RESEARCH ARTICLE

A Pilot Study on Screening of BRCA1 Mutations (185delAG, 1294del40) in Nepalese Breast Cancer Patients

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Abstract

Background: Breast cancer is the second most common malignancy among Nepalese women, accounting for 60% of the total cancer cases in females. Women diagnosed with germline mutations in BRCA1 like 185delAG, 1294del40 develop breast and/or ovarian cancer with a lifelong likelihood of up to 85% whereas presence of a mutation increases the risk for mutations to occur in other genes. The major objective of this study was to find the prevalence of these mutations in Nepalese cancer patients. Materials and Methods: This prospective study was carried out at two cancer hospitals in the Kathmandu valley over a period of 11 months. Irrespective of age group and stage of canceran appropriate amount of blood was withdrawn from 50 breast cancer patients and 20 controls. DNA was extracted manually and subjected to PCR using primers for 185delAG and 1294del40 mutations. PCR products were then digested with restriction enzyme (DdeII) followed by electrophoresis. Results: Prevalence of 185delAG in reference breast cancer patients was found to be 4/50 (8%) but no 1294del40 was apparent. Conclusions: Several mutations occurring in different exons of BRCA1 as well as mutations in other genes like BRCA2, for example, should also be taken in account.

Keywords: Breast cances - susceptibility genes - BRCA1 - 185delAG - mutation - Nepal

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Introduction

Since the localization (the long arm (q) of chromosome 17 (17q12-21))(Miki et al., 1994) and isolation of the breast cancer susceptibility gene BRCA1, various studies have revealed that highly penetrant mutations in BRCA1 constitute very high risk of breast cancer (Peto et al., 1999; Papelard et al., 2000; Stegel et al., 2011). BRCA1 is a large gene with 22 coding exons encoding a 220 kD protein that functions in maintaining genomic integrity and in transcriptional regulation (Belogianni et al., 2004). Frameshift and nonsense mutations are the predominant germ line mutations of BRCA1 gene, which causes either loss or mutation of the remaining allele leading to total inactiveness of BRCA1 gene in the infected cell(Shattuck-Eidens et al., 1995). Thus engendered truncated proteins may lack the biochemical functions when equated to its prototype, may be non-functional due to aberrant sub cellular localization or physically absent due to rapid degradation (Miki et al., 1994; Kainu et al., 1996).

Breast cancer is the leading cancer in Asian females and its incidence is further uphill(Kim and Choi, 2013). The global burden of breast cancer is expected to cross 2 million by the year 2030, with growing proportions from developing countries(Jemal et al., 2011). In Nepal it is the 2nd most common cancer in women (7.2% of all malignancy) (Piya and Acharya, 2012). It is high in perimenopausal women (41 to 50 yrs) i.e. the risk of breast cancer increases exponentially up to the age of menopause, and then the rate of increase in the risk slows significantly (Smigal, 2006). In addition, BRCA1 mutations also appear to predispose to a range of other cancer types (prostate, pancreatic, colon cancer etc.) (Capalbo et al., 2006). In 2012, the total number of breast cancer cases in Nepal was 1716, Age Specific Incidence Rate (ASIR) was 13.7/100000 and Age Specific Mortality rate (ASMR) was 2.7/100000 respectively(Ghoncheh et al., 2015).

BRCA1 mutation analysis is a method of genetic testing that evaluates individual risk status for breast or ovarian cancer. It may be considered after studying family history, providing pre-test genetic counselling. Genetic testing predicts the likelihood of developing cancer and does not mean that the cancer will or will not occur. There are 1536 distinct mutations, polymorphisms and variants in BRCA1 and although less than 5% of total breast cancer patients have mutations in the BRCA1 gene, but the carrier have a 60%-80% probability of developing breast cancer (Saxena et al., 2006; Kim and Choi, 2013). Screening of

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these germline mutations serves as a critical diagnostic tool to identify the cancer in its inchoate state so as to constrict the carcinomal invasion and its complications. Although for genetic testing it's advantageous to know founder and/or recurrent mutation but since little have been studied about the mutation screening and profiling in Nepal, so this study was carried out to gauge the contribution of BRCA1 mutation (185delAG, 1294del40) to the incidence of breast cancer in reference Nepalese population or patients.

Materials and Methods

Prospective study was carried out at a cancer hospital in the Kathmandu valley for a period of 11 months. Preceding the study, approval was obtained from the institutional research ethical review board. Irrespective of age group and stages of cancer appropriate amount of blood was withdrawn from 50 breast cancer positive patients and 20 controls (normal womens). DNA was extracted manually, subjected to PCR using primers for 185delAG and 1294del40 mutations. PCR products were then digested with restriction enzyme (DdeII) followed by 4% Gel Electrophoresis for detection of former 185delAG mutation. Finally bands were visualized over UV-trans-illuminator.

Sample Collection And Storage

After obtaining written consent form from each individual, 5 ml peripheral blood sample was collected into EDTA vials and stored at 4°C until further processing.

DNA extraction

DNA extraction was carried out using "Lahiri and Nurnberger" protocol(Lahiri and Nurnberger, 1991). The quality of the DNA was checked by 0.8 % agarose gel electrophoresis. The quantity of the DNA was measured spectrophotometrically.

Table 1. Description of Primers Used

Mutations	Exon	Primers set used	
		Foreward Primer	Reverse Primer
185DelAG	2	5'-AAA ATG AAG TTG TCA TTT TAT AAA CC-3'	5'-CTG ACT TAC CAG ATG GGA CAT T-3
1294Del40	11	5'-GAA TAA GCA GAA ACT GCC ATG C-3'	5'-CTC TAC TGA TTT GGA GTG AAC TC-3'

Table 2. PCR Conditions

95 ºC for 10 minute followed by 35 cycles of denaturation, annealing and extension depending on mutation primer sets used.			
Mutation	Denaturation	Annealing	Extension
185DelAG	30s at 94ºC	30 s at 52ºC	1.0 min at 72ºC
1294Del40	30s at 94ºC	30s at 54ºC	1.0 min at 72ºC
R1443X	30s at 95ºC	30 s at 56ºC	1.0 min at 72ºC

The reaction is ended with 10 min incubation at 72 ºC. PCR Product put at 4 ºC on Hold.

PCR amplification

For amplification Mastermix was prepared with 7.5ul PCR Buffer, 6ul dNTPs and 0.9ul Taq Polymerase. 4.8ul of this mastermix was taken and in it 1ul Template DNA, 1ul Foreward and 1ul Reverse Primer (Specific to the Mutation) was added followed by 17.2ul of Nuclease Free Water to obtain final volume of 25ul in PCR tube. The PCR Primer sets and the condition for amplification for the different mutation detection were varied and is shown below Table1 and Table 2 respectively:

Restriction digestion and visualization

For 185delAG the PCR products were digested with 1U of restriction enzymes and were detected by resolving over 4% agarose gel containing Ethidium Bromide (EtBr). For 1294del40 mutation, 500ml of Running Buffer was prepared50ml of 5X TAE buffer was mixed with distilled water to make the final volume 500ml. 50ml of that buffer was mixed with 1gm of Agarose, mixed well and boiled till clear (2% agarose gel). 40µl of Ethidium bromide was mixed with the molten gel in a flask and poured onto the Gel-cast.

Electrophoresis was performed at 80V-50mAmp for 45minutes. The resolved bands were visualized over an UV-trans-illuminator. Summary of restriction digestion enzyme and its restricted product can be seen in Table 3:

Results

The mean age of onset of cancer in our study was found to be 42.59. For 185delAG mutation, out of 50 samples tested, 4 samples (8%) showed undigested band in the range of 176bp meaning positive for this mutation, while the other 75 samples showed digested bands, with one on the range of 150bp and the other on the range of 26bp as shown in the Figure 1:

For 1294del40 mutation, the PCR products after electrophoresis and visualization under UV showed

Table 3. Restriction Enzymes Used and their Products

Mutation	Restriction Enzymes	Restricted Product in base pair (bp)		
		Wild type	Mutant	Heteroduplex
185delAG	DdeII	150, 26	176	181, 189
1294del40	None	300	260	2300

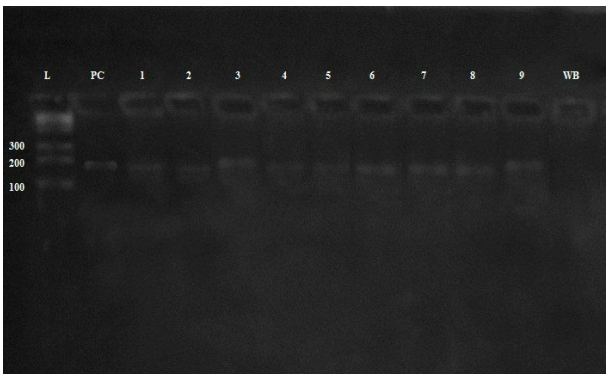

Figure 1. L : Ladder, PC :Positice control, WB : WaterBlank, Sample 1-2;4-8 show digested band(wild type) Sample 3&9 show undigested band(mutated)

distinct band of amplified DNA sequence. The band sizes were found to be 300bp that is of wild type and no samples showed two bands that would appear for mutated type at 260bp (mutated).

Discussion

The mean age of onset of cancer in our study was found to be 42.59 which was slightly lower than the age (46.5±12.8: Range 25 to 80 years) revealed by other study in Nepal (Singh and Sayami, 2009). A study done on different ethinicities showed that the mean age at the time of testing was 50±11.9 years among European women, whereas the mean age was 45.2 ±10.9 years for women of African descent, and 47.1± 12.5 years for women of Asian descent(Hall et al., 2009). Although we tested for different founder mutations, our study only revealed the presence of 185delAG mutation in our reference population. 8% of our study population showed 185delAG mutation which was relatively lower than the result shown by a study done at India, which revealed an alarming prevalence of 16.3%(Karami and Mehdipour, 2013). Another study in India found 185delAG mutation in 4 familial breast cancer patients with an age of diagnosis of 35, 37, 40 and 50 years (Rashid et al., 2006). In a similar study done in eastern India there was found to be no prevalence of 185delAG mutation(Chakraborty et al., 2014). Hansa et al found the presence of 185delAG mutation in his study which was conducted in north east India(Hansa et al., 2012).The mutation 185delAG has been coined as the "Ashkenazi Mutation" because it was predominantly detected among Ashkenazi Jews having attained a 1% carrier frequency within the population since origin of the ancestral mutation(Struewing et al., 1995).Its prevalence have shown a diversity among different parts of world. A study done in Korea didn't showed the prevalence of 185delAG mutation as well as 5382insC mutation in BRCA1(Ahn et al., 2004). Among non-Jewish Americans of Spanish ancestry from the San Luis Valley,185delAG mutation have been reported to be present in high frequency of 31.6% but contrastingly it has been found at a very low prevalence rate (1.13–5.9%) among white Americans, the Spanish from Spain, Polish, Iranian, Pakistani and Turkish women(Vaidyanathan et al., 2009).

A frequency of nearly 1% among the general population has been reported for a 40-bp deletion, 1294del 40, within large exon 11 of the BRCA1 gene, which leads to the translation termination(Walker and Rapley, 2005). 1294del40 mutation sought to be important in causation of early onset of breast cancer in women. Hence, the detection of this particular mutation could give an insight into the relative risk of breast cancer in pre-symptomatic relatives of the breast cancer patients, especially in their first degree relatives in our region where this mutation has not been tested/ researched yet.

The variation in our study may be due to small reference population that we included in our study. In addition it may also be possible that the mutation may be occouring in other exons of BRCA1 or even in other genes like BRCA2, p53 etc which thus initiated an importance to screen those mutation as well.

In conclusion, The assumption behind screening high risk women for genetic cancer susceptibility is that, in those individuals affected, the cancers anticipated can be prevented or their negative health impact reduced by early diagnosis. However, it would be interesting to investigate the carrier frequency of these mutations among diversity (religious/ethnic/geographical groups) of Nepalese population with a larger sample size to further understand its prevalence and origin.

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