British Microbiology Research Journal
12(1): 1-5, 2016, Article no.BMRJ.22295
ISSN: 2231-0886, NLM ID: 101608140

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The p53 Codon 72 Polymorphism in Moroccan Women and the Risk of Ovarian Cancer

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Authors' contributions

This work was carried out in collaboration between all authors. Author MB designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Author SZA managed the analyses of the study and literature searches. Authors LMAB, SB, MNB and MME critically revised the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/22295
Editor(s):
(1) Alok K. Upadhyay, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.
Reviewers:
(1) Anthony Cemaluk C. Egbuonu, Michael Okpara University of Agriculture Umudike, Nigeria.
(2) V. Harshini, ESI Post Graduate Institute of Medical Science and Research, India.
Complete Peer review History: http://sciencedomain.org/review-history/12525

Original Research Article

Received 27th September 2015
Accepted 24th October 2015
Published 2nd December 2015

ABSTRACT

The aim of this study was to examine the association of the p53 codon 72 polymorphism with ovarian carcinoma development in Moroccan women.
Samples from 44 women with ovarian cancer and 80 healthy controls, were used. p53 codon 72 polymorphism determination was performed by allele-specific PCR assay. The distribution of

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Arg/Arg, Arg/Pro and Pro/Pro genotypes was 33/44 (75%), 10/44 (22.7%) and 1/44 (2.3%) in the cervical cancer group and 43/80 (53.8%), 27/80 (33.7%) and 10/80 (12.5%) in the control group. No significant association was found between polymorphism of p53 and risk of development of ovarian cancer among Moroccan women. Thus, the polymorphism of p53 codon 72 in exon 4 does not seem to play a role in the development of ovarian cancer among Moroccan women.

Keywords: Ovarian cancer; p53 codon 72; polymorphism; Moroccan women.

1. INTRODUCTION

Worldwide, ovarian cancer is the most lethal among female genital tract disease [1]. In Morocco, ovarian cancer is the third female cancer (represent 5% of gynecological cancer) with more than 70% of cases discovered in advanced stages of the disease [2]

The factors contributing to the onset of ovarian carcinomas are well known. As with most cancers, age is one of the most important risk factors of ovarian cancer. In addition there are also two other major types of risk factors for this cancer: hormonal and genetic risks [3]. The genetic factors include alterations associated with a family history of ovarian cancer as well as mutations and modifications at the level of tumor suppressor genes such as p53.

Since the last decade, a single nucleotide polymorphism at codon 72 of the p53 gene encoding either an arginine (Arg) or a proline residue (Pro) has been a suitable candidate in the risk of developing various carcinomas such as cervical [4] and lung [5-6] cancers and many studies tried to confirm this hypothesis in different cancer [7-9]. Moreover, a correlation between this polymorphism and ovarian cancer has been also suggested [10-11] However, results are controversial. In the present study, we investigated a possible role of the codon 72 p*53* gene polymorphism for ovarian carcinoma risk in Moroccan women.

2. MATERIALS AND METHODS

2.1 Samples Collection

The study included 44 fresh biopsies of women with ovarian cancer obtained after surgical intervention in the department of gynecology and obstetrics "A", Ibn Rochd University Hospital, Casablanca, Morocco as test group and 80 Pap smears from healthy women diagnosed with normal cytology as control group. At the moment of recruitment, informed consent was obtained from all participants and the study protocol was approved by the local ethics committee of the University Hospital Ibn Rochd.

2.2 DNA Extraction and Quantification

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 lL of ultrapure water and stored at -20℃ until use.

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

2.3 p53 codon 72 Polymorphism Detection

Before the detection of the Arg and Pro alleles, the quality of DNA and the absence of inhibitors for the further PCR was check by b-globin amplification with the PC04/ GH20 primers. Then, for all amplifiable samples, 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 ll 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℃ for 10 min; then 40 cycles of 30 s at 95℃, 50 s at 59℃ (p53Pro and p53Arg), 30 s at 72℃ and

finally, 1 cycle of 72℃ for 7 min for chain elongation. The amplifi- cation 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).

2.4 Statistical Analysis

Hardy–Weinberg equilibrium in the studied groups was examined by 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.

3. RESULTS

The distribution of the homozygous (Arg/Arg or Pro/Pro) and heterozygous (Arg/Pro) genotypes of codon 72 in exon 4 of p53 in cases and controls were: 33/44 (75%) versus 43/80 (53.8%) for Arg/Arg; 1/44 (2.3%) versus 10/80 (12.5%) for Pro/Pro and 10/44 (22.7%) versus 27/80 (33.7%) for Arg/Pro.

The frequencies of alleles were: 0.86 for Arg and 0.14 for Pro in cases, 0.71 for Arg and 0.29 for Pro in controls. All groups were in Hardy–Weinberg equilibrium.

There was no statistically significant association in p53 genotype distribution between the ovarian cancer patients and the healthy women (Table 1).

4. DISCUSSION

In the basis of several previous studies, the current consensus is that the arginine form of the p53 gene codon 72 is more efficient in inducing apoptosis than the proline form [12]. Thus, many studies had tried to confirm this hypothesis in different cancer sites including ovarian cancer. Some previous studies have reported no correlation between the polymorphism of p53 gene in codon 72 and increased risk of ovarian cancer. However, only a few studies have reported that the arginine form increased the risk of development of ovarian cancer [13-14] other associated the increase of development of ovarian cancer to the proline form in codon 72 of the p53 gene [15-16].

In our study, we tried to examine the association between p53 codon 72 exon 4 polymorphism and the risk of ovarian cancer among 44 Moroccan women with ovarian cancer and 80 healthy women. Our present results revealed that the difference in the polymorphic frequency of p53 Arg/Arg, Arg/Pro and Pro/Pro genotypes between control subjects and ovarian cancer patients were statistically not significant. A study conducted by Agorastos et al. [9] among Greek women with ovarian cancer and healthy status did not find an association between Arg/Arg or Pro/Pro genotype and increased risk of cervical cancer. Another study conducted among Japonese women had similar results [17].

However, some studies have found an association between the homozygous genotype proline and the development of ovarian cancer. Dholariya et al. in India [18] and Ktivokuca et al. in Serbia [19] 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 ovarian cancer. Difference may be due to variation of laboratory performance, sample size, DNA quality and source, variation in ethnic background, and methodological errors [3,20].

Table 1. Genotypes frequencies in 44 patients with ovarian cancer and 80 healthy women in Morocco

Parameters	Total	Genotypes		
		Arg/Arg	Arg/Pro	Pro/Pro
Cases	44	33 (75)	10 (22.7)	1 (2.3)
Controls	80	43 (53.8)	27 (33.7)	10 (12.5)
Cases vs Controls				
OR (95% CI)		7.7 (0.9-63)	3.7 (0.4-32.8)	1 (ref)
p value		0.06	0.2	

5. CONCLUSION

In conclusion, this is the first study in Moroccan population to estimate the risk factor for the polymorphism of p53 codon 72 in the development of ovarian cancer in women. Our study showed no association between p53 codon 72 polymorphism and risk of development of ovarian cancer among Moroccan female. This suggests that the distribution of p53 codon 72 genotypes may not play a role in the development of ovarian cancer among Moroccan women.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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DOI:10.1590/S1415-47572004000400005

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12525