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Research Article

Genetic polymorphisms of CYP3A4*1B of cervical cancer patients in Bangladeshi population, Bangladesh

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ABSTRACT

Background: Cervical cancer incidence rate in Bangladesh is 15.9 and age-standardized incidence rate is 19.2 (rates per 1,00,000 women per year). Polymorphisms of different genes have been affirmed to be associated with cervical cancer. Our prime purpose is to observe whether *CYP3A4*1B* polymorphisms are related with increasing cervical cancer risk in Bangladeshi population. The compilation of precise clinical information allows the clarity of different clinical phenotypes, which play a vital role in genetic studies of cervical cancer.
Methods: The study was a case-control study carried out between patients and volunteers matched by age, sex, height, weight and smoking status. Daly's chemical method was used to isolate genomic DNA from venous blood. The demographic variables of cases and controls were put side by side using chi-square tests and Student's t tests.
Results: Odds ratio and 95 % confidence interval were assigned to estimate the risk of cervical cancer. *CYP3A4*1B* polymorphisms and cervical cancer risk do not show any considerable relationship. The polymorphic frequencies of *CYP3A4*1B* allele (normal homozygote, heterozygote and mutant homozygote**)** in cervical cancer were 60%, 40% and 0% respectively; frequencies in control were 66.67%, 33.33% and 0% respectively ($p<0.05$). Finally, we conclude that *CYP3A4*1B* polymorphisms is not associated in susceptibility to developing cervical cancer, at least in Bangladeshi population.
Conclusions: In brief, the consequences of our study demonstrated that CYP3A4*1B polymorphism is not allied in susceptibility to develop cervical cancer, at least in Bangladeshi women.

Keywords: Genetic polymorphism, *CYP3A4*1B*, Cervical cancer, DNA, Restriction enzyme

INTRODUCTION

Cancer in uterine cervix is one of the most frequent neoplasms among women in worldwide. [1] Among female cancers, it ranks as the 4th in the World and 2nd in Bangladesh. Besides it is the 2nd most frequent female cancer having aged 15 to 44 years in the World as well as in Bangladesh. In global aspect 5,27,624 new cervical cancer cases are diagnosed in each year and 2,65,653 died (estimated for 2012).[2] In Africa, the majority of cervical cancer patients present with delayed stage disease (59.3% stage III vs. 5.2 % stage 1B). Although in first world countries like the United States, Canada, and Scandinavia a declination of cervical cancer incidence and mortality has been recognized.[3] Every year in Bangladesh about 11,956 new cervical cancer cases are examined (estimations for 2012). Worldwide, prevalence rates are higher than mortality rates having a proportion of prevalence to mortality is 100 to 50.3.[2] Epidemiological, Clinical, and molecular data have also clearly conformed

that several human papilloma viruses (HPVs) types, are responsible for cervical cancer.[4] Indeed, they have been related with more than 90% of cervical cancers.[5] HIV affected women possess 6 times more risk of cervical cancer compared to general population.[6] There is a likely of this cancer for women who use oral contraceptive for more than 5 years in comparison to never users.[7]

Parous women possess 64% chance having 7+ full term pregnancies versus women with 1 or 2. Current smokers have higher cervical cancer risk as they come in contact of HPV infection or HPV-infected cells tend to cancerous progression by smoking.[8]

Cervical cancer risk is privileged in those of vaginal, kidney, urinary tract, or skin cancers.[9,10] Carcinoma risk in squamous cell in cervix is 74-80% privileged in women with their relative like mother, sister, daughter in comparison with general women.[11] In high-income countries the incidence rates were similar as those in the developing world since 1960 and 1970s. Efficient screening programs turn down incidence and mortality rates in first world countries.[12] CYP3A4*1B gene notably increased lung cancer risk was only found for homozygous CYP3A5*1 allele but not for heterozygotes.[13]

Moreover CYP3A4*1B polymorphism might raise the risk of cancer prostate cancer among African populations.[14] This allele is not related with breast or ovarian cancer. To maintain of this negative result, in-vitro functional analysis shows that NFSE genotype is not a vital factor in the transcriptional activity of the CYP3A45-flanking region.[15] Another study recommended that CYP3A4*1B was related with markers of complex disease. These observations maintain the belief that progress of strong, conservative molecular epidemiologic case-control studies to deal with gene-gene and gene-environment interactions, will be timely.[16] No samples were positive in Bangladeshi tuberculosis patients for CYP3A4*2, CYP3A4*4, CYP3A4*5, CYP3A4*6, CYP3A4*10, and CYP3A4*18 alleles. A particular sample was establish to be heterozygous for CYP3A4*1B (1.11%).[17]

METHODS

National Institute of Cancer Research and Hospital Mohakhali, Dhaka approved this hospital-based case-control study. Thirty diagnosed, histologically established cervical cancer patients were successively recruited between August 2014 and December 2014.

A total of 30 healthy control subjects were selected matching for age, marital status, previous cancer history, former screening experience were collected for comparison. Control and patients were not relative to each other. Interviews were done by qualified nurses in the company of proficient physicians. All patients were announced about the study, and their assent to participate in this study was achieved on a predesigned feedback form.

DNA extraction and primer construction

3 ml venous blood of all patients and control were collected in sterile tubes containing EDTA-Na_2 and preserve at -80°C until DNA isolation. Daly's chemical method was used to isolate Genomic DNA.[18] 150 µl of blood mixed up with 1ml cell lysis buffer, centrifuged at 4,000 rpm for 7.5 min, and the resultant precipitate (nucleus) was mixed with 100 µl nuclear lysis buffer and 25-µl of 5 M Sodium perchlorate.

To dissolve the precipitate accurately, the mixture was incubated at 37°C for 30 min. To precipitate the protein Chloroform (125 µl) was added. After precipitation, the mixture was rotated in a rotary mixture (10 min, 30 rpm, at room temperature). Collected clear aqueous supernatant was mixed with two times amount of ethanol in order to disclose the white cotton like pellet (DNA) and collected the white cotton like pellet using disposable microbiology loop. DNA was preserved in 200 µl TE buffer and kept the tube at 65°C overnight. The total DNA solution preserved at -20°C freezer until PCR.

Polymerase chain reaction-restriction fragment length polymorphism

Briefly, 26µl PCR mixture consisted of 1µl genomic DNA samples (50–70 ng/µl), 20µl of ×10 standard Taq reaction buffer (with $MgCl_2$), 4.0 µl dNTPs (2.5 mM), 2.0µl of each primer (10 µM), 1.0 µl of Taq DNA polymerase (5.0 U/µl) (Jena Bioscience, Germany), and 180µl nuclease free water. The typical program was followed for PCR amplification as follows:

One initial denaturation step at 94°C for 1 min, accompanied by 35 denaturation cycles of 30s at 94 °C, 30s of annealing at 60°C, and 30s of extension at 72°C, accompanied by a final elongation cycle at 72°C for 1 min. By staining the PCR product with ethidium bromide, it was analyzed on a 3% agarose gel. For RFLP, the PCR products of *CYP3A4*1B* were digested with *Mbo* II (New England Bio Labs). For resolution DNA fragments were analyzed through 5% agarose gel (Figure 1).

Statistical analysis

Using chi-square tests and Student's t tests, the demographic variables of cases and controls were compared. The association between CYP3A4*1B allele and cervical cancer risks were assessed using the odds ratio (OR) and 95% CI (confidence intervals) (Table 4). Odds ratio (OR) and 95% CI (confidence intervals) was determined applying conditional logistic regression analysis with correction for age, smoking status, and sex by means of the statistical software package SPSS version 20.0. $p<0.05$ was measured statistically significant.

RESULTS

Case and control characteristics

A total of 30 cervical cancer patients and 30 controls were recruited into the study. Of the 30 cervical cancer cases, 23 (76.67%) had no family history of cancer and 07 (23.33%) possessed previous cancer history in the patients group compared to 27 (90.00%) and 03 (10.00%) with control group respectively. Only 02 (6.67%) patients were smoker and the other 28 (93.33%) were non-smoker in comparison with 01 (3.33%) smoker and 29 (96.67%) non-smoker correspondingly. Most of the patients (70.00%) were poor since their monthly incomes were less than 4500 taka (Table 2). Moreover 24 (80.00%) had no former cancer screen experience in contrast to control group 14 (46.67%). For each variable both of the groups do not show any significant result.

Polymorphism analysis

Compared with the NH (Normal Homozygote) genotype of CYP3A4*1B heterozygous (HE), mutant homozygote (MH) and combined heterozygous in addition to mutant variants (HE+MH) is not significantly associated with cervical cancer risk (OR=1.33, 95% Cl=0.47 to 3.82, p=0.5925; OR=1.11, 95% Cl=0.02 to 58.72, p=0.9596 and OR=1.33, 95% Cl=0.47 to 3.82, p=0.5925 respectively).

Heterozygote and Mutant Homozygote genotypes have increased risk of cervical cancer 1.33 and 1.11 times respectively compared with normal homozygote genotype, whereas HE+MH combined genotype has 1.33 times more risk of cervical cancer compared with NH genotype. The obtained results are not statistically significant (p>0.05).

Table 1: Demographic and selected variables.

Variables	Sample (n= 30)	Control (n= 30)	Chi-square (χ^2)	*P*
	N (%)	N (%)		
Age, y (Mean ± SD)	47.37±8.16	48.20±8.52		0.350[u]
Age at first menstruation [y (Mean ± SD)]	14.92±1.06	15.19±1.00		0.173u
Age at menopausal [y (Mean ± SD)]	40.31±2.41	40.83±2.16		0.173u
Age at first child birth [y (Mean ± SD)]	20.34±1.65	20.72±1.58		0.183u
Smoking status				
Smoker	02 (6.67)	01 (3.33)	0.351[c]	0.554[c]
Non-smoker	28 (93.33)	29 (96.67)		
Parity				
0-2	06 (20.00)	08 (26.67)	2.242[c]	0.326[c]
3	14 (46.67)	17 (56.67)		
> 3	10 (33.33)	05 (16.67)		
Family history of cancer				
No	23 (76.67)	27 (90.00)	1.92[c]	0.166[c]
Yes	07 (23.33)	03 (10.00)		
Monthly Income (Taka)				
< 4,500	21 (70.00)	15 (50.00)	2.5[c]	0.287[c]
4,500 - 6,000	06 (20.00)	10 (33.33)		
>6,000	03 (10.00)	05 (16.67)		
Education				
None	07 (23.33)	05 (16.67)	0.428[c]	1.717[c]
Primary	19 (63.33)	16 (53.33)		
Secondary	05 (16.67)	09 (30.00)		
No. of previous Screen				
None	24 (80.00)	14 (46.67)	2.5[c]	0.287[c]
03-Jan	04 (13.33)	11 (36.67)		
> 5	02 (6.67)	05 (16.67)		

[u] Unpaired t-Test, C= Chi-Square Test, SD= Standard deviation, P =Probability value.

Table 2: Primer sequence.

Allele		Primer Sequence	T_m (°C)	Size (bp)
CYP3A4*1B	**FP**	5'-GGAATGAGGACAGCCATAGAGACAAGGGGA-3'	69.5	30
CYP3A4*1B	**RP**	5'-CCTTTCAGCTCTGTGTTGCTCTTTGCTG-3'	66.6	28

Table 3: Genetic analysis of cervical cancer patients.

Sample					
Allele	**PCR product size (bp)**	**RE**	**Expected fragments (bp)**	**Observed fragments (bp)**	**Conclusion about samples**
CYP3A4*1B	385	*Mbo* II	NH 175, 169, 41	175, 169, 41	NH=18
			HE 210, 175, 169, 41	210, 175, 169, 41	HE=12
			MH 210, 175	Not found	MH=0

NH = Normal homozygote, HE= Heterozygote, MH = Mutant homozygote.

DISCUSSION

In this hospital base case-control study, we analyzed the relationship of cervical cancer risk and CYP3A4*1B polymorphism in Bangladeshi women. Finally, we found that CYP3A4*1B did not interact to impart to the risk of cervical cancer.

A number of possible efficient polymorphisms of the CYP3A4 gene have been concerned in cancer risk, but independently published studies have revealed unconvincing outcome. Subgroup investigation by cancer category explain that CYP3A4*1B polymorphism had considerable links with enhanced risk of prostate cancer, but not with breast cancer, leukemia, or other cancers.[14] Still no published study have probe to the relationship between CYP3A4*1B polymorphisms and cervical cancer risk. Some analysis have assessed to ascertain the involvement between CYP3A4*1B polymorphisms and small cell lung cancer risk, breast, prostate and ovarian cancer.[13,15,16]

Table 4: Odds ratio calculation.

Genotype	Cases	Control	OR	95% CI	*P*
NH	18	20	1	-	-
HE	12	10	1.33	0.47 to 3.82	0.5925
MH	0	0	1.11	0.02 to 58.72	0.9596
HE+MH	12	10	1.33	0.47 to 3.82	0.5925

OR= Odds ratio, Cl= Confidence interval

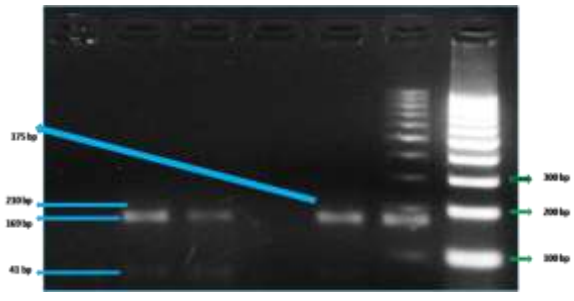

Figure 1: Restriction enzyme (*Mbo II*) digestion for CYP3A4*1B

Our study gone through several limitations. First, we could not appraise the associations between genotypes and phenotypes of CYP3A4*1B. Subsequent, the quantity of issues in our study was restrained, and the statistical supremacy of the study was narrow. Third, we did not able to collect tissue sample due to lacking of such facilities.

Tissue sample is necessary for HPV typing. HPV infection is a crucial pathogenic factor for cervical cancer. It is expected that associations may be firmer with one particular subtype than another. Consequently, the study subjects, mainly cancer cases, may not be delegated for the target population, and our conclusions might not be unrealizable to the general population. Lastly, our findings require to be interpreted with care, and the associations need to be simulated in bigger, preferably population-based studies.

CONCLUSION

In brief, the consequences of our study demonstrated that CYP3A4*1B polymorphism is not allied in susceptibility

to develop cervical cancer, at least in Bangladeshi women.

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