HPV INFECTION AND ENDOMETRIOSIS: A SYSTEMATIC REVIEW AND META-ANALYSIS

INFECÇÃO POR HPV E ENDOMETRIOSE: UMA REVISÃO SISTEMÁTICA E METANÁLISE

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ABSTRACT

Introduction: Recent research has focused on the role of persistent ascending bacterial infections and sexually transmitted infections (STI) as a factor associated to endometriosis. Indeed, some studies investigated the possible role of HPV in endometriosis, but this topic remains inconclusive. **Objective:** The present study aims to meta-analyze research that assessed the presence of HPV infection in patients with endometriosis. **Methods:** MEDLINE, Embase, Scopus, LILACS, Cochrane Library, and OpenGrey were searched until February 10th, 2020. Search terms included "endometriosis" and "HPV" without language restrictions. The combined relative risks and 95% confidence interval (95%CI) were calculated, and heterogeneity was assessed with I-square (I^2). **Results:** Meta-analysis with low heterogeneity found a relative risk of twice as much in women exposed to HPV in relation to the unexposed control. **Conclusion:** Results indicate that HPV could be a risk factor for developing endometriosis.

Keywords: papillomaviridae; endometriosis, papillomavirus infections; sexually transmitted diseases; PCR; DNA probes, HPV.

RESUMO

Introdução: A pesquisa recente enfocou o papel de infecções por bactéria ascendente persistente e infecções sexualmente transmissíveis (IST) como um dos fatores associados à endometriose. Na verdade, alguns estudos investigaram o possível papel do HPV na endometriose, mas esse tópico permanece inconclusivo. **Objetivo:** O presente estudo tem como objetivo fazer uma metanálise de pesquisas que avaliaram a presença de infecção por HPV em pacientes com endometriose. **Métodos:** As bases de dados MEDLINE, Embase, Scopus, LILACS, Biblioteca Cochrane e OpenGrey foram pesquisadas até 10 de fevereiro de 2020. Os termos de pesquisa incluíram "endometriose" e "HPV" sem restrições de idioma. Os riscos relativos combinados e intervalo de confiança de 95% (IC95%) foram calculados, e a heterogeneidade foi avaliada usando o I-quadrado (I^2). **Resultados:** A metanálise com baixa heterogeneidade encontrou um risco relativo duas vezes maior em mulheres expostas ao HPV em relação ao controle não exposto. **Conclusão:** Os resultados indicam que o HPV pode ser um fator de risco para o desenvolvimento de endometriose.

Palavras-chave: papillomaviridae; endometriose; infecções por papillomavírus; doenças sexualmente transmissíveis; reação em cadeia da polimerase; sondas de DNA de HPV.

INTRODUCTION

The pathogenesis of endometriosis remains a challenging task for science[1]. Established theories cannot explain all the phenotypes known of the disease, leading to understanding endometriosis as a disease of multiple causes and manifestations[2].

Reflux of menstrual tissue into the abdominal cavity is an event that occurs in 90% of women of childbearing age. Under normal conditions, the peritoneal immune system eliminates the refluxed tissue, and dysregulation of this clearance mechanism could implicate in the predisposition to implantation and growth of ectopic lesions[3]. Both macrophage M2 polarization, and dysfunction of Natural Killer cells and T lymphocytes occur in patients with endometriosis[4].

Female upper genital tract is not aseptic, like it was previously supposed[5]. Proliferative changes in the uterine microbiota favor diseases such as endometriosis, due to immune desensitization, escape from apoptosis, and oxidative stress[6]. Contamination by *Escherichia coli* in refluxed menstrual blood could promote ectopic endometrial growth mediated by toll-like receptors (TLRs)[7]. The microbiota and ascending infections of female genital tract play a critical role in favoring the immunological and inflammatory changes of endometriosis[8]. Human papillomavirus (HPV), which also uses escape mechanisms from innate and adaptive immune responses, is present in endometriotic lesions[9].

OBJECTIVE

The objective of this systematic review and meta-analysis was to study and to describe the presence of HPV in endometriosis tissues in comparison with controls, bringing evidence that can support or deny the association between HPV and endometriosis.

METHODS

Search strategy

This systematic review followed the guidelines for meta-analysis of observational studies in epidemiology (MOOSE)[10], intending to review literature on HPV and endometriosis. For this, we used the following databases: MEDLINE, Embase, Scopus, LILACS, and Cochrane Central Register of Controlled Trials. Besides that, additional relevant references were searched using the OpenGrey databases (http://www.opengrey.eu/) for non-indexed trials. The research strategy was carried out using the following keywords without field restrictions: "Human Papillomavirus" OR "HPV" AND "endometriosis"

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OR "ectopic endometrium." The search was limited to studies in humans but without geographical or language restrictions. Electronic literature was searched until February 10th, 2020.

Study selection

Duplicate articles were excluded. Each article was examined based on its title and summary by all researchers. Those with irrelevant titles or abstracts were excluded. A complete copy of references considered for analysis, by at least one of the researchers, was obtained and read by the reviewers. Any disagreements during review were resolved by consensus. Inclusion criteria were observational studies that detected HPV by any method of detection in tissues of patients with endometriosis and compared to the control patients, without the disease. Exclusion criteria were:

- case reports and systematic reviews;
- studies conducted with the same patients;
- HPV detection by cytology;
- studies in which the control tissue was from the same patient of the case group. A flow chart of the selection process is available in **Figure 1**.

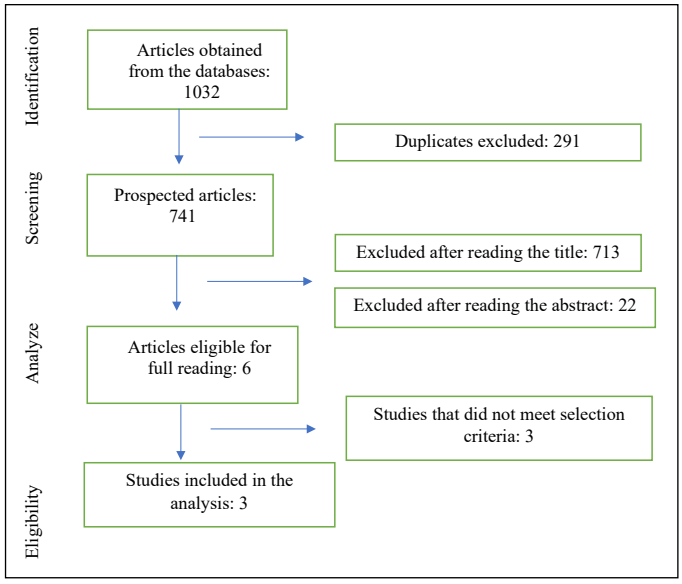


Figure 1 – Flow chart of the selection process of studies on the association of HPV and endometriosis.

Data extraction

The authors examined full-text articles assessed for eligibility, and evaluated the content of texts, according to the data extraction protocol. Any disagreement was resolved by consensus. Data of interest extracted from the eligible references formed the following analysis subgroups: authors; year of publication; country; study design; diagnostic method; type of tissue studied in the case; type of control; HPV positivity for case group; HPV positivity for the control group.

The risk of a bias assessment tool for non-randomized studies of Cochrane interventions (ROBINS-I)[11] was used to assess the methodological quality of studies, and the two authors analyzed risk of bias. The domains evaluated were:

- bias due to confusion;
- bias when selecting participants for the study;
- bias in the classification of interventions;
- bias due to deviations from intended interventions;
- bias due to missing data;
- bias in measuring results;
- bias in the selection of the reported result.

The categories for analyzing risk of bias are "Low risk", "Moderate risk", "Serious risk", "Critical risk", and "No information on which to base a judgment about risk of bias". The result of analysis is available in **Table 1**.

Statistical analysis

Statistical analysis and graph generation were performed using the software R-3.6.1 (R Foundation for Statistical Computing, 2019). *Odds ratio* (RR) and 95% confidence interval (95%CI) were calculated for each article. The presence of heterogeneity in the meta-analysis was assessed with the value of the percentage I square (I^2%) and Cochran's Q test. Low heterogeneity was considered when I^2 reached 25%, moderate when I^2 was close to 50%, and high when I^2 was close to 75%, according to the Higgins and Thompson classification[15]. Fixed effects models were used for low and moderate heterogeneity, whereas random effect model was used for high heterogeneity. The result of meta-analysis was reproduced graphically in a forest plot. Egger's linear regression test[16]; funnel plot was also used to check possible publication bias. In case Egger's test returned with a significant result, Duval and Tweedie's trim-and-fill method was used. There were no analyzes of pre-planned subgroups. The Mantel-Haenszel method was used to analyze combined data,

Table 1 – Studies to assess quality and risk of bias with ROBINS-I tool in studies on the association between HPV and endometriosis.

First author	Bias due to confusion	Bias when selecting participants for the study	Bias in the classification of interventions	Bias due to deviations from the intended interventions	Bias due to lack of data	Bias in measuring results	Bias in the selection of the reported result	Medium-Risk
Vestergaard et al.[12]	Moderate	Moderate	Moderate	Low	Moderate	Low	Low	Moderate
Heidarpour et al.[13]	Low	Moderate	Low	Low	Low	Low	Low	Low
Rocha et al.[14]	Moderate	Low	Moderate	Moderate	Low	Low	Low	Low

HPV: human papillomavirus.

extracted from the studies selected in this meta-analysis. The estimator described by DerSimonian-Laird was used to calculate the variance between studies (tau^2). A value of $p<0.05$ was considered statistically significant.

RESULTS

Database searching generated 1,032 entries, of which 291 were duplicated, leaving 741 titles for choosing by title, and only 28, by summary. Six studies were considered for complete reading, and only three met all the eligibility criteria (**Table 2**).

After applying all the selection criteria, studies were subjected to risk analysis of critical bias, as described in the methodology (**Table 2**), in which a low risk of accumulated bias was observed. The selected studies evaluated a total of 211 samples, 111 cases, and 100 controls. The overall HPV positivity in the case group was 34.2% (n=38), whereas 17% (n=17) of controls were positive.

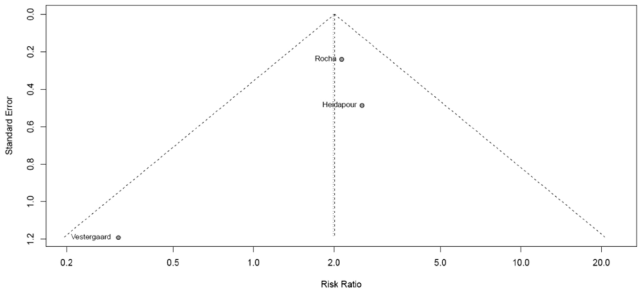
Meta-analysis obtained a relative risk of 2.0112 (1.3126 to 3.0815) with a p=0.0013, considering a fixed effect. Heterogeneity of meta-analysis was 27.1%, with tau ^ 2=0.0938, indicating low heterogeneity, and a p=0.2536 for the heterogeneity test (**Figure 2**).

Funnel graph (**Figure 3**) showed an apparent asymmetric distribution. After applying Egger's test, a p=0.473 and a residual standard error of 2.11 in one degree of freedom were obtained, suggesting the absence of publication bias. Because of this, an adjustment analysis with the trim-and-fill method was not needed. Due to the low number of existing studies that met the selection criteria, no meta-regression was performed.

DISCUSSION

To the best of our knowledge, this is the first meta-analysis on the association between HPV and endometriosis. Data show that there is a relative risk of twice as much for HPV detection in tissues of patients with endometriosis than in patients who do not have the disease. It may mean that a genital HPV infection would play a role in the disease's genesis. However, the mechanism of this participation remains speculative.

Ascending bacterial and viral infections promote a series of changes in the peritoneal microenvironment, which favor the formation of endometriosis[17,18]. Interestingly, the DNA of endometriotic lesions



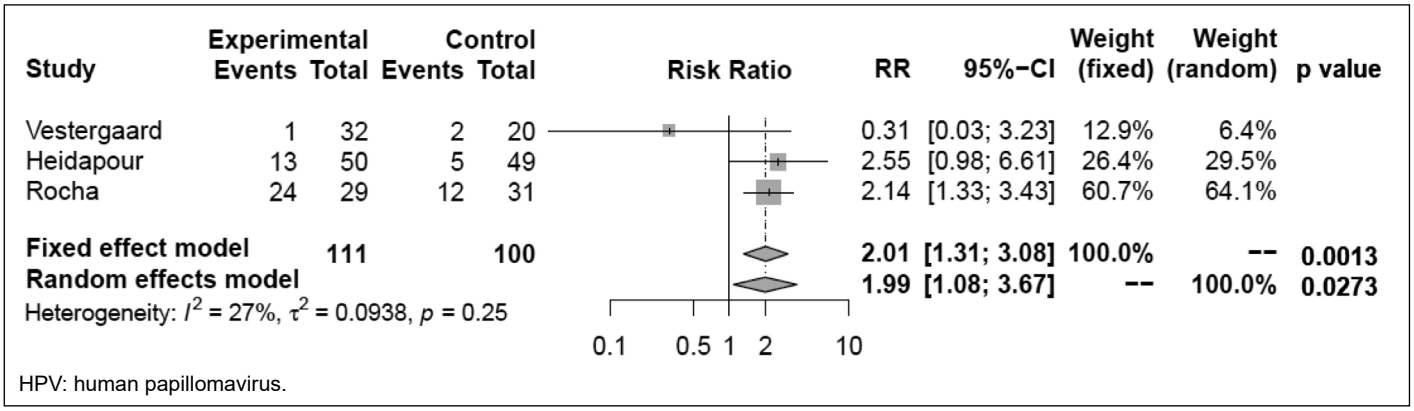
HPV: human papillomavirus.

Figure 3 – Funnel plot of observational studies on the association between HPV and endometriosis eligible for meta-analysis (Egger's test with p=0.473).

Table 2 – Synthesis of observational studies on the association between HPV and endometriosis eligible for meta-analysis.

Study	Country	Year	N	Method	Tissue	Result
Vestergaard et al.[12]	Denmark	2010	52	PCR	Case: Endometriosis Control: Endometrium	No association
Heidarpour et al.[13]	Iran	2017	99	PCR	Case: Ovarian Endometriosis Control: Ovaries without endometriosis	p=0.041
Rocha et al.[14]	Brazil	2019	60	PCR	Case: Tissues of the genital tract and peritoneum of patients with endometriosis Control: The same tissues in patients without endometriosis	p=0.001

HPV: human papillomavirus; PCR: polymerase chain reaction.



HPV: human papillomavirus.

Figure 2 – Forest plot of observational studies on the association between HPV and endometriosis eligible for meta-analysis.

has 96% homology with Shigella's DNA[19]. Various etiologies that affect the female lower genital tract have been reported as potentially involved, such as *Ureaplasma Urealyticum*[20], *Mycoplasma sp*.[21], and *Chlamydia trachomatis*[22]. Medium- and high-risk HPVs were not only detected colonizing peritoneal fluid in patients with endometriosis, but also representing the second largest population in ectopic endometrial lesions[9].

A few studies sought to investigate the association of HPV in the development of endometriosis. Vestergaard et al.[12], when studying the relation of endometriosis with several viruses, obtained a low prevalence of detection, and did not observe an association between HPV and endometriosis. These authors worked only with a few cases, and selected only endometrial tissue as the control, whereas cases of endometriosis brought together different tissues. As an ascending infection, the viral load and HPV detection are overestimated in the endometrium, concerning peritoneal cavitary tissues[14]; therefore, the groups were not homogeneous.

Pioneering work by Oppelt et al.[23], studied HPV, herpes virus, and *Chlamydia trachomatis* in several endometriosis sites. Even though there was no statistical significance, HPV could be associated to endometriosis lesions. Nonetheless, the study was excluded for two reasons: the control group had healthy tissues from patients with endometriosis, and the control group had patients diagnosed with cancer. We understand that there was an overlap of group selection and selection bias, which would compromise the study result.

Two studies, both using the PCR technique, performed only on endometriotic tissue, found an association between HPV and endometriosis. Heidarpour et al.[13] studying only ovarian endometriosis and high-risk HPV, and Rocha et al.[14], later, reached the same result, suggesting a role for HPV in the disease's genesis or maintenance. However, these are still studies with few cases. Heidarpour et al.[13] studied only ovarian endometriosis, whereas Rocha et al.[14] studied several sites intending to demonstrate ascending infection, and not explore the association between HPV and endometriosis.

Interestingly, some studies found a significant lesser association between cervix cancer, mouth, and pharynx in patients with endometriosis, pathologies in which HPV is highly associated[24,25]. We believe that these conditions manifest themselves in different age groups than those in which pelvic endometriosis occurs. Endometriosis and malignancy, however, could result from an infection with an oncovirus, such as HPV, which deserves to be the subject of future studies[23].

A strength of this meta-analysis was to obtain significant relative risk and low heterogeneity between studies, despite including studies with different methodologies. Moreover, there was no significant publication bias. However, it was only possible to include three studies, which weakened the reach of our meta-analysis. Further studies, preferably with designs in which only endometriotic tissues are compared to similar healthy tissues, are required.

CONCLUSION

The meta-analysis of three selected studies, which investigated the detection of HPV in tissues of patients with endometriosis and compared them with controls, supports the hypothesis that infection with this virus may be an independent risk factor for the development of endometriosis.

Participation of each author

The authors declare that all authors were active participants.

Funding

The authors declare no grants or other funding for all authors were received.

Conflict of interests

Nothing to declare.

REFERENCES

1. Mehedintu C, Plotogea Mn, Ionescu S, Antonovici M. Endometriosis still a challenge. J Med Life. 2014;7(3):349-57.
2. Gordts S, Koninckx P, Brosens I. Deep endometriosis: Pathogenesis. Fertil Steril. 2017;108(6):872-885.e1. https://doi.org/10.1016/j.fertnstert.2017.08.036
3. Burney R, Giudice L. Pathogenesis and Pathophysiology of Endometriosis. Fertil Steril. 2012;98(3):511-9. https://doi.org/10.1016/j.fertnstert.2012.06.029
4. Symons LK, Miller JE, Kay VR, Marks RM, Liblik K, Koti M, et al. The Immunopathophysiology of Endometriosis. Trends Mol Med. 2018;24(9):748-62. https://doi.org/10.1016/j.molmed.2018.07.004
5. Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, et al. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. Nat Commun. 2017;8(1):875. https://doi.org/10.1038/s41467-017-00901-0
6. Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine Immunity and Microbiota: A Shifting Paradigm. Front Immunol. 2019;10:2387. https://doi.org/10.3389/fimmu.2019.02387
7. Khan KN, Kitajima M, Hiraki K, Yamaguchi N, Katamine S, Matsuyama T, et al. Escherichia coli contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. Fertil Steril. 2010;94(7):2860-3.E3. https://doi.org/10.1016/j.fertnstert.2010.04.053
8. Lin WC, Chang CY, Hsu YA, Chiang JH, Wan L. Increased Risk of Endometriosis in Patients With Lower Genital Tract Infection: A Nationwide Cohort Study. Medicine (Baltimore). 2016;95(10):e2773. https://doi.org/10.1097/MD.0000000000002773
9. Koninckx PR, Ussia A, Tahlak M, Adamyan L, Wattiez A, Martin DC, et al. Infection as a potential cofactor in the genetic-epigenetic pathophysiology of endometriosis: a systematic review. Facts Views Vis Obgyn. 2019;11(3):209-16.
10. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283(15):2008-12. https://doi.org/10.1001/jama.283.15.2008
11. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016;355:i4919. https://doi.org/10.1136/bmj.i4919
12. Vestergaard AL, Knudsen UB, Munk T, Rosbach H, Bialasiewicz S, Sloots TP, et al. Low prevalence of DNA viruses in the human endometrium and endometriosis. Arch Virol. 2010;155(5):695-703. https://doi.org/10.1007/s00705-010-0643-y
13. Heidarpour M, Derakhshan M, Derakhshan-Horeh M, Kheirollahi M, Dashti S. Prevalence of high-risk human papillomavirus infection in women with ovarian endometriosis. J Obstet Gynaecol Res. 2017;43(1):135-9. https://doi.org/10.1111/jog.13188
14. Rocha RM, Souza RP, Gimenes F, Consolaro MEL. The high-risk human papillomavirus continuum along the female reproductive tract and its relationship to infertility and endometriosis. Reprod Biomed Online. 2019;38(6):926-37. https://doi.org/10.1016/j.rbmo.2018.11.032

15. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-58. https://doi.org/10.1002/sim.1186
16. Egger M, Smith GD, Altman DG. Systematic reviews in Health Care: Meta-analysis in context. London: BMJ; 2001.
17. Khan KN, Fujishita A, Hiraki K, Kitajima M, Nakashima M, Fushiki S, et al. Bacterial contamination hypothesis: a new concept in endometriosis. Reprod Med Biol. 2018;17(2):125-33. https://doi.org/10.1002/rmb2.12083
18. Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. BJOG. 2020;127(2):239-49. https://doi.org/10.1111/1471-0528.15916
19. Kodati VL, Govindan S, Movva S, Ponnala S, Hasan Q. Role of Shigella infection in endometriosis: a novel hypothesis. Med Hypotheses. 2008;70(2):239-43. https://doi.org/10.1016/j.mehy.2007.06.012
20. Noh EJ, Kim DJ, Lee JY, Park JH, Kim JS, Han JW, et al. Ureaplasma Urealyticum Infection Contributes to the Development of Pelvic Endometriosis Through Toll-Like Receptor 2. Front Immunol. 2019;10:2373. https://doi.org/10.3389/fimmu.2019.02373
21. Campos GB, Marques LM, Rezende IS, Barbosa MS, Abrão MS, Timenetsky J. Mycoplasma genitalium can modulate the local immune response in patients with endometriosis. Fertil Steril. 2018;109(3):549-60.e4. https://doi.org/10.1016/j.fertnstert.2017.11.009
22. Dragic M, Posteraro P, Marani C, Natale M, Vecchioni A, Sanguinetti M, et al. Assessment of Chlamydia trachomatis, Neisseria gonorrhoeae, and Mycobacterium tuberculosis infections in women undergoing laparoscopy: the role of peritoneal fluid sampling. Microbiol Med. 2016;31(4):6038. http://dx.doi.org/10.4081/mm.2016.6038
23. Oppelt P, Renner SP, Strick R, Valletta D, Mehlhorn G, Fasching PA, et al. Correlation of high-risk human papilloma viruses but not of herpes viruses or Chlamydia trachomatis with endometriosis lesions. Fertil Steril. 2010;93(6):1778-86. https://doi.org/10.1016/j.fertnstert.2008.12.061
24. Saavalainen L, Lassus H, But A, Tiitinen A, Härkki P, Gissler M, et al. Risk of gynecologic cancer according to the type of endometriosis. Obstet Gynecol. 2018;131(6):1095-102. https://doi.org/10.1097/AOG.0000000000002624
25. Gandini S, Lazzeroni M, Peccatori FA, Bendinelli B, Saieva C, Palli D, et al. The risk of extra-ovarian malignancies among women with endometriosis: A systematic literature review and meta-analysis. Crit Rev Oncol Hematol. 2019;134:72-81. https://doi.org/10.1016/j.critrevonc.2018.12.009

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Received on: 11.13.2019
Approved on: 12.28.2019