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The preliminary study of p53 codon 72 polymorphism and risk of cervical carcinoma in Gabonese women

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Abstract Cervical cancer is the leading cause of cancer-related death in Africa and the first most common cancer in Gabonese women due to infection of high-risk human papillomavirus (HPV). However, other cofactors such as genetic factors also come into play. A common polymorphism of the p53 codon 72 in exon 4 with two alleles encoding arginine or proline is known at this locus. The homozygous arginine form of this polymorphism has been associated with the development of cervical cancer as an increased genetic risk factor. However, the results are still controversial. This study aims to investigate whether the genotype distribution of p53 codon 72 may be a risk factor for cervical cancer among Gabonese women. Samples from 102 Gabonese women, 31 diagnosed with cervical cancer and 71 healthy controls, were used. HPV detection was done by nested PCR with MY09/11 and GP5+/6+ primers followed by sequencing for HPV genotyping. p53 codon 72 polymorphism determination was performed by allele-specific PCR assay. Viral DNA was detected in 87.1 % of cases and in 54.93 % of control. HPV 16 was the most predominant in cancer and controls cases. The distribution of Arg/Arg, Arg/Pro and Pro/Pro genotypes was 35.5, 51.6 and 12.9 % in the cervical cancer group and 22.5, 62 and 15.5 % in the control group. No significant association was found between polymorphism of p53 itself as well as in combination with HPV16/18 infection and risk of development of cervical cancer among Gabonese women. Thus, the polymorphism of p53 codon 72 in exon 4 does not seem to play a role in the development of cervical cancer among Gabonese women.

Keywords p53 codon 72 · Polymorphism · Cervical cancer · Human papillomavirus · Gabonese women

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide and the first type of cancer among Gabonese women. Infection with high-risk oncogenic human papillomavirus (HPV) is closely linked to the development of cervical cancer. Indeed, HPV DNA was found in more than 90 % of cervical cancer cases [1]. Among the high-risk HPV, genotype 16 and 18 are most frequently detected in cervical cancer worldwide [2].

The oncogenic potential of high-risk HPV is due to the activity of two major oncoprotein E6 and E7 [3]. The E7 oncoprotein can bind and inactivate the retinoblastoma cellular tumor suppressor protein pRb. On the other hand, E6 oncoprotein binds to the p53 cellular tumor suppressor protein and induces its degradation through the ubiquitin pathway [4].

HPV is the first sexually transmitted infection but only a small fraction of women infected develop cervical cancer.

Thus, infection with HPV is not sufficient for development of cervical cancer. It is admitted that other factors also come into play. Candidate factors include smoking, diet, hormonal exposure, immunologic status, sexual behavior and genetic attributes [5, 6]. Among the genetic factors, the effect of p53 polymorphism in cervical cancer has been studied extensively [5].

In fact, a common polymorphism at codon 72 in exon 4 of the p53 gene is known, encoding either arginine (CGC) or proline (CCC). Preliminary study about this polymorphism reported that individuals with homozygous Arg were seven times more susceptible to E6 degradation than those with the proline form [7]. Thus, several studies have been conducted in different countries to confirm or refute this hypothesis, but the results are still controversial.

In the present study, we assess the p53 codon 72 polymorphism among Gabonese women with cervical cancer compared to a health control population and examine the association with the cervical cancer carcinogenesis.

Materials and Methods

Samples collection

The study included 102 samples collected from the "Département d'Anatomie et de Cytologie Pathologiques de l'Université des Sciences de la Santé" and from the "Institut de Cancérologie d'Agondjé" in Libreville.

Thirty-one paraffin-embedded tissues of women diagnosed with cervical cancer served as test group and 71 Pap smears from healthy women diagnosed with normal cytology as control group. The selection criteria for the control group included no individual history of cervical cancer. At the moment of recruitment, oral consent was given by all women. Ethical approval was obtained from the "Ministère de la Santé de Libreville" in the N° 00287/MS/SG.

DNA extraction and quantification

Paraffin-embedded formaldehyde-fixed tissues were deparaffinated with heat as described by Steinau et al. [8]. Then, DNA from all specimens was collected after lysis with a digestion buffer (Tris–HCl 0.5 M pH 8.0, EDTA 0.1 M, NaCl 2.5 M and SDS 5 %) containing proteinase K (10 mg/ml). DNA isolation was performed with phenol/chloroform methods and precipitated with absolute ethanol. Then, DNA was resuspended in 20 µL of ultrapure water and stored at −20 °C until use.

DNA quantification was performed through Nanodrop 8000 spectrophotometer (Thermo Scientific, Wilmington, USA).

HPV detection and Genotyping

To check the quality of DNA and the absence of inhibitors for the further PCR, β-globin amplification with the PC04/GH20 primers was done. Then, for all amplifiable samples, the presence of HPV was carry out by nested PCR with the couples MY09/11 and GP5+/6+ primers that amplified, respectively, a 450 and 150 bp in a highly conserved region of L1 HPV gene. Both PCRs were performed in a total volume of 25 µl of the reaction mixture containing 10 mM of dNTP, 2.5 mM of Mgcl2, 0.2 U of GoTaq® DNA Polymerase (Promega, Madinson, USA), 1X colorless GoTaq® Flexi Buffer and 10 µM of MY09/11 primer or 20 pmol of GP5+/6+ primers. PCR was performed as follow: 95 °C for 10 min, and 40 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, and final extension of 7 min at 72 °C. For GP + PCR, 2 µL of the MY PCR products was use as template. The amplification conditions for the GP PCR were the same as the MY PCR, only the hybridization temperature was 48 °C. For every reaction, ultrapure water was used as a negative control and Siha cell lines was used as positive control. The amplification reactions were performed in a Perkin Elmer 2400 GeneAmpR® PCR thermal Cycler (Scientific Support, Inc, Hayward, CA).

The amplified fragments were detected by electrophoresis on a 2 % agarose gel and revealed by UV transilluminators gel Doc (Life Science, Cambridge, UK) coupled to software UPV-Doc-It-LS Version 7.1 RC 3.54 after an Ethidium bromide staining (Bioline, UK).

For HPV genotyping, PCR products from all HPV positive samples were purified by using the ExoSaP-IT clean up system (USB, USA) and the sequencing reaction was performed using GP6 + primer [9] as the sequencing primer with the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI 3130 XL DNA analyzer (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol.

The BLAST server (http://www.ncbi.nlm.nih.gov/blast/) was used to match all sequences available in GenBank database. At least 90 % identities matching between the query and subject sequences were required for genotyping.

p53 polymorphism study

p53 Arg72Pro polymorphism was determined by PCR with allele-specific primers that selectively detect either the Arg or Pro p53 allele. The primer pair used to detect the Pro sequence was: p53 Pro1: 5′GCCAGAGGCTGCTCCCCC3′; p53 Pro2:5′CGTGCAAGTCACCAGACTT3′ (177 bp) and the primer pair used to detect the Arg sequence was:

p53a1:5′TCCCCCTTGCCGTCCCAA3′, p53a2:5′CTGGTG CAGGGGCCACGC3′ (141 bp).

Briefly, PCR amplification was done in a volume of 25 µl using 1 unit of Taq DNA polymerase (Promega), 1.5 mM of MgCl2, 10 mM of DNTP, 1X PCR buffer and 20 pmol of each primer (p53Pro primer or p53Arg primer). DNA was amplified in separate room.

Reactions were cycled as follows: 94 °C for 10 min; then 40 cycles of 30 s at 95 °C, 50 s at 59 °C (p53Pro and p53Arg), 30 s at 72 °C and finally, 1 cycle of 72 °C for 7 min for chain elongation. The amplified fragments were detected by electrophoresis on a 2 % agarose gel and revealed by UV transilluminators gel Doc (Life Science, Cambridge, UK) coupled to software UPV-Doc-It-LS Version 7.1 RC 3.54 after an Ethidium bromide staining (Bioline, UK).

Statistical analysis

Hardy–Weinberg equilibrium in the studied groups was examined by χ^2 test. Association between p53 exon 4 codon 72 polymorphism and cervical cancer was expressed by odds ratio (OR) and 95 % confidence interval (95 % CI). All analyses were done using Epi info6 software (www.epivf.fr); $P < 0.05$ was considered statistically significant.

Results

DNA from 31 patients and 71 healthy women was included in this study. The mean age was 43.46 years for the control group and 44.32 years for the case group. Histologically, all cancer cases were squamous cell carcinomas.

Overall, detection of HPV DNA by nested PCR showed the viral signature in 87.10 % of cases (27/31) and in 54.93 % of control (39/71). The HPV genotype 16 was found in 66.67 % of cancer samples followed by genotype 33 and 18 respectively in 22.22 % and 7.41 % of cancer cases. In control group, oncogenic HPVs were found in 43.59 % of samples and non-oncogenic HPV in 56.41 % of samples. Distribution of HPV genotype in case and control group is presented in Table 1.

Figure 1 shows the PCR products obtained after amplification with p53 Arg- or p53 Pro-specific primers.

The genotype distributions of p53 codon 72 in cases versus controls (Table 2) were: 11/31 (35.5 %) versus 16/71 (22.5 %) for Arg/Arg, 16/31 (51.6 %) versus 44/71(62 %) for Arg/Pro and 4/31 (12.9 %) versus 11/71 (15.5 %) for Pro/Pro. The frequencies of alleles were: 0.61 for Arg and 0.39 for Pro in cases, 0.54 for Arg and 0.46 for Pro in controls. All groups were in Hardy–Weinberg equilibrium ($P > 0.05$).

Table 1 Distribution of HPV genotypes in case and control groups

	Cases		Control	
	n	%	*n*	%
HPV−	4	13.90	32	55.03
HPV+	27	87.10	39	54.93
Oncogenic HPVs				
HPV16	18	66.67	11	28.21
HPV18	2	7.41	2	5.13
HPV33	6	22.22	3	7.69
HPV31	1	3.70	1	2.56
Non-oncogenic HPVs (6, 81)	0	0.00	22	56.41

There was no statistically significant association in p53 genotype distribution between the patient and the healthy women. The distribution of p53 genotypes was not statistically significant when cases and control groups were restricted to HPV 16/18 positive status (Table 2).

Discussion

HPV infection is one of the most common sexually transmitted infections worldwide. Approximately 10 % of women worldwide with normal cytology are carriers of HPV infection [10]. However, in Africa, the prevalence of HPV is higher in women with a normal cervical cytology than in women in other regions of the world [11]. In the same way, in this study conducted in Gabon, a central Africa country, the prevalence of HPV infection in women with normal cytology results was also high, representing 54.93 % (39/71) of the studied population.

In cervical cancer cases, it is admitted that HPV is a necessary cause [12]. The worldwide HPV prevalence in cervical carcinomas is 99.7 %, and the case of cervical cancer without HPV is extremely rare [12, 13]. In this study, HPV DNA was detected in 87.10 % (27/31) of samples. HPV 16 and HPV 33 were the more frequent genotypes found in this study, respectively, with 66.67 % (18/27) and 22.22 % (6/27).

Since 15 years, an effect of the p53 codon 72 polymorphism on the risk of cervical cancer was reported [7]. Indeed, Storey and collaborators reported that the p53 homozygous arginine was more susceptible to HPV degradation than the p53 homozygous proline. Thus, many studies had tried to repeat this experience in order to confirm this hypothesis. Many of them have failed to confirm this finding [14–19], and only a few studies has reported the same observation [5, 20–22]. In the same way, we also tried to examine the association between the p53 codon 72 polymorphism and the risk of cervical cancer

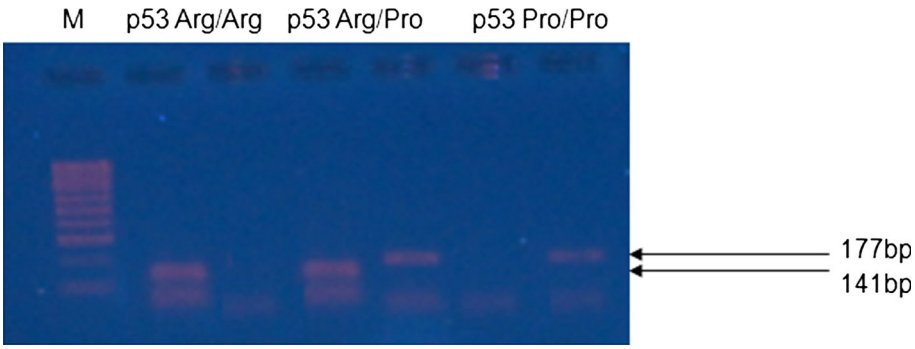

Fig. 1 Analysis of p53 codon 72 exon 4 polymorphism by PCR using allele-specific primers. M: 100-bp ladder. Arg allele-specific primer: 141 bp and Pro allele-specific primer: 177 bp

Table 2 Distribution of p53 codon 72 genotypes and its association with cervical cancer

Parameters	Total	Genotypes			Alleles	
		Arg/Arg *n* (%)	Arg/Pro *n* (%)	Pro/Pro *n* (%)	Arg *n* (%)	Pro *n* (%)
Cases	31	11 (35.5)	16 (51.6)	4 (12.9)	0.61	0.39
Controls	71	16 (22.5)	44 (62)	11 (15.5)	0.54	0.46
Cases versus controls						
OR (95 % CI)		1.89 (0.48–7.50)	1.00 (0.29–3.59)	1 (Ref)		
P value		0.37	1.00			
Cases with HPV 16/18	20	8 (40)	10 (50)	2 (10)		
Controls with HPV 16/18	13	5 (38.46)	7 (53.85)	1 (7.69)		
Cases with HPV 16/18 versus controls with HPV 16/18						
OR (95 % CI)		0.80 (0.05–11.3)	0.71 (0.05–9.50)	1 (Ref)		
P value		0.87	0.80			

among Gabonese women by analyzing p53 genotypes from 31 cervical carcinoma samples and Pap smears from 71 healthy women. Our results did not confirm the initial findings of Storey and co-workers. In fact, polymorphism in p53 codon 72 was not associated with an increased risk for development of cervical cancer in Gabonese women. There was also no statistically significant difference in the distribution of p53 codon 72 genotypes between women with HPV16/18 infections and healthy women. Our findings confirm the studies that refute the association between the p53 codon 72 exon 4 polymorphism and the risk of development of cervical cancer. There were also similarities to those conducted in Italy, Japan and in a Korean cohort [23–25].

In the same way, El Khair et al. [26] who studied Moroccan women with cervical cancer and healthy cytological status did not find an association between Arg/Arg genotype and increased risk of cervical cancer. Another Africa study conducted in Black women of South Africa had the same results [27]. However, some studies enriched the findings of the preliminary study of Storey and Colleagues. Thus, Dokianakis et al. [28]. have found an association between the homozygous genotype arginine and the development of cancer. Saranath et al. in India [29] and Eltahir et al. in Sudan [30, 31] also reported the same results.

There is a clear discrepancy between the results found all over the world in the study of the association of p53 codon 72 exon 4 polymorphism and the risk of development of cervical cancer. Difference may be due to variation of laboratory performance, sample size, DNA quality and source, variation in ethnic background, and methodological errors [32, 33]. All these characteristics may explain the variability reported even in same population. Those explications reflect the two different results found in two studies conducted in the same population in South Africa [27, 34].

In conclusion, this is the first study in Gabonese population to estimate the risk factor for the polymorphism of p53 codon 72 in the development of cervical cancer in women. Our study showed no association between p53 codon 72 polymorphism itself or combined with HPV 16/18 infection and risk of development of cervical cancer among Gabonese female. This suggests that the distribution of p53 codon 72 genotypes may not play a role in the development of cervical cancer among Gabonese women. The small size of our sample may explain this result, suggesting the need to undergo a study with a more large size samples. However, attention should be also focusing to other risk factors such

sexual behavior, smoking, diet, hormonal exposure, immunologic status and other genetic attributes.

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Conflict of interest None.

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