International Journal of TROPICAL DISEASE & Health
15(3): 1-6, 2016, Article no.IJTDH.24676
ISSN: 2278–1005, NLM ID: 101632866

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P53 Codon 72 Polymorphism and the Risk of Cervical Cancer in Zimbabwean Women

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Authors' contributions

This study was carried out in collaboration between all the authors. Authors VK, NC, AM and CM designed the study. Author WK collected samples from patients. Authors VK and NC carried out laboratory experiments. Authors VK and GN performed the statistical analysis. All authors wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2016/24676
Editor(s):
(1) Thomas Britt, Chair, Department of Health Studies, College of Health Sciences, Chicago State University, USA.
Reviewers:
(1) Diana C. Tapia-Pancardo, National Autonomous University of Mexico, Mexico.
(2) Maria Cristina de Melo Pessanha Carvalho, University Federal do Rio de Janeiro–UFRJ, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/13931

Short Research Article

Received 29th January 2016
Accepted 17th March 2016
Published 30th March 2016

ABSTRACT

Aims: To investigate the prevalence of p53 codon 72 polymorphism in Zimbabwean women and risk of cervical cancer
Study Design: Case-control study
Place and Duration of Study: Parirenyatwa Referral Hospital in Harare in between January and December 2014.
Methodology: Genomic DNA from cervical cancer patients and women without cancer was extracted from whole blood and genotyping of the p53 codon 72 polymorphism was performed using the polymerase chain reaction followed by restriction fragment length polymorphism method.
Results: The frequencies of the Arg/Arg; Arg/Pro and Pro/Pro genotypes in cervical cancer patients

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were reported as 15.07%, 43.84% and 41.10% respectively. An unconditional logistic regression model was used to calculate odds ratios (ORs) with a corresponding 95% confidence interval [95%CI] as measure of risk and association between the p53 polymorphism and the development of cervical cancer. The p53 Arg/Arg was found to be at increased risk for the development of cervical cancer (OR= 1.78). However its association with the development of cervical cancer was not statistically significant (p-value= 0.29; 95% CI, 0.54-6.12).
Conclusion: The prevalence of the Arg/Arg genotype in our study was low in women with cervical cancer. The genotype was poorly associated with the disease.

Keywords: p53; codon 72; polymorphism; cervical cancer; Zimbabwe.

1. INTRODUCTION

Cervical cancer is a very devastating disease in women, especially those in developing countries [1]. It has been estimated that more than 80% of all global deaths due to cervical cancer occur in low-and middle-income countries [2]. In Africa, cervical cancer is the most common cancer and remains a leading cause of mortality [3,4]. In Zimbabwe, cervical cancer is also the most common cancer in women and accounts for about 30% of all cancers in females [5]. Human papillomavirus (HPV) infection is now known to be associated with the cancer in Zimbabwe [6]. The development of cervical cancer due to HPV infection is now believed to be associated with host genes such as tumour suppressor gene (p53) of the host. The single nucleotide polymorphism (SNP) of the codon 72 of p53 which results in translation to either arginine or proline has been linked to the development of cervical cancer [7]. The SNP is the variation in a single nucleotide (C or G) at codon 72 (CCC or CGC) of the p53 gene that results in the translation to either proline (Pro) or arginine (Arg) amino acid residue [7]. Other studies have however failed to show any association between the polymorphism and cervical cancer [8-10]. The main aim of this study was to investigate the prevalence of genetic variants of p53 codon 72 polymorphism and risk of cervical cancer women at Parirenyatwa Hospital in Harare in Zimbabwe.

2. METHODOLOGY

2.1 Study Subjects

The study design was a case-control investigation in which cervical cancer cases and healthy controls were enrolled. Cervical cancer cases comprised consenting women participants aged between 30-84 years old with histologically-confirmed cervical cancer. The age-group 30-84 years was chosen because that is the group with the highest number of cervical cancer cases in Zimbabwe. These included the newly-diagnosed and those under treatment and monitoring at the Radiotherapy Centre (RTC) of Parirenyatwa Hospital in Harare in Zimbabwe. The controls were undiagnosed healthy group comprising women aged between 30-84 enrolled from the National Blood Transfusion Services of Zimbabwe (NBSZ). The controls were apparently healthy individuals who however had not undergone screening for cervical cancer. They had been screened for infectious diseases including HIV and viral hepatitis as per NBSZ protocols.

2.2 Collection of Blood Samples and DNA Extraction

Five millilitres (5 mls) of blood from each participant were collected by venepuncture and dispensed into a tube with ethylene diamine tetra acetate (EDTA) anticoagulant. The blood specimen was then stored at -20°C pending genomic DNA extraction. Genomic DNA was extracted from whole blood using Gene JET Whole Blood Genomic DNA purification mini Kit (Thermo Scientific) according to manufacturer's recommendations. Initial volume of 200 µl of whole blood was used for DNA extraction. The DNA was eluted in 100 µl of elution buffer from the kit and stored at -20°C until genotyping.

2.3 Genotyping p53 codon 72

The genotyping of the p53 codon 72 polymorphisms was done using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods as previously described [11]. A PCR amplicon of the gene was amplified using primers, 5′-TTG CCG TCC CAA GCA ATG GAT GA-3′ (forward) and 5′- TCT GGG AAG GGA CAG AAG ATG AC-3′ (reverse). The amplicon was then digested using restriction enzyme BstUI and 2% gel electrophoresis was used for analysis of the different gene variants.

2.4 Statistical Analysis of Data

Genotype and allele frequencies of the p53 codon 72 polymorphism in cervical cancer cases and controls were calculated using an online statistical tool GENEPOP (www.genepop.com) / Stata 11.2 (StataCorp LP, Texas USA). Genotype frequencies were calculated by counting the number of individuals carrying a particular genotype in a group and dividing by the total number of individuals in the group. To determine the minor allele frequencies, the number of homozygotes for the allele was multiplied by two, added to the number of heterozygotes and divided by twice the number of individuals in the group. The Hardy Weinberg Equilibrium (HWE) or exact probability test (p-value) was tested using the Markov chain method. Chi-squared (X2) and Fisher's exact tests with one degree of freedom were used to determine particular genotype associations. Patients' demographic data was presented as mean for normally distributed continuous variables and as median for non-normally distributed continuous variables. Odd ratios with respective 95% confidence interval (95% CI) were calculated to estimate the association between the p53 polymorphism and risk of developing cervical cancer.

2.5 Ethical approval and Considerations

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Ethical approval for the study was sought and granted by the Joint Research Ethics committee (JREC) of Parirenyatwa hospital and the University of Zimbabwe College of Health Sciences and the Medical Research Council of Zimbabwe (MRCZ). All participants were informed about the method, read and signed a written consent for their participation in accordance with the recommendations of the ethical review committee. The participant's confidentiality was maintained throughout the research process.

3. RESULTS

3.1 Demographic Characteristics of the Study Participants

The mean age in years for the 73 cervical cancer cases was 57.1 (SD; 13.3) whereas the mean age in years for the 62 controls was 41.9(SD; 9.06). The majority of the cervical cancer cases were in stage 2b with 45.21% (n=33) whilst 34.24% (n=25) had stage 3b. The most observed type of cervical cancer was squamous cell carcinoma (SCC), with 89.04% (n=65) compared to adenocarcinoma (ADC), 8.22% (n=6) and mixed cervical cancer, 1.37% (n=1). Out of the 73 cases, only 5 participants had metastasis whilst 26 were HIV positive.

3.2 Frequencies of p53 codon 72 Variants

Analysis of PCR products digested with BstUI restriction enzyme showed the presence of three genetic variants of p53 codon 72 (Fig. 1). The 199 bp PCR product remained undigested in Pro/Pro variant. The 199 bp PCR product generated 113 bp and 86 bp bands in Arg/Arg variant and the heterozygote (Pro/Arg variant) showed three bands (199 bp, 113 bp and 86 bp).

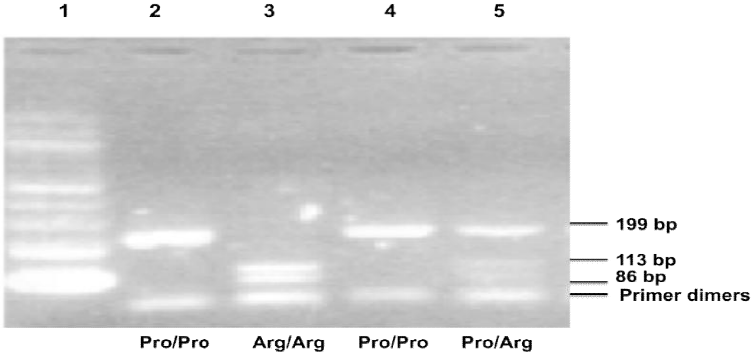

Fig. 1. Representative gel showing the p53 codon 72 variants

Lane 1 has the DNA marker. Lanes 2 and 4 show the Pro/Pro variant (199 bp band); Lane 3 shows Arg/Arg variant (113 bp and 86 bp bands) and Lane 5 shows Pro/Arg variant (199 bp, 113 bp and 86 bp bands). The lower band was for the primer dimers

The genotype frequencies of p53 polymorphism were calculated (Table 1). The frequencies of the polymorphism conformed to the Hardy Weinberg equilibrium in both groups. The frequencies of the p53 GG (15.05%) GC (43.84%) and CC (41.105%) in the cervical cancer cases were not significantly different from those in the controls reported as GG (11.29%), GC (33.87%) and CC (54.84%) respectively (Table 2). Similarly, there was no statistical difference between the frequency of p53 G allele 36.99 % compared to 28.23% in the undiagnosed group (p=0.1270). With respect to the p53 C allele, there was also no statistically significant difference (p=0.1270) in frequencies between the cervical cancer diagnosed group (63.01%) and the undiagnosed group (71.77%).

3.3 Association of p53 codon 72 Polymorphism and Risk of Cervical Cancer

An unconditional logistic regression model was used to calculate odds ratios (ORs) and 95%CI as measure of association between p53 polymorphism and cervical cancer. The association between the p53 Arg/Arg genotype (GG) and the development of cervical cancer was not statistically significant (p-value= 0.29; OR=1.78; 95% CI, 0.54-6.12). Crude and Adjusted odds ratios (OR) and their 95% confidence intervals (CI) were ascertained for the age risk factor between cervical cancer cases and undiagnosed participants control (Adjusted OR= 1.63; 95%CI: 1.32-1.99). These results showed that there was still no association between p53 Arg/Arg genotype and cervical cancer.

4. DISCUSSION

The single nucleotide polymorphism of the codon 72 of the tumour suppressor gene p53 resulting in translation to either arginine or proline has been investigated as a genetic marker for the risk of developing cervical cancer [7]. In the original study, an association between the majority allele, arginine (G) form of codon 72 of p53 and cervical cancer development was shown [7]. Since then, the association between p53 codon 72 polymorphism and cervical cancer has received considerable attention, but with inconsistent results. The main objective of this study was to establish the frequency of the single nucleotide polymorphisms (SNP) of the codon 72 (G/C) of the tumour suppressor gene p53 in patients with histologically confirmed cervical cancer and estimate the association between p53 polymorphism and risk of developing cancer of the cervix. There was no significant difference in frequencies between the Arg/Arg (GG) genotype among the cervical cancer cases 15.07% compared to 11.29% in the undiagnosed group (p=0.5197). The low prevalence of the Arg/Arg genotype compared to the Pro/Pro genotype reported in this study confirmed the results found in other Black African populations [12]. The association between the p53 Arg/Arg genotype (GG) and the development of cervical cancer was not statistically significant (OR= 1.78; 95% CI, 0.54-6.12; P-value= 0.29). These results demonstrated that women with GG genotype did not have an increased risk for developing cervical cancer. Our findings are in disagreement with the initial findings of Storey and colleagues. Our results were similar with what has been found in other studies. A study in Gabonene women showed a distribution of p53 codon 72 Arg/Arg, Arg/Pro and Pro/Pro genotypes as 35.5, 51.6 and 12.9% in the cervical cancer patients respectively, with no significant association with cervical cancer [13]. Studies of Moroccan, Gambian and Portuguese women also showed no association of the p53 polymorphism and cervical cancer development [14-16]. However, studies in Chinese women showed a strong association of the polymorphism and cervical cancer [17,18]. Differences among the findings may result from variation in allele frequency among different ethnic populations [19]. Inter-laboratory variation in the methods used to determine allele frequencies could also be a factor [20].

This study had few limitations. The HPV status of the cervical cancer cases was not established. The sample sizes of cases and controls were not

Table 1. Genotypic frequencies of the cervical cancer cases and controls

Genotype	Genotypic frequency of cases (n=73)	Genotypic frequency of controls (n=62)	p-value
GG	11 (15.07%)	7 (11.29%)	0.5197
GC	32 (43.84%)	21 (33.87%)	0.2371
CC	30 (41.10%)	34 (54.84%)	0.1111

very big. The controls were not screened for cervical cancer although they were healthy blood donors. In future studies to establish the association of cervical cancer and codon 72 polymorphism of p53, the HPV status or genotypes of subjects should be established, the sample sizes of the subjects should be big enough and controls should be checked for the absence of pre-cancer or cancer.

Table 2. Allelic frequencies of the cervical cancer cases and controls

Allele	Allelic frequency of cases (n=73)	Allelic frequency of controls (n=62)	p-value
G	54 (36.99)	35 (28.23)	0.1270
C	92 (63.01)	89 (71.77)	0.1270

5. CONCLUSION

The prevalence of the Arg/Arg genotype in our study was low in women with cervical cancer and the genotype was poorly associated with the disease. Future studies should attempt to correlate the HPV status, the genotype and the risk of developing cervical cancer.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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