PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	PREVALENCE AND GENOTYPE DISTRIBUTION OF CERVICAL HUMAN PAPILOMAVIRUS INFECTION IN THE PRE-VACCINATION ERA: A POPULATION-BASED STUDY IN THE CANARY ISLANDS
AUTHORS	ANDUJAR, MIGUEL; ROURA, ESTHER; TORRES, ALEJANDRA; VEGA, BEGOÑA; PAVCOVICH, MARTA; SANCHEZ, MIGUEL ANGEL; LUBRANO, AMINA; TRUJILLO, JOSE LUIS; ALMEIDA, LUCIA; SANTANA, MILAGROS; HURTADO, ROSAURA; ARENCIBIA, OCTAVIO; BENITO, VIRGINIA; MEDINA, NORBERTO; CARBALLO, SONIA; CAMACHO, MARIA DEL CARMEN; RUIZ DEL POZO, ARANCHA; QUESADA, ALFOSO; SALIDO, EDUARDO; De Sanjosé, Silvia; Bruni, Laia

VERSION 1 – REVIEW

REVIEWER	Julio Cesar Teixeira University of Campinas Brazil
REVIEW RETURNED	29-Feb-2020

GENERAL COMMENTS	Some suggestions: 1. Keywords: I missed the following words: cervical cancer; precursor lesions/CIN; human papillomavirus. 2. Abstract: Methods: the period of the study. 3. Methods: the name of the Ethics Committee (line 28). 4. Methods: Statistical... Line 33: "number of women of women" 5. Discussion: HPV genotypes and HPV vaccine protection offered: Your results point HPV-51, -53, and -59 as the more prevalent after HPV-16 and these types are not in the composition of the vaccines. I suggest discussing about this find.

REVIEWER	Leith León-Maldonado CONACYT-Instituto Nacional de Salud Pública. México.
REVIEW RETURNED	04-Mar-2020

GENERAL COMMENTS	Manuscript ID: bmjopen-2020-037402 Title: Prevalence and genotype distribution of cervical human papillomavirus infection in the pre-vaccination era: a population-based study in the Canary Islands. Authors: M Andújar et al. Date: March 3, 2020 Journal: BMJ Open Summary: The study provides estimates of HPV infection prevalence in Canary Islands, this community has one of the highest prevalence

	in Spain. In addition, provides information on HPV distribution of 20 high-risk and 14 low-risk genotypes for this region, what will allow comparisons with other cities in Spain. Finally, the results of this study will allow to assess the impact of HPV vaccination. Keywords: Perhaps the authors should include human papillomavirus infection as an additional keyword. Comments: This is a well-written document. The issue addressed is relevant, mostly for the Canary Islands and Spain. Abstract: 1) Methods: it is suggested to add the period in which this study was carried out. 2) Conclusions: Could the authors mention something very brief about genotypes? Methods: 2.1 Participants 1) It is suggested to describe the difference between the initial probabilistic sample of 2,276 and the 665 who were volunteers. For example, the differences in age distribution and gynecological characteristics. 2) Page 8, line 4: How many women were registered in the database of the General Population of Gran Canaria? How was the sample selected? What was the sample fraction? 3) Page 8, line 21: How many volunteers were included? What were their characteristics? What was the procedure for recruitment volunteers? It may be convenient to discuss if the volunteers were similar to the random sample? 4) Recruitment: It may be good to explain what happened if the women did not attend the scheduled visit, what actions were taken? What was the recruitment rate? 5) There is quality control of the registers of the regional Health Administration database? e.g. duplicate registers. 6) Page 8, line 26-28: It is suggested to mention the date and number of approbation as well as the name of the hospital. 2.3. Procedures 1) Page 8, line 44-46 What was the sample collection procedure? What kind of brush and which vial was used to preserve the sample? Results: Study population 1) Table 1. It would be interesting to see the data of Tenerife and Canaria separately, in order to show the characteristics of each population. 2) The first graph of the supplementary tables may better explain the genotyping results, I would suggest to changing this for the Table 2. Prevalence of cervical HPV infection 1) The discussion of quality control of samples should be addressed in the methods section. 2) Page 13, line 30-32 it is mentioned “Although volunteers from Gran Canaria showed higher prevalence of any-type HPV infection…” Can the authors present the prevalence’s data?

	Discussion: Prevalence of cervical HPV infection 1) Page 19, lines 34-39: Is there any reference for this fact? 2) Page 20, lines 1-19: Which could be the explanation for differences in HR-HPV prevalence's among European countries? Is the prevalence of HR-HPV in Spain similar than other European countries? Which? Prevalence of cervical HPV infection per age group 1) Page 20, lines 31-33: Authors mention "This finding was also observed in previous Spanish and European studies", it is suggested to add the reference for this fact. Risk factors and cervical HPV infection I suggest to further discuss the lack of association with the level of education, as observed in many other studies. References: It is suggested to unify the use of et al., according to the guidelines of the journal. Examples: Ferlay J, Ervik M, Lam F, et al. (3 authors) Bray F, Colombet M, Mery L, Piñeros M, et al, (4 authors)

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Julio Cesar Teixeira
Institution and Country: University of Campinas
Brazil

1. Keywords: I missed the following words: cervical cancer; precursor lesions/CIN; human papillomavirus.
We have included human papillomavirus, cervical cancer and prevalence.

2. Abstract: Methods: the period of the study.
It have been included: 2002-2007.

3. Methods: the name of the Ethics Committee (line 28).
It does not have any specific name.

4. Methods: Statistical... Line 33: "number of women of women" Corrected.

5. Discussion: HPV genotypes and HPV vaccine protection offered: Your results point HPV-51, -53, and -59 as the more prevalent after HPV-16 and these types are not in the composition of the vaccines. I suggest discussing about this find.
We have included the following text: However, the frequency of HPV types 51, 53, 59, frequently found in our population, indicate the need to continue the cytological screening population.

Reviewer: 2
Reviewer Name: Leith León-Maldonado
Institution and Country: CONACYT-Instituto Nacional de Salud Pública. México.

Abstract:
1) Methods: it is suggested to add the period in which this study was carried out.
It have been included: 2002-2007.

2) Conclusions: Could the authors mention something very brief about genotypes?
It have been included: “being HPV 16 the most frequent genotype”

Methods:
2.1 Participants
1) It is suggested to describe the difference between the initial probabilistic sample of 2,276 and the 665 who were volunteers. For example, the differences in age distribution and gynecological characteristics.

We have included Supplementary table 1 to show this differences and we have added the following text: “Regarding the subgroup of Gran Canaria volunteers, they were younger, with a high level of education, more divorced or separated, ex-smokers and with more previous cervical pap smears compared with the general population of the island” (page 9).

2) Page 8, line 4: How many women were registered in the database of the General Population of Gran Canaria? How was the sample selected? What was the sample fraction?

Participants were randomly selected from the regional Health Administration databases, stratified and selected with a probability proportional to the different healthcare areas on both islands. Selected women were stratified into nine age groups (18-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59 and 60-65 years). The data available at the time of study design (data from 1996) from the Canary Institute of Statistics were used. Gran Canaria with a population of 713,768 had 358,039 women between 18 and 65 years old and Tenerife with a population of 665,611 had 339,219 women between 18 and 65 years old. This represents approximately 6 women out of 10,000. An estimated prevalence of HPV infection of 16% was assumed with a maximum assumable difference of 1.5% for a confidence interval of 95%.

3) Page 8, line 21: How many volunteers were included? What were their characteristics? What was the procedure for recruitment volunteers? It may be convenient to discuss if the volunteers were similar to the random sample?

- We have included the following text: “a group of 934 women from Gran Canaria asked to participate in the study (volunteers) of which 665 finally attended the arranged appointment.

- There was no active recruitment. The population was aware of the study and voluntarily made contact with the study's administrative office in Gran Canaria.

- We have included Supplementary table 1 to show this differences and we have added the following text: “Regarding the subgroup of Gran Canaria volunteers, they were younger, with a high level of education, more divorced or separated, ex-smokers and with more previous cervical pap smears compared with the general population of the island” (page 9).

4) Recruitment: It may be good to explain what happened if the women did not attend the scheduled visit, what actions were taken? What was the recruitment rate?

- We have included the following text: “For each age group, four reserve groups were obtained to supply the absences or refusals to participate”. Women who did not attend the visit were called by phone to schedule another visit. If the women finally refused to participate, another woman from the consecutive group was invited by letter.

- A total of 24,345 postal letters were sent, 15,577 in Gran Canaria and 8,768 in Tenerife, of which 23.3% of the women agreed to participate and were scheduled with an appointment, in addition to 934 women from the group of volunteers from Gran Canaria they asked to participate in the study.

5) There is quality control of the registers of the regional Health Administration database? e.g. duplicate registers.

Standardized quality control methods are implemented to ensure the quality data, such as the use of standardized data collection forms, adequate training of staff, creation of a database, or depuration of data collection.

6) Page 8, line 26-28: It is suggested to mention the date and number of approbation as well as the name of the hospital.

We have added de following text: “This study was favorably evaluated by the Ethics and Clinical Trial Committee of our hospital Complejo Hospitalario Universitario Insular Materno Infantil”.

2.3. Procedures

1) Page 8, line 44-46 What was the sample collection procedure? What kind of brush and which vial was used to preserve the sample?

We have added de following text: “For cytological collection, the wooden Ayre spatula and endocervical brush (cytobrush) were used, stained with the Papanicolau technique and the cytological diagnosis was made by a single pathologist according to the criteria of the Bethesda system. For the molecular study, a sterile cotton-tipped polystyrene swab without culture medium (Deltalab®, Spain) was used. The obtained cell pellet was subjected to enzymatic digestion with stirring for 2 hours at 55°C with proteinase K following the inactivation of the process with incubation for 10 minutes at 90°C and subsequent centrifugation, obtaining DNA from the sample supernatant.”

Results:

Study population

1) Table 1. It would be interesting to see the data of Tenerife and Canaria separately, in order to show the characteristics of each population.

We have included Supplementary table 1 to show this differences.

2) The first graph of the supplementary tables may better explain the genotyping results, I would suggest to changing this for the Table 2.

We think both (figure 1 and table 1) are complementary.

Prevalence of cervical HPV infection

1) The discussion of quality control of samples should be addressed in the methods section.

The following text appears in Procedures section: “Samples that were negative for both HPV DNA and β-globin were excluded from the final analysis” (page 7).

2) Page 13, line 30-32 it is mentioned “Although volunteers from Gran Canaria showed higher prevalence of any-type HPV infection…” Can the authors present the prevalence’s data?

We have included Supplementary table 2 to show this differences.

Discussion:

Prevalence of cervical HPV infection

1) Page 19, lines 34-39: Is there any reference for this fact?

We have included reference number 16.

2) Page 20, lines 1-19: Which could be the explanation for differences in HR-HPV prevalence’s among European countries?

The different screening policies, the different types of HPV tests used and the different sexual behavior of the population could explain the differences between different European countries.

Is the prevalence of HR-HPV in Spain similar than other European countries? Which?

Studies carried out on women from different European screening programs showed prevalences of HPV infection of 13.4% in the UK (Hibbitts S, et al. Br J Cancer 2006;95:226-232) and 13.7% in France (Heard I, et al. PloS ONE 2013;8: e79372).

Prevalence of cervical HPV infection per age group

1) Page 20, lines 31-33: Authors mention “This finding was also observed in previous Spanish and European studies”, it is suggested to add the reference for this fact.

We have included references numbers 9, 16 and 17.

Risk factors and cervical HPV infection

I suggest to further discuss the lack of association with the level of education, as observed in many other studies.

We have included de following test with several references: “The evidence is controversial regarding the association between HPV infection and level of education. 26, 27, 31”

References:
It is suggested to unify the use of et al., according to the guidelines of the journal.
Examples:
Ferlay J, Ervik M, Lam F, et al. (3 authors)
Bray F, Colombet M, Mery L, Piñeros M, et al, (4 authors)

Corrected.

VERSION 2 – REVIEW

REVIEWER	Leith León-Maldonado Cátedra CONACYT-Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública. Cuernavaca, Morelos, México.
REVIEW RETURNED	21-Apr-2020

GENERAL COMMENTS	Reviewer report: Manuscript ID: bmjopen-2020-037402 Title: Prevalence and genotype distribution of cervical human papillomavirus infection in the pre-vaccination era: a population-based study in the Canary Islands. Authors: M Andújar et al. Date: April 16th, 2020 Journal: BMJ Open The revised version of the manuscript improved significantly; the authors reviewed each suggestion and incorporated most of the comments satisfactory.