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

INFECÇÃO POR HPV E ENDOMETRIOSE: UMA REVISÃO SISTEMÁTICA E META-ANÁLISE

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ABSTRACT

Introduction: Recent research has focused on the role of persistent ascending bacterial infections and Sexually Transmitted Infections (STI) as an associated factor of 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. Pooled relative risks and 95% confidence interval (95%CI) were calculated, and heterogeneity was assessed with I-squared (I^2). **Results:** The meta-analysis with low heterogeneity found a twice as much relative risk in women exposed to HPV in relation to the unexposed control. **Conclusion:** The results indicate that HPV could be a risk factor for the development of endometriosis.

Keywords: papillomaviridae; endometriosis; papillomavirus infections; STI; PCR.

RESUMO

Introdução: Pesquisas recentes enfocaram o papel das infecções bacterianas ascendentes persistentes e das infecções sexualmente transmissíveis (IST) como um fator associado à endometriose. De fato, alguns estudos investigaram o possível papel do HPV na endometriose, embora o tópico permaneça inconclusivo. **Objetivo:** O presente estudo tem como objetivo meta-analisar pesquisas que avaliaram a presença de infecção por HPV em pacientes com endometriose. **Métodos:** Foram realizadas buscas nas bases MEDLINE, Embase, Scopus, LILACS, Biblioteca Cochrane e *OpenGrey* até 10 de fevereiro de 2020. Os termos de pesquisa incluíram "endometriose" e "HPV" sem restrição de idioma. Riscos relativos agrupados e intervalo de confiança de 95% (IC 95%) foram calculados, e a heterogeneidade foi avaliada com I-quadrado (I^2). **Resultados:** A meta-análise 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; DST; PCR.

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 the understanding of 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].

The female upper genital tract is not aseptic, as it was previously supposed[5]. Proliferative changes in the uterine microbiota favor diseases such as endometriosis, with immune desensitization, escape from apoptosis, and oxidative stress[6]. Contamination by *Escherichia coli* in refluxed menstrual blood could promote ectopic endometrial growth mediated by the toll-like receptor (TLR)[7]. Microbiota and ascending infections of the female genital tract play a critical role in favoring the immunological and inflammatory changes of endometriosis[8]. The *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 comparing to controls, bringing evidence that can support or deny the association between HPV and endometriosis.

METHODS

Search strategy

This systematic review followed the guidelines of the meta-analysis of observational studies in epidemiology (MOOSE)[10], intending to review the literature on HPV and endometriosis. For this, we use 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 eith the following keywords, without field restrictions: "Human Papillomavirus" OR "HPV" AND "endometriosis" OR "ectopic endometrium". The search was limited to studies in humans but without geographical or language restrictions.

Study selection

Duplicate articles were excluded. Each article was examined based on its title and summary by all researchers. Titles or abstracts which were not relevant were excluded. A complete copy of

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references considered for analysis by at least one of the researchers was obtained and read by the reviewers. Any disagreements during the 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 of patients without the disease. Exclusion criteria were:

- case reports and systematic reviews;
- studies that used the same patients;
- HPV detection with cytology;
- studies in which the control tissue was from the same patient in the case group.

A flow chart of the selection process is available in **Figure 1**.

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. The 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 group of cases; HPV positivity for the control group.

The risk of a biased 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 judged the risk of bias. The domains evaluated was:

- Bias due to confusion;
- Bias in selection of study participants;
- Bias in the classification of interventions;
- Bias due to deviations from intended interventions;
- Bias due to missing data;
- Bias in measurement of outcomes;
- Bias in the selection of the reported result.

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

Statistical analysis and graph generation were performed using the software R© Version 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 using 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 would be used for low and moderate heterogeneity, whereas the random effect model was used for high heterogeneity. The meta-analysis result was reproduced graphically with a forest plot. Egger's linear regression test[16] and the funnel plot were also used to check for possible publication bias. If Egger's test returned a significant result, we would use the Duval & Tweedie cut and fill procedure. There were no analyzes of pre-planned subgroups. Mantel-Haenszel method was used to analyze the combined data 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.

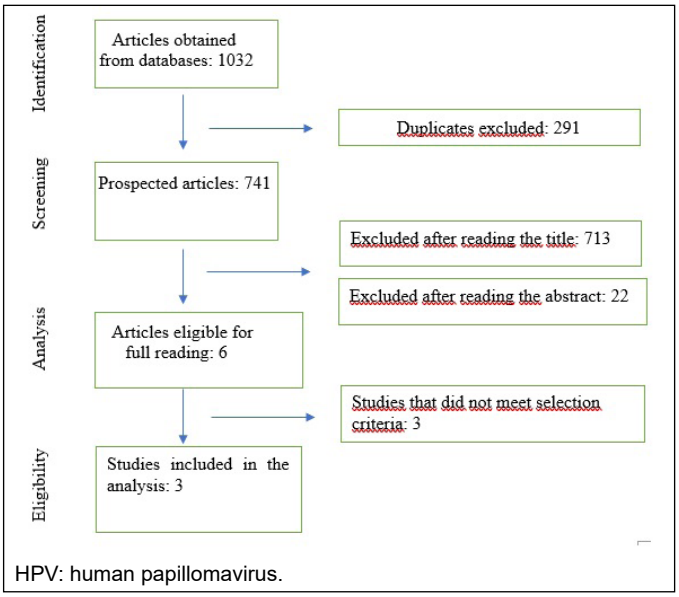

Figure 1 – Flow chart of the selection process of studies on the association of HPV and endometriosis.

RESULTS

Search on database generated 1,032 entries, of which 291 were duplicated, leaving 741 titles for sorting by title, and only 28 for sorting by abstract. Only six studies were considered for complete reading, and only three met all the eligibility criteria (**Table 2**).

Table 1 – Studies to assess quality and risk of bias using the Robins I instrument in studies on the association between HPV and endometriosis.

Authors	Bias due to confusion	Bias in the selection of study participants	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.

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-value=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-value=0.001

HPV: human papillomavirus; PCR: polymerase chain reaction.

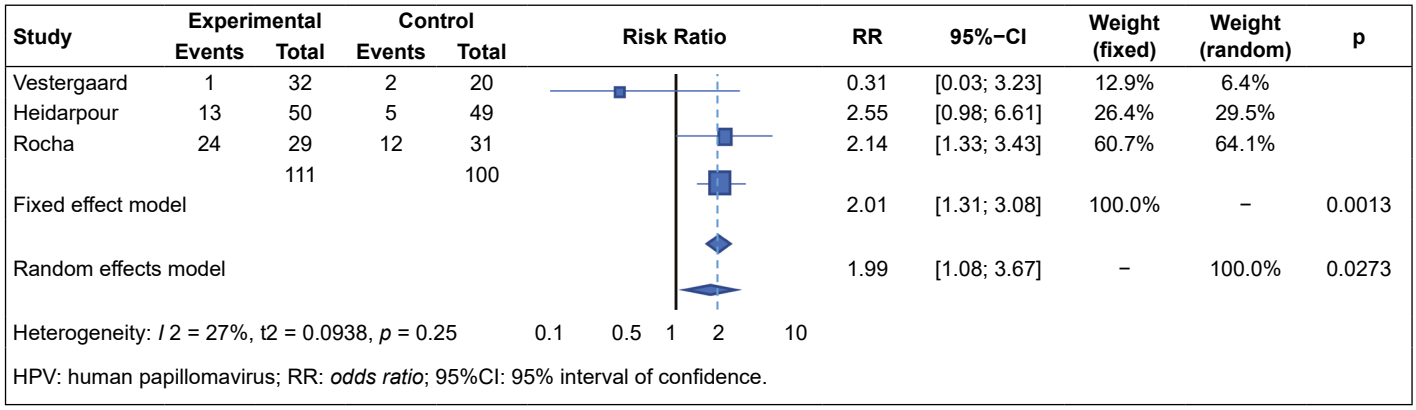

Figure 2 – Forest plot of observational studies on the association between HPV and endometriosis eligible for meta-analysis.

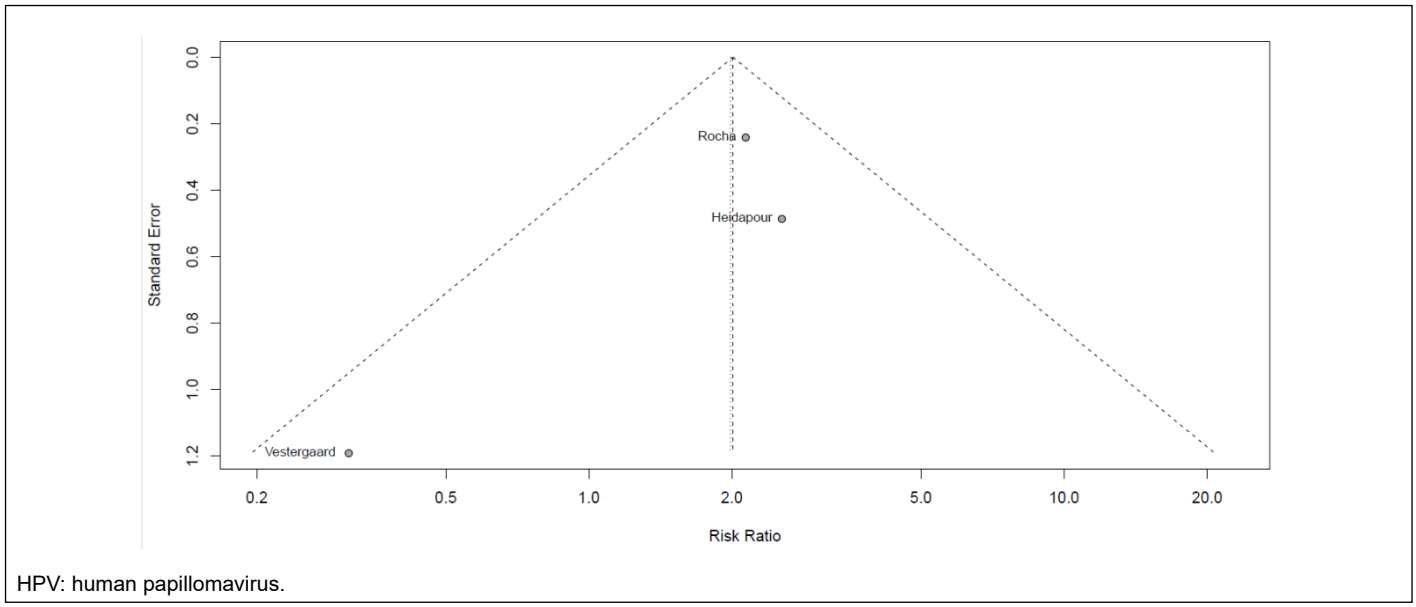

Figure 3 – Funnel plot of observational studies on the association between HPV and endometriosis eligible for meta-analysis (Egger's test with p=0.473).

After applying all the selection criteria, studies were subjected to risk analysis of critical bias, as described in the methodology (**Table 2**), in whcih 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 the controls were positive.

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

The funnel graph (**Figure 3**) showed an apparent asymmetric distribution. Egger's test applied returned p=0.473 and a residual

standard error=2.11 in one degree of freedom, 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 two-fold relative risk of HPV detection in tissues of patients with endometriosis than in patients without this disease. It may mean that a genital HPV infection plays some role in the disease genesis of this disease. The mechanism of this participation, however, remains speculative.

Ascending bacterial and viral infections promote a series of changes in the peritoneal microenvironment that favor the formation of endometriosis[17,18]. Interestingly, the DNA of endometriotic lesions 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 detected for colonizing not only peritoneal fluid in patients with endometriosis but also the second largest population in ectopic endometrial lesions[9].

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

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

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 some role for HPV in the genesis or maintenance of the disease. 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 have found a significant lesser association between cancer of the cervix, 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 when compared to those in which pelvic endometriosis occurs. Endometriosis and malignancy, however, could result from an infection with an oncovirus, such as HPV, a topic 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. There was also no significant publication bias. Nonetheless, 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 needed.

CONCLUSION

The meta-analysis of three selected studies, which investigated the detection of HPV in tissues of patients with endometriosis and compared them to 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.

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Conflict of interests

Nothing to declare.

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