Clinical and epidemiological study of human papillomavirus infection in women with systemic lupus erythematosus in eastern brazilian amazon

Amaral JLA[1], Araújo MVA[2], Dias GAS[1], Ledebur EICF[3], Quaresma JAS[1], Fuzii HT[1]

ACTA REUMATOL PORT. 2017;42:47-54

ABSTRACT

Cervical cancer, the second most common cancer affecting women in Northern Brazil, is strongly associated with human papillomavirus (HPV) infection. Diseases affecting the immune state of a patient, including autoimmune diseases like systemic lupus erythematosus (SLE), can lead to persistent HPV infection and cancer. We evaluated cervical HPV prevalence and the associated risk factors in 70 women with SLE in the city of Belém, located in Brazilian Amazon. HPV DNA was detected by PCR using primers MY9 and MY11. HPV subtypes were determined by real-time PCR, using specific probes. Overall, prevalence of cervical HPV in women with SLE was 22.8%. HPV prevalence was significantly higher in younger women (aged 18–25 years; 75%, p< 0.0001), in women who had not given birth (50%, p=0.01), and in women with no prior pregnancy (57%, p=0.003). Five women (7.1%) had Pap smears with a cytopathological outcome suggestive of HPV; four of these women were HPV-positive. No women with both, SLE and HPV, had a normal Pap smear. Of the women diagnosed with SLE in the last 1–5 years, 75% was HPV-positive (p=0.04). The two most prevalent HPV subtypes were HPV 58 (37.5%) and HPV 31 (31.3%). These results are important for understanding HPV infection in women with SLE and are valuable tools for developing cervical cancer prevention strategies and health management policies.

Keywords: HPV; Systemic lupus erythematosus; Prevalence; Autoimmunity; Cervical cancer.

1. Immunopathology Laboratory of the Center of Tropical Medicine. Universidade Federal do Pará, Belém-Pará, Brazil
2. Institute of Health Sciences. Universidade Federal do Pará, Belém-Pará, Brazil
3. Institute of Biological Sciences, Universidade Federal do Para, Belém – Pará, Brazil

INTRODUCTION

Worldwide, cervical cancer is the third most common cancer found in women. In Brazil, it is the third most prevalent form of cancer in women (excluding non-melanoma skin cancer), surpassed only by breast and colorectal cancer. However, in northern Brazil, cervical cancer is the second most common type of cancer affecting women, after non-melanoma skin cancer[1-3]. It is a well-documented fact that human papillomavirus (HPV) is the etiological agent of cervical cancer, but is not sufficient to cause it[4,5]. Other factors, including socioeconomic status, behaviour, and genetic factors play an important role in the natural history of cervical cancer[1,6].

HPV infection is transitory in most individuals, and is normally eliminated in 1 or 2 years by the immune response. Conditions that change the host's immune state, including autoimmune diseases, such as systemic lupus erythematous (SLE), can change the natural response to the virus, leading to persistent infection and cancer[7-9]. SLE is a rheumatic, systemic, and chronic disease of unknown etiology, and is diagnosed more frequently in young women. It has been linked to genetic and environmental factors (i.e., sun exposure)[10,11]. Many SLE patients require immunosuppressive agents to control disease manifestations, which in turn can decrease immune responses towards viral agents, including HPV. SLE alone or in association with immunosuppressive therapy can lead to a higher susceptibility to infection and cancer development[12].

The HPV prevalence in the city of Belém, located in the Brazilian Amazon, is around 15% in healthy women with normal cervical cytology[13]. The aim of the present study was to evaluate the prevalence of cervical HPV infection among SLE patients and the risk factors associated with this infection.

The seven most frequent HPV types found in invasive cervical cancer are HPV type 16, 18, 31, 33, 35, 52 and 58[14]. Low-risk HPV types 6 and 11 are associated with about 90% of genital warts, low-grade cervical abnormalities, and recurrent respiratory papillomatosis, a condition in which warts grow in the respiratory tract[15,16].

MATERIALS AND METHODS

PATIENTS

All 165 female SLE patients, who were routine patients at the Rheumatology Outpatient Clinic at Fundação Santa Casa de Misericórdia do Pará (FSCMPA), Belém-PA, Brazil, were invited to participate in this study; of these, 70 patients agreed and provided informed consent. The Patients underwent a Pap smear examination; cell samples for HPV detection were collected using endocervical brush. All patients were evaluated based on Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).

Study protocol was reviewed and approved by the regional ethics committee, Comitê de Ética em Pesquisa da FSCMPA, following the national resolution 196/96, in June 2010 (CAAE 0069.0.440.000-10, protocol number 056/10). Sociodemographic, sexual behavior, gynecological history, and contraception use data were collected using a structured questionnaire.

PCR FOR HPV DETECTION

DNA from cervical cells was extracted using the PureLink® Genomic DNA kit (Invitrogen, Carlsbad, CA, USA), according to manufacturer's instructions. Polymerase chain reaction (PCR) using primers for the globin gene was performed to test whether extracted DNA was suitable for subsequent reactions. PCR was also used to identify HPV DNA by using primers MY9 and MY11[17]. Each PCR mixture contained 100 ng of DNA sample, a reaction mix (10pmol of each primer (MY9/11), 10 µl GoTaq® Green Master Mix (Promega, Madison, WI, USA)), and distilled water to a final volume of 20 µl. Amplification conditions included one cycle of initial denaturation at 94ºC for 5 min, followed by 35 cycles at 94ºC for 30s, 56ºC for 30s, and 72ºC for 30s, and a final extension cycle at 72ºC for 5 min. PCR products were subjected to electrophoresis on a 1% agarose gel in Tris/Borate/EDTA (TBE) buffer. Positive samples were characterized by a 440-bp fragment. HPV subtypes were determined by Real-time PCR.

HPV TYPING

Samples were typed for HPV subtypes 6, 11, 16, 18, 31, 33, 35, 52 and 58. Reaction mix contained 100ng of DNA, 10pmol of probes/primers (IDT - Integrated DNA Technologies, Inc. Coralville, IA, USA), 0.2µl ROX Reference Dye (Invitrogen, Carlsbad, CA, USA), 10µl Platinum® qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA, USA), and distilled water; final volume of the mixture was 20µl. Amplification was carried out in a StepOnePlus real time thermocycler (Applied Biosystems® Life Technologies™, Foster City, CA, USA) and involved 40 cycles at 95ºC for 15 s and at 60ºC for 60s. The results were analyzed using StepOnePlus software (Applied Biosystems® Life Technologies™, Foster City, CA, USA).

CYTOPATHOLOGY

Uterine cervix material was evaluated by Papanicolau staining of cytology slides. Slides were read following a routine laboratory protocol, and cytological classification was reported based on the Bethesda nomenclatural system[18].

STATISTICAL ANALYSIS

Pearson's Chi-square or G test and Fisher's exact test were performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego CA, USA) to evaluate the association between prevalence of HPV DNA and cervical cancer risk factors in the samples studied. 95% confidence interval (CI) was established to estimate prevalence of HPV in this population, and $p \leq 0.05$ was considered statistically significant.

RESULTS

This study evaluated cervical HPV prevalence in 70 women with SLE in Belém, Brazil. Women were aged 18–57 years; 48.6% were married, 94.2% had some form of formal education, and 67.1% had 1–3 children (Table I).

Cervical HPV prevalence was 22.8% (16/70), with a 95% confidence interval between 13% and 32.7%. A sociodemographic factor analysis showed marital status and educational level did not correlate with HPV infection. However, prevalence of HPV infection in young women (aged 18–25 years) was 75%, higher than observed in other age groups ($p < 0.0001$). No difference was found in sexual behaviour between women infected with HPV and uninfected women.

TABLE I. HPV PREVALENCE IN PATIENTS WITH SLE ACCORDING TO RISK FACTORS

	SLE HPV- N (%)	SLE HPV+ N (%)	Total N	p-value
Age (years)				
18–25	3 (25)	9 (75)	12	<0.0003[a]*
26–45	40 (87)	6 (13)	46	
≥46	11 (92)	1 (8)	12	
Marital status				
Married	28 (82)	6 (18)	34	0.30[a]
Divorced	7 (87)	1 (13)	8	
Single	19 (68)	9 (32)	28	
Education (years)				
≤8	4 (100)	0 (0)	4	0.40[b]
8–11	47 (76)	15 (24)	62	
≥12	3 (75)	1 (25)	4	
Number of children				
None	8 (50)	8 (50)	16	0.01[a]*
1–3	40 (85)	7 (15)	47	
>4	6 (86)	1 (14)	7	
Pregnancy				
None	6 (43)	8 (57)	14	0.003[a]*
1–3	37 (86)	6 (14)	43	
>4	11 (85)	2 (15)	13	
Abortion				
None	37 (73)	14 (27)	51	0.14[a]
1	11 (100)	0 (0)	11	
2 or 3	6 (75)	2 (25)	8	
Age at first sexual intercourse (years)				
<15	25 (76)	8 (24)	33	1[c]
>15	29 (78)	8 (22)	37	
Number of sexual partners in life				
1–3	41 (75)	14 (25)	55	0.38[c]
≥4	13 (87)	2 (13)	15	
Sexual partners in the last 12 months				
0	9 (69)	4 (31)	13	0.39[b]
1	44 (79)	12 (21)	56	
2	1 (100)	0 (0)	1	
Contraceptive methods				
None	50 (79)	13 (21)	63	0.31[b]
Oral contraceptives	0 (0)	1 (100)	1	
Barrier	4 (67)	2 (33)	6	

[a]Chi-square test; [b]Chi-square test for trend; [c]Fisher's exact test; *Statistically significant
HPV – Human Papillomavirus; SLE – Systemic Lupus Erythematosus

Women who had not given birth presented a significantly higher HPV prevalence (50%) than did those with children (p=0.01). Likewise, women who had never been pregnant had a higher prevalence of HPV (57%; p=0.003; Table I).

Pap smear results revealed all 16 women infected by HPV had inflammatory cytology or varying grades of lesion. Five women (7.1%) had cytopathological out-

TABLE II. HPV PREVALENCE IN PATIENTS WITH SLE ACCORDING TO PAP SMEAR RESULTS

Cervical cytology	SLE HPV- N (%)	SLE HPV+ N (%)	Total N	p-value
Normal	4 (100)	0 (0)	4	0.0001[b]*
IP + IP, with/or suggestive of infection with *Candida sp.* IP with/or suggestive of infection with *Gardnerella* and *Mobiluncus sp.* IP with/or suggestive of infection with *Trichomonas vaginalis*	43 (84)	8 (16)	51	
IP with ASCUS (inflammation w/ non-specific atypical squamous cells)	6 (67)	3 (33)	9	
IP and/or infection with cytopathological outcome of HPV or presence of intraepithelial lesion.	1 (20)	4 (80)	5	
CIN 1	0 (0)	1 (100)	1	

[b]Chi-square test for trend; *statistically significant
HPV – Human Papillomavirus; SLE – Systemic Lupus Erythematosus, Pap smear – Papanicolaou smear, IP – Inflammatory Process; ASCUS – atypical squamous cells of undetermined significance; CIN-1 – Cervical Intraepithelial Neoplasia grade 1

TABLE III. HPV GENOTYPES FOUND IN THE 16 HPV-POSITIVE PATIENTS WITH SLE

HPV subtype	N	%
6	1	6.3
31	5	31.3
35	1	6.3
52	1	6.3
58	6	37.5
11, 16, 18, or 33	0	0.0
Other subtypes	4	25.0
Multiple infection		
(31+58 and 35+6)	2	12.5

HPV – Human Papillomavirus; SLE – Systemic Lupus Erythematosus; N – number of patients

comes suggestive of HPV, four of these women were HPV-positive. Among the HPV-negative women, 7.4% showed normal cytology (Table II). The most prevalent HPV subtype found was HPV 58 (37.5%), followed by HPV 31 (31.3%). Two patients were infected by more than one subtype (Table III).

Association analysis of HPV infection and time of SLE diagnosis demonstrated that 75% of women who had been diagnosed with SLE in the last 1–5 years were also HPV-positive (p=0.04; Table IV).

Analysis of clinical symptoms reported at the moment of data collection indicated musculoskeletal symptoms association with HPV infection by bivariate analysis (p=0.0463). In another analysis, using Poisson Regression, the results showed that musculoskeletal symptoms (prevalence ratio (PR)=2.40 (IC95% 1.05;5.51, p=0.039)) and hematological manifestations (PR= 2.44 (IC95% 1.05;5.67, p=0.039)) are associated to HPV genital infection.

The association between immunosuppressive therapy and HPV infection was also analyzed. No association was found between HPV infection and use or dose of immunosuppressive drugs (cyclophosphamide or methylprednisolone pulse therapy, azathioprine >50 mg; prednisone >20 mg; small doses of prednisone), or type of intravenous pulse during the last 2 years (Table IV).

DISCUSSION

The association of high-risk HPV infection with cervical intraepithelial neoplasia and development of cervical cancer is well established[3,4,19]. However, for cancer to develop, other host factors are necessary[20]. Women with SLE have suppressed immune systems due to disease itself or immunosuppressive medications; as a result, they are more susceptible to HPV infections and cervical cancer[7,8,10,11,21,22]. We evaluated which risk factors contribute to HPV infection in women with SLE.

In this study, 70 women with SLE in Belém, a major city in the Eastern Brazilian Amazon, were evaluated for cervical HPV infection. Prevalence of HPV infection was found to be 22.8%. A previous study published by our group found that prevalence of cervical HPV in

TABLE IV. HPV PREVALENCE IN PATIENTS WITH SLE ACCORDING TO SLE CLINICAL FEATURES AND THERAPY

	SLE HPV- N(%)	SLE HPV+ N(%)	Total N	p-value
SLE – Time since diagnosis (years)				
<1	7 (87)	1 (13)	8	0.04[a]*
1–5	21 (64)	12 (36)	33	
≥6	26 (90)	3 (10)	29	
SLEDAI – at the time of data collection				
0 – 7	52 (79)	14 (21)	66	0.22[c]
≥ 8	2 (50)	2 (50)	4	
Undergoing immunosuppressive[&]				
Yes	48 (75)	16 (25)	64	0.32[c]
No	6 (100)	0 (0)	6	
Type of immunosuppressant[&]				
None	6 (100)	0 (0)	6	0.31[b]
Pulse therapy (methylprednisolone or CPA)	3 (100)	0 (0)	3	
Azathioprine > 50 mg/day	3 (75)	1 (25)	4	
Prednisone > 20 mg/day	10 (67)	5 (33)	15	
Azathioprine+prednisone>50/20mg/day	2 (67)	1 (33)	3	
Low doses of prednisone and others	30 (77)	9 (23)	39	
Underwent pulse therapy in the last 2 years				
None	44 (79)	12 (21)	56	0.85[a]
CPA only	5 (71)	2 (29)	7	
Methylprednisolone only	5 (71)	2 (29)	7	

[a]Chi-square test; [b]Chi-square test for trend; [c]Fisher's exact test; *statistically significant; & at the time of sample collection
HPV – Human Papillomavirus; SLE – Systemic Lupus Erythematosus; SLEDAI – Systemic Lupus Erythematosus Disease Activity Index; CPA – cyclophosphamide

healthy women, also from Belém was 15%[13]. Similarly, a study in Rio de Janeiro, a major city in Southeast Brazil with a population four times larger than Belém[23,24], found HPV infection among women with SLE to be 20.2% and 7.3% among healthy women (173 women with SLE and 217 healthy women were analyzed)[8]. In Hong Kong, prevalence of HPV infection among women with SLE was 11.8%, significantly higher than in healthy women, who had a prevalence of around 7.3%[22].

In most populations, prevalence of HPV is higher in younger age groups (aged 15–25 years) and decreases gradually with increasing age. An increase in HPV infections in older women (second peak) has been observed in some studies (after 45 years of age)[25]. We analyzed prevalence of HPV infection in three separate age groups: 18–25, 26–45, and ≥46 years. In our study only the first peak occurred, women aged between 18--25 years had a HPV prevalence of 75%, much higher than what we found in the two other age groups analyzed (26–45 years, 13% and ≥46 years, 8%; Table I).

Pinto et al.[13] studied HPV prevalence in healthy women living in two different cities in the State of Pará – Brazil (Belém and Tucuruí). Similar to our observations, in Belém the highest HPV prevalence was in young women (13-25 years old, 19,0%; 26-44 years old, 13.5%; ≥45 years old, 15,5%), and the same was reported in Tucuruí (13-25 years old, 17.2%; 26-44 years old, 13.9%; ≥45 years old, 10.9%)[13]. Higher prevalence of HPV infection has been previously found in young women with and without SLE[26,27]. Higher prevalence of HPV infection in younger women is attributed to some biological mechanisms, including cervical immaturity, inadequate production of protective cervical mucus and increased cervical ectopy. In addition, women with SLE have immune response dysregulation that could contribute to the high rate (75%) HPV prevalence found in our study.

Our results show that women recently diagnosed (≤5 years) with SLE are at higher risk of HPV infection (Table IV). In the first 5 years of SLE, severe symptoms, including lupus nephritis and neuropsychiatric symptoms including seizures, cerebrovascular accident (CVA), transverse myelitis, and psychosis are common. These manifestations require appropriate management with high doses of immunosuppressant drugs and corticoids to prevent renal insufficiency and neuropsychiatric damage[28]. Subsequently, the immune response is impaired, leading to increased susceptibility to genital HPV infection and interfering with its resolution.

Many studies have shown a higher incidence of HPV infection to be associated with parity[29]. Contrary to this, in the present study, HPV infection was associated with women with SLE who did not have children or who had never been pregnant. 75% of the women with SLE who tested positive for HPV infection and had no children were younger than 25 years and/or had been diagnosed with SLE in the last 5 years. Both of these risk factors were found to be associated with an increased rate of HPV infection in our study. It is important to note that in the early stages of SLE, pregnancy is often discouraged to avoid exacerbating the symptoms of SLE. Moreover, in these early stages, medications prescribed are high doses of immunosuppressive-like corticoids such as cyclophosphamide, which have high gonadotoxicity and can lead to higher risk of infertility[30].

Some studies have shown that treatment with immunosuppressive drugs can make patients more susceptible to infection, particularly HPV, and cervical cytological alterations[31-33]. Our results did not indicate any association between HPV infection and the use of immunosuppressants. However, patients undergoing immunosuppressive therapy and patients who received pulse therapy in the last 2 years had a higher incidence of HPV, although the difference was not statistically significant (Table III).

Other factors such as marital status, education level, number of abortions, age at first sexual intercourse, number of sexual partners in life, number of sexual partners in the last 12 months, and type of contraceptive methods used were not associated to an increased risk of HPV infection (Table I), which is consistent with the findings of other published studies[32].

HPV prevalence, evaluated by PCR techniques in patients with normal Pap smears, varies from 10.4% to 24.5% internationally, and from 10% to 15.9% in Brazil[34]. In the present study, no women with both SLE and HPV had a normal Pap smear. This is consistent with findings in other studies that demonstrated an increased prevalence of cervical cytological alterations in SLE patients[7,35,36]. A systematic review of the literature indicates a general 9-fold increase in the risk of high-grade cervical squamous intraepithelial lesions in SLE women, compared to healthy controls; however this varies among different populations[12].

In the present study, HPV subtype prevalence was analyzed; in this population of women with SLE, low-risk subtype 6, high-risk subtypes 31, 35, 52, and 58 occurred[37]. High-risk subtypes 31 and 58 occurred at the highest frequency (Table IV). The presence of low-risk subtypes (6, 40, 61 and 70), probable high-risk subtypes (26, 53), and high-risk subtypes (58, 45, 66, 33, 16 and 68) in women with SLE were also found in women from Rio de Janeiro, Brazil. Subtype 58 was also among the most prevalent subtypes in the Rio de Janeiro study[8]. Another study, also using PCR techniques, found that out of 134 sexually active women with SLE, 24.6% were infected with high risk HPV subtypes[36].

This study has some potential limitations. No healthy women were enrolled in the study. However, HPV prevalence and its associated risk factors have already been well documented in several international studies. There are also strong indications in the literature that HPV infections are more prevalent in patients with SLE. Our results give some insight into the risk factors associated to HPV infection in women with SLE.

We faced some difficulties during the enrolment of the women with SLE because it is a rare illness, and patients resisted to take part in the study. Despite its limitation, this study provides further evidence that women with SLE are at increased risk of cervical HPV infection, and indicates that younger women (18-25 years) and recently (< 5 years) diagnosed with lupus women are more vulnerable to HPV infection.

CONCLUSION

In conclusion, our results demonstrate that HPV prevalence in SLE women was 22%, and the highest prevalence was found in young women (75%). The risk factors associated to HPV infection were age (18-25 years old), recent diagnosis of SLE (less than 5 years) and no pregnancy. The high HPV prevalence found in women with SLE reinforces the importance of HPV infection prevention through the use of existing vaccines at ear-

ly ages. In Brazil, vaccination policy against HPV started recently. The Brazilian Health Care System (SUS - Sistema Único de Saúde) began offering the Quadrivalent vaccine against HPV 6, 11, 16 and 18 free of charge for girls aged between 11-13 years in march 2014. This means that all women who took part in this study did not benefit from the vaccination[38,39]. Understanding the risk factors associated with HPV infection and SLE will contribute to disease management.

These results can also contribute to the enhancement of current public health strategies and the Brazilian national health policy for the control and treatment of HPV and cervical cancer, and can help maximize the therapeutic efficacy of drugs and provide a basis for the development of new therapeutic schemes under the Sistema Único de Saúde (SUS, Brazilian National Health Service).

ACKNOWLEDGEMENTS

This work received financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors are thankful to Fundação Santa Casa de Misericórdia do Pará (FSCMPA) as well as to patients who participated in this study.

CORRESPONDENCE TO

Hellen Thais Fuzii
Universidade Federal do Pará,
Núcleo de Medicina Tropical, Laboratório de Imunopatologia,
Av. Generalíssimo Deodoro, 92, Belém-PA CEP: 66055-240.
E-mail: hellenfuzii@gmail.com

REFERENCES

1. INCA - CÂNCER - Tipo - Colo do Útero. http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home++/colo_utero/definicao (accessed 13 May 2015)
2. INCA - Ações e Programas no Brasil - Controle do Câncer do Colo do Útero. http://www2.inca.gov.br/wps/wcm/connect/acoes_programas/site/home/nobrasil/programa_nacional_controle_cancer_colo_utero/conceito_magnitude (accessed 14 May 2015)
3. Rosa MI, Medeiros LR, Rosa DD, Bozzeti MC, Silva MR, Silva BR. Human papillomavirus and cervical neoplasia. Cad Saúde-Pública. 2009;25:953–964.
4. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nat Rev Cancer. 2010;10:550–560.
5. Pirog EC, Kleter B, Olgac S, et al. Prevalence of Human Papillomavirus DNA in Different Histological Subtypes of Cervical Adenocarcinoma. Am J Pathol. 2000;157:1055–1062.
6. Bosch FX, Lorincz A, Muñoz N, Meijer CJLM, Shah KV. The causal relation between human papillomavirus and cervical cancer.J ClinPathol.2002; 55:244–265.
7. Lyrio LDC, Grassi MFR, Santana IU, et al. Prevalence of cervical human papillomavirus infection in women with systemic lupus erythematosus. Rheumatol Int. 2013; 33:335–340.
8. Klumb E, Pinto A, Jesus G, et al. Are women with lupus at higher risk of HPV infection? Lupus.2010; 19: 1485–1491.
9. Gonçalves MAG, Donadi EA. Immune cellular response to HPV: current concepts. Braz J Infect Dis. 2004; 8: 1–9.
10. Klumb EM, Silva CAA, Lanna CCD, et al. Consensus of the Brazilian Society of Rheumatology for the diagnosis, management and treatment of lupus nephritis. Rev Bras Reumatol. 2015; 55: 1–21.
11. Cooper GS, Gilbert KM, Greidinger EL, et al. Recent Advances and Opportunities in Research on Lupus: Environmental Influences and Mechanisms of Disease. Environ Health Perspect. 2008 ;116: 695–702.
12. Zard E, Arnaud L, Mathian A, et al. Increased risk of high grade cervical squamous intraepithelial lesions in systemic lupus erythematosus: A meta-analysis of the literature. Autoimmun Rev. 2014;13: 730–735.
13. Pinto D da S, Fuzii HT, Quaresma JAS. Prevalence of genital HPV infection in urban and rural women in the Eastern Brazilian Amazon.Cad Saúde Pública. 2011; 27: 769–778.
14. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. The Lancet Oncology. 2010 Nov;11(11): 1048–56.
15. Wiley, Dorothy, and Emmanuel Masongsong. Human Papillomavirus: The Burden of Infection. Obstetrical & Gynecological Survey 61, no. 6 Suppl 1 (June 2006): S3–14. doi:10.1097/01.
16. Lacey, Charles J. N., Catherine M. Lowndes, and Keerti V. Shah. "Chapter 4: Burden and Management of Non-Cancerous HPV--Related Conditions: HPV-6/11 Disease."Vaccine 24 Suppl 3 (August 31, 2006): S3/35–41. doi:10.1016/j.vaccine.2006.06.015.
17. Bauer HM, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA.1991; 265: 472–477.
18. Apgar BS, Zoschnick L, Wright TC. The 2001 Bethesda System terminology. Am Fam Physician. 2003; 68: 1992–1998.
19. Faridi R, Zahra A, Khan K, Idrees M. Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. Virol J. 2011; 8: 269.
20. Pai SI. Adaptive immune resistance in HPV-associated head and neck squamous cell carcinoma.OncoImmunology. 2013; 2: e24065.
21. Growdon WB, Del Carmen M. Human papillomavirus-related gynecologic neoplasms: screening and prevention. Rev Obstet Gynecol. 2008; 1: 154–161.
22. Tam L-S, Chan AYK, Chan PKS, Chang AR, Li EK. Increased prevalence of squamous intraepithelial lesions in systemic lupus erythematosus: association with human papillomavirus infection. Arthritis Rheum.2004; 50: 3619–3625.
23. IBGE | Cidades | Pará | Belém. http://www.cidades.ibge.gov.br/xtras/perfil.php?lang=&codmun=150140&search=para|belem (accessed 19 May 2015)
24. IBGE | Cidades | Rio de Janeiro | Rio de Janeiro http://www.cidades.ibge.gov.br/xtras/perfil.php?lang=&codmun=330455&search=rio-de-janeiro|rio-de-janeiro (accessed 19 May 2015)
25. Gonzalez P, Hildesheim A, Rodriguez AC, et al. Behavioral/Lifestyle and Immunologic Factors Associated with HPV Infection among Women Older Than 45 Years. Cancer Epidemiol Biomarkers Prev. 2010;19: 3044–3054.
26. Bernatsky S, Ramsey-Goldman R, Clarke AE. Malignancy in systemic lupus erythematosus: what have we learned? Best Pract Res ClinRheumatol. 2009; 23: 539–547.
27. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-Specific Prevalence of Infection with Human Papillomavirus in Females: A

Global Review. J Adolesc Health. 2008; 43: S5.e1–S5.e62.
28. Ramsey-Goldman R, Gladman D. Epidemiology and Diagnosis: desease development and outcome. In: Tsokos GC, Gordon C, Smolen JS, editors. Systemic lupus erythematosus: a companion to Rheumatology. 1st ed. Philadelphia: MosbyElsevier; 2007. pp. 24-31.
29. Muñoz N, Franceschi S, Bosetti C, Moreno V, Herrero R, Smith JS, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. The Lancet. 2002 359(9312):1093–101.
30. Oktem O, Guzel Y, Aksoy S, Aydin E, Urman B. Ovarian Function and Reproductive Outcomes of Female Patients With Systemic Lupus Erythematosus and the Strategies to Preserve Their Fertility: ObstetGynecolSurv. 2015; 70: 196–210.
31. Bernatsky S, Kale M, Ramsey-Goldman R, Gordon C, Clarke AE. Systemic lupus and malignancies: CurrOpinRheumatol. 2012; 24: 177–181.
32. Tam L-S, Chan PKS, Ho SC, et al. Risk factors for squamous intraepithelial lesions in systemic lupus erythematosus: A prospective cohort study. Arthritis Care Res. 2011; 63: 269–276.
33. Klumb EM, Araújo ML, Jesus GR, et al. Is Higher Prevalence of Cervical Intraepithelial Neoplasia in Women With Lupus Due to Immunosuppression?: JCR J ClinRheumatol. 2010; 16: 153–157.
34. Ayres ARG, Silva GA e. Cervical HPV infection in Brazil: systematic review. Rev Saúde Pública. 2010; 44: 963–974.
35. Santana IU, Gomes A do N, Lyrio LD, Rios Grassi MF, Santiago MB. Systemic lupus erythematosus, human papillomavirus infection, cervical pre-malignant and malignant lesions: a systematic review. ClinRheumatol. 2011; 30: 665–672.
36. Lee Y-H, Choe J-Y, Park S-H, et al. Prevalence of human papilloma virus infections and cervical cytological abnormalities among Korean women with systemic lupus erythematosus. J Korean Med Sci. 2010; 25: 1431–1437.
37. ZurHausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat RevCancer. 2002; 2: 342–350.
38. "Vacinação Contra o HPV - Ministério da Saúde". (accessed 8 March 2016). http://portalarquivos.saude.gov.br/campanhas/hpv/oquee.html.
39. "SUS oferta vacina contra HPV para meninas de 9 a 11 anos". Portal da Saúde – Ministério da Saúde – www.saude.gov.br. (accessed 8 March 2016). http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/16959-sus-oferta-vacina-contra-hpv-para-meninas-de-9-a-11-anos.