

Introduction

Persistent infection with high-risk human papillomavirus (HPV) is necessary for cervical cancer to develop. Subtype 16 and 18 are responsible for 70% of cervical cancer [1]. Prevalence of HPV infection in Spain in recent studies is about 14.3% for women aged between 18-65 years old [2-4].

The risk factors for HPV acquisition are: early onset of sexual intercourse and a high number of sexual partners, smoking, multiparity, combined hormonal contraceptive use during long periods, immunosuppression and other sexually transmitted diseases such as HIV, Chlamydia trachomatis and Herpes Simplex Virus [5-7].

An important inverse relation between Intrauterine Device (IUD) use and risk of cervical cancer has been proved. Women who used IUD had half the risk of developing cervical cancer. Compared with never users, the risk was reduced nearly by half in the first year of use and was maintained with longer durations of use [8]. Also, there is an inverse relation between IUD use and cervical adenocarcinoma [8].

In 2017, a meta-analysis was published studying the relation between IUD and cervical cancer risk, concluding that invasive cervical cancer was one third less frequent in women using IUD [9].

To date, it is still unclear the mechanism how IUD is a protector factor of cervical cancer. It may act helping to decrease the HPV infection persistence or stopping progression to cancer from preneoplastic lesion.

The primary aim of this study was to check if IUD increases HPV cervical infection clearance, that is, checking if after one year HPV infection disappears in more patients that used IUD compared with those that do not used it.

Methods

This is a cohort case-control prospective study, carried out in Universitary Hospital La Zarzuela in Madrid, Spain, performed between October 2015 and April 2018.

All the participants have been informed of the study and signed informed consent was obtained from them. The protocol has been approved by an independent ethic committee for clinical research at Universitary Hospital Puerta de Hierro Majadahonda in Madrid, Spain; code number 314.3

Study population and procedure

We included in the study patients between 25-50 years, all of them with known HPV cervical infection, who had to agree to participate in the study and provide their informed consent. We assigned participants into two groups: an IUD group, which included women starting using IUD and non IUD group (control group), which included women using any other contraceptive method or none. Subjects completed a behavioural questionnaire including tobacco exposure, contraceptive use and previous gestations.

HPV genotyping of cervical samples were performed initially on enrolment day (day 1) and one year after. Real-time human papillomavirus type-specific multiplex PCR assays has been used for the diagnosis of the HPV infection. Cytological examination using the Bethesda system was performed at the beginning of the study. A colposcopy was performed in all patients with HPV 16 or 18, or smear test informing ASCUS, L SIL or H SIL.

Participants who became pregnant during the year of the study, stopped using IUD before the end of the first year or did not complete the post year follow up process, were excluded from the study. We also excluded patients who chose copper IUD and patients using IUD and preservative (double contraceptive method). We excluded IUD copper users because there were only 5 patients and could affect the results of clearance as we know the clearance is different in levonorgestrel IUD and copper IUD.

Two kinds of IUD have been used: *Levonorgestrel IUD Mirena®* and *Levonorgestrel IUD Jaydess®*. Each patient chose accordingly.

Clearance was considered when a type-specific negative HPV DNA sample was obtained after a twelve month interval, following a positive sample at day 1.

Statistical analysis

Categorical variables were presented as lists of frequencies and proportions. Quantitative variables were described as means and standard deviations or as medians with maximum and minimal values. All tables, figures or graphs were calculated from the number of valid cases (n). Categorical variables were analysed by Pearson's Chi-Square test for categorical nominals and U Mann-Whitney test for categorical ordinals and the effect size of clearance was measured by binary logistics regression (Odds Ratio, CI 95%).

Results

254 participants were enrolled at the beginning of the study, 85 in the IUD group and 169 in the control group (non IUD). At the end, 174 participants completed the study, 54 (31%) in IUD group and 120 participants in non IUD group (69%). 80 women has been excluded, 31 in IUD group (38.8%) and 49 in non IUD group (61.3%).

The most frequent cause of the exclusion of the study was: missing the second visit representing 76.9% in the IUD group and 81.6% in the non IUD group. In the IUD group 23.1% of the patients removed IUD before the year of follow up and in non IUD group 18.4% of the patients became pregnant during the follow up so they were excluded from the study. Also 8.4% patients chose copper IUD so they were excluded.

Population characteristics are showed in Table 1

Different contraceptive methods in the control group are shown in Table 2 with the number of patients who used them.

About IUD preference in the IUD group, 30 patients have chosen a *Mirena*® IUD (55.6%) and 24 patients *Jaydess*® IUD (44.4%).

At the beginning of the study a citology was performed in all the patients. 137 of the patients had a normal citology (76.5%) and 42 of the patients (23.5%) had an abnormal result in the citology. In IUD group 56.25% of the patients with of abnormal citology had L-SIL, 37.50% had ASCUS and 6.25% H-SIL. In the non-IUD group 46.15% of the patients with abnormal citology had ASCUS, 34.61% had L-SIL, 11.53% had H-SIL, 3.84% had AGUS and 3.84% ASC-H.

At the beginning of the study, the most frequent HPV subtypes founded in the IUD group were 16 (16.7%) and 52 (9.3%) and most frequent HPV subtypes in non IUD group were 16 (13.2%), 51 (13.2%) and 52 (13.2%).

38 women from IUD group cleared HPV infection (70.4%) and 65 women from non IUD group cleared it (54.2%) ($p=0.044$).

In subgroups análisis, 38 women from IUD group cleared HPV infection (70.4%) while 30 women from non IUD group using preservative cleared it (52.6%) ($p=0.052$). 38 women from IUD group cleared HPV infection (70.4%) and 18 women from non IUD group not using any contraceptive (66.7%) cleared HPV infection ($p=0.734$). Finally, 38 women from IUD group cleared HPV infection (70.4%) and 17 women from non IUD group using hormonal contraception (47.2%) cleared HPV infection ($p=0.022$). Also, we compared HPV clearance between IUD users and non hormonal contraceptive users (preservative and none contraceptive): 38 women from IUD group cleared HPV infection (70.4%) and 48 women from non IUD group using no hormonal contraception (57.1%) cleared HPV infection ($p=0.118$) (Table 3).

The HPV clearance using condom was 52.6%, hormonal contraceptive 47.2% and not using any 66.7% ($p=0.293$).

HPV clearance in patients younger than 35 years old in both groups was 55.4% and in patients older than 35 years old was 61.5% ($p=0.430$). HPV clearance in patients with normal results in smear test was 62.7% versus in abnormal results 47.5% ($p=0.086$). Clearance in smokers was 57.6% and in non-smokers 59% ($p=0.882$).

HPV clearance between high-risk HPV infection (55%) and low-risk HPV infection (76.5%) showed statistical differences ($p=0.022$).

We compared HPV clearance between patients with only one HPV type (68.1%) or more than one (28.2%), showing statistical differences ($p<0.001$) (Table 4).

An association in logistic regression was observed in HPV clearance with different factors. Firstly comparing IUD and non IUD, higher percentage of patients with IUD clear the HPV infection, significantly associated (OR=0.698, CI 95%; 0.251-0.998,

p=0.046). Clearance was higher in patients with low-risk HPV infection comparing with high risk HPV (OR=1.078, CI 95%; 1.126-6.281, p=0.026) and in patients with only one HPV subtype than those with more than one subtype (OR=1.695, CI 95%; 0.084-0.403, p<0.001) (Table 5).

Comments

HPV 16 is the most frequent high-risk HPV type in Spanish women, as we also obtained in our study. Previous studies have shown that the proportion of high and low-risk HPV prevalence in the female population is similar, while in men the prevalence of low-risk HPV is higher than the high-risk HPV [10], but in our study, the majority of women have high-risk HPV infection (80.4%).

HPV persistence is necessary for cervical cancer development and is defined by finding the same HPV type in two or more samples separated by 1 or 2 years. Persistence is different in each HPV type and infection period is longer in high-risk HPV than low-risk HPV. HPV types more persistent are: 16, 31, 33 and 52. The average infection period in women with HPV and non-pathological smear tests is 11.5 months, and for HPV 16 type is 12.4 months.

In general, 53% of HPV infections clear in 12 months, 79% in 24 months, 87% in 36 months and 89% in 48 months [11]. Our study revealed a similar result in non IUD group, where 54.2% women cleared the infection in 1 year, considering the beginning of infection when the first HPV sample is obtained..

Smoking, high parity and using hormonal contraceptives increased the risk of having a persistent HPV infection [12]. However, we didn't get any significant differences for persistent infection between smokers and non-smokers, as well as condom users or other contraceptive methods.

Several studies have found a relation between age and HPV persistence, older patient have longer period infection [13] [14], but in our study we didn't find any difference in HPV clearance in the group of women younger than 35 years old and older than 35 years old.

We also found that women older 30 years had HPV infection for longer periods, according by previous work published by us but we were unable to obtain significant

differences after separating the patients into 2 groups, namely 25-35 years old and 35-50 years old.

Besides, 25% of women with HPV infection have a pathological result in their citologies, similar to our study, where 23.5% of women shown pathological citology. Most of the patients had high-risk HPV infection (80.4%) and only 19.6% had low-risk HPV infection subtypes.

Using preservative is recommended for patients with HPV infection. In older patients the use is less, where we know it's more common to have persistent infections, which lead to the development of pre-neoplastic lesions.

Our study proved that the clearance is higher when there is only one type of HPV, which has been confirmed too by Skinner et al study [15].

The relation between HPV and host is complex. The virus replication process provides for itself an evasive mechanism inhibiting and delaying the host immune response against the viral infection. At the beginning of infection, HPV induces an immune response activating cytokines and promoting cellular migration of macrophages, Langerhans cells, natural killer cells, lymphocyte T. These cytokines and Langerhans cells stimulate lymphocytes T, which is the main mechanism for HPV clearance [16]. The increase of lymphocytes Th2 cytokines (interleukin 10) and decrease of Lymphocytes Th1 cytokines (interferon gamma, interleukin 12, interleukin 2 and tumoral necrosis factor) have been found in cervical samples of patients with high-risk HPV.

IUD induces histological and immunological changes in cervix and endometrium. Initially, when IUD is inserted, it produces an inflammatory reaction and increases pro-inflammatory cytokines. *Levonorgestrel* IUD increases inflammatory cytokines $TNF\alpha$, Interleukin 1 beta, Interferon gamma, interleukin 12 and interferon $\alpha 2$, which show a strong local immune response [17]. In citology results found in IUD users after 1 year of insertion, an increase of leucocytes have been shown in 80% of patients with high proportion histiocytic.

Cells CD 69 positive: macrophages, neutrophil and dendritic cells, increase two months after copper IUD insertion, being less important with *Levonorgestrel* IUD. Also, hyperplasia in endocervial epithelium and squamous metaplasia have been seen [18].

The increase of inflammatory cells after IUD insertion, which are also the same participating in HPV clearance made us think that IUD could improve HPV clearance stimulating immune response.

Several studies have shown an important inverse relation between IUD use and risk of cervical cancer [19-21].

A study in China comparing the risk of cervical cancer in between IUD users and patients with tubal sterilization, showed a decrease in cervical cancer with IUD use [22].

However, the HPV infection had not been included in any of these studies, so the possible effect of IUD improving HPV clearance had not been confirmed.

Another possibility could be that IUD stopped the progression of pre-invasive lesions to cervical cancer, but we know that in case of HPV persistent infection, 20-30% of women with pre-invasive lesion will develop cervical cancer [23].

In our study we included HPV infection in order to check if IUD improved HPV clearance. Our results show than more patients cleared the HPV infection using IUD, which can explain why IUD is a protector factor in cervical cancer.

We have shown results with statistical significance between groups IUD and control ($p=0.044$) for HPV clearance. The results were statistically significant for HPV clearance between low-risk HPV patients and high risk HPV patients ($p=0.002$) and between patients with one subtype of HPV and more than one HPV subtype ($p<0.001$).

We did not compare HPV clearance between copper IUD and levonorgestrel IUD because we only had 5 patients using copper IUD. In Lekovich et. al study [24] the clearance was higher with copper IUD with statistical significance ($p=0.04$), so it could be interesting study the difference in further investigations.

HPV infection is the most frequent sexually transmitted disease in the world today, with easier transmission than HIV or herpes. The probability of HPV 16 transmission from man to woman is 60-80% in each act of sexual intercourse [25].

The knowledge that condoms are not totally effective against HPV infection and currently, no treatment exists, it's essential to discover a mean to eradicate HPV infection. Also, combined hormonal contraceptives are not recommend in patients with HPV infection because they have shown an association with HPV persistent infection [5, 12, 26]. IUD could be an important contraceptive option in women with HPV infection, even with the use of condoms.

Conclusion

In Spanish women diagnosed with HPV, the clearance between IUD and no IUD groups show a difference with statistical significance. This could explain how IUD is a protector factor in cervical cancer, improving the HPV clearance. There were differences between suffering one subtype and more than one, as well as the role of low-risk HPV with respect to high-risk HPV, showing the relevance of HPV subtypes in clearance and in the prevention of development of cervical cancer in patients with VPH infection.

This study contributes to evaluate contraceptive conseil in women with cervical human papillomavirus infection, and open research lines to investigate methods which could decrease HPV infection.

Conflicts of interests

None

Authorship & contributorship

All authors have approved the final version of the manuscript.

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