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ASSOCIATION OF TISSUE INHIBITORS OF MATRIX METALLOPROTEINASE 2 (TIMP2) -481 G/C POLYMORPHISM IN BREAST CANCER FROM SOUTH INDIAN POPULATION: A CASE CONTROL STUDY

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Abstract

Tissue inhibitors of matrix metalloproteinases (TIMPs) are a class of proteins that regulate extracellular matrix remodeling and cellular functioning in both normal and diseased conditions.TIMP-2 is component of the extracellular matrix in normal tissues, may have direct and systemic antitumor and metastasis-suppressing actions, indicating a possible role in the clinical management of breast cancer progression. Our study aims to examine the association of TIMP-2 -418G/C single nucleotide promoter polymorphism with breast cancer progression and invasion. The study involves 300 breast cancer patients and age matched healthy controls. The SNP TIMP2 -418G/C (rs8179090) in TIMP2 promoter region was examine by polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) method and gel electrophoresis. The obtained genotype data was compared between controls and patients, the influence of polymorphic variants was analyzed on clinical data of breast cancer patients. Our results suggest GC genotype is found to be associated with positive axillary lymph node status in breast cancer patients.

Index terms – Breast cancer, TIMP2 gene, Genotyping, PCR-RFLP.

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I. INTRODUCTION

Breast cancer (BC) has recently taken the lead among all other types of cancer that affect women around the world, and it is currently the type of cancer that is expanding at the quickest rate in India. The rate of breast cancer incidence is rapidly increasing in developing countries, with an annual percentage rise ranging from 3 to 4 percent [1]. The development of breast cancer is a complicated process that involves both genetic and non-genetic risk factors playing. Genetic manifestations of breast cancer are known to include breast cancer-related single nucleotide polymorphisms (SNPs) as well as pathogenic mutations in genes that predispose individuals to develop cancer. SNPs have only moderate risks on their own, but when combined with additional non-genetic risk variables, their polygenic risk score becomes crucial and helps to predict the etiology of breast cancer [2-3].

Matrix metalloprotinases, zinc-dependent endopeptidases, can degrade extracellular matrix proteins (ECM) and affect cell proliferation, differentiation, migration, programmed cell death, blood vessel growth, tissue repair, and immunological control. MMPs regulate several biological and signaling pathways and are linked to cancer, osteoarthritis, cardiovascular disease, and musculoskeletal disorders. [4-5].Endogenous tissue inhibitors of metalloproteinases inhibit metalloproteinases (TIMPs) and control extracellular matrix turnover, tissue repair, and cell conductance [6].

The TIMP family consists of four members (TIMP 1, 2, 3, and 4). Members of the TIMP family are commonly recognized to suppress the malignant phenotype by inhibiting matrix metalloproteinases (MMPs) from degrading the surrounding matrix which results in an inhibition of cell motility and invasion [7]. TIMPs are essential for maintaining the homeostasis of the ECM, mostly as a result of the MMP-inhibiting actions that they possess. In order to ensure that cells are able to function normally, it is necessary to preserve a vital physiological equilibrium between MMPs and TIMPs [8].

TIMP2 is a secretory protein located at 17q25 that regulates the proteolytic activity of matrix metalloproteinase 2 (MMP2), primarily accountable for the degradation of the extracellular matrix [9]. In addition, TIMP2 affects cell proliferation and programmed cell death [10].Several single-nucleotide polymorphisms (SNPs) in the human TIMP2 genes have been discovered to have a functional effect on TIMP2 production leading to a wide range of disease susceptibility, including breast cancer. The current case control study aims to understand the genotype-phenotype associations of the TIMP-2 -418G/C single nucleotide polymorphism with breast cancer disease susceptibility in the south Indian population.

I. MATERIALS AND METHODS

Study subjects

The present study comprised 300 patient and 300 control female subjects. BC patients were consecutively recruited from Mehdi Nawaj Jung Institute of Oncology and Regional Cancer Center (MNJIO&RCC), Hyderabad, Telangana, INDIA. All the BC cases were diagnosed based on clinical, radiological and histological parameters by medical and surgical oncologists. Women with any other malignancy and with any systemic inflammatory diseases were excluded from the present study. All the subjects were undergone interview using a detailed questionnaire for collecting clinical, pathological, demographical data and informed consent was obtained from each subject. Control subjects The present study was approved by institutional ethics committee of Mehdi Nawaj Jung Institute of Oncology and Regional Cancer Center, Hyderabad.

Genotyping

About 4 ml of venous blood was drawn from each participant and subjected to genomic DNA isolation, which was carried out by non enzymatic salting out method [11].Genotyping of TIMP2 -418G/C polymorphism was carried out by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method. The primer pair 5-CGTCTCTTGTTGGCTGGTCA-3 (forward) 'and 5'-CCTTCAGCTCGACTCTGGAG-3' (reverse) was used for the amplification with the following conditions; one cycle of 95^0C-5 minutes for initial denaturation,35 cycles of 95^0C-30 seconds for denaturation,58^0C-30 seconds for annealing,72^0C-30 seconds for extension followed by one cycle of 72^0C-5 minutes for final extension. The resulted 150 base pairs amplicon was subjected to RFLP analysis using *AvaI* restriction enzyme (*New England Bio labs*).The results were validated by blind repeat of 10% samples and the concordance of the genotypes were 100%

Statistical analysis and data presentation

The baseline characteristics of the subjects were examined using the chi-square test (for categorical variables) and the Student's t-test (for continuous data). To determine if the TIMP2 -418G/C SNP deviated from the Hardy-Weinberg equilibrium, a goodness-of-fit Chi-square test was performed (HWE). Odds ratios (ORs) and 95% confidence intervals were calculated as a result of logistic regression analysis (CIs).SNPstats tool was used to derive risk estimates for co-dominant, dominant, and recessive genetic models. Regression analysis of the variables was conducted using the SPSS software package, version 22.0 (SPSS Inc., Chicago, IL, USA). P-values ≤ 0.05 were regarded as statistically significant for all two-sided statistical tests.

II. RESULTS

The control population has a mean age of 46.34 ±5.94 years, while the patients diagnosed with breast cancer have a mean age of 48.33 ±10.1 years. When it comes to the clinical characteristics of breast cancer patients, 65% of the subjects were in the early stage of the cancer, while 35% are in the late stage. In terms of the steroid harmone receptor status, out of the total patients, 59% of patients were positive for ER receptors, while 56% of patients were positive for PgR receptors, and 57% of patients were positive for HER2/neu receptors. Histopathologically, 83% of the patients have been identified as having ductal carcinoma, while 17% have been identified as having invasive lobular carcinoma, and 34% of the total patients have been identified as having positive metastasis involvement, as shown in table 1.The summary of the allele and genotype frequencies, as well as the Chi-square, Odds ratio, and 95% confidence interval for the TIMP2 -418 G/C polymorphism is represented in Table 2. The distribution frequencies of GG, GC, and CC genotypes are 64%, 31%, 5% in control and 69%, 28%, and 3% in cases, respectively. The genotype and allele frequencies in both the control and patient populations are following HWE, suggesting that the study population was heterogeneous. Correlation between TIMP2 -418 G/C polymorphism and clinical features of breast cancer is shown in table 3. The stratified analysis of TIMP2 -418 G/C polymorphism with clinical characteristics of breast cancer patients revealed GC genotype to be associated with positive lymph node status.

were gender matched and genetically unrelated individuals recruited from same region having no evidence of any malignancies. Demographic data of control subjects was obtained in a prescribed format.

Table 1:Baseline clinicopathological characteristics of breast cancer patients

Characteristics	BC Cases [N=300] N (%)
Stage of the cancer	
T_0-T_2 **Early stage**	197 (65.67)
T_3-T_4 **Late stage**	103 (34.33)
Histological subtype	
Ductal Carcinoma	247 (82.33)
Lobular Carcinoma	53 (17.67)
Axillary Lymph nodal status	
Negative	80 (26.67)
Positive	220 (73.33)
ER[a] status	
Positive	177 (59)
Negative	123 (41)
PgR[b] status	
Positive	168 (56)
Negative	132 (44)
HER2/neu[c] receptor status	
Positive	171 (57)
Negative	129 (43)
Triple Receptor status	
Other combinations	239 (79.66)
Negative	61(20.33)
Metastasis	
Absent	197 (66.67)
Present	103 (34.33)

[a] Estrogen receptor
[b] Progesterone receptor
[c] Human epidermal growth factor receptor 2/neu receptor

Table 3: Association between TIMP2 -418G/C polymorphism and clinical features of breast cancer

Characteristics	Genotype distribution		
TIMP2 -418G/C	**GG**	**GC**	**CC**
Cancer stage T_1-T_2/T_3-T_4	125/82	63/21	9/0
OR (95% CI); P-value	1.00 (reference)	0.99(0.60-1.64);0.99	1.03(0.61-1.73); 0.89
ER Status ER+/ER-	122/85	50/34	4/4
OR (95% CI); P-value	1.00 (reference)	1.00 (0.59-1.6); 1.0	1.47 (0.35-6.04); 0.50
PR Status PR+/PR-	111/96	53/31	4/5
OR (95% CI); P-value	1.00 (reference)	0.67(0.40 to 1.13); 0.14	1.44(0.37-5.53); 0.59
Her2/Neu Status Positive/Negative	127/80	83/39	4/5
OR (95% CI); P-value	1.00 (reference)	0.74(0.46-1.90); 0.36	1.98(0.51-7.06); 0.31
Triple Negative Positive/Other	40/167	18/66	3/5
OR (95% CI); P-value	1.00 (reference)	0.87(0.47-1.64); 0.68	0.39(0.09-1.74); 0.22
Histological Subtype Ductal/Lobular	154/53	140/0	9/0
OR (95% CI); P-value	1.00 (reference)	0.01(0.0006-0.168);0.013*	0.15(0.008-2.65);0.19
Lymphnode status Positive/Negative	53/154	21/63	6/3
OR (95% CI); P-value	1.00 (reference)	8.70(4.86-15.6); <0.0001*	1.45(0.35-6.01; 0.60
Metastasis Positive/Negative	63/138	32/52	1/8
OR (95% CI); P-value	1.00 (reference)	0.74(0.43-1.26; 0.27	3.65(0.44-29.0); 022

Significant at *p<0.05

Table 2: Genotype and Allele frequencies distribution for TIMP2 -418G/C polymorphism

TIMP-2 -418 G>C rs8179090	Genotype	Controls N=300 (%)	Cases N=300 (%)	Age adjusted odds ratios With χ^2 *p-values*	
				OR (95% CI)	**χ^2 p-value**
Co-dominant	G/G	192 (64)	207 (69)	1.00	
	G/C	93 (31)	84 (28)	0.84 (0.59-1.19)	0.28
	C/C	15 (5)	9 (3)	0.56 (0.24-1.30)	
Dominant	G/G	192 (64)	207 (69)	1.00	0.19
	G/C-C/C	108 (36)	93 (31)	0.80 (0.57-1.12)	
Recessive	G/G-G/C	285 (97.7)	291 (97)	1.00	0.21
	C/C	15 (2.3)	9 (3)	0.59 (0.25-1.36)	
Over dominant	G/G-C/C	207 (62.7)	216 (72)	1.00	0.42
	G/C	93 (37.3)	84 (28)	0.87 (0.61-1.23)	
Allele	G	477 (0.80)	498 (0.83)	1.00	
	C	123(0.20)	102 (0.17)	0.79 (0.59-1.06)	0.12
Log-additive				0.80 (0.60-1.07)	0.13
HWE(p)		0.38	0.84		

Significant at *p<0.05

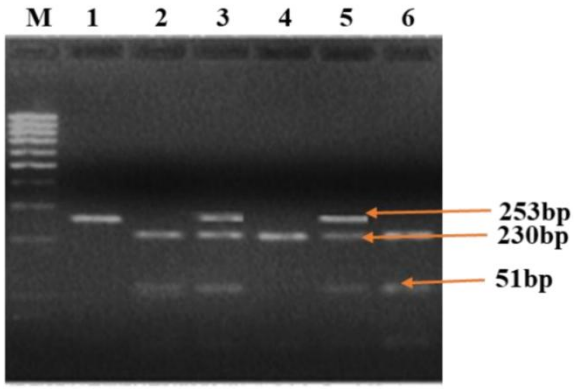

Figure 1: Electrophoresis of TIMP2 -418 G/C SNP PCR products
on 2.5% agarose gel
Lanes 1 Homozygous: CC (253+51 bp); Lanes 2,4 and 6: Homozygous: GG (230+51+23 bp); Lanes 3 and 5 Heterozygous: GC (235+230+51 bp) ;Lane M: 50 bp molecular marker

III. DISCUSSION

Breast cancer is the most prevalent cancer in the world. In the year 2020, approximately 2.3 million new cases of breast cancer were identified, and 7.8 million women had a history of breast cancer in last five years [12]. A cascade of numerous intracellular mechanisms, including genetic changes and signal transduction pathways, are involved in the progression of breast cancer [13]. Matrix metalloproteinases (MMPs), a class of zinc-dependent endopeptidases, and their physiological inhibitors, such as TIMPs, are crucial for numerous physiological processes, including tumor metastasis and invasion [14-15].

TIMP-2 is the only protein that interacts with a cell-membrane bound MMPs among TIMP family.TIMP-2 is ubiquitously expressed in the majority of cell types, where it works as an endogenous MMP inhibitor. In addition to its MMP-2-dependent actions, TIMP-2 can influence signaling pathways through direct interaction with non-cancerous and cancer cell surface receptors [16]. Multiple studies indicate that a high MMP2/TIMP2 ratio is related with an advanced stage of tumor development and a bad prognosis. Additionally, greater TIMP2 production has been related with enhanced survival odds [17].The current study aimed to evaluate the association of TIMP2 -418 G/C single nucleotide promoter polymorphism with epidemiological and clinical characteristics of breast cancer patients from south India.

In recent years, a substantial amount of studies were focused on the role of the TIMP2 gene in the progression of breast cancer; several functional single nucleotide polymorphisms (SNPs) were identified that affect transcriptional activity of human TIMP gene which in turn leads to the disease susceptibility [18]. TIMP2 gene promoter activity was found in a 519 base pair stretch upstream from the primary transcription initiation site. According to *Yves A De Clerck et al.,1994* a functional single nucleotide polymorphism in the TIMP2 promoter at position -418 bp that includes a G to C substitution (rs8179090) disrupts the Sp1 binding at its 5' end resulting in a decrease in the transcriptional activity of the TIMP2 gene [17].

A study by Mikołajczyk-Stecyna et al., 2015 [19] had shown TIMP2 -418 G/C single nucleotide promoter polymorphism as an independent risk factor for abdominal aortic aneurysm (AAA) patients.

According to Gai et al., 2010 [20] the -418 G/C polymorphism affects the tissue TIMP2 levels in hyper sensitive heart disease in patients of Han Chinese cohort and I was also found that subjects with C alleles had a decreased TIMP2 enzyme concentration than GG homozygotes. In addition, Klaus et al., 2017 [21] observed a decrease in the expression of the TIMP2 gene in AAA patients with -418 G/C polymorphism.

Peterson et al., 2009have shown polymorphisms in TIMP-2 to play a major role in susceptibility to breast cancer and survival, five SNPs rs4789932 (promoter), rs8064344, rs2277698, rs9916809 and rs11654470 (intron) were found to be associated with modest increase in breast cancer risk with Odds Ratios s ranging from 1.2 - 1.4, which was statistically significant in dominant models, but TIMP2 -418 G/C (rs8179090) was not their part of analysis [22].

A meta analysis by Egeblad and Werb, 2002 on TIMP2 -418 G/C revealed no significant association with acne risk and no heterogeneity was identified [23]. Further, Mandal et al., 2014 conducted a meta analysis with several molecular epidemiological factors to evaluate the probable association between the TIMP2 -418 G/C polymorphism and cancer susceptibility in various human cancers from different populations and have concluded that is no significant increased or decreased risk with the cancer progression [24]. The distribution of TIMP2 -418 G/C polymorphism in controls and cases in our study found no significant association of TIMP2 -418 G/C genotype and allele frequencies with breast cancer.

Similarly, Zhou, 2003 identified a moderately reduced risk of breast cancer in those carrying the TIMP2 -418 GC or CC genotype, with an adjusted OR of 0.76 compared to those carrying the GG genotype [25]. A study by Banday and Sameer, 2019 on ethnic kashmiri population have shown that heterozygous genotype (GC) of TIMP2 -418 G/C polymorphism to be significantly associated with an increased risk of colorectal cancer but the frequency of the variant genotype CC was completely absent in both controls and patient group which was the limiting factor for the study [26]. Further, in the present study the stratified analysis of breast cancer patient's clinical features revealed that heterozygote GC of TIMP2 -418 G/C polymorphism is found to be associated with positive lymph node status, Witzel et al., 2016 has suggested that axillary lymph node status may lead to the metastatic breast cancer during the progression of the disease[27].

The low number of homozygote individuals who possessed rare alleles was a significant shortcoming of our research, since it led to estimates of odds and hazard ratios that were not statistically reliable. In order to validate our findings, further research with larger sample number is required.

IV. CONCLUSION

Our results do support the hypothesis that TIMPs play a important role in the development and progression of breast cancer but our study on has found no association with BC. However there is need to understand the functional significance of the TIMP2 -418 G/C genetic variables that define breast cancer risk and disease outcome.

V. CONFLICT OF INTEREST

Authors declare no conflict of interest.

VI. ACKNOWLEDGEMENTS

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