concern among newborns, in whom ocular infection constitutes the most common clinically recognized manifestation of neonatal infection (*ophtalmia neonatorum*) and a significant cause of blindness in developing countries[7,10]. Routine recommendation of prophylactic

ocular silver nitrate solutions or erythromycin (0.5%) ophthalmic ointment at birth for all neonates has been shown as an efficient and safe measure[7,10].

Although the overriding risk factor for acquiring NG infection is having sex with an infected partner, non-sexual transmission of this agent should not be disregarded. Goodyear-Smith[10] reported that gonococcus can survive up to 48 to 72 hours on moistened towels or surfaces, being able to grow after inoculation of contaminated material in experimental animals. Moreover, gonococcal infection outbreaks described in prepuberal institutions in the late 19th century were considered dependent on the shared use of rectal thermometers and towels, and could be contained with implementation of hygiene measures and contact isolation. Even though sexual transmission cannot be completely ruled out in retrospective studies, the fact that they resulted in ocular infection only, with no increased occurrence of genital infection, strongly suggests non-sexual transmission. It is noteworthy that children aged 0 to 4 years have a 27-fold higher risk of eye infection due to NG than adults[10]. In the case presented here, NG isolation from an endocervical sample of the child's mother, in the absence of any symptoms, raised the possibility of sustained exposure to intrafamilial transmission.

According to international guidelines, nucleic acid amplification test (NAAT) in vaginal secretions, urine or endocervical samples are sufficiently sensitive and specific to provide an adequate approach to diagnose adolescents and adults, enabling appropriate therapeutic interventions to be made[1,7]. However, when it comes to prepuberal children, or in the context of suspected SV, it is necessary to obtain gonococcal isolation in culture for differentiation of NG from other Neisseria isolates. Moreover, NAAT is not validated for the diagnosis of gonococcal infection in anorectal and oropharyngeal sites, and NG isolation in culture allows for the assessment of drug sensitivity, before new molecular techniques for detection of antimicrobial resistance are developed[7,8]. In the reported case, gonococcal isolation and drug sensitivity assessment were obtained from both the child and the mother, and enabled successful therapy with ceftriaxone. In fact, the frequency of gonococcal resistance to extended-spectrum cephalosporins in Brazil is still below 0.1%[5,8]. Nevertheless, the unusual presentation of NG infection in this 7-year-old girl hindered early diagnosis and therapy, which may have contributed to the development of permanent visual impairment as sequel.

Finally, one must also consider the emotional impact and psychosocial consequences of this 7-year-old girl's family having experienced the suspicion of SV. In previously reported cases, gonococcal infection was diagnosed in the absence of further evidence of SV, leading to separation of the suspected under aged victims from their parents and families and consequently triggering secondary emotional losses that could otherwise have been averted[6,7,9]. Joint assessment and counseling by a well-trained multidisciplinary team of care providers encompassing the evaluation of the structural dimensions of the child's vulnerability, particular behavioral issues of those involved, assessment of the psychological suffering of the child and its caregivers is crucial in these cases, to allow investigation of suspected SV perpetration with a nonjudgmental attitude as to mitigate harm.

Conflict of interests

The authors declare no conflict of interests.

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A NEW STRATEGY TO PREVENT AND TREAT PRECANCEROUS CERVICAL LESIONS AMONG HPV-POSITIVE WOMEN

UMA NOVA ESTRATÉGIA PARA PREVENIR E TRATAR LESÕES CERVICAIS PRÉ-CANCEROSAS EM MULHERES POSITIVAS PARA O HPV

Luis Serrano[1], Silvia González[1], Santiago Palacios[2], Josep Combalia[3], Carine Emsellem[3], Bruna De Vita[3], Javier Cortés[4]

There is a group of women in which usual human papillomavirus (HPV) viral clearance does not occur and this is considered high risk for early development of precancerous lesions of the cervix[1]. Experts speculate four circumstances that may be related to the clearance and persistence of HPV.

The viral genotype is the best-known variable that determines the capacity of cellular integration and associated oncogenic risk. Genotypes 16 and 18 are critical types[2].

The second variable is the histological structure of the ectocervix. HPV needs cells in mitotic activity to integrate[3]. Cells in metaplastic re-epithelization process in the transformation zone of the cervix are ideal targets for anchoring HPV. Consequently, well-epithelized cervix with squamous epithelium and limited or non-existent transformation areas do not provide a suitable environment for inclusive colonization of HPV.

Further, recent data clearly demonstrates vaginal microbiota imbalance may determine the pathogenesis of HPV cervical cancer. This circumstance is attracting more and more interest, due to the growing evidence of the direct relationship between the alteration of the microbiota (with decrease in lactobacilli and increased diversity), and the greater frequency and severity of the HPV-dependent cervical lesions[4]. The alteration of the vaginal microbiota can affect the other circumstances, deteriorating the epithelial integrity of the cervix and affecting the vaginal immune status.

Finally, there is the immune status of the host. It is known that immunocompromised patients have a high risk of developing HPV related lesions[5]. Recent data has shown a significant correlation between the immune status of women and the persistence of high-risk HPV genotypes[6]. Then, improving the local immune status, in the area of action of HPV, can be presumed a good strategy to facilitate the clearance of the virus.

Therefore, we hypothesize that interfering positively in the three modifiable circumstances outlined earlier could be a new strategy to prevent and treat precancerous lesions on HPV-positive women.

Based on this concept, the effect of a vaginal gel based on natural ingredients with repithelizing, moisturizing and microbiota balancing actions (Papilocare, Procare Health, Spain) on both cervical epithelization and vaginal microbiota in HPV-positive women without cytology/colposcopy lesions was evaluated in an exploratory, prospective and observational study.

Twenty-one patients were treated for 21 days. Changes in cervical epithelization degree were evaluated by standard colposcopy and measured by Likert scale (from 5, indicating no cervical ectopy, to 1, i.e., severe ectopy and bleeding). Changes in the microbiota composition were evaluated by 16S ribosomal ribonucleic acid (rRNA) gene pyrosequencing, and proportion of both bacterial phyla and species was assessed. The ectopy degree was significantly improved (mean score baseline 3.79 vs. final 4.47, T-test, $p<0,006$). Of all, 52.6% of the patients improved their cervix epithelization degree, and the score of 5 was observed in 63% of the patients. Also, reduction on vaginal diversity and increased proportion of *Firmicutes phylum*, which lactobacilli belongs, were observed. Specifically, significant increase of *Lactobacillus crispatus* and *iners* was shown (T-test, $p<0.005$)[7].

In conclusion, Papilocare has demonstrated significantly improvement to the epithelization degree, and in the vaginal microbiome status, reducing diversity and significantly increasing the concentration of specific species of *Lactobacillus*. These results suggest interfering in the modifiable factors of HPV persistence may provide positive results on normalizing HPV-dependent cervical lesions.

Conflict of interests

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