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Research Article

Association of Il10 Gene Polymorphisms (Rs 1800896, Rs1800872) in Breast Cancer Patients

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Abstract: During course of cancer, the cytokine profile plays a critical role in tumor fate. The multi-functional cytokines with diverse applications are involved in cancer initiation, progression and elimination. The cytokine synthesis inhibitor molecule IL-10 with immune suppression and anti-angiogenic functions is considered to be prominent marker in cancer immunology. The SNPs in IL10 gene -1082 and -592 are linked with differential levels of IL-10 expression. The present case-control study deals with IL10 gene selected polymorphisms (rs180896 A/G and rs1800872 A/C) to explore their relation with breast cancer susceptibility and progression in south Indian patients.

We observed a significant association of rs1800896 with BC in our study group, where AA genotype showed a two fold increased risk towards BC and three fold risk towards metastasis. In-silico analyses strengthened our observation revealing the alteration in transcription binding site in the IL10 promoter by the mutant allele G. The IL10 rs1800872 polymorphism showed an association with the susceptibility. The AA genotype showed a seven fold increased risk towards BC. The haplotype A-A was found to be two fold risk towards breast cancer (OR=1.94). The study suggests varied roles of different polymorphisms of IL10 in the aetiopathogenesis of BC. Understanding the mechanism may help in the IL10 based immunotherapy for BC treatment.

Key words: cytokines. anti-angiogenic, breast cancer, immune suppression.

I. INTRODUCTION

Breast cancer (BC) is the most common malignancy threatening the health and life of women and it's incidence has increased in recent years in both developed and developing countries (**Meraj, 2015, Parveen, 2013**, **Miller, 2012**, **Chopra, 2001**). It is characterized as non-cutaneous malignancy associated with uncontrolled growth of ducts (ductal carcinoma) or lobules (lobular carcinoma) of the mammary gland (**Gomez, 2013**). These tumors can invade the surrounding tissues of the breast in later stages.

The etiology of breast cancer is extremely complex and its onset and progression is a multi step process resulting from a series of epigenetic, genetic, endocrine and external environmental factors like infectious agents (**Indu, 2009, Welch, 2000**). The role of genetic factors in epidemiology and pathogenesis of both sporadic breast cancer (genetic mutations) and familial breast cancer (inherited defects in DNA repair genes BRCA1 and BRCA2) (**Easton, 2007, Smith, 2006, Pharoah, 2002**), and failure of immune surveillance (increased numbers of tumor associated macrophages, agiogenic factors and imbalance in cytokine profile) is now well established (**Julian, 2008**). Site of tumors often found with cytokines and inflammatory mediators that influences immune suppression, growth of cancer cells, tissue remodeling and angiogenesis (**Bostjan, 2008**). Multifunctional cytokines which are related to development of immunological and inflammatory responses play an important role in the pathogenesis of cancer (**Smyth, 2004, kurzrock, 2001**)**.** Many reports have appeared which describe positive, equivocal or negative associations between cytokines such as tumor necrosis factor α (TNF-α) , interlukin-1β (IL-1β), interlukin-6 (IL-6) and interlukin-10 (IL-10) with human diseases, in respect of susceptibility, severity, or clinical outcome **(Morse, 1999)**. One such molecule IL-10 (Interleukin-10) is a key regulator of immune responses described as cytokine synthesis inhibitor, immune suppressive and anti agiogenic factor produced by Th2 cells and inhibits Th1 cells by inhibiting pro-inflammatory cytokines. In addition IL-10 can inhibit monocyte/macrophage functions including monokine synthesis, nitric oxide production, and major histocompatibility complex (MHC) class II and CD80/CD86 co stimulatory expression. In vitro and in vivo studies revealed pleotropic activities of IL-10 on B and T cells and , taken together, that a critical function of IL10 is to suppress multiple immune responses through individual actions on T cells and B cells, antigen presenting cells and other cell type, and skew the immune response from Th1 to Th2. In malignancy, IL-10 might promote tumor development, by acting to suppress anti- tumor immune responses. However, a number of other findings suggest that the biological properties of IL-10 are more complex may have immunostimulatory or

immunosuppressive effects, depending upon the assay used, cell type involved and other concomitant immune events, therefore the actions of IL-10 on tumor development may be more complex. In particular, animal models suggest that IL-10 can induce NK cell activation and facilitate anti-tumor responses, leading to tumor cell destruction (**Zhen, 1996, Kundu, 1996**).

Anti- tumor effects of IL-10 explained with various in vitro and transgenic studies in different tumors such as in prostate tumor cells showed that IL-10 stimulates tissue inhibitors of metalloproteinase (TIPPs) and inhibits matrix metalloproteinase (MMP) expression affecting induction of angiogenesis, similarly in malignant melanoma down regulation of agiogenic factors take place such as vascular endothelial growth factor (VEGF), Tumor necrosis factor- α (TNF-α), Interluekin-6 (IL-6), matrix metalloproteinase-9 (MMP-9) in tumor associated macrophages (**Fortis, 2003, Huang, 1999**). IL-10 over expression as well as deficiency was found under different pathological conditions depending on the cancers analyzed (**Qi Ding, 2013, Asadullah, 2003**). Some clinical researchers suggest that serum IL-10 levels might be a useful marker for monitoring the progression of breast cancer (**Ke-DA Yu, 2012, Howell, 2007, Garcia, 2000, Merendin, 1996)** which may assist in cancer cells escape from immune surveillance. IL10 gene comprises of 5 exons, spans; 5.2 kB, and is located on chromosome 1 at 1q31–1q32 (**Martin Howell, 2007**). Genetic variations that affect the production of IL10 could have an important effect on tumor genesis. The SNPs in the -1082 and -592 in the IL10 gene are linked with differential levels of IL-10 expression (**Turner et al., 1999**) and well studied in various malignancies, including Cutaneous malignant melanoma (**Howell , 2007**), Prostate cancer (**Michaud, 2001**), Cervical cancer (**Roh, 2002**) and Breast cancer (**Pooja, 2012, Funjun, 2010, Langsenlehner, 2005**) some showed positive and other are negative associations . Above observations indicate that the IL10 gene and haplotypes and its levels play a critical role in cancer patho-physioloy. We aimed to evaluate the individual and combined effect of IL10 SNPs G-1082A (rs1800870), C-592A (rs1800872) and associated serum levels in BC pathogenesis.

II. Materials and Methodology

Subjects

Blood samples were collected from a total of 570 individuals comprising of 285 BC patients and 285 healthy controls. The patients were recruited from the Osmania General Hospital and Railway Central Hospital, Hyderabad, India. The entire patient's were either histopathologically or cytologically confirmed as BC by pathologists of two hospitals. Patient's clinical and demographical information was obtained from medical reports and through personal interaction. The controls were healthy volunteers who are age-matched women who visited for general health check-up division at the two hospitals. The individuals entered into the study were unrelated residents of South India. The study has been approved by institutional ethical committee for Biomedical Research, Osmania University, Hyderabad, India, in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. written informed consent was obtained from all participating individuals at the time of sample collection.

Inclusion and Exclusion criteria

Patients were recruited based on the lump in the breast and confirmed by FNAC (Fine needle aspiration cytology) test. Patients who have other breast anomalies like abscess, phyllodes tumor and fibroids are excluded from the study. Control group was selected on the basis of age-matched healthy women with no family history of cancer and social habits like alcohol consumption and chewing tobacco.

III. Polymorphism analysis

Genomic DNA was isolated by salting out method using standard protocol (Tippisetty, 2011). Genotypes were assayed with Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (PCR-RFLP) method. The primers were obtained from previous literature (F:5-GTCAGTGTTCCTCCCAGT-3 and (R:5-TTACCTATCCCTACTTCCTC-3_ for rs1800896 polymorphism Lucia, 2003 and (F:5GGTGAGCACTACCTGACTAGC-3' and R:5'-CCTAGGTCACAGTGACGTGG-3') for rs1800872 (A-592C) Fanjun, 2010 . PCR was performed with a final volume of 10-µl reaction mixture containing 100 ng of genomic DNA, 0.2 µmol/l of each primer, 0.2 µmol/l of each dNTP, 0.2 µl of Taq DNA polymerase (5U Labpro India), 10× PCR buffer supplied by Labpro India. The PCR profile consisted of an initial melting step of 5 min at 94°C, followed by 27 cycles and annealing temperature 54°C; 55 s for rs1800872 and 52°C for rs1800896; and a final elongation at 72°C for 5 min. The restriction endonucleases *EraI* for rs1800896 and *RsaI* for rs1800872 SNP (New England Biolabs, UK) was used to distinguish the IL10 genotypes. The amplified products were run on 4% for rs1800896 and 2% agarose gel for rs1800872, containing ethidium bromide at 100V for 20 minutes. Post PCR amplification product of 370 base pairs (bp) was digested with *Era I* restriction endonuclease to observe fragment sizes of 175 bp for (GG), 195 for (AA) and 195, 175 for (GA) heterozygote for -1082 polymorphism and 412 base pairs (bp) was digested with *RsaI* and for -592 SNP 412 bp for wild type (CC), 236 bp, 176 bp for mutant type (AA) and 412 bp, 236 bp, 176 bp bands for heterozygous (CA) genotypes and under gel documentation system (UV tech, UK) which was shown in figure 1. Serum samples of 180 patients and 190 controls subjected to estimate the IL-10 levels by sandwich ELISA method (eBIOsciences).

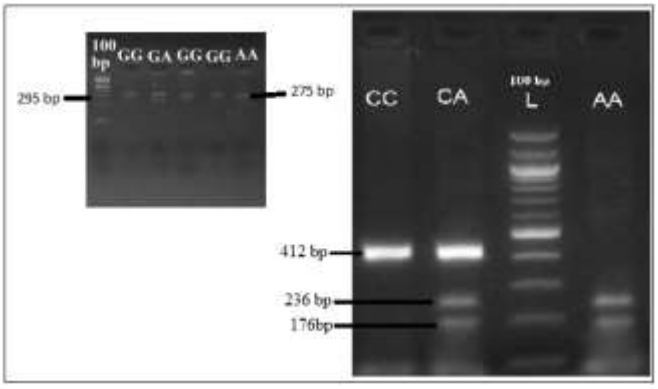

Figure 1:Gel images representing snps of IL10 gene rs 1800896 and rs1800872

IV. Statistical analysis

Descriptive statistics were used to calculate percentages, mean, and standard deviation. Chi- square test was used to compare the allele and genotype frequencies between patients and controls and further within the subgroups. The risk associated genotypes was calculated using odds ratio at 95 % confidence interval (CI) with 2-tailed level of significance (≤0.05). The total patients were stratified according to duration of disease (DOD) i.e <6 months and >6 months, but we did analysis for only <6 months DOD. Multi logistic regression analysis was performed for clinical parameters. Medical software tool was used to correlate the serum IL-10 levels in patients and healthy controls with SNPs. Data analysis was carried out by SPSS version 21.

V. Results

Demographic and clinical data The demographic and clinical information about the cases and the controls enrolled in the present study is summarized in table I&II. Significant variation was noted between patients and controls with respect to parity, consanguinity, BMI, breast feeding, area of living and occupation.

Table I: Demographical and clinical information of Breast cancer patients and healthy controls

*t test- Mean age, Age at onset of breast cancer (years), Body mass of index (BMI kg/m^2)

z test- Age at marriage(years), Age at first pregnancy, Parity, Consanguinity, Familial incidence, Diet, Lactation, Area of living, Occupation

Category	Controls (N %)	Breast cancer patients (N %)	t, Z test (p – Value)
*Mean age	(285) 48.45±13.49	(285) 51.29±10.75	0.005
Range (years)	18-90	25-86	
***Age at onset of breast cancer (years)**	-	48.5±11	
≤48	-	40.50±5.68 142(50)	-
>48		57.64±7.09 143(50)	
Age at marriage(years)	179(62)	185 (64)	0.69
≤18	105(37)	93 (33)	0.55
19-30	1(0.3)	7 (3)	-
>30	0(0)	0(0)	-
unmarried			
Age at first pregnancy	138(48)	107 (37)	0.08
≤18	144(50)	157 (54)	0.48
19-30	3(2)	9 (3)	0.92
>30	0(0)	12 (4)	-
0			
Parity	285(100)	273 (95)	<0.01
Parous	0(0)	12 (5)	-
Nulliparus			
Consanguinity	0(0)	29(10)	-
Yes	285(100)	256(98)	<0.01
No			
Familial incidence	-		-
No		206(72)	-
BC		15(5)	-

Other cancer		29(10)	-
Life style related diseases		35(12)	
Variables			
Diet	35(12)	40(14)	0.79
Vegetarians	250(88)	245(86)	0.50
Non-vegetarians	24.5±3.8	24.9±5	0.23
***Body mass of index(BMI(kg/m²))**	176(61)22.2±1.9	140(49)±21.3±2	<0.01
<25	108 (39), 28.27±3.1	145(51)±27.8±3.2	0.19
≥25			
Breast Feeding	200(100)	279(98)	<0.01
Yes	0(0)	6(2)	-
No			
Area of living	95(33)	66 (23)	0.16
Rural	190(66)	219 (77)	<0.01
Urban			
Occupation	107(37)	165(58)	<0.01
House wifes	177(62)	120(42)	<0.01
Agriculture+workers			

Table II: Clinical characteristics of BC patients:

Clinical parameters	Values
Tumor stage	
T_{1-2}	160(56)
T_{3-4}	125(44)
Lymph node status	
LN-ve	48(17)
LN+ve	237(83)
Metastatic	
M0	237(82)
M1	48(18)
Duration of Disease	
<6 months	150(52)
7-12 months	83(29)
>12 months	52(19)
DOD	
<6months	
T_{1-2}	100(66)
T_{3-4}	50(44)
LN-ve	34(22)
LN+ve	116(78)
M0	130(87)
M1	20(13)

Genotype and allele distribution

The genotype and allele frequencies of IL10 gene polymorphism rs 1800896 in the BC patients, subgroups and healthy controls are shown in table III. The genotype frequencies were AA, GA and GG 13%, 56%, 31% in controls 21%, 51%, 28% in patients respectively. There was statistically significant difference was observed in the distribution of rs1800896 genotypes between patients and controls ($\chi 2$=6.07; p<0.01). Though we find statistical significant association of homozygous AA genotype showed 1.74 fold risk with BC patients compared to others (OR: 1.74(CI: 1.11-2.74) p<0.01). Further, the genotype frequencies were compared in subgroups after categorizing according to mean age at onset viz..≤49 and ≥50 years of patients. There was no significant difference was observed in genotypes and allele frequencies ($\chi 2$=0.09; p>0.05). In addition, the homozygote mutant genotype AA showed a significant association with metastatic cases ($\chi 2$=11.79; p<0.01) and odds ratio value were significant (OR: 3.05 (CI: 1.54-6.02) p<0.01), same results are revealed in multiple logistic regression analysis (MLR) showed (OR: 4.23 (CI: 1.77-10.07) p<0.05) data not shown.

Table III**:** Genotype distribution and allele frequencies of IL10 rs1800896 in Breast cancer patients, healthy controls and

subgroups

rs no.1800896	AA N(%)	AG N(%)	GG N(%)	χ^2 value (p-value)	Minor Allele frequency G	Group Comparison	OR (95% CI)	p-value
Controls	37(13)	159(56)	89(31)	6.07(<0.01)	0.59	AA vs. others	1.74(1.11-2.74)	<0.01
						GA vs. others	0.83(0.59-1.15)	0.31
Patients	59(21)	146(51)	80(28)		0.54	GG vs. others	0.85(0.59-1.23)	0.46
AAO								
≤48 years (143)	30(21)	72(50)	41(29)	0.09(0.95)	0.54	AA vs. others	1.03(0.58-1.83)	1
						GA vs. others	0.93(0.58-1.48)	0.81
≥49 years (142)	29(20)	74(52)	39(27)		0.58	GG vs. others	1.06(0.63-1.78)	0.89
Clinical parameters								
								1
T1-2(160)	33(20)	81(50)	46(29)	0.08(0.95)	0.54	AA vs. others	1.01(0.56-1.80)	0.90
						GA vs. others	1.05(0.66-1.68)	0.79
T3-4(125)	26(21)	65(52)	34(27)		0.53	GG vs. others	0.92(0.54-1.560	
								0.84
LN-ve(48)	9(18)	26(55)	13(27)	0.22(0.89)	0.54	AA vs. others	1.15(0.52-2.55)	0.75
						GA vs. others	0.86(0.46-1.6)	1
LN+ve(237)	50(21)	120(51)	67(28.2)		0.54	GG vs. others	1.06(0.52-2.12)	
M0(237)	40(16)	125(53)	72(31)	11.79(<0.01)	0.57	AA vs. others	3.05(1.54-6.02)	<0.01
						GA vs. others	0.72(0.38-1.35)	0.34
M1(48)	19(38)	21(44)	8(17)		0.39	GG vs. others	0.45(0.20-1.0)	0.07
H.W.E: Controls- $\chi 2$= 6.7 (p<0.05) , Patients –$\chi 2$= 0.25 (p>0.05)								

The stratified data on duration of the disease (DOD) ≤ 6 months revealed significant difference in genotypes with metastatic cases. The heterozygote genotype GA showed reduced risk (OR: 0.32(CI: 0.11-0.89) p<0.05) and with homozygote wild genotype increased risk (OR: 5.43(1.96-15.0) p<0.01) table V.

Another SNP of IL10 gene rs 1800872 shown a statistical significant difference in distribution of genotypes between patients and controls ($\chi 2$=38.9; p<0.01). The observed CC, CA and AA genotype frequencies were 21%, 76%, 3%, in controls 11%,71%, 18%, in patients respectively showed in table IV. Elevated frequency of AA genotype in patients was noted and the odds ratio values were significant (OR:

6.48 CI: 3.30-14.18 p<0.01). The CC genotype find to be more in controls (OR: 0.48 CI: 0.30-0.77 p<0.05). However, we did not observe any variation with respect to allele frequencies between patients and controls (p>0.05).

Table IV**:** Genotype distribution and allele frequencies of IL10 gene rs1800872 in Breast cancer patients, healthy controls and subgroups

rs no.1800872	CC N (%)	CA N (%)	AA N (%)	χ^2 value (p-value)	Minor Allele frequency (A)	Group Comparison	OR (95% CI)	p-value
Controls	59(20.8)	217(76.1)	9(3.2)	38.9(0.01)	0.41	CC vs. others	0.48(0.30-0.77)	<0.05
						CA vs. others	1.16(0.77-1.74)	0.4
Patients	32(11)	201(71)	52(18.2)	3.23(0.19)	0.54	AA vs. others	6.84(3.30-14.18)	<0.01
AAO							1.76(0.82-3.75)	0.18
≤48(143)	20(15)	101(70)	22(15)	5.07(0.07)	0.51	CC vs. others	1.01(0.60-1.68)	1
						CA vs. others	0.67(0.36-1.24)	0.22
≥49(142)	12(9)	100(70)	30(21)	0.52(0.76)	0.56	AA vs. others		
Clinical parameters				0.27(0.87)			0.99(0.47-2.08)	1
							0.61(0.36-1.02)	0.06
	18(11)	120(75)	22(14)		0.51	CC vs. others	1.98(1.07-3.64)	0.03
T1-2(160)						CA vs. others		
	14(11)	81(65)	30(24)		0.56	AA vs. others		
T3-4(125)							0.9(0.34-2.37)	0.99
							0.85(0.42-1.70)	0.73
	6(13)	35(73)	7(14)			CC vs. others	1.37(0.57-3.25)	0.54
LN-ve(48)					0.51	CA vs. others		
	26(11)	166 (70)	45(19)			AA vs. others	0.47(0.17-1.31)	0.18
LN+ve(237)					0.54		0.90(0.46-1.76)	0.86
							1.20(0.56-2.64)	0.68
	27(11)	168(71)	42(18)		0.53	CC vs. others		
M0(237)						CA vs. others		
	5(10)	33(69)	10(20)		0.55	AA vs. others		
M1(48)								
H.W.E: Controls- $\chi 2$= 92.2 (p<0.05) , Patients –$\chi 2$= 49.6 (p<0.05)								

Further, the stratification of data based on Age at onset of patients showed no significant variation of genotypes between age at onset ≤49 years and ≥50 years of patients. However, no genotypic significant variation was observed in tumor size, lymph node, metastasis and DOD (p>0.05) showed in table V.

Table V**:** Genotype distribution of IL10 gene rs1800896, rs1800872 within <6months DOD

rs1800896	AA N (%)	AG N (%)	GG N (%)	χ^2 value (p-value)	Minor Allele frequency G	Group Comparison	OR (95% CI)	p-value
DOD <6months					0.54	AA vs. others	1.07(0.43-2.61)	1
T1-2(100)	17(17)	57(57)	26(26)	1.91(0.38)		GA vs. others	0.64(0.32-1.27)	0.22
					0.59	GG vs. others	1.60(0.77-3.3)	0.25
T3-4(50)	9(18)	23(46)	18(36)					
LN-ve(34)	4(12)	21(62)	9(26)	1.49(0.47)	0.57	AA vs. others	1.77(0.56-5.49)	0.44
						GA vs. others	0.64(0.29-1.40)	0.32
LN+ve(116)	22(19)	59(51)	35(30)		0.56	GG vs. others	1.36(0.57-3.2)	0.53
M0(130)					0.58	AA vs. others	5.43(1.96-15.0)	<0.01
	17(13)	74(57)	39(30)	12.69(0.01)		GA vs. others	0.32(0.11-0.89)	<0.05
M1(20)	9(45)				0.40	GG vs. others	0.77(0.26-2.28)	0.79
		6(30)	5(25)					
rs1800872	CC N (%)	CA N (%)	AA N (%)	χ^2 value (p-value)	Minor Allele frequency A	Group Comparison	OR (95% CI)	p-value
DOD <6months						CC vs. others	1.62(0.63-4.15)	0.32
T1-2(100)	12(12)	76(76)	12(12)	1.04(0.59)	0.50	CA vs. others	0.80(0.33-1.72)	0.69
						AA vs. others	0.82(0.27-2.40)	0.79
T3-4(50)	9(17.5)	36(72.5)	5(10)		0.46			
<6months						CC vs. others	0.93(0.31-2.75)	
LN-ve(34)	5(14.2)	25(74.2)	4(11)	0.03(0.98)	0.49	CA vs. others	1.07(0.45-2.55)	1
						AA vs. others	0.95(0.28-3.12)	1
LN+ve(116)	16(13)	87(75)	13(12)		0.49			1
<6months					0.49		1.72(0.51-5.75)	
M0(130)	17(13)	98(75)	15(11)			CC vs. others	0.73(0.25-2.06)	0.48
	4(20)				0.45	CA vs. others	0.88(0.18-4.1)	0.58
M1(20)		14(70)	2(10)	0.69(0.70)		AA vs. others		1

Haplotype analysis

The estimated haplotype frequencies of IL-10 polymorphisms in breast cancer patients, controls shown in table VI. Four possible haplotypes were demonstrated in our population. The most frequent haplotype in both patients and controls was AA haplotype (harboring wild type and mutant alleles of all two positions and with 0.26 % frequency in patients vs. 0.20 % in controls. The frequencies of haplotype were investigated and significant differences were observed between patients and healthy controls. Analysis of IL-10 promoter SNPs and breast cancer revealed that AA haplotype was associated with a significantly increased two fold risk compared with other haplotypes (OR: 1.94 (CI: 1.27 - 2.95) p<0.05). In addition, we not found any association between age at onset, tumor size, lymph node and metastasis, DOD.

Table VI: Haplotype of IL10 (rs1800896) and (rs1800872) gene polymorphisms association and odds ratios with clinical parameters in south Indian Breast cancer patients

SNO	HAPLO TYPE		PATIENTS Vs CONTOLS	AAO <48 YEARS VS ≥50YEARS PATIENTS	TUMOR SIZE	LN -STATUS	METASTASIS	DOD<6 months vs >7 months
C1	C	G	1.00	1.00	1.00	1.00	1.00	1.00
C2	A	A	**1.94 (1.27 - 2.95) p<0.05**	1.26 (0.74 - 2.14) P>0.39	0.83 (0.49 - 1.41) P>0.49	0.85 (0.42 - 1.71) P>0.64	1.66 (0.83 - 3.31) P>0.15	1.09(0.65-1.84) p>0.05
C3	A	G	1.23 (0.67 - 2.26) P>0.51	1.83 (0.87 - 3.85) P>0.11	0.48 (0.23 - 1.02) P=0.05	0.78 (0.27 - 2.27) P>0.65	0.58 (0.18 - 1.87) P>0.37	1.47(0.70-3.08) p>0.05
C4	C	A	0.92 (0.53 - 1.59) P>0.76	1.40 (0.71 - 2.78) P>0.34	0.53 (0.26 - 1.06) P>0.07	0.90 (0.30 - 2.71) P>0.85	1.40 (0.64 - 3.08) P>0.4	0.99(0.50-1.97) p>0.05

Cytokine levels analysis

In our study we found that there was singnificant variation in IL-10 concentration between healthy controls and BC patients (F=11.94, p<0.01, Table 9). However, IL-10 concentration not correlated to SNPs and clinical parameters not showed significant association table VII.

Table VII: Mean serum levels of IL10 gene in Breast cancer patients, healthy controls and subgroups.

Source	Sum of Squares	DF	Mean Square	F	P
Age	20.052	1	20.052	1.319	0.252
Controls Vs patients	181.616	1	181.616	11.947	0.001
il10_1082	35.838	2	17.919	1.179	0.309
il10_592	50.054	2	25.027	1.646	0.194
il10-1082, il10-592	59.676	4	14.919	0.981	0.418
Tumor	22.466	1	22.466	1.281	0.260
Lymph node	4.506	1	4.506	0.257	0.613
Metastasis	6.776	1	6.776	0.386	0.535

LD and Hap map analysis

Linkage disequilibrium plot of IL10 gene shown SNPFs rs1800896 and rs1800872 are not in linkage they independently act on disease susceptibility, showed in figure 2. Hap map analysis revealed that the alleles in IL10 gene rs1800896 and rs1800872 showed in various studies conducted in different ethnic groups and Asian population studies and our study indicates nearly same in frequency, showed in figure 2.

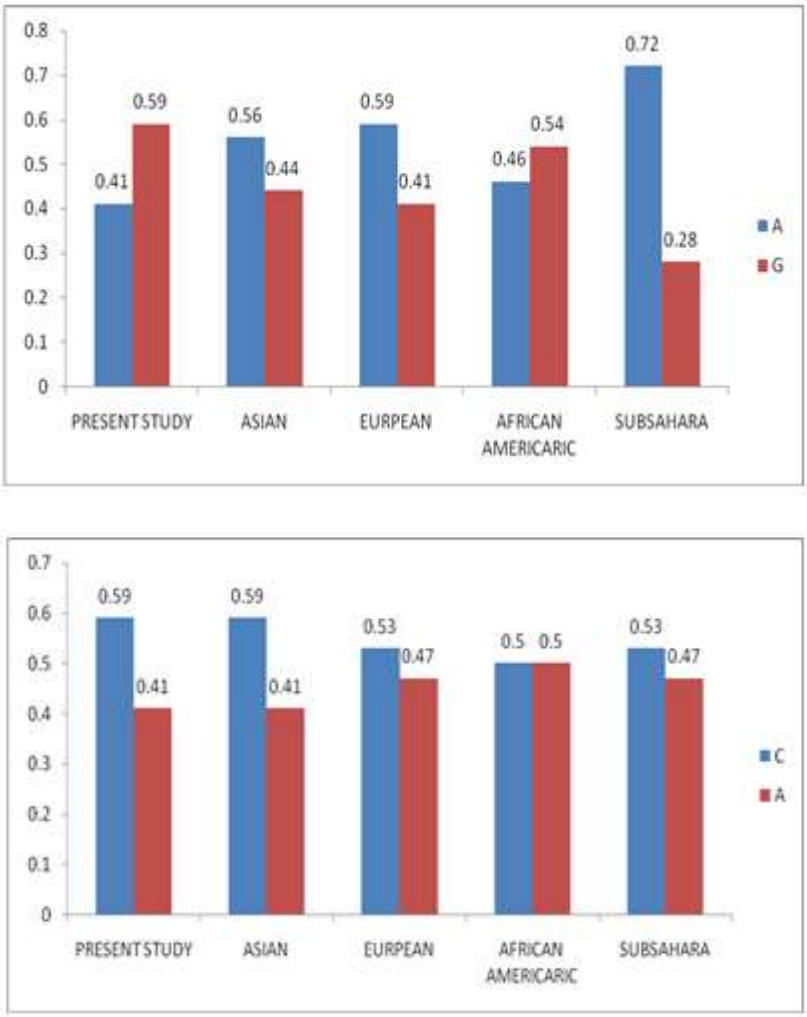

figure2: Graphical Representation of IL10 Gene Polymorphisms rs1800896 and rs1800872 Hapmap association

In-silico analysis: we used transcription binding finder tool (Alibaba 2.1) to IL10 SNPs rs1800896 and rs1800872 which was located promoter region of IL10 gene. The results are mentioned in figure-3.

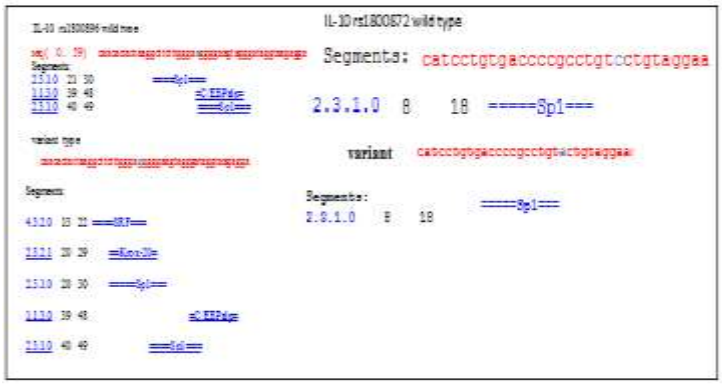

Figure 3: Transcription factors binding sites for IL10 gene rs SNPs 1800896 and rs1800872

VI. Discussion

Recent data suggest that polymorphic variations in the promoter sequences of the IL-10 gene may influences the gene expression and consequently play a certain role in susceptibility and clinical course of BC **(Gibson, 2001)**. Our data on rs1800896, a promoter polymorphism had revealed significant difference in genotype, but not with allele frequencies between BC patients and control subjects. However, the low producing genotype AA showed two fold risk seen in patients. **Turner, 1997** demonstrated that IL10-1082 A allele, AA genotype is responsible for lower IL10 production and associated with increased tumor growth.

These results were consistent with case control study conducted by **Lucia, 2003** reported that IL10 AA genotype is correlated with a marked increase in breast cancer risk. However, another study from western region of China found that IL10-1082 GG was associated with an increased malignancy risk (**Lu, 2005**). In analysis of DOD <6 months revealed that the heterozygote GA genotype acts as protective and AA showed five fold risk towards fast progression to metastasis. One more report on IL10-1082 polymorphism G allele is responsible for higher IL10 production which facilitate development of tumors and viral infections by suppressing the expression of MHC class I and II antigens and preventing tumor antigen presentation to CD8-cytotic T lymphocytes **(Susanne, 2003, Matsuda, 1994).** However, a recent report from south Indian population should IL-10-1082 AA genotype was associated with BC (**Cingeetham, 2015)**. Above observations clearly indicate that in our study G and A alleles of rs1800896 are involved in BC susceptibility and progression.

Another, IL10 SNP rs1800872, had revealed a significant difference in genotype frequencies, but not with respect to alleles between BC patients and healthy women. The seven-fold increased risk of BC seen in patients with low producing genotype (AA) and elevated frequency of low producing AA genotype in patients suggests that predisposing role of this polymorphism in South Indian women. Mata analysis study conducted by Zhi-jun Dai et al 2014 indicated that the average minor allele frequencies of IL-10 -1082 and -572 was 0.41 and 0.72, in our study minor allele frequencies not exceed the average of minor alleles so may got deviation from hardy Weinberg equilibrium. The present results are supported by the experimental studies with tumor models that have shown the association of the lower IL10 levels with increased tumor growth (**Karjalainen, 2003, Sharma, 1999, Halak, 1999**). Further**, Elizabeth et al .,2003** demonstrated that AA genotype of rs1800872 to be correlated with decreased levels of IL10. **Qi Ding, 2013** stated that AA variant genotype was associated with a decreased risk in smoking-related cancers, but not for BC, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, melanoma, lung cancer, hepatocellular carcinoma, Non-Hodgkin lymphoma or prostate cancer. On the contrary, an Austrian study by **Langsenlehner, 2010** reported AA genotype is associated with reduced BC risk. Further, several other studies from different populations have shown lack of association between this polymorphism and BC risk, including a study from North India (**Pooja, 2012, Langsenlehner, 2005 , Ke-Da, 2012, Erika, 2015**). Discrepant results of IL10 rs1800872 polymorphism in relation to breast cancer suggest a possible role of population difference in genetic background and the environment they lived in.

Further, Four possible haplotypes were demonstrated in our population. The most frequent haplotype in both patients and controls was AA haplotype (harboring wild type and mutant alleles of all two positions. The frequencies of haplotype were investigated in various populations had similar results with small differences. The CA haplotype (-1082 C and -592 A alleles) of IL10 polymorphisms was associated with younger age of BC diagnosis in European population (**Erika, 2015)**. Conversely, in Turkish population observed that haplotypes AC/AA frequency is high in BC patients than controls and lower frequency in lymph node negative BC cases **(Guzin, 2007)**. Similarly, the IL-10 (-1082) AA genotype and the ATA/ATA and ACC/ACC homozygous haplotypes were more frequent among patients with rapid fibrosis in HCV (Hepatitis C virus) infection **(Knapp, 2003).**

The IL-10 -1082 -819and -572 ACC/ATA 'low expression' haplotype and GCC/GCC 'high expression' haplotype producers (**Turner, 1997)**. In our study we noted low producing haplotype AA was found to be in the susceptibility. In vitro and in vivo studies revealed pleotropic activities of IL-10 on B and T cells and , taken together, that a critical function of IL10 is to suppress multiple immune responses through individual actions on T cells and B cells, antigen presenting cells and other cell type, and skew the immune response from Th1 to Th2. IL-10 differential promoter activity was observed between alleles of position -1082 , -592 and transcription factor PU.1 (**Reuss, 2002**), which encoded by the *SPI1* gene in humans and produces ETS-domain transcription factor that activates gene expression during myeloid and B-lymphoid cell development which may alter the anti tumor effect on BC.

The increase of IL-10 production with in tumor microenvironment might be protective and conversely, that low IL-10 producing capability makes individuals susceptible to more aggressive course of the disease **(Roba, 2014, , Cervenak, 2000, Groux, 1999)**. In diffuse large B cell lekemia (**Roba, 2014)**, SLE **(Ahmad , 2015)** observed that significantly increased serum IL-10 levels in patients. A study from china in AML patients confirmed that increased IL-10 levels was observed by performing RT-PCR analysis (Chen, 2013).

Some limitations, present study should be taken into account: potential confounding such as Estrogen, Progestron and herceptin 2 receptor status are not available and expression of IL-10 levels with RT-PCR may give us exact genotype dependant expression of IL-10. We suggest that screening of genetic variants in IL-10 gene among ethnic groups may help in find out the risk individuals in general population and understanding the disease patho-physiology as well as assesses the chemotherapy response, disease out come. several investigators have suggest therapeutic use of IL-10 in cancer patients **Huang, 1999**, **Zheng, 1996, Kundu, 1996,** but at present no clinical trails have been performed.

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