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Editorial

The introduction of HPV vaccines in Brazil: advances and challenges

INTRODUCTION

The impact of the human papillomavirus (HPV) infection all over the world is considerable. Its importance as a public health issue is demonstrated by its high frequency, association to uterine cancer (mostly), and clinical implications, besides the psychological impact on the affected people[1].

More than one of two women have been exposed to HPV throughout life; 10% have the virus in its chronic form, and, among these, one of five women has a chance of developing uterine cancer without screening tests or in case, the examination is flawed[2,3]. It is worth mentioning that uterine cervix cancer usually affects women aged around 40 years, at a time when their family, professional, and social responsibilities are significant[4].

It is estimated that annually, around the world, there are about 530 new cases of uterine cervix cancer, and 266,000 deaths associated with HPV[5]. Because of the growing population, if changes are not made in prevention and control, the projections for the next few years reveal an important increase in cases of invasive uterine cervix cancer attributed to HPV 16 and 18 (responsible for 70% of the cancer cases), from 391,016 new cases in 2012 to 444,167 cases in 2025[6]. Therefore, it is currently known that the high disease burden caused by HPV is a global health issue.

According to a report by the World Health Organization (WHO), from September 15, 2010, the epidemiological data regarding the prevalence and incidence of HPV and associated conditions in Brazil are similar to those of other countries, especially in South America, where uterine cervix cancer and genital warts are a major problem for governments and the economically active population[7]. All over the world, 32 million new cases of genital warts are estimated every year; in Brazil, around 1.9 million/year, and most are associated with HPV 6[8,9].

It is important to mention that in Brazil, uterine cervix cancer is the third most common type of cancer affecting the female population; it is estimated that 17,000 new cases will be registered, and that about 5,000 women will die from it every year[10].

Therefore, the availability of vaccines preventing HPV has been a powerful tool to prevent uterine cervix cancer and other HPV-associated diseases. After 2006, these vaccines were licensed in more than 130 countries, and introduced to more than 60 vaccination programs. Australia, the United Kingdom, the United States, and Canada were the first countries to introduce this vaccine[11]. Its safety and efficacy are well established and recognized. Ever since its approval, in June 2006, more than 190 million doses were distributed all over the world[12].

IMPLANTATION OF THE HPV VACCINE IN BRAZIL

In Brazil, the decision to incorporate the vaccine in the National Immunization Schedule of the National Immunization Program (PNI) was preceded by a cost-effective study analyzing different scenarios for its introduction, and the recommendation from the Technical Advisory Group (CTAI) of PNI, which sustained its implantation. After technical analysis, the National Commission of Technology Incorporation in the Unified Health System (CONITEC) approved the introduction of this vaccine to PNI.

The sustainability of this vaccine was guaranteed by a partnership for technology transfer established between the national laboratory Butantan and Merck Sharp & Dohme[13].

Thus, in 2014, the Ministry of Health introduced the quadrivalent HPV vaccine in the Unified Health System (SUS). The vaccine, together with the current actions to screen uterine cervix cancer will allow this disease to be prevented in the next decades[13]. This vaccine protects against the viral types 6, 11, 16, and 18. Viruses 6 and 11 are responsible for 90% of anogenital warts, and types 16 and 18 are in charge of 70% of the uterine cancer cases[14,15]. The target group selected for vaccination included adolescents aged from 9 to 13 years, because this vaccine is highly effective among girls in this age group not exposed to HPV, before sexual initiation, leading to the production of antibodies ten times higher than that found in a naturally acquired infection in a 2-year period[13].

The implantation in Brazil was gradual: in 2014, the HPV vaccine was offered to teenagers aged from 11 to 13 years in the vaccination routine, preferably in public and private schools and in basic health units. The target audience was 5.2 million adolescents in this age group, and the goal was to vaccinate 80% of the target group, which represented 4.16 million girls[13].

In 2015, the HPV vaccine was offered to girls aged from 9 to 11 years. In 2016, it will be available for girls aged between 9 and 13 years[16].

The initial strategy used by the Ministry of Health was the extended one: 1st dose, 2nd dose 6 months later, and 3rd dose 5 years after the 1st dose (0, 6, and 60 months)[13]. In 2016, as in the United Kingdom and other countries, this scheme was changed to two doses (0 and 6 months), because some studies showed that the two-dose scheme presented noninferior antibody response (among healthy girls aged from 9 to 14 years) when compared with women aged from 15 to 25 years who were administered three doses[17]. This recommendation is already in the vaccine information.

In 2015, women aged from 14 to 26 years living with human immunodeficiency virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS) were incorporated to the target population of

the vaccination, considering that the complications resulting from HPV are more frequent among patients with HIV and AIDS[18]. For this population, the HPV vaccines were available not only in the health units, but also in the Reference Centers for Special Immunobiologicals (CRIEs) and in the Specialized Care Services (SAE), which have vaccination facilities. In this population group, the three-dose scheme was maintained (0, 2, and 6 months)[16].

Regarding the safety of the HPV vaccine, since its licensing in 2006, the Advisory Committee on Vaccine Safety from the WHO (GAVCS) has systematically investigated safety matters about HPV vaccines, and has issued many reports about this subject.

Until the present time, there is no evidence to change the recommendations for the use of the vaccine. This conclusion is also restated by the institution in charge of pharmacovigilance in the United States (Centers for Disease Control and Prevention – CDC) and the European Medicines Agency (EMA)[19].

In 2015, scientific societies such as the Brazilian Society of Pediatrics, Infectious diseases, Immunology, and the Brazilian Federation of Obstetric Gynecology elaborated reports supporting the use of the HPV vaccine in the country, reaffirming its safety and importance to prevent uterine cervix cancer.

As the vaccine, in terms of global health, will only have an impact by reaching at least 70% of vaccine coverage, generating "global immunity" (reducing the transmission even among people who have not been vaccinated), a series of initiatives were adopted by the Ministry of Health, involving state and city health secretariats, scientific societies, field of education, and churches. Media campaigns (electronic, spoken, and written) were conducted, and online courses were provided to health and education professionals. Educational materials addressed to health and education teams, to teenagers, and their families were elaborated and distributed.

It is important to mention that the HPV vaccine is available in the public network all year long, for girls aged between 9 and 13 years who have not been vaccinated or who have not completed the scheme (0 and 6 months), and for women aged between 14 and 26 years living with HIV/AIDS[16].

RESULTS

Vaccination data are recorded in real time, in the website pni.datasus.gov.br. There, it is possible to accompany the evolution of the vaccine per federation unit, city, and age group.

Monitoring of such data allowed verifying that the Brazilian strategy used for the first vaccine dose in schools, in 2014, was successful. In less than 3 months, it was possible to overcome the goal of vaccinating 80% of the Brazilian adolescents aged between 11 and 13 years. It is known that countries such as Australia and Denmark have also used schools as a vaccination site, reaching vaccine coverage of 85%, whereas the United States, Sweden, New Zealand, and Germany, which used only the health units, reached less than 40% of the coverage; therefore, it is concluded that this strategy was positive in Brazil[20].

Another successful factor in the first stage of the Brazilian vaccination was the intensive participation of states and cities in the vaccination process, as well as the well-coordinated strategy between health and educational sectors. There was also an efficient communication that provided professionals from these two fields, families, and specially teenagers, with information that sensitized and advised about the importance of this vaccine.

All of these factors allowed Brazil to reach the vaccine coverage goal of 100% for the first dose of the vaccine (D1) by the end of 2014, considering that other countries took much longer to be able to immunize such a large portion of the population; for instance, the United Kingdom vaccinated 4.5 million girls in 2 years[21].

Considering the vaccine coverage data accumulated in Brazil for girls aged from 9 to 11 years vaccinated with the second dose (D2) of the HPV vaccine (**Table 1**), it is possible to observe vaccine coverage of 84%, overcoming the preestablished goal of 80%, in 2014 and 2015. However, in 2015, for the group aged between 9 and 11 years, vaccine coverage for D1 was 63.8% (**Table 2**) and for D2 (**Table 3**) was 36.7%[21]. In this sense, it is essential that local managers can identify strategies and partners they consider to be more adequate to search for higher adherence of this population, also assessing the possibility to promote the vaccination in the school environment, both for D1 and D2. The objective is to improve the vaccination results in this age group, to reach high and homogeneous rates of vaccine coverage, as it happened for the target population aged from 11 to 13 years[21].

The data presented next are preliminary and represent the records of the information system in the PNI until the date this document was elaborated.

ADVERSE EVENTS

In Brazil, in 2014, 1,727 adverse events related to the HPV vaccine were registered, and 91.0% of those were not considered as severe — local reactions (pain at the application site, edema, and moderate erythema) and systemic manifestations (migraine, 100.4ºF fever or higher, syncope, or fainting). Of the 32 severe events, 9 cases of anaphylaxis, 10 neurological events, and 13 reactions of anxiety associated with immunization were observed[22].

As demonstrated, most events associated with the HPV vaccine were classified as mild (not severe). The most frequent syncope among adolescents and young adults was the vasovagal syncope, particularly common among people with emotional lability. There is usually a triggering factor such as intensive pain, expectation of pain, or sudden emotional shock[23]. Many factors, such as prolonged fasting, fear of the injection, warm or crowded places, standing up for a long time, and fatigue can increase the probability of its occurrence. This type of event may occur with any type of injection. Therefore, the Ministry of Health recommends that girls are vaccinated while sitting down.

It is important to clarify that many vaccines (and other injections) rarely produce reactions, and only the cases of anaphylaxis and anxiety reactions were classified as consistent with the HPV vaccine in the evaluation of causality. According to the records, all of these adolescents fully recovered and felt good. In the first stage, 4,987,416 doses of the vaccine were applied, presenting an incidence anaphylaxis rate of 0.7/100,000 applied doses. The analysis

by Brotherton et al.[24] found an anaphylaxis incidence rate in Australia of 2.6/100,000 applied doses, and this country also presented high vaccine coverage, demonstrating that the cases registered in the country were below expectations.

Every event classified as severe is investigated to check if there is a causal relation between the event and the vaccine, or if it is just a temporal relation. When millions of people are vaccinated in a short period of time, diseases and events that would naturally occur with those people can be mistakenly attributed to the vaccine. Therefore, it is necessary to investigate each case.

Vaccination against HPV in the school environment is a strategy used by many countries to reach high vaccine coverage. This strategy was essential for Brazil to have reached 100% of the vaccine coverage in the target population with the first dose—considered to be one of the highest coverage rates in the world. However, the close relationship of the students may favor the occurrence of adverse events related to the anxiety reaction. One adolescent presented signs and symptoms after being vaccinated. Afterward, a group of colleagues began to present, at the same time, an unexpected behavior or apparent sickening without the presence of an apparent cause. Facts like these have happened in Brazil and in other countries, and may be related with any vaccine, as they are associated with the fear of the injection, and not to the composition of the vaccine.

In 2014, 23 cases of psychogenic reaction were notified after the adolescents were vaccinated in the school environment. Psychogenic reactions after vaccination have been reported in other countries, such as Australia. There, in 2007, 720 girls aged between 12 and 17 years were vaccinated (same HPV vaccine used in Brazil) in the same school, and, 2 hours later, 26 girls presented with symptoms including dizziness, syncope, and neurological complaints, such as difficulty in walking. Without evidence of organic etiology after laboratory and imaging examinations, or similar reports of adverse events in another place using the same vaccines, the conclusion was that this was a mass psychogenic response to the vaccination[25].

In Colombia, in August 2014, about 280 adolescents from the same school who were vaccinated against HPV presented symptoms such as fainting, headaches, numbness, and pricking sensation in many parts of the body. After being taken to the hospital, no clinical causes were found to justify these symptoms. In the country, there was speculation that the HPV vaccine had been the cause of these problems, which led the Colombian minister of health to state that these cases had been a psychogenic reaction[25].

CHALLENGES

The Brazilian experience with the operation of major vaccination campaigns was essential to effectively include the HPV vaccine in the National Vaccination Schedule, especially because the target group was a population that is not used to

Table 1 – Vaccine coverage, HPV quadrivalent dose 1, females aged from 11 to 13 years old, 2014.

UF	9 years old			10 years old			11 years old			12 years old			13 years old			Total		
	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)
AC	–	–	–	–	–	–	9,062	9,558	105.5	9,134	8,399	92.0	7,492	8,212	109.6	25,688	26,169	101.9
AM	–	–	–	–	–	–	40,995	30,170	73.6	41,193	6,710	16.3	33,963	4,461	13.1	116,151	41,341	35.6
AP	–	–	–	–	–	–	8,236	9,911	120.3	8,357	7,766	92.9	6,918	7,896	114.1	23,511	25,573	108.8
PA	–	–	–	–	–	–	85,088	88,300	103.8	85,826	82,052	95.6	71,111	78,265	110.1	242,025	248,617	102.7
RO	–	–	–	–	–	–	15,468	15,234	98.5	15,752	14,750	93.6	13,222	13,797	104.4	44,442	43,781	98.5
RR	–	–	–	–	–	–	5,392	5,923	109.9	5,425	4,897	90.3	4,463	4,704	105.4	15,280	15,524	101.6
TO	–	–	–	–	–	–	14,409	17,049	118.3	14,628	14,107	96.4	12,211	13,555	111.0	41,248	44,711	108.4
AL	–	–	–	–	–	–	34,007	35,346	103.9	34,609	31,954	92.3	28,669	31,566	110.1	97,285	98,866	101.6
BA	–	–	–	–	–	–	132,156	130,935	99.1	134,619	119,243	88.6	112,753	122,953	109.1	379,528	373,131	98.3
CE	–	–	–	–	–	–	83,579	94,458	113.0	86,452	85,911	99.4	72,779	87,505	120.2	242,810	267,874	110.3
MA	–	–	–	–	–	–	73,299	75,859	103.5	74,144	66,730	90.0	61,438	66,519	108.3	208,811	209,108	100.1
PB	–	–	–	–	–	–	34,368	36,311	105.7	35,014	33,262	95.0	29,468	29,587	100.4	98,850	99,160	100.3
PE	–	–	–	–	–	–	83,392	82,030	98.4	84,939	78,527	92.5	70,844	81,374	114.9	239,175	241,931	101.2
PI	–	–	–	–	–	–	30,671	30,724	100.2	31,296	28,552	91.2	26,120	27,369	104.8	88,087	86,645	98.4
RN	–	–	–	–	–	–	29,040	26,817	92.4	29,750	25,627	86.1	25,007	27,537	110.1	83,797	79,981	95.5
SE	–	–	–	–	–	–	20,759	22,158	106.7	21,292	19,456	91.4	17,790	20,132	113.2	59,841	61,746	103.2
ES	–	–	–	–	–	–	30,099	33,309	110.7	30,801	28,440	92.3	25,775	27,809	107.9	86,675	89,558	103.3
MG	–	–	–	–	–	–	165,947	187,600	113.1	170,648	159,765	93.6	143,809	159,636	111.0	480,404	507,001	105.5
RJ	–	–	–	–	–	–	129,745	131,210	101.1	133,511	120,133	90.0	111,451	129,402	116.1	374,707	380,745	101.6
SP	–	–	–	–	–	–	330,791	384,440	116.2	339,546	331,474	97.6	283,411	336,248	118.6	953,778	1,052,162	110.3
PR	–	–	–	–	–	–	88,845	93,434	105.2	91,504	76,611	83.7	77,245	79,676	103.2	257,594	249,721	96.9
RS	–	–	–	–	–	–	84,048	81,170	96.6	86,567	77,144	89.1	72,981	81,486	111.7	243,596	239,800	98.4
SC	–	–	–	–	–	–	51,094	53,452	104.6	52,763	47,975	90.9	44,705	51,647	115.5	148,562	153,074	103.0
DF	21,157	16,889	79.8	21,606	19,730	91.3	22,119	11,160	50.5	22,484	5,701	25.4	18,840	2,663	14.1	106,206	56,143	52.9
GO	–	–	–	–	–	–	53,104	58,676	110.5	54,233	52,359	96.5	45,486	49,965	109.9	152,823	161,000	105.4
MS	–	–	–	–	–	–	22,045	27,936	126.7	22,565	24,804	109.9	19,014	24,549	129.1	63,624	77,289	121.5
MT	–	–	–	–	–	–	27,947	28,868	103.3	28,473	25,283	88.8	23,918	25,226	105.5	80,338	79,377	98.8
Brazil	21,157	16,889	79.8	21,606	19,730	91.3	1,705,705	1,802,038	105.7	1,745,525	1,577,632	90.4	1,460,913	1,593,739	109.1	4,954,906	5,010,028	101.1

Source: http://pni.datasus.gov.br

Pop: Population of the federation unit; D1: first dose; Cov (%): vaccine coverage.

Note: Vaccination data (doses and coverage) among girls aged between 9 and 10 years made available only for the Federal District.

Table 2 – Vaccine coverage, HPV quadrivalent dose 1, females aged between 9 and 11 years old, 2015.

UF	9 years old			10 years old			11 years old			Total		
	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)	Pop	D1	Cov (%)
AC	8,669	7,452	86.0	8,853	6,821	77.1	9,062	5,040	55.6	26,584	19,313	72.7
AM	39,826	26,931	67.6	40,378	23,154	57.3	40,995	16,743	40.8	121,199	66,828	55.1
AP	7,787	5,599	71.9	7,999	4,971	62.2	8,236	3,872	47.0	24,022	14,442	60.1
PA	82,101	41,885	51.0	83,547	40,366	48.3	85,088	30,382	35.7	250,736	112,633	44.9
RO	14,687	11,266	76.7	15,071	10,337	68.6	15,468	7,666	49.6	45,226	29,269	64.7
RR	5,193	4,705	90.6	5,286	3,613	68.4	5,392	2,673	49.6	15,871	10,991	69.3
TO	13,696	11,744	85.8	14,036	9,379	66.8	14,409	6,129	42.5	42,141	27,252	64.7
AL	31,843	28,326	89.0	32,878	23,327	71.0	34,007	19,356	56.9	98,728	71,009	71.9
BA	124,991	74,982	60.0	128,467	67,757	51.2	132,156	47,983	36.3	385,614	188,722	48.9
CE	75,760	68,950	91.0	79,479	58,148	73.2	83,579	46,301	55.4	238,818	173,399	72.6
MA	70,124	52,178	74.4	71,624	46,780	65.3	73,299	34,254	46.7	215,047	133,212	62.0
PB	32,675	23,822	72.9	33,505	21,971	65.6	34,368	16,376	47.7	100,548	62,169	61.8
PE	78,606	72,637	92.4	80,892	62,345	77.1	83,392	49,508	59.4	242,890	184,490	76.0
PI	28,752	19,088	66.4	29,654	17,460	58.9	30,671	11,007	35.9	89,077	47,555	53.4
RN	27,132	16,953	62.5	28,040	15,207	54.2	29,040	11,747	40.5	84,212	43,907	52.1
SE	19,196	16,767	87.4	19,926	13,988	70.2	20,759	9,459	45.6	59,881	40,214	67.2
ES	28,088	30,252	107.7	29,033	25,152	86.6	30,099	18,690	62.1	87,220	74,094	85.0
MG	153,328	131,601	85.8	159,404	110,950	69.6	165,947	72,613	43.8	478,679	315,164	65.8
RJ	118,424	80,266	67.8	123,755	72,516	58.6	129,745	58,074	44.8	371,924	210,856	56.7
SP	304,971	299,622	98.3	316,943	254,813	80.4	330,791	158,506	47.9	952,705	712,941	74.8
PR	82,037	57,554	70.2	85,300	49,845	58.4	88,845	35,758	40.3	256,182	143,157	55.9
RS	77,272	57,977	75.0	80,532	53,161	66.0	84,048	40,550	48.3	241,852	151,688	62.7
SC	46,948	47,296	100.7	48,919	37,803	77.3	51,094	23,340	45.7	146,961	108,439	73.8
DF	21,157	3,157	14.9	–	–	–	–	–	–	21,157	3,157	14.9
GO	50,007	33,400	66.8	51,475	29,793	57.9	53,104	23,133	43.5	154,586	86,306	55.8
MS	20,762	17,143	82.6	21,374	14,438	67.6	22,045	10,859	49.3	64,181	42,440	66.1
MT	26,579	20,210	76.0	27,228	16,810	61.7	27,947	12,152	43.5	81,754	49,172	60.2
Brazil	1,590,611	1,261,763	79.3	1,623,598	1,088,905	67.1	1,683,586	772,151	45.9	4,897,795	3,122,819	63.8

Source: http://pni.datasus.gov.br

Pop: Population in the federation unit; D1: first dose; Cob (%): vaccine coverage.

Table 3 – Vaccine coverage, HPV quadrivalent dose 2, females aged between 9 and 12 years old, 2015.

UF	9 years old			10 years old			11 years old			12 years old			Total		
	Pop	D2	Cob (%)	Pop	D2	Cob (%)	Pop	D2	Cob (%)	Pop	D2	Cob (%)	Pop	D2	Cob (%)
AC	4,334	1,423	32.83	8,853	2,632	29.73	9,602	3,512	38.76	4,567	1,794	39.28	26,816	9,361	34.91
AM	19,913	6,511	32.70	40,378	8,872	21.97	40,995	9,874	24.09	20,596	4,071	19.77	121,882	29,328	24.06
AP	3,893	405	10.40	7,999	972	12.15	8,236	2,084	25.4	4,178	1,573	37.65	24,307	5,034	20.71
PA	41,050	5,706	13.90	83,547	12,834	15.36	85,088	18,433	21.66	42,998	10,326	24.02	252,683	47,299	18.72
RO	7,343	2,667	36.32	15,071	4,956	32.88	15,468	5,810	37.56	7,876	3,581	45.47	45,758	17,014	37.18
RR	2,596	833	32.08	5,286	1,684	31.86	5,392	2,418	44.84	2,712	1,607	59.24	15,987	6,542	40.92
TO	6,848	2,001	29.22	14,036	4,211	30.00	14,409	5,907	41.00	7,314	2,387	32.64	42,607	14,506	34.05
AL	15,921	5,614	35.26	32,878	11,333	34.47	34,007	14,212	41.79	17,304	7,708	44.54	100,111	38,867	38.82
BA	62,495	10,932	17.49	128,467	25,377	19.75	132,156	34,106	25.81	67,309	20,167	29.96	390,428	90,582	23.20
CE	37,880	19,002	50.16	79,479	34,305	43.16	83,579	40,167	48.06	43,226	20,342	47.06	244,164	113,816	46.61
MA	35,062	8,484	24.20	71,624	17,612	24.59	73,299	24,932	34.01	37,072	14,328	38.65	217,057	65,356	30.11
PB	16,337	6,043	36.99	33,505	10,669	31.84	34,368	12,743	37.08	17,507	4,818	27.52	101,717	34,273	33.69
PE	39,303	14,738	37.50	80,892	28,459	35.18	83,392	37,008	44.38	42,469	21,210	49.94	246,056	101,415	41.22
PI	14,376	3,400	23.65	29,654	7,605	25.65	30,671	9,344	30.47	15,648	3,638	23.25	90,349	23,987	26.55
RN	13,566	2,035	15.00	28,040	4,673	16.67	29,040	6,837	23.54	14,875	5,237	35.21	85,521	18,782	21.96
SE	9,598	2,626	27.36	19,926	5,894	29.58	20,759	7,288	35.11	10,646	3,539	33.24	60,929	19,347	31.75
ES	14,044	7,504	53.43	29,033	14,743	50.78	30,099	17,181	57.08	15,400	6,199	40.25	88,576	45,627	51.51
MG	76,664	28,718	37.46	159,404	59,893	37.57	165,947	74,604	44.96	85,324	24,735	28.99	487,339	187,950	38.57
RJ	59,212	13,932	23.53	123,755	29,572	23.90	129,745	41,411	31.94	66,755	22,400	33.56	379,467	107,345	28.29
SP	152,485	79,887	52.39	316,943	180,428	56.93	330,791	180,647	54.61	169,773	72,843	42.91	969,992	513,805	52.97
PR	41,018	9,976	24.32	85,300	21,316	24.99	88,845	31,254	35.18	45,752	14,455	31.59	260,915	77,001	29.51
RS	38,636	14,289	36.98	80,532	31,869	39.57	84,048	38,842	46.21	43,293	13,566	31.34	246,509	98,566	39.98
SC	23,474	11,049	47.07	48,919	23,853	48.76	51,094	27,104	53.05	26,469	9,699	36.64	149,956	71,705	47.82
DF	10,578	117	1.11	10,803	162	1.50	–	–	–	–	–	–	21,381	279	1.30
GO	25,003	5,365	21.46	51,475	11,154	21.67	53,104	17,298	32.57	27,116	11,322	41.75	156,699	45,139	28.81
MS	10,381	2,706	26.07	21,374	5,584	26.13	22,045	7,758	35.19	11,300	4,947	43.78	65,100	20,995	32.25
MT	13,289	3,127	23.53	27,228	7,055	25.91	27,947	9,021	32.28	14,236	5,388	37.85	82,701	24,591	29.73
Brazil	795,305	269,090	33.83	1,634,401	567,717	34.74	1,683,586	679,825	40.38	861,720	311,880	36.19	4,975,013	1,828,512	36.75

Source: http://pni.datasus.gov.br

Pop: population in the federation unit; D2: second dose; Cob (%): vaccine coverage.

attending health services to be vaccinated. The articulation of the three management spheres: city, state, and union, both in the health and in the education field, with the acknowledged positive response of the Brazilian population to the initiatives involving the promotion of health, was essential to reach the short-term goal in the first year of the vaccine implantation.

However, it is necessary to constantly mobilize the society toward vaccination, and the attention of the teams regarding its technical and operational aspects, as well as to sensitize the girls and to provide immediate responses to the negative rumors resulting from the mistaken information in the media and social network, without any scientific evidence supporting this news, especially spread by antivaccine groups. The latter is the biggest and the hardest challenge faced to be successful when it comes to high coverage rates and, consequently, the effective protection of the Brazilian adolescents against uterine cervix cancer.

Another important matter is the necessary discussion about the possible adverse events with the health teams, so it is possible to conduct adequate monitoring and notification, and that the diagnosis and care addressed to these possible adverse events can be conducted as fast as possible.

In this context, the involvement of scientific societies is fundamental, asking their members to take part as essential parties in terms of raising awareness about the importance of this vaccine for the Brazilian population.

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Cytotoxic and antiviral activity of extracts and compounds isolated from *Clusia fluminensis* Planch. & Triana (Clusiaceae)

Atividade citotóxica e antiviral de extratos e compostos isolados de Clusia fluminensis Planch. & Triana (Clusiaceae)

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ABSTRACT

Introduction: The worldwide distribution of herpes simplex virus type 1 (HSV-1) allied to the emergence of resistant strains makes necessary and urgent the search and development of new substances capable of preventing and treating HSV-1 infections. Studies demonstrate synergy between genital herpes and human immunodeficiency virus type 1 (HIV-1), which represents a major concern for global public health. **Objective**: The objective of this study was to evaluate the activity of crude extracts and isolated substances from *C. fluminensis* in the *in vitro* replication of the HSV-1 virus and HIV-1-RT activity. **Methods**: This study evaluated the activity of extracts and isolated compounds from *Clusia fluminensis* Planch. & Triana against HSV-1 using Vero cells in culture and against HIV-1 using a recombinant reverse transcriptase enzyme (HIV -1 RT). The percentage of inhibition against HSV-1 was determined from viral lysis plaque reduction assay, and the anti-HIV-1-RT test was performed by a fluorimetric assay. It was also evaluated the cytotoxic activity of the samples using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. **Results**: The crude extracts showed high percentage of inhibition against HSV-1, reaching 81.4 to 100.0% inhibition in non-cytotoxic concentration (50 µg/mL). The isolated compounds, lanosterol and clusianone, demonstrated 100% inhibition in non-cytotoxic concentration (50 µg/mL). We also examined the effects of the extracts and isolates on the activity of the HIV-1-RT. Among the crude extracts, only the methanolic extract of leaves and methanolic extract of stems showed inhibitory activity against HIV-1-RT. Regarding the isolated compounds, lanosterol showed a moderate activity. **Conclusion:** Our data demonstrate that extracts and isolates compounds *Clusia fluminensis* Planch. & Triana have promising antiviral activity inhibiting HSV-1 replication and HIV-1 by inhibiting the anti-RT activity.

Keywords: *Clusia fluminensis*; Clusiaceae; cytotoxic activity; antiviral activity; HSV-1; HIV-1 RT; lanosterol.

RESUMO

Introdução: A distribuição mundial do vírus herpes simplex tipo 1 (HSV-1) aliada ao surgimento de cepas resistentes torna necessária e urgente a busca e o desenvolvimento de novas substâncias capazes de prevenir e tratar infecções HSV-1. Estudos demonstram sinergia entre herpes genital e vírus da imunodeficiência humana tipo 1 (HIV-1), o que representa uma grande preocupação para a saúde pública global. **Objetivo:** O objetivo deste estudo foi avaliar a atividade de extratos brutos e substâncias isoladas de *Clusia fluminensis Planch. & Triana* na replicação *in vitro* do vírus HSV-1 e na atividade anti HIV-1-RT. **Métodos**: Este estudo avaliou a atividade de extratos e substâncias isoladas de *Clusia fluminensis Planch. & Triana* contra o HSV-1 utilizando células Vero em cultura e contra o HIV-1 utilizando a enzima transcriptase reversa recombinante (HIV-1 RT). A porcentagem de inibição contra o HSV-1 foi determinada a partir do ensaio de redução de placas de lise viral, e o ensaio anti-HIV-1 RT foi realizado por um ensaio fluorimétrico. Também foi avaliada a atividade citotóxica das amostras utilizando MTT [brometo de 3- (4,5-dimetiltiazol-2-il) -2,5-difeniltetrazólio]. **Resultados**: Os extratos demonstraram elevada percentagem de inibição contra o HSV-1, atingindo 81,4 a 100,0% de inibição em concentração não citotóxica (50 µg/mL). Os compostos isolados, lanosterol e clusianona, demonstraram 100% de inibição em concentração não citotóxica (50 µg/mL). Examinamos também os efeitos dos extratos e isolados sobre a atividade anti-HIV-1 RT. Entre os extratos brutos, apenas o extrato metanólico das folhas e caules apresentaram atividade anti-HIV-1 RT. Em relação aos compostos isolados, lanosterol mostrou uma atividade moderada. **Conclusão:** Nossos dados demonstram que os extratos e compostos isolados de *Clusia fluminensis Planch. & Triana* possuem atividade antiviral promissora inibindo a replicação do HSV-1 e HIV-1 através da inibição da atividade anti-RT.

Palavras-chave: *Clusia fluminensis*; Clusiaceae; atividade citotóxica; atividade antiviral; HSV-1; HIV-1 RT; lanosterol.

INTRODUCTION

Herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2), two serious human pathogens, are members of the *Herpesviridae* family, a large family of DNA viruses that cause diseases in animals. Both are alpha-herpes viruses that are neurotropic, have a rapid replication cycle and are able to infect a wide variety of cells and hosts. These viruses establish latent infections in sensory neurons and can be reactivated by factors such as stress, fatigue, overexertion, fever, sun exposure, trauma, prolonged use of antibiotics and

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menstruation. Latency allows the maintenance of the viral genome in a non-pathogenic and nonreplicate form and serves as a reservoir for later viral attack of the host, which may increases the pathogenicity of HSV, and can become particularly severe in immunocompromised patients, to whom recurrent HSV-infection may be more extensive and/or aggressive, slow healing and extremely painful[1,2].

HSV-1 is commonly associated with orofacial infections and encephalitis. The public health impact of orolabial herpes caused by HSV-2 is probably small, being this virus primarily responsible for genital infections; however, recent studies have shown that HSV-1 infections also account for a substantial proportion of genital herpes infections[3].

Prevalence of HSV-1 infection is higher in most geographic areas worldwide and more prevalent than HSV-2 infection in non-high-risk populations. Exceptions are groups of immunocompromised patients, such as HIV-positive individuals. Most studies show that HSV-1 infection is acquired during childhood and adolescence, and HSV-1 prevalence increases consistently with age across the age spectrum or stabilizes after age 30[4].

Several clinical and epidemiological studies demonstrate synergy between genital herpes and human immunodeficiency virus type 1 (HIV-1). HIV-1 is the etiologic agent of acquired immunodeficiency syndrome (AIDS) and is still a major concern for global public health. HSV infections are regularly associated with transient depression of cell-mediated immunity, since there is a close relationship between viruses infections and cellular immunological competence of the host. Currently, there is no treatment capable of curing genital herpes and AIDS, and there is no effective vaccine for HSV and HIV-1 yet. Furthermore, only few antiviral drugs are able of shortening the disease and prevent breakouts. These antiviral drugs decrease the rate of virus replication, giving more opportunity for the immune system interference[5].

Although antiviral drugs such as acyclovir, famciclovir and valacyclovir show to be efficient and safe, the emergency of resistant HSV strains has been documented, and an aggravating situation is the fact that HSV is usually resistant to the three of them simultaneously, which decreases treatment options. In fact, the only tools to reduce morbidity and mortality associated with AIDS and its complications are still the prevention and the use of antiretroviral drugs. The antiretroviral drugs act by interacting with major viral proteins of the replication cycle of the virus. The resistance to antiretroviral drugs is largely unavoidable due to the error-prone nature of HIV reverse transcriptase (RT) and its lack of a proofreading function[6].

The need for search and development of new substances capable of preventing and treating infections with HSV-1, HSV-2 and HIV-1 makes natural products interesting targets for assays involving evaluation of antiviral activity.

Among the botanical families, Clusiaceae was chosen because of its great representativeness in Brazil and the description of the use of its species in folk medicine. It comprises 14 botanical genera and is chemically characterized mainly by the presence of xanthones, polyisoprenylated benzophenones, flavonoids and terpenes[7-11].

Clusia fluminensis Planch. & Triana is a native species from the Brazilian coast, with few studies exploring its chemical and biological aspects as a part of the investigation of the biological activities of this species, and in the search of substances with potential to become new drugs for the treatment of herpes and AIDS.

OBJECTIVE

The objective of this study was to evaluate the activity of crude extracts and isolated substances from *C. fluminensis* in the *in vitro* replication of the HSV-1 virus. Singh[12] demonstrated the anti-HIV-1 activity of polyisoprenylated benzophenones isolated from fruits of *Clusia torresi*, with EC_{50} values in the range between 0.02 and 0.8 μM. These results prompted us to also analyze the activity of the extracts and the isolated compounds on the enzyme reverse transcriptase of HIV-1.

METHODS

Plant material

Leaves, stems and flowers from a male individual and fruits from a female individual of *Clusia fluminensis* Planch. & Triana were collected at Forte Barão do Imbuhy, Niterói, in Rio de Janeiro State, Brazil. Flowers were collected in the summer, leaves, stems and partially ripe fruits were collected in the autumn, and completely ripe fruits were collected in the winter. The plant material was identified by Dr. Marcelo Guerra Santos, and a voucher specimen was deposited at the herbarium of the Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro (RFFP), Brazil, registered under the number 9213.

Preparation of plant extracts

Leaves, fruits and stems of *C. fluminensis* were dried in an oven at 40°C and subsequently fragmented. The flowers were not subjected to the drying process. The crude extracts were obtained by static maceration of plant organs with the respective solvents at room temperature for 30 days for methanolic and hexanic extracts, and 15 days for acetonic extracts. The solvent was renewed at each seven days and followed by evaporation under reduced pressure.

Isolated substances

Clusianone (**Figure 1**) was previously isolated from the hexanic extract of the flowers of *C. fluminensis* by countercurrent chromatography using the solvent system *n*-hexane-acetonitrile-methanol (2:1.25:0.5, v/v/v)[13]. A new fractionation of this extract using the same procedure described by Silva *et al.*[13] now allowed the isolation of lanosterol (**Figure 1**), for the first time from the flowers of *C. fluminensis*. The chemical structure of lanosterol was determined by Gas Chromatography-Mass Spectrometry (GC-MS) and The Nuclear Magnetic Resonance (NMR) and spectra were compared with literature data[14]. GC-MS was performed on an Agilent model 6890N gas chromatograph equipped with a mass selective detector, model 5973N with data base. Conditions: carrier gas, helium at 2.0 mL.min^{-1}, split ratio 5:1, injector temperature 300ºC, ion source temperature 230 ºC. Oven temperature was programmed from 150 – 300 ºC at 10 ºC.min^{-1} and held at 300 ºC for 15 min. Sample injection volume was 1 μL. The NMR spectra were acquired using a BRUKER DRX 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts were measured relatively to an internal tetramethylsilane (TMS) reference.

Lanosterol (C30H50O) characterization is made by 1H-NMR (400MHz, CDCl3) and 13C-NMR (100 MHz, CDCl3). 1H-NMR (400MHz, CDCl3): δH: 0.76 (s, 3H, 18-Me), 0.81 (s, 3H, 29-Me), 0.88 (s, 3H, 30-Me), 0.86 (d, 3H, 21-Me), 0.96 (s, 3H, 19-Me), 1.01 (s, 3H, 28-Me), 1.13 (m, 1H, 5-H), 1.31 (m, 1H, 20-H), 1.52 (m, 1H, 17-H), 1.61 (s, 3H , 27-Me), 1.69 (s, 3H, 26-Me), 3.25 (dd, J = 4.4, 11.6 Hz, 1H, 3-H), 5.10 (s, 1H, 24-H); 13C-NMR (CDCl3): δC: 15.7 (C-18), 15.8 (C-29), 17.9 (C-27), 19.1 (C-6), 19.2 (C-21), 20.4 (C-19), 21.7 (C-11), 24.7 (C-30), 25.0 (C-23), 25.9 (C-26), 27.9 (C-7), 28.2 (C-2), 28.3 (C-28), 28.4 (C-16), 30.0 (C-15), 31.1 (C-12), 35.5 (C-20), 35.6 (C-22), 36.1 (C-1), 37.5 (C-10), 39.2 (C-4), 44.3 (C-13), 49.9 (C-14), 50.3 (C-5), 51.2 (C-17), 79.2 (C-3), 125.4 (C-24), 131.1 (C-25), 133.8 (C-9), 134.3 (C-8).

Cell culture and virus

Vero cells were cultured in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 5% fetal bovine serum (FBS; HyClone, Logan, Utah), 100 U/mL penicillin, 100 µg/mL streptomycin, and amphotericin B (25 mg/mL; Cultilab, São Paulo, Brazil), at 37 ºC in a humidified 5% CO_2 atmosphere. Herpes simplex virus type 1 (HSV-1, strain KOS) obtained from Departamento de Virologia Universidade Federal de Santa Catarina, Brazil, was routinely grown. Vero cells and virus stock cultures were prepared from supernatants of infected cells and stored at -80 ºC until use.

Cell viability test

Monolayer of Vero cells in 96-multiwell plates were treated with 25, 50, 100 and 200 µg/mL of the plant extracts, and 50, 250, 500 and 1000 µM of the pure compounds, for 72 h/37 °C. After that, 1 mg/mL solution of 3-(4-5 dimethylthiazol-2yl)-2-5- diphenyl tetrazolium bromide (MTT; Sigma) was added (50 µL/well) to evaluate cell viability according to procedures described elsewhere[15]. The 50% cytotoxic concentration (CC_{50}) was calculated by linear regression analysis of the dose-response curves.

Viral inhibition percentage

The antiviral effect was evaluated by plaque reduction assay[6]. Substances and extracts at 50 µg/mL were added 1 h after addition of viral dilution and left for 20 h. After 20 h, cells were lysed, and the title of supernatant virus was determined by adding different dilutions on plate to verify the viral plaque reduction. The result was obtained by counting PFU, comparing cells treated with the substances and cells of viral control[16,17].

Bacteria and Plasmids

Escherichia coli strain BL21 (DE3) was used as a recipiente for DNA transformations. *E. coli* cells transformed with the plasmid containing RTp66 and RTp51 HIV-1 gene were cultured in Luria-Bertani (LB) containing ampicillin (100 µg/mL) under shaking, at 220 rpm at 37 °C; overnight. This culture was used as inoculum for 1L of LB medium containing 100 µg/mL of ampicillin. Cells were grown for 6 h at 37 °C with vigorous shaking, then induced with isopropyl-b-D-thiogalactopyranoside (IPTG) (1 mM) for 2 h. Cells were harvested by centrifugation (5,000 g, 15 min), and bacterial lysates were prepared using a lysis buffer (50 mM Tris-HCl (pH 7,9 at 4 °C), 60 mM NaCl, 1 mM EDTA and using lysozyme/DNAse I treatment. Clarified lysates were used for the isolation of the p51/p66 heterodimeric RT. The active RT heterodimer was purified by using MagneHis™ Protein Purification System according to the manufacturer's instructions.

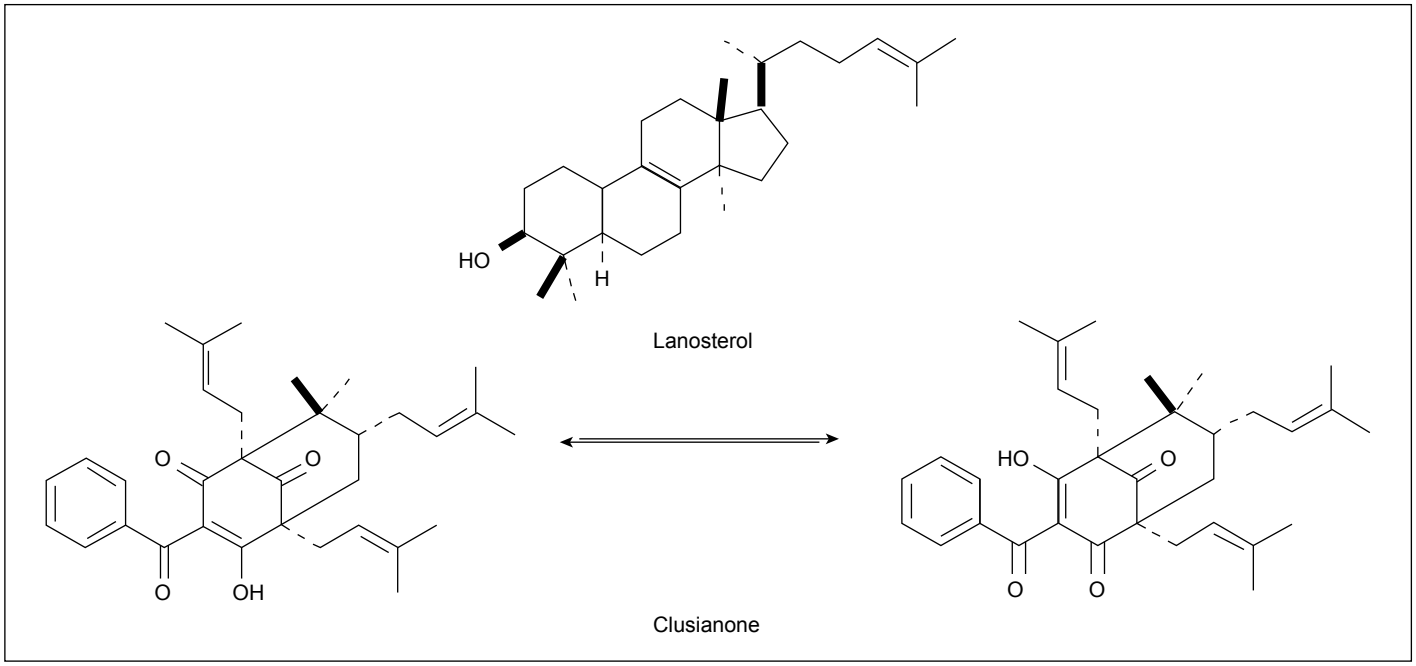

Figure 1 – Chemical structures of lanosterol and clusianone (as a tautomeric pair).

Fluorometic assay of HIV-1-RT activity

An EnzChek RT Assay Kit (Molecular Probes) was used for detecting the RT activity. The mixture of 350 base-poly (A) ribonucleotide template and oligo d(T)16 primer was incubated at room temperature for 1 h. Then, the mixture was added into the tube containing polymerizing buffer (63 mM Tris-HCl, pH 8.1, 8 mM MgCl2, 132 mM NaCl, and 13 mM DTT). The reaction was started by adding the purified enzyme (1 µg/mL) into reaction mixture. The samples were incubated at 37 °C for 30 minutes and stopped by adding 200 mM EDTA. Then, the polymerizing activity was measured using a fluorometric assay by adding PicoGreen in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) to the EDTA-terminated reaction mixture and incubated for 10 minutes on ice and in the dark. The background fluorescence was normalized by subtracting a control reaction value, and the fluorescence intensity was measured in microplate reader Thermomax (Molecular Devices) by using excitation and emission at 502 and 523 nm, respectively.

Statistical analysis

Results of anti-HIV-1 RT activity were expressed as means ± SD of three determinations. The IC_{50} values were calculated using the Microsoft Excel program. Results were considered significant if the $p < 0.05$.

RESULTS

Isolation of lanosterol

A portion (1.0956 g) of the crude hexanic extract from flowers of *C. fluminensis* was fractionated through high speed counter-current chromatography as described by Silva *et al*.[13]. A fraction constituted of white crystals in needle shape was analyzed though GC-MS and revealed the presence of a major substance with the retention time of 18.85 min. and 71.55% purity. The mass spectrum showed the molecular ion of 426 Da and triterpene characteristic mass fragmentation. The ion fragments with m/z = 411 and 393 correspond, respectively, to [M+ - CH_3] e [M+ - (CH_3+H_2O)]. The fragment with m/z = 69 corresponds to the isopentenyl group. The major signal was accompanied by three minor signals, with the same fragmentation pattern of the major component, suggesting that the sample is a mixture of isomers. A portion of the sample was analyzed through ^{1}H and ^{13}C NMR, and the spectral data were compared to those described in literature for lanosterol[14], confirming its structure.

Cell viability, viral inhibition and anti-HIV-1-RT activity

The crude extracts showed high percentage of inhibition against the HSV-1 virus, reaching 81.4 to 100.0% inhibition in non-cytotoxic concentration (50 µg/mL) (**Table 1**). The isolated substances, lanosterol and clusianone, also showed some cytotoxicity compared to acyclovir and both demonstrated 100% inhibition in non-cytotoxic concentration (50 µg/mL) (**Table 2**). The crude extracts showed some cytotoxicity compared to acyclovir, except the hexanic extract of fruits (CFFRH) and methanolic extracts of leaves (CFLM) and fruits (CFFRM) (**Table 1**).

The anti-HIV-1 activities of extracts and isolated products from *C. fluminensis* were investigated using RT fluorimetric assay, where the enzyme activity is determined after treatments in the presence or absence (untreated control) of drugs. Inhibition of HIV-1 RT enzyme was evaluated based on their percentage of inhibition, compared to a negative control and with a standard drug. Only the methanolic extracts of leaves (CFLM) and stems (CFSM) showed inhibitory activity against HIV-1-RT. Among the isolated compounds, lanosterol showed a moderate inhibitory effect (**Tables 1** and **2**).

Previous studies showed that the hexanic extracts of leaves and stems of *C. fluminensis* (CFLH and CFSH, respectively) are composed primarily of terpenes, especially friedelin and epifriedelinol[18]. CFLH and CFSH showed 100% inhibiton of viral replication at 50 µg/mL, making these extracts interesting targets for the search of antiviral substances. However, in a study with friedelin and epifriedelinol, the terpenes did not inhibit herpes simplex virus 1 and 2[19], which means that the antiviral activity of CFLH and CFSH may be due to other substances present in the extracts, and these samples need to go through further chemical investigations.

The hexanic extract of the flowers of *C. fluminensis* showed a CC_{50} of 78 µg/mL and 100% inhibition of the virus in the concentration evaluated. Chemical investigations of the floral resins of *Clusia* species revealed that they are mainly constituted by polyisoprenylated benzophenones[13,20] which are also present in their fruits[21-23]. These substances have a broad spectrum of biological activities, which includes antiviral activity against HIV[20,10].

Table 1 – Anti-HSV-1, anti-HIV-1-RT and cytotoxic activities of extracts from *Clusia fluminensis.*

Samples	Code	CC_{50} (µg/mL)	HSV-1 Inhibition (%)[a]	HIV-1-RT Inhibition (%)[a]
Hexane extract of leaves	CFLH	100	100	Inactive
Hexane extract of stems	CFSH	131	100	Inactive
Hexane extract of fruits	CFFRH	303	95,5	Inactive
Hexane extract of flowers	CFFLH	78	100	ND
Acetone extract of stems	CFSAC	138	100	Inactive
Acetone extract of fruits	CFFRAC	154	100	ND
Methanol extract of fruits	CFFRM	304	81,4	Inactive
Methanol extract of leaves	CFLM	325	100	41,75 ± 11,19
Methanol extract of stems	CFSM	149	100	20,24 ± 6,24
Acyclovir	ACV	216	100	–
Efavirenz	EFV	–	–	92,16 ± 2,34

[a]Determined at 50 µM; ND: not determined.

Table 2 – Anti-HSV-1, anti-HIV-1-RT and cytotoxic activities of isolated substances from *Clusia fluminensis.*

Samples	Code	CC_{50} (µM)	HSV-1 Inhibition[a]	HIV-1 RT Inhibition[a]
Lanosterol	CFFLH16	74	100	77,31 ± 10,74
Clusianone	CFFLH35	121	100	37,6 ± 1,73
Acyclovir	ACV	960	100	–
Efavirenz	EFV	–	–	92,16 ± 2,34

[a]Determined at 50 µM.

Clusianone is the major component of the flowers of *C. fluminensis*, representing about 37% of the composition of the resin from male flowers of this species[24]. It has been isolated from the hexanic extract of the flowers of *C. fluminensis* by Silva *et al*.[13]. Lanosterol has already been isolated from the hexanic extract of fruits of *C. fluminensis*[25]; here we describe the isolation of this triterpene from the hexanic extract of flowers of this species. Both substances showed 100% inhibition of viral replication at 50 µg/mL. Lanosterol was more cytotoxic than clusianone, and this one less cytotoxic than the original crude extract.

In the study conducted with clusianone isolated from the fruits of *Clusia torresii*, this substance was evaluated for its activity against HIV-1 in C8166 cells (human T-lymphoblastoid cells), as well as other benzophenones. The substance was active in a very low concentration, 0,02 µM, however showed high citotoxicity. The benzophenone was more effective when added before or during the viral infection period and also neutralized more than 99% of viral infection when incubated with the virus in the concentration 0,05 µM for 60 min. at 37 °C. The study of the mechanism of action showed that clusianone inhibited the interaction gp120-sCD4, suggesting its interference with virus binding to CD4 cell receptor, preventing infection[23]. In the present study, clusianone showed a weak inhibition on HIV-1-RT. This result complements the information on the mechanism of action of clusianone on HIV, showing that the inhibition of the enzyme reverse transcriptase is not the main mechanism of action of this substance.

The acetonic extracts of fruits and stems are expected to be composed by substances with medium to high polarity, such as benzophenones, while the methanolic extracts of leaves, fruits and stems are expected to have a high content of substances with higher polarity, such as flavonoids and flavonoids glucosydes. Silva and Paiva[26] determined the flavonoid content (as flavones and flavonols) of these extracts, together with their antioxidant activity. The extracts evaluated showed a relative high percentage of these substances, which may also be responsible for their antiviral activities[27,28].

CONCLUSION

The results corroborate that plant extracts are a valuable source of substances with antiviral activities. This study showed that *C. fluminensis* is a promising target for studies in the search for new substances with anti-HSV and anti-HIV-1 activity, including *in vitro* studies of their action mechanisms. Also, the relative low cytotoxicity of the samples makes them potential candidates for *in vivo* studies.

Conflict of interests

The authors have declared no conflict of interest related to the manuscript.

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ONCOGENIC HIGH-RISK HUMAN PAPILLOMAVIRUS DETECTION AND EVALUATION OF RISK FACTORS IN THE CERVICAL INTRAEPITHELIAL NEOPLASIA I

DETECÇÃO DO PAPILOMAVÍRUS HUMANO DE ALTO RISCO ONCOGÊNICO E AVALIAÇÃO DOS SEUS FATORES DE RISCO NAS NEOPLASIAS INTRAEPITELIAIS CERVICAIS GRAU I

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ABSTRACT

Introduction: The cervical intraepithelial neoplasia grade I (CIN I) shows an important percentual of spontaneous regression (80%) and there is no universal consensus if these lesions should be treated or only monitored. Lesions at risk for progression are precisely those associated with high-risk HPV. Knowing which CIN I are related to these viruses may be one of the determining factors for the decision to treat or not. **Objective:** To determine the prevalence of high-risk HPV in women with CIN I and to evaluate if there is an association between the presence of the virus and some risk factors. **Methods**: Cross-sectional descriptive study. We evaluated 55 women with histological diagnosis of CIN I. All of them were previously submitted to HPV-DNA testing. **Results:** 25 out of 55 (45.5%) women analyzed were positive for high-risk HPV. The virus infection was related with higher level of education, smoking and history of sexually transmitted diseases. Other risk factors like age, number of sexual partners, age at first sexual intercourse, use of hormonal contraceptives, and immunosuppression condition did not show a relation to the high-risk HPV infection. **Conclusions:** The prevalence of high-risk HPV in women with histological diagnosis of CIN I was 45.5%. HPV-DNA detection was associated with smoking, history of sexually transmitted diseases, and higher level of education.

Keywords: Papillomaviridae; DNA probes, HPV; cervical intraepithelial neoplasia.

RESUMO

Introdução: As neoplasias intraepiteliais cervicais grau I (NIC I) apresentam um percentual importante de regressão espontânea (cerca de 80%) e não há um consenso universal se essas lesões devam ser tratadas ou apenas acompanhadas. As lesões que apresentam risco de progressão são justamente aquelas associadas aos HPV de alto risco oncogênico. O conhecimento de quais lesões estão relacionadas a esses vírus pode ser um dos fatores determinantes para a decisão de se tratar ou não tais lesões. **Objetivo:** Determinar a prevalência do papilomavírus humano (HPV) de alto risco oncogênico em mulheres com resultado histológico de NIC I e verificar a existência da associação entre a presença do vírus e alguns fatores de risco. **Métodos:** Trata-se de um estudo transversal, de caráter descritivo, onde foram avaliadas 55 mulheres com diagnóstico histopatológico de NIC I. Todas foram submetidas ao teste de DNA-HPV previamente. **Resultados:** Das 55 mulheres analisadas, 25 (45,5%) apresentaram HPV de alto risco positivo. A infecção pelo vírus foi associada às mulheres com mais anos de estudo, tabagismo e à história de alguma doença sexualmente transmissível. Outros fatores de risco, como a idade, o número de parceiros sexuais, a faixa etária em que ocorreu a coitarca, o uso de anticoncepcionais hormonais e o estado de imunossupressão, não mostraram relação com a infecção pelo HPV. **Conclusão:** A prevalência do HPV de alto risco oncogênico nas mulheres com diagnóstico histológico de NIC I foi de 45,5%. A detecção do vírus foi associada ao tabagismo, à história de doença sexualmente transmissível e a um grau maior de escolaridade.

Palavras-chave: Papillomaviridae; sondas de DNA de HPV; neoplasia intraepitelial cervical.

INTRODUCTION

Cervical cancer is the second most common malignancy in the female population wordlwide (about 471,000 new cases per year). About 80% of them occur in developing countries, where in some regions, it becomes the most frequent one. In Brazil, the cervical cancer is the third most common malignancy among women, being preceded only by breast cancer and colorectal cancer, and the fourth cause of death by cancer among women. The estimate of cervical cancer for 2016 is 16,340 new cases and a mean of 5,000 deaths/year[1].

The invasive cervical cancer is preceded by premalignant or precursory lesions, represented by cellular atypia of the cervical epithelium[2]. As for the etiology, it is currently well established that the human papillomavirus (HPV) is the cause of cervical cancer and their precursory lesions, the high-grade cervical intraepithelial neoplasias (CIN II and III)[3-5]. There are over 45 different genotypes of these viruses infecting the anogenital area, both among men and women, which associate to benign lesions and invasive cancers, though most cases are caused by HPV 16 and 18[6,7].

The HPV infection is common, especially among young women, and their prevalence is related, mainly, to sexual behavior[8,9]. The infection alone is not enough to cause cervical cancer. Other factors associated to the host (genetic and immunologic) or environmental (smoking, high-dosage oral contraceptive, diet low in

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vitamins and oligoelements, sexual activity, pregnancies, among others) also contribute to the progression of HPV lesion inducing carcinoma[10]. A compulsory phase in the development of this cancers is the integration of the viral DNA to the DNA of host cells, leading to an uncontrolled cell division and the onset of tumor[11]. This integration happens almost exclusively with the oncogenic high-risk (HR) virus[2,12-15]. It is the persistence of the infection by these specific HPV types, especially the 16 and the 18, the factor responsible for the development, maintenance and progression of a CIN for the invasive cancer[10,12,13,16].

As the cervical carcinoma is preceded by preinvasive lesions, the best prevention is the diagnosis and treatment of these lesions. There is no doubt that all the cases of high-grade lesions (mainly CIN III) should be treated, since they have great chances of evolving into the invasive form[13]. However, the low-grade lesion (CIN I) presented an important percentage of spontaneous regression (about 80%)[17,18] and there is no universal consensus if these lesions should be treated or just monitored[13]. The lesions that represent the risk of progressions are precisely those associated to oncogenic HR-HPV[13,19]. The knowledge of which lesions are related to this virus may be one of the determining factors for the decision of treating or not such lesions.

OBJECTIVE

In order to evaluate the prevalence of the oncogenic HR-HPV in the CIN I and to relate this finding to other possible cofactors associated with the progression of these lesions. It is expected that this study may contribute to establish strategies for a more suited therapeutic planning, avoiding, thus, the treatment of unnecessary cases (overtreatment) or the absence of treatment of the cases of risk for progression into cancer (undertreatment).

METHODS

It is a prospective, observational, descriptive, and cross-sectional study carried out at the Genital Oncology Clinic of the University Hospital of the Universidade Federal de Santa Catarina (HU/UFSC), evaluating 55 women who had histopathological results for CIN I and who performed the DNA test for oncogenic HR-HPV by the hybrid capture method II (Digene & Co.).

The age of those women were stratified into ≤30 years or >30 years of age, because the presence of oncogenic HR-HPV persisting among women aged over 30 years represents a risk factor for the development of cervical carcinoma, according to various studies [9,16,18].

The educational level was stratified into elementary school and high school/college degree in a same group, since there was only one case with college degree and, for statistical reasons, these two variables were combined. There was no discrimination in relation to the level being complete or incomplete.

The number of sexual partners women had in their lives was subdivided into <3 or ≥3 partners. Several studies have showed that the higher the number of sexual partners, the higher is the prevalence of oncogenic HR-HPV[16,18].

Other variables considered to be risk factors for infection by HPV were analyzed such as parity, age at the first sexual intercourse, and current and previous history of sexually transmitted diseases (STDs)[9,18,20].

When we analyzed the variable smoking, women who smoke or who have smoked in the past were considered smokers. Similarly, women who use or who have used hormonal contraception were considered users of this method.

Regarding their immunosuppression status, only the presence or absence of HIV infection was considered, since there were no other cases of immunosuppression among these women.

The results of the oncotic colpocytology were based on the Cytological Classification of Bethesda 2001[21].

The result of the colposcopic evaluation was divided into atypical transformation zone (ATZ) with smaller and greater cervical alterations, depending on the images observed[22].

The anatomopathologic results of the biopsy of cervix were classified into CIN I and alterations consistent with HPV.

The data necessary to conduct this study were obtained by means of the information registered in the medical charts of patients who underwent some kind of follow-up or treatment of cervical intraepithelial lesions in the Genital Oncology Clinic of the HU/UFSC. The data collection was based on a semistructured research protocol.

The colposcopy and the cervical biopsy were carried out in the Ginecology Service and the histopathological tests at the Pathological Anatomy Service of the HU/UFSC. The test to detect oncogenic HR-HPV DNA was performed at the *Laboratório DNAnálise*, in Florianópolis, Santa Catarina. The types of HPV tested were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 68, corresponding to 99% of the HR-HPV. The test was considered positive when the rate of relative light units (RLU) of the test on two positive controls equals 1 pg/ml of DNA-HPV or more. According to recent studies, this cut-off value is what represents greater sensitivity and specificity to the test[23-27].

The analysis of the data was carried out by the Epi Info 6.0 software. In order to verify the possible associations between the presence of HR-HPV and the variables, the chi-square (χ^2) test was used. The result was considered significant if the probability of error was <5% ($p<0.05$). When the p-value did not show significance, it was presented as p=NS.

This study was approved by the Human Being Research Ethics Committee of the UFSC.

RESULTS

The HR-HPV was detected in 25 (45.5%) women with CIN I.

The age of the women varied from 14 to 45 years of age. By correlating age and the presence of HR-DNA-HPV, we observed that there was no significant difference between them. However, on analyzing women with positive HR-HPV, a higher prevalence (60%) of the virus among the youngest ones (≤30 years) was observed.

As for the level of education, there was a prevalence twice as high (68.4%) among women with more years of study (high school to college degree) than among those with lower schooling (33.3%), achieving statistical significance, with p=0.012 (**Table 1**).

The sexual behavior was assessed as the beginning of sexual activity and the number of partners. Regarding the first sexual intercourse, the prevalence of the virus was 1.7 times higher among women who began their sexual activities after 18 years of age; however, the statistical value was not significant.

With regard to the number of sexual partners, despite not being statistically significant, a greater prevalence of DNA-HPV was observed among the women with three or more partners (58.8%) than among women with less than three (39.5%).

Contrary to what was expected, we found greater HR-HPV detection among women who had two pregnancies or less (50%). This infection was present in 36.8% of the women with more than two pregnancies. However, this difference was not significant.

In relation to the habit of smoking, there was a statistically significant difference between smoking women, who had double the prevalence of HR-HPV (63.6%) in relation to nonsmokers (33.3%) (p=0.027) (**Table 2**).

There was no difference regarding the use of oral contraception (OC) and the presence of HR-HPV. More than half of the positive cases (51.4%) were among women using OC.

Of the 55 women, only 8 had the history of STDs (syphilis, anogenital HPV, Chlamydia and Ureaplasma). Of those, seven of them had oncogenic HR-HPV (87.5%), observing a significant difference with p=0.0097 (**Table 3**).

When evaluating the presence of immunosuppression, there was no statistical difference, despite the two cases of HIV infection being positive for HR-HPV (100%). The negative HIV women were positive for HR-HPV in 43.4%.

In relation to the previous colpocytologic test of women diagnosed with CIN I, only 16 (29.1%) had agreeable diagnosis. We also observed the subdiagnosis in 58.2% of them and overdiagnosis (high grade lesion) in 12.7%. As the distribution of the cases was small for each result, these data were not subjected to statistical calculation (**Table 4**).

We observed great correlation between the colposcopic result of ATZ with minor changes and the histologic result of CIN I (87.3%). There was no statistical difference between women with minor alterations (43.8%) or major alterations in the colposcopy (57.1%) (**Table 5**).

Table 1 – Prevalence of the oncogenic high-risk human papillomavirus according to the education level.

Education	Oncogenic high-risk HPV		Total
	Positive	Negative	
	n (%)	n (%)	n (%)
Elementary	12 (33.3)	24 (66.7)	36 (65.5)
High School/ College	13 (68.4)	6 (31.6)	19 (34.5)
Total	25 (45.5)	30 (54.5)	55 (100.0)

Source: *Serviço de Arquivo Médico HU/UFSC.*
HPV: human papillomavirus; χ^2=6.18; p<0.05.

Table 2 – Prevalence of the oncogenic high-risk human papillomavirus according to the habit of smoking.

Smoking	Oncogenic high-risk HPV		Total
	Positive	Negative	
	n (%)	n (%)	n (%)
Yes	14 (63.6)	8 (36.4)	22 (40.0)
No	11 (33.3)	22 (66.7)	33 (60.0)
Total	25 (45.5)	30 (54.5)	55 (100.0)

Source: *Serviço de Arquivo Médico HU/UFSC.*
HPV: human papillomavirus; χ^2=4.89; p<0.05.

DISCUSSION

The relation between the HPV and cervical cancer is already well established[3,4,6,8]. However, there is a considerable variation in the literature about the frequency of detection for DNA-HPV among women with precancerous lesions (CIN). This may be explained by the differences of the evaluated populations and by the kinds of studies, which have varying designs, in addition to the difference on sensitivity and specificity of the tests used[9]. Thus, a great variability in the prevalence of HR-HPV in CIN I is observed, varying from 41[25] to 86%[2,9,12,28-30]. Borges *et al.*[29] observed the oncogenic virus in all ten CIN I cases studied. In our study, we found a prevalence of 45.5%, similar to what was found by Santos *et al.*[25] (41%). Another study carried out in our city showed a very similar prevalence of 47%[30]. Quite different from most studies, Cavalcanti *et al.*[15] found a lower rate, with 16.9% of prevalence for this type of virus.

Table 3 – Prevalence of the oncogenic high-risk human papillomavirus according to previous or current history of sexually transmitted diseases.

STDs	Oncogenic high-risk HPV		Total
	Positive	Negative	
	n (%)	n (%)	n (%)
No	18 (38.3)	29 (61.7)	47 (85.5)
Yes	7 (87.5)	1 (12.5)	8 (14.5)
Total	25 (45.5)	30 (54.5)	55 (100.0)

Source: *Serviço de Arquivo Médico HU/UFSC.*
STDs: sexually transmitted diseases; χ^2=6.68; p<0.05.

Table 4 – Prevalence of the oncogenic high-risk human papillomavirus according to the result of the previous pap smear.

PAP	Oncogenic high-risk HPV		Total
	Positive	Negative	
	n (%)	n (%)	n (%)
Negative	3 (17.6)	14 (82.4)	17 (30.9)
Inflammatory	7 (53.8)	6 (46.2)	13 (23.6)
ASCUS	2 (100.0)	–	2 (3.7)
LSIL	9 (56.3)	7 (43.7)	16 (29.1)
HSIL	4 (57.1)	3 (42.9)	7 (12.7)
Total	25 (45.5)	30 (54.5)	55 (100.0)

Source: *Serviço de Arquivo Médico HU/UFSC.*
PAP: pap smear; ASCUS: atypical squamous cells of undetermined significance; LSIL: Low grade squamous intraepithelial lesion; HSIL: High grade squamous intraepithelial lesion

Table 5 – Prevalence of the oncogenic high-risk human papillomavirus according to the colposcopic findings.

Colposcopy	Oncogenic high-risk HPV		Total
	Positive	Negative	
	n (%)	n (%)	n (%)
Minor alterations	21 (43.8)	27 (56.2)	48 (87.3)
Major alterations	4 (57.1)	3 (42.9)	7 (12.7)
Total	25 (45.5)	30 (54.5)	55 (100.0)

Source: *Serviço de Arquivo Médico HU/UFSC.*
χ^2=0.44; nonsignificant p-value.

Several studies showed that the CIN I is more prevalent among young women[3,9,12]. The same occurs with the infection by HR-HPV. Many studies found this greater prevalence among women with less than 30 years of age[3,13-15,20,31]. However, with aging, despite there is a decline of this prevalence, infection by HPV becomes more persistent[9,13,14,32,33]. In our study, we observed 50% positive results for the HR-HPV for younger women (≤30 years) and 40% for those aged over 30 years. Cohort studies indicate that most infections by oncogenic HPV are transitory and that its lower prevalence in older age may be due to an acquired immunity to the virus through previous exposures and their elimination[34]. Dalstain *et al.*[13] suggest that this greater prevalence of HR-HPV among young women is due mainly to new acquisitions of the virus through sexual relations or reactivations of preexisting infections. Also, since some women with negative HPV have altered cytology, they defend the hypothesis that floating infections, false-negatives or low concentrations of the virus, which were not detected is supported. Woodman *et al.*[20] also confirm these hypothesis and add that it is not possible to distinguish these alternatives.

Our study was a cross-sectional observation, in which a single sample was used to characterize the women with HR-HPV; this can mean only transitory infections or reactivation of latent infections. Also, there might have been false-negatives due to an inappropriate sample or due to low levels of the virus, which were undetectable by hybrid capture.

Many sociodemographic and behavioral factors are classically described as risk factors for cervical cancer. Most authors show significance when comparing data related to education, demonstrating that the risk of cervical lesions is increased among women with lower schooling[12,35,36]. Leal *et al.*[35] suggest that this relation may be explained by the lack of knowledge about the Pap smear and its benefits, in addition to other risk factors associated to low socioeconomic levels. However, Adam *et al*.[9] did not find an association between the level of education and HPV infection. However, we observed that women with higher schooling had greater prevalence of HR-HPV, which was statistically significant (68.4 *versus* 33.3%). However, this finding may not be considered isolated, because there might be an association with other risk factors, which were not surveyed.

According to Hernández-Hernández *et al.*[12], the early onset of sexual activity is associated to infections by HPV and precursor lesions of cervical cancer. Brito *et al.*[37] add that the early onset of sexual activity may increase the sensitivity of the cervix to the effects of a sexually transmitted agent. In our cases, there was no statistical difference when comparing the first sexual intercourse with higher or lower age than 18 years.

The relation between the number of sexual partners and the risk of infection by HPV for the development of precursor lesions of cervical cancer is considered an important risk factor by many authors[12,34,35,37,38]. Ho *et al.*[34] demonstrated that women who had four or more partners, in a period of 6 months, presented a risk almost four times higher of acquiring HR-HPV when compared to women with three partners or less. Leal *et al.*[35] observed greater alterations of the cervical epithelium among women with eight or more sexual partners, with a prevalence of 1.6 times higher when compared to women with only one partner. Our study showed a higher rate of HR-HPV among women who had three or more partners in life; however, this relation was not statistically significant.

Great part of the literature also shows a strong association between the multiple pregnancies and the risk of infections by HR-HPV, as well as risk of invasive cervical cancer[9,10,12,39]. Adam *et al.*[9] demonstrated that three or more pregnancies were considered an independent risk factor for HPV infection. Hernández-Hernández *et al.*[12] also found similar results, demonstrating that the number of pregnancies (three or more previous pregnancies) was the most important cofactor for infection by HPV and the development of a CIN. However, Beby-Defaux *et al.*[14] found a significant decrease in the prevalence of HR-HPV among women with more pregnancies. Although not statistically significant, these data were also observed in our sample, in which women with two pregnancies or less had a higher rate of viral infection (50 *versus* 36.8%), perhaps due to age.

As expected, we observed a significantly higher prevalence of HR-HPV (almost double) between female smokers and former smokers. Smoking is one of the most important risk factors for cervical cancer[5,9,14,35,40]. Moore *et al.*[40] suggest that the components of cigarettes, when secreted through the cervical mucus, cause a local immunosuppressive effect and/or a carcinogenic effect directly on the uterine cervix. Beby-Defaux *et al.*[14] showed an important association between smoking and the HPV infection, once that female smokers had a prevalence of HPV four times as high as nonsmokers. However, some authors do not demonstrate relevance of smoking as a risk factor for the disease[12,15]. In contrast, Ho *et al.*[34] considered it as a protection factor against persistent infections.

The relation between the use of OC and cervical cancer is not a consensus. Most authors consider that the hormonal factor plays a role of cofactor for both the infection by HPV and the progressions of lesions related to the virus[3,14]. More recent researches demonstrate that the risk for these lesions increase significantly among users of OC for more than 5 years[39,41]. However, some authors described that there was no correlation between the use of OC with infection by HPV and their manifestations[12,15]. In the present study, the prevalence of HR-HPV also showed no association with the use of OC, despite a higher rate of the virus (51.4%) being present among its users. Although the HPV has estrogen receptors, it is likely that the relation of OC with this infection and cervical lesions is associated with hormonal concentration.

SDTs have been described as a great risk factor for cervical lesions caused by HPV[35]. Cavalcanti *et al.*[15] when prospectively studying Brazilian women, reported an important contribution of STDs in the progressions of these lesions, suggesting they could act out as cofactors in the activation of the mechanisms of cellular transformation or in the decreased local immunity of the genital tract. Also, when analyzing the different diseases, they found that *Chlamydia trachomatis* and *Neisseria gonorrhoeae* showed greater association. In our study, a greater prevalence of the HR-HPV was observed among women with a history of STDs, with an important statistical value (p=0.0097). Beby-Defaux *et al.*[14], however, did not find a significant relation between HR-HPV and STDs.

Women with immunodeficiency, such as the HIV infection, have increased rates of HPV infection and are more likely to develop high-grade cervical lesions, once that there is a greater tendency to

persistently keep the DNA of the HPV[15,42-44]. Branca *et al.*[42] suggest that the increased risk for high-grade lesions among women with HIV is due to the different risk factors that these women are exposed to, the direct effects of HIV or to the molecular interactions between the HIV and the HPV. In our casuistic, we found just two patients infected by HIV, considering both of them had infections by oncogenic HR-HPV.

The favorable evolution of the low-grade lesions depends on proper handling. Thus, it is important that the investigative methods are reliable, in order to diagnose them at an early stage. However, the citopathologic test is not a perfect screening method. Agorastos *et al.*[45] mention some of the problems involved, such as limitations on the population coverage, technical limitations in relation to the sample, and laboratory error in the test and in the interpretation. They also add that many false-positive cytologic tests lead to frequent and unnecessary invasive procedures. On the other hand, the false-negative could cause serious problems to women. In our study, we observed a high rate of false-negatives (CIN I with cytology without atypia) comprising 54.5% of the case. In the colposcopy, however, there were no false-negatives, once that most findings corresponded to the ATZ of minor lesions. This way, it is important to emphasize that the cytologic screening is only one part of the approach to cervical cancer, considering there should be conducted, at least, one good speculum examination associated to the use of acetic acid and Lugol solution, or ideally, a colposcopy.

The ideal handling of the CIN I lesions is still not a consensus, once that great part of them regresses without treatment. Another part, however, may persist or even progress[17,18,46,47]. Most of these studies show that 80% of these lesions will spontaneously regress within 1 year and just 20% of them will persist. Therefore, it is important to identify which are the persistent lesions, once that they are the higher-risk ones for progression to a high-grade lesion[13,16]. Thus, the use of techniques for the detection of DNA-HPV has been proposed by various authors[31,48,49], once that this test has showed increased sensitivity when detecting high-grade lesions with high negative predictive value[2,23,24]. So, it is argued that those exam would have an important role in the populations with low prevalence of HPV and low risk of cervical cancer, and it may be associated to the cytopathological exam, or even being performed alone[26,45].

Sherman *et al.*[50] demonstrated that when the result of the combined test (cytology and DNA-HPV) is negative, the interval of the cytopathologic tests among these women could be longer. Whereas, if the combined test is positive, this would identify a small group that would need more appropriate further examinations, such as the colposcopy. In face of this evidence, it would be useful to use the DNA-HPV test in screening. With a longer interval between the HPV tests (5 years, for example), we would have a reduction in costs. The use of HPV test could result in improvement of the detection of cervical lesions, with consequent reduction of the incidence of mortality by cervical cancer. **Figure 1** presents a proposed screening of cervical cancer using HPV test, which could be applied in Brazil and could even use a self-collection method.

CONCLUSION

The prevalence of oncogenic HR-HPV among women with histologically confirmed CIN I was of 45.5%, those being the cases with risk for progression and which should be conducted more cautiously. The detection of the virus was associated to smoking, the history of STDs and a higher schooling level.

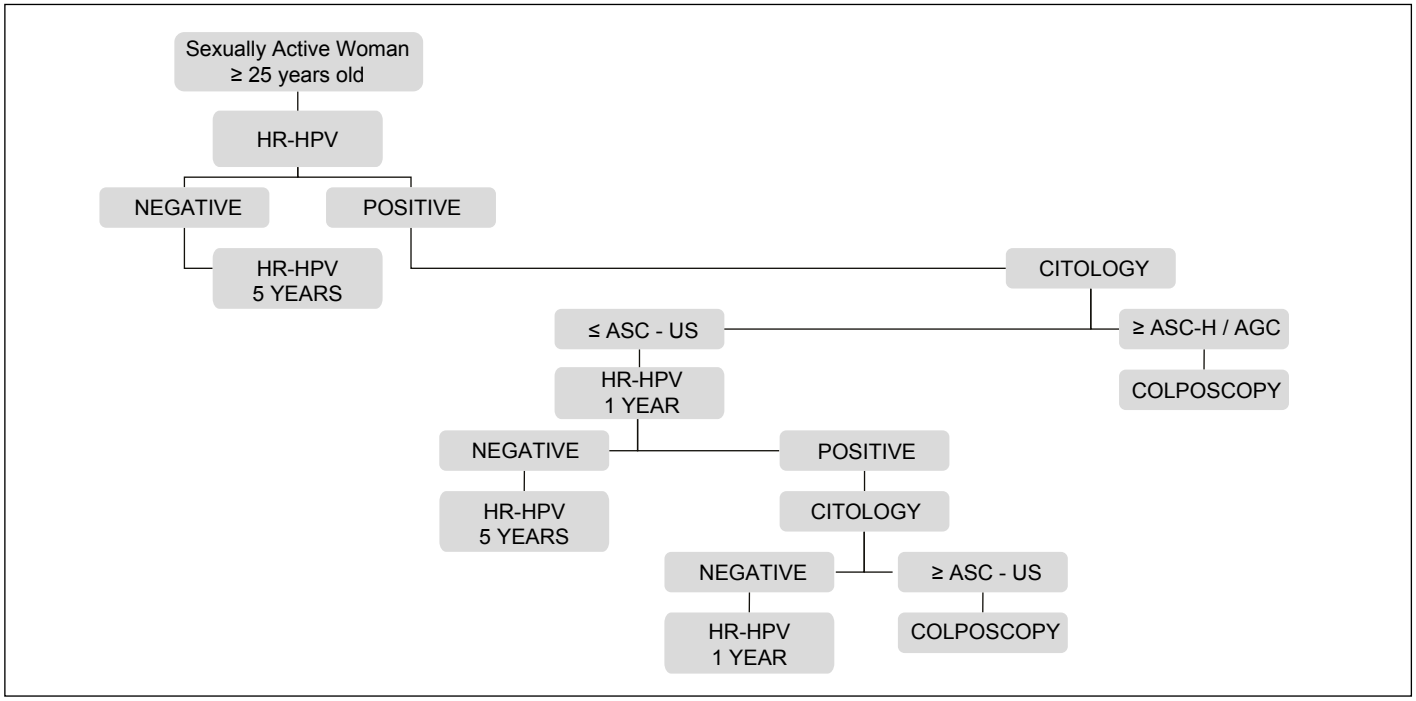

Figure 1 – Strategy of prevention of cervical cancer in Brazil, based on primary screening with DNA test for HR-HPV and secondary screening by means of cytology.

Conflict of interests

The authors reported no conflict of interests.

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Trichomonas Vaginalis infection among women attending in the public service in Rio Grande do Sul, Brazil: frequency, risk factors and clinical signs

Infecção por Trichomonas Vaginalis em mulheres atendidas pelo serviço público no Rio Grande do Sul, Brasil: frequência, fatores de risco e sinais clínicos

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ABSTRACT

Introduction: Trichomoniasis is a cosmopolitan disease that can affect the female fertility, and is commonly underdiagnosed, both in private practices and in public health services, because of the low sensitivity of the wet mount exam used routinely. **Objective:** To understand the occurrence of *T. vaginalis* infection by means of *in vitro* culture in women receiving care in a public health unit, in the city of Pelotas, Rio Grande do Sul, Brazil, as well as to identify the possible risk factors associated with this infection. **Methods:** Cross-sectional study was carried out, which included 201 women undergoing interview and gynecological exam, with the collection of vaginal discharge in the Gynecology Ambulatory at the School of Medicine of Universidade Federal de Pelotas. The material collected was examined in the form of fresh smears and cultivated in Diamond's medium. Epidemiological data were obtained by means of patient interviews and clinical trials, from the medical records. The results were statistically analyzed through χ^2 and Fisher's exact tests, using version 9.0 of the Statistix program. **Results:** The occurrence of *T. vaginalis* infection was 7% (14/201). In the wet mount exam, used routinely for the diagnosis of this agent, only 42.85% of the infected women (6/14) were positive. It was noted that 21.4% of the infected women were asymptomatic, and 89.05% were not aware of the existence of the *Trichomonas vaginalis* infection. Factors independently associated with the infection were the smoking habit (odds ratio [OR] = 11.8), not having a stable sexual partner (OR = 6.36), presence of vaginal discharge with odor (OR = 5.65), and altered vaginal microbiota (OR = 5.31). **Conclusion**: *T. vaginalis* infection was present among the women studied, being underestimated because of the diagnostic technique, and because many of them were asymptomatic. The smoking habit, not having a stable sexual partner, having fetid discharge, and altered vaginal microbiota are the risk factors for infection.

Keywords: *Trichomonas vaginalis*; diagnosis; Trichomonas infections; risk factors.

RESUMO

Introdução: A tricomoníase é cosmopolita, pode afetar a fertilidade feminina, e geralmente é subdiagnosticada, tanto em consultórios particulares, quanto em serviços públicos, devido à baixa sensibilidade do exame a fresco, usado rotineiramente. **Objetivo:** Conhecer a ocorrência de infecção por *Trichomonas vaginalis*, através de cultivo *in vitro*, em mulheres atendidas em unidade pública, na cidade de Pelotas, Rio Grande do Sul, além de identificar os possíveis fatores de risco associados a essa infecção. **Métodos:** Estudo de corte transversal que incluiu 201 mulheres, submetidas a entrevista e exame ginecológico, com coleta de conteúdo vaginal, no Ambulatório de Ginecologia da Faculdade de Medicina da Universidade Federal de Pelotas. O material coletado foi examinado a fresco e cultivado em meio de Diamond. Foram obtidos dados epidemiológicos através de entrevista, e clínicos, nos prontuários. Os resultados foram analisados estatisticamente através dos testes do χ^2 e exato de Fisher, utilizando o programa Statistix versão 9.0. **Resultados:** A ocorrência da infecção por *T. vaginalis* foi de 7% (14/201). No exame a fresco, usado como rotina para o diagnóstico desse agente, apenas 42,85% das infectadas (6/14) foram positivas. Constatou-se que 21,4% das mulheres infectadas são assintomáticas, e que 89,05% desconhecia a existência da tricomoníase. Os fatores independentemente associados com a infecção foram o hábito de fumar (OR=11,8), não ter companheiro fixo (OR=6,36), apresentar corrimento vaginal fétido (OR=5,65) e microbiota vaginal alterada (OR=5,31). **Conclusão:** A infecção por *T. vaginalis* está presente entre as mulheres estudadas, e sendo subestimada, devido à técnica de diagnóstico e por muitas serem assintomáticas. O hábito de fumar, não ter companheiro fixo, ter corrimento fétido e microbiota vaginal alterada são fatores de risco para a infecção.

Palavras-chave: *Trichomonas vaginalis*; diagnóstico; tricomoníase; fatores de risco.

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INTRODUCTION

Trichomonas vaginalis as well as trichomoniasis were described nearly 200 years ago (1836) and trichomoniasis remains the most prevalent nonviral sexually transmitted disease (STD) in the world. The protozoan causes a disease that has a relatively simple and low-cost treatment, but factors such as populational misinformation and the fact that most of the infected individuals are asymptomatic and underdiagnosed explains its wide geographic distribution. Its importance has recently been recognized, as it can cause reproductive complications and facilitate infection with human immunodeficiency virus (HIV)[(1)].

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The World Health Organization estimates that there are 276.4 million new cases per year worldwide[2]. Prevalence studies indicate that data vary according to the diagnostic techniques used, the presence of other STDs, and the socioeconomic conditions of the population studied among others. In Brazil, these prevalences range from 1.9 to 37.5%[3].

The infection by *T. vaginalis* can cause vulvovaginitis, characterized by the presence of yellowish-white discharge, often fetid with the presence of bubbles, and erythematous vaginal and cervical walls. However, about half of the women infected are asymptomatic, and the infection can persist for an average of 3 to 5 years. These characteristics aggravate the problem by allowing the transmission to subsequent partners, and the fact that about a third of them become symptomatic in up to 6 months makes it difficult to establish the source of infection[4].

The diagnostic routine, performed both in private gynecological clinics and in public health services, is usually based on clinical examination, which is difficult as the symptoms are common to other STDs. Laboratory investigation is needed, and is made through direct examination of the vaginal exudate collected during the pelvic examination under light microscopy, during which the motility of living protozoa is perceived[4]. However, the sensitivity of this technique is low, diagnosing the infection in only 30 to 60% of the women infected[5]. Thus, in many undiagnosed and untreated women, the infection may be aggravated, and they become more susceptible to infection by HIV[1], spreading the protozoan to sexual partners, as well as being more likely to have reproductive complications.

In clinical centers, where more improved diagnostic techniques such as *in vitro* culture are offered, which are 70–80% more sensitive than the wet mount technique, the visualization of the parasite is facilitated because of its multiplication[6]. In this technique, the samples are incubated under optimal conditions for 5 to 7 days, and majority of clinics do not have easy access to this diagnosis[7]. On the other hand, a commercial kit, known as InPouch TV, representing the traditional culture method, is so efficient and sensitive that it can be used in laboratories[2]. Molecular techniques, such as the nucleic acid amplification test (NAAT), have 100% sensitivity, but are still not viable as a routine, because it requires special equipment and reagents, technical expertise, and has high cost[2].

Studies conducted in different countries found different factors related to *T. vaginalis* infection in women. Among them are older age, black ethnicity, higher number of sexual partners in the previous year, prostitution, having same-sex partners, using illicit drugs, not using condoms, having other STDs, and having low education levels[8-12].

OBJECTIVE

This study aimed at assessing the occurrence of infection with *T. vaginalis* through *in vitro* culture in women seeking care in a public unit in the city of Pelotas, Rio Grande do Sul, as well as at identifying the possible risk factors associated with this infection and the main related medical conditions.

METHODS

The prospective study was conducted at the Clinic of Gynecology and Obstetrics in the Infant and Maternal Department of the School of Medicine (FAMED) of the Universidade Federal de Pelotas (UFPel), which is a reference center for the diagnosis of STDs in the region. This center provides service to an average of 40 to 50 cases daily, through the Unified Health System. These correspond to the gynecologic procedures (including wet mount test with vaginal content for the diagnosis of *trichomonas* infections), prevention of gynecologic cancers, surgical pathologies, mastology, and the prenatal care for low- and high-risk pregnancies. A sample of each patient was taken to the Parasitology Laboratory of the Biology Institute of the same university, for the diagnosis of *T. vaginalis* through *in vitro* culture. The study was approved by the Research Ethics Committee of the FAMED of the UFPel (Protocol No. 873.180).

A total of 201 patients were analyzed during the period from January to October 2015. Patients awaiting consultation were invited to participate. After clarification of the project and acceptance by the patients, an informed consent was signed, based on Resolution No. 466/12 of the National Health Council of the Brazilian Ministry of Health, for the authorization of the collection of vaginal content during gynecological examination, as well as for the access to the data contained in the medical records. Afterward, they were interviewed by a researcher in a private environment, without witnesses, in secrecy and confidentiality. They answered a questionnaire covering demographic data, sexual habits, symptoms, knowledge of trichomoniasis, and use of condoms, among other details.

In addition to gynecological clinical examination, they were subjected to the collection of material for cytological diagnosis for the prevention of cervical cancer, routinely performed by the accredited laboratory at the clinic. During some consultations, rapid detection tests were also performed for the diagnosis of STDs, such as HIV, hepatitis B and C, and syphilis. In this study, we used cotton swabs and Ayre spatula, with the aid of a speculum, to collect vaginal and cervical content. The diagnosis of *T. vaginalis* was done through direct examination of wet mounts, used routinely in the clinic, and also by *in vitro* culture of the parasite. To perform the wet mount test, a training was given to perform the readings, which were read alternately by two responsible researchers. The examination was performed immediately after the collection of the material that was smeared on to a microscope slide with Brilliant Cresyl Blue solution (to facilitate observation), and covered with a coverslip, then observed through a binocular optical microscope under 40x objective lens. For culture, the swab with the collected material was immediately introduced into an Eppendorf tube containing the Diamond culture medium and transported to the Parasitology Laboratory in an isothermal box. Samples were cultured in Diamond's medium (trypticase–yeast extract maltose – TYM), pH 6.0, supplemented with inactivated 10% adult bovine serum, plus antibiotic and antifungal agents. In the laboratory, the material was homogenized and transferred to 15 mL Falcon tubes containing 9 mL of the same medium, incubated vertically at 37°C; daily observations were made in the microscope, and only after 7 days, the material was considered negative. In positive samples, the motility of protozoa was clearly observed.

Patients for whom the wet mount test was positive, and for those whose result was negative but who showed clinical signs characteristic of trichomoniasis during the consultation, received free treatment with oral or vaginal metronidazole to treat the infection. The patients

whose wet mount was negative, but the culture was positive, were called to receive treatment. Information relating to the positive result of the culture was transferred to the medical records of these patients.

Data were organized using spreadsheets in Excel (Microsoft). First, the independent variables (risk factors for *T. vaginalis*) were subjected to univariate analysis to assess the association of each of the possible risk factors and the dependent variable (positive culture for *T. vaginalis*) through the χ^2 and accurate Fisher's tests. The cut-off point adopted at this stage of the analysis was $p<0.25$, and the associated factors were then evaluated in the logistic regression or multivariate analysis. The model was built using elimination in descending order according to the p-value. The variables with $p\leq0.05$ were maintained until the end of the multivariate analysis. For the study, the Statistix 9.0 software was used. The odds ratio (OR) was calculated with a 95% confidence interval (95%CI).

RESULTS

Among the 201 women surveyed, 14 (7%) were infected with *T. vaginalis*. This result was obtained by *in vitro* culture. In the wet mount test (routinely used), the parasite was detected in only 42.85% of the infected patients (6/14).

The data on the sociodemographic profile and the relationship with trichomoniasis of the patients in the study are shown in **Table 1**. This is a sample population with low levels of income and education, and in most cases, they were 21 to 50 years of age, white, and had a stable partner.

Factors analyzed and their respective frequencies, which were the significant predictors of *T. vaginalis* infection in the univariate analysis ($p<0.25$), are shown in **Table 2**. It was found that 89.05% of the women reported no knowledge of the disease.

After application of the multivariate logistic regression model, it was found that the factors that have a statistically significant relationship ($p=0.05$) with the infection were smoking, not having a steady partner, having vaginal discharge with foul odor, and altered vaginal microbiota because of the decrease or absence of Döderlein bacilli. The risk factors associated with the infection by *T. vaginalis* and their respective OR are shown in **Table 3**.

As for the symptoms presented, it was found that 78.6% (11/14) of the infected women had clinical signs of the disease. The most common signs in these patients were white or yellow vaginal discharge (90.9%), vaginal itching (54.5%), vaginal discharge with foul odor (45.5%), and painful urination (9.1%). **Figure 1** shows the difference in frequency of these symptoms among women infected and uninfected by *T. vaginalis*.

Other infections have been diagnosed during the study, such as those caused by fungi (*Candida*), viruses (HIV, human papillomavirus [HPV], hepatitis C, and herpes infections), or bacteria (syphilis and *Gardnerella vaginalis* according to Amsel's criteria), through the wet mount test or through quick detection tests. However, there was no statistically significant difference between the frequency of these infections in women infected or uninfected by *T. vaginalis*. In the population studied, 48.3% (97/201) were infected by at least one of the above said agents.

DISCUSSION

Most studies held in Brazil on the prevalence of infection by *T. vaginalis* were carried out through the diagnosis by wet mount test or cytopathology, the routine techniques for the diagnosis of parasitosis. Prevalence rates in these studies vary from 0.6% in women of Alta Sorocabana, São Paulo[13] to 10.5% in women of

Table 1 – Sociodemographic profile of 201 patients surveyed at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, between January and October 2015, and its relationship with infection by *Trichomonas vaginalis*.

Characteristics	Frequency		Tv positive (n=14)	Tv negative (n=187)
	n	%	n (%)	n (%)
Age				
>40 years	90	44.77	5 (35.7)	85 (45.5)
≤40 years	111	55.22	9 (64.3)	102 (54.5)
Ethnicity				
White	143	71.14	7 (50.0)	136 (72.7)
Black/brown	58	28.85	7 (50.0)	51 (27.3)
Marital status				
Married	141	70.14	7 (50.0)	134 (71.7)
Single, separated, divorced, widowed	60	29.85	7 (50.0)	53 (28.34)
Education				
Complete high school or more	72	35.82	4 (28.6)	68 (36.4)
Incomplete high school or less	129	64.17	10 (71.4)	119 (63.6)
Income				
>1 minimum wage	31	15.42	5 (35.7)	26 (13.9)
≤1 minimum wage	170	84.57	9 (64.3)	161 (86.1)
No. of people living in the same residence				
≤5 people	185	92.03	13 (92.9)	172 (92.0)
>5 people	16	7.96	1 (7.1)	15 (8.0)

Tv: *Trichomonas vaginalis.*

Table 2 – Frequency of significant risk factors predictive of *T. vaginalis* infection in the univariate analysis (p<0.25) in women treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, in southern Rio Grande do Sul (n=201).

Variables	Frequency		Diagnosis				p-value
	n	%	Positive		Negative		
			n	%	n	%	
Being a smoker	59	29.35	9	15.25	50	84.74	0.0029
Itching	40	19.90	6	15.00	34	56.66	0.0257
Unawareness of trichomoniasis	179	89.05	11	6.14	168	93.85	0.1927
Black or mixed ethnicity	58	28.85	7	12.06	51	87.93	0.0703
Vaginal discharge	92	45.77	9	9.78	83	90.21	0.1494
No fixed sexual partner	60	29.85	7	11.66	53	88.33	0.0876
Altered vaginal microbiota	120	59.70	12	10.00	108	90.00	0.0397
Income up to 1 minimum wage	170	84.57	9	5.29	161	94.70	0.0293
Being in menopause	45	22.38	1	2.22	44	97.77	0.1560
Having fetid odor	39	19.40	5	12.82	34	87.17	0.1096
Having other STDs	86	42.78	3	3.48	83	96.51	0.0940

STDs: sexually transmitted diseases.

Table 3 – Factors significantly associated with infection by *T. vaginalis* in patients treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, between January and October 2015 (multivariate analysis, p≤0.05).

Characteristics	Positive		Negative		OR	95%CI	p-value
	n	%	n	%			
Being a smoker	9	64.3	50	26.7	11.84	2.5–55.8	0.0018
No fixed sexual partner	7	50.0	53	28.3	6.36	1.4–28.9	0.0166
Vaginal discharge with foul smell	5	35.7	34	18.2	5.65	1.2–26.2	0.0270
Altered vaginal microbiota	12	85.7	108	57.8	5.31	1.0–29.2	0.0545

OR: odds ratio; 95%CI: 95% confidence interval.

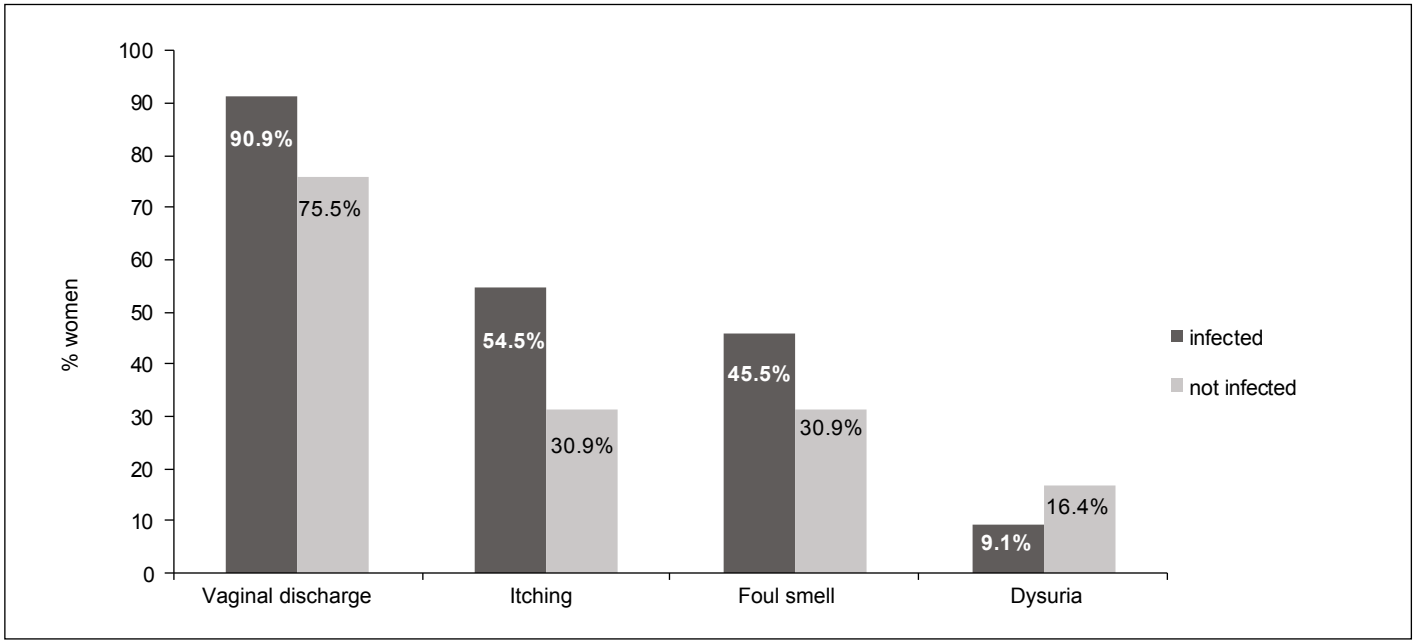

Figure 1 – Frequency of major symptoms in women treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, infected and not infected with *T. vaginalis*, from January to October 2015.

Vitória de Santo Antônio, Pernambuco[14]. In this study, the infection by protozoan was detected in 3% (6/201) of women through a wet mount test. This is a low-sensitivity diagnostic technique, as it needs a higher concentration of the parasite in the vaginal content so that they can be observed. However, when *in vitro* culture was performed for this diagnosis, it was found that 7% (14/201) of the women were infected. This result corroborates with the results of the studies by other authors in Brazil, who, using this technique, found prevalence rates ranging from 2.5[15] to 13.5%[16], with an average of 5.8%. But when female prisoners[8] or women living in extreme poverty[17] were examined, these rates reached between 30 and 20%, respectively, showing the importance of hygiene, cultural, and behavioral habits to fight this parasitosis. Infection rates can also be influenced by factors such as the stage of menstrual cycle at which the collection is made[3].

The *in vitro* culture is considered the gold standard for the diagnosis of trichomoniasis, for its high sensitivity, and for the difficulty and cost of molecular techniques[6]. However, it is still not used as routine diagnosis, as trichomoniasis is a neglected disease that receives little attention from health agencies, despite being widely disseminated and representing a public health problem.

The sociodemographic profile of the women studied, most with low education and income levels, is due to the fact that the study was conducted in a clinic from the public health service. The most frequent age group, 21 to 50 years, is exactly that of sexually active women who often seek gynecological care. The large percentage of women who are unaware of the disease (89.05%) makes them more vulnerable to acquiring the infection, as well as other STDs.

The risk factors, most often associated with the infection as cited in the literature, in the order of frequency are having more than one sexual partner in the last 3 months, not using condoms, black ethnicity, older than 40 years, low education, illegal drug use, smoking, alcoholic, and partner with low education level[8,12,18,19]. In this study, it was found, by bivariate analysis, that the factors that were significant predictors of infection by *T. vaginalis* were smoking, being black or brown, not having a steady partner, income below the minimum wage, being in the menopause, unaware of the disease, presenting vaginal discharge, itching or foul odor, having altered vaginal microbiota, and other STDs, such as herpes, HPV, syphilis, hepatitis B and C, and HIV. The clinical and pathological factors, already reported by other authors, were the existence of other STDs, altered vaginal microbiota, symptoms such as itching, vaginal discharge, foul smell, dysuria, dyspareunia, and pelvic pain[11,14]. It is evident in this and other studies that the economic factor is relevant, as it interferes with education, ignorance of STDs, and consequent oversights in prevention.

Among the risk factors, the one that is statistically associated with infection (logistic regression) is smoking, which increases the risk of infection up to 11.48 times, as observed by other authors[20-23]. It was also found that women who have no steady partner had 6.36 times greater risk of acquiring the infection than those who have, a factor of disagreement between authors, because although some agree with this information[24], as the first group of women generally have a greater number of partners making them more exposed to STDs, other authors have found higher prevalence rates among those with a steady partner[18] and who claim to trust their partner and who do not use condoms. The presence of vaginal discharge with foul smell is a strong clinical evidence of this infection, and women with this symptom were 5.65 times more likely to be infected, confirming with the results of other findings[14,20]. Another associated factor was the presence of altered vaginal microbiota (OR=5.31), as the protozoan causes vaginitis. It has also been reported as a risk factor by other authors[25]. The inflammatory reaction caused by *T. vaginalis* generally favors the installation of anaerobic bacterial vaginitis, responsible for the release of amines that produce foul odor. Also because of the inflammatory reaction, there is the accumulation of leukocytes in the vaginal content, observed even in the wet mount test.

Many patients infected with *T. vaginalis* are asymptomatic, which further complicates the control of the disease, as they are a source of infection, being exposed to the complications of the infection, and not being induced to seek medical help. In the group studied, 21% of the infected women were asymptomatic. This percentage may vary from 10 to 50%, even if *in vitro* culture is used as a diagnostic technique[20]. Several factors can interfere in these indices, such as the time between the collection and the culture of material, its precision, the absence of secondary contamination that may prevent the growth of protozoa, and the population studied.

The most common symptoms found in women infected with *T. vaginalis* (yellowish-white discharge, foul odor, and itching) coincide with those described by other authors[6,14,20]. Although they occur in other gynecological infections, they are strong indications for the clinical diagnosis of trichomoniasis, which was confirmed in this study by the higher frequency of these symptoms among patients infected by the protozoan.

The fact of being infected with trichomoniasis or not did not significantly alter the frequency of other infections diagnosed during the study (candidiasis, HIV infections, HPV infections, hepatitis C, syphilis, and *Gardnerella vaginalis*). However, in other studies, it was found that women with genital itching and those diagnosed with syphilis, herpes, *Chlamydia trachomatis,* or *Mycoplasma genitalium* were at a risk for infection with *T. vaginalis*[22]. It has also been found that the HIV infection facilitates the infection of *T. vaginalis* and vice versa[5]. In fact, the presence of an STD indicates exposure to the diagnosed agent and also to other agents that can be transmitted in the same way.

CONCLUSION

Infection by *T. vaginalis* is prevalent among the women studied, many of whom were asymptomatic. This is an underdiagnosed infection because, besides the fact that there is a great ignorance among the patients about the infection, the routine diagnostic technique (wet mount test) is not very sensitive, and the culture is still a laborious and not a very accessible technique. Smoking, not having a steady partner, presenting vaginal discharge with foul odor, and abnormal vaginal microbiota are associated with the increased risk for this infection. Ignorance of the disease, high number of infected women, asymptomatic patients, and difficulties in the deployment of more sensitive diagnostic techniques reveal an alarming picture on trichomoniasis, which requires public educational measures and changes in the routine diagnosis.

Conflict of interests

The authors report no conflict of interests.

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Comparison between knowledge, behavior and risk perception about the STD/AIDS in medicine and law students from PUC-GO

Comparação entre conhecimento, comportamento e percepção de risco acerca das DST/AIDS nos estudantes de medicina e direito da PUC-GO

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ABSTRACT

Introduction: There has been a rise in the incidence of STIs/AIDS in the young Brazilian population; therefore, college students are a major focus for change of risky sexual behaviors. **Objective:** To analyze knowledge, sexual behavior, and risk perception of students in different years of the medical and law programs at the Pontifical Catholic University of Goiás (PUC-GO), Brazil, with regard to STIs/AIDS. **Methods:** Administration of anonymous questionnaires to students in their first, third, and last years of the medical and law programs at PUC-GO, using probability-proportional-to-size sampling and with margin of error set at 5%. **Results:** Medical students answered 201 questionnaires and law students 441 questionnaires. The comparison of both programs revealed that 40.3% of law students and 19.6% of medical students believe that HIV is transmitted through kissing and that 39.9% of law students and 29.3% of medical students believe that this virus is also transmitted through utensils. Consistent condom use was reported by 21.2% of medical students and 30.1% of law students. Medical students have greater risk perception of sexual behavior, and 83.8% claim they have been exposed to STIs; furthermore, 72.6% of law students believe they are at risk. **Conclusion:** There was an increase in medical students' knowledge about STIs/AIDS throughout the program. Nevertheless, medical students adopt riskier sexual behavior, which is caused by the lower frequency of condom use. Medical students have, however, increased risk perception regarding sexual behavior.

Keywords: acquired immune deficiency syndrome; sexually transmitted infection; health vulnerability; sexual behavior; knowledge; disease prevention.

RESUMO

Introdução: Vem ocorrendo um aumento da incidência de DST/AIDS na população jovem brasileira, assim, os universitários são um importante foco para mudança de comportamento sexual de risco. **Objetivo:** Analisar o conhecimento, o comportamento e a percepção sexual de risco acerca das DST/AIDS dos estudantes de diversos anos dos cursos de Medicina e Direito da Pontifícia Universidade Católica de Goiás (PUC-GO). **Métodos:** Aplicação de questionário anônimo a alunos do primeiro, terceiro e último anos dos cursos de Medicina e Direito da PUC-GO, considerando amostra probabilística proporcional e com margem de erro de 5%. **Resultados:** Foram respondidos 201 questionários pelos estudantes de Medicina e 441 pelos de Direito. Quando comparados os dois cursos, 40,3% dos estudantes de Direto e 19,6% da Medicina consideram que o HIV é transmitido pelo beijo e 39,9% do Direito e 29,3% da Medicina acreditam que esse vírus também seja transmitido por utensílios. O uso consistente do preservativo foi referido por 21,2% dos alunos de Medicina e 30,1% dos de Direito. Os estudantes de Medicina possuem maior percepção sexual de risco, com 83,8% considerando estarem sujeitos às DST; no Direito, 72,6% dos estudantes se consideram sob esse risco. **Conclusão:** Houve aumento do conhecimento acerca das DST/AIDS pelos acadêmicos de Medicina ao longo do curso. Apesar disso, os acadêmicos de Medicina adotam maior comportamento sexual de risco no que diz respeito à menor frequência de uso do preservativo. Os acadêmicos de Medicina apresentam, entretanto, maior percepção sexual de risco.

Palavras-chave: síndrome da imunodeficiência adquirida; doenças sexualmente transmissíveis; vulnerabilidade em saúde; comportamento sexual; conhecimento; prevenção de doenças.

INTRODUCTION

The university years are a transitional period between adolescence and adulthood, presenting big transformations in social, cognitive, and emotional aspects. Among such changes, sexuality and romantic relationships are highlighted. During the university years, a young person comes into contact with new ideas, practices, and thoughts, which awaken their curiosity about trying different experiences, especially sexual ones. Such practices make this group vulnerable to the adoption of risky behaviors, making them susceptible to infections such as sexually transmitted infections (STIs) and acquired immune deficiency syndrome (AIDS)[1].

Young people's sexual behavior must be assessed while considering social inequalities, culture, sex, and education, which are important determinants for risky behavior, as acquired infections resulting therefrom may increase mortality in this population. Therefore, it is important to understand the reality of this group in the different settings in which they are situated, as the use of preventive measures in sexual intercourse not only is scientifically standardized but also supported by behavior[2-4].

Several factors explain young people's increased vulnerability to infection by STIs/AIDS[5], such as misinformation, overconfidence with regard to vulnerability, social and family taboos on sexuality, and information from unqualified sources, which may interfere negatively on their sexual behavior. Adolescents and young adults are also exposed to the risk of acquiring HIV because they often engage in intercourse with multiple partners, and many do not use condoms in all sexual relations[6]. Another common risky behavior for this age group is the beginning of a new relationship, in which many young people unrealistically idealize their partners, deeming

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them perfect, healthy, and incapable of maintaining relationships with other people; thus, these young people conclude they are not at risk[7,8]. In the presence of an HIV/AIDS epidemic, it becomes necessary to monitor the incidence and prevalence of STIs, because genital ulcers facilitate HIV penetration, increasing the risk of infection by up to 18 times if one of the partners is a carrier of an untreated STI. Another reason to monitor STIs is that they reflect a presumed lack of concern with the consequences of unprotected sexual relations, as they are transmitted during sexual intercourse[6,9,10].

According to the Joint United Nations Program on HIV/AIDS (UNAIDS), the use of condoms during sexual intercourse is the most effective method available to prevent sexual transmission of HIV and other STIs. It is a relatively cheap method, with little or no use restrictions; they can be used safely without requiring any particular skills; furthermore, it is an effective method of contraception and prevention of other STIs[11,12].

According to the *Epidemiological Bulletin on HIV/AIDS* published in 2013, 39,185 cases of AIDS were reported in Brazil in 2012. The national detection rate was 20.2 cases per 100,000 inhabitants. The highest detection rate was observed in the South region (39.9/100,000), followed by the North region (21.0/100,000), the Southeast region (20.1/100,000), the Midwest region (19.5/100,000), and the Northeast region (14.8/100,000). Between 1980 and June 2013, 686,478 cases of AIDS were reported, of which 64.9% are male subjects and 35.1%, female subjects[13].

The *2013 Epidemiological Bulletin of the State of Goiás* reported 12,109 cases of AIDS in people over 13 years of age since the beginning of the AIDS epidemic, in 1984, to September 5, 2013, of which 67.4% were in male subjects and 32.6%, in female subjects. From the total, 1,492 cases were young people aged 15 to 24 years (12.3%). The distribution of AIDS cases by sex highlights its feminization from 1988 onward, when the sex ratio, which was 13 male cases to one female case, reached the ratio of 1:8 in 2011, according to the Information System on Diseases of Compulsory Declaration (SINAN). In terms of age, the largest number of cases is in individuals aged between 20 and 49 years, represented by 84% of all cases[3,10,14].

According to estimates from the Department of STIs, AIDS, and Viral Hepatitis, approximately 718,000 people live with HIV/AIDS in Brazil. In the young population, the prevalence rate of HIV infection has a tendency to increase. In the age group between 17 and 21 years, which coincides with the start of university life, the prevalence of HIV infection increased from 0.09% in 2002 to 0.12% in 2007, with the most significant increase in the population of young men who have sex with men (MSM)[10]. In view of that fact, it is necessary to place this population at the top of the public policy priorities.

It is important that universities recognize their role in implementing strategies for the prevention of STIs/AIDS through teaching, research, and extension programs. Studies show that university students are knowledgeable about STI/AIDS transmission. However, some research shows that knowledge does not create change in behaviors related to disease prevention, as students do not have the perception of vulnerability in this regard[3,4,10,15].

As medical students are naturally exposed to specific knowledge owing to more extensive contact and, often, assistance to patients who are carriers of such *pathoses*, a different behavior is expected in comparison with programs outside the biological field. Although law programs do not provide technical knowledge about these infections, it provides information to support change in people's risky behaviors, as the human sciences seek to understand human beings and everything that surrounds them, addressing various aspects related to them as individuals, people, and subjects as well as their behavior in society. Therefore, by studying the human being and understanding their behavior, it is possible to change their actions.

OBJECTIVE

To identify and compare knowledge about STIs/AIDS and the sexual behavior associated with the risk of HIV infection or other STIs among students in their first, third, and last years of the medical and law undergraduate programs.

METHODS

We conducted a descriptive and analytical cross-sectional study that operated under the assumption that there is a variation between risky behaviors related to STIs/AIDS in different university programs. We administered a modified questionnaire[2,7,15] during classes from May to June 2015, clarifying and explaining the purpose of the survey to students. It was a self-administered questionnaire with 38 objective questions related to knowledge, behavior, and risk perception regarding STIs/AIDS, which were answered after participants read and signed the informed consent form. Sample comprised students in their first, third, and last years of the medical and law programs at the Pontifical Catholic University of Goiás (PUC-GO) who were duly enrolled and attending school during the data collection period. Classes were randomly selected, respecting the proportion of the sample number per period.

Precisely 4,430 students were enrolled in the first, third, and fifth years of the law program. With a margin of error set at 5%, the sample consisted of 441 students. Precisely 271 students were enrolled in the first, third, and sixth years of the medical program. With the same margin of error, the sample consisted of 201 students.

We analyzed and compared the results from the three years within the same program and the results from both programs. For the analysis of categorical and nonparametric variables, we used the χ^2-test with 5% significance level.

This study was approved by the Research Ethics Committee of the PUC-GO on May 11, 2015, with the Certificate of Presentation for Ethical Appreciation (CAAE) No. 42351915.4.0000.0037.

RESULTS

Among the 201 students of the medical program at PUC-GO who answered the questionnaire, 81 were enrolled in the first year, 61, in the third year, and 59, in the sixth year. Of the total, 127 were female and 74 male students. There was a significant difference in the age group studied: 85.3% of the first-year students and 63.3% of the third-year students were aged between 18 and 21 years, while 52.2% of the last-year students were aged between 22 and 26 years ($p<0.001$). In the law program, 441 students answered the questionnaire: 158 were enrolled in the first year, 103, in the third year, and

180, in the sixth year. Most first- and third-year students were aged between 18 and 22 years (60.8 and 73.3%, respectively), while most fifth-year students were aged between 22 and 26 years (62.6%). By contrasting students from both programs, there was a significant difference in age, and the majority of law students was over 26 years.

With regard to the characteristics analyzed in medical and law students, respectively, there was no significant difference in gender and former higher education. There was a significant difference in the number of people living with each student and in family income among students in varied years of the medical and law programs. In the law program, there was a significant difference in student's marital status: the majority of the first- and third-year students was single, and the majority of fifth-year students reported being in a serious relationship. By contrasting both programs, there was as a statistically significant difference in relation to marital status, and medical students showed a higher percentage of single individuals. There was also a difference in the employment status during the programs, both between the various years of the law program and between the medical and law programs, as most law students work and most medical students do not.

As to the aforementioned knowledge about STIs/AIDS, there was a difference between programs, and 13% of law students reported not having knowledge about them. In the medical program, there was a significant difference regarding which of the infections in question (AIDS, hepatitis B, syphilis, gonorrhea, candidiasis, chlamydia, genital herpes, Gardnerella, and HPV) were considered STIs, as 77% of third-year students and 77.6% of sixth-year students recognized hepatitis B as an STI, while 50% of first-year students stated otherwise. The same happened with candidiasis, which most first-year students (72.5%) considered an STI.

Among law students, there was a statistical difference in relation to knowledge on gonorrhea and on hepatitis B and candidiasis. Among first-year students, a higher percentage (73.9%) did not consider hepatitis B an STI; this percentage was lower among students enrolled in the remaining years. However, the prevailing answer in all analyzed years was that hepatitis B is not an STI. The opposite happened with gonorrhea, and 87.3% of first-year, 91.2% of third-year and 83.1% of fifth-year students considered it an STI.

By contrasting specific knowledge on STIs between the two programs, there was statistical difference between all infections, with greater knowledge among medical students, except on genital herpes and Gardnerella, as shown in **Table 1**.

First-, second-, and last-year students from both programs were well-informed about the ways HIV is transmitted, as most stated that HIV is transmitted both sexually and vertically and through needle sharing, in addition to being prevented by condom using and not prevented by the sole use of combined oral contraceptive pill (COCP). However, by contrasting the medical and the law programs with regard to HIV transmission through kissing and utensils, there was a significant difference, in which 40.3 and 39.9% of law students believed that AIDS can be acquired through kissing and utensils, respectively, and 19.2 and 28.4% of medical students believed they were transmission routes, respectively. To acquire knowledge on STIs/AIDS, students reported using the following media: the Internet, the media, university, friends, and family.

Most first-year (62%), second-year (69%), and third-year (81%) medical students reported having active sex lives; most revealed the sexarche (age when a person first engages in sexual intercourse) between 16 and 19 years. In the law program, there was a significant difference with regard to having active sex lives because, although most students in all years studied were sexually active, this percentage was higher in fifth-year students (85.8%). The majority of students in all these years and from both programs reported sexual intercourse with people of the opposite sex. In contrast to what happened in the medical program, the majority of law students revealed the sexarche between the ages of 20 and 24 years (55.3%).

By contrasting both programs with regard to risky behavior, there was statistical difference related to active sex life, sexarche, condom use at first intercourse, use of COCP, number of partners throughout life, and when to use condoms and with which type of partner. There was no difference in clinical manifestations of STIs.

In the medical program, 60.8% of first-year students and 78.6% of sixth-year students used a condom at sexarche, while that only happened with 45.9% of third-year students, revealing a significant difference between students in different years. The same happened in the law program, in which 66.9% of first-year students, 55.9% of third-year students, and 68% of fifth-year students used this contraceptive method. However, 27% of first-year medical students always use condoms. That percentage is even smaller in comparison

Table 1 – Sample distribution with regard to knowledge about STIs/AIDS in Medical and Law programs.

	Medical program		Law program		p-value
	n	%	n	%	
HIV					
Yes	195	98.0	396	90.6	0.001
No	4	2.0	41	9.4	
Hepatitis B					
Yes	132	66.3	131	30.0	< 0.001
No	67	33.7	305	70.0	
Syphilis					
Yes	199	99.5	410	93.8	0.004
No	1	0.5	27	6.2	
Gonorrhea					
Yes	190	95.5	378	86.5	0.001
No	9	4.5	59	13.5	
Candidiasis					
Yes	88	44.2	244	55.8	0.007
No	111	55.8	193	44.2	
Chlamydia					
Yes	121	60.8	150	34.3	< 0.001
No	78	39.2	287	65.7	
Genital herpes					
Yes	167	83.9	365	83.5	0.900
No	32	16.1	72	16.5	
Gardnerella					
Yes	40	20.1	64	14.6	0.085
No	159	79.9	373	85.4	
HPV					
Yes	180	90.5	308	70.5	< 0.001
No	19	9.5	129	29.5	

to third- and sixth-year students (88.5 and 26.8%, respectively), who reported using condoms with casual partners more frequently. In the law program, 35.7% of first-year students always use condoms, while the highest percentage of third- and fifth-year students (27.2 and 27.0%, respectively) use condoms in all sexual relations.

Regarding the use of COCP, 60.8 and 54.2% of first- and third-year medical students, respectively, reported not using it, and 29.3% of last-year students did not use this contraceptive method. As for first- and third-year law students, 62.7 and 57.9%, respectively, did not use COCP, in addition to 57.9% of last-year students; thus, the comparison of both programs presents a significant difference. Regarding the number of sexual partners, the majority of students in all years and from both programs reported sexual activity with one to five partners.

When asked about the type of partner with whom they use condoms, 25.7% of first-year medical students answered they only used them with casual partners, while condom use prevailed with steady partners with third- and last-year students; therefore, there is a significant difference between these data. The same situation occurred in the law program as 30.7% of first-year students also used condoms with casual partners, while condom use also prevailed with steady partners with students in other years (**Chart 1**).

When asked about the presence of vaginal discharge, there was a statistically significant difference in both programs; a greater number of discharge reports occurred with last-year students (30.5% in the medical program and 18.5% in the law program). There was no statistical difference with regard to genital warts and sores. However, by contrasting both programs, we found no significant difference between these clinical manifestations.

With regard to the perception of risk, the majority of students in all analyzed years and from both programs deemed necessary to use condoms throughout the entire relationship, whether it lasts for a few months or it is a stable relationship/marriage. There was concordance among the majority of students (83.8% of medical students and 72.6% of law students) when they were asked about the possibility of either them or another person acquiring STIs (98.5% of medical students and 98.8% of law students); there was a significant difference between both programs only with regard to the possibility of acquiring one STI.

DISCUSSION

The majority of medical students, regardless of the year they were in, appropriately responded to questions on the knowledge about the ways HIV is transmitted, as they stated that HIV is transmitted both sexually and vertically and through needle sharing. They also agreed that it is prevented by condom use and not prevented by the sole use of COCP, in addition to not being acquired through kissing and utensils. Although the majority of law students also demonstrated satisfactory knowledge about HIV transmission routes, a higher percentage of them, in comparison with medical students, believed that AIDS can be transmitted through kissing and utensil sharing. This data reveal vestiges of taboos related to the ways the infection is transmitted, which may create separation from and prejudice toward people living with HIV/AIDS. Therefore, it is necessary that students acquire greater elucidation about transmission routes, including those enrolled in programs that are not related to health.

First-year medical and law students proved to reveal less knowledge than those in other years about hepatitis B, as they do not consider it an STI, and candidiasis, as they consider it as such. According to the *Manual de Controle das DST* (Manual STI Control) by the Ministry of Health[17], sexual intercourse is not considered the main mode of transmission of *Candida*, since these organisms may be part of the endogenous vaginal flora in up to 50% of asymptomatic women, while sexual contact is an important transmission route of hepatitis B. Such divergence of knowledge may occur because third-year and sixth-year students have already acquired a greater amount of knowledge throughout the program and through their experiences. It is also possible to assume that more emphasis is being given to AIDS than to other STIs. Werne and Grusin[18] have been discussing the overvaluation of social and educational measures related to AIDS, which neglects other STIs, since 1984. Attempts to control the spread of HIV, without the implementation of parallel measures aimed at the prevention and control of other STIs, are less likely to be effective, because STIs are risk factors for HIV infection. Corroborating the fact that knowledge about AIDS is more valued than knowledge about other STIs, last-year law students did not consider gonorrhea an STI[13].

Most medical and law students are sexually active; medical students showed earlier sexarche (between 16 and 19 years of age) in comparison with law students, which is different from what is seen in the Brazilian population, according to the *Mosaico Brasil* (Brazil's Mosaic) study, conducted by the Sexuality Studies Program, in which the average age of first sexual intercourse occurs at 15 years, indicating that socioeconomic and cultural factors affect sexarche age.

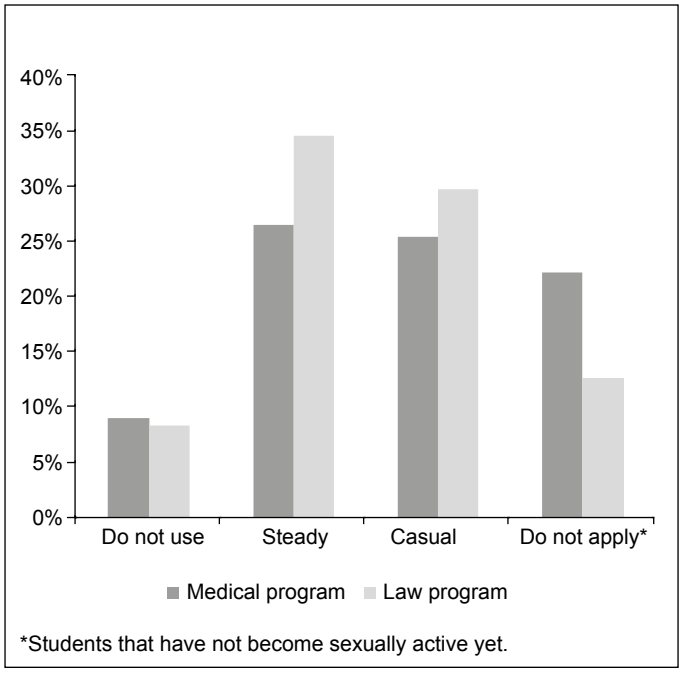

Chart 1 – Sample distribution with regard to the type of partner with whom medical and law students use condoms.

There was a higher prevalence of condom use in the first year of both programs in comparison with the other years. We found a higher percentage of single students in these program years, and condom use is more frequent with casual partners. The majority of students in the last program years are in steady or stable relationship; thus, they use condoms more often with a steady partner. Nevertheless, this frequency is still lower when compared with the frequency of condom use by single students. Studies show that there is a downward trend in condom use throughout relationships as an emotional and trusting bond is established, in which one believes in the partner's fidelity and their incapability of transmitting any infection[3,5,6,19]. There is an increase in the use of oral contraceptives, indicating a greater concern for contraception at the expense of STI/AIDS prevention.

With regard to the frequency of condom use, there was a difference between medical and law students, as the highest percentage of medical students used condoms only occasionally while the highest percentage of law students always used it. Such difference was significant in third-year students from both programs. We expected students from the health-care program field to have less risky behaviors because they have more experience, greater access to information on STIs/AIDS, and additional contact with patients who are carriers of such *pathoses* in hospitals, foreseeing they would have a greater understanding of the consequences of such diseases on the life of carriers. According to Andrade and Tanaka[20], health-care professionals, despite working with people who were diagnosed with STIs/AIDS, are not necessarily more careful in their sexual activities, as they apparently act as patients in their personal lives. Thus, positive behaviors are not only related to social and relational environments but with one's life story and needs. Unequivocally, behavioral changes happen when people perceive themselves at risk of acquiring infections.

The increased exposure corroborates the percentage of abnormal discharge among last-year students from both programs. Through these data, it is clear that last-year students, despite having greater theoretical knowledge about the various sexually transmitted infections, show increased risky sexual behaviors in comparison with first-year students.

The majority of medical and law students, in all years, stated they were vulnerable to STIs/AIDS, demonstrating they have perception of risk. This finding is in line with a study by Dessunti and Reis[3], in which first- and last-year medical students at the State University of Londrina showed low perception with regard to the probability of acquiring STIs or AIDS. Although the majority of law students also show such perception, the related percentage is lower than that found in the medical program.

Different studies show that, although students have more knowledge about STIs/AIDS, preventive measures are seldom adopted. That indicates that knowledge in itself is not enough to change behaviors and make students protect themselves from such infections[3-5,21]. Regardless of being an essential aspect to raise awareness and change behaviors, knowledge is probably not the only factor that influences students' practices. Condom use is determined not only by individual factors but also by sociocultural factors, such as different sexuality concepts and gender, belief, and habit differences. Therefore, we understand that it is necessary to further investigate these factors aiming at conducting specific programs that address the needs of each group, thus creating more effective preventive measures.

Information must reach all university students, regardless of the program, in its entirety because they are the future opinion leaders, and the university is the place where changes in perception and behavior happen.

CONCLUSION

Throughout the medical program, there was an increase in knowledge about STIs/AIDS; however, that did not happen with the law program, as last-year students revealed having less knowledge about some STIs than those enrolled in different years. Despite having greater specific knowledge, medical students adopt riskier sexual behaviors than law students with regard to lower frequency of condom use. Nevertheless, they have fewer sexual partners over a lifetime, perhaps because they are younger. In both courses, there was a lower frequency of condom use among students who were in a steady/stable relationship, indicating a greater concern for contraception at the expense of STI prevention.

We found that medical students have a higher perception of risk than law students, demonstrating that knowledge, despite not changing risky sexual behaviors, alters the perception of risk. Such perception not followed by change in sexual behavior is troubling and raises questions about the reasons to adopt such behavior, indicating the need for more studies related to students' health, especially of those enrolled in medical programs.

It is necessary to better understand the factors that determine the gap between knowledge and risky sexual behaviors by promoting more debate and discussion on the subject, as a paradigm shift is essential to foster the change of habits and beliefs since childhood, while respecting the individual and cultural aspects of each group.

Conflict of interests

The authors report no conflict of interests.

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Comparative study on sexual and reproductive health of adolescents with and without AIDS: is there a difference in knowledge between the two groups?

Estudo comparativo sobre a saúde sexual e reprodutiva dos adolescentes com e sem AIDS: há diferença de conhecimento entre os dois grupos?

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ABSTRACT

Introduction: Currently we are facing the challenge to deal with the first generation of adolescents HIV+, infected by vertical transmission. This is new, and creates the need to improve attention to self-care and knowledge on sexual and reproductive health. Issues like the exercise of sexuality, contraception, pregnancy, sexually transmitted diseases (STD), are not enough debated among professionals, families and adolescents, despite their importance, concerning the affective and sexual discoveries typical of that age. **Objective:** To assess and compare the knowledge among adolescents HIV+ and HIV-, the guidance received on sexual and reproductive health and their sexual behavior, in order to better assist an integral health attention. **Methods:** A prospective, quali-quantitative, observational, analytical and cross-sectional study that took place during one year at the public hospital of an university in Curitiba, interviewing 61 adolescents HIV+ and 61 adolescents HIV- after their outpatient attendance. A questionnaire with objective multi-choice questions, as well as open-ended questions thought to stimulate free narratives was the base of data acquisition. Statistical analysis have considered the adolescents HIV+ and HIV- matched by age, gender and education. To evaluate differences on continuous variables, Student's *t*-test for normal distribution and Mann-Whitney test for asymmetric distribution were the tools. For categorical variables: Fisher exact tests and chi-square of Pearson. The analysis of answers for the open-ended questions was based on categorization of semantic equivalence. Significance level of 5% for all tests. Main variables of study in the amount enough to allow comparisons have driven the sample estimation, with less than 5% of significance level and minimum test power of 95%. **Results:** The study showed that adolescents don't have good enough knowledge about reproductive health in both groups (p=0.01). They have initiated sexual activity at about 15 years old, they report using condoms, but not the habit of picking them up. The group of HIV + have expressed more opinions about the sexual and reproductive rights, they have received less guidance on emergency contraception (p<0.001); they "hook-up" and dated less than HIV- group. **Conclusion:** The lack of knowledge of adolescents on reproductive health is greater than about sexual health and both groups reported the habit of not getting condoms. The HIV+ group had more opinions about sexual and reproductive rights, received less guidance on emergency contraception, "hooked-up" and dated less than the HIV- group. The knowledge about sexual rights and sexuality, and the guidance provided to both groups of teenagers, didn't seem to be adequate to make protective sexual attitudes preventing STD.

Keywords: acquired immune deficiency syndrome; adolescent; sexual health; reproductive health; sexual and reproductive rights.

RESUMO

Introdução: A sociedade está enfrentando o desafio de lidar com a primeira geração de adolescentes HIV+, infectados por transmissão vertical. Isso cria a necessidade de melhorar a atenção ao autocuidado e o conhecimento sobre saúde sexual e reprodutiva. Questões como sexualidade, contracepção, gravidez e doenças sexualmente transmissíveis (DST) são pouco debatidas entre profissionais, famílias e adolescentes, apesar de sua importância diante das descobertas afetivas e sexuais típicas dessa idade. **Objetivo:** Avaliar e comparar o conhecimento de adolescentes HIV+ e HIV- e as orientações recebidas sobre saúde sexual e reprodutiva, seus comportamentos sexuais, visando auxiliar na Atenção Integral à Saúde. **Métodos:** Estudo prospectivo, quali-quantitativo, observacional, analítico e transversal, realizado por um ano em hospital público de uma universidade de Curitiba, entrevistando 61 adolescentes HIV+ e 61 adolescentes HIV-, após seu atendimento ambulatorial. Foi elaborado questionário com perguntas objetivas de múltipla escolha e perguntas abertas, para estimular a livre narrativa sobre as temáticas. Foram pareados por idade, gênero e escolaridade. Para avaliar as diferenças em variáveis contínuas, foram utilizados os testes *t* de Student, para distribuição normal, e de Mann-Whitney, para distribuição assimétrica; para variáveis categóricas, os testes exato de Fisher e do χ^2 de Pearson. A análise das respostas para as perguntas abertas baseou-se na categorização de equivalência semântica. Para os testes, foi considerado um nível mínimo de significância de 5%. A amostra foi estimada considerando as principais variáveis do estudo, sendo suficiente para as comparações um nível de significância inferior a 5%, com poder de teste mínimo 95%. **Resultados:** O estudo mostrou que ambos os grupos de adolescentes não têm conhecimento suficiente sobre saúde reprodutiva (p=0,01), iniciaram atividade sexual com cerca de 15 anos, relataram uso de preservativos, mas não o hábito de buscá-los. O grupo HIV+ expressou mais opiniões sobre os direitos sexuais e reprodutivos, recebeu menos orientações sobre contracepção de emergência (p<0,001), "ficou" e namorou menos que o grupo HIV-. **Conclusão:** O desconhecimento dos adolescentes sobre a saúde reprodutiva é maior do que sobre a saúde sexual e ambos os grupos disseram não ter o hábito de adquirir preservativos. O grupo HIV+ expressou mais opiniões sobre os direitos sexuais e reprodutivos, recebeu menos orientações sobre contracepção de emergência, "ficou" e namorou menos que o grupo HIV-. O conhecimento sobre direitos sexuais e sexualidade e as orientações fornecidas, para ambos os grupos de adolescentes, não pareceram ser suficientes para atitudes sexuais protetivas diante das DST.

Palavras-chave: síndrome da imunodeficiência adquirida; adolescente; saúde sexual; saúde reprodutiva; direitos sexuais e reprodutivos.

INTRODUCTION

The acquired immune deficiency syndrome (AIDS) has affected young individuals since the beginning of the epidemic. In Brazil, about 3,500 new cases among adolescents and young people aged 12–24 years are notified every year[1]. Mainly because of the availability of antiretroviral therapy, a large proportion of children with the human immunodeficiency virus (HIV) acquired during perinatal period survived and reached adolescence[2-5], representing a challenge for self-care and exercise of sexuality. It is necessary to clarify all the changes in personal and relational life caused by the disease in addition to explaining the diagnosis. A person affected should reach adolescence aware of the disease, of their responsibilities, and of their rights[6].

One of the risks and vulnerabilities of this phase is related to sexual and reproductive health because an open channel for teenagers to communicate and acquire knowledge about these issues is not always available. It is known that one of the main AIDS risk behavior is sexual intercourse without condoms[7,8]; therefore, the approaches that aim at reducing the vulnerable conditions, the adoption of safer sex practices such as the use of condom in all sexual intercourses, the promotion of human rights, and the reduction of stigma remain as central strategies of the Brazilian policy to prevent sexually transmitted diseases (STDs) and HIV[9]. The context of teenagers growing up affected by a chronic sexually transmitted infection with the implications and the stigma associated with HIV is still not much known in the professional practice and literature. There are also few references to the knowledge of adolescents infected with HIV by means of vertical transmission on sexual and reproductive rights and sexual behaviors. These information could support policies and services directed to this population[10].

OBJECTIVE

To evaluate and compare the knowledge and guidance regarding sexual and reproductive health and the rights of the HIV-positive and HIV-negative adolescents as well as the patterns of sexual behavior to contribute to the integral health attention of this population.

METHODS

This is an observational, analytical, and cross-sectional study, with prospective data collected by means of a quantitative- and qualitative-type questionnaire, conducted in the Infectious Diseases and Adolescents Services of the Clinical Hospital of the Universidade Federal do Parana (UFPR), from June 2010 to August 2011. The research project was approved by the Ethics Committee on Human Research of the institution, under No. 2163.058/2010-03.

The population of this study consisted of 61 patients infected with HIV (HIV-positive group) and 61 uninfected (HIV-negative group), in a total of 122 adolescents. Patients of the HIV-positive group were selected among the 110 patients infected with HIV, aged 12–19 years, who were monitored in the hospital at the time of the survey, and who were eligible for the study. The sampling technique used was the convenience sampling, according to the inclusion criteria. The individuals were selected in order of attendance at medical appointments at the hospital. Adolescents responded to the questionnaire without the interference of parents or guardians.

Inclusion criteria for HIV-positive group were adolescents of both genders infected with HIV by means of vertical transmission, aged 12–19 years, who knew their HIV status, without neurological, visual, or intellectual impairment, and who agreed to participate in the study by signing the informed consent form (ICF). In HIV-negative group, the inclusion criteria were the same, except that these adolescents should have been referred to the clinic for clinical conditions different from infection or suspected HIV infection. In case of the adolescents younger than 18 years, parents or caregivers signed the ICF. Questionnaires with incomplete information, inconclusive, or divergent of the criteria for pairing were excluded in both the groups. The pairing criteria of HIV-positive and HIV-negative groups were gender, age, and education, with a margin of 1 year more or less.

Two data collection tools with open-ended and objective questions with multiple choices were prepared. Open-ended questions aimed at encouraging the free narrative around the themes of the study. We considered as the base the Survey of Knowledge, Attitudes and Practices in the Brazilian Population conducted by the Brazilian Ministry of Health in 2004[11].

Questionnaires had four blocks of questions regarding the sociodemographic profile, knowledge about rights and sexual and reproductive health, sexual and social practices, and guidance received by the adolescents. Later, keywords from the open-ended answers were selected taking into consideration their behavior, attitude, and concepts. They were also grouped and analyzed based on the semantic content categorization. The definition of an event in a given category followed the guidelines of Danna and Matos, and should be:

a) objective, clear, and precise;
b) expressed in direct and affirmative modes;
c) include only elements that are relevant; and
d) explicit and complete[12].

The tables found in the "Results" section, related to the open-ended responses contain a qualitative (response categories) and

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quantitative synthesis, illustrated with sentences written by teenagers, even with Portuguese errors.

Statistical analysis was performed using the statistic software (Stasoft®). The estimation of the difference in continuous variables with normal distribution was performed by the Student's *t*-test; for asymmetric distribution variables, we used the nonparametric Mann–Whitney U test. The estimated difference between categorical variables was performed by Fisher's exact test and Pearson χ^2 test. The sample was estimated based on the main variables of the study, and a significance level lower than 5% was sufficient for the comparisons, with a test power of 95%.

RESULTS

The sociodemographic profiles of both HIV-positive and HIV-negative adolescents studied were similar, except for religion, city where they lived, and who they lived with ($p<0.05$) (**Table 1**). In HIV-positive group, there was a high frequency of individuals who lived in two nongovernmental organizations (31.1%), which served as shelter homes where they could stay up to the age of 18 years. More than half of the institutionalized individuals reported no religion ($p<0.001$). All HIV-positive adolescents met the definition criteria for AIDS according to the Ministry of Health[13].

In the analysis of the responses to the open-ended questions "What is your understanding of sexual health?" and "What is your understanding of reproductive health?" the responses were categorized according to the central idea (focus) on the behavior. Regarding sexual health, responses were grouped into four categories; and regarding reproductive health, they were grouped into three (**Table 2**).

With regard to the responses to the first question, the groups differed in those categorized as "Sex/pleasure" with higher frequency in HIV-positive group, and "Condoms/STD" with higher frequency in HIV-negative group ($p=0.01$). Examples of responses categorized as "Condoms/STD": "Always in sexual encounters you must always use condoms because you can get a disease like AIDS" (12-year-old HIV-negative female) and as "Sex/pleasure": "To know if you want a life with sex, have sex to have children or do not intend to have children, just sex" (12-year-old HIV-positive female). In the analysis of the responses related to reproductive health, it was observed that the frequencies were similar between HIV-positive and HIV-negative adolescents ($p>0.05$). Examples of responses categorized as "Reproduction/Sexual intercourse": "Sexual activity of a couple who reproduces through sex" (15-year-old HIV-positive female) and as "Prevention/Awareness": "If you take care using condoms and contraceptives to avoid become pregnant, or take care not to transmit to the child" (16-year-old HIV-positive female).

Responses "Blank/do not know" from both groups to both questions showed that the frequency of ignorance about the reproductive health was higher than the frequency of ignorance about the sexual health ($p=0.01$) (**Table 2**).

As the sexual and reproductive rights serve as references for the exercise of sexuality, individuals were asked whether they read about those issues, what their sources of information were,

Table 1 – Sociodemographic profile of the HIV-positive and HIV-negative adolescents.

Categories	HIV-positive group (n=61)		HIV-negative group (n=61)		p-value
	n	%	n	%	
Age (in years)	14.6±2.1	–	14.8±1.9	–	0.92*
Gender					
Male	31	50.8	31	50.8	0.85#
Female	30	49.2	30	49.2	
Sexual orientation					
Heterosexual	31	50.8	35	57.4	0.62#&
Homosexual	1	1.6	00	0.0	
Bisexual	00	0.0	00	0.0	
Did not disclose	2	3.3	3	4.9	
Did not understand	27	44.3	23	37.7	
Color of skin					
White	29	47.5	42	68.9	0.08#&
Black	3	4.9	1	1.6	
Yellow	2	3.3	00	0.0	
Brown	25	41.0	17	27.9	
Indigenous	2	3.3	1	1.6	
Marital status					
Single	59	96.7	61	100.0	0.47#
Married	02	3.3	00	0.0	
Religion					
Evangelical	21	34.4	20	33.0	0.01#&
Catholic	19	31.1	32	52.0	
Spiritist	2	3.3	1	2.0	
Buddhist	00	0.0	00	0.0	
No religion	17	27.9	6	9.8	
Other	2	3.3	1	1.6	
Did not answer	00	0.0	1	1.6	
Birth place					
Curitiba	39	63.9	40	65.6	1.00#&
Metropolitan region	9	14.8	5	8.2	
Country region	8	13.1	9	14.7	
Other states	3	4.9	5	8.2	
Did not answer	2	3.3	2	3.3	
Living place					
Curitiba	41	67.2	58	95.1	<0.001#&
Metropolitan region	15	24.6	2	3.3	
Country region	5	8.2	1	1.6	
Living with					
Parents	27	44.3	55	90.2	<0.001$
Institutions	19	31.1	1	1.6	
Others	15	24.6	5	8.2	
Schooling					
Incomplete elementary school	41	67.2	33	54.1	0.37$
Complete elementary school	6	9.8	7	11.5	
Incomplete high school	9	14.8	16	26.2	
Complete high school	5	8.2	5	8.2	
Occupation					
Student	58	95.1	59	96.7	1.00#
Other	3	4.9	2	3.3	
Total	61	100.0	61	100.0	

*Student's *t*-test; #Fisher's exact test; &among the two categories with higher frequencies; $Pearson χ^2 test.

whether they had received guidance, and what their opinion on the subject was.

When asked whether they had read about sexual and reproductive rights, 34.4% of HIV-positive and 42.6% of HIV-negative adolescents responded yes (p=0.45). In HIV-positive group, the source of information most widely used was the Internet (p=0.08), and in HIV-negative group, the source was the materials from the Ministry of Health (p=0.05).

In relation to the guidance received about these rights, 63.9% of HIV-positive and 59.0% of HIV-negative group said yes (p=0.70). Most of the individuals did not express their opinions about those rights when asked, and this frequency was higher in the HIV-negative group (73.8 versus 54.1%; p=0.07). The following questions were asked to both groups: "Should adolescents infected with HIV relate to their 'date'/boyfriend or girlfriend in the same manner that the adolescents who are not infected with HIV?" and "Why?"

In HIV-positive group, most of the adolescents responded yes (61.0%) and justified their responses similarly. In the group HIV-negative, the majority of the adolescent did not express an opinion (43.0%) or responded no (39.0%), justifying their opinion with prejudiced and discriminatory statements (**Table 3**). Examples of "yes" categorized responses as "Juvenile omnipotence/Magical thinking": "Because we forget that we have AIDS" (13-year-old HIV-positive female) and as "Care/Magical thinking": "Because if you love you cannot infect the other, be more careful" (12-year-old HIV-positive female). In these two categories of responses, "Magical thinking" in the HIV-positive group was a frequent finding, on the other hand, it was not possible to classify any of the responses of the HIV-negative group.

Responses equivalent to "no", categorized as "Care/Prejudice": "Because the others care and have responsibility and those infected with HIV do not" (16-year-old HIV-negative female) and categorized as "Transmission/Prejudice": "Because the infected teenagers do not use prevention" (18-year-old HIV-negative female). It was not possible to classify any responses of the HIV-positive group in these two categories.

In adolescence, sexual games such as "hook up," "play the field," "casual relationship," and "steady relationship" are part of the discovery and experience of sexuality and have different names and meanings according to the period in time and culture. In relation to "hook up"/"steady relationship," we observed a higher frequency in the HIV-negative group as compared with HIV-positive group ("hook up": 74.6 versus 60.7%; p=0.15) ("steady relationship": 50.8 versus 35.0%; p=0.11). The frequency of adolescents who reported having had sex was similar in both groups (p=1.00) (**Graph 1**).

Table 2 – Distribution of the responses of HIV-positive and HIV-negative adolescents to the questions: "What is your understanding of sexual health?" and "What is your understanding of reproductive health?"

Question: "What is your understanding of sexual health?"

Response categories*	HIV-positive group (n=61)		HIV-negative group (n=61)	
	n	%	n	%
Blank/do not know	30	49.2	22	36.1
Condoms/STDs	7	11.5	21	34.4
Sex/pleasure	11	18.0	5	08.2
Care/knowledge	13	21.3	13	21.3
Total	61	100.0	61	100.0

Question: "What is your understanding of reproductive health?"

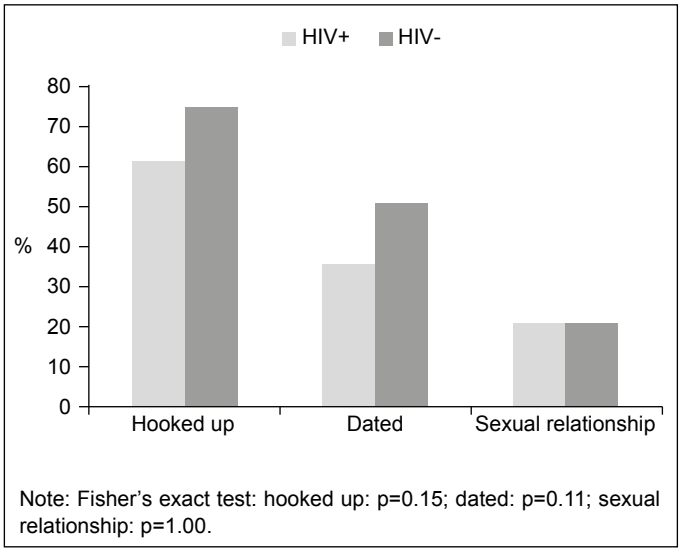
Response categories#	HIV-positive group (n=61)		HIV-negative group (n=61)	
	n	%	n	%
Blank/do not know	43	71.0	36	59.0
Reproduction/sexual intercourse	10	16.0	17	28.0
Prevention/awareness	8	13.0	8	13.0
Total	61	100.0	61	100.0

*Pearson χ^2 test: p=0.01.
#Pearson χ^2 test: p=0.48.

Table 3 – Distribution of the responses of HIV-positive and HIV-negative adolescents to the question: "Should adolescents infected with HIV relate to their 'date'/boyfriend or girlfriend in the same manner that adolescents who are not infected with HIV?" and "Why?"

Response categories	HIV-positive group (n=61)		HIV-negative group (n=61)	
	n	%	n	%
"Yes" responses(1)	37	61.0	11	18.0
Do not know	5	14.0	5	45.0
Juvenile omnipotence/Magical thinking	11	30.0	00	00.0
Care/Magical thinking	12	32.0	00	00.0
Transmission/Sex	9	24.0	6	55.0
"No" responses(1)	8	13.0	24	39.0
Do not know	2	25.0	2	8.0
Caution/fear	6	75.0	2	8.0
Caution/prejudice	00	00.0	4	17.0
Transmission/prejudice	00	00.0	16	67.0
"Do not have opinion" responses (1)	16	26.0	26	43.0
Total	61	100.0	61	100.0

Source: Data from the research (2013).
(1)Pearson χ^2 test: p<0.01.



Note: Fisher's exact test: hooked up: p=0.15; dated: p=0.11; sexual relationship: p=1.00.

Graph 1 – Distribution of the frequencies of social and sexual practices reported by HIV-positive and HIV-negative adolescents.

With regard to the onset of sexual activity among those who responded and in both groups, six initiated before the age of 15 years and seven initiated after that age (p=0.95). In both the groups, the sex partner was the "date"/boyfriend or girlfriend and in almost all cases (except one in each group) the use of condoms was reported. The majority of the individuals (84.6% HIV-positive and 61.5% HIV-negative) reported using condoms in all sexual encounters (p=0.21). However, most teenagers said they do not usually pick condoms up (61.7% of the HIV-positive and 63.3% of the HIV-negative groups; p=1.00).

The reproductive health care includes the right to information, access to contraceptive methods and techniques, and services that contribute in preventing and solving reproductive health problems of men and women[14]. For this reason, adolescents were asked if they received guidance on contraception, emergency contraception, pregnancy, abortion, and STDs.

Table 4 shows that most of the adolescents were guided on contraceptive methods, and no difference was observed in relation to the knowledge of the various contraceptive methods. With regard to emergency contraception, it was observed that 21.7% of the HIV-positive group and 52.5% of the HIV-negative group received guidance, and this difference was statistically significant (p<0.001). HIV-positive girls received less guidance on emergency contraception in relation to HIV-negative girls (22.6 versus 54.8%; p=0.01). The same pattern was observed among boys (20.7 versus 50.0%; p=0.02). The groups received similar guidance on preventing pregnancy, abortion, and STDs.

Comparing boys and girls, it was observed that HIV-positive boys received more guidance on STD (89.3 versus 60.0%; p=0.01).

A higher frequency of guidance on STDs received from education professionals was observed for both the groups.

DISCUSSION

Advances in medicine have been decreasing morbidity and mortality rates related to AIDS, and the risk of vertical transmission can be reduced to less than 2%. Children with perinatal HIV infection are reaching adolescence and young adulthood, bringing new challenges to health services, society, and public policies. Attention needs to encompass the aspects of sexuality, reproductive health, and mental health, and also discussion on the educational and professional projects of these adolescents in coordination with governmental and nongovernmental organizations should be carried out.

By analyzing the sociodemographic profile of these groups, it was observed that the majority of the HIV-positive lived with "Others" or in "Institutions," as a consequence of the death or illness of their parents in most cases, evidencing that these adolescents were exposed to orphanhood[3-5].

Both groups showed more lack of knowledge about reproductive health. A multicenter study in Brazil showed that only few professionals discussed the fertility and family planning as options for HIV-positive people. Probably, this happened to avoid stimulating women to consider pregnancy. Those professionals also admitted of being uncomfortable in discussing sexuality issues while counseling on reproductive health, particularly for HIV-positive people[15]. The reports of adolescents in this study confirm the limited access to advice on sexual and reproductive health, a fact that has hindered the knowledge acquisition on these issues and, consequently, the conscious and responsible decisions, including those related to drug interactions between antiretrovirals and contraceptives.

In HIV-positive group, the number of adolescents who responded "Blank/do not know" for questions related to sexual and reproductive health was higher than in the HIV-negative group. The lack of knowledge on these issues of the HIV-positive adolescents brings vulnerabilities to the hetero- and homosexual relationships, being a risk factor for the vertical transmission of HIV and other STDs. Both seroconcordant and serodiscordant couples wishing to have children should undergo an interdisciplinary assessment and receive information on reproductive planning to reduce the risks of transmission and to prevent the worsening of the immunodeficiency[16]. The vulnerability of females to HIV infection among the population aged 15–64 years was identified in all risk practices related to HIV[17]. Given women's vulnerability, the health services need to

Table 4 – Guidance received by HIV-positive and HIV-negative adolescents.

Guidance received	HIV-positive group (n=61)				HIV-negative group (n=61)				p-value
	Yes		No		Yes		No		
	n	%	n	%	n	%	n	%	
Sexual and reproductive rights	39	63.9	22	36.1	36	59.0	25	41.0	0.70
Masturbation	17	27.9	44	72.1	26	42.6	35	57.4	0.12
Menstruation	38	64.4	21	35.6	37	62.7	22	37.3	1.00
First ejaculation	13	21.3	48	78.7	21	34.4	40	65.6	0.15
Contraceptive methods	36	60.0	24	40.0	43	71.7	17	28.3	0.24
Emergency contraception	13	21.7	47	78.3	31	52.5	28	47.5	<0.001
Pregnancy prevention	38	64.4	21	35.6	38	63.3	22	36.7	1.00
Risk of miscarriage	29	47.5	32	52.5	33	54.1	28	45.9	0.58
STD	45	76.3	14	23.7	40	65.6	21	34.4	0.23
Vaccines	30	49.2	31	50.8	36	60.0	24	40.0	0.50
Drug consumption	11	19.0	47	81.0	13	21.3	48	78.7	0.92
Daily life	42	72.4	16	27.6	52	85.2	9	14.8	0.13

Note: Fisher's exact test.
STD: sexually transmitted disease.

carefully analyze the profile of their patients and their social and educational contexts to assess the need of specific care for women. Thus, the debate about gender may equip them to build or rebuild the relationship with their companions, also contributing to the reduction of gender violence in the next generations.

The responses of infected adolescents about sexual health were more related to "Sex/pleasure," with no relation to the prevention actions even after being aware of their HIV serostatus, while HIV-negative adolescents showed greater association between the use of condoms and prevention of STD and pregnancy. These results point to a sexuality exercised with little protection and information, adding another risk factor for both groups of adolescents. However, specifics should be considered in the attention to sexual and reproductive health of people living with HIV and AIDS, to better understand their behavior. The fear of infecting partners or being rejected by their HIV serostatus, the stigma associated with AIDS, their negative effects on self-esteem, and the possible difficulties in using condoms are the various aspects to be considered in the care offered by the health services, both for the teenagers and their caregivers[15-19].

Less than half of the adolescents of both groups responded "yes" to the question whether they had read about sexual and reproductive rights. In HIV-positive group, the most used source of information was the Internet, and in the HIV-negative group, it was the materials from the Ministry of Health. If HIV-positive adolescents have regularly attended both health services and schools, why have the materials of the Ministry of Health hardly constituted as a source of information for them, especially for such a subject? Teenagers from the HIV-positive group read less, but received more guidance than the teenagers in the HIV-negative group. More than half of the adolescents of both groups paradoxically had no opinion or did not know about these issues, when asked about their opinion on these rights.

The results in HIV-positive group related to health and sexual, and reproductive rights showed that despite existing guidelines and materials, the content, approaches, and reflections directed to these groups need to be reviewed and the health professionals need to be trained. Patients infected with HIV have major challenges to overcome during their adolescence such as learning how to deal with their biopsychosocial transformations, with their chronic disease, and with stigmas and prejudices associated with it. In this scenario, the exercise of sexual and reproductive rights is an important tool in relationships and self-care. On the other hand, the responses of HIV-negative adolescents also revealed the need to improve information on these issues and to reduce the prejudice and stigma associated with the health and rights of HIV-positive adolescents.

The opinions of the adolescents related to the following question showed significant differences: "Should adolescents infected with HIV relate to their 'date'/boyfriend or girlfriend in the same manner that adolescents who are not infected with HIV?" and "Why?" Among those who responded that the relationships were similar, thoughts and behavior of the HIV-positive group indicated lack of knowledge of risk and protective factors, of sexual transmission of HIV, and of self-care and care for the partner. Another aspect may be the willingness or necessity of these adolescents to deny their illness and the reality imposed by the disease, so as to simply live and feel like teenagers. We also noticed that the feeling of invulnerability and the "magical thinking" (abstract thought) predominated in both genders, regardless of age and education.

The study by Benincasa et al.[20] showed the importance of psycho-emotional characteristics of the adolescents, a finding that was similar to this study. They identified the predominance of the "magical thinking," the belief that something bad could hardly happen and a feeling of immunity against the risks involved in unprotected sex. They noted that although the majority had knowledge about the means of transmission of STDs and prevention, the information was often incorrect or inconsistent. Many of the findings of that study were similar to those found in the HIV-positive group of this study. Also in HIV-negative group, some opinions pointed to the "strength" of the adolescence, as if it could be stronger than the disease. Studies have found that the lack of opportunity for young people to reflect on the risks to which they are daily exposed prevents them from reviewing their opinions, habits, and in finding possible protective solutions for such risks. Although they have heard about the potential damage caused by unprotected sex, they reported that they never thought about how much their attitude left them exposed[21].

It is necessary to develop professionals to prepare educational programs for a sexual life that includes pleasure, self-care, and care for others. The information should include sexual development, the emotions, the types of sexual practices, the prevention of pregnancy, and the discussions on the STDs, and it should mainly empower adolescents to negotiate the use of condom, by listening to them without judgment, and making them confortable to expose their conflicts, fears, and doubts. It is also important to develop their self-esteem, resilience, body image, and affectivity[8,9,22]. With regard to the 24 HIV-negative adolescents who responded that relationships were not similar, the prejudice and lack of knowledge of these adolescents were evident. Other studies have also found that young people generally think about AIDS by the biomedical aspect and associates HIV infection with the lack of concern with prevention and the irresponsibility of others[22,23].

The frequency of "hook up" and "steady relationship" was a little higher in HIV-negative group, but the groups were similar in age with regard to onset of sexual activity and the use of condoms in all sexual intercourses. A study involving students also showed that girls initiated sexual activity between 15.2 and 16 years of age and boys between 13.9 and 14.5 years of age[24,25].

Abramovay and colleagues found that young people justify the lack of prevention in a steady relationship by the trust in their partner. They reported that condoms should be used only when you do not know "very well" the other person and also mentioned the sensation of decreased pleasure while using them[25]. The reasons for nonuse and negotiation about condoms were related to a number of factors that are still present nowadays, representing a challenge to public health. Despite the information available on AIDS and the free distribution of condoms by the Unified Health System (SUS) since 1994[6], there are a considerable number of young people, infected or not, who do not use condoms regularly. In relation to the responses about the habit of getting condoms, more than half of the adolescents reported they were not used to getting condoms. How do they use condoms in all sexual intercourses but are not accustomed to picking them up? Did they only provide a socially acceptable response while reporting the use of condoms in all sexual intercourses? Therefore, understanding the

challenges related to the formation and maintenance of intimate and romantic relationships can be used to improve the public health efforts to reduce HIV transmission, particularly if this population is able to disclose their status to their partners with less suffering[26].

With regard to the guidance received, HIV-positive adolescents received less guidance on emergency contraception. This finding is alarming, because adolescents and caregivers regularly attended the health services, and such orientation is part of the recommendations of the Brazilian Society of Pediatrics and the Brazilian Federation of Societies of Obstetrics and Gynecology since 2004[27].

The results showed that the physical, cognitive, and emotional characteristics that accompany adolescence and the brain transformation[21], seem to overcome the serious and chronic aspect of a disease like AIDS. Chronic diseases of long evolution impose to patients and caregivers a suffering that surpasses symptoms, restrictions, and treatments. This may interfere in adolescents' feelings, making them more vulnerable to emotional stress. To develop or strengthen skills and knowledge in these adolescents is a protective factor for their quality of life[4,10,28], and the exercise of sexual and reproductive rights is an important means to acquire the skills to take care of their sexual and reproductive health throughout life[10,14,28].

It is necessary to conduct more research on affective and sexual behaviors that allow developing various prevention strategies for the health services that care for adolescents infected with HIV, strengthening their role in the daily coping with the disease. Thus, it is mainly the responsibility of the caregivers and health professionals to increase the awareness about AIDS as a serious disease that impacts romantic and social relationships and self-care, without developing a sense of inferiority in relation to the HIV-negative adolescents.

CONCLUSION

The lack of knowledge of the adolescents on reproductive health is greater than the lack of knowledge on sexual health. HIV-positive adolescents expressed more opinions on the sexual and reproductive rights, received less guidance on emergency contraception, "hooked up" and dated less than HIV-negative adolescents, but started the sexual life in the same age range as the HIV-negative group (approximately 15 years of age). Despite reporting the use of condoms, they were not accustomed to picking them up. The knowledge about sexual rights and sexuality, and the guidance provided for both groups of adolescents, did not seem to be sufficient to stimulate protective sexual attitude for STDs. It is necessary to conduct more research on affective and sexual behavior that allows developing prevention strategies, strengthening the skills to care for sexual and reproductive health.

Conflict of interests

The authors reported no conflict of interests.

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Factors associated with condom use in women of a testing and advice center for STD/AIDS of Bahia, Brazil

Fatores associados ao uso de preservativo em mulheres usuárias de um centro de testagem e aconselhamento para DST/AIDS da Bahia, Brasil

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ABSTRACT

Introduction: The feminization process of the human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) infection in Brazil is evidenced by the expressive increase in the number of infected women and by mortality rates, which is increasing in females, contrary to what occurs among men. Despite the diffusion of condoms, consistent use is still considered low, even in relations with non-regular partners. **Objective:** To investigate factors associated with the use of condoms with non-regular partners, in women users of a Testing and Counseling Center for sexually transmitted disease (STD)/AIDS of Bahia, from 2006 to 2012. **Methods:** This is a transversal observational analytical study. Secondary data of 790 women users of a Testing and Counseling Center, obtained from forms and clinical records, were used. Statistical analysis, crude and adjusted, used the prevalence ratio as the measure of association and a 95% confidence interval. **Results:** The consistent use of condom in relations with non-regular partners was low (27.97%). The main reasons appointed to justify the absence of use were not liking it (35.71%) and difficulty in negotiating with the partner (21.22%). The outcome was associated statistically to the absence of use of condom in the last relation with a regular partner and to the age of first sexual relation lower than 16 years. **Conclusion:** The findings contribute to the understanding of the behavior of women in relationships with non-regular partners and provide a base in planning coping actions against the diseases resulting from unprotected sex, both in specialized care and in the primary health care network.

Keywords: sexually transmitted infections; sexually transmitted disease; AIDS serodiagnosis; hepatitis; syphilis.

RESUMO

Introdução: O processo de feminização da infecção por HIV/AIDS, no Brasil, é evidenciado pela elevação expressiva no número de mulheres infectadas e também pelas taxas de mortalidade, que vem aumentando no sexo feminino, ao contrário do que ocorre entre os homens. Apesar da difusão do preservativo, o uso consistente ainda é considerado baixo, mesmo em relações com parceiros não fixos. **Objetivo:** Investigar fatores associados ao uso de preservativo com parceiros não fixos, em mulheres usuárias de um Centro de Testagem e Aconselhamento (CTA) para DST/AIDS da Bahia, de 2006 a 2012. **Métodos:** Trata-se de um estudo analítico observacional transversal. Foram utilizados dados secundários de 790 mulheres usuárias de um CTA, obtidos de formulários e prontuário clínicos. A análise estatística, bruta e ajustada, adotou a medida de associação razão de prevalência com intervalo de confiança de 95%. **Resultados:** O uso consistente de preservativo nas relações com parceiro não fixo foi baixo (27,97%). Os principais motivos apontados para justificar a ausência de uso foram não gostar (35,71%) e dificuldade de negociação com o parceiro (21,22%). O desfecho associou-se estatisticamente com ausência de uso de preservativo na última relação com parceiro fixo e idade da primeira relação sexual menor que 16 anos. **Conclusão:** Os achados contribuem para a compreensão do comportamento de mulheres em relações eventuais e trazem subsídios para o planejamento de ações de enfrentamento dos agravos decorrentes de relações sexuais desprotegidas, tanto na atenção especializada quanto na Atenção Primária à Saúde.

Palavras-chave: infecções sexualmente transmissíveis; doenças sexualmente transmissíveis; sorodiagnóstico da AIDS; hepatite; sífilis.

INTRODUCTION

Current epidemiological patterns show a trend of heterosexualization and feminization in acquired immunodeficiency syndrome (AIDS)[1]. In Brazil, the incidence among women rose very quickly and the sex ratio rose from 18.9 men per 1 woman, in 1984, to 1.5:1 in 2004, reaching 0.9:1 at the age group of 13 to 19 years[2]. From 2004 to 2008, the sex ratio has remained stable; from 2009 to 2013, there was an increased incidence among men and a downward trend among women, which caused an increase in the sex ratio, reaching 1.8:1 in 2013[3], but maintaining the process of feminization from the beginning of the epidemic. The AIDS mortality coefficient follows the same upward trend in women, with an increase in 0.8% per year between 2002 and 2011, unlike observed in males, which showed a reduction of around 0.5% per year over the same period[4].

The use of condoms during sexual intercourse, essential for the prevention of AIDS and other sexually transmitted infections (STIs), collides with complex issues whose approach requires overcoming social, cultural, and emotional barriers[5]. Addressing STIs involves the understanding of the social relations of gender and its implications for affective and sexual interactions[5].

Study carried out at the Referrence center for STDs/AIDS in Juazeiro (BA), Brazil.

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In adolescence, sexuality has a huge importance in the life of the young, as it is when, in general, sexual life begins, and behaviors assumed in this phase permeate attitudes that can establish behavior patterns throughout life[6]. However, since adolescence, women seem to have more difficulties with the use of condoms. A study conducted in São Paulo showed that 73.3% of boys reported knowing how to use condoms, whereas only 56.4% of the girls knew it; 66.7% boys and 52.4% girls claim to use condoms in all sexual relations; 19.8% girls claim to be ashamed to carry a condom, whereas among boys, the percentage was 4.7%[6].

The use of the female condom, which could provide greater autonomy and freedom to women, has many obstacles. The near-zero outreach to the general population, the absence of free distribution at health centers (usually, when there is, it is limited to sex workers and to women living with the human immunodeficiency virus – HIV), and the high cost compared with male condoms, are some of the difficulties for the spread of the female condom use in the population[7]. A study found that 84% of women knew about the female condom, but only 8% have used it at least once in their life, which shows that the frequency of use is still low[8].

The condition of poverty can make women more vulnerable to STIs because the socioeconomic and cultural issues interfere with their power to make decisions, including the choice of how and when to have sex. Moreover, these issues are directly linked to the ability to process information about ways to prevent HIV infection[9]. Poverty, coupled with violence, increases the risk of early and unprotected sexual initiation, and hence STIs, especially in adolescence[10].

The role of Testing and Counseling Centers (TCCs) is of utmost importance in this context, to identify the most vulnerable segments of the population in its coverage area, keeping in mind the epidemiological, socioeconomic, and cultural aspects of the location. In addition to serological screening and distribution of prevention materials, counseling is conducted in TCCs. Its performance is important for the diagnosis of STIs and for health prevention and promotion in the community[11].

OBJECTIVE

To investigate the factors associated with the condom use with non-regular partners, in women users of a TCC for sexually transmitted disease (STD)/AIDS in Bahia, between 2006 and 2012.

METHODS

A cross-sectional observational, analytical, exploratory study was conducted using secondary data from the health services. The study was developed in the TCC of the Reference Center for STD/AIDS in Juazeiro (BA), which also runs the Specialized Care Service (SCS) in STD/HIV/AIDS, responsible for processing and monitoring seropositive users for STIs tested on the TCC.

The data sources were the TCC Information System Entry Forms, the clinical records of the SCS (for users with positive serology), and the Service Description Sheet, in which the service enters complementary sociodemographic data. The target population consisted of women registered between 2006 and 2012 in the previously mentioned TCC. The exclusion criteria used were age lower than 10 years, exclusively stable (regular) partner, blank forms/records, or no information about condom use with non-regular partners. In situations where the user had filled more than one form, the latest was adopted, observing the date of the interview.

The total number of users seen at the TCC in the period between 2006 and 2012 was 10,125. Of these, 3,029 were excluded because of blank forms and 48 by being younger than 10, leaving 7,048. Of these, 3,726 (52.87%) were women, and 2,465 (67.3%) reported having exclusively steady partner whereas 65 (1.7%) did not report the type of partnership. Both groups were excluded.

A total of 1,196 women with no regular partner were eligible for the study: 378 (31.6%) with exclusively non-regular partner and 818 (68.4%) with regular and non-regular partner. To define the outcome of the use of condoms with non-regular partner, 406 forms (33.0%) that did not contain this information (ignored or not reported) were excluded. Finally, the study was conducted with 790 participants. Data were collected in the service, during operational hours, by directly typing them. Before the beginning of the collection, there was a team training and calibration to standardize the procedures.

Statistical analysis identified the prevalence of condom use with non-regular partner (always, never, sometimes) and the main reasons reported for not using. The variable considered as the outcome was the use of condoms with non-regular partners (no, when never used or used eventually; yes, when always used, representing routine/consistent use).

The independent variables studied were sociodemographic (age, self-reported skin color, education level, marital status, employment status, and population group) and related to individual vulnerability (partner type, stable partner, number of sexual partners in the last year, contracted STDs in the last year, condom use during the last intercourse with a steady partner, condom use during the first intercourse, age during the first intercourse, and the serology results of the STI tested at the TCC). For the independent variables, the same categories used in the collection instruments were adopted, grouping those with very low frequencies to allow statistical analysis.

Data were entered in Microsoft Office Excel 2007 and they were submitted to a quality control process with coherence and consistency analysis to identify typos or errors in filling. Statistical analysis was performed using Stata 9.0 software, by means of bivariate and multivariate analyzes, using Poisson regression with robust variance, adopting the prevalence ratio as a measure of association with a 95% confidence interval (5% significance level). For association analysis, the ignored/not applicable categories in all variables were excluded.

This subproject is part of the study "Epidemiological survey in Testing and Counseling Center and Reference Unit for STD/AIDS in Juazeiro-BA," approved by the Ethics Committee on Human and Animal Research of Universidade Federal do Vale do São Francisco (Registration No. 0006/301111). Because this is a study with secondary data, the approval of the health service was obtained; secrecy and confidentiality were assured by the main researcher.

RESULTS

The average age of the participants was 27 years (standard deviation 10.3 years), ranging from 11 to 64 years. **Table 1** shows the

prevalence of condom use with non-regular partners and the main reasons reported for not using them. Consistent condom use was low and was used by little more than one-fourth of the participants (27.97%). The main reasons given were "not liking it," "partner not accepting/being unable to negotiate with the partner," "trusting the partner," and "not having one at the time."

Table 2 shows the prevalence of condom use with non-regular partners according to the sociodemographic characteristics and the result of the crude analysis between this group of variables and their outcome. There was a predominance of individuals with brown skin color, with 8 to 11 years of schooling, single, self-employed/employed, and that were part of the population group "general population". There was a statistically significant association between the marital status and the outcome. The other variables were not associated with the outcome.

Table 3 shows the distribution of the variables related to the individual vulnerability and the result of the gross analysis with the outcome. Most reported two to four partners in the last year, sexual initiation between 16 and 19 years of age, and not using a condom during the first intercourse. Among those who had positive serological results, syphilis was the most prevalent disease. Statistically significant association was observed between the outcome and the use of condom during the last intercourse with a regular partner, the number of sexual partners in the last year, STD in the last year, condom use during the first intercourse, and the age during the first intercourse.

The result of the adjusted analysis of the outcome and independent variables associated in the crude analysis can be seen in **Table 4**. The following variables maintained a statistically significant association with the outcome: condom use during the last intercourse with a steady partner, and age at the first intercourse.

With regard to condom use during the last intercourse with a steady partner, women who reported not having used it presented a 49% lower frequency of use with non-regular partners, in relation to those who reported using condoms with a steady partner.

As for the age at first intercourse, subjects who initiated sexual life under the age of 14 years or between 14 and 15 years reported the use of condoms with a non-regular partner 57% (p=0.05) and 73% (p=0.012) lower, respectively, compared to those who initiated with 19 years of age or older.

The other variables have lost association. The age during the first sexual intercourse was identified as a confounder of the association between condom use during the first intercourse and the outcome.

Table 1 – Prevalence of condom use with non-regular partner and main reasons reported for not using, in women users of the Testing and Counseling Center. Juazeiro, Bahia (n=790).

Variables	n (%)
Condom use with non-regular partner	
Always	221 (27.97)
Never	295 (37.34)
Sometimes	274 (34.68)
Reason for not using condoms*	
Does not like it	170 (35.71)
Partner does not accept it/cannot negotiate with partner	101 (21.22)
Trusts in partner	61 (12.82)
Did not have one at the time	60 (12.61)
Thinks she won't catch anything	10 (2.10)
Use of alcohol/drugs	12 (2.52)
Other reasons	62 (13.02)

*Considering only those who did not routinely use condoms (n=569).

DISCUSSION

The study results show that the use of condoms with non-regular partner is not a routine behavior and is associated with the use

Table 2 – Prevalence of condom use with non-regular partners, according to sociodemographic characteristics and results of the crude analysis with the outcome in women users of the Testing and Counseling Center in Juazeiro, Bahia (n=790).

Variables	n (%)	Condom use with non-regular partner		
		n (%)	PR_{crude} (95%CI)	p- value
Age (years)				
≤19	204 (25.82)	58 (28.43)	1.00	–
20–30	341 (43.16)	104 (30.50)	1.07 (0.82–1.40)	0.611
31–40	140 (17.72)	37 (26.43)	0.93 (0.65–1.32)	0.684
41–50	75 (9.50)	18 (24.00)	0.84 (0.53–1.33)	0.468
≥51	30 (3.80)	4 (13.33)	0.47 (0.18–1.20)	0.114
Skin color (489*)				
White	47 (15.61)	11 (23.40)	1.00	–
Black	37 (12.29)	15 (40.54)	1.73 (0.90–3.31)	0.097
Brown	217 (72.09)	56 (25.81)	1.10 (0.63–1.94)	0.735
Years of study (100*)				
None	19 (2.75)	4 (21.05)	1.00	–
1 to 3	56 (8.11)	16 (28.57)	1.36 (0.52–3.56)	0.535
4 to 7	249 (36.09)	55 (22.09)	1.05 (0.42–2.58)	0.917
8 to 11	253 (36.67)	113 (31.01)	1.52 (0.63–3.68)	0.353
12 and over	113 (16.38)	33 (29.20)	1.38 (0.55–3.47)	0.484
Marital status				
Married/civil union	238 (30.13)	47 (19.75)	1.00	–
Single	471 (59.62)	150 (31.85)	1.61 (1.21–2.15)	0.001
Separated/ widow	81 (10.25)	24 (47.70)	1.50 (0.98–2.28)	0.060
Professional situation (6*)				
Self-employed/employed	351 (44.43)	104 (29.63)	1.00	–
Unemployed	117 (14.81)	33 (28.21)	0.95 (0.68–1.32)	0.771
Student	150 (18.99)	44 (29.33)	0.99 (0.74–1.33)	0.947
Housewife	172 (21.77)	40 (23.26)	0.76 (0.57–1.08)	0.133
Population group (31*)				
General population	533 (70.22)	162 (30.39)	1.00	–
STD carriers	170 (22.40)	40 (23.53)	0.77 (0.57–1.04)	0.095
Pregnant women	37 (4.87)	6 (16.22)	0.53 (0.25–1.12)	0.098
Health professionals	7 (0.92)	1 (14.29)	1.37 (0.69–2.71)	0.365
Sex workers	12 (1.58)	5 (41.67)	0.47 (0.08–2.90)	0.416

PR: prevalence ratio; p: significance level (5%); 95%CI: 95% confidence interval; STD: sexually transmitted disease.
*Number of observations lost (ignored/not informed).

Table 3 – Prevalence of condom use with non-regular partners, according to variables related to individual vulnerability, and results of the Poisson regression with the outcome, in women users of the Testing and Counseling Center in Juazeiro, Bahia (n=790).

Variables	Condom use with non-regular partner			
	n	n (%)	PR_{crude} (95%CI)	p-value
Type of partner				
Man	703	202 (28.73)	1.00	–
Woman	65	18 (27.69)	0.96 (0.64–1.45)	0.860
Man and woman	22	1 (4.55)	0.15 (0.23–1.08)	0.060
Stable partner				
Exclusively steady partner	149	36 (24.16)	1.00	–
Stable partner and not exclusive	641	185 (28.86)	1.19 (0.88–1.63)	0.260
Number of sexual partners in the last year (30*)				
1	239	54 (22.59)	1.00	–
2 to 4	407	130 (31.94)	1.41 (1.07–1.86)	0.013
5 to 10	83	22 (26.51)	1.17 (0.76–1.80)	0.465
>10	31	6 (19.35)	0.86 (0.40–1.82)	0.688
Contracted an STD in the previous year (129*)				
Yes	441	108 (24.49)	1.00	–
No	220	79 (35.91)	1.47 (1.15–1.86)	0.002
Condom use at last intercourse with steady partner (182*)				
Yes	174	95 (54.60)	1.00	–
No	434	79 (18.20)	0.33 (0.26–0.42)	0.000
Condom use at first intercourse (530*)				
Yes	97	36 (37.11)	1.00	–
No	163	40 (24.54)	0.66 (0.45–0.96)	0.030
Age at first intercourse (133*)				
>19 years	72	24 (33.33)	1.00	–
Between 16 and 19 years	263	84 (31.94)	0.95 (0.66–1.38)	0.822
Between 14 and 15 years	222	54 (24.32)	0.72 (0.48–1.09)	0.124
<14 years	100	16 (16.00)	0.048 (0.27–0.84)	0.010
Seropositivity**				
HIV	20	6 (30.00)	1.00	–
Hepatitis B	10	1 (10.00)	0.33 (0.04–2.43)	0.279
Hepatitis C	4	2 (50.00)	1.67 (0.50–5.50)	0.402
Syphilis	47	9 (19.15)	0.64 (0.26–1.56)	0.326

PR: prevalence ratio; p: significance level (5%); 95%CI: 95% confidence interval; STD: sexually transmitted disease; HIV: human immunodeficiency virus.
*Number of observations lost (ignored/not informed); **considering only those who were tested for HIV (n=81).

Table 4 – Results of the Poisson regression adjusted between the independent variables and the outcome in women users of the Testing and Counseling Center in Juazeiro, Bahia (n=790).

Variables	Condom use with non-regular partner	
	$PR_{adjusted}$ (95%CI)	p-value
Marital status		
Married/civil union	1.00	–
Single	1.32 (0.73–2.39)	0.362
Separated/widow	1.96 (0.88–4.35)	0.100
Professional situation		
Self-employed	1.00	–
Unemployed	0.57 (0.28–1.17)	0.126
Student	1.37 (0.81–2.31)	0.242
Housewife	0.95 (0.49–1.85)	0.890
Number of sexual partners in the last year		
1	1.00	–
2 to 4	1.70 (0.97–2.99)	0.065
5 to 10	1.00 (0.38–2.68)	0.990
>10	0.54 (0.44–2.30)	0.732
Contracted an STD in the previous year		
Yes	1.00	–
No	1.38 (0.89–2.16)	0.150
Condom use at last intercourse with a steady partner		
Yes	1.00	–
No	0.51 (0.33–0.79)	0.003
Condom use at first intercourse		
Yes	1.00	–
No	0.77 (0.50–1.20)	0.250
Age at first intercourse		
>19 years	1.00	–
Between 16 and 19 years	0.60 (0.36–1.10)	0.060
Between 14 and 15 years	0.43(0.23–5.77)	0.005
<14 years	0.27 (0.10–0.75)	0.012

PR: prevalence ratio; p: significance level (5%); 95%CI: 95% confidence interval; STD: sexually transmitted disease.

during the last relationship with a regular partner and the age during the first sexual intercourse.

Although low, the prevalence of condom use observed in this population was higher than in the users of the TCC in Canoas, Rio Grande do Sul, considering non-regular partners (17%)[12]. In the same region of that study, a population-based study showed a condom use frequency similar to this study (29%), but almost half of those who used it aimed at preventing pregnancy, and not STDs [13]. Another study carried out in São Paulo found that 33.5% of the women reported to be always using condoms[14], but it should be considered that the participants were users of the SCS and already had positive serologic results for any of the STI tested at TCC, which may have led to an increase in their use.

Major factors contributing to justify the lack of use were "not liking it," "the partner not accepting/not being able to negotiate with the partner," and "trusting the partner," which were different from the study by Maciel and Bizanni, which points "partner did not have the time," "not liking it," and "thinking that the partner does not have HIV," as the main reasons reported for not using condoms, with confidence in the partner not being reported by any of the women with non-regular partners[12].

The fact of not liking to use condoms may be related to misinformation, myths, or annoyance, which shows that it is still necessary to raise awareness on the importance and effectiveness of use, especially among the most vulnerable populations. The decision to not to use condom is influenced by the belief that condom interferes with sexual pleasure and that it compromises the male–female interaction[15]. There is still the idea that pleasure is directly linked to the naturalness of sexual practice and, as condoms are artificial devices,

people are conditioned to refuse to use them[16]. In this scenario, the Popular Education in Health constitutes a great tool to demonstrate that the benefits of condom use outweigh the annoyances and thus spread the practice.

Low adherence to the consistent use of condoms during the sexual intercourse is also linked to nonacceptance by the partner and the difficulty that women have to negotiate, as pointed out by previous studies[5]. Inequalities in gender relations, with the submission of women to men, are still very much present in our society, and this, coupled with the fact that many men do not like to use condoms, results in the predominance of the male desire. One must understand the relationships of gender and their social implications, because while women do not find ways to act as protagonists during sexual relations, it will be continuously difficult to convince or reach an agreement with their partners. Also in this context, the use of the female condom can offer women a different method of protection, in addition to giving them more autonomy. However, the female condom potential is still untapped, because of its high cost and almost nonexistent disclosure to the general population[7].

In this study, the fact that trusting the partner was one of the main reasons for not using condoms is worrying, as all the women analyzed had no fixed partner. Research indicates that women consider monogamy as an STD protection factor, leading to neglect the use of condom because of their trust in the partner[17], which cannot be applied among users with no fixed partner. However, confidence in the partner can also be explained by the affective marital issues. A study conducted on adolescents, addressing the aspects of love/passion, demonstrated the difficulty in making informed decisions in situations involving feelings[18]. This can reflect directly on the practice of condom use, as emotions are more intense at the time of the sexual activity because of the hormones and psychological factors, and it may overlap reason and knowledge of the risks.

Women who did not use condoms in relations with a steady partner also made less use with casual partners, compared with those who used them. This may be showing that women have a tendency to reproduce, in their casual relationships, the same behavior adopted with their regular partners, which ends up creating a situation of great vulnerability to STI for all those who are involved. It is also important to consider that women who do not use condoms with their regular partners may have difficulty negotiating in any relationship, because they are not accustomed to its use and probably to arguing with their partners about the need to use it.

The study also showed that the age of onset of sexual life can have an influence on subsequent behavior, as those who started early (before the age of 16 years) made less use of condoms with non-regular partners than those who started later (after the age of 19 years). Early sexual initiation, when women have no maturity, information, and skills to make their own decisions for a healthy sexual practice, implies difficulty to address gender issues, and increase exposure to STIs[19]. There is evidence of an increase in early sexual activity among women mainly because of the need to adapt to the social universe in which she is inserted, contributing to exposure to unprotected sex[20].

It is worth pointing out that, since the beginning of sexual life, women are at a disadvantage because, while boys need to prove their masculinity, girls take on a more submissive role, which makes the partner's will as to whether to use or not use a condom to prevail[6]. Another factor that exposes women who initiate sex life early to unprotected sex is the relationship with older men, which also reduces the possibility of negotiation by women[16]. Because adolescence is a period of intense physical, psychological, and social changes, when the young are defining their values, behaviors assumed at this stage tend to be reproduced in future relationships. This makes this age group a strategic one for the development of health promotion activities to raise awareness for the adoption of self-preservation sexual practices[21].

Currently, some strategies have been adopted for the control of HIV, such as postexposure prophylaxis, which, when indicated, expands the possibilities of action to prevent new HIV infections[22]. Preexposure prophylaxis is also promising to help reduce transmission in groups at high risk of HIV infection[23]. Moreover, the promotion of early access to diagnosis has been increased more incisively, so that the individuals infected with HIV can initiate antiretroviral therapy earlier, thereby reducing the risk of transmission.

However, the use of condoms is the safest method to prevent transmission of both HIV as well as other STIs, which reinforces the need to encourage their use and, where necessary, to associate it with these strategies, which should be viewed as complementary, not substitutive. In addition, other alternatives than the biomedical ones should be valued, including the approach of gender, social, and economic inequalities and combating stigma and discrimination[25].

It is important to mention the limitations in this cross-sectional study, such as timelessness, which does not establish causal relationships, and the homogeneity of the sample, which was composed only of TCC users, making it difficult to identify significant differences between the groups. In addition, the use of secondary data may have compromised its reliability, as the turnover of professionals who make the records creates a lack of standardization in filling. The large amount of blank or uninformed responses, representing a loss of information, and the lack of clarity of some questions contained in the forms, which made them difficult for users to understand, can also be seen as limitations.

However, the multivariate analysis that allowed the control of confounding factors gives greater reliability to the results. By including only women who have relationships with non-regular partners, this study provides new and relevant information for the understanding of the issue, as in general, surveys do not distinguish between the type of partnership, although the aspects involved in relationships with regular and non-regular partners are different.

CONCLUSION

This study found low prevalence of condom use with non-regular partners and the existence of association of this outcome with the age of sexual initiation and the condom use during the last intercourse with a steady partner.

The findings provide valuable information about the knowledge and understanding of the reality of women receiving care in TTCs, generating subsidies for the planning of coping actions against diseases resulting from unprotected sex, both in specialized care and in primary health care, through the enhancement of individual and collective empowerment strategies.

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Conflict of interests

The authors report no conflict of interests.

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Chlamydia trachomatis INFECTIONS AND THEIR IMPACT IN THE ADOLESCENT POPULATION

Infecciones por Chlamydia trachomatis y su repercusión en adolescentes

Martín Santalucía[1], Alicia Farinati[2]

ABSTRACT
Chlamydia trachomatis is an obligate intracellular organism and can only replicate inside eukaryotic host cells. It has a unique developmental cycle, with metabolically inert, spore-like elementary bodies that infect host cells and develop into metabolically active, replicative or reticulate bodies (RBs) within a membrane-bound inclusion. RBs are divided once more into elementary bodies 24 to 48 hours after infection and they are eventually released through lysis of the host cell. The chlamydial infection, like the gonococcal infection, is the possibility of severe sequelae in both the eye and the genital tract. *Chlamydia trachomatis* infects epithelial cells in the eye and genital tract. The early stage of infection can present with a mucopurulent discharge, but infections are often asymptomatic at this stage. In most infected women, the infection has a resolution, but in women with persistent or repeated infections, it can spread upwards from the endocervix to the fallopian tubes, leading to infertility or ectopic pregnancy because of tubal occlusion by scar tissue. It is a common etiologic agent in acute salpingitis, mainly in the adolescent's population. With the exception of the lymphogranuloma *venereum* strains, which cause systemic illness and infect regional lymph nodes, *Chlamydia trachomatis* infection usually remains confined to mucosal surfaces, and it continues to produce enormous social and economic consequences despite advances in prevention, screening, and treatment.
Keywords: *Chlamydia trachomatis*; adolescent; biology; immunity.

RESUMEN
Chlamydia trachomatis es una bacteria intracelular obligada que solo se puede replicar en células eucarióticas. Tiene un ciclo de desarrollo que es único dentro de la microbiología. Consta de un cuerpo inicial o elemental metabólicamente inerte que ingresa en la célula eucariótica y allí se transforma en un cuerpo reticular o replicativo que es él que se divide. Luego, cada uno de estos cuerpos reticulares se transforma después de 24 a 48 horas en cuerpos elementales o iniciales que son los que se liberaran posteriormente a la lisis de la célula. La infección por *Chlamydia* y la infección gonocócica pueden dejar severas secuelas a nivel del tracto genital y ocular. En el estadio temprano de la infección, *Chlamydia trachomatis* puede presentar descarga mucopurulenta, pero a menudo es asintomática. En la mayoría de las mujeres, se resuelve favorablemente, pero en muchas de ellas, la infección puede ser persistente y/o recidivante. También puede diseminarse hacia el aparato genital superior (endometrio, trompas de Falopio), generando lesiones cuyas cicatrices dejan secuelas como esterilidad e infertilidad. Es la causa más común de la salpingitis aguda, principalmente en las adolescentes. Con excepción de las cepas responsables del linfogranuloma venéreo que pueden causar enfermedades sistémicas e infectar los ganglios regionales, *Chlamydia trachomatis* usualmente permanece confinada a las mucosas. Además, continúa produciendo consecuencias sociales y económicas con trascendencia a nivel mundial a pesar de los avances en su prevención, detección y tratamiento.
Palabras clave: *Chlamydia trachomatis*; adolescente; biología; inmunidad.

INTRODUCTION

Chlamydia trachomatis (CT) is an obligate intracellular organism. This is an important fact, as CT is not developed in conventional culture media.

Similarly to *Neisseria gonorrhoeae*, its importance is based on its possible complications and sequelae both for women and for their contacts and descendants[1,2].

This text begins with a brief listing of the important points that will be developed in this article about CT, which will allow us to better understand the biology and pathogenesis of the infections produced by such elusive bacterium.

CT is a human pathogen, one of the leaders among the microorganisms that most produce sexually transmitted infections (STI) in the world, with more than 90 million new cases of genital infections every year. Around 70% of the infected women are asymptomatic, thus chronic infections may be established for months or even years. Although CT does not present symptoms, it can damage the reproductive organs and the conventional treatments, many times, are not able of completely eliminating it; therefore, it remains a persistent infection[3-5]. Recently, researchers from the Max Planck Institute for the Study of Biology Infections showed that CT infections might cause mutations in the DNA of the host, thus preventing the growth regulation mechanisms of damaged cells, which could be the path toward the development of tumors[6].

EPIDEMIOLOGY

CT is an obligate intracellular Gram-negative microorganism, because it is unable of making metabolic energy and requires live cells for its development.

The prevalence of CT is estimated in 6.8% among sexually active women in the age range of 14 to 19 years[1,2].

The increase in reports about CT infection in the last years reflects the expansion of screening activities that were conducted in many communities, the use of more sensitive tests, the emphasis put in the detection of asymptomatic cases, and the use of statistical diffusion tools.

Study carried out in the *Cátedra de Microbiología y Parasitología*, School of Medicine, *Universidad del Salvador* – Buenos Aires, Argentina.
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When prevalence was compared between both the genders, the outcomes were higher in women, which was also seen in our experience[6].

It is worth mentioning that CT is part of a great group of microorganisms that currently form the *Chlamydiales* order. There are two important genera: *Chlamydia* y *Chlamydophila*[7]. While *Chlamydia* infects only mammals such as humans, rodents, and swine, the specificity of species for *Chlamydophila* is less strict and includes amphibians, reptiles, birds, and mammals. Both *Chlamydia* and *Chlamydophila* include some important species like human pathogens, as explained in **Table 1**[8,9].

CT causes several infections in humans and animals. It infects the epithelial cells and possesses 15 serovares, some of which are responsible for trachoma. Trachoma is the leading disease of blindness that can be preventable similarly to the STI. When the natural history of the disease is analyzed, it is found that there is limited knowledge regarding what happens in human infections without antibiotic treatment. There are two studies including females suggesting that it disappears after 1 year in 45 to 55% of them, reaching until 94%. In women, nontreated genital infections can have devastating consequences, such as inflammatory pelvic disease and its sequelae: ectopic pregnancy, infertility, and chronic pelvic pain[1,2,10].

GENOMICS

The genome of a relative number of *chlamydiae* has been extensively studied since 1998[11,12].

As most of the microorganisms, CT has a chromosomic and plasma DNA. Its genome is small, and it only has 1.042.519 pb (58.7% of A-T). CT has a cryptic plasmid of 7.493 pb. The genomic analysis showed it codifies for around 875 proteins, which are not necessarily expressed and 70 of which are exclusively of CT. It is worth noting that in the area near the origin of the chromosome replication, there is a higher genetic diversity. This region includes genes that control tryptophan synthesis, and its use has been associated with the intervention of gamma interferon in the development of persistent infection. CT recovered from the human genital tract have a homologous gene of cytokines reported in the 0157 enterohemorrhagic *Escherichia coli* and in *Clostridium*[13], in such area.

Table 1 – Genera division of the *Chlamydiaceae* family and its division into two genera, *Chlamydia* y *Chlamydophila.*

Family I. Chlamydiaceae	
	Genus 2. Chlamydophila
	Chlamydophila psittaci.
Genus 1. Chlamydia	*Chlamydophila abortus**
Chlamydia trachomatis	*Chlamydophila felis**
C/PK-2 Trachoma biovar	*Chlamydophila caviae**
LGV L2/434/BU Biovar	*Chlamydophila pecorum*
*Chlamydia muridarum**	*Chlamydophila pneumoniae*
*Chlamydia suis**	TWAR Biovar
	Equine Biovar
	Koala Biovar
Families II, III, and IV	
Family II. Simkaniaceae fam.nov	
Simkania negevensis sp.nov	
Family III. Parachlamydiaceae fam.nov	
Genus 1	**Genus 2**
Parachlamydia gen.nov	*Neochlamydia* gen.nov
Parachlamydia acanthamoebae sp.nov	*Neochlamydia hartmannellae* sp.nov
Family IV. Waddliaceae fam.nov	
Waddlia chondrophila sp.nov	

Based on the genetic structure, different genotypes, which vary according to the areas and pathologies, are found. In a study carried out in Spain, the most frequent genotypes were E (45.3%), D (15.3%), G (10.2%), and F (9.6%). Other genotypes included B, H, I, J, K, and LGV II[14]. This study found that the genotype E was more prevalent among asymptomatic adolescents. This genotype is precisely associated with the asymptomatic infection[15,16].

Chlamydophila abortus, which has been considered a new species of *Chlamydophila psittaci*, is one of the causing agents of the real epizootic of abortion in the ovine cattle, resulting in economic damage. However, recently, the possible participation of this microorganism in an abortion condition has been described in a woman presenting abdominal pain and vaginal secretion, and culture or molecular studies were negative for *N. gonorrhoeae* and CT. The suspicion of a disease or of a pelvic inflammatory syndrome resulted in the determination of antibodies (Ac) study, which was consistently higher in the lipopolysaccharide (LPS) gender-specific antigen (Ag) of *C. psittaci*. A retrospective investigation found that the patient had Ac titers that were seen in the heat-shock protein 60 (HSP60), demonstrated in the ELISA test. The nested PCR reaction for the specific ompA of *Chlamydiaceae* spp. conducted in the liquid obtained by puncture of the Douglas pouch was positive.

IMPORTANCE OF ENVIRONMENTAL CHLAMYDIAE

Recently, some intracellular microorganisms similar to that of the *Parachlamydiaceae* with new families were recently described. The use of techniques such as PCR together with phylogenetic techniques based on rARN allowed a substantial accumulation of genetic sequences associated with *Chlamydia.* Therefore, Chlamydia-like organisms are mentioned, in which some of them are much unknown and others have been associated with zoonosis. There is evidence that they might be the cause of several pathologies in the cattle that remained underdiagnosed.

Some species of *Parachlamydiaceae* replicate in different amoebae of free lives and can have a lithic action. Their DNA has been detected as antibodies in the materials of patients[17].

These bacteria might be currently considered as emerging pathogens, and thus they need to be investigated. *Simkania negevensis* (*Simkaniaceae* family)[9,17] and *Parachlamydia* (*Parachlamydiaceae* family)[18] could be essential human respiratory pathogens, whereas *Waddlia chondrophila* (*Waddliaceae* family) seems to be a new agent of abortions in ruminants[19].

STRUCTURE

The elementary bodies (EB) of CT are microscopic rounded structures that are infectious, rigid, resistant to rupture because of disulfide bond of the proteins from the outer layer of the membrane, and they are released after lysis of the infected host cell. The size of EB

ranges from 200 to 400 nm. They are stained purple with Giemsa stain, and red with Macchiavello stain, which is different from the color stained by the cytoplasm of the host cell.

DNA and RNA are found in EB. The largest part of the DNA is found in the central nucleoid electron density and the largest part of the RNA, in the ribosomes. The EB show species-specific and serotype-specific Ag that lead to phagocytosis, they have no metabolic activity, cannot replicate, and are infective (**Figure 1**).

Reticulate bodies (RB) are the result of the EB differentiation after suffering phagocytosis, they have a bacillary morphology, do not have a dense nucleoid and their size ranges from 600 to 1,000 nm, but they are not infectious. They are stained blue with Giemsa stain, they can replicate, have metabolic activity and their DNA is disperse.

The membrane presents extracellular proteins rich in cysteine, including main protein of the outer membrane (MOMP) or 60-Kda protein, which is the biggest protein ; 12- to 15-Kda protein, which is present in the RB; and HSP, i.e., 60 and 70 HSP found in women with upper genital tract infections or pelvic inflammatory disease (PID).

In women with infertility and ectopic pregnancies, high Ac levels were found in the HSP 60 (anti-HSP60), in contrast with the Ac anti-HSP 70 that was reported in women with protective immunity[20-22].

VIRULENCE FACTORS

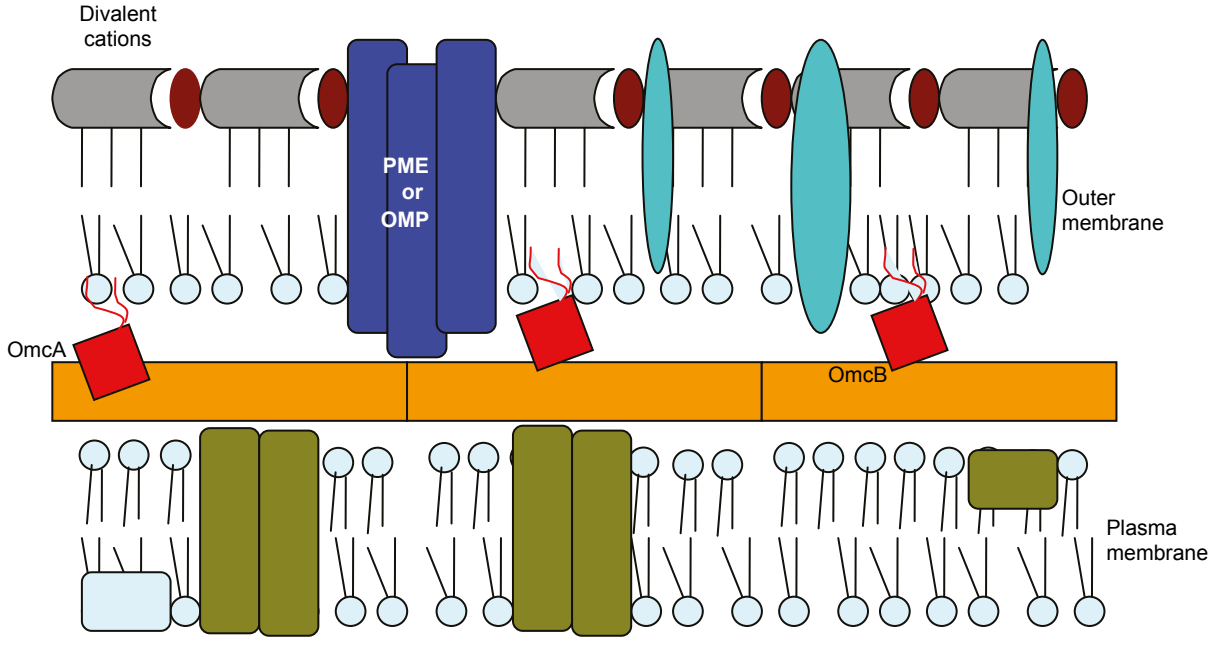
CT has been divided into 18 serotypes: A, B, Ba, C, D, E, F, G, H, I, Ia, J, K, L1, L2, L2a, L3, and L3a. This division is based on the analysis of the MOMP, which has four variable domains (VDs) that are flanked and interspaced through five constant domains. Three of four VD (VD1, VD2, and VD4) are found in the surface and contain antigenic epitopes, which are blank sites for serum typification. Both the LPS and the HSP60 have stimulated the innate response through the TLR4. However, the CT LPS has low endotoxic power[23,24], and the lack of significant expression of TLR4 in the cervix and in the upper genital tract suggests that the contribution of this molecule as a proinflammatory signal during the genital tract infections can be small[25]. Its union with the TLR2 seems more significant, and to be connected to the presence of a plasmid. When this plasmid is lost, the inflammatory response, the glycogen accumulation, and the infectivity are decreased[26-28].

The CT introduces proteins in the host cells through many mechanisms, including type III secretion[29], while some of them are translocated to the outer part upon the Sec-dependent pathway[30,31].

INTERACTION OF *CHLAMYDIA* WITH THE HOST CELL

In order to understand the pathogenesis of any infection, attention should not only be toward the pathogen, but also to the interaction between the pathogen and the cells. This is even more notorious in the case of CT, because this is an obligate intracellular microorganism that depends on such interaction to replicate and survive.

CT uses the mononuclear cells for reproduction. The EB invades the cells through endocytosis and actively multiplies as a RB inside the phagosome. Then, each body is transformed to EB, and they are eliminated as such.



OmcA: former Omp2; OmcB: functional protein associated with the outer membrane complex (former Omp3).

Figure 1 – Cell wall of the elementary body.

When the EB infects the cells, it induces the centrosome to form an apparatus and damages the replication. The infection begins in the vaginal cells and climbs to the upper genital tract. The alterations in the cells require replicating cells. Thus, its preferential location is inside the metaplasic area of the lower genital tract between the ectocervix and the vagina, where the cells actively replicate themselves.[1,2]

Cervical dysplasia that originated in experimental injuries with rats were seen, which can be progressive. CT manipulates the cells in several ways:

- acknowledging the cell (adhesion);
- penetrating;
- constituting phagosome;
- transforming the EB to RB;
- dividing the RB;
- transforming the RB to EB;
- releasing the EB or persisting on it.

ADHESINS AND ADHESION

We will further see more details regarding the analysis of the cell cycle, but, in each step, modifications and interactions of the microorganism with the compatible cell are being made.

CT is a Gram-negative microorganism, and thus it has an outer membrane with proteins, called MOMP, that work as adhesins. The characteristic of PME is that it promotes nonspecific interactions (electrostatical and hydrophobic) with the host cell. They are also known for having structures named VD I to IV, because they are separated by four symmetrical spaces whose sequence varies between the serum types. The importance of CT, on a clinical point of view, is that it is an in vivo determinant of the neutralizing antibody activity, because it works as a target[31].

In order to promote the adherence to the host cells, CT uses a trimolecular mechanism and requires the heparan sulfate similar to Glycosaminoglycan (GAG), that is, Heparan Sulfate Like Glycosaminoglycan (HSLG), in its surface. It seems CT elaborates a unique HSLG. However, an exogenous heparan sulfate, working as an adhesion analog, restores the suppressed CT infectivity through its early treatment with the heparan sulfate lyases.

DEVELOPMENTAL CYCLE OF *CHLAMYDIA TRACHOMATIS*

All chlamydiae have a biphasic cycle. The infection begins when an infectious particle named EB invades the host cell. It is around the cytoplasmic membrane and constitutes the phagosome. This intracellular EB is differentiated in the RB, thus it is divided into two cycles: the early and the late cycle. A series of events is produced, and one of the most important ones is the modification of the RNA:DNA relation, which was 1:1 and becomes 3:1, thus indicating the synthesis of proteins (**Figure 2**).

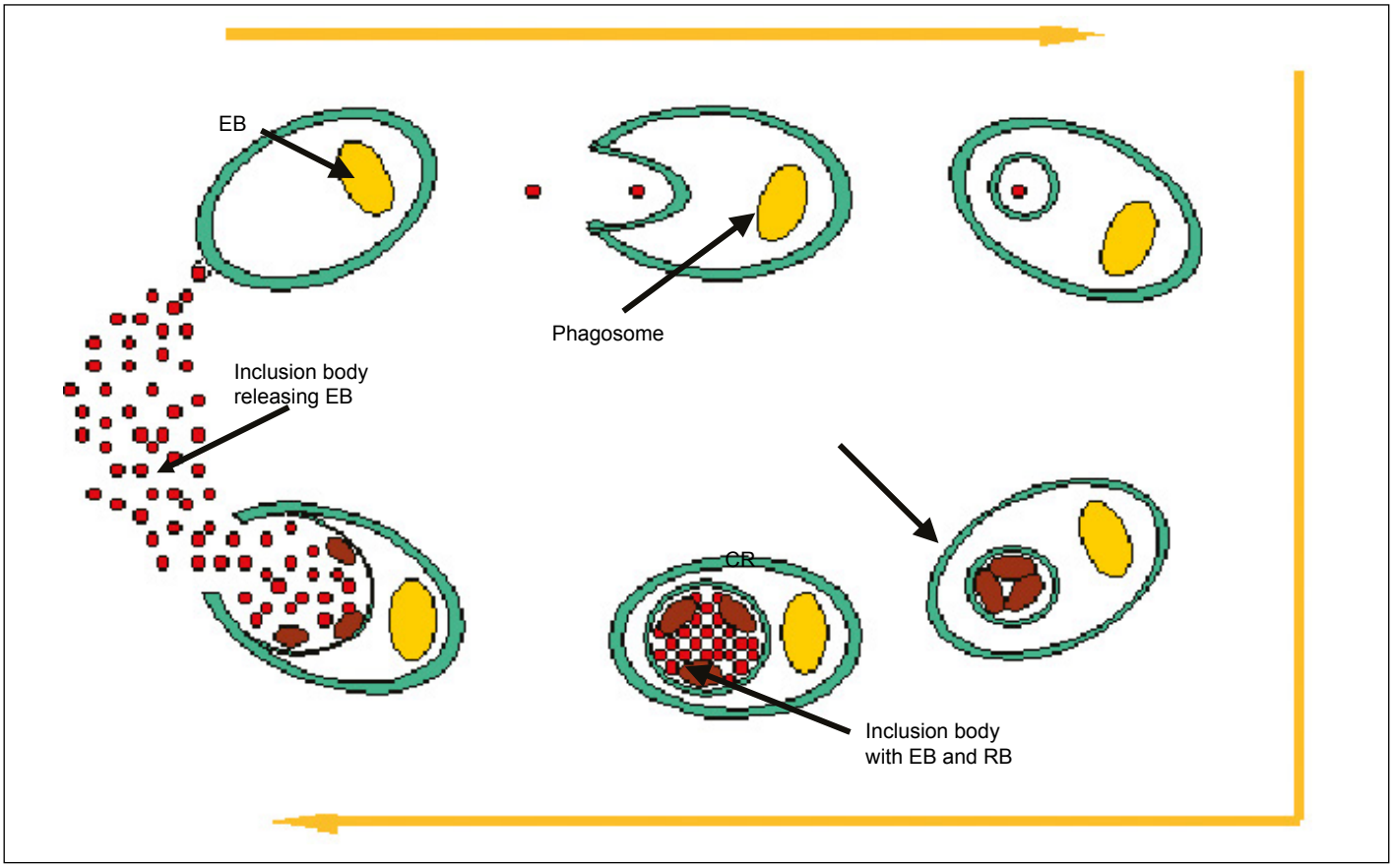


Figure 2 – Scheme of the development cycle of *Chlamydia trachomatis* in the inner part of mononuclear cells.

PENETRATION AND CONSTITUTION OF THE PHAGOSOME OR PARASITOPHOROUS

In order to develop its intracellular life, CT needs to scheme its penetration and create an adequate niche, which is done through a non-flagellar type III secretion system that is an improved virulence factor commonly found in Gram-negative bacteria, but it is not located in the pathogenicity islands of CT because it is dispersed in the entire chromosome. The type III secretion collaborates to virulence, as already expressed, and consists in the introduction of proteins inside the cell, while the steps that allow the exportation from the host or the vacuole refer to translocation. These denominations have been standardized.

All chlamydiae have a biphasic cycle of development. It has been studied that the ingression is produced as an EB and, inside the vacuole, it is divided into RB, which is not infectious. This is carried out in the so-called early cycle, during which cellular modifications are done (**Figures 3 and 4**)[32,33]. After and during the medium and late cycles, the RB transforms to EB and leaves the cell.

CHLAMYDIA TRACHOMATIS PRODUCES CELL ALTERATIONS

In cell cultures, the new inclusion migrates along the microtubules toward the perinuclear area that corresponds to the microtubule organizing center (MTOC) or centrosome. Many secretory organelles reside around the MTOC and possibly facilitate the interaction with lipids and with the compartments enriched by nutrients. Although CT cannot synthesize most of its requirements, it can, however, synthesize its own lipids, even though it is preferable to acquire them through the host cell. It was possible to show the presence of lipids in CT development areas in cellular lines (**Figures 5 and 6**) using cytochemical techniques[34,35]. It has been seen that the cell acquires neutral lipids through a nonvesicular pathway[36], interacting with lipid droplets and interfering with the energetic metabolism[37].

With regard to the alterations that CT produces in the cell[33], we can mention (**Figures 7 and 8**):

- fragmentation of Golgi apparatus;
- mitochondrial dysfunction;
- possible creation or facilitation of the supernumerary centrosome (abnormal mitosis)[38,39] appearance;
- exercise of an anti-immune strategy, thus suppressing cellular protection systems like apoptosis[40,41].

ABERRANT BODIES

The formation of aberrant persistent bodies that prevent the CT cycle completion might occur.

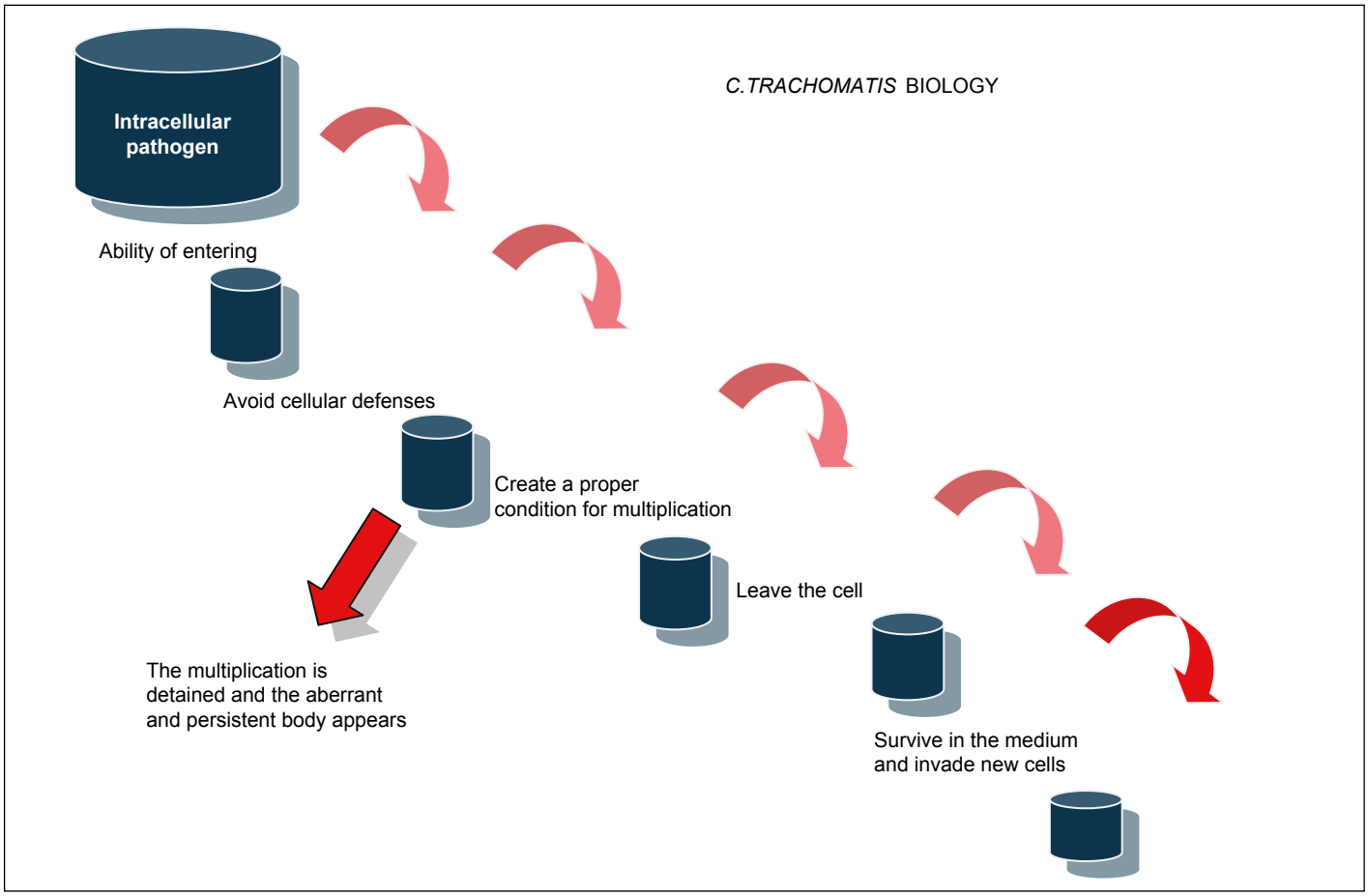


Figure 3 – Step followed by *Chlamydia trachomatis* to reach the intracellular inclusion body.

The in vitro persistence of large aberrant bodies follows several factors, among them the IFN-γ can remain in this condition for long period.[21] It is similar to what happens to other microorganisms such as *Toxoplasma gondii* and *Plasmodium*. The factors mentioned as facilitators of aberrant bodies and their persistence are fundamentally: penicillin use, depletion of tryptophan, and IFN-γ activity.

These aberrant bodies by remaining in the cells replicate with them and, based on a biological point of view, behave as bacterial plasmids; thus they remain inside the cells around 2 or 3 years. When they are not active, they are insensitive to the action of antimicrobials, even in the presence of those with the capacity of intracellular penetration.

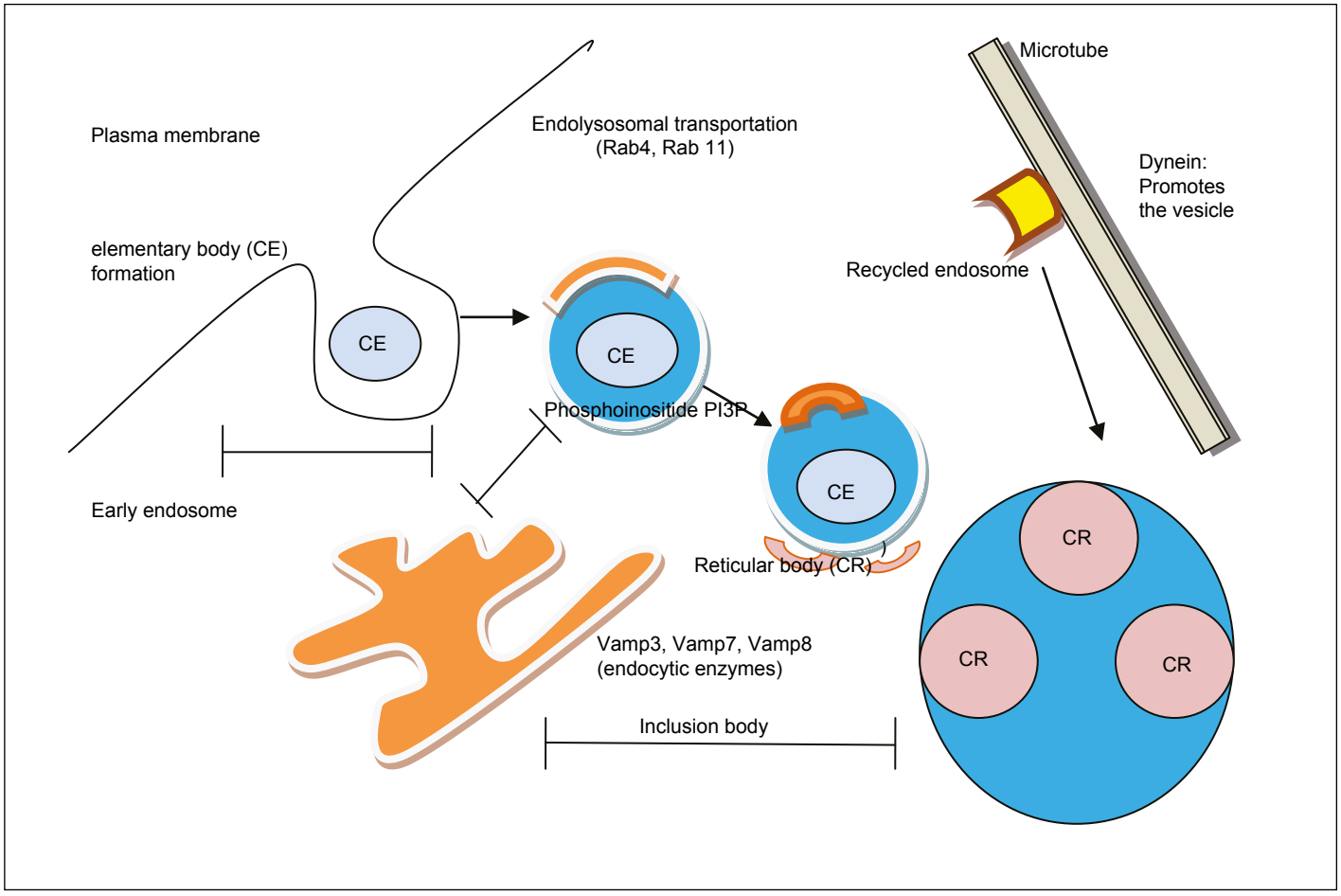


Figure 4 – Early intracellular cycle of *Chlamydia trachomatis*[33].

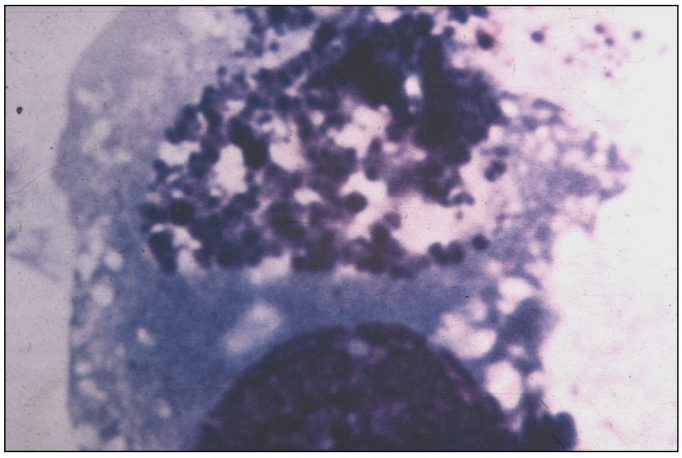


Figure 5 – Inclusion body releasing elementary body.

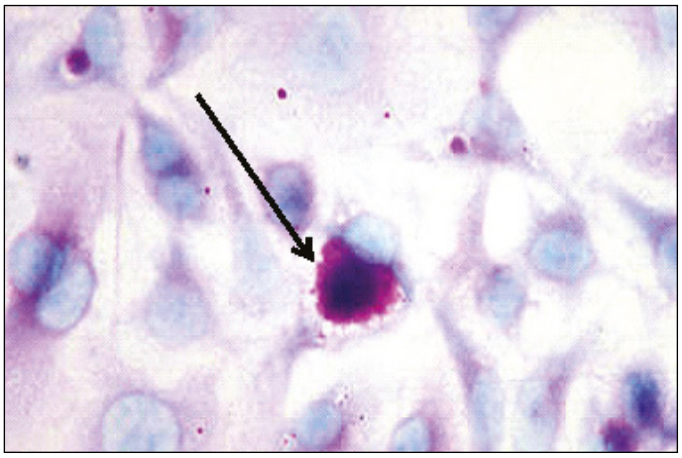


Figure 6 – Inclusion body in HeLa cells.

The detection of *Chlamydia* after a treatment with beta-lactam antibiotics suggests that its division is detained upon the incapacity of cell wall synthesis, and the presence of large aberrant bodies is seen. It is unknown how long it takes them to appear after exposure. The availability of biomarkers that might indicate this persistence is important[42].

Some points should be emphasized regarding the aberrant bodies of *Chlamydia* (suspended development):

- Cultures in cell lines that seem to be not infected, but the presence of *Chlamydia* with anomalous RB is found.
- The infection persists, because (1) the aberrant bodies can be transferred from a cell to another during cell division, (2) the aberrant bodies are capable of persisting during 2 or 3 years, (3) the infection can be induced through the immune response in the organism, (4) the aberrant bodies are relatively inert regarding the biochemical processes and less sensitive to the antimicrobials.

IMMUNOPATHOGENESIS

The innate immunity is critical, since it is the early component of the host response. It was first identified by Rasmussen et al.[43] and Stephens[44], who demonstrated that the in vitro infection of cervical and colonic cells with CT induced the secretion of a great amount of chemoattractive cytokines and with proinflammatory activity. The CT internalization is not enough to promote a response, opposite to what happens with other microorganisms. The response is developed during the CT cycle. The endocervical cells release interleukin 1α (IL-1α) after the infection and the anti-IL-1α-specific antibodies inhibit the inflammatory cascade (Figure 9). Stephens' paradigm named "cell paradigm of clamidial pathogenesis" theorizes that "the inflammatory process of the pathogenesis is caused by infected cells and is necessary and enough for the development of a chronic inflammation and for the promotion of cell proliferation, tissue remodeling, and sequelae lesions"[45].

Observations using the electronic microscope reveal that the EB is associated with the epithelial cell 3 hours after the infection (HPI). After 12 hours of insertion, the transition to RB and the active division begins. However, the host response is early manifested at three HPI. There is also the appearance of 11 genes that codify receptors for CCR2 and CCR6 chemokines, the CCL3 chemokines (MIP-1 α), CCL20 (MIP-3 α), CCL24, CCL25 and CXCL15, the cytokines IL-1F8, IL-13, and the tumor necrosis factor alfa (TNF-α) in the cervical tissue. The CCL3 (MIP-1 α) and the CCL24 (eotaxin 2) are chemotactical for the immature dendritic cells and CXCL15 is chemotactical for polymorphonuclear. These are seen infiltrating the infected epithelium at 12 HPI, and the activity of Natural Killer (NK) cells has been early seen at 12 HPI, thus confirming that the chemokine gradient quickly develops after the infection[46]. The predictive value of the inflammatory response was seen in 1996[47], and then the migratory capacity of polymorphonuclear through endothelial cells infected with *C. pneumoniae* and stimulated with TNF-α[48,49].

INFECTIONS

Recently, symptomatic and asymptomatic infections produced by CT in the area of gynecology and obstetrics were recognized: urethritis, cervicitis, PID, and perinatal infections. The asymptomatic condition and the latent infections constitute a real challenge for their research and prevention[50]. **Table 2** presents a summary of the infections and complications originated by CT.

Cervicitis

Cervicitis can be asymptomatic or symptomatic. In general, it is seen as mucopurulent cervicitis (**Figure 10**). There may be hypertrophic, edematous, and bleeding ectropion. The presence of immature squamous metaplasia in the ectropion area has also been described

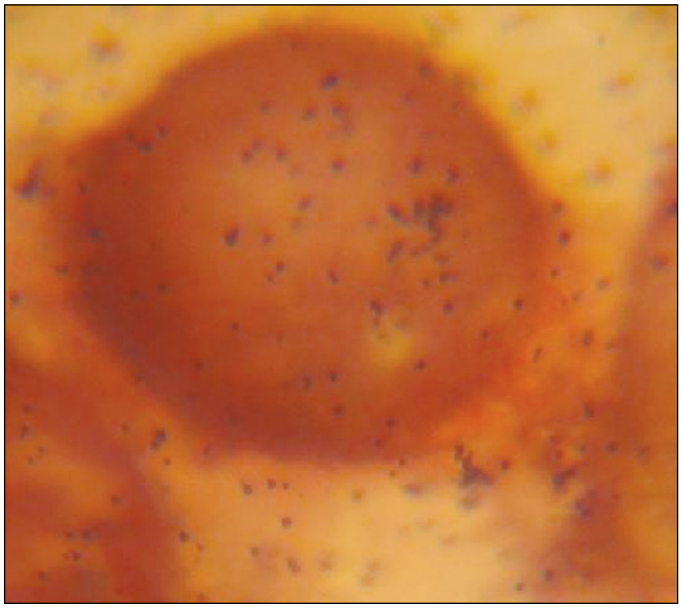


Figure 7 – *Chlamydia trachomatis* culture in HeLa. The black-colored intracellular lipids (LIC) are observed.

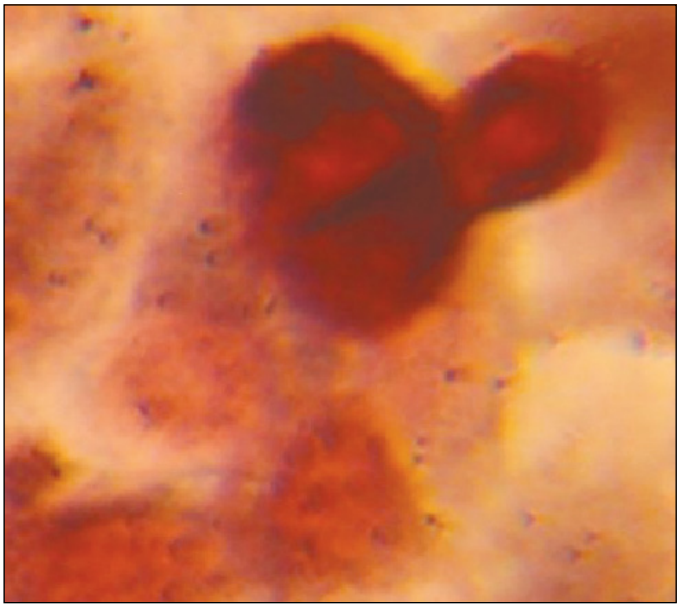


Figure 8 – Accumulation of lipid material.

by Paavonen and Eggert-Kruse[51] as an association with Chlamydia infection. Chlamydia infection can be related to the inflammatory response. The number of leucocytes can be a good index, although it is not exclusive.

The prevalence of cervical infection seems to be higher in women with ectropion, which predisposes to the acquisition of *Chlamydia*, since several columnar cells are exposed to the receptors or adhesins of the microorganism. This would explain the high proportion of adolescents with Chlamydia, because cervical ectropion is present in 60 to 80% of the sexually active female adolescents.

Oral contraceptive pills also promote the presence of ectropion; therefore, they are also a risk factor.

Clinical diagnosis of the mucopurulent discharge through Chlamydia is not conclusive. The differentiation with gonococcal cervicitis, salpingitis, endometritis, intrauterine device-induced inflammation, among other causes, should also be investigated. Thus, the clinical diagnosis of Chlamydia by professionals with a few or no training has a small correlation with laboratory data.

Almost none of the women with cervical infection develop antibodies against Chlamydia, and the presence of local antibodies has been reported in only 20 to 50% of the cases. In nontreated women, the sequential cultural study showed that the Chlamydia infection can persist for many weeks or months without showing any symptoms, or it can be spontaneously solved.

The detection of CT cervicitis in women with high risk of STIs and its treatment have showed a decrease of the PID incidence, which was also seen for *N. gonorrhoeae*.

Urethritis

About 50% of the women studied by cultures conducted in the cervix and urethra show positivity in both areas, and 25% in one or the other area. The causes of dysuria syndrome include frequency of infectious urinary sediment and negative urine common culture.

Table 2 – Summary of *Chlamydia trachomatis* infections.

In men	In women
Urethritis	Cervicitis
Prostatitis	Urethritis
Epididymitis	Endometritis
Vesiculitis	Salpingitis
Orchitis	Oophoritis
Proctitis	Abortion
Reiter syndrome	Reiter syndrome (less frequent)
	Perihepatitis
Perinatal infections: conjunctivitis, pneumonitis, pneumonia.	

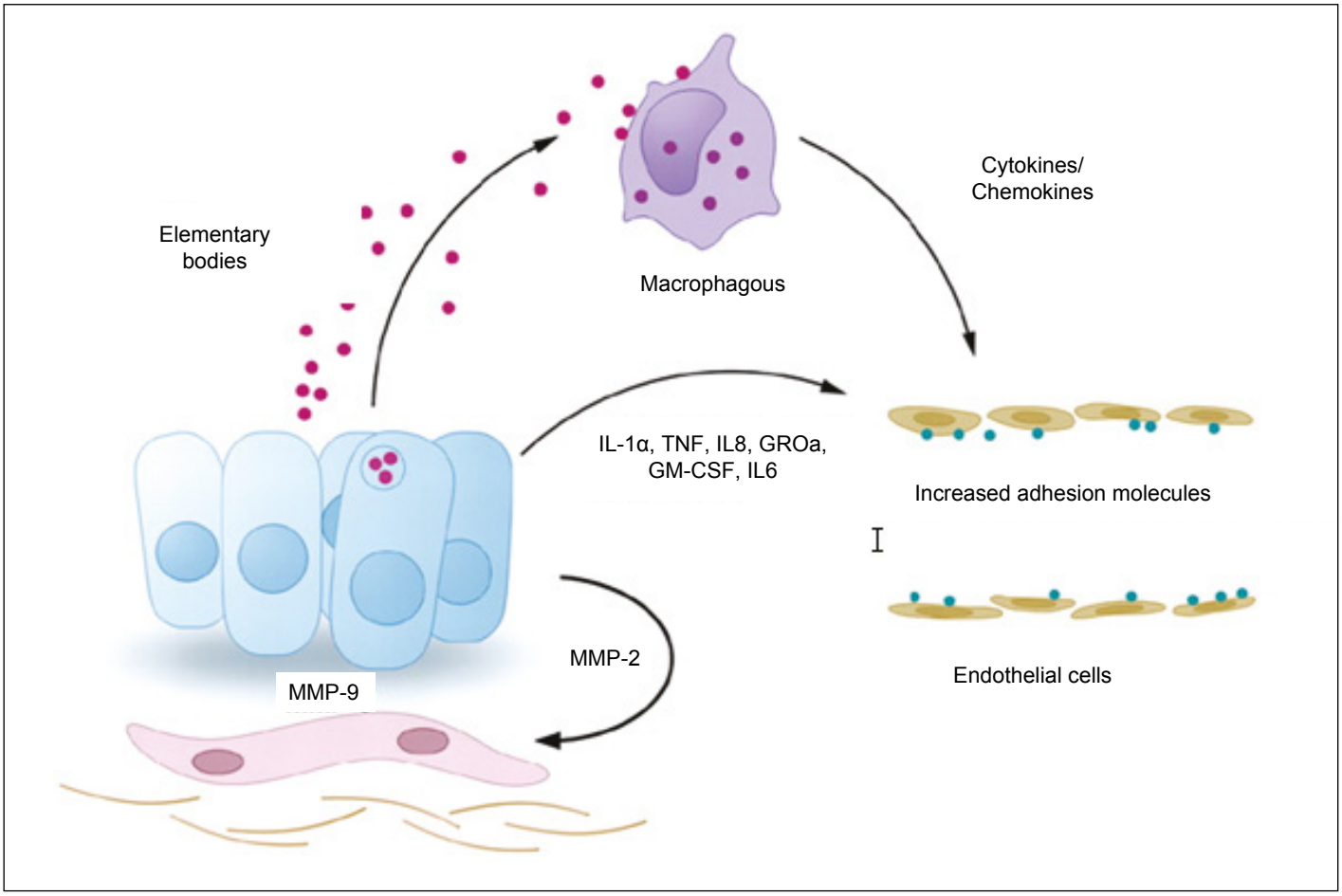


Figure 9 – Events in the early infection.

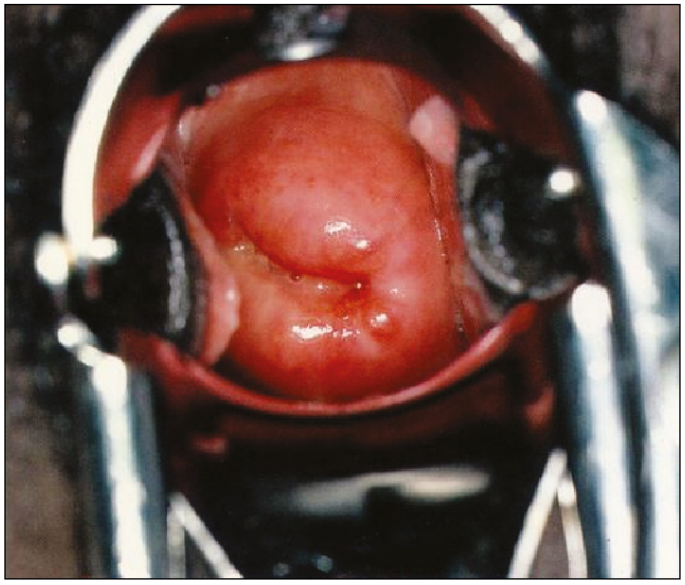


Figure 10. Cervicitis.

Bartholinitis

The purulent infection of Bartholin's gland can be due to both *N. gonorrhoeae* and/or CT or other facultative or anaerobic microorganisms, which cannot be distinguished on a clinical point of view. The possibility of infection happens due to the existence of columnar epithelium in the glandular ducts. The concrete diagnosis is only achieved by means of a laboratory study.

Endometritis

The disease is present in around half of the patients with mucopurulent cervicitis, and it is not frequent during pregnancy, although it can produce complications after such period.

It is characterized by the infiltration of the stroma with plasma cells and of the endometrial surface with neutrophil leukocytes.

Lymphogranuloma Venereum

This is the only CT infection that leads to compromise and multisystemic manifestations. Many phases can be noticed: there is a transitory lesion followed by a secondary stage with suppurated regional lymphadenopathy. This is the period when the most important symptomatology is found, that is, the secretion, the painful adenopathy, and the fistulae. Sequelae associated with fibrotic changes and lymphatic drainage appear in the last or late phase.

Pelvic inflammatory disease

Although the etiology of salpingitis remains uncertain, after improving the use of laparoscopy as a diagnosis method that allows to directly approach the tube to obtain samples destined to the etiological investigation, in the last decade, it has been established that CT is probably the most common cause, together with *N. gonorrhoeae*, of acute salpingitis cases or PID. As in *N. gonorrhoeae*, neither PID nor CT is frequent during pregnancy.

Perihepatitis

Perihepatitis is a common manifestation associated with salpingitis because its observation in pregnant women is less usual.

Proctitis

Chlamydia proctitis represents a disease that is not commonly diagnosed in women with digestive or rectal manifestations. It is a cause of abdominal pain in adolescents. One should carry out lab tests to avoid subdiagnosis of these infections. It is important to remember that the intestinal tract might change to a reservoir for CT[52].

Infections associated with arthritis

We must distinguish septic arthritis that has microorganisms in the joint and that is generally the product of systemic dissemination, as seen in the cases of disseminated gonococcal infection, from reactive arthritis, like in most of the cases, which are multifactorial. The most common form of arthritis associated with *Chlamydia* is the one developed after a urethritis condition that has been named: sexually acquired reactive arthritis (SAR).

Participation in preterm birth

The CT infections in pregnant women vary from 2 to 30% based on the studied population. There is no reliable information regarding the influence of pregnancy in the physiopathology of Chlamydia infections, or about its role in perinatal prematurity and mortality. The association of premature birth with IgM antibody titer and CT-positive culture was confirmed by Sweet et al.[53]. This correlation is expected, but more prospective studies with a larger number of patients should be carried out.

The same happens regarding the relation between CT and spontaneous abortion. In animals, as explained, it has been seen that *C. psittaci* is an important cause of abortion. Some authors[54] have reported the relation of CT with spontaneous abortion. However, we believe it is still early to provide a definite role of this microorganism in the spontaneous abortion pathology. With regard to the post-partum or post-abortion infections, endometritis and salpingitis were the most significant ones. Some studies show these complications happen in a significantly higher level among women who were infected with Chlamydia during pregnancy. Several authors have found cervicitis due to Chlamydia in 6.7% of women during the 19th week or a lower stage of pregnancy. Women who are early infected have higher risks of giving birth to dead fetuses than women who are not early-infected (6 of 18.33% versus 8 of 23.34%, respectively). Some changes in the duration of pregnancy among positive women and 238 noninfected women ($p < 0.001$) were seen. However, in other studies including 9,000 patients, there was no significant association between cervical infection by Chlamydia and preterm birth. Techniques that are more sensitive might be needed to conduct studies in pregnant women, although all information should be

reviewed and the prevalence of the infection in the community and the risk factors for women to have STI should be considered as well.

Neonatal and perinatal infections

A child can acquire Chlamydia through aspiration of the infected secretions that pass though the birth canal or if he/she is born via C-section, by infection of the membranes that suffered spontaneous early rupture before birth. The infections include conjunctivitis; nasopharyngitis; pneumonia or pneumonitis, and vaginitis.

Other complications

CT can participate in oncogenic processes, thus increasing the risk of cervical cancer, causing the duplication of the host cell genome due to alteration and rupture of cell spindle and other alterations, as already seen [39,40].

It is worth mentioning that adolescents, mainly female ones, are under risk of acquiring this preventable infection, but most of the times, they do not know about the care that should be adopted. Therefore, if the infection is not seen early, it could cause severe damage to their lives.

DIAGNOSIS

The diagnosis methods are based on:

- CT isolation in cell cultures;
- direct detection of different Ag in the clinical sample;
- molecular tests like PCR or LCR;
- serological studies that allow investigating antibodies, which are limited only to some pathologies, since they involve the antigenic persistence in the mucosa, during a relatively long period.

The used technique must be based on the characteristics of the studied population and such purpose (**Table 3**).

Detection of antibodies

The techniques used are establishment of complement, micro-immunofluorescence (MIF), immunofluorescence against the inclusion body, enzyme immunoassay, and detection of CT HSP. These techniques must be carefully evaluated and have limited value (**Table 4**).

We shall remember that for definite diagnosis, more or less complex methods are required, such as culture in cell lines. Currently, there are some molecular techniques available based fundamentally on the amplification of nucleic acids that provide great sensitivity and are very valuable for epidemiological studies, since they allow carrying out a study in the urine. The techniques that find Ag, ELISA, direct immunofluorescence (IFD), or chromatographic ones should not be used to evaluate the treatment in patients that had a positive clinical response, since Ag can continue for several months.

Several teams use different supports and methodologies to detect CT Ag and Ac. When a comparison evaluation is done on the same populations, notable differences are found and, based on it, one or the other method is used with different results. This can cause the adoption of erroneous measurements in the epidemiological research or in the establishment of therapeutic standards (**Tables 5 and 6**)[55-57].

Table 4 – Techniques for antibody detection.

Test	Number of organisms/sample
DNA/RNA amplification	1–10
Culture	10–102
DFA	10–103
EIE	103–105
Probes	103–104

DFA: direct immunofluorescence antibody; EIE: enzyme immunoassay.

Table 3 – Diagnostic guidelines of *Chlamydia trachomatis* in women.

Diagnosis	Clinical criteria	Laboratory criteria	
		Assumption	Certainty
Mucopurulent cervicitis	Mucopurulent cervical exudate, cervical ectropion, spontaneous, or induced easy bleeding.	Inflammatory response (>10 L/400´) in the absence of an acknowledged etiological agent (*T. vaginalis*, *C. albicans*, among others).	Positive culture or positive direct test in cervical smear test material.
Dysuria syndrome	Miccional difficulty with or without pain, including polyuria and frequent urination, in sexually active women without neither a structure change of the urinary apparatus nor immune deficiency, which is usually associated with a new partner.	Infectious sediment without conventional bacteriuria.	Positive culture or positive direct test in urethral and/or cervical swab.
Pelvic inflammatory disease	Pain around the lower abdomen, painful uterine or adnexal mobilization, palpable mass in the rectouterine pouch. Frequent presence of purulent cervical discharge.	It is similar to that of the mucopurulent cervicitis. Presence of purulent material in the puncture of the pouch of Douglas or in a direct tube laparoscopic sample.	Positive culture or positive direct test in endocervix. Tubal, endometrial, or rectouterine pouch material (rare in the latter).
Perihepatitis	Right upper quadrant pain, vomits, fever, sexually active female individual, evidence of recent or concurrent pelvic inflammatory disease.	It is similar to mucopurulent cervicitis or pelvic inflammatory disease.	IgM or IgG antibody titers against high *Chlamydia trachomatis*.

CT screening in sexually active young population has low cost and great effectiveness when the population prevalence of the infection exceeds 3 to 6%. A nondiagnosed or treated STI can result in severe complications; therefore, we should pay attention to the asymptomatic characteristic that CT infection has in adolescents.

A very common error is to request the diagnosed and treated patients a new test to prove the treatment efficacy immediately. This is a problem since it could provide false-positive results that lead to unexplainable re-treatments. The Center for Diseases Control and Prevention (CDC) does not recommend the "healing" test in patients with CT continuously treated, but they recommend conducting a new monitoring after 3 or 4 months (**Chart 1**)[10]. The currently recommended treatment for cervical infection is the single dose of 1 g of azithromycin (the PID doses have not yet been reliably established).

Today, the use of azithromycin is recommended in other pathologies to avoid resistance to this microorganism.

For a successful treatment, these guidelines should be followed:

- complete the entire treatment, even if the symptoms disappeared before it is cured;
- sexual partners must be controlled and treated;
- no sexual intercourses until the infection is totally healed.

Today there are several efficient antibiotics to be used in short-term treatments, including single-dose antibiotics. Other antibiotics require a longer treatment, yet also effective, but they are usually abandoned after the symptoms disappear before the treatment conclusion or due to their side effects.

Some common aspects in the prevalence of CT infection include:

- it could be conducted without a speculum;
- screening is important to control PID;
- it can be conducted using enzyme immunoassay (EIE) if the prevalence is around 5 to 7%;
- universal screening if the prevalence corresponds from 10 to 12%, which is selective if below 5 to 6%;
- screening should be conducted every 6 months;
- diagnosis and treatment of CT infections seemed to significantly reduce the ectopic pregnancy.

Table 5 – Related limits of detection with different technologies[56].

	Culture (%)	EIE (%)	DFA (%)	NAA (%)
S	40–60	65–75	70–75	90–98
E	100	90–95	90–97	98–99

S: sensitivity; E: specificity; EIE: enzyme immunoassay; DFA: direct immunofluorescence antibody; NAA: nucleic acid amplification.

Table 6 – Techniques for the Ag detection of *Chlamydia trachomatis*[56].

Techniques	Prevalence (%)
Immunofluorescence	14.3
Ag: MOMP	14.3
IgG detection with EIA	11.9
Ag: HSP 60 (cHSP60) (Medac) with EIA	23.2
Immunoassay PID-fluorescence (InoDiag)	26.2

MOMP: Major Outer Membrane Protein; EIA: Enzyme Immuno Assay; HSP: Heat Shock Protein; PID: Pelvic Inflammatory Disease.

PERSISTENT INFECTIONS DUE TO *CHLAMYDIA TRACHOMATIS*

Bear in mind what was explained about aberrant bodies that create persistent infections. It is very hard to establish an adequate therapy for the habitual behavior of these CT forms. It is possible that, despite the correct treatments, the infection persists. When these aberrant bodies persist on the cell, they multiply with them and remain inside them for 2 or 3 years.[55]

VACCINES: PROGRESSES AND CHALLENGES

Still no vaccines are available for CT or *C. pneumonia*, which can be used in humans. However, the existence of some successful vaccines in the veterinary field might hold some expectations. They include mitigated or EB established as immunogens, they produce short-term immunity and, therefore, they require reinforcement doses to maintain a proper protection[58].

The Ag that develop protective immunity have been studied around three decades. Therefore, based on the conventional techniques, the findings were limited. When the CT genome was available, it was possible to find some advances. Around 900 proteins are codified, and each one can be cloned for the analysis of specific Ag based on the response that is manifested during the chlamydial infection. The potentially useful Ag for vaccinations are presented in **Figure 11**.

Chart 1 – Guide of general therapeutics[10].

There are several therapeutic proposals regarding antimicrobial doses and time of administration. Based on our experience, we are in favor of a 15-day average for most of the medications used nowadays, with the exception of azithromycin. In each scheme, we will mention the useful drugs that have been commercialized in our environment:
Noncomplicated male urethritis, cervicitis, or proctitis Minocycline or doxycycline: 100 mg in 12 hours. Tetracycline: 500 mg, four times a day. Erythromycin: 500 mg, four times a day. Roxithromycin: 150 mg every 12 hours. Ofloxacin: 400 mg in 12 hours*. Ciprofloxacin: 500 mg in 12 hours*. Levofloxacin: 500 mg daily orally, for 7 days*. If the clinical evolution has a favorable scenario, the recovery evaluation using a laboratorial diagnosis test will not be necessary.
Chlamydia trachomatis infections in pregnancy Erythromycin: 500 mg, four times a day. Roxithromycin: 150 mg in 12 hours. The administration of erythromycin estolate during pregnancy period is not recommended because of the hepatotoxicity it might cause (thus, the azithromycin use is established).
Pelvic Inflammatory Disease The treatment implies the association of an antimicrobial on activity over aerobic, anaerobic, and facultative bacteria, which are associated with an anti-chlamydia antimicrobial.
Dysuria syndrome: with macrolides or azalides.

It should not be administered to subjects younger than 14 years old.

Efforts should be given to correctly identify the protective Ag causing vigorous response of T CD8+ cells.

Stary et al.[59] commented that CT infection induces a protective immunity that depends on interferon and on the T CD4 cells. We know, in general, that subcutaneous or intramuscular vaccination could create a systemic and cutaneous effective immunity to several pathogens, but the vaccination through these nonmucosae routes usually do not induce, or induce very little, the protection in mucosae surfaces like the one that CT requires. In very interesting experiments done by the investigators, the inactivated CT mucosa exposure through ultraviolet light (UV-CT), with charge-switching synthetic adjuvant particles (cSAP), creates an extended protection in rats. The UV-CT-cSAP vaccine induces a memory in T cells, regardless of the route that is employed, but only the mucosa route induces effective T cells that will be needed together with the T cells of resident memories (TRM) to produce the clearance or elimination of CT.

MAIN POINTS

1. CT is a very common STI, more frequently reported in the United States, estimated in 3 million cases every year.
2. The cost estimated by the CDC of CT consequences is around two million or more dollars and that of the screening and treatment programs is around 175 million dollars.
3. Each dollar invested in the control saves 12 million in the complications of the nontreated cases.
4. It is the most common STI among female sexually active adolescents and young adults: 1 in every 10 subjects will be infected at some time. The most frequent age group is between 15 and 19 years. This group represents 46% of all infections.
5. It can be asymptomatic and its beginning indolent, and causes cervicitis, urethritis, and endometritis. Up to 40% of nontreated women develop the upper genital tract infection or PID, which can be silent, especially among adolescents.
6. The nontreated PID can cause sterility, chronic pelvic pain, and ectopic pregnancy.
7. Fifty percent of the children exposed to the birth canal develop conjunctivitis and from 10 to 16% develop pneumonia.
8. Diagnosis can be directly made in clinical samples by means of several immunological and molecular techniques.
9. These techniques should not be used for controlling the therapy and should never be used immediately after the treatment.
10. Culture in cell lines should be considered for conflicting cases.
11. Do not forget about the possibility of persistent cases, which do not respond to therapy.

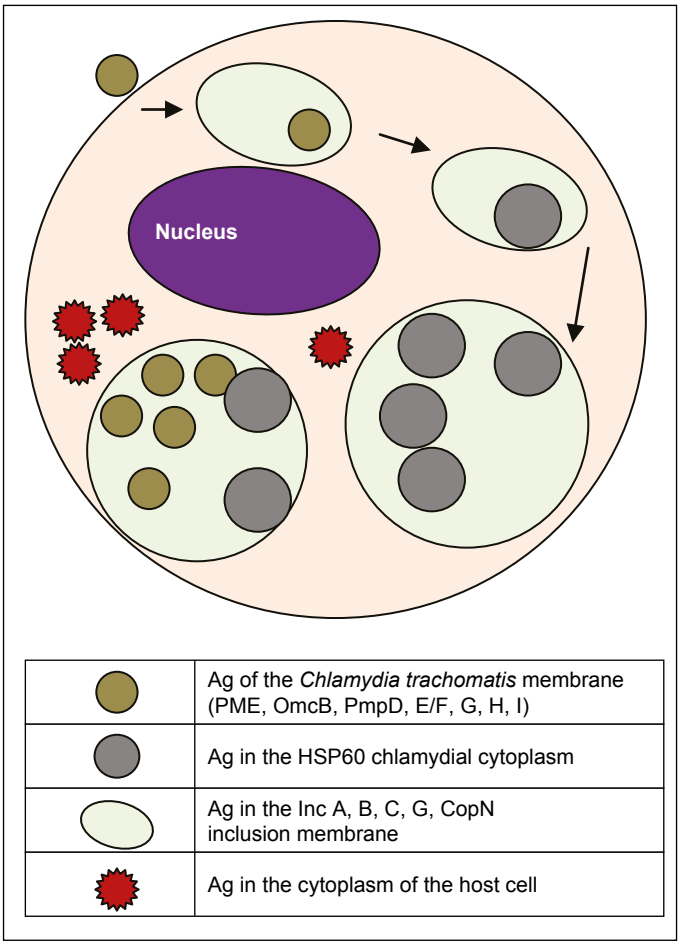


Figure 11 – Useful antigens for vaccines.

Conflict of interests

The authors report no conflict of interests.

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Transmission of Human Papilloma virus among couples: match between the subtypes and different sites of infection

Transmissão do Papilomavírus humano entre casais: concordância entre os subtipos e sítios diferentes de infecção

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ABSTRACT

Introduction: Among the sexually transmitted diseases, the highest world incidence is infection by the human papillomavirus (HPV). It affects 65% of men and 80% of women. At the moment, it is only the viral infection associated with development of cancer. **Objective:** To review the aspects related to the transmission of the HPV in men and women, collecting studies that analyze their transmission among couples, comparing the correlation between the partners and the transmission rates among different sites. **Methods:** The study was designed based upon literature review, using medical search tools like Pubmed/Medline, with the keywords: "Human Papillomavirus"; and combinations, "Human Papillomavirus" and "couples," "HPV infection in men," "transmission of papilloma virus." The studies selected were those that included HPV detection through HPV-DNA. **Results:** The concomitance of at least one viral genotype between genders showed great variation from 2.63 to 100%, the latter being specifically related to high-grade and consistency in HIV-positive groups. **Conclusion:** Men play a decisive role in this mechanism, both as a transmitter and as a carrier, thus they should also be the focus of HPV vaccination and treatment policies .

Keywords: Papillomaviridae; sexually transmited diseases; pathogenicity.

RESUMO

Introdução: Dentre as doenças sexualmente transmissíveis, a de maior incidência mundial, é a infecção pelo Papilomavírus humano (*HumanPapillomavirus* — HPV). Atinge 65% dos homens e 80% das mulheres. No momento, é única infecção viral que está associada desenvolvimento de um câncer. **Objetivo:** Revisar os aspectos relacionados à transmissão do papilomavírus humano em homens e mulheres, reunindo estudos que analisam sua transmissão entre casais, comparam a concordância entre os parceiros e as taxas de transmissão entre sítios diferentes. **Métodos:** O estudo foi concebido a partir de revisão bibliográfica usando a ferramenta de busca médica pubmed/medline, com as palavras-chave: "papilomavírus humano"; e as combinações: "papilomavírus humano" e "casais", "infecção HPV no homem", "transmissão do papilomavírus". Foram selecionados aqueles em que a presença do HPV foi comprovada pela detecção do HPV-DNA. **Resultados:** A concomitância de pelo menos um genótipo viral entre os gêneros mostrou grande variação desde 2,63% até 100,00%, especificamente este relacionado ao alto grau e concordância em grupos HIV-positivo. **Conclusão:** O homem desempenha um papel decisivo nesse mecanismo, tanto como transmissor quanto como reservatório, devendo ser alvo também das políticas de vacinação e tratamento do HPV.

Palavras-chave: Papillomaviridae; doenças sexualmente transmissíveis; patogenicidade.

INTRODUCTION

Among the sexually transmitted diseases, the one with the highest incidence worldwide is the infection by the human papillomavirus (HPV). It affects 65% men and 80% women[1]. It is currently the only viral infection linked with the development of cancer[2].

HPV is largely responsible for cervical neoplasia, estimated at 450,000 new cases per year worldwide[3]. It was identified in 46–100% cases of cancer of squamous cells in the anal region.

Despite affecting both genders, there are few studies concerning couples or male individuals.

The infection and transmission among men and women have distinct patterns, with significant differences in the persistence of the virus.

After a year of investigation, a Dutch study showed the persistence of the virus among women of 20% whereas only 6% among men[5]. In Finland, the majority of women presented an average clearance time (the capacity to eliminate the virus) of 6 years, an average of 62.5 months. However, among 75% men the average was merely 12 months[6,7]. In addition, when comparing the rate of infection and the manifested disease, higher rates of infection were found among men than women, in contrast to the rates of the disease, which are higher among women[8].

The consistent findings between sexual partners are still poorly understood. They seem to depend upon the type of sexual relation,

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the time since the act, the subtype of HPV involved, and the site in which the virus is found[9].

OBJECTIVE

Review the aspects related to the transmission of the HPV among men and women, collecting studies that analyze the transmission among couples, comparing the consistency among partners, and the rates of transmission in different sites.

METHODS

The study was designed based on a literaturereview utilizing the medical search tool Pubmed/Medline, with the keywords: "human papillomavirus"; and combinations: "human papillomavirus" and "couples," "HPV infection in men," "transmission of papillomavirus." The studies selected were those that included HPV detection through HPV-DNA. In all of them, the collection was carried out from the site of the female genitalia using the Papanicolaou test and the male by swabbing the penile regions, such as the glans, urethra, and foreskin.

RESULTS

Consistency among couples

when assessing the existence of the HPV virus in partners, we can locate corresponding characteristics in its presence or lack thereof as well as in the similarity between viral subtypes.

Huang et al. found a rate of 5.26% of concomitant presence of the virus in partners, with this being the lowest rate found among the studies. In the HIM (HPV in men) study, conducted in the USA, which assessed 88 couples, the concomitance of the viral infection was of 62.5%.

A similar rate was found by Widdice, in an assessment of 25 couples through 116 examinations, with correspondence in 77 of them (61%). Each participant was examined five times, each at distinct times after sexual intercourse. The highest rate of consistency was of 95%, with the material being collected 24 h after sexual intercourse. The rate was of 74% after 48 h of sexual intercourse. The variation found was of 61–91% in all sites and all examinations.

In Brazil, Afonso et al. conducted a study in two population groups; the first, in which the woman presented cervical intraepithelial neoplasia (CIN), presented 50% of consistency between the couples; and the second, conducted with asymptomatic couples, resulted in 15%[8].

When the comparison is made between African couples, specifically those who are carriers of the human immunodeficiency virus (HIV), the correspondence in the detection of HPV was of 56%, whereas in patients who were not carriers of the HIV virus, the correspondence was of 22%[13].

Parada et al.[14] obtained the highest percentage (79%) among the analyzed studies, perhaps due to the sample being bigger and due to the method for collecting material in men having included numerous anatomical sites on the penis, increasing the chance for detection of HPV[14] (**Table 1**).

CONSISTENCY OF VIRAL SUBTYPE

Another assessment of the analyzed studies was carried out in relation to the consistency among couples and the subtype of the virus present in the individual, both related to oncogenic as well as non-oncogenic subtypes.

The concomitance of at least one viral genotype among the gendersreveals a great variance from 2.63%, in a Chinese study, to 100%, a value obtained in an African study, with the latter being specifically conducted on an HIV positive group.

Subtype 16 is the most common among oncogenic individuals, being found in a higher prevalence, both in the studies by Huang *et al*. as well as in the Brazilian study[8,10].

Subtypes 31 and 89 can also be considered important as they are predominant in the HIM study, where 23.9% partners had equivalence in at least one viral genotype; another relevant finding was the total correspondence of subtypes in merely 2 of 88 couples[19].

Subtypes 39, 54, 59, and 62, found by Parada et al.[14], are highlighted due to their viral similarities of 61.8% and subtype 84, prevalent in the Widdice et al.[12] study. This last study, unlike the others, utilized anogenital consistency among the genders, obtaining 15% rate in 116 examinations.

Despite not citing the specific subtypes of HPV involved, the Canadian study, known as HITCH, revealed that 42% couples presented at least one similar subtype[15].

Finally, it is worth noting that the correspondence of genotype of the HPV virus was higher when the participants presented diseases such as CIN, in the Brazilian study, and HIV, in the African study[8,13] (**Table 2**).

FACTORS RELATED TO TRANSMISSION

Some factors seem to influence the transmission of the virus and the rate of consistency.

The rate of consistency, taking the specific subtypes into consideration, was higher among couples with a similar age and who had

Table 1 – Consistency among couples in the various studies.

Study	Number of couples	Rate of consistency
Huang et al.[10]	76	5.26%
Giuliano et al.[19]	88	62.5%
Widdice et al.[12]	25	61.00%
Afonso et al.[8]	60	15–50%
Vogt et al.[13]	34	22–56%
Parada et al.[14]	504	79%

Table 2 – Consistency of viral subtypes.

Study	Subtype consistency	Most common subtype
Huang et al.[10]	2.63%	16
Giuliano et al.[19]	23.9%	31 and 89
Widdice et al.[12]	15%	84
Afonso et al.[8]	42%	
Vogt et al.[13]	61.8%	59. 62.54, and 39
Parada et al.[14]	3–26%	16

been in a relationship for less than a year[11], and in the presence of the HIV virus[13].

Although not statistically significant, the presence of non-monogamous relationships seems to have a connection with the transmission of the virus, which was not found in relation to variables such as race, ethnicity, education level, and marital status[11].

The inconsistency of subtype was related to a lower number of partners for each individual during their life ($p<0.0001$)[11].

Other positive variables for the increase in the transmission of the virus are the following: men having had a higher number of partners throughout his life; the couples involved maintaining an active sex life; not using condoms; and having recently had vaginal intercourse[14,15]. It is imperative to emphasize that the main risk factor for the transmission of the virus appears to be the presence of the same partner, increasing the rate of transmission up to 5.1 times[12,14].

RATE OF TRANSMISSION

Some authors investigated the rate of transmission between different sites of the human body. In 2013, Widdice[12] assessed each partner during five examinations and obtained the following values: detection of female genital HPV on the partners had varied from 48% to 60%;in the partner's perianal area, from 48 to 60%; and in the partner's oral cavity, from 0 to 5%. The consistency between the female anal site and the male genitalia was between 56 and 76%; male hand, from 33 to 63%; male perianal area, from 13 to 38%; and the oral cavity, 0% — showing that in addition to the genitals, the hand and perianal areas are important vehicles of transmission, contrary to the oral cavity which did not present a significant role. In all the examinations, the rate of transmission of the women to men was higher than the inverse, the same occurring when non-genital sites were considered[12].

A similar result was found by Burchell et al.[15], in the HITCH study, and by Hernandez et al.[16]. The former found a rate of transmission of 4.0 from the women to the men whereas the inverse was 3.5, when a calculated variable of 100 people/months (number of events divided by the number of people exposed per month, multiplied by 100)[15]. The latter shows the transmission of women to men as approximately 3.5 times higher than the inverse; they also attempt to statistically define the main sources of transmission for each sex, verifying the penis as the main vehicle for transmission in men and the cervix as the main vehicle of transmission in women. In addition, the hand and anus of women are also relevant as they can transmit HPV to men. In the same study, self-inoculation was noted in both sexes, but mainly in men[16].

DISCUSSION

Based on the cited studies, it can be inferred that the HPV is highly transmissible, which is corroborated by the average of consistency among 58% couples. In the study by Huang et al.[10], which presented lower rates (5.26%), the authors believe that this fact is due to the characteristics of the couples studied, such as long-term monogamous relationships and the men over 35 years of age, which likely decreases the chance of recent contamination of the individuals. In addition, the clearance among men is more efficient and the faster elimination of the virus in men decreases its identification after a long period has passed since the sexualrelationship[10].

The finding that the HPV genotype 16 presents higher consistency among couples is in line with what had been shown in previous studies: infection by high-risk HPV, attributed to this genotype, is long-lasting and seems to have a higher potential for transmission[17].

The presence of HIV seems to be an important cofactor in the transmission and infection of the HPV, as the African study demonstrated. People with reduced immunity, such as those infected by HIV or with transplants, present more persistent infection and a higher prevalence of cervical cancer[13,17].

The viral difference between the men and women can be due to numerous factors. Beginning with the greater difficulty in collecting material from men, who present various sites for collection in the genitals, such as the glans, prepuce, and urethra. In case of women, there is a greater ease in detecting the virus, since cervical smear provides greater cellularity than the urethral swab, which is performed on men. There is also a relevant difference between the cervical and penile epithelium, which helps to emphasize this difference[10,14,18].

The fact that these studies demonstrate the presence of HPV-DNA, which does not necessarily mean viral infection, is another important aspect that should be discussed. Furthermore, even stable sexual partners present low consistency of genotypes. Detection of viral DNA and the high rate of transmission may not be as significant for the infection and the diseases caused by them[12].

Despite the analyzed studies had a high degree of relevant consistency, more of them should be conducted, especially those which involve more significant samples, with more heterogeneous population groups.

There is a clear barrier imposed by the difficulty in treating sexually transmitted diseases among couples that are important to emphasize. They conflict with preconceived cultural and religious notions, in addition to a lack of communication between partners and between the patient and the doctor.

CONCLUSION

Transmission of HPV involves various factors and its study can help to unveil and help its prevention.

Men play a decisive role in this mechanism, both as transmitters as well as carriers, thus they should also be the focus of HPV vaccination and treatment policies.

New studies can better clarify this relationship and contribute to the decrease in the dissemination of this virus.

Conflict of interests

The authors reported no conflict of interests.

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DNA METHYLATION: A REVIEW OF NEW PERSPECTIVES FOR EARLY DETECTION OF CERVICAL CANCER

METILAÇÃO DE DNA: UMA REVISÃO SOBRE NOVAS PERSPECTIVAS PARA DETECÇÃO PRECOCE DO CÂNCER DO COLO DO ÚTERO

Maria Cristina de Melo Pessanha Carvalho[1], Maria Paula Leite da Rocha[2]

ABSTRACT

Introduction: Cervical cancer is a serious public health problem and the fourth most common type of cancer in the female population. Persistent human papillomavirus infection is a risk factor for this tumor. However, epigenetic abnormalities occur in the carcinogenic process, in which DNA methylation is an important mechanism for gene silencing. That tends to lead to premalignant changes, a fact which can be investigated through the use of biomarkers for early detection and prevention of cervical cancer. **Objective:** To identify studies that analyzes epigenetic science, DNA methylation, and research on molecular biomarkers as new perspectives for early detection of cervical cancer. **Methods:** This is a literature review including articles published in the last 5 years in English and Portuguese, using the LILACS, Medline, SciELO, Cochrane Library, and PubMed databases. **Results:** The study determined a process of convergence in the analyzed subject. After reading various studies on DNA methylation and cervical cancer, the team highlighted the inactivation of tumor suppressor genes and the activation of oncogenes, which have an important role in cervical carcinogenesis, caused by epigenetic changes found in women with cervical intraepithelial neoplasia and cervical cancer. **Conclusion:** This review showed several screening strategies associated with cytological and molecular tests; however, there are knowledge gaps and the need for further investigation. Potential biomarkers are suggested to allow the monitoring of molecular events that complement the program of cervical cancer control, reducing mortality in the female population.

Keywords: DNA methylation; papillomaviridae; cervical intraepithelial neoplasia; biological markers.

RESUMO

Introdução: O Câncer do colo do útero é um sério problema de saúde pública e o quarto tipo mais comum na população feminina. A infecção persistente por HPV é um dos fatores de risco para o desenvolvimento deste tipo de tumor. No entanto, anormalidades epigenéticas ocorrem no processo carcinogenico, em que a metilação do DNA é importante mecanismo para silenciamento gênico que tende a levar a alterações pré malignas, fato que pode ser explorado com a utilização de biomarcadores para detecção precoce do cancer cervical. **Objetivo:** Identificar estudos que exploram a ciência epigenetica, metilação de DNA e investigações acerca de biomarcadores moleculares como novas perspectivas para detecção precoce do câncer cervical. **Métodos:** Trata-se de uma revisão bibliográfica em que foram incluidos artigos publicados nos últimos 5 anos em ingles e portugues, utilizando as bases de dados LILACS, Medline, SciELO, Biblioteca Cochrane e Pubmed. **Resultados:** Encontrou-se convergência sobre a temática estudada. Após a leitura de vários estudos sobre a metilação do DNA e câncer cervical, destacou-se a inativação de genes supressores tumorais e ativação de oncogenes, que desempenham um importante papel na carcinogênese cervical causada por alterações epigenéticas encontrados em mulheres com neoplasia intraepitelial cervical e câncer cervical. **Conclusão:** Esta revisão mostrou várias estratégias de triagens associadas com testes citológicos e moleculares, no entanto ainda existem lacunas de conhecimento e necessidade de maiores investigações. Propostas de biomarcadores foram ilustradas permitindo monitorar eventos moleculares que complementam programa de controle do câncer cervical, reduzindo a mortalidade na população feminina.

Palavras-chave: metilação de DNA; papillomaviridae; neoplasia intraepitelial cervical; marcadores biológicos.

INTRODUCTION

This study is based on the increasing research on epigenetic science, human papillomavirus (HPV), cervical cancer, and molecular markers, indicating new perspectives for early detection of cervical cancer. This topic is relevant in view of the carcinogenic effects that may be associated with DNA methylation and molecular changes in the development of this type of tumor[1].

The carcinogenic process comprises a series of modifications that are accumulated in the cell and eventually allow unregulated growth. The presence of mutations in key genes is involved in the cell-cycle regulation and cell growth and they are among the genetic exchanges that take place[2].

Persistent high-risk HPV infection is essential, but not a prerequisite, for cervical carcinogenesis. There are genetic and epigenetic changes that also operate in the development of precursor lesions and invasive cancer[2].

Thus, cervical cancer is a serious public health problem in women worldwide. It is the second most common type of cancer among the women and has higher incidence in less developed countries. This difference is also seen in relation to survival, as, in poor countries, diagnosis is most often made in advanced stages[3].

According to the statistical data, 528,000 new cases and 266,000 deaths were estimated in 2012. In Brazil, 15,590 new cases of cervical cancer were estimated in 2014, with an estimated risk of 15.33 cases per 100,000 women. Other factors related to immunity, genetics, and sexual behavior also influence the mechanisms that determine the regression or persistence of the infection[3].

Therefore, understanding epigenetic events of cervical cancer, such as the methylation of several genes, becomes relevant, which promises to aid the diagnosis and prognosis of cervical tumors. DNA

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methylation is an important epigenetic mechanism for gene silencing, which tends to cause cervical cancer and premalignant changes[2].

In addition, epigenetic abnormalities occur in the carcinogenic process, a fact that can be investigated through the use of molecular markers for diagnosis. Tumor biomarkers are indicators of the physiological state and the changes that occur during the neoplastic process[4]. Accordingly, the identification of a set of hypermethylated genes in cytological smears may offer new means for the detection of cervical cancer[4].

DNA methylation and cervical cancer

Research advances in genetics have brought new discoveries and approaches in the context of molecular and cellular biology. Epigenomic investigations are leading to a better understanding of factors associated with complex disease processes and have elucidated revelations and definitions for diseases such as cancer. Malignant neoplasms contain genetic and molecular bases which, because of genomic instability, may cause certain complications and may lead to the development of malignant tumors[5].

Therefore, epigenetics is the study of DNA and histone modifications that are inheritable and do not alter the sequence of DNA bases. In other words, epigenetic changes are chemical alterations in the genome that do not change the DNA sequence; they may lead, however, to a specific phenotype, disease state, or other observable characteristics[5].

In this regard, methylation is based on a methyl grouping (CH3), which is transferred from S-adenosylmethionine to a cytosine at the carbon 5 position (5-MeC), usually preceded by a guanine (CpG dinucleotides), by the action of a family of enzymes named DNA methyltransferase (DNMT)[6].

Methylation is an epigenetic process that prevails during development and may be modulated along the postnatal life. In addition, methylation is an important event because it leads to gene silencing[1].

With regard to cervical cancer, genetic and epigenetic changes favor the progression of precancerous changes to invasive cancer. A number of changes occur during all stages of cervical carcinogenesis, affecting the expression of the HPV as well as the host genes that represent stages of the process in carcinogenesis. These include global DNA hypomethylation, hypermethylation of key tumor suppressor genes, and histone modifications[7]. The increase in DNA methylation occurs in the promoter region of genes during the progression of the lesion[8]. In cervical cancer, methylation of various genes has been described. DNA methylation has been established as deregulatory in the development of cervical cancer[8].

According to Lodi et al.[9], although high-risk HPV infection is necessary for the development of cervical cancer, epigenetic changes must also be considered when occurring in the viral genome. They can also influence the carcinogenic process directed by the virus and epigenetic changes in the host genome. HPV can silence genes activation, reducing the host defense and enabling the persistent infection[9].

In view of that fact, epigenetic science emerges as an essential factor to foster interest in research and discoveries in the field of molecular genetics, leading to diagnostics and prognostics as well as creating new ways to care for women's health. The control of persistent HPV infection should be a priority, benefiting women with prevention by HPV vaccination, early detection, and treatment of precursor lesions and cervical cancer.

Thus, detection and the use of tools that can complement both diagnosis and screening of precursor lesions of cervical cancer are suggested by several studies, which make them highly relevant. The choice for this study is justified by the importance of focusing on stimulating the research on new means of early detection for this type of tumor.

OBJECTIVE

Considering the situation, the objective is to identify studies that explore epigenetic science, DNA methylation, and research on molecular biomarkers as new perspectives for early detection of cervical cancer.

METHODS

This is a literature review of articles published between 2010 and 2015 to search for the information on the topic and to gather information for the survey of relevant publications. We used the LILACS, MEDLINE, SciELO, Cochrane Library, and PubMed databases. We used the following keywords as the search strategy: *methylation, cervical cancer, cervical intraepithelial neoplasia, DNA, human papillomavirus, genetics*. To combine the terms, we used the words *and, or, and not* as identifiers. As limits, we used articles published in English and Portuguese. We found 74 bibliographical productions, of which 69 were in English; from this total, 25 met the criteria established to achieve the objectives of the study. Some of the selected articles are outlined in **Chart 1**. The highlighted inclusion criteria are the publication period, the suggestion approach to biomarkers as means of cervical cancer detection, as well as the study of the DNA methylation of the human genome and the HPV genome. We excluded articles that did not investigate and/or suggest molecular biomarkers for the detection of precursor lesions and cervical cancer from the study. The team read scientific publications, classified them by author and year of publication, method used, relevant findings and suggestions of genes as biomarkers.

RESULTS

The team conducted an analytical and a selective reading as the criteria to provide data on the subject to meet the proposed objective. This study indicates some suggested biomarkers in **Chart 1** as new perspectives for early detection of cervical cancer.

DISCUSSION

HPV methylation with different approaches and specific methodologies to identify biomarkers that may contribute to the early detection of cervical cancer has been widely investigated in the studies.

We found a significant convergence of several authors consulted on the subject studied. The analysis of several studies on DNA methylation and cervical cancer provides a comprehensive overview revealing that the inactivation of tumor suppressor genes and the activation of oncogenes have an important role in cervical carcinogenesis caused

Chart 1 – Highlight of some studies found in this review on DNA methylation and cervical cancer.

Authorship (year)	Method	Relevant findings	Suggested biomarkers
Yang[2] (2014)	Review that summarized DNA methylation in the HPV genome and identified its clinical implications.	Methylation of the HPV long control region (LCR) and the L1 gene is common during cervical carcinogenesis and increases with the severity of the neoplasia.	Hypermethylation of the L1 gene of HPV16 and HPV18
Nye et al.[12] (2013)	Study with 213 women with CIN I, II, or III or cervical cancer. Data collected from the questionnaire, biopsies peripheral blood, cervical smears, HPV, and HIV infections. Assessed methylation status of PEG3 by bisulfite sequencing and treatment.	The data confirm that the methylation of the PEG3 gene is a potential molecular target for inclusion in the screening of cervical intraepithelial neoplasia, to assess disease progression.	Methylation of the PEG3 gene
Carestiato et al.[14] (2013)	The study assessed 141 cervical samples from patients. HPV detection and genotyping were performed using the PCR technique and methylation of the p16(INK4A) gene, through nested methylation-specific PCR (MSP).	HPV infections and epigenetic alterations showed strong statistical association with cervical carcinoma.	Methylation of the p16(INK4A) gene
Xiong et al.[16] 2014	Studies eligible at PubMed, Web of Science, EMBASE, and CNKI were systematically assessed using meta-regression, subgroup analysis, and sensitivity analysis. Sources of heterogeneity were analyzed. The odds ratio (OR) and the 95% confidence interval (95% CI) were calculated by meta-analysis in R.	Association between methylation of the DAPK1 promoter gene.	Methylation of the DAPK1 gene
Murakami et al.[17] (2013)	Cervical cells were collected from 54 HPV52-positive and 41 HPV58-positive women. The HPV genome was examined using bisulfite modification as well as amplification and sequencing of the polymerase chain reaction.	The increase in methylation of the CpG regions of the L1 gene of HPV52/58 L1 was correlated with the severity of the cervical neoplasia, similar to HPV16 and HPV18.	Methylation of the HPV 52L1 and 58L1 genes
Louvanto et al.[19] (2015)	The methylation status was examined of selected loci in HPV16 and human genes in DNA extracted from exfoliated cervical cell samples from 244 women with cancer who were HPV16-positive or had cervical intraepithelial neoplasia (CIN) or who tested negative for intraepithelial lesion and malignancy (NILM). The methylation of the CpG regions of the L1 gene of HPV16 (CpG 6367 and 6389) as well as of the EPB41L3 (CPG 438, 427, and 425) and LMX1 (CPG 260, 262, 266, and 274) human genes was quantified after bisulfite treatment and sequencing.	The methylation of the LMX1 and EPB41L3 genes of the host and the viral loci of the HPV16L1 viral gene has the potential to distinguish between precancerous lesions and invasive diseases.	LMX1 and EPB41L3 host genes HPV16L1 viral gene
Brebi et al.[20] (2014)	Cervical smear samples were assessed using methylation-specific PCR (MSP) and quantitative MSP (qMSP).	Brebi et al. tested the methylation of regions of the ZAR1 and SFRP4 promoter genes as potential biomarkers for the diagnosis of cervical preneoplastic and neoplastic lesions.	Methylation of the ZAR1 and SFRP4 genes
Vasiljević et al.[21] (2014)	Methylation of 26 genes: APC, CADM1, CCND2, CDH13, CDKN2A, CTNNB1, DAPK1, DPYS, EDNRB, EPB41L3, ESR1, GSTP1, HIN1, JAM3, LMX1, MAL, MDR1, PAX1, PTGS2, RARB, RASSF1, SLIT2, SOX1, SPARC, TERT, and TWIST1 was measured by sequencing in cytology samples from a group of women with normal histological results or CIN III results.	High methylation of the EPB41L3 gene in CIN II and III neoplasias. ($p < 0.0001$)	Methylation of the EPB41L3 gene
De Strooper et al.[22] (2014)	We analyzed the methylation of the FAM19A4 gene through quantitative methylation-specific PCR (qMSP). Cervical hrHPV-positive smears from 43 women with cervical intraepithelial neoplasia III (CIN III) and 135 with cervical intraepithelial neoplasia I (CIN I) were used.	Highly efficient methylation of the FAM19A4 gene in the detection of cervical carcinomas, CIN II and CIN III.	Methylation of the FAM19A4 gene
Van der Meide et al.[25] (2011)	Methylation of the promoter gene of nine Wnt-antagonists (APC, AXIN2, DKK3, SFRP2, SFRP4, SFRP5, SOX17, WIF1, and WNT5A) was assessed through methylation-specific PCR (MSP) in a small number of cervical samples, including cervical adenocarcinoma and adenocarcinoma in situ. To assess the diagnostic potential of more frequently methylated genes, they were analyzed through quantitative MSP (qMSP).	The frequency of methylation of the DKK3 and SFRP2 promoter genes establishes promising screening markers for HPV-positive women at risk cervical adenocarcinoma and adenocarcinoma in situ.	Methylation of the DKK3 and SFRP2 genes

Continue...

Chart 1 – Continuation.

Authorship (year)	Method	Relevant findings	Suggested biomarkers
Zuo et al.[26] (2014)	Methylation-specific PCR (MSP) and HPV-specific (HPV16 and HPV18) PCR were performed in 110 cervical samples: 40 normal cervical tissues, 10 CIN I tissues, 10 CIN II tissues, 10 CIN III tissues, and 40 cervical cancer tissues. The expression of both genes was determined by reverse transcription PCR (RT-PCR) for CIN III and 40 cervical cancer tissues.	The CCNA1 and HS3ST2 genes may have an important role in cervical cancer induced by HPV. Patients with specific hypermethylated genes may be at increased risk of progression to invasive cervical cancer.	Methylation of the CCNA1 and HS3ST2 genes
Chujan et al.[27] (2014)	A histopathological method was used as the gold-standard method for samples separated into the following groups: negative (n=31), low-grade squamous intraepithelial lesion (n=34), and high-grade intraepithelial lesions (n=32). High-risk HPV was detected by Hybrid Capture 2 (HC2), and the methylation of the CCNA 1 promoter gene was analyzed through CCNA 1 methylation-specific duplex-PCR.	The assessment of the prevalence of the methylation of the cyclin-A1 promoter gene (CCNA 1) in residual cervical cells isolated from cytology in liquid medium enable to distinguish negative histology, low-grade lesions, and high-grade lesions.	Methylation of the CCNA 1 gene
Jung et al.[28] (2011)	Methylation levels for the vimentin promoter (VIM) and the expression of the VIM gene were analyzed in seven cervical cancer cell lines and 50 samples of human tissues with a different grade of malignant formation.	The hypermethylated promoter gene in cervical cancer cells and epigenetic alterations of the VIM gene are associated with the development of cancer cells as well as gene silencing.	Methylation of the vimentin promoter gene (VIM)

CIN: cervical intraepithelial neoplasia; PCR: polymerase chain reaction; CpG: phosphate-cytosine-guanine; hrHPV: high-risk human papillomavirus.

by genetic and epigenetic changes[7]. According to the survey, we observe that some methylated genes have an important role in the development of cervical cancer, suggesting the benefits of using relevant biomarkers in the carcinogenic process. According to a study by Clarke et al.[10], the detection of methylated genes in cervical samples is technically feasible and it is a source to identify biomarkers for cervical carcinogenesis.

Therefore, there was an increase in information sources, with concatenated approaches. We expanded concepts and redefined them and thus, contributing to new proposals for the detection of cervical cancer.

The Papanicolaou test, also called colpocytology or Pap smear test, is considered a more appropriate, practical, and cost-effective instrument to screen cervical cancer. However, there is still a need to search for new alternatives for early detection of this type of tumor among women[11].

Considering the various epigenetic change in profiles, many researchers seek to propose new, more sensitive, and more specific alternatives for cervical cancer screening[7]. As a result, we understand that even with the evidence of the success of the program of cervical cancer control and with a substantial reduction in the incidence of cervical cancer, there are still few limitations. A frequent example of that is the inadequacy and poor quality of smears for the cytological analysis, which lead to increased costs and higher sensitivity when the collection is associated with the HPV test.

For Nye et al.[12], cytology screening for invasive cervical cancer (ICC) requires sensitivity and specificity to distinguish a process of persistent cervical intraepithelial neoplasia (CIN) from a regression process[12].

Cytology through the Papanicolaou method has good sensitivity and high specificity when used as a screening method. However, method sensitivity and specificity are reduced if analyzed in patients with cervical alterations[13]. In terms of prevention, HPV prophylactic vaccine currently offers the possibility of action at the primary level, because, thus far, prevention only occurred at secondary level.

In the field of epigenetics, for example, the study on other screening methods for cervical cancer is being conducted in order to detect the tumor in an early stage. In view of that context, it is clear that the issue is being addressed as a priority in recent years. We emphasize the finding of several studies aimed at reducing morbidity and mortality caused by cervical cancer.

The review by Termini and Villa[4], for example, stresses some of the main lines of research on biomarker identification and their possible use in the screening of cervical cancer and its precursor lesions. For that reason, there are many studies that seek to identify biomarkers associated with cervical neoplasia, which may be used to perform the screening of women who are carriers of HPV. This article only discusses the most outstanding biomarkers in scientific production.

Among the studies included in this review, we highlighted the use of possible biomarkers; some mention the hypermethylation of the p16(INK4A) gene. Carestiato et al.[14] indicated that the hypermethylation of the p16(INK4A) gene is an important cofactor of cervical carcinogenesis, eliminating the tumor suppressor function of the p16 protein in malignant lesions. Methylation was identified only in 10.7% of the normal epithelium samples, 22.9% of the low-grade lesions, 57.1% of the high-grade lesions, and 93.1% of the carcinomas ($p<0.0001$)[14].

There was an association between the completion of the p16(INK4A) gene and the infection by different types of HPV. The most prevalent type was HPV16 (37%), followed by HPV18 (16.3%), and HPV33/45 (15.2%). We found a correlation between methylation and HPV infection ($p<0.0001$), high-risk genotypes ($p=0.01$), high-grade lesions ($p<0.0007$), and cancer ($p<0.0001$)[14]. Considering such findings, we established a strong statistical association with cervical carcinoma.

Mirabello et al.[15] added that, from their age-stratified analysis, women who are above the average age of 28 years have an increased risk of developing precancerous lesions associated with high methylation. Therefore, high levels of HPV16 DNA methylation can be useful for the early diagnosis of precursor lesions of cervical cancer[15].

By demonstrating the detection of the overexpression of p16(INK4a) as an indirect test of the expression of E6/E7, which is used to confirm cervical neoplasia, Grce et al.[13] reported that the detection of viral oncogenes transcribed from E6/E7 as a marker of productive infection is a promising tool for the follow-up of women with HPV.

In addition to such findings, Nye et al.[12], studying 213 women diagnosed with CIN I, II, and III, outlined as the first evidence an important cofactor of the risk of developing invasive cancer; they stressed a 5% increase in the 5% DNA methylation of the PEG3 gene, which is associated with an increased 1.6-fold risk of cervical cancer and is useful as a biomarker.

With regard to the DAPK1 promoter gene, Xiong et al.[16], from a systematic review comprising 818 samples of tumor tissues and 671 samples of normal tissue, established the methylation frequency of the DAPK1 promoter gene, which ranged from 30.0 to 78.6% (median of 59.3%) in cervical cancer tissue and from 0.0 to 46.7% (median of 7.8%) in normal cervical tissue, indicating that DAPK1 methylation may be a biomarker during carcinogenesis and may serve as an early indication of cervical cancer[16].

The review by Barbaresco et al.[6] supplemented the data for the DAPK1 gene by the report that the hypermethylation of the DAPK1 gene was observed in 33.3% of CIN I cases, 50% of CIN III cases, and 71.4% of cervical samples. It was observed that methylated genes may have a significant role in the onset of cancer and that the methylation of some genes is associated with a more advanced stage of the disease. The authors consider that this information may be useful to predict cervical neoplasia, to prevent disease progression, and to be used as a tool in the disease treatment[6].

Yang[2] showed that the L1 gene of HPV16 and HPV18 is consistently hypermethylated in ICC and may be used as a marker in the clinical progression of this type of cancer.

Corroborating that study, Murakami et al.[17] introduced a research with 54 women with HPV type 52 and 41 women with HPV type 58. There was a hypomethylation in the long control region of HPV52/58; the methylation of the L1 gene of HPV52 had a correlation with the prognosis of CIN I and II, with a percentage mean of 15% and 35% for regression and persistence, respectively ($p < 0.05$). Moreover, the methylation state of the L1 gene of HPV58 had a correlation with the severity of cervical neoplasia, with a percentage mean of 12%, 38%, and 61% for CIN I, II, and III, respectively[17].

Johannsen and Lambert[18] summarized that in the case of HPV16, it is documented that the methylation status of the viral genome changes not only in the context of the viral life cycle but also in the context of the progressive neoplasia that culminates in cancer. They also specify the recent implementation of the methodologies of ChIP-seq and RNA-seq analysis to study cervical cancer, which offer a new opportunity to identify epigenetic markers for tumors with viral genomes and correlate such signs with the expression of viral genes in the context of neoplastic diseases caused by these viruses[18].

Louvanto et al.[19] examined the methylation status of selected loci in HPV16 and human genes in the DNA extracted from the cervical cell samples of the 244 women with cancer with HPV16 or CIN or who tested negative for intraepithelial lesion and malignancy. The methylation of the CpG regions of the L1 gene of HPV16 as well as of the EPB41L3 and LMX1 human genes was quantified. Methylation in all loci significantly increased according to the severity of the lesion ($p<0.0001$)[19].

Brebi et al.[20] also reported from their study that the methylation frequency of the ZAR1 and SFRP4 genes increased as the grade of the lesions increased, and the differences between normal and cervical cancer are statistically significant ($p<0.0001$). Therefore, the more severe the lesion, the greater the possibility of identifying DNA methylation.

There is, in addition, the research by Vasiljević et al.[21], which measured the methylation of 26 genes originating from the material obtained from two previous studies. That study highlighted the methylation of the EPB41L3 gene, which was significantly high in CIN, reporting it as a potential and significant diagnostic biomarker for CIN in high-grade HPV[21].

De Strooper et al.[22], assessing the marker FAM19A4 in the screening of women with high-risk HPV based on women with CIN I and III, found that all carcinomas and advanced intraepithelial neoplasias had methylation of the FAM19A4 gene in comparison with 42.1% of CIN II and III of early lesions. Furthermore, Steenbergen et al.[23] assessed the increase in methylation levels for six genes, that is, FAM19A4, LHX1, NKX2-8, NPTX-1, PHACTR3, and PRDM14, with disease progression in cervical tissue samples. All six methylated genes frequently occurred in cervical carcinomas; however, they assessed higher frequencies of up to 100% for FAM19A4, PHACTR3, and PRDM14[23].

Regarding the LDOC1 tumor suppressor gene, Buchholtz et al.[24] concluded that after the study of four of the six cervical cancer cell lines, the LDOC1 expression was silenced. Gene methylation analysis revealed a significant association between the methylation of the tumor promoter gene, indicating that LDOC1 silencing is a frequent event in cervical cancer and may be of interest as a molecular marker for it[24].

Van der Meide et al.[25] presented new biomarkers from the Wnt/β-catenin protein, which are activated during the carcinogenesis of cervical cancer. After the analysis of samples of cervical tissue to assess the diagnostic potential of more methylated genes, the study established the frequency of DKK3 and SFRP2 methylation, which was significantly higher in adenocarcinomas in comparison with squamous cell carcinomas, that is, 82% against 18% ($p < 0.01$) and 84% against 39% ($p < 0.01$), respectively. That fact reveals promising screening biomarkers for women who are carriers of HPV[25]. Zuo et al.[26], after assessing the suitability of HS3ST2 and CCNA1 genes as biomarkers for early detection of cervical cancer, found that hypermethylated genes were correlated with HPV16 and HPV18 infection in high-grade lesions and cervical cancer (both at <0.05). They also reported that the expression of the HS3ST2 and CCNA1 genes was lower in cervical tissues with positive methylation than in cervical tissues with negative methylation[26].

It is important to outline that CCNA1 methylation is a marker that can distinguish negative, low-grade, and high-grade results through cervical cytology samples. Results of a study by Chujan et al.[27] showed that the methylation frequencies of the promoter gene were 0.00, 5.88, and 83.33%, whereas the high-risk HPV percentages were

66.67, 82.35 and 100.00% in the negative, low-grade, and high-grade groups, respectively[27].

Some studies have addressed the methylation of the VIM promoter gene (vimentin). Jung et al.[28] investigated the correlation between the methylation levels of the VIM promoter gene and the effect of methylation in the expression of the VIM gene (vimentin) during the development of cervical cancer.

Vimentin is a protein-coding gene, known for being associated with several biological processes, including cell maintenance as well as cytoskeletal interactions and stabilization. Methylation of the VIM promoter gene appears in CIN I and II, during relatively early stages of carcinogenesis. Therefore, methylation of the VIM promoter gene is suggested as an effective biomarker for cervical diagnosis.

Some studies also indicate new treatment perspectives for cervical cancer through molecular events. That is exemplified by Nogueira-Rodrigues and Melo[29], who presented an opinion piece while studying the search for molecular alterations to find new therapeutic strategies for the group of tumors with HPV-dependent molecular signature. These changes, which are influenced by HPV infection, have been studied as cellular targets for the development of new treatment technologies. These examples demonstrate that there is the commitment not only to find innovative ways to achieve better results in early detection of cervical cancer, but also to research on new therapeutic perspectives for cervical cancer.

CONCLUSION

This literature review is a foundation for conducting further studies on new perspectives for the early detection of cervical cancer precursor lesions and cervical cancer. Considering the strategies for the control of cervical cancer, such as HPV vaccination for prevention and oncologic colpocytology for early detection of precursor lesions, technological advances may be used, reducing the number of cases of the disease in women.

In that scenario, this study establishes that most of the research reports that methylation begins in the early stages of the carcinogenic process. We observed an increase in the methylation pattern as the lesion progresses. A variety of different markers were analyzed from several studies and the most important criterion for the potentiality of a biomarker is its reliability.

Therefore, from this broader view, the relevance of the knowledge and discoveries from studies being conducted worldwide is proved, as they seek new alternatives to benefit the female population with additional care toward their health. Generally, having a Pap smear test proves insufficient. The proposal of complementary strategies for the detection of premalignant changes and cervical cancer is necessary. Despite the advances and discoveries in the field of epigenetics, there are still gaps in the knowledge about the relationship of DNA methylation in HPV and in the host, which need to be clarified.

Factors associated with the complex process of cervical carcinogenesis and new proposals for the detection of cervical cancer through biomarkers will enable the monitoring of such molecular events, redefining and complementing the program of cervical cancer control, thus reducing the morbidity and mortality caused by the disease in the female population.

Therefore, we stress the importance on studies that analyze the topic, to aid the reduction of the incidence of cervical cancer, and to introduce the concepts and definitions that lead to the implementation of preventive actions for this type of tumor in women.

Conflict of interests

The authors report no conflict of interests.

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ABSTRACTS OF PRESENTED PAPERS

Intratype genetic diversity of HPV35, HPV45, and HPV58 in the states of Rio de Janeiro and Pará

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Introduction: Cervical cancer is the fourth most common cancer in women worldwide (528,000 new cases/year and 266,000 deaths), and the human papillomavirus (HPV) plays a fundamental role being present in more than 95% of the cases. More than 200 types of HPV were described, and 15 types are considered a high carcinogenic risk. Studies suggest that the intratype variants may differ biologically in development and behavior of the tumor, but the majority of the studies examines strains of most prevalent types: HPV16 and 18. Data on other high-risk types, not 16 and 18, are rare and inconsistent. **Objective**: To analyze the intratype genetic diversity of HPV types 35, 45, and 58 in cervical cancers and the prevalence of variants in patients in the states of Rio de Janeiro and Pará. **Methodology:** The study included biopsies from 73 women with cervical cancer enrolled in the Instituto Nacional do Câncer (n = 49, in the state of Rio de Janeiro) and the Ophir Loyola Hospital (n = 24, in the state of Pará) which had HPV35 (n = 21), HPV45 (n = 40), and HPV58 (n = 12). The samples were submitted to the Polymerase Chain Reaction and sequencing of the long control region (LCR) and E6 and E7 genes of the viral DNA. For the identification of the variants, a maximum likelihood tree was constructed using the software *PhyML* using as reference sequences described in the literature for the strains of each type. The analysis of median-joining networks were made using the software Network and for the population analysis, the software Arlequin. **Results:** For HPV35, 11 haplotypes were identified, 17 patients with the A1 strain and 4 patients with A2, being that the A1 strain was the most frequent in both states. For HPV45, 27 haplotypes were identified, 11 patients with the A1 strain, 3 with A2, 1 with A3, 4 with B1, and 21 with B2. For HPV45, the most common strain was B2 in both regions studied. For HPV58, nine haplotypes were found, eight patients identified with strain A2, two with A3, and two with C, A2 being the most common in both regions studied. The population of HPV35 in Rio de Janeiro has a higher gene and nucleotide diversity compared to the population of Pará. HPV35, on the other hand, has a smaller gene and nucleotide diversity compared to the other types of HPV. In the population studied, the presence of haplotypes unique to each region was observed, but there was not a geographical structure in any of the HPV types studied. **Conclusion:** The results show that, for each HPV type, there is a strain which is frequent in both states studied, which indicates that the prevalence of some strains are more recurrent than others. The intratype gene diversity between both populations was virtually the same for all types with exception of HPV35 that showed different gene diversity between the two populations.

Keywords: Human Papillomavirus; HPV35; HPV45; HPV58; strains; haplotypes.

Condilomatosis in childhood

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Introduction: The human papillomavirus (HPV) is usually identified by means of its form of clinical manifestation, the condylomata acuminata. Its location can be on the entire lower genital tract, in addition to the urethra, perianal, and anal regions. In pediatric patients, a possible history of sexual abuse should always be investigated if the history of the current disease does not evidence the origin of the condyloma. However, other means of viral transmission may be responsible for the majority of pediatric cases. Potential means for acquisition of HPV in children are described as: heteroinolucação defined by HPV transmission during nonsexual contact with a caregiver while showering or changing diapers; for example, the autoinoculation in which children acquire anogenital injuries from other mucocutaneous sites of themselves and HPV infection in newborns during vaginal birth by contact with the infected maternal genital tract. In addition, HPV DNA was detected in amniotic fluid, in the blood of the umbilical cord, suggesting that the appearance of infection in the uterus and hematogenous dissemination of the virus can be ways of prenatal transmission of HPV. Estimates of the proportion of children with condyloma acuminatum which were sexually abused vary widely from <10 to 90%. Once diagnosed, imiquimod may be chosen as a form of treatment. **Objective:** The objective of this study was to demonstrate the efficacy and safety of the use of imiquimod in childhood. **Case Description:** B. I. S. J., 1 year and 8 months, white, born by cesarean section was referred to the Vulvar Pathology Service of the Hospital Universitário Antônio Pedro (HUAP) in March 2015 due to verrucous lesions in genitalia noticed by her grandmother, with

progressive evolution of their size in the past 4 months. There were reports of treatment for maternal vulvar warts during pregnancy. Physical examination revealed condylomatous lesion, of approximately 4 cm, covering the bottom of the mons Venus, extending to the labia majora in its upper thirds. Imiquimod was prescribed for a month, in alternate days. In the third-day follow-up, regression was observed in 90% of the lesion in mons Venus and labia majora, still remaining a lesion of approximately 0.5 cm in the anterior commissure of the labia majora and reactional erythema at the application site. **Results:** There was improvement, with remission of the lesions only by use of imiquimod. It is assessed, therefore, that this method is a great option for pediatric patients for being safe, noninvasive, and painless. **Conclusion:** The use of imiquimod for the treatment of genital and perianal warts has shown to be efficient. Its administration in childhood is safe and guided by several studies in the medical literature, but there is still a limitation in the data on its safety and efficacy in children under 12 years. The indicated treatment is of application three times a week, from 1 to 4 months. It is usually well tolerated by children. Its most common side effect is skin irritation at the application site.

Keywords: Human Papillomavirus; condyloma acuminatum; vulva; child; therapy.

Sexually transmitted infections and deafness: the production of didactic material to approach important issues for women's health

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Introduction: Currently, sexuality is presented heterogeneously in the literature; and experienced since childhood, it is a search for pleasure and may or may not be related with the sexual organs. Sexuality is directly linked to cultural aspects and is a component of personal relations. In this context, the deaf community is directly affected by its bilingual characteristic established by law, in which their first language, the Brazilian Sign Language (LIBRAS), is not guaranteed by the government bodies for dissemination of important issues for women's health. **Objective:** To develop informative and communicative materials in Portuguese and LIBRAS addressing female sexuality, its characteristics and relation with sexually transmitted infections (STIs), focusing on human immunodeficiency virus, human papilloma virus (HPV), and syphilis for being STIs belonging to the group of higher incidence, according to the Ministry of Health. **Methods:** The signs on the topic of choice in LIBRAS were found in the Accessibility Brazil Dictionary in multimedia format and the Illustrated Trilingual Encyclopedic Dictionary of the Brazilian Sign Language (LIBRAS), edited by Fernando César Capovilla and Walkiria Duarte Raphael. **Results:** We performed a search on the three signs: Acquired Immunodeficiency Syndrome (AIDS), condylomata acuminata (HPV), and syphilis. In this work, we identified two signs, AIDS present in both sources and syphilis present only in the Illustrated Trilingual Encyclopedic Dictionary of the Brazilian Sign Language (LIBRAS), edited by Fernando César Capovilla and Walkiria Duarte Raphael. **Conclusion:** The final product will be the development of the website Sexually Transmitted Infections and Deafness at the Universidade Federal Fluminense (UFF) in partnership with the Santa Casa de Misericordia of Rio de Janeiro (SCMRJ). The technological progress for society and individuals in general plays an important role in the life of Brazilian citizens, because with the use of Internet, information is transmitted and received in real time and worldwide in several languages, including in sign language. To promote access to information is to provide education, culture, and experience, which are essential bases for the development of a full citizen in society.

Keywords: Sexually transmitted diseases; teaching; women's health; deafness.

Immunohistochemical analysis of CD45RA$^+$ and CD45RO$^+$ cells in high-grade cervical intraepithelial neoplasia

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Introduction: The viral infection by human papillomavirus (HPV) is the most prevalent sexually transmitted disease in the young female population. The primary factor for the development of cervical intraepithelial neoplasia (CIN) is the infection by HPV types considered of high risk for malignant progression. The majority of these infections occurs in a transient mode and are generally asymptomatic. Viral persistence is required for the development and progression of precursor lesions of cervical cancer. The immune response is actively involved in the control of HPV infection and progression to malignancy. Intratumoral lymphocytes are clearly identified in cervical lesions and play an important role in carcinogenesis. **Objective:** This research had as main objective the evaluation of the activation of T lymphocytes in cervical lesions diagnosed with high-grade carcinogenic evolution (High-grade Squamous Intraepithelial Lesion – HSIL). **Methods:** Twelve women with HSIL were included, and cervical samples were obtained after diagnostic confirmation by histopathology. Immunohistochemical reactions using anti-CD45RA antibodies (naïve cells) and anti-CD45RO (activated cells) were performed. The positive cells were detected in epithelial, subepithelial, chorion, and perivascular layers. **Results:** At the epithelial layer, the focus of viral replication, it was found an increased frequency of activated T cells (CD45RO + 1.62 ± 7.7 cells/mm^2) than naïve cells (CD45RA + 3.38 ± 3.36 cells/mm^2), demonstrating an active inflammatory profile in the lesions. The same profile was observed in the subepithelium (2.06 ± 2.04 and 4.2 ± 2.61 cells/mm^2), chorion (7.43 ± 9.28 and 19.07 ± 15.13 cells/mm^2), and perivascular regions (2.86 ± 4.00 and 10.39 ± 15.79 cells/mm^2), showing the recent migration of these cells. **Conclusions:** The migration of lymphocytes to sites of infection by HPV is important to induce an inflammatory environment and eliminate cells infected by HPV. Other studies are necessary to evaluate the involvement of these cells in the control of precursor lesions of cervical cancer and the evolution of the carcinogenesis.

Keywords: HPV; immunohistochemistry; CD45RA; CD45RO.

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Immune regulatory profile "in situ" in anal lesions of patients coinfected with HPV/HIV-1

Awarded best Poster

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The human papillomavirus (HPV) is the main etiologic agent of anogenital tract cancer. Higher prevalence and incidence of development of carcinoma and diseases associated with HPV have been observed in HIV-infected individuals. The natural history of HPV infection is not yet fully elucidated, as well as the immune response in HIV/HPV coinfection, especially in Anal Intraepithelial Neoplasia (AIN). **Objective:** To analyze the immune response *"in situ"* of biopsies of HIV-infected individuals monitored at the Instituto Nacional de Infectologia da Fundação Oswaldo Cruz (IOC/Fiocruz). **Materials and Methods:** In all, 114 biopsies were analyzed using the Tissue Microarray format, being 15 of individuals not

infected by HIV, all without lesion, and 99 of HIV-infected individuals: 21 without lesion, 39 with AIN1, and 39 with AIN2/3. Polymerase Chain Reaction and sequencing for genotyping of HPV and immunohistochemical analysis of immune markers CD4, CD8, Foxp3, T-bet, IL-10 and SLPI were performed. The statistical analysis was performed using the software SPSS, version 15.0 by applying the tests: Kruskal–Wallis, χ^2-test and Fisher's Exact Test. **Results:** HIV-infected patients with AIN2/3 presented CD4+ nadir <50 cells/mm³ compared to normal patients ($p = 0.01$). As for the immune markers, HIV-infected individuals showed higher expression of FoxP3 and IL-10 according to the severity of the lesion ($p = 0.002$). A positive correlation coefficient was observed between FoxP3 and IL-10 ($r = 0.34$; $p = 0.027$). In all, 93.4% (101/107) of the samples presented HPV DNA, being the most prevalent types: HPV 16 (26,9%), HPV 6 (15,7%), HPV 59 (13%), and HPV 18 (10.2%). Samples of individuals with high-oncogenic risk HPV were negative for SLPI, as well as there was lower expression in samples with AIN2/3 compared to the group without lesion of HIV-infected individuals, showing an inverse correlation with the HPV type and degree of lesion. **Conclusion:** The results of this study suggest that individuals infected with HIV present a greater risk to develop the anal cancer, due to the high prevalence of HPV 16 and the increase of IL-10 (Th2) and FoxP3 (T-Reg), which show an immune response regulatory profile, emphasized by the decrease of T-bet cells (Th1), in these high-grade lesions. The majority of HIV-infected individuals have been exposed to the four types of HPV of the quadrivalent vaccine, suggesting that HPV vaccination should be regarded as a prophylactic measure to reduce the risk of anal intraepithelial lesions in HIV-infected individuals. Our study seems to be the first to describe the SLPI protein as possible biomarker in samples of anal lesions and to describe the T-bet transcription factor in anal lesions.

Keywords: HPV; HIV-1; anus; neoplasms; immunology.

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Analysis of the nucleotide sequences of ORF L1 of papillomavirus of animal species compared with the sequences of HPV deposited in gen bank

Poster Awarded Honorable Mention

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The papillomavirus genome consists of a circular, double-stranded, nonsegmented DNA molecule, with approximately 8 Kbp and infects a broad spectrum of hosts. Several techniques of molecular biology have recently been employed in the detection of human papillomavirus (HPV). The objective of this study was to analyze the homology of the nucleotide sequences of ORF L1 of various animal species compared with the sequences of HPV deposited in *Gen Bank*. The procedures for sample collection followed the standards of local research units according to each analyzed species. The DNA of the blood samples and biopsies was extracted using the QIAamp DNA blood mini kit (QIAGEN). Samples of the species *Bos taurus*, *Canis familiaris*, *Felis concolor*, *Panthera onca,* and *Struthio camelus* presented sequences of the papillomavirus DNA, using the pair of degenerated oligonucleotides FAP59 (sense: 5´ - TTACWGTIGGICAYCCWTATT - 3`) /FAP64 (antisense: 5'- CCWATATCWVHCATITCICCATC- 3') for the amplification of ORF L1 in PCR assays. All samples were tested for β-globin gene (sense: 5` - AACCTCTTTGTTCACAACCAG - 3`) / (antisense: 5´ - CAGATGCTTAACCCACTGAGC - 3`). For the sequencing reaction, 5 µL were used of the PCR product purified using the GFX PCR DNA *Purification Kit (GE Healthcare).* Subsequently, 2 µL of the purified PCR product were quantified. The sequences were analyzed by the *Sequence Analyser* using *Base Caller Cimarron* 3.12. The quality of the sequences obtained was evaluated by the software *Chromas,* version 2.33 and/or *Biological Sequence Alignment Editor* (BioEdit). The identities of the products were searched using the *Basic Local Alignment Search Tool* (BLAST) for comparison with the sequences deposited in *Gen Bank*. By the analysis of the nucleotides sequence of the ORF L1, it was possible to demonstrate that the genome of different animal species is strictly related with the genotypes of HPV-5, 7, 8, 12, 14, 28, 36, 40, 47, 74, 82, 84, 97, and 107 in different nucleotide positions. *Bos taurus*: Through the bovine papilloma sample amplified by PCR analysis (RMA 28), we found 100% similarity with the HPV-97 papillomavirus isolate W15189 (Access: EF202168.1 - nt:215-228 and 6529-6516), with the HPV-97 isolate 624 complete genome (Access: EF436229.1 - nt:215-228 e 6529-6516), and with HPV-97 (Access: DQ080080.1 - nt:215-228 e 6529-6516). The nucleotides sequence of the same sample also revealed 90% genetic similarity with the partial gene E1 of HPV-7 isolate alb (Access: AF322243.1 - nt:151-170 e 32-12). *Canis familiaris*: It was found a 91% similarity by alignment of the nucleotides sequence of ORF L1 of the RMA 75 sample of canine with the L1 gene of HPV-14 (Access: AF054874.1 - nt:224-245 and 23-45). It was possible to detect by the alignment of the sequences of the nucleotides of sample RMA 78, 100% similarity in nucleotide positions (nt:639-655 and 394-410) of the L1 parcial gene of HPV clone vs75-3 (Access: X79945.1), as well as 100% alignment of the nucleotide positions (nt: 642-656 and 133-147) of the L1 gene of HPV viral capsid clone vs203-2 (Access: X89880.1). By the analysis of the nucleotide sequence of the sample of canine RMA 95, it was possible to verify 100% homology with the complete genome of HPV-5 (Access: M17463.1); HPV-8 (Access: M12737.1); HPV12 (Access: X74466.1); HPV-36 (Access: U31785.1); and HPV-47 (Access: M32305.1). Through the RMA 96 sample, a 94% similarity was found with the genomic DNA of HPV-40 (X74478.1). The analyzed sample also presented a close relationship with the complete genome of HPV-82 (Access: AB027021.1), showing 100% of alignment (NT:161-175 and 971-985). The RMA 97 sample showed a 94% similarity with the HPV-28 complete genome (Access: U31783.1) and 85% with the complete genome of HPV-74, subtype AE10 (Access: AF436130.1). By the alignments of the nucleotides from the adenocarcinomatous lesion (RMA98 sample), 90% homology was identified with the complete genome of HPV-8 (Access: M12737.1), 88% with HPV-40 (Access: X74478.1), and 86% with HPV-31 (Access: EF422120.1). *Felis concolor*: Were found in the nucleotide positions (nt: 346-365 and 346-363) of RMA 47 sample of feline wild puma, 90% and 94% of similarity with the sequence of the complete genome of HPV-84 (Access: AF293960.1); and HPV-87 (Access: AJ400628.2), respectively. *Panthera onca*: Through the RMA 46 sample of the jaguar, it was possible to identify a 100% similarity with HPV-7 (Access: X74476.1 - nt: 848-863 and nt: 2319-2274), a viral type of a tropical common skin wart. *Struthio camelus*: The sample RMA 42 of the ostrich showed a 100% similarity at nucleotide position (nt: 64-81 and 3623-3640), with the complete genome of HPV-74, subtype AE10 (Access: AF436130.1). Through the alignment of the nucleotides sequence of sample RMA 43, 81% of homology was found with the HPV-34 genomic DNA (Access: X74476.1) and 94% with HPV-107 (Access: EF422221.1). Thus, given such diversity in the Papillomaviridae family, it was possible to demonstrate through the similarity between the sequences that share the same ancestry the phylogenetic proximity between the investigated hosts, strictly related with the different genotypes of HPV in different nucleotide positions.

Keywords: Sequence alignment; homology of genes; Papillomarividae; polymerase chain reaction.

Diversity of methylation patterns in 3'LCR of HPV16, HPV18, HPV35, and HPV45 in cervical cancer biopsies

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Introduction: In cervical cancer (CC) has been suggested that viral integration is an essential event during malignant transformation due to the loss of E2 repressive functions over E6 and E7 oncogenes. However, in up to 40% of cases showed presence of HPV DNA in an episomal form. On these cases, HPV DNA methylation would play a fundamental role by deregulating E6/E7 during oncogenesis. The characterizing and understanding of these mechanisms will allow answering questions such as why only few HPV infections can lead to cancer. **Objective:** To describe the methylation pattern of CpG sites of promoter regions, 3'LCR, of HPV16, HPV18, HPV35, HPV45, and HPV16 coinfected with others HPV types, associating with tumor characteristics, HPV genetic diversity, and the viral DNA physical state. **Methodology:** Samples analyzed were obtained from biopsies of patients attended at INCA ambulatory and diagnosed with invasive CC. By means of bisulfite treatment followed by PCR and pyrosequencing, CpGs of 3'LCR were analyzed for methylation presence in 103 samples infected with HPV16 (n = 35), HPV18 (n = 37), HPV35 (n = 06), HPV45 (n = 14), and coinfections (N = 11). CASKI and HeLa lineage were used as methylation control. PCR combining 21 pairs of primers was performed to assess disruption status of E1 and E2 genes for HPV16 and HPV18. **Results:** The 3'LCR segment of HPV16 showed increased level of average methylation (12%), mainly in coinfections (17%) when compared to the other HPV types (6% for HPV18, 5% for HPV35, and 5% for HPV45). Adenocarcinoma tumors infected with HPV16 were more methylated (24%) than squamous cell carcinoma ones (6%); however, this difference was not significant ($p = 0.126$). In the same way, European variants of HPV16 were more methylated (27%) when comparing with non-European ones (8%), but

the difference was not significant (p = 0.245). Moreover, average methylation at 3' LCR of HPV16 was positively associated with patient age (r = 0.437; p = 0.008). Regarding the HPV DNA physical state, disruptions in E1 and/or E2 were more frequent in tumors with HPV18 than in HPV16 positives (p = 0.005). For HPV16 and HPV18, the disruption of E1/E2 was associated to a low level of average methylation (3% vs. 22% for HPV16, p = 0.004; and 4% *versus* 13% for HPV18, p = 0.023). **Conclusion:** It was observed a higher average methylation for HPV16 (in single and coinfections) in comparison with HPV18, HPV35, and HPV45. For HPV16 and HPV18 were found differences in methylation level between viruses with intact and disrupted E1/E2, and between intratype variants of HPV16, a finding not observed for tumors associated to HPV18.

Keywords: Papillomavirus Human; DNA methylation; Cervix Uteri.

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Infection by human papillomavirus concomitant to psoriasis

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Introduction: The human papillomavirus (HPV) is a DNA virus of the papovavirus group with tropism for epithelial cells, causing infections of the skin and mucous membranes. HPV replication occurs in the nucleus of squamous cell, and its life cycle is directly related to the differentiation program of the host cell. In addition to being responsible for benign verrucous lesions of the skin and mucous membranes, it is also involved in the development of several tumors in these areas. On the other hand, psoriasis is a chronic inflammatory disease characterized by hyperproliferation of the epidermis, of multifactorial origin with interaction of genetic conditions and immune responses to external factors, which are necessary for both the onset and evolution. It is known that there are several predisposing factors, among them, the infections caused by viruses, including the HPV. It often affects the extensor surface of the limbs, trunk, sacral region, and scalp. The form of inverted psoriasis is characterized by more humid lesions in areas of flexure (such as the axillary, inguinal, inframammary, and perigenital regions), being more common in individuals infected by HIV and melanodermics. In psoriasis, there is an accelerated evolutive cycle of keratinocytes and an inappropriate immune activation. The HPV penetrates the epithelium from the basal and parabasal cells, causing a genetic mutation and predisposing the development of psoriasis. In addition, the involvement of the HPV in psoriasis pathogenesis is still uncertain, being postulated that the virus may act as a superantigen or is activated from a latent state by inflammatory cytokines. There is a hypothesis that replication of HPV in psoriatic keratinocytes can cause the epidermal hyperproliferation, as well as the stimulation by the antigen, which induces the autoimmune phenomena. **Objective:** To report a case of HPV infection in a site previously affected by psoriasis in an immunocompetent patient. **Case Report:** A male patient, 38 years old, mullato, coming from Rio de Janeiro, sought the outpatient clinic reporting the presence of hyperchromic verrucous lesions on the bilateral inguinal region, perineum, and testicular region a year ago. He reported discrete local itching, denied previous treatments, and informed having had a previous diagnosis of inverted psoriasis, being treated with acitretin 30 mg/day and phototherapy. The patient had undergone inguinal hernia surgery before. At physical examination, a brownish verrucous lesion was evidenced, with linear arrangement, bilateral, on a basis of erythematous scaling on the perineal area. Presence of erythematous-violaceous plaques as well, with silver desquamation on the trunk, genital region, and limbs. Tests were performed, in which VDRL serology and anti-HIV 1 and 2 were negative. Biopsy was performed of the cutaneous lesion of the perineum, and the findings were of epithelial hyperplasia verrucosa compatible with viral etiology (HPV). The patient began treatment with biweekly application of 90% trichloroacetic acid (TCA) on the HPV lesions, associated subsequently with the use of imiquimod cream (5%) three times a week. The patient evolved with complete improvement of the cutaneous lesion related to HPV. After 3 months, in its follow-up, he presented new verrucous cutaneous lesions in the genital region, in a different area of the previously affected, but also in the area of inverted psoriasis. The biweekly application of 90% TCA was restarted, and the patient showed significant improvement. **Discussion:** The involvement of the HPV in psoriasis uncertain, and the virus may act as a superantigen or is activated from a latent state by inflammatory cytokines. Furthermore, phototherapy can be an underlying factor that predisposes patients with psoriasis for infectivity by HPV due to induced immunosuppression in these cases. The appearance of new lesions of HPV in a different area previoulsy affected by the virus, but in a topography affected by psoriasis, reinforces this thesis. **Conclusion:** The reported patient presented HPV infection in the area previously affected by psoriasis (inverted form) and evolved with satisfactory therapeutic response combined with application of TCA and use of imiquimod in both moments. The patient remains in treatment for psoriasis with acitretin and phototherapy. The relevance of the case described is by the rarity of the association of a immunoinflammatory diseases, psoriasis with another infectious (viral) disease in a same topography.

Keywords: Condyloma acuminatum; Papillomaviridae; psoriasis.

Evaluation of the viral load of high risk human papillomavirus detected by hybrid capture 2 test (HC2) in the screening of cervical cancer patients treated by the sistema único de saúde (SUS)

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Introduction: Persistent infection with one or more types of high-risk human papillomavirus (HR HPV) is the main factor for the development of cervical cancer. Currently, biomolecular tests for HR HPV detection are used as an adjunct to Pap smear or as an independent method for screening of cervical-uterine precancerous lesions in developed countries. However, in Brazil, the Ministry of Health recommends only performing a Pap smear. **Objective:** To evaluate the positivity and the viral load (VL) of the HPV test for its insertion in the screening program of cervical cancer in Brazil by the Sistema Único de Saúde (SUS). **Methods:** Samples from 509 patients from the Vale do Ribeira (SP) region were analyzed, the cytopathological tests were performed in material collected in Sure Path® liquid medium, and in its remainder the hybrid capture 2 test (CH2) for HR HPV was performed. Histopathological examination was performed in 204 patients and colposcopy in 202. The Kruskal–Wallis test with 5% significance was applied for evaluating VL. The VL values were compared between the cases of cervicitis, CIN 1 (and CIN not graded), and CIN 2+ (CIN 2 and more severe lesions). The cytopathologic evaluation, the HC2, histopathological examination, and statistical analysis of the results were carried out at the Instituto Adolfo Lutz de São Paulo. The project was funded by the Políticas Públicas para o SUS/Fundação de Amparo à Pesquisa do Estado de São Paulo (PPSUS/FAPESP) (nº2012/51738-7). **Results:** Were positive for HC2, among the cytopathologic findings: 23/270 (9%) negatives; 1/8 (13%) AGC; 40/125 (32%) ASC-US; 18/32 (56%) ASC-H; 32/45 (71%) LSIL; 21/28 (75%) HSIL e 1/1 (100%) CEC. Were positive for HC2, among the histopathological diagnoses: 37/130 (29%) cervicitis; 20/34 (64%) CIN 1 and CIN not graded 16/18 (89%) of CIN 2; 14/17 (82%) CIN 3 and 1/2 (50%) CEC. The sensitivity of the HC2 test was 91% and the Negative Predictive Value (NPV) of 97% for the detection of a CIN 2+ lesion. The Kruskal–Wallis test of the distribution of the results of VL according to the cytopathologic result showed that an elevated VL in lesions cytopathologically classified as Low-Grade Squamous Intraepithelial Lesion (LSIL) and atypical squamous cells of undetermined significance, not excluding high-grade squamous intraepithelial lesion (ASC-H) are associated with the histopathological diagnosis of CIN 2+. **Conclusion:** The HC2 exhibited sensitivity and NPV desirable values for a technique for the screening of CIN 2+ lesions. The evaluation of VL in patients with cytopathological results of LSIL and ASC-H may assist in the follow-up of patients, and, in this way, the indication of colposcopy in patients with LSIL and high HL for HR HPV may contribute to a better effectiveness of early detection of high-grade lesion or carcinoma.

Keywords: Screening programs; uterine cervical neoplasms.

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