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Methylenetetrahydrofolate Reductase (*MTHFR*) and Angiotensin Converting Enzyme (*ACE*) Gene Variations in Link with Breast Cancer in Jammu Region of Jammu and Kashmir State

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ABSTRACT Molecular genetics of cancer has revealed a probable linkage of both folate-metabolizing enzymes and angiogenic processes generated by the renin-angiotensin system (RAS) with mammary gland tumorigenesis. *MTHFR* (C677T) and *ACE* I/D polymorphism are considered as candidate markers for breast cancer. The objective of the present study was to evaluate the association of *MTHFR* (C677T) and *Alu ACE* I/D gene polymorphisms in females with both benign breast disease (BBD) and breast cancer (BC) in Jammu region of the J&K state. The polymorphisms were genotyped by PCR and RFLP technique. Significant association of *MTHFR* (C677T) polymorphism was observed with BC but not with BBD. The "T" allele of *MTHFR* (C677T) polymorphism was adding nearly 11 folds risk towards of BC (p=0.0003). Regarding *ACE* gene polymorphism, heterozygous genotype (ID) was conferring approximately 2.5 folds risk for BBD (p=0.003). On contrary, *ACE* polymorphism was projecting a protective role towards BC susceptibility. The study concludes *MTHFR* gene to be a potential candidate for breast tumorigenesis.

INTRODUCTION

Breast cancer is the most prevalent malignancy and the primary cause of female death worldwide. It accounts for 25 percent of all cancers among women worldwide (Malvia et al. 2017) and is currently transcending cervical cancer among Indian women (Gupta 2016). The etiology of breast malignancy is uncertain and involves the interplay of both genetic and non-genetic risk factors. Genetic variations in genes controlling folate metabolism and Renin-angiotensin system (RAS) have been identified to play a plausible role in carcinogenesis (Hedayatizadeh-Omran et al. 2017; Singh et al. 2017).

Methylenetetrahydrofolate reductase (*MTHFR*) is one of core enzyme of folate and homocysteine metabolism. The *MTHFR* gene is localized on chromosome 1 (1p36.3), composed of 11 exons and encodes a protein of 656 amino acids (Hedayatizadeh-Omran et al. 2017). The biological function of *MTHFR* enzyme is to reduce 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), which is the main circulatory form of folate and at the same time, it acts as a carbon donor for remethylation of homocysteine back to methionine. A common C to T missense mutation at 677 in exon 4 of the gene is responsible for alanine (A) to valine (V) substitution at codon 222 and is associated with reduced *MTHFR* enzyme activity (Raina et al. 2016). The polymorphism has controversial results regarding different cancers. It has been proposed that there occurs a lower susceptibility risk for acute lymphoblastic leukemia and colorectal cancer with mutant *MTHFR* variant (Krajinovic et al. 2004; Wang et al. 2006). The possible mechanism behind this phenotype is an increased pool of 5,10-MTHF leading to a reduction in chances of uracil (causative agent for DNA double-strand breaks) misincorporation during DNA synthesis (Pooja et al. 2015). Reports from cohort studies showed that polymorphic *MTHFR* variant has relation to breast cancer in women (Waseem et al. 2016). The positive influence of *MTHFR* mutant variant with risk of breast cancer may be due to DNA hypomethylation occurred in response to depletion of 5-MTHF (Pooja et al. 2015).

Angiotensin-converting enzyme (*ACE*), a dipeptidyl carboxypeptidase that converts Ang I

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to the octapeptide angiotensin II (Ang II) is another emerging candidate marker for tumorigenesis (Singh et al. 2017; Zhang et al. 2014). The human *ACE* gene is located on chromosome no.17 at locus 17q23.3 and spans 45kb in size, consisting of 26 exons and 25 introns. The function of the *ACE* gene is to convert the inactive form of angiotensin i.e. angiotensin I to its active form angiotensin II which controls fluid-electrolyte balance and blood pressure. One of the imperative polymorphism of *ACE* gene is characterized by the presence (I) or absence (D) of 287bp *Alu*Ya element inside intron 16. Studies have shown that highest serum *ACE* activity was seen in DD genotypes which significantly increases the risk of breast cancer and lowest in II genotype which is associated with lower risk of cancer (Koh et al. 2003; Wang et al. 2013; Singh et al. 2017). Elevation in serum *ACE* leads to an increase in the production of Ang II which is believed to be the direct cause of breast cancer via the binding to its receptors, that is, angiotensin II receptor type 1 (AGTR1) and type 2 (AGTR2). Several pieces of evidence generated by studies on animal model and human cancer cell lines have suggested a probable role of Ang II in neovascularization and angiogenesis in neoplastic growth (Koh et al. 2003; Fernandez et al. 1985). It also acts as pro-mitotic (Koh et al. 2003), cell proliferative (Muscella et al. 2002) and tumor metastatic agent (Fujita et al. 2002).

Objectives

In the above context, the researchers performed a case-control study on genotyping and evaluation of *MTHFR* (C677T) and *Alu ACE* I/D gene polymorphisms in females with benign breast disease and breast cancer in Jammu region of J&K state.

MATERIAL AND METHODS

Study Population, Inclusion and Exclusion Criteria

The study sample comprised of 69 patients of surgically and histopathologically confirmed Breast Cancer (BC), 106 cases with benign breast disease (BBD) and 150 healthy unrelated females as a control group. BC patients on Chemotherapy were excluded. The patient samples were collected from Department of Pathology and Oncology, Government Medical College (GMC), Jammu. The control sampling was done from premises of the University of Jammu, Jammu and females attending OPD, GMC, Jammu with minor ailments without having any history of breast cancer and breast related complications/BBD or other major diseases (cardiovascular disease, hypertension, diabetes). The study was conducted after having an informed written consent from all participants. The study was approved by the Animal and Human Experimentation Ethical Committee (AHEEC), University of Jammu, Vide letter. No. JU/SBT/13/1598.

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using the Phenol-Chloroform method. PCR amplification of the DNA samples was performed using locus-specific primers (Table 1). The PCR products of *MTHFR* were subjected to restriction digestion with *HinfI* restriction enzyme. The restriction digestion products were analyzed on 3% agarose gel under UV- transilluminator. Amplified products of *Alu ACE* I/D were directly visualized under UV- transilluminator on 1.5% agarose gel.

Table 1: Primer sequence

S. No.	*Marker*	*Primer sequence*	*Annealing temperature*	*Band size (bp)*
1	*MTHFR* (C677T)	FP: 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' RP: 5'-AGG ACG GTG CGG TGA GAG TG-3'	62R^{0}C	198-CC 175, 23- TT 198,175, 23-CT
2	*Alu ACE* I/D	FP: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' RP: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T–3'	58R^{0}C	490-II 190-DD 490,190-ID

Statistical Analysis

The power of the present study was calculated using the CaTS power calculator (Skol et al. 2006) and it was found to be 80 percent. The allele frequencies were calculated by allele counting for each genetic marker. Hardy-Weinberg equilibrium and the differences in genotypic frequencies were examined by using Chi-square test. Odds ratios (ORs) were used to measure the association of breast cancer risk with both *MTHFR* and *ACE* genotypes. All analyses were performed using SPSS v.11.5 (SPSS Inc., Chicago, USA) statistical analysis software. A two-tailed p-value of $p < 0.05$ was considered statistically significant.

RESULTS

Genetic Association of *MTHFR* (C677T) polymorphism with Benign Breast Disease (BBD) and Breast Cancer (BC)

The genotype frequencies for both BBD cases (χ^2=0, p=0.05) and controls (χ^2= 0.01, p= 0.05) were following with Hardy Weinberg equilibrium (HWE) except for breat cancer cases (χ^2= 6.57, p= 0.01) thereby signifying disease association. Among cases, the observed prevalence for CC genotype was higher in BBD group in comparison to BC patients (99.06% vs 86.96%) whereas in controls it was almost comparable to BBD subjects (that is, 98.44%). The researchers were not able to locate TT genotype in both BBD and control subjects whereas in BC patients it was found to be 2.9 percent. Overall, the frequency of wild C-allele was higher in both BBD and control subjects (that is, 0.99%) and variant T-allele was higher in BC patients (0.08%). The genotypic and allelic frequencies for both *MTHFR* gene polymorphisms among cases and controls are presented in Tables 2 and 3. In order to draw association of *MTHFR* gene variation with both BBD and BC different genetic models viz. Co-dominant, dominant, recessive and allelic models were applied (Tables 2 and 3). No significant association was observed for *MTHFR* (C677T) polymorphism under any of the tested genetic models with BBD. The variant T-allele was not shown to project risk towards BBD in the study population. Further, *MTHFR* (C677T) polymorphism was associated with significant increased risk of BC under heterozygous co-dominant [CC vs CT: OR= 7.35, 95% CI (1.48-36.45), p= 0.005] and allelic model [C vs T: OR= 11, 95% CI (2.40-50.38), p= 0.0003]. The T-allele was conferring nearly 11-folds risk for the susceptibility of BC in study population which was quite high, the plausible reason behind this includes very low frequency of T-allele in general population of Jammu region.

In Table 4 association analysis was also carried out between BC and BBD in order to reach more authenticate conclusion. For *MTHFR* C677T variation, it was observed that variant genotype (TT) was present only in BC patients and also a higher frequency of T-allele was reported in BC individuals in contrast to BBD group thereby suggesting a probable association of the said polymorphism in etiology of BC. Since the frequency of variant allele was quite lower in BBD group it was not possible to apply any genetic models.

Genetic Association of *ACE* I/D polymorphism with Benign Breast Disease (BBD) and Breast Cancer (BC)

Deviation from HWE was shown by BBD group (χ^2= 12.44, p= 0.001) whereas both breast cancer group (χ^2= 0.02, p= 0.05) and controls (0.07, p= 0.05) were obeying HWE. The frequency of II genotype was observed to be higher in BC group (42.03%) followed by healthy controls (35.16%) and lower in individuals with BBD (18.87%). The prevalence of heterozygous (ID) genotype was higher in BBD patients and the genotype was found to be significantly associated with BBD outcome thereby providing nearly 2.54 folds risk towards the disease progression (Table 2). The frequency of risk DD- genotype was almost similar all the study groups (BC=13.04%, BBD=14.15%, Controls=15.62%). Overall, the frequency of variant D-allele was slightly higher in BBD cases (0.5) than in BC (0.4) and controls (0.4). The distribution of genotype and allele frequencies for *ACE* gene polymorphisms among cases and controls were presented in Tables 2 and 3. Logistic regression analysis for *ACE* I/D polymorphism showed that co-dominant model II vs ID [OR=2.54, 95% CI (1.35-4.74), p= 0.003] and dominant model II vs ID+DD [OR= 2.33, 95% CI (1.27-4.28), p= 0.006] were having significant association with BBD whereas none of the applied genetic models has shown any association with susceptibility to breast cancer in studied population. The results of the present study

Table 2: Association of *MTHFR* (C677T) and *ACE* I/D polymorphism with benign breast disease (BBD)

Genetic model	*Cases with benign breast disease (n=106)*	*Controls (n=128)*	*OR (95% CI)*	*p-value*
MTHFR C677T				
Co-dominant				
CC	105 (99.06%)	126 (98.44%)	**1 (Reference)**	
CT	1 (0.94%)	2 (1.56%)	0.60[0.05-6.71]	0.68
TT	0	0	Not possible†	_
Dominant				
CC	105 (99.06%)	126 (98.44%)	**1 (Reference)**	
CT+TT	1 (0.94%)	2 (1.56%)	0.60[0.05-6.71]	0.68
Recessive				
CC+CT	106 (100%)	128 (100%)	**1 (Reference)**	
TT	0	0	Not possible†	_
Allelic				
C	211 (0.99)	254 (0.99)	**1 (Reference)**	
T	1 (0.01)	2 (0.01)	0.60[0.05-6.68]	0.68
ACE I/D				
Co-dominant				
II	20 (18.87%)	45 (35.16%)	**1 (Reference)**	
ID	71 (66.98%)	63 (49.22%)	2.54[1.35-4.74]	0.003*
DD	15 (14.15%)	20 (15.62%)	1.69[0.72-8.95]	0.23
Dominant				
II	20 (18.87%)	45 (35.16%)	**1 (Reference)**	
ID+DD	86 (81.13%)	83 (64.84%)	2.33[1.27-4.28]	0.006*
Recessive				
II+ID	91 (85.85%)	108 (84.38%)	**1 (Reference)**	
DD	15 (14.15%)	20 (15.62%)	0.89[0.43-1.84]	0.75
Allelic				
I	111 (0.5)	153 (0.6)	**1 (Reference)**	
D	101 (0.5)	103 (0.4)	1.35[0.94-1.95]	0.11

†Some genotype combinations were not observed, so it was not possible to calculate odds ratio.

showed that D-allele was not involved in the development of both BBD and BC in the populace of Jammu region.

On comparing BC group with BBD group (Table 4), it has appeared that the heterozygous (ID) genotype and variant allele (D) was projecting a protective role against BC in this study population in a statistically significant manner. Further on applying genetic models, it was found that the ID+DD genotype was conferring nearly 1.10 folds protection towards BC risk (p=0.001).

Association of Non- genetic Factors with Benign Breast Disease (BBD) and Breast Cancer (BC)

Since females of Jammu region (J&K) are conservative regarding habit of smoking/alcohol intake and use of oral contraceptives (OC). Majority of the females of both study groups were married and were involved in the practice of breastfeeding. So, in the present study, none of the risk factors taken into account have shown any significant association with BBD and BC (Table 5).

DISCUSSION

Breast cancer is clinical, a heterogeneous disease and its pathophysiology involve the participation of both environmental risk factors and the genetic factors. The genetic susceptibility to breast cancer is prompted in several ways that induce malignant transformation (both genetic and epigenetic changes) and finally, results in uncontrolled cellular proliferation and/or abnormal programmed cell death or apoptosis. It has been suggested that the *MTHFR* and *ACE* genes are promising candidate markers for breast-related complications including breast cancer.

Folate deficiency is one of the known trigger for carcinogenesis and increases the risk of malignancy via two mechanisms: (1) by causing DNA hypomethylation and proto-oncogene activation; and/or (2) by inducing uracil misincorporation during DNA synthesis that induces catastrophic DNA repair, DNA strand breakage and chromosome damage (Pooja et al. 2015). The C to T transition at nucleotide position 677 of

Table 3: Association of *MTHFR* (C677T) and *ACE* I/D polymorphism with breast cancer (BC)

Genetic model	*Cases with benign breast disease (n=69)*	*Controls (n=128)*	*OR (95% CI)*	*p-value*
MTHFR C677T				
Co-dominant				
CC	60 (86.96%)	126 (98.44%)	1 (Reference)	
CT	7 (10.14%)	2 (1.56%)	7.35[1.48-36.45]	0.005*
TT	2 (2.9%)	0	Not possible†	_
Dominant				
CC	60 (86.96%)	126 (98.44%)	**1 (Reference)**	
CT+TT	9 (13.04%)	2 (1.56%)	Not possible†	_
Recessive				
CC+CT	67 (97.1%)	128 (100%)	**1 (Reference)**	
TT	2 (2.9%)	0	Not possible†	_
Allelic				
C	127 (0.92)	254 (0.99)	**1 (Reference)**	
T	11 (0.08)	2 (0.01)	11[2.40-50.38]	0.0003*
ACE I/D				
Co-dominant	29 (42.03%)	45 (35.16%)	**1 (Reference)**	
II	31(44.93%)	63 (49.22%)	0.76[0.40-1.44]	0.40
ID	9 (13.04%)	20 (15.62%)	0.70[0.28-1.74]	0.44
DD				
Dominant				
II	29 (42.03%)	45 (35.16%)	**1 (Reference)**	
ID+DD	40 (57.97%)	83 (64.84%)	0.75[0.41-1.36]	0.3
Recessive				
II+ID	60 (86.96%)	108 (84.38%)	**1 (Reference)**	
DD	9 (13.04%)	20 (15.62%)	0.81[0.35-1.89]	0.6
Allelic				
I	89 (0.6)	153 (0.6)	**1 (Reference)**	
D	49 (0.4)	103 (0.4)	0.82[0.53-1.26]	0.4

the *MTHFR* gene of folate metabolism pathway produces *MTHFR* enzyme with retarded functional capacity. The "677T" variant of *MTHFR* gene is responsible for elevated plasma homocysteine levels and affects methionine synthesis by reducing DNA methylation in cancer patients (Sharp et al. 2002). In the present case-control study, the researchers genotyped individuals for *MTHFR* (C677T) variation to evaluate its association with benign breast disease and sporadic breast cancer. The frequency of "C" allele was higher and comparable in BBD cases (0.9) and controls (0.99), showing lack of association of the said polymorphism with BBD (C vs T, p= 1.06). On contrary, the mutant "T" allele frequency was higher in breast cancer cases (0.08) when compared to controls (0.01) depicting significant association of "T" allele with CA breast (p=0.0003). Genetic association analysis between BC and BBD group confirmed *MTHFR* gene as a putative gene involved in the susceptibility of BC in present study population. The genotype and allele frequencies observed in the present study were comparable to frequencies reported earlier in Jammu region by Raina et al. 2016. Similarly, Gershoni-Baruch and associates reported that the *MTHFR* 677T allele was significantly more common in cases of bilateral breast cancer or combined breast and ovarian cancer (Gershoni-Baruch et al. 2000). Chen et al. (2005) found the significant role of TT genotype with risk of breast cancer [CC vs TT; OR= 1.34, 95% CI (1.04-1.73), p=0.04]. A recent investigation on *MTHFR* C677T polymorphism and BC risk has supported a positive significant association of 677T allele with the disease susceptibility in North Indians (Waseem et al. 2016). However, Campbell et al. (2002) although, reported a higher frequency of the "T" allele among the breast cancer cohort (0.34), than that of the controls (0.30) but they did not find any significant association. According to a meta-analysis report, 677 C>T substitution did not appear to be a prominent candidate for affect breast cancer (Pooja et al. 2015). Another study on North Iranian women was also in support of non-involvement of this polymorphism in breast cancer (Hedayatizadeh-Omran et al. 2017). Low significant association of TT genotypes with

Table 4: Genetic association analysis between breast cancer (BC) and benign breast disease (BBD)

Genetic model	*Cases with benign breast disease (n=69)*	*Controls (n=106)*	*OR (95% CI)*	*p-value*
MTHFR C677T				
Co-dominant				
CC	60 (86.96%)	105 (99.06%)	**1 (Reference)**	
CT	7 (10.14%)	1 (0.94%)		
TT	2 (2.9%)	0	Not possible†	
Dominant				_
CC	60 (86.96%)	105 (99.06%)	**1 (Reference)**	
CT+TT	9 (13.04%)	1 (0.94%)	Not possible†	_
Recessive				
CC+CT	67 (97.1%)	106 (100%)	**1 (Reference)**	
TT	2 (2.9%)	0	Not possible†	_
Allelic				
C	127 (0.92)	211 (0.99)	**1 (Reference)**	
T	11 (0.08)	1 (0.01)	Not possible†	_
ACE I/D				
Co-dominant	29 (42.03%)	20 (18.87%)	**1 (Reference)**	
II	31(44.93%)	71 (66.98%)	0.30[0.15-0.61]	0.001
ID	9 (13.04%)	15 (14.15%)	0.41[0.15-1.13]	0.1
DD				
Dominant				
II	29 (42.03%)	20 (18.87%)	**1 (Reference)**	
ID+DD	40 (57.97%)	86 (81.13%)	0.32[0.16-0.63]	0.001
Recessive				
II+ID	60 (86.96%)	91 (85.85%)	**1 (Reference)**	
DD	9 (13.04%)	15 (14.15%)	0.91[0.37-2.21]	0.8
Allelic				
I	89 (0.6)	111 (0.5)	**1 (Reference)**	
D	49 (0.4)	101 (0.5)	0.61[0.39-0.94]	0.02

sporadic breast cancer was shown by Akram et al. (2012) in Pakistani females. Chou and co-workers also found decreased risk susceptibility of breast cancer with "T" allele [OR= 0.81, 95% CI (0.54–1.21)] carriers in Taiwanese population (Chou et al. 2006). In view of other cancers viz. colorectal and prostate cancer, some researchers have suggested *MTHFR* as a low penetrance susceptible gene because the C677T polymorphism was imparting a protective effect towards the cancer risk in the study population (Huang et al. 2007; Guo et al. 2015). The possible justification for these conflicting results may be due to genetic heterogeneity as well as clinical heterogeneity in different studies.

Variation in *ACE* gene imbalances plasma *ACE* level which further affects Ang II level and the fluctuating levels of these metabolites in together contribute to breast carcinogenesis. It has been found that Ang II provokes the expression of a proto-oncogene (fos, c-jun, c-myc, erg-1, VL-30 and the activator of the protein 1 complex) in rats and humans and it also acts as a bi-func-tional apoptosis modulator (Mendizábal-Ruiz et al. 2010). The present study which was aimed to evaluate the role of *ACE* I/D polymorphism with BBD and BC depicted that Indel polymorphism was associated with risk of BBD but not with BC. The frequency of wild (low risk) allele I in both BC and controls was same (0.6) and was higher than mutant (high risk) allele (0.4). The frequency of D allele in BBD (0.5) was higher than in controls (0.4). The dominant model showed significant association ID+DD genotype with risk of BBD in this study population. Similar findings were observed by Mendizábal-Ruiz et al. (2010) as they also reported the significant role of the dominant model [OR=0.32, 95% CI (0.09-0.99), p=0.029] in BBD. On comparing association analysis between BC and BBD group, the researchers have noticed a protective role of *ACE* I/D polymorphism towards BC outcome but Mendizábal-Ruiz et al. (2010) reported both dominant and recessive models to be associated with BC. Yaren et al. (2006) reported that *ACE* genotypes were not associated with breast cancer patients except that

Table 5: Distribution of clinical demographics and non-genetic factors in study participants

Factors	*BBD cases (n=106)*	*BC cases (n=69)*	*Controls (n= 128)*
Age (Mean±SD)	45.32±23.551	47.14±14.43	52.31±13.67
Age at menarche (Mean±SD)	14.12±1.13	14.03±1.31	14.77±1.42
Age at menarche, Years			
>13	82(77.4%)	45(65.2%)	94 (73.4%)
<13	24 (22.6%)	24 (34.8%)	34 (26.6%)
OR (95% CI), p-value	0.81(0.44-1.47), 0.5	1.47 (0.78-2.77), 0.2	_
History of Smoking			
Yes	1 (0.9%)	3 (4.4%)	4 (3.1%)
No	105 (99.1%)	66 (95.6%)	124 (96.9%)
OR (95% CI), p-value	0.29(0.03-2.68), 0.3	1.41 (0.31-6.48), 0.7	_
Breast Feeding			
Yes	98 (92.5%)	67(97.1%)	125(97.7%)
No	8 (7.5%)	2(2.9%)	3(2.3%)
OR (95% CI), p-value	3.40 (0.88-13.16), 0.1	1.24 (0.20-7.63), 0.8	_
Use of OC			
Yes	Nil	Nil	Nil
No	106 (100%)	69 (100%)	128 (100%)
OR (95% CI), p-value	_	_	_
FH of BC in mother/sister			
Yes	1(0.9%)	2(2.9%)	2 (1.6%)
No	105(99.1%)	67(97.1%)	126 (98.4%)
OR (95% CI), p-value	0.60 (0.05-6.71), 0.7	1.88 (0.26-13.65), 0.5	_
Tumor Stage			
I & II	_	3 (4.3%)	_
III & IV	_	66 (95.7%)	_
Tumor Grade			
Grade 1	_	42 (60.86%)	_
>Grade 1	_	27 (39.13%)	_
Lymph Node Grade			
N0	_	11 (15.94%)	_
N1+N2	_	58 (84.06%)	_
Metastasis			
Yes	_	31 (44.93%)	_
No	_	38 (55.07%)	_

DD genotype was significantly associated with tumor size (p=0.02). Likewise, in a meta-analysis study conducted by Sun et al. (2011) proclaimed that *ACE* I/D polymorphism may not be a genetic risk factor for breast cancer. A study from India on Kashmiri population declared that *ACE* activity could be a protective tool against breast cancer as their results showed significant association of II genotype with the breast cancer [DD vs II, OR=1.54, 95% CI (0.82-2.93); p=0.001] (Syeed et al. 2010). According to Singapore Chinese Health Study, women with DD genotype had a significantly higher risk of developing breast cancer as compared to ID and II genotypes respectively (Koh et al. 2003). Roy and researchers observed the higher frequency of DD genotype in Hindu females of West Bengal with BC (57.4%) than in controls (25.0%) and declared a significant association with breast cancer risk [OR= 4.232, 95% CI(0.083-0.229), p< 0.0001] (Roy et al. 2015). Recently, it was proclaimed that individuals harboring DD genotype or D-allele showed an increased risk of BC (Singh et al. 2017). However, present study denied any such association regarding BC risk in this study population.

CONCLUSION

The findings of the present study declared a significant linkage of *MTHFR* (C677T) polymorphism in the pathophysiology of BC whereas *Alu ACE* I/D polymorphism was suggesting a protective role towards BC risk in the studied females of Jammu region (J&K).

RECOMMENDATIONS

This study strongly recommends genetic screening based on the polymorphisms of folate-homocysteine metabolism and RAS pathway for evaluating the likelihood of breast-related diseases and cancer in high-risk females.

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