

Prevalence and Genotype Distribution of Oncogenic-Risk Human Papilloma Virus in the Cervix of Climacteric Women with Normal Pap Smears

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Abstract

Objective: To find out the incidence of high-risk HPV infections on climacteric women within our area of influence; and to typify HPV genotypes on women with normal cytology that come to our hospitalary unit of menopause. **Material and Method:** Cross-sectional study; with a random sample of 140 cases of climacteric women of ≥ 50 years of age, with normal Pap smears for the last 12 months. HPV determination was carried out by PCR for screening, and by hybrid capture for genotype typification. **Results:** The percentage of climacteric women who are carriers of oncogenic HPV and a normal Pap smear was of 11.43% (16/140 cases). The genotype found most frequently was HPV-16, followed by HPV-58, 51 and 18. **Conclusions:** We found a high prevalence of women who were carriers of oncogenic HPV in climacteric women with normal Pap smears (latent infections) in our health region. We consider that cervical cancer screening, either by PCR or conventional Pap smear, should not be minimized or ignored from 50 years of age onwards.

Keywords

Prevalence, Oncogenic Human Papilloma Virus, Climacteric

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1. Introduction

Human papilloma virus (HPV) infection is presently very frequent and contagious, it being considered a very prevalent and multi-focal STD. In Europe, this prevalence is of around 30% in young women and 18% in adults [1]. In the USA, the mean prevalence, from 14 to 59 years of age is of around 26.8% [2].

Nowadays, it is widely accepted that this infectious (viral) disease, with a transmission that is mainly due to sexual practices, is an inducer or participant in the genesis of certain types of anal-genital cancers [3]. Currently, over 120 types of HPV have been described (40 have tropism for the lower genital tract), for 12 of which there is scientific evidence that they are of high oncogenic risk (HR) [4]. In 6 more HPV types, the evidence is still inconclusive (of a probable high risk) [5].

Most of these infections affect young women, and fortunately, they are transient and cause no lesions (sub-clinical infection), 70% resolving spontaneously by one year and 90% by two years; but they still have great repercussions in Public Health [6].

The oncogenic risk appears in women over 30 years of age (with HPV 16 - 18, and with normal Pap smears), with a possibility of 15% - 30% of developing high-grade intraepithelial lesions (CIN II+) in the next 5 - 10 years [7].

The prevalence of HPV-DNA (+) HR in patients with normal Pap smears varies greatly worldwide, in the USA it is of 7.8% - 8.4% (mean of 8.2%). Conversely, in South America, it ranges from 12.4% to 13.5% (mean of 30%); in Africa, it reaches up to a mean of 22.1% and on the other hand, in Asia, it appears in between 7.5% - 8.5% (mean of 7.9%) [8].

Summarizing data from the WHO, the rate in the developed world is of 10.0%, and in developing and under-developed countries it is of 15.5%. This difference is possibly due to the precocity in marriage in these countries, usually being comprised of older males with more than one previous partner, but above all, it is associated with their poor hygienic conditions [9].

A recently-published meta-analysis [1] shows a prevalence of HPV with oncogenic risk (with normal Pap smears) of 10.4%, increasing significantly with climacteric (45 - 55 years).

As we were unaware of the HPV infection incidence rates in our female population ≥ 50 years of age, with normal Pap smear results and asymptomatic (from the oncological standpoint), we designed this study in collaboration with the menopause unit at the hospital.

For this, we set out to find out the results of applying the molecular biology techniques for the determination and typification of the human papilloma virus (HPV), together with colposcopic (with 5% acetic acid) and cytologic (conventional exfoliative Pap smear) studies, by means of a sample that is representative of our reference menopause consult for the central district of Malaga, Western Costa del Sol and Guadalhorce Valley.

2. Material and Methods

A cross-sectional study was carried out, by means of a sample that was random (for this, the first and last user where included who, fulfilling the required specifications, had an appointment at our Unit every Monday and Wednesday during the 12 consecutive months that the enrolment period lasted) and significant ($n = 140$ cases).

The inclusion criteria were: Climacteric women (≥ 50 years of age) with normal cervical-vaginal Pap smear results (in the last 6 months) who were also asymptomatic. Hysterectomized patients were excluded, as were those having a history of diagnosis and/or treatment of Cervical Intraepithelial Neoplasm (CIN) or cervical carcinoma.

Informed consent was requested from the patients. As it was an epidemiological, non-interventionist study, the approval of a hospital committee was not necessary, although it was informed thereof.

The enrolment period lasted from 01.02.2012 until 01.02.2013. All the women summoned to our Unit came from our health region of the Andalusian Health Service (Servicio Andaluz de Salud, SAS), which is comprised of the central district of Malaga, Western Costa del Sol and Guadalhorce Valley (Spain). This would be comprised of a population of approximately 750,000 inhabitants, of which 51.09% are women, according to the last census by the National Statistics Institute (Instituto Nacional de Estadística, INE) for 2005.

HPV-DNA determination was carried out by our hospital's Anatomic Pathology Department, by means of the polymerase-chain-reaction technique (PCR) for screening and by means of hybrid capture for viral genotype typification. In clinical practice, the probe that we use is called VPH-AR "Híbrido Capture II[®]" (HC2) [10].

The statistical sample was obtained by applying the mean prevalence of the series published worldwide on the

female population that is registered in the census for our province, having an age of ≥ 50 years, with a 95% confidence interval.

To carry out the statistical analysis, we used the program SPSS® (SPSS® for Windows, version 18.1) of the University of Malaga (Spain). For the analysis of the frequency tables of the analyzed variables, we used the Chi-Square test. We applied the statistical significance for p -values < 0.05 .

3. Results

The age mean of the sample was 53.1 years (RR: 50 - 71 years of age).

Regarding their source, the majority (non-significant) was comprised of an urban population (Malaga, central district), with 9 cases (55%); 7 cases (45%) came from the rural area, divided into 25.7% from Western Costa del Sol, and 19.3% from Guadalhorce Valley. This confirms that there are practically no differences between some communities if they are within a same region or country, and they possess the same cultural, geographical and economical development [11].

Most (60%) were married, healthy and the reasons for which they attended the menopause unit were: Hormone replacement therapy revisions, neoplasm prevention, screening for osteoporosis, etc. Of the 140 patients of the sample: 97 (69.28%) were Spanish; 22 (15.72%) were Central-South American, 10 cases (7.14%) were British; 9 cases (6.43%) Central European and 2 cases (1.43%) came from elsewhere.

The percentage of climacteric women (≥ 50 years) who were carriers of high-oncogenic risk HPV DNA and who had normal Pap smear results was **11.43%** (16/140 cases); where as the percentage of those with negative results was 88.57% (124/140 cases).

The most frequently found genotype was HPV-16 in 7 of the cases (43.75%), followed at a great distance by HPV 58, 51 and 18, with 12.5% (2 cases, respectively). After that, HPV-45, 31 and 39 appeared, with a 6.25% (1 case each) (**Figure 1**).

Seventy-five percent of these (12 cases) were Spanish, 18.75% (3 cases) came from South America and 6.25% (1 case) were from Central Europe. Most cases (6 cases) with HPV-16 were Spanish and 1 case was from Argentina. Cases with HPV-58 corresponded to 1 case from Colombia and 1 from Argentina. All the recorded cases of HPV 51-18-39-45 were of Spanish women. The only case of HPV-31 came from Romania.

4. Discussion

These data on the prevalence of subclinical HPV infection (11.43%), at these ages and carrying normal Pap smear results, are greater than what is expected for a Spanish population, as we are not a leading country in terms of cervical cancer (which has a global mean of around 6.8%), and the European mean is around 8.2% [1], although they are well above the data published in Holland, with 4% [12], and those from the Soviet Union, of around 2.4% [13].

In order to explain this high incidence of asymptomatic carriers of high-oncogenic-risk human papilloma virus in climacteric women with normal Pap smear results, several scientific hypotheses have been proposed, seeing that it could be due to multiple factors: Reactivation of infections that were latent until this time, or rather due to hormonal changes that happen in this age cluster (atrophic colpitis, hypoestrogenism, etc.), or also due to ageing of the immune system, which would allow for the expression of infections that were latent until then, and that are practically undetectable by methods such as the conventional triple-sampling Pap smear.

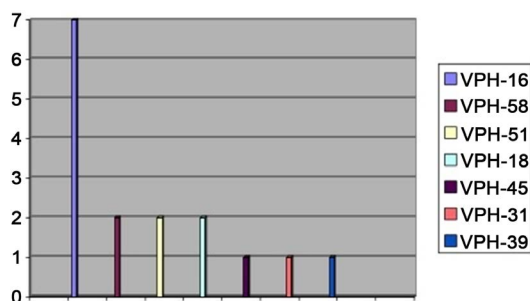


Figure 1. High-oncogenic risk HPV genotypes distribution and frequency.

There are authors that point at causes for the increase in oncogenic HPV carriers at these ages, the simple increase in the acquisition of “de novo” infections, regarding the changes in the climacteric women’s sexual behavior [14]: High divorce rate in our country, new partners (according to the recently-published Afroditá[®] study about the Spanish woman’s sexual profile [15], 77.9% of Spanish women between 45 and 55 years of age only have one sexual partner, 17.3% between 2% and 4%, and 4.8% of these women have ≥ 5 sexual partners), practices of unprotected sex (according to data from the same published study [15] 15.1% of women between 45 - 55 years do not use any contraceptive method, and in those who do use it, the most frequent one is oral contraceptives, followed by condoms). For these reasons an easier transmission of HPV is acquired at these ages. These Spanish data could justify the 11.43% of HPV subclinical infections that we have found in our study.

Although we concur with Fusté [16] in that the genotypes found in climacteric women with normal Pap smears are similar to those found when Pap smears are pathological, we believe that the percentages of HPV-16 and 18 are somewhat lower, and hence, we disagree with him regarding the other five more common HPV genotypes found in our hospital menopause unit.

We provide, as an epidemiologic curiosity, the finding of a greater proportion of HPV-58 in these users mainly coming from countries in Western Africa (such as Nigeria or Senegal), as well as from South America.

On the contrary, we are seeing a lesser proportion of HPV-31, coming from Central America and Eastern Europe.

Our results, although congruent with the worldwide meta-analysis, confirm that although the HPV infection is more frequent in young women of around 25 years of age, there is a second peak of HPV prevalence from 45 - 55 years onwards in all the regions of the planet, except for Asia [1]. This prevalence peak is seen at 54 years of age in Africa and Europe, and after 44 years in America [1].

Cervical cancer screening, either by conventional Pap smear or by HPV-DNA PCR, in light of these findings, cannot be minimized or ignored in this age group of climacteric women. This is why we point out the need to present clinical studies or trials that support the convenience of broadening the indication of preventive vaccines for oncogenic-risk HPV, even in ages closed to menopause.

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