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Original Articles

Risk factors to develop multicentric lesions of the lower genital tract

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Summary

Purpose of investigation: To analyze which are the risk factors in developing multicentric lesions of lower genital tract. *Materials and Methods:* A prospective study of 1,011 patients was conducted at the low genital tract pathology clinic of Sant Joan de Deu Hospital between 2003-2011. A complete assessment of cervix, vagina, and vulva was carried out including HPV-DNA testing, cytology study, colposcopy, and biopsy in case of atypical findings. The statistical analysis was done with SPSS v.19 software. Differences between groups were considered statistically significant at $p < 0.05$. *Results:* Twenty-two patients presented multicentric lesions (2.2%). The average age was 43 years. Most of the lesions were bicentric affecting cervix and vagina and cervix and vulva. Only in two cases (9%) there were three sites of genital neoplasia. The authors found four cervical cancer, 17 high grade, and one low grade lesions of the cervix. Eighteen vaginal intraepithelial neoplasia (VAIN), six high grade, 14 low grade, and four vulvar intraepithelial neoplasia (VIN) were found. HPV infection, age > 35 years, multiparity, contraceptive method, immunodeficiency, and level of studies were significantly correlated with multicentric lesions. High percentage of affected margins were found. VIN cases were treated with surgical excision and in two cases microinvasion was found. VAIN III cases were treated with surgical excision or with laser and one case progressed to vaginal cancer. Recurrence after treatment was 27%. *Conclusion:* Age, multiparity, contraceptive method, immunodeficiency, and level of studies were significantly correlated with multicentric lesion. Multicentric lesions had an increased risk of recurrence and progression to cancer.

Key words: Multicentric lesions; HPV; Intraepithelial lesions; Recurrence; Cancer.

Introduction

The most common site of the lower genital tract to develop a premalignant or malignant lesion is the cervix [1]. Vulvar and vaginal premalignant or malignant lesions are less frequent [2]. Multicentricity is defined as the presence of premalignant or malignant lesions in two or three sites of the lower genital tract (cervix, vagina, and vulva) [3]. These lesions could appear concomitantly or not. Diagnosis of multicentric lesions is not easy because screening is focused mainly on the cervix. In other words, these patients with multicentric lesions have an increased risk of failure of the treatment and recurrence [4]. HPV infection by high-risk genotypes seems to play a important role in the development of these multicentric lesions affecting all lower genital tract [5].

The aim of the study was to analyze which are the risk factors presented by these women to develop multicentric lesions of lower genital tract and also to describe the clinical pictures, present the different managements, and results of treatment of these types of lesions.

Materials and Methods

This is a prospective study including 1,011 patients seen at the low genital tract pathology clinic of Sant Joan de Déu University Hospital in Barcelona (Spain) between January 2003 and March 2011. All women were referred to the present hospital because of cytological alterations or vulvar lesions. Cytological studies were done for all patients and the findings were classified according to the Bethesda classification. All women were also examined by colposcopy with an aqueous 3%–5% acetic acid solution. The authors used the classification proposed in Barcelona by the International Federation for Cervical Pathology and Colposcopy in 2002 [6]. A complete assessment of cervix, vagina, and vulva was carried out. Colposcopy-directed biopsies were performed. The biopsy specimens were fixed in formalin and analyzed by a two pathologists. Prior to cytological study and colposcopy, the patients underwent HPV-DNA testing. Cervical scrapes were obtained with a cotton brush and transported at room temperature to the molecular microbiology department for HPV genotyping. During the study period, two techniques: line probe assay (LiPA) and microarray, were used consecutively. For LiPA assays, cervical swabs for DNA extraction were obtained with a commercial kit and eluted to a final volume of 200 μL. For microchip array assays, DNA was extracted with a proteinase K lysis solution (20 mg/ml). The purified DNA extracts were stored at -20 °C. The LiPA assay was based on the reverse hybridization principle and provides type-specific genotype

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information for 25 different HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) simultaneously. Amplification of HPV DNA was based on the SPF10 PCR primer set, which amplifies a fragment of only 65 bp within the L1 open reading frame (ORF) region. Part of the human beta-globin gene (268 bp) was amplified in each sample as a control. Line probe assays with SPF10 were done with ten μL of the DNA extract in a final reaction volume of 100 μL. The microchip array assay detected infections and coinfections up to 35 of the most relevant HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85, and 89) in different sample types. The system was based on a low-density microarray attached to the bottom of a classical two-ml Eppendorf tube. For DNA amplification a reaction mixture was used which amplifies a 450-bp fragment within the L1 ORF region. A 892-bp fragment of the human CFTR gene was amplified in each sample as a genomic DNA control. To avoid false negative results, an amplification control was added to the reaction mixture.

Multicentric disease included CIN or invasive cervical cancer coexisting with vaginal intraepithelial neoplasia (VAIN) and/or vulvar intraepithelial neoplasia (VIN). All lesions were confirmed by biopsy and the authors included low grade, high grade, and invasive lesions. Multicentric lesions were divided in three groups: cervix+vagina, cervix+vulva, and cervix+vagina+vulva. For each patient, following data were noted: age, age of first sexual intercourse, contraceptive method, number of sexual partners, parity, immunosuppression, tobacco, HPV infection, HPV genotype, and coinfection by various HPV genotypes.

Different therapeutic modalities were used: surgery (conization, hysterectomy, and surgical excision) and non-surgical (CO_2 laser). Conization, an extensive loop excision of the transformation zone in the form of a cone which also included part of the endocervical canal, was performed on all women with a cytological diagnosis of HSIL at the present hospital, or those whose biopsy results yielded a diagnosis of HSIL independently of the result of cervical cytology. Hysterectomy was performed in case of invasive lesion, recurrent lesion or affected margin of the conization. Surgical excision of vagina or vulva was performed in case of high grade lesions (VAIN II-III or VIN II-III) under colposcopy control. Vaginal high grade lesions were also treated by CO_2 laser vaporization previous multiple biopsies to discard invasive lesions. In case of low grade lesions of vulva or vagina, non-surgical procedures were done and close follow-up was performed. Follow-up of these patients include a complete examination of vulva, vagina, and cervix every three or six months depending on the type of the lesion. Residual lesion was defined as persistent histological lesion after primary treatment and recurrence as histological lesion occurring after at least two negative post-treatment controls. In case of invasive cervical cancer, the authors only includes the case if vaginal lesion appeared five years after the primary treatment.

All data were analyzed with SPSS software (v. 19). The authors used Student's *t*-test for quantitative variables when the data were distributed normally, and the Mann-Whitney U test when normal distribution could not be confirmed. Comparisons for qualitative variables were analyzed with the chi-squared test. Analysis of variance was used for comparisons involving more than two samples. The results were considered statistically significant if the *p*-value was < 0.05.

Results

From January 2003 to March 2011, 1,011 patients were referred to the present low genital tract pathology clinic. Out of 1,011 patients, 22 presented multicentric lesions (2.2%). For these 22 patients, the average age was 43 years (range 26-67 years). When comparing the age of the group with multicentric lesions with the rest of the group, the authors found that the patients with multicentric lesions were older, 72.7% *vs.* 44.5% were older than 35 years, respectively ($p = 0.009$).

Table 1. — *Analysis of risk factors in multicentric and unicentric disease groups.*

	Multicentric disease	Unicentric disease	*p* value
HPV infection	21/22 (95%)	717/985 (72.8%)	**0.014**
Age > 35 years	16/22 (72.7%)	438/985 (44.5%)	**0.009**
High grade lesion cervix	16/22 (72.7%)	463/985 (47%)	**0.001**
Not user of contraceptive method	3/14 (21.4%)*	75/751 (10%)*	**0.02**
Previous pregnancy	18/22 (90%)	646/975 (66.3%)	**0.029**
Without studies	16/19 (84.2%)*	532/933 (57%)*	**0.048**
Positive cone margin	12/22 (54.5%)	182/985 (18.5%)	**0.000**
HIV	3/22 (13.6%)	6/985 (0.6%)	**0.000**
Smoking cigarettes	8/18 (48.1%)*	470/959 (49%)*	0.81

Significant values are in **bold**. *Patients with evaluable data.

HPV infection was more frequent in patients with multicentric lesions (95.5%) than in patients with unicentric diseases (72.8%) and the difference was statistically significant ($p = 0{,}014$). The authors did not find differences between the two groups when they analyzed the type of HPV, high o low risk for cancer; in both groups high risk HPV were predominant. Infection by more than one HPV genotype was also similar in both groups. Patients with multicentric lesions had 72.7% of high grade lesion of the cervix *vs.* 47% in patients with only cervical lesions ($p = 0.001$)

The age of first sexual intercourse range from 14 to 20 years (average 20). There was no statistical difference between the two groups according to the age of first intercourse and the number of couples.

The authors found differences ($p < 0.02$) between contraceptive method in both groups; in patients with multicentric lesions, the percentage of women not using any method were higher (Table 1).

Women having at least one children and without studies had a higher risk of multicentric lesion (Table 1). Smoking cigarettes was not related in this study with multicentric lesions (Table 1). Immunodeficiency (HIV patients) was more frequent in multicentric disease group (3/22 cases) than in patients with unicentric disease (6/979).

Most of the multicentric lesions were bicentric (cervix+vulva-2- and cervix+vagina-18-) in 90.9%. Only 9% had three sites (cervix,vagina, and vulva) of genital neoplasia (2/22). Lesions were concomitant in 86.3% (19/22). In case of non-concomitant lesions, the mean delay between the first two locations was nine years (range 3-18).

Eighteen CIN, 20 VAIN, four VIN, and four cervical cancers were reported. Regarding CIN, 17 were high grade (94.4%) and one was low grade (5.6%). Regarding VAIN, six were high grade (31.6%) and 14 were low grade (68.4%). Regarding VIN, three were high grade (75%) and one was low grade (25%). Regarding management of cervical lesions, all the patients were treated; most common treatment was conization in 81.8% (18/22). Margins were positive in 54.5% of multicentric disease women if these were compared with 18.5% of patients with unicentric disease ($p < 0.05$). Hysterectomy was performed in 16 cases (72.7%) of the multicentric group, in four cases due to cervical cancer.

Most of the vaginal lesions (68.5%) had no treatment and were followed-up. These lesions were low grade VAIN. High grade lesions of vagina were treated by surgical excision and by CO_2 laser. One case of vaginal high grade lesion developed vaginal cancer.

For vulvar lesions, most common treatment was surgery (75%; 3/4). Rate of positive margins was 66.6% (2/3); in two cases there was an occult micro-invasive lesion. Recurrent rate was 27.3% (6/22) in the multicentric group. Five patients had vaginal recurrence and one patient had vaginal and cervical recurrence at the same time.

Discussion

It is difficult to find a review in the literature, studies about multicentric intraepithelial neoplasias of the genital tract associated with HPV infection. If we compare the present data with a large study published, the frequency of multicentric disease of the genital tract is 4.4% [3], which is two times the prevalence observed in this study (2.2%).

Most of the lesions in the present study involved two sites (90.9%); this result is also reported by others authors [1, 7]. The two sites more frequently involved were cervix and vagina. The percentage of concomitant lesions in the present study (86.3%) was higher than the percentage reported in other large studies (52.3%) [3]. When we analyze the risk factors of the patients in the current study to present a multicentric lesions, HPV infection was more frequent in patients with multicentric lesion (95.5%) than in women with unicentric disease, similar to other studies (93%) [8].

Most of the risk factors observed were associated with multicentric lesions and related with HPV infection. Contraceptive method, multiparity, and level of studies are related in the study and in the general population with a higher risk to by infected by HPV [9]. Some of them are also related to a risk of persistent infection by HPV like immunodeficiency observed by other authors [10-13] and detected in the present study. Smoking cigarettes was not related in the present study with multicentric lesions (Table 1).

The present authors also observed that women with multicentric lesion were in a great percentage older than 35 years, which is an age if the HPV infection is not cleared, then the risk of persistent infection is higher.

The present authors observed a high incidence of positive margins similar to that observed by Ait Menguet *et al.* [3]: 54.5% and 46.2%, respectively, after surgical treatment in cervical lesions and vulvar lesions 66.6% and 30%, respectively. These data confirm the higher risk of these patients to develop persistence of lesions after treatment and the need for a close follow-up [3, 14]. It is important to mention that two cases in the present study, a microinvasive lesion was found after the excision of the VIN III lesion. The recurrence rate in this study was 27.2% - a little lower than other authors (43%) [3].

Conclusion

All the patients with multicentric lesions have an increased risk of persistent HPV infection with a lower response to the treatments, and hence include an increased risk of recurrence and progression of the disease into an invasive lesion [15, 16]. Therefore these women with the risk factors described, especially those who develop multicentric lesions, should be closely followed up because they are at risk to develop invasive lesions.

References

[1] Torné Bladé A.: "Patología premaligna del cuello uterino". *In:* González-Merlo J., González-Bosquet E., González-Bosquet J., (eds). *Ginecologia* 9th ed. Barcelona: Elsevier Masson, 2014, 416.

[2] Cardosi RJ, Bomaslasky JJ, Hoffman MS.: "Diagnosis and management of vulvar and vaginal intraepithelial neoplasia". *Obstet. Gynecol. Clin. North. Am.*, 2001, *28*, 685.

[3] Ait Menguellet S., Collinet P., Houfflin Debarge V., Nayama M., Vinatier D., Leroy J.L.: "Management of multicentric lesions of the lower genital tract". *Eur. J. Obstet. Gynecol. Reprod. Biol.* ,2007, *132*, 116.

[4] Houfflin Debarge V., Collinet P., Vinatier D., Ego A., Dewilde A., Boman F., Leroy J.L.: "Value of human papillomavirus testing after conization by loop electrosurgical excision for high-grade squamous intraepithelial lesions". *Gynecol. Oncol.*, 2003, *90*, 587.

[5] van Beurden M., ten Kate F.W., Tjong-A-Hung S.P., de Craen A.J., van der Vange N., Lammes F.B., *et al.*: "Human papillomavirus DNA in multicentric vulvar intraepithelial neoplasia". *Int. J. Gynecol. Pathol.*, 1998, *17*, 12.

[6] Walker P., Dexeus S., DePalo G., Barrasso R., Campion M., Girardi F., *et al.*: "International terminology of colposcopy: An updated report from the International Federation for Cervical Pathology and Colposcopy". *Obstet. Gynecol.*, 2003, *101*, 175.

[7] Audet-Lapointe P., Body G., Vauclair R., Drouin P., Ayoub J.: "Vaginal intraepithelial neoplasia". *Gynecol. Oncol.*, 1990, *36*, 232.

[8] Hampl M., Wentzensen N., Vinokurova S., von Knebel-Doeberitz M., Poremba C., Bender H.G., Kueppers V.: "Comprehensive analysis of 130 multicentric intraepithelial female lower genital tract lesions by HPV typing and p16 expression profile". *J. Cancer Res. Clin. Oncol.*, 2007, *133*, 235.

[9] Castellsegue X, Muñoz N.: "Co-factors in human papillomavirus carcinogénesis role of parity, oral contraceptives, and tobacco smoking".: *J. Natl. Cancer. Ins. Monogr.*, 2003, *31*, 20.

[10] Rapose A.: "Human papillomavirus and genital cancer". *Indian J.*

Dermatol. Venereol. Leprol., 2009, *75*, 236.
[11] Hørding U, Daugaard S, Iversen AK, Knudsen J, Bock JE, Norrild B.: "Human papillomavirus type 16 in vulvar carcinoma, vulvar intraepithelial neoplasia, and associated cervical neoplasia". *Gynecol. Oncol.*, 1991, *42*, 22.
[12] Lindeque BG.: "Management of cervical premalignant lesions". *Best. Pract. Res. Clin. Obstet. Gynaecol.*, 2005, *19*, 545.
[13] Frega A., Sesti F., Sopracordevole F., Biamonti A., Votano S., Catalano A., *et al.*: "Multiple intraepithelial neoplasias of the lower female genital tract: the reliability of HPV mRNA test". *J. Low. Genit. Tract. Dis.*, 2014, *18*, 174.
[14] Fallani M.G., Fambrini M., Zipoli E., Marchionni M.: "CO2 laser surgery for vulvar intraepithelial neoplasia. Excisional, destructive and combined techniques". *J. Reprod. Med.*, 2002, *47*, 913.
[15] Melkert P.W., Walboomers J.M., Jiwa N.M., Cuesta M.A., Kenemans P., Meijer C.J.: "Multiple HPV 16-related squamous cell carcinomas of the vulva, vagina, anus, skin and cervix in a 31-year-old woman". *Eur. J. Obstet. Gynecol. Reprod.Biol.*, 1992, *46*, 53.
[16] Henning E.M., Di Lonardo A., Venuti A., Holm R., Marcante M.L., Nesland J.M.: "HPV 16 in multiple neoplastic lesions in women with CIN III". *J. Exp. Clin. Cancer. Res.*, 1999, *18*, 369.

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