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The risk of developing cervical cancer in Mexican women is associated to *CYP1A1 MspI* polymorphism

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ABSTRACT

The aim of the study was to evaluate the association of two *CYP1A1* polymorphisms (*Msp1* and exon 7) with cervical cancer in Mexican women considering their smoking habit. The polymorphisms were determined in 310 individuals (155 with cervical cancer and 155 healthy controls). Women with *MspI T/C* or *C/C* showed increased risk of developing cervical cancer (3.7- and 8.3-fold increase, respectively) compared to women with *T/T* genotype. When smoking habit was considered, the risk for non-smokers with *T/C* and *C/C* genotypes was similar (5.2 and 4.1, respectively), whereas smoking women with *C/C* genotype showed a 19.4-fold increase of cervical cancer. Number of child births, number of sexual partners and marital status were strong risk factors for developing cervical cancer in women with *T/T* genotype; however, in women with *T/C* genotype, only the number of child births and sexual partners had a significant influence. These results suggest an important role of the *CYP1A1 MspI* polymorphism in the risk of developing cervical cancer.

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1. Introduction

Cervical cancer is the result of a process that starts with a human papilloma virus (HPV) infection. This virus has more than 100 types, but types 16, 18, 33 and 45 have been identified as the cause of 80% of the cases of cervical cancer diagnosed throughout the world.[1] This disease is more common in Latin American women than in Jewish or European women, and its prevalence is greatest amongst women in the lowest socio-economic level.[2,3] The major risk factors associated are multiple sexual partners, beginning sexual relations at a young age, cervical inflammatory processes[4–7] and smoking. Many of these factors are surrogate markers of HPV infection. Smoking has been studied importantly in association with the disease for the toxic, mutagenic and carcinogenic compounds found in smoke.[8,9]

The P450 cytochrome system (CYPs) is a group of enzymes that have an important role in activating or detoxifying carcinogenic elements found in tobacco and other compounds.[10,11] CYP1A1 is the main metabolising enzyme of the aromatic polycyclic hydrocarbons (PAHs), which include benzo(a)pyrene and dimethylbenzoanthracene. These compounds are carcinogenic and are found in tobacco smoke.[12] This enzyme is located in several tissues such as lungs, mammary glands and placenta, and several studies demonstrate its relationship with cancers.[12–14] It is encoded by a polymorphic gene located in chromosome 15. Two polymorphic sites located on the *CYP1A1* gene (*Msp1* and exon 7) have been associated with a genetic susceptibility to several types of cancer. The first position is located in the 3′- flanking region of the gene (*T6235C* position). The presence of *C* at this position (**m2* allele) has been associated with genetic susceptibility to lung cancer and it has also been reported that individuals with this susceptible genotype are at high risk of developing squamous cell carcinoma. [15] A previous study pointed out that the homozygous variant (**m2*) of *CYP1A1-Msp1* is associated with 3.4-fold increased risk of developing cervical intraepithelial lesions.[16] The second polymorphism, *A4889G*, located in the heme-binding region of exon 7 at codon 462, alters the protein structure by replacing an isoleucine for a valine (**Val* allele)[17] and may render the carriers more susceptible to lung cancer, cigarette-induced severe coronary atherosclerosis and diabetes.[18,19] The aim of the present study was to evaluate the association of the *CYP1A1* polymorphisms with the risk of developing cervical cancer in Mexican women. We also evaluated the potential effect of the smoking habit on this association.

2. Materials and methods

2.1. Studied population

Between January 2002 and January 2003, patients with the diagnosis of cervical cancer who attended the Oncology Hospital of the Instituto Mexicano del Seguro Social (IMSS), the Instituto Nacional de Cancerología and the Hospital General de México were included in the study. Only Mexico City residents were selected. All cases presented invasive cancer and positive HPV-16 infection. The cases were evaluated by two pathologists and only the confirmed ones were included. Women without intraepithelial cervical dysplasia or cancer were selected as controls from first stage medical units. They had class I or II Papanicolau study negative to high-risk HPV infection and had a normal colposcopy.

The whole population was Mexican Mestizo with a history of three previous generations being born in Mexico.

The present study was approved by the Bioethics and Research Committee and all study subjects signed an informed consent letter.

2.2. HPV sampling procedure

A sample was taken from endocervix using a cytobrush (Digene Cervical Sampler™, Digene Corporation, Gaithersburg MD, USA). Specimens for HPV DNA testing were stored at −4 °C and were sent to the Molecular Biology Laboratory for masked high-risk HPV test using Hybrid capture II method with specific primers for HPV16 at the L1 region (nt 6028–6179).

Specimens were denatured, and liberated single-stranded DNA was hybridised in a solution with a bionucleic acid (RNA) probe mix consisting of HPV type 16. Each mixture reaction containing any RNA/DNA hybrid forms was transferred to a capture tube coated with antibodies against RNA/DNA hybrids. Unreacted material was removed by washing and a dioxetane-based chemiluminescent substrate, which binds to alkaline phosphatase, was added. Light produced by the ensuing reaction was measured by a luminometer (DML 2000™, Digene Corp.). Light measurements were expressed as relative lights units (RLU). Solutions of HPV16 at 10 pg/ml served as positive controls. All RLU measurements of specimens were divided by the RLU of appropriate positive controls to a yield ratio. A specimen ratio of $\geqslant 1.0$ was regarded as positive for HPV DNA, while a ratio <1.0 was regarded as negative. Because the amount of light produced by the HC capture assay is proportional to the amount of target DNA in each specimen, results are quantitative: the higher the ratio, the greater the amount of target HPV DNA in the specimen.

2.3. CYP1A1 polymorphism detection

Genomic DNA from whole blood containing EDTA was extracted by standard techniques (Kit Genomic DNA Isolation BD tact™).

2.3.1. Exon 7 polymorphism detection

Exon 7 genotype was determined by allele-specific polymerase-chain-reaction (PCR) as described by Hayasi et al.[19] Each DNA sample was amplified in two separate reactions using one of the 5′ primers: even 5′-GAAGTGTATCGGTGAGACCA-3′ or 5′-GAAGTGTATCGGTGAGACCG-3′. All reactions included the 3′ primer 5′-GTAGACAGAGTCTAGGCCTCA-3′. The samples were amplified in a Perkin–Elmer thermocycler model 9700 (Foster City, CA, USA) with an initial denaturalisation temperature of 95 °C followed by 30 cycles of 95 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min, with a final extension temperature of 72 °C for 5 min. Products were analysed by electrophoresis of a 1.5% agarose gel and visualised by ethidium bromide stain on a UV transilluminator.

2.3.2. *MspI polymorphism detection*

Previously reported primers P1: 5′-CAGTGAAGAGGTGTAGCCGCT-3′ (forward primer) and P2: 5′-TAGGAGTCTTGTCTGATGCCT-3′ (reverse primer) were used to amplify the polymorphic site.[18] Amplification conditions were denaturalisation at 95 °C for 1 min, followed by 30 cycles of 95 °C for 30 s, 57 °C for 1 min and 72 °C for 1 min, and a final extension of 72 °C for 5 min. Products were digested using MspI restriction enzyme at 37 °C for 3 h and separated by electrophoresis in an 1.5% agarose gel stained with ethidium bromide and visualised on an UV-transilluminator.

2.4. *Statistical analysis*

Statistical analysis was carried out with Stata 8.0 for windows software. In the exploratory analysis, numerical data showed a different distribution from normal standard (Gaussian distribution) (Test of normality Shapiro Wilk's $p > 0.05$), nominal and categorical variables were tabulated in order to estimate their proportions and to identify suitability of each category. Smoking habit, condom use, occupation and marital status, each one has two categories, yes or no for the first two; occupation was tabulated as employed and house keeping, and the last one as married (married or living together) or single (divorced, widowed or separated). Socio-economical level index was a three category variable (poor, fair and good) and was constructed from six socio-economical variables.[20] Each genotype of exon 7 as well as *Msp1* also had three categories (*A/A*, *A/G*, *G/G* and *C/C*, *C/T*, *T/T*, respectively). The number of sexual partners was categorised as one, two and three or more partners. Comparison of numerical variables such as age, age at first intercourse, and number of child births between cases and controls were done with Mann Whitney *U* test, data are presented as median and percentile 25 and 75. Categorical variables were analysed with Chi square or Fisher tests as required and presented as absolute frequencies and proportions. Statistical significance was set at an alpha level $\leqslant$ 0.05. Logistic regression analysis was used in a bi-variable way to estimate the risk of developing cervical cancer in each genotype category between cases and controls, and smokers and non-smokers. Multiple logistic models were constructed in order to identify the variables which better explained the risk of developing cervical cancer between cases and controls and within each genotype group. Models were constructed including one variable at a time in order to identify confounding bias, changes in estimated ORs were less than 10%, smoking habit was added to the models and Likelihood-ratio test after estimation was done, no significant changes were identified in two of the three models. When a principal effect model was reached, the effect modification was also tested. Interaction terms were constructed: one with the age at first intercourse and the number of sexual partners, and another with smoking habit and genotype, both were included in this model. No significant ORs (p value $\leqslant$ 0.20) were identified and therefore these terms were not included in the final models. Hosmer–Lemeshow Goodness of fit test was performed for each multiple logistic model.

3. Results

A total of 340 women with and without cervical cancer were included in the present study. Thirty of these women were excluded (9.2%) because of a shortage of biological sample for the determination of the *CYP1A1* polymorphisms (27 with cancer and 3 without cancer). Population for data analysis was conformed by 155 women with cervical cancer of recent diagnosis and 155 women without this cancer.

The results identify differences between women with and without cervical cancer in relation to various risk factors. Cases were older, had their first intercourse while they were young and had more child births than controls (Table 1).

Marital status, occupation, number of sexual partners and smoking habit had different distributions among studied groups. While most controls (72%) were married, cases were divided into two groups with 50% of the women in each one (married and single) ($p < 10^{-3}$). Most women in both groups were house keepers; however, this occupation was more frequent in cases (81%) than in controls (68%) ($p = 0.006$). In relation to the number of sexual partners more than 50% of the women in the case group informed that they have had two or more sexual partners, while most controls (68%) have had one sexual partner ($p = 0.002$). Smoking habit was more frequent among cases (51%) than controls (38%) ($p = 0.015$) (Table 2).

Analysis of genotype frequency of *CYP1A1* gene polymorphisms showed that exon 7 genotype had a similar distribution between cases and controls (data not shown). However, for *MspI* polymorphism, *T/C* genotype was more frequent in cases (51%) and *T/T* in controls (63%). Adjusted ORs showed that genotype *T/C* has 3.7-fold increased risk of developing

Table 1 – Comparison of general characteristics between study groups[a]

	Cases				Controls				p
	Median	Percentile 25	Percentile 75	Range	Median	Percentile 25	Percentile 75	Range	
Age (Years)	51	43	55	27–55	45	40	55	31–55	$<10^{-3}$
Age at first intercourse (years)	17	16	21	12–32	18	17	21	12–33	0.001
Child birth (Number)	5	3	5/6	0–6	3	2	5	0–6	$<10^{-4}$

[a] *U* Mann Whitney test

Table 2 – Comparison of social and demographic characteristics between study groups

	Cases (*n* (%))	Controls (*n* (%))	*p*
Marital status			
Single	77 (50)	44 (28)	$<10^{-3}$
Married	78 (50)	111 (72)	
Socio-economic level			
Poor	31 (20)	27 (17)	NS
Fair	39 (25)	41 (27)	
Good	85 (55)	87 (56)	
Occupation			
House keeping	126 (81)	105 (68)	0.006
Employed	29 (19)	50 (32)	
Sexual partners			
One	75 (48)	106 (68)	0.002
Two	54 (35)	34 (22)	
Three or more	26 (17)	15 (10)	
Condom use			
Yes	2 (1)	8 (5)	NS
No	153 (99)	147 (95)	
Smoking			
Yes	79 (51)	59 (38)	0.015
No	76 (49)	96 (62)	

NS: not significant.

cervical cancer than T/T ($p < 10^{-3}$) and for C/C genotype, this probability is even larger (8.3 times) ($p < 10^{-3}$) (Table 3).

Table 4 shows the adjusted ORs for cases and controls considering the *MspI* polymorphism and the smoking habit. For non-smokers the risk of developing cervical cancer is very similar between the T/C and C/C genotypes (5.2 and 4.1, respectively), but greater than the T/T risk ($p < 10$-3 for T/C and $p = 0.026$ for C/C genotypes). Smokers showed a different behaviour; T/C had a 2.4-fold increased risk than T/T, however this comparison had borderline statistical significance; on the other hand, the C/C genotype had a 19.4-fold increased risk than the T/T, unfortunately the sample was very small and therefore these estimations have a limited significance.

Multiple logistic analysis identified those variables with statistical and biological influence such as child birth, marital status and occupation, and those with a biological mining like age, age at first intercourse and number of sexual partners in relation to the risk of developing cervical cancer. Smoking habit in this analysis showed limited significance and therefore was not included in the final models. Adjusted logistic models for each genotype of *MspI* polymorphisms in cases and controls with the T/T genotype showed that child birth, number of sexual partners and marital status are strong risk factors for developing cervical cancer. On the other hand, cases and controls with the T/C genotype showed a different behaviour since only two variables (child birth and number of sexual partners) had a significant influence on the risk for cervical cancer (data not shown). The sample of women with the C/C genotype was small ($n = 40$) and therefore estimations were inconclusive, confidence intervals were very wide and no significant association was found (model was not included).

4. Discussion

Genetic variations in the *CYP1A1* gene have been associated with genetic susceptibility to several types of cancers, including colorectal carcinoma, prostate cancer and breast cancer; however, few data have been reported for cervical cancer.[21–23] These associations are mainly due to the important role of the enzyme encoded by this gene in the bioactivation of procarcinogenic xenobiotic constituents of tobacco smoke. *CYP1A1* encodes aryl hydrocarbon hydrolase (AHH), an enzyme involved in the production of reactive epoxide intermediates from polycyclic aromatic hydrocarbons.[24] Several polymorphisms have been identified in *CYP1A1*, some of which lead to a more highly inducible AHH activity.[25–27] On the other hand, important differences in the allele distribution of these polymorphisms have been reported among different populations including the Mexicans.[28] Distribution

Table 3 – Msp1 genotype distribution between cases and controls and estimated risk probability

MspI genotypes	Cases (*n* (%))	Controls (*n* (%))	OR	95% CI	*p*
T/T (140)	43 (28)	97 (63)	1	–	–
T/C (130)	80 (51)	50 (32)	3.7	2.1–6.5	$<10^{-3}$
C/C (40)	32 (21)	8 (5)	8.3	3.2–21.5	$<10^{-3}$

OR: Odds ratio adjusted for age, age at first intercourse, child birth, number of sexual partners, marital status and occupation; 95% CI: 95% confidence intervals.

Table 4 – Estimated OR between case and controls with Msp1 genotype by smoking habit

MspI genotypes	Non-smokers ($n = 172$)					Smokers ($n = 138$)				
	Cases	Controls	OR	95% CI	*p*	Cases	Controls	OR	95% CI	*p*
T/T (140)	22	67	1	–	–	21	30	1	–	–
T/C (130)	42	23	5.2	2.3–11.6	$<10^{-3}$	38	27	2.4	1.0–5.7	0.056
C/C (40)	12	6	4.1	1.2–14.2	0.026	20	2	19.4	3.6–105.8	0.001

NS: not significant; OR: odds ratio adjusted for age, age at first intercourse, child birth, number of sexual partners, marital status and occupation; 95% CI: 95% confidence intervals.

of exon 7 and *Msp1* polymorphisms in Caucasian and African populations is very similar with high frequencies of *Isoleucine* at exon 7 polymorphism (94.8% in Caucasians and 97.5% in Africans) and **m1(T)* allele at *Msp1* polymorphism (90.6% in Caucasians and 76.1% in Africans). Contrary to these populations, the Mexicans together with other Latin populations showed the highest frequency of *Valine* (*G*) (exon 7 polymorphism) and **m2(C)* allele (*Msp1* polymorphism). For this reason it is mandatory to perform association studies in order to define the relationship between *CYP1A1* polymorphisms and several types of cancers in Latin populations. Therefore, we investigated the associations between two *CYP1A1* polymorphisms (*Msp1* and exon 7) and cervical cancer risk in Mexican patients. Several studies suggest that the presence of **m2* (*C*) allele increased the risk of developing cancer in some populations. Thus, the high frequency of **m2* allele in the Mexican populations could explain the high frequency of this type of cancer in Mexican and other Latin American women. The present case-control study corroborates this hypothesis because women who presented the **m2/m2* (*C/C*) genotype had an increased risk of developing cervical cancer. The association was evident in our results and suggests that the genetic polymorphism in the non-codifying region 3 of the *CYP1A1* gene, which has been called *Msp1*, can increase the risk of developing cancer although the mechanism for this relation is still unclear. According to the previous studies there is a 3.4-fold risk of presenting cervical intraepithelial lesions in women who have the *C/C* genotype for the *Msp1* polymorphism.[16] In our study, the estimated ORs showed that women with the *C/C* genotype have 8.3-fold increased risk of developing cervical cancer than women with the *T/T* genotype. There are epidemiological data that show inconsistencies in the identification of the interaction between smoking habit and the risk of developing this neoplasm, whereas there are others that have reported a positive association between the number of cigarettes smoked per day and the risk of developing a cervical intraepithelial lesion in the presence of HPV infection.[29,30]When the ORs estimation in both cases and controls with the *MspI* polymorphism was made taking into consideration the smoking habit, the smokers with the *C/C* genotype had an increased risk of developing cervical cancer (19.4-fold increased risk than women with the *T/T* genotype). Unfortunately the sample was very small, therefore this estimation is only of a limited significance. On the other hand, and considering that the controls used in the present study were HPV negative subjects with cervical cancer, we cannot avoid any confounding effect of smoking in the HPV acquisition. On the other hand, our results suggest that the prevalence of cervical cancer is greatly influenced by marital status, occupation, number of sexual partners and smoking habit. In our study, house keeping and smoking habit increased the risk of developing cancer, whereas being married was a protective factor. Finally, women with a small number of sexual partners had a smaller risk than those with a larger number of sexual partners. Child birth, number of sexual partners and marital status were strong risk factors for developing cervical cancer in women with the *TT* genotype; however, in women with the *T/C* genotype, only two variables (child birth and number of sexual partners) had a significant influence.

In summary, the present study suggests that *CYP1A1* gene has an important role in the risk of developing cervical cancer, and the risk is increased in smoking women. Studies including HPV positive control groups could help to define the true role of the smoking habit in the risk of developing cervical cancer in individuals with a specific *CYP1A1* polymorphism. Also, an additive effect between *CYP1A1* gene polymorphisms and several social and reproductive factors was observed. The results of the present work will prove valuable to the current preventive health programmes, allowing improvement and optimisation of their design to address the population with a high risk of cancer. However, in order to avoid false alarms, a call for consistency is necessary before genetic testing of smokers is recommended.

Conflict of interest statement

None of the authors had competing interests.

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