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Association of human papillomavirus (HPV) infection and potentially pathogenic vaginal microorganisms in women attending at primary care nursing gynecological consultation

Associação de infecção por papilomavírus humano (HPV) e microrganismos vaginais potencialmente patogênicos em mulheres atendidas em consulta ginecológica de enfermagem de atenção primária

Asociación de infección por virus del papiloma humano (VPH) y microorganismos vaginales potencialmente patógenos en mujeres atendidas en consulta ginecológica de enfermería de atención primaria

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Abstract

To identify the incidence of human papillomavirus (HPV) with the presence of other microorganisms. This is a cross-sectional study composed of 128 women who were attended in the basic health units, between 2014 and 2018, to perform the Pap smear. The presence of papillomavirus was investigated using conventional PCR with primers MY09/11 and GP5 +/6+ (echo and endocervix cells) and the smear bacterioscopy of vaginal secretions was performed to identify the microorganisms. Statistical was performed using the square test, including a risk ratio or 95% confidence interval. HPV infection was identified in 48.4% (n = 62) women. *Corynebacterium sp* (39.8%) was the most prevalent microorganism, followed by *Escherichia coli* (21.1%), *Staphylococcus aureus* (14.0%), *Gardnerella vaginalis* (13.2%) and *Candida albicans* (7%), respectively. According to statistical analysis or HPV and the group of potentially pathogenic microorganisms had a significant association p <0.02. *Corynebacterium sp* showed an effective protection (0.356 [0.170-0.744] <0.05) in relation to sociodemographic, gynecological and obstetric factors. Only vaginal discharge and pH had p <0.05 when comparing the presence of infection. The other variables showed no statistical differences. This study revealed an association between the presence of pathogenic microorganisms with HPV virus infection, confirming a need for a better understanding of the female genitals' microbiota. Thus, research involving the vaginal infectious agents, methods of protection and rapid diagnosis to contribute to the reduction of the number of lesions in the cervix and cervical cancer are needed.

Keywords: Papillomavirus infections; Microbiota; Women's health; Nursing consultation.

Resumo

Identificar a incidência do papilomavírus humano (HPV) com a presença de outros microrganismos. Trata-se de um estudo transversal composto por 128 mulheres atendidas nas unidades básicas de saúde, entre 2014 e 2018, para realização do exame de Papanicolaou. A presença de papilomavírus foi investigada por PCR convencional com os primers MY09/11 e GP5 +/6+ (células de eco e endocérvice) e a baciloscopia de secreção vaginal foi realizada para identificação dos microrganismos. A estatística foi realizada por meio do teste do quadrado, incluindo uma razão de risco ou intervalo de confiança de 95%. A infecção pelo HPV foi identificada em 48,4% (n = 62) mulheres. *Corynebacterium sp* (39,8%) foi o microrganismo mais prevalente, seguido por *Escherichia coli* (21,1%), *Staphylococcus aureus* (14,0%), *Gardnerella vaginalis* (13,2%) e *Candida albicans* (7%), respectivamente. De acordo com a análise estatística, o HPV e o grupo de microrganismos potencialmente patogênicos tiveram associação significativa p <0,02. *Corynebacterium sp* apresentou proteção efetiva (0,356 [0,170-0,744] <0,05) em relação aos fatores sociodemográficos, ginecológicos e obstétricos. Apenas o corrimento vaginal e o pH tiveram p <0,05 na comparação da presença de infecção. As demais variáveis não apresentaram diferenças estatísticas. Este estudo revelou uma associação entre a presença de microrganismos patogênicos com a infecção pelo vírus HPV, confirmando a necessidade de um melhor entendimento da microbiota dos órgãos genitais femininos. Assim, pesquisas envolvendo os agentes infecciosos vaginais, métodos de proteção e diagnóstico rápido que contribuam para a redução do número de lesões no colo do útero e câncer cervical são necessárias.

Palavras-chave: Infecções por Papillomavirus; Microbiota; Saúde da mulher; Consulta de enfermagem.

Resumen

Identificar la incidencia del virus del papiloma humano (VPH) con la presencia de otros microorganismos. Se trata de un estudio transversal compuesto por 128 mujeres atendidas en unidades básicas de salud, entre 2014 y 2018, para realizar la citología vaginal. Se investigó la presencia de virus del papiloma mediante PCR convencional con los cebadores MY09/11 y GP5 +/6+ (células eco y endocervicales) y se realizó microscopía de frotis de esputo vaginal para identificar los microorganismos. Las estadísticas se realizaron mediante la prueba del cuadrado, incluido un índice de riesgo o intervalo de confianza del 95%. Se identificó infección por VPH en 48,4% (n = 62) mujeres. *Corynebacterium sp* (39,8%) fue el microorganismo más prevalente, seguido de *Escherichia coli* (21,1%), *Staphylococcus areus* (14,0%), *Gardnerella vaginalis* (13,2%) y *Candida albicans* (7%). respectivamente. Según el análisis estadístico, el VPH y el grupo de microorganismos potencialmente patógenos tuvieron una asociación significativa p <0.02. *Corynebacterium sp* mostró protección efectiva (0.356 [0.170-0.744] <0.05) en relación a factores sociodemográficos, ginecológicos y obstétricos. Solo el flujo vaginal y el pH tuvieron p <0.05 al comparar la presencia de infección. Las demás variables no mostraron diferencias estadísticas. Este estudio reveló una asociación entre la presencia de microorganismos patógenos y la infección por el virus del VPH, lo que confirma la necesidad de un mejor conocimiento de la microbiota de los órganos genitales femeninos. Por tanto, son necesarias investigaciones que involucren agentes infecciosos vaginales, métodos de protección y diagnóstico rápido que contribuyan a la reducción del número de lesiones cervicales y cáncer de cuello uterino.

Palabras clave: Infecciones por Papillomavirus; Microbiota; Salud de la mujer; Enfermería de consulta.

1. Introduction

The vaginal microbiota is complex and dynamic. Vaginal microbiota is also exposure to internal and external factors, which can lead to major changes in the vaginal environment. Hormonal, histophysiological and immunological aspects influence the maintenance of vaginal health conditions (Souza, 2009). Several pathogenic microorganisms can infect the vaginal tract, causing imbalance in its dynamics. The main changes related to pathogenic microorganisms can observed in vaginal secretion, which ceases to be a physiological response of the female organism. In the infectious process, changes in appearance, quantity and color, becoming something nuisance and harmful to the woman's health. This is one of the most frequent concerns among women in reproductive age (Cunha, Salazar, Lopes, & Mira, 2017).

Bacterial communities, natural inhabitants of the vaginal microbiota, are an important protector against pathogenic agents (Dominguez-Bello et al., 2016; Gomes Martins, L., 2010). The *Lactobacillus sp*. are known to provide this broad-spectrum protection through lactic acid production, which maintains acidic pH (3.8 to 4.5) and prevents the growth of other bacterial species (Allsworth & Peipert, 2011; Reddy & Ramakrishna, 2007). In addition, they produce bacteriocins, bacteriocinamic antagonistic substances and biosurfactants that have the ability to adhere to mucus, potentiating the defense against pathogens (Kovachev, 2018). Bacterial vaginosis (VB) is characterized by a loss of lactobacillus species, resulting in an increased proliferation of anaerobic bacteria, have been associated with vaginal discharge syndrome, pelvic inflammatory disease, postoperative wound infections, and endometritis, after elective abortions (Gomes Martins, L., 2010; Malaguti, 2015).

The vaginal microbiota is sensitive to more abrupt changes, with potential interference in its constitution, such as the use of vaginal lubricants, hormonal therapy (contraceptive or not), sexual activity, phase of the menstrual cycle, vaginal showers, antibiotherapy, immune changes of the female organism and age physiological changes (Ma, Forney, & Ravel, 2012; O'Hanlon, Moench, & Cone, 2013). Studies suggest that a co-infection between the HPV virus and bacteria in female genital tract facilitates access of the virus to basal epithelial cells through lesions, micro abrasions or by changing the characteristics of epithelial cells, which may increasing the viral load of the infection and facilitating persistence (Gillet et al., 2012). Considering this scenario, the aim of this study was to characterize the vaginal microbiota in women assisted in primary care in a sample of Brazilian population.

2. Methodology

Human Papilloma Virus (HPV) is an etiological factor and an important prognosis for cervical cancer (Carter, Ding, & Rose, 2011; Hellen, Costa, Anne, & Pereira, 2020). HPV infection is among the most common sexually transmitted infections (STI) in the world and one in five women carries the virus. It is estimated that about 80% of sexually active women will be infected per one or more types of HPV at some moment of your life (WHO, 2017). There is emerging evidence that leads us to conclude that increased diversity of the vaginal microbiota combined with the reduced relative abundance of *Lactobacillus sp*. it is involved in the acquisition and persistence of HPV and the development of cervical pre-cancer and cervical cancer (Bautista et al., 2016).

A descriptive study was conducted with a cross-sectional analytical approach, from November 2014 to December 2018, including women between 18 and 59 years old that were attended in primary health centers - Arapiraca, State of Alagoas, Brazil. The study was approved by the National Research Ethics Committee, nº 931.700. All study participants signed the informed consent form (TCLE). Data were collected during gynecological nursing consultations in patients who sought health services to perform the oncotic colpocytology. All care was provided following the steps of systematization of nursing care. The nursing profession of the unit accompanied by an academic in the area performed the clinical evaluation. During anamnesis and interview, they conducted the survey of information on epidemiological and clinical antecedents. Subsequently, the patients were submitted to routine gynecological examination and collection of material for viral detection, fungal, bacterial and parasitic infections was made. The microorganisms identified were classified as "potentially pathogenic" and "non-pathogenic". The group of potentially pathogenic included the following microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Gardnerella, vaginalis*, *Klebsiella sp*, *Candida albicans* and *proteus*. In addition, the presence of protozoan *Trichomonas vaginalis* was investigated but it was not found in any of the samples collected. The group of nonpathogenic included: *Lactobacillus sp* and *Corynebacterium sp*.

Contents of the lateral wall of the women's vagina were collected using "swab" and stored in a sterile test tube containing Stuart's means of transport. The material was sent to Laboratory of Clinical analysis. To identify human papillomavirus (HPV), biological fluids of ecto and endocervical epithelium cells were used. Genomic DNA extraction was performed using intraepithelial cells from endocervix, following instructions from the manufacturer PROMEGA® (Company Eppendorf, Hamburg, Germany). Samples that demonstrated a satisfactory concentration (>20 ng/μL) and an adequate DNA state in gel electrophoresis were submitted to PCR amplification according to SIMOANTO et.al. (2007), using specific primers for human β-actin gene - FW (5’- AGCGGGAAATCGTGCGTG – 3’) and RV (5’- GGTGATGACCTGGCCGTC – 3’). These primers amplify a fragment of 134 bp, which consists of a structural gene used to verify DNA quality and also as an internal control of the reaction. Positive samples for the human β-actin gene were destined to PCR with primers MY09 and GP5+/GP6+ for amplification of the *L1* gene located in the genetic material of HPV virus. Identification of the presence of HPV in cervical samples was performed by conventional PCR. The results from amplification reactions were submitted to gel electrophoresis (5%). The data was stored in Microsoft spreadsheets® Office Excel 2010. SPSS (Statistical Package for Social Sciences) version 23.0 was used to perform the statistical analyses. The power of the sample size was calculated using G*power version 3.0 (Faul et al., 2007). Association between frequencies and was performed using the chi-square test (X2) and Fisher's exact test. The Odds ratio (OR) with a 95% confidence interval (OR <1 associated with protection and OR> 1 associated with susceptibility / risk). P values less than 0.05 were considered statistically significant.

3. Results

A total of 128 women participated in this study (mean age of 42.33 years) and 48.4% (n = 62) were positive for HPV infection. A higher frequency of positive HPV was observed in women over 25 years of age (36.72%). Regarding education level, 32% (n = 41) concluded high school, 22.7% (n = 29), concluded elementary School I, 18.8% (n = 24) concluded elementary School II, 14.8% (n = 19) were graduated and 11.7% (n = 15) did not applied for school. For binary statistical analysis these variables were categorized into two groups (1) < 8 years at school (61.56%) from of these 25% were positive for HPV and (1) 23.4% > 8 years at school, presented p > 0.05 (p=0.991), represented according to Table 1. Regarding to ethnicity/color, we observe that non-white population showed more positive HPV 77.3%. However, not significant statistical was observed (p=0.095). Taking into account marital status, 28.1% (n=36) reported having no partner and 64.1% (n=92) of the women reported having a sexual partner (32.0% (n = 41) had positive HPV), with a result of p= 0.161 (Table 1).

Table 1. Bivariate analysis of HPV presence according to women's sociodemographic variables.

Variables Sociodemographic	**HPV Present**		**HPV Absent**		**Total**		X^2	**OR**	**CI 95%**	***p***
	n	**%**	**n**	**%**	**n**	**%**				
Skin color										
White	18	14	11	8.59	29	22.6	2.79	0.57	0.29-1.11	0.09
Not white	44	34.3	55	42.9	99	77.3		1.17	0.96-1.42	
Education										
≤ 8 yers	32	25	34	26.5	66	51.5	0.00	0.99	0.71-1.39	0.99
> 8 yers	30	23.4	32	25	62	48.4		1.00	0.70-1.43	
Age range										
≤ 25 yers	15	11.7	9	7.03	24	18.7	2.33	0.56	0.26-1.19	0.12
> 25 yers	47	36.7	57	44.5	104	81.2		1.13	0.96-1.35	
Marital status										
With partner	41	32	51	39.8	92	71.8	1.96	1.16	0.93-1.45	0.16
No partner	21	16.4	15	11.7	36	28.1		0.67	0.38-1.18	

Legend: OR=Odds Ratio; Pearson p=chi-square; CI= Confidence interval (95%).
* = Inadequate test pattern. Source: Search data (2020).

Eight different types of microorganisms were identified in the total sample: *Lactobacillus sp* 2.3 % (n = 3), *Corynebacterium sp* 39.8% (n =51), *Klebsiella sp* 0.7% (n = 1), *Candida proteus* 1.5% (n = 2), *Staphylococcus aureus* 14.0 % (n = 18), *Escherichia coli* 21.1 % (n = 27), *Gardnerella vaginalis* 13.3 % (n = 17), and *Candida albicans* fungus 7% (n = 9) (Table 2). The most frequent pathogenic microorganism related to HPV infection was *Escherichia coli*, *Staphylococcus sp* and *Gardnerella Vaginalis*, respectively. *Corynebacterium sp* was significant correlated with the absence of HPV virus [X^2=7.74; OR= 0.356; CI=0.170-0.744; *p*= 0.005]. No association was found for the other microorganisms (Table 2).

Table 2. Prevalence of microorganisms when associated with the presence or absence of HPV.

Microorganisms Identified	**HPV (+)**		**HPV (-)**		**Total**		***X^2***	**OR**	**CI 95%**	***p***
	N	**%**	**N**	**%**	**N**	**%**				
Lactobacillus sp	1	0.78	2	1.5	3	2.3	0.28	*	*	*
Corynebacterium sp	17	13.2	34	26.5	51	39.8	7.74	0.35	0.17-0.74	0.005
Escherichia coli	15	11.7	12	9.3	27	21	0.69	1.43	0.61-3.37	0.40
Staphylococcus Aureus	11	8.5	7	5.4	18	14	0.24	0.73	0.22-2.46	0.62
Gardnerella Vaginalis	10	7.8	7	5.4	17	13.2	0.84	1.62	0.57-4.56	0.35
Klebsiella sp	1	0.7	0	0	1	0.7	1.07	*	*	*
Candida albicans	6	4.6	3	2.3	9	7.0	1.28	*	*	*
Candida Proteus	1	0.7	1	0.7	2	1.5	0.002	*	*	*
Total	62	48.4	66	51.5	128	100				

Legend: OR=Odds Ratio; Pearson p=chi-square; CI= Confidence interval (95%).
* = Inadequate test pattern. Source: Search data (2020).

During the gynecological nursing consultation, 79.7% women did not present complaints related to sexual health condition. Only 13.3% presented complaints of abdominal cramps and 7% burning during sexual intercourse. During gynecological examination was observed that 67.9% (n = 87) women had abundant vaginal discharge, while. Regarding the menstrual cycle, 38.28% (n = 49) women had an irregular period, 41.41% (n = 53) regular periods and 20.31% (n =26) women were in menopause. When asked about the duration of the menstrual period, 35.9% (n = 46) women reported lasting from 1 to 3 days, 39.1% (n = 50) from 4 to 6 days and 4.7% (n = 6) from 7 to 10 days, 20.3% (n=26) in menopause. In relation to the use of contraceptive methods, 19.5% (n = 25) women confirmed the use of barrier contraceptives and 80.4% (n=103) other types or none. The results regarding the pH analysis, the group with the more acidic pH showed less frequency of HPV. There was a higher prevalence of the virus in women who admitted give birth to < 3 children (a) 38.2% (n=49), only vaginal discharge and pH had $p < 0.05$ when compared to the presence of infection, data presented in the Table 3.

Table 3. Bivariate analysis of the presence of HPV according to gynecological and obstetric variables of women.

Variables gynecological and Obstetric	**HPV Present**		**HPV Absent**		**Total**		**X^2**	**OR**	**CI 95%**	***p***
	N	**%**	**N**	**%**	**N**	**%**				
Parity										
≤ 3 children	49	38.2	58	45.3	107	83.5	1.82	1.1	0.95-1.30	0.17
> 3 children	13	10.1	8	6.2	21	16.4		0.5	0.25-1.29	
Contraceptives										
Barrier	10	7.81	15	11.7	25	19.5	0.08	1.4	0.68-2.89	0.34
Other types	52	40.6	51	39.8	103	80.4		0.9	0.77-1.09	
Gynecological complaints										
Yes	19	14.8	14	10.9	33	25.7	1.4	0.6	0.38-1.25	0.22
No	43	33.5	52	40.6	95	74.2		1.1	0.92-1.39	
Vaginal discharge										
Yes	49	38.2	38	29.6	87	67.9	6.7	0.7	0.57-0.92	0.009
No	13	10.1	28	21.8	41	32.		2.0	1.15-3.53	
vaginal pH (*)										
3.5 – 5 .0	22	25.2	32	36.7	54	62.0	5.50	1.48	1.05-2.09	0.019
6.0-9.0	22	25.2	11	12.6	33	37.9		0.51	0.28-0.92	

Legend: OR=Odds Ratio; Pearson p=chi-square; CI= Confidence interval (95%).
* = Inadequate test pattern. Source: Search data (2020).

Women with non-pathogenic microorganisms was more frequent in the absence of HPV infection, demonstrating a significant association for protection of HPV virus [X^2=9.69; OR=0.626; CI= 0.46-0.85; *p*= 0.002].

Table 4. Potentially pathogenic microorganisms in patients with and without HPV.

Microorganisms Identified	**Infection**									
	Presence		**Absent**		**Total**		**X^2**	**OR**	**CI 95%**	***p***
	n	**%**	**N**	**%**	**n**	**%**				
Potentially Pathogenic	17	13.2	36	28.1	53	41. 4	9.69	0.62	0.46-0.85	0.002
Non-pathogenic	45	35.1	30	23.4	75	58.6		1.98	1.25-3.15	
Total	62	48.4	66	51.5	128	100		0.31	0.15-0.65	

Legend: OR=odds Ratio; Pearson p=chi-square; CI= confidence interval (95%); Descriptive level for fisher's exact test.
Source: Search data (2020).

4. Discussion

Corynebacterium sp are Gram-positive bacilli belong to vagina normal microbiota, predominantly nonpathogenic. However, when vaginal immunity is impaired, some specific species have been associated with the occurrence of urinary tract infections (Guerreiro, Barbosa, Conceição Filho, Tishchenko, & Hagge, 1986; Noyes, Cho, Ravel, Forney, & Abdo, 2018; Shukla, Bernard, Harney, Frank, & Reed, 2003). In our study there was a significant association with relation to the protection for the acquisition of HPV virus when this bacillus was present. Corroborating with this result the study by Kenyon et al. (2009) presented a higher frequency of this bacterium in the absence of HPV. Studies conducted in South Africa and Korea did not find an association of this microorganism and HPV (Lee, Kim, Sung, Song, & Ko, 2016; Onywera, Williamson, Mbulawa, Coetzee, & Meiring, 2019).

Pathogenic microorganisms represented 41.4% of the samples of population investigated, in contrast, a previous study showed a lower incidence in its study population, only 1.4%. Several studies differ in the incidence of this association in the vaginal tract (Al-Zaiti et al., 2019). There was an association between the group of potentially pathogenic microorganisms (*Escherichia coli*, *Candida proteus*, *Staphylococcus aureus*, *Gardnerella vaginalis*, *Klebsiella sp*, *Candida albicans*) and the increased risk for co-infection with HPV virus. An unbalanced vaginal microbiome containing a low number of lactobacilli and a high amount of potentially pathogenic microorganisms, especially bacterial vaginosis, increase susceptibility to HPV infection. This interaction can increase the evolution from cervical lesions to cervical cancers. However, there is no knowledge about which bacterial species are most involved in this process or molecular mechanisms mechanism underlying this association (Veldhuijzen, Snijders, Reiss, Meijer, & van de Wijgert, 2010).

The most risk factors related to the presence of HPV were sexual behavior, smoking, family history, parity, co-infections, contraceptives, immune system and sociodemographic factors (Manga et al., 2015; Renata, Lessa, Karina, & Pinheiro, 2020). Co-factor analysis is an important evaluation strategy, considering the associations with susceptibility/resistance of HPV virus infection. The viral detection of HPV was compared with demographic, behavioral and reproductive variables. The variables, age, schooling, ethnicity, partner, contraceptives, gynecological complaints and parity presented $p>0.05$. The variables pH and vaginal discharge had $p < 0.05$, showing a significant association with HPV.

Studies indicate that the prevalence of HPV virus infection was higher in women aged between 31 and 40 years, coinciding with the findings of this study. The mean age of HPV-infected women was 40.54 years with standard deviation

(SD) of 10.6, similar to the study by Manga et. al. (2015), in which the mean age of positive HPV was 40.79 years, as well as data from the National Institute of Disease Science and Technology (INCT-D), which has a age range of 35 to 55 years of age (Santos, 2018). Virus infection was more prevalent in women over 25 years of age 36.7% (n = 47), although not statistically significant [X^2=2.33; OR=1.139; CI= 0.96-1.35; p= 0.126], this data may suggest only in percentages that the higher the age, the more susceptible the infection by the virus the woman will be, this is due to the fact that the virus has the ability to remain latent for years, becoming evident only at the beginning of the climacteric period, where there is a great physiological change in the female body , which may or may not favor infection (Dalla Libera, 2016). It was observed in the population the time of school years reached, although not statistically significant about 51.% had < 8 years of studies, of these 25% were positive for HPV. Similar to this research, studies state that women with a lower level of education were at higher risk of genital infections. These results are consistent with those conducted in northwestern Tanzania (Diadhiou et al., 2019).

The results regarding the pH analysis, specifically, the group that corresponded to the most acidic values were more frequent in the absence of HPV, this association was significant [X^2=5.50; OR=1.488; CI= 1.05-2.09; p= 0.019]. According to studies, the analysis of pH in the most acidic vaginal environment favors protection, when avoiding the growth of other pathogenic bacterial species in the normal microbiome (Allsworth & Peipert, 2011). Regarding the obstetric profile, it is emphasized that the majority reported that they had 1 to 3 pregnancies, although we found no significant association between parity and HPV infection, a study conducted in Texas, United States, showed that women with three or more pregnancies were considered at risk for HPV infection (Thomas et al., 2000) according to the literature this is due to hormonal changes during this period contributing to increased exposure to the virus (Fedrizzi, 2011; Jensen et al., 2013).

Regarding the existence or not of sexual partners, the prevalence of HPV infections had similar frequencies among the category of those who had a partner, about 32.0% had the virus and 39.8% did not present, non-significative in statistical analysis (p= 1.161), considering only an analysis of the percentages, the condition of not living with the partner is related to the risk for infection due to the multiplicity of partners , when taking into account that the greater the number of sexual partners, the more vulnerable and exposed the woman will be to possible infections (Lima-Costa & Barreto, 2003). Other studies evaluate the non-use of barrier methods as a risk to exposure to infectious diseases through unprotected sexual intercourse, the non-use of these types of methods, becomes a risk condition for contracting infections (Chiconela & Chidassicua, 2017). In this study, HPV infection was present in 40.6% (n = 52) of women who did not use barrier methods, a statistically non-statistically significant variable (p=0.347). Retrospective studies report that condoms are the most recommended barrier contraceptive against HPV infection (Rocha, Barbosa Filho, de Queiroz, & Dos Santos, 2013).

During the nursing consultation it was possible to observe that approximately 68% of the women presented abundant vaginal discharge, of these 56.3% were positive for HPV, this cofactor had a statistically significant analysis [X^2= 6.76; OR= 0.729; CI=0.57-0.92; p= 0.009], demonstrating that secretion becomes a risk factor for HPV infection. According to the literature, vaginal discharge consists of an infectious process, causing inflammation and irritation of the genital mucosa (Cunha et al., 2017). Compared to the previous data, only 18% of the women registered complaints about gynecological problems, this is due to the embarrassment caused by vaginal secretion, being one of the information most omitted by women during the consultation. It is the professional's duty at this time to investigate the health situation of women, as well as to guide and sensitize them regarding their own health care and encourage women to report doubts, inabilities and complaints (Marques, Pivetta, Freitas, & Gerk, 2011).

Genital pathogens are associated with infection because they cause inversion in the vaginal microbiota, the main ones being bacterial vaginosis, *Candida albicans, Gardnerella vaginalis* and *Trichomonas vaginalis*. Of these, *Gardnerella vaginalis* was identified in 13.8% of the samples, but there was no statistically significant difference in our study (p=0.357). It preferentially colonizes the female genital tract and presents some well-established virulence factors (Marisleidys Llanes

Rodríguez Oscar González Reyes, Lilian Sánchez Miranda, 2014). Some studies describe that VB is associated with HPV acquisition. A meta-analysis conducted research in the Medline and Web of Science database until 2009, electing 12 studies, and these indicated a positive association between VB and HPV infection (Gillet et al., 2012). According to the Pan American Health Organization (Organization, 2019) the protozoan *Trichomonas vaginalis* is the most commonly incident curable sexually transmitted infection worldwide, but our sample did not present its incidence, thus preventing the evaluation of its relationship with HPV infection.

It is estimated that up to 75% of sexually active women develop Candidiasis and that at least 5% and 10% of them present recurrently (Marisleidys Llanes Rodríguez Oscar González Reyes, Lilian Sánchez Miranda, 2014). In our results 7.0% of the women presented Candida albicans, although they did not present significant difference p>0.05, other studies showed significant results in the association of HPV infection (Voog, Bolmstedt, Olofsson, Ryd, & Lowhagen, 1995). The bacterium Escherichia coli appeared 11.7% of the HPV-positive samples in our population, although not significant in the statistical analysis (p=0.405), according to the literature, which are responsible for extra intestinal infections caused by expected strains that become pathogenic by adaptation or the acquisition of virulence determinants.

Its performance in the dynamics in the vaginal tract has not been as enlightening in the literature, than in other places of the body. These are capable of causing various infections and presenting themselves asymptomatically (Obata-Yasuoka, 2002). Another microorganism detected in the population was *Staphylococcus aureus*, a gram-positive coconut that can occur as a dinner, but in other regions, such as the vaginal microbiota, it may be associated with the mechanism of tumor formation, with production of a chronic inflammation, which is a classic mechanism of carcinogenesis In this study there was no statistical difference (p=0.622), present in 14.0%, of this and 8.5% samples were positive for HPV (Balkwill & Mantovani, 2001).

5. Conclusion

The presence of potentially pathogenic microorganisms in the vaginal microbiome is related to a higher risk of HPV virus infection. Regarding the stratification of microorganisms present in the vaginal microbiome, there were no significant results due to a drop in the sample number when comparting each microorganism separately with the presence of the HPV virus, which does not allow determining which of these were more associated with co-infection with the virus. Our study has some limitations, the samples represent only a part of Brazilian women, being limited to the Agreste of Alagoas, this is due to the fact that the country in question has several ethnic groups, due to its historical process of development and great territorial extension, making it difficult to obtain a sample that represents the entire population without having exacerbated financial cost. In view of this scenario, it is necessary to emphasize the importance of knowing better the female genital tract through new research that elucidates the vaginal dynamics on the possible associations between factors, genetic, hormonal, behavioral and infectious, in order to contribute to the reduction of the number of lesions and cervical cancer.

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