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Polymorphic variations in IL-1β, IL-6 and IL-10 genes, their circulating serum levels and breast cancer risk in Indian women

Singh Pooja [a], Preeti Chaudhary [a], Lakshma V. Nayak [a,1], Singh Rajender [a], Karan Singh Saini [a], Debashish Deol [b,2], Sandeep Kumar [b,3], Hemant Kumar Bid [a,4], Rituraj Konwar [a,*]

[a] Endocrinology Division, CSIR-Central Drug Research Institute, Lucknow, India
[b] Department of Surgery, Chhatrapati Sahuji Maharaj Medical University (CSMMU), Lucknow, India

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ABSTRACT

Background: Cytokines are known as important regulators of the entire gamut of cancer from initiation, invasion and metastasis. This fact and plethora of gene polymorphism data prompted us to investigate cytokine gene polymorphisms in breast cancer (BC) patients.
Methods: Selected polymorphisms in the IL-1β [−511 T > C (rs16944) and +3954 C > T (rs1143634)]; IL-6 [−174 G > C (rs1800795)]; IL-10 [−1082 A > G (rs1800896), −819 T > C (rs1800871) and −592 A > C (rs1800872)] genes were genotyped in 200 BC patients and 200 healthy volunteers in a case-control study using PCR-RFLP and direct DNA sequencing techniques. Peripheral cytokine levels were measured using ELISA. Allele and genotype data were analyzed for significance of differences between cases and controls using Chi-Square [χ^2] test. Two sided *P*-values of less than 0.05 were considered to be statistically significant.
Results: Peripheral level of all three cytokines did not show any significant difference between cases and controls. Allele and genotype frequency of IL-1β [−511 T > C (rs16944)] did not show any difference between cases and controls. On the other hand mutant allele and genotype at IL-1β [+3954 C > T (rs1143634)] associated with increased risk of BC. This was also true for pre-menopausal cases and for mutant genotype in post-menopausal cases. Mutant allele and genotypes at IL-6 [−174 G > C (rs1800795)] appeared to be protective in nature such that controls had a higher frequency of both mutant alleles and genotypes. None of the three SNPs in IL-10 gene associated with risk of BC, except significant association of mutant allele and genotypes of −1082 A > G (rs1800896) polymorphism with post-menopausal BC.
Conclusions: Mutant allele and genotype at IL-1β [+3954 C > T (rs1143634)] site associated with increased BC risk, while mutant allele and genotypes at IL-6 [−174 G > C (rs1800795)] polymorphism appeared to be protective. Also, there was significant association of mutant allele and genotypes of IL-10 [−1082 A > G (rs1800896)] with postmenopausal BC. None of the other polymorphisms investigated appear to affect BC risk.

1. Introduction

BC is the most common malignancy in women and a family history of BC is one of the most important and consistent risk factors. BRCA1 and BRCA2 gene mutations are inherited in an autosomal dominant manner and confer a high risk [1,2] but account for only a small percentage of BC cases [3]. It is highly likely that a number of low penetrance genes contribute to BC susceptibility, thus accounting for a higher proportion of the disease burden [3–6]. Complex diseases such as BC do not have ideal co-segregation of specific loci due to incomplete penetrance, phenocopy, genetic heterogeneity and polygenic inheritance. Genetic polymorphisms have been recently considered to significantly affect the development

Abbreviations: BC, breast cancer; IL, interleukin; SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio; ELISA, enzyme linked immunosorbant assay; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

* Corresponding author. Endocrinology Division, CSIR- Central Drug Research Institute, Lucknow, U.P. 226 001, India. Tel.: +91 9451408487; fax: +91 522 2623938.
E-mail address: r_konwar@cdri.res.in (R. Konwar).
[1] Present address: Pharmacology Division, Indian Institute of Chemical Technology (IICT), Uppal Road, Tarnaka, Hyderabad 500 007, Andhra Pradesh.
[2] Present address: Department of Surgical Oncology, Dr. B.R.A Institute-Rotary Cancer Hospital (IRCH), All India Institute of Medical Sciences (AIIMS), New Delhi.
[3] Present address: Director, All India Institute of Medical Sciences (AIIMS), Bhopal 462 026, Madhya Pradesh, India.
[4] Present address: Center for Childhood Cancer (WA-5109), The Research Institute at Nationwide Children's Hospital, 700 Children's Drive, Columbus, Ohio 43205.

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and progression of common complex diseases [7]. Cytokines are small molecules secreted by cells in response to specific stimuli and change the behavior of the same or other cells. Multi-functional cytokines are related to the development of inflammatory and immunological responses which play a crucial role in the pathogenesis of autoimmune and malignant diseases [8,9], making them important candidate genes in BC.

IL-1 family consists of cytokines IL-1α, IL-1β and IL-1RA (receptor antagonist) that participate in various physiologic and patho-physiologic processes [10]. IL-1α and IL-1β are produced by monocytes, macrophages and epithelial cells and are involved in various processes such as modifying host response to microbial invasion, tissue injury and inflammation [11]. IL-6, a phosphorylated glycoprotein containing 185 amino acids, is involved in processes like inflammation, bone metabolism, synthesis of C-reactive protein, and carcinogenesis [12,13]. As a growth factor it plays a significant role in cell differentiation and is believed to be involved in tumor progression [14]. But its role in tumorigenesis of different cancer types is nonetheless still controversial, especially in BC [15]. IL-10 is a multifunctional immuno-regulatory cytokine with anti-angiogenic functions involved in the development and progression of various tumors [16]. It may act as a two-edged sword. On one hand it exhibit cancer promoting immunosuppressive effect and on the other hand it has cancer inhibiting anti-angiogenic property.

Considering the diverse roles of cytokines in cell growth, proliferation, differentiation and migration in inflammation and cancers, we proposed that polymorphisms in the cytokine genes could affect the risk of BC. We, therefore, analyzed selected SNPs of the interleukin cytokine gene family in BC patients. In a case-control design, we analyzed IL-10 [−1082 A > G (rs1800896)], [−819 T > C, (rs1800871)], [−592 A > C (rs1800872)]; IL-1β [−511 T > C (rs16944)], [+3954 C > T (rs1143634)] and IL-6 [−174 G > C (rs1800795)] polymorphisms in BC patients from India.

2. Subjects and methods

2.1. Patient and healthy subjects

This study was undertaken with prior approval from the Institutional Ethical Committee of the Chhatrapati Sahuji Maharaj Medical University (CSMMU), Lucknow. Subjects were recruited from the clinic of Department of Surgery, CSMMU, Lucknow, India. A total of 200 patients and 200 healthy volunteers were recruited for the study. Healthy controls were recruited from the out-patient department and staff members of the Department of Surgery. The controls had no family history of BC and all had been clinically confirmed and/or had a recent mammogram confirming that there was no detectable BC at the time of sampling. Staging of the tumor was done according to the standard TNM classification [17]. Tumors were graded using the Bloom-Richardson grading system [18].

2.2. Peripheral levels of IL-1β, IL-6 and IL-10

Circulating peripheral levels of IL-1β, IL-6 and IL-10 were measured with commercially available ELISA kits according to the manufacturers' instructions. ELISA kits were procured from Diaclone (Gen-probe) and Ray-Biotech Inc. For ELISA, 2 ml peripheral blood of the patients and controls was collected followed by isolation of serum. Samples with inadequate serum quantity or quality due to heamolysis were excluded from biochemical measurements. Serum samples measured in triplicate showing more than three time variation from mean value were excluded from the study.

2.3. Genotyping

Genomic DNA was isolated from 2–4 ml of peripheral blood from subjects with already reported method [19]. The primers for PCR amplification of the candidate polymorphisms were designed using GeneTool software and synthesized commercially (Eurofins, India). Primer sequences for IL-1-β [−511(T > C), +3954 (C > T)] polymorphic sites were, Forward: 5′-GCCTGAACCCTGCATACCGT-3′ and reverse: 5′-GCCAATAGCCCTCCCTGTCT-3′; forward: 5′-CCCCACTCCCAGCTTCATCCC-3′ and reverse: 5′-CGTCTAATTTCT CAGGGTCACACTC-3′ respectively. PCR reactions were performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, and polymerization at 72 °C for 45 s, with a final polymerization step at 72 °C for 7 min. Primers sequence for IL-10[−1082(A > G), −819(T > C), −592(A > C)] polymorphic sites were, Forward : 5'-GCCTGAACCCTGCATACCGT-3' and reverse:5'-GCCAATAGCCCTCCCTGTCT-3'; forward: 5′-CGCAACCCA ACTGGCTCCCCTTAC-3′ and reverse: 5′-GTGGGTTCTCATTCGCGTG TTC-3′ respectively. PCR reactions were performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 20 s and polymerization at 72 °C for 45 s, with a final polymerization step at 72 °C for 7 min. The PCR products thus generated were purified and directly sequenced using dideoxy cycle sequencing chain termination method [Big Dye V3.1, Applied Biosystems, Foster City, Ca, USA] on ABI 3730 DNA analyzer [19]. Sequence editing and multiple alignments were carried out using AutoAssembler software [Applied Biosystems, USA].

IL-6 genotyping for [−174(G > C)] site was carried out using already reported PCR-RFLP method. Primers used for PCR amplification were forward: 5′-ATGCCAAGTGCTGAGTCACTA-3′ and reverse: 5′-TCGAGGGCAGAATGAGCCTC-3′. PCR reactions were performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, and polymerization at 72 °C for 30 s, with a final polymerization step at 72 °C for 7 min. For variant specific restriction digestion, 10 μl of PCR product was incubated with 2 unit of *Nla III* enzyme as per manufacturer's recommended protocol. The digested PCR products were visualized on 2% Agarose gel to make out differences in the digestion pattern [20]. Randomly selected samples were subjected to direct DNA sequencing to validate the results of PCR-RFLP.

2.4. Statistical analyses

The control group genotype data for all SNPs were analyzed for fitness in Hardy Weinberg equilibrium with no significant deviation observed in any case. The allele and genotype percentage for all the polymorphisms were calculated and analyzed using Chi-Square [χ^2] test for significance of differences between cases and controls. The chi-square, odds ratio and 95% confidence interval [CI] values were calculated using the online Vassar Stats Calculator [www. faculty.vassar.edu/lowry/VassarStats.html]. Two sided *P*-value of less than 0.05 was considered to be significant. We also compared the allele and genotype distribution for pre- and post-menopausal patients with that of all controls.

3. Results

3.1. Subject demographics

The age of the control subjects was between 28 and 90 years with a mean age of 39 years (SD, 11.30) (Table 1). The patients comprised of 107 pre-menopausal and 93 post-menopausal wo-

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Table 1
Demographics of study subjects.

Variables	Cases	Controls
Age (mean ± SD)	44.92 ± 13.56	39 ± 11.30
BMI (kg/m^2, mean ± SD)	22.34 + 5.37	24.21 ± 4.92
Age at menarche (years, mean ± SD)	13.29 ± 1.54	13.31 ± 1.48
Menopausal status		
Pre-menopausal	107	112
Post-menopausal	93	88
Family history		
Positive	10	0
Negative	190	200
Tobacco chewing/smoking habit		
Yes	20	10
No	180	190

men. Out of the total, 96 (48%) patients had cancer in right breast, 98 (49%) in left breast and only 6 (3%) had bilateral BC. The mean age of the patient cohort was 44.92 years [SD 13.56] with range of 22–90 years. The mean tumor size was found to be 125.93 cm^3 [SD, 324.43] with a range of 3–1150 cm^3. Majority of patients (N = 101, 50.5%) were presented in stage 3, followed by stage 2 (N = 75, 37.5%) and stage 4 (N = 24, 12%). None of the patients was found to be in stage 1.

3.2. Association between IL-1β and BC risk

The promoter polymorphism of IL-1β (−511 T > C) did not show any significant difference between cases and controls; neither in overall nor in sub-group comparison (Tables 2 and 3). However, allele and genotype frequency at IL-1β (exon 5, +3954 C/T) polymorphic site were significantly different between cases and controls (P = 0.009 and 0.0002, respectively) such that the presence of the mutant allele or genotype increased the risk of BC (Table 4). Analysis as per menopause status showed strong association of mutant allele and genotypes with pre-menopausal group (Table 5). Similarly, mutant allele and genotypes were significantly more frequent in the post-menopausal group, however; only genotype frequency reached statistical significance (Table 5).

3.3. Association between IL-6 and BC risk

Mutant genotypes at IL-6 (−174 G > C) polymorphic site appeared to be protective such that patients had significantly lower frequency of mutant genotypes; however, allele frequency was at the borderline (Table 6). In sub-group analysis, presence of both, mutant alleles and genotypes appeared to be protective, though the difference was significant only in the case of genotypes (Table 7).

3.4. Association between IL-10 and BC risk

None of the three polymorphisms of IL-10 gene (−1082 A > G, −819 T > C, −592 A > C) showed statistically significant difference between cases and controls. The only significant difference was observed at −1082 A > G polymorphic site between post-menopausal group and controls (Tables 8–13) such that over-representation of mutant allele and genotypes increased the risk of BC.

3.5. Serum Cytokine levels in BC patients and normal subjects

The cytokine level in a random subset of BC patients (n = 20) and normal subjects (n = 20) were measured using ELISA kits. We did not observe any significant difference in the mean levels of interleukins, IL-1β (P = 0.23), IL-6 (P = 0.86) and IL-10 (P = 0.0836) between cases and controls (Table 14).

4. Discussion

Very few studies have analyzed IL-1β gene polymorphism in breast cancer till now. We could find only three previous reports analyzing this polymorphism. Two studies on Caucasian women showed no association with BC [21,22]. In contrast, a case-control analysis on a Chinese population suggested that −511 TT genotype was a risk factor for BC [23]. Similarly, the earlier report on risk associated with IL-1β (+3954 C > G) polymorphism is inconsistent. The presence of mutant allele and genotypes at +3954 C > T site is associated with increased BC risk in our population. Only two previous studies have analyzed this polymorphism, of which one reported a highly significant association between the mutant allele and the aggressive phenotype of breast carcinoma defined by the high histological grade, axillary lymph node metastasis and large tumor size [24]. The second study analyzed its correlation with survival in BC patients [22]. It was found that presence of homozygous mutant genotype associated with overall survival in univariate but not in multivariate analysis. Our study is the first report suggesting association of mutant allele and genotypes of IL-1β (+3954 C > G) with BC risk.

The IL-6 polymorphism (−174 G > C) has been reported to influence plasma concentration of the corresponding cytokine in response to different inflammatory stimuli [25–27]. In this connection, it has been studied for association with many inflammatory and neoplastic diseases including BC; however, the association status of mutant allele and genotypes across populations is largely contradictory. Most of the previous studies have analyzed this polymorphism in different context. A study on a French Caucasian population showed no association with BC diagnosis or prognosis [15]. A similar study conducted on Caucasian women found increased risk with mutant allele; however, the difference was not significant [28]. A meta-analysis conducted on 25,703 subjects suggested that this polymorphism was not associated with BC risk [29]. DeMichele et al. [30] found that −174G/C polymorphism is associated with clinical outcome in a cohort of node-positive BC patients who received high–dose adjuvant therapy [30]. Similarly, a study conducted by Iacopetta et al. [31] on Australian BC subjects concluded that −174 C allele was associated with a more aggressive BC phenotype [31]. A dose dependent correlation between −174C allele and BC was also reported [21]. We are the first to report protective effect of this polymorphism in BC risk. However,

Table 2
Allele and genotype distribution of IL-1B Promoter (−511) locus among cases and controls.

Alleles	Wild type (T)	Mutated (C)	Comparison	OR	CI (95%)	
Cases (200)	263 (65.75%)	137 (34.25%)	χ^2 = 0.27, P = 0.603	0.926	Lower	Upper
Controls (200)	256 (64%)	144 (36%)			0.692	1.238
Genotype	Wild type (TT)	Mutated (CT + CC)	Comparison	OR	CI (95%)	
Cases (200)	93 (46.5%)	107 (53.5%)	χ^2 = 1.46, P = 0.226	0.783	Lower	Upper
Controls (200)	81 (40.5%)	119 (59.5%)			0.527	1.163

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Table 3
Comparison of allele and genotype data of IL-1B Promoter (−511) variants between cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95% CI)	POSTMENOPAUSE		P	OR (95% CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Genotypes								
T/T	52 (48.6%)	81 (40.5%)	0.172	0.719	41 (44.0%)	81 (40.5%)	0.559	0.863 (0.52–1.41)
CC + CT	55 (51.4%)	119 (59.5%)		(0.44–1.15)	52 (56.0%)	119 (59.5%)		
Alleles								
T	147 (68.7%)	256 (64.0%)	0.243	0.810	116 (62.4%)	256 (64.0%)	0.698	1.072
C	67 (31.3%)	144 (36.0%)		(0.56–1.15)	70 (37.6%)	144 (36.0%)		(0.74–1.53)

Table 4
Allele and genotype distribution of IL-1β (exon 5 + 3954) locus among cases and controls.

Alleles	Wild type (C)	Mutated (T)	Comparison	OR	CI (95%)	
Cases (200)	340 (85%)	60 (15%)	$\chi^2 = 6.82$, $P = 0.009$	1.784	Lower	Upper
Controls (200)	364 (91%)	36 (9%)			1.150	2.767
Genotype	Wild type (CC)	Mutated (CT + TT)	Comparison	OR	CI (95%)	
Cases (200)	147 (73.5%)	53 (26.5%)	$\chi^2 = 13.53$, $P = 0.0002$	2.644	Lower	Upper
Controls (200)	176 (88%)	24 (12%)			1.556	4.490

Table 5
Comparison between allele and genotype data of IL-1β (exon 5 + 3954) in cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95%CI)	POSTMENOPAUSE		P	OR (95%CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Alleles								
C	178 (83.2%)	364 (91.0%)	0.004	2.044 (1.25–3.36)	160 (86.0%)	364 (91.0%)	0.068	1.64 (0.96–2.81)
T	36 (16.8%)	36 (9.0%)			26 (14.0%)	36 (9.0%)		
Genotype								
C/C	75(70.1%)	176 (88.0%)	0.000	3.128	71 (76.3%)	176 (88.0%)	0.010	2.27 (1.19–4.31)
CT + TT	32 (29.9%)	24 (12.0%)	1	(1.73–5.67)	22 (23.7%)	24 (12.0%)		

Table 6
Allele and genotype frequencies at IL-6-174 (G > C) locus in cases and controls.

Alleles	(G)	(C)	Comparison	OR	CI (95%)	
Cases (200)	270 (67.5%)	130 (32.5%)	$\chi^2 = 3.68$, $P = 0.05$	0.753	Lower	Upper
Controls (200)	244 (61.0%)	156 (39.0%)			0.563	1.006
Genotype	(GG)	(GC + CC)	Comparison	OR	CI (95%)	
Cases (200)	80 (40.0%)	120 (60.0%)	$\chi^2 = 8.86$, $P = 0.002$	0.527	Lower	Upper
Controls (200)	52 (26.0%)	148 (74.0%)			0.3449	0.8054

Table 7
Comparison allele and genotype variants of IL-6-174 (G > C) locus between cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95%CI)	PREMENOPAUSE		P	OR (95%CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Alleles								
G	145 (67.7)	244 (61.0)	0.097	0.744	125 (67.2)	244 (61.0)	0.147	0.763 (0.52–1.10)
C	69 (32.2)	156 (39.0)		(0.52–1.05)	61 (32.7)	156 (39.0)		
Genotypes								
G/G	44 (41.1)	52 (26.0)	0.006	0.503	36 (38.7)	52 (26.0)	0.02	0.556 (0.32–0.93)
CC + GC	63 (58.8)	148 (74.0)		(0.30–0.82)	57 (61.2)	148 (74.0)		

this polymorphism is a subject for further investigation before a consensus could develop.

We observed no association of any of the three polymorphisms in IL-10 gene with BC. A previous study on −1082 G > A polymorphism found that AA genotype is correlated with a marked increase in BC risk [32]. However, relatively higher numbers of studies support lack of correlation between this polymorphism and BC [33–35]. Bulpitt et al. [33] using a large sample size (2000 cases and

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Table 8
Allele and genotype frequencies at IL-10 (−1082) locus in cases and control.

Alleles	Wild type (A)	Mutated allele (G)	Comparison	OR	CI (95%)	
Cases (200)	324 (81%)	76 (19%)	$\chi^2 = 2.27$, $P = 0.131$	1.329	Lower	Upper
Controls (200)	340 (85%)	60 (15%)			0.917	1.926
Genotype	Wild type (AA)	Mutated (AG + GG)	Comparison	OR	CI (95%)	
Cases (200)	132 (66%)	68 (34%)	$\chi^2 = 1.98$, $P = 0.159$	1.358	Lower	Upper
Controls (200)	145 (72.5%)	55 (27.5%)			0.886	2.080

Table 9
Comparison allele and genotype data of IL-10(−1082) variants between cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95%CI)	POSTMENOPAUSE		P	OR (95%CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Genotype								
A/A	77 (72%)	145 (72.5%)	0.920	1.027	55 (59.1%)	145 (72.5%)	0.022	1.821 (1.08–3.05)
AG + GG	30 (28%)	55 (27.5%)		(0.60–1.73)	38 (40.8%)	55 (27.5)		
Alleles								
A	180 (84.1%)	340 (85%)	0.777	1.070	144 (77.4%)	340 (85%)	0.024	1.652
G	34 (15.8%)	60 (15%)		(0.67–1.69)	42 (22.5%)	60 (15%)		(1.06–2.56)

Table 10
Allele and genotype frequencies at IL-10 (−819) locus in cases and controls.

Alleles	Wild type (T)	Mutated (C)	Comparison	OR	CI (95%)	
Cases (200)	200 (50%)	200 (50%)	$\chi^2 = 0.32$, $P = 0.571$	1.083	Lower	Upper
Controls (200)	208 (52%)	192 (48%)			0.821	1.429
Genotype	Wild type (TT)	Mutated (CT + CC)	Comparison	OR	CI (95%)	
Cases (200)	54 (27%)	146 (73%)	$\chi^2 = 1.45$, $P = 0.228$	1.301	Lower	Upper
Controls (200)	65 (32.5%)	135 (62.5%)			0.846	2.001

Table 11
Comparison allele and genotype data of IL-10 (−819) variants between cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95%CI)	POSTMENOPAUSE		P	OR(95%CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Genotype								
T/T	29 (27.1%)	65 (32.5%)	0.327	1.295	25 (26.9%)	65 (32.5%)	0.332	1.309
CC + CT	78 (72.9%)	135 (67.5%)		(0.77–2.17)	68 (73.1%)	135 (67.5%)		(0.75–2.26)
Allele								
T	111 (51.9%)	208 (52.0%)	1.000	1.005	89 (47.8%)	208 (52.0%)	0.348	1.180
C	103 (48.1%)	192 (48.0%)		(0.72–1.40)	97 (52.2%)	192 (48.0%)		(0.83–1.67)

Table 12
Allele and genotype frequencies at IL-10(-592) locus among cases and controls.

Alleles	(A)	(C)	Comparison	OR	CI (95%)	
Cases (200)	157 (39.25%)	243 (60.75%)	$\chi^2 = 0.05$, $P = 0.823$	1.031	Lower	Upper
Controls (200)	160 (40%)	240 (60.0%)			0.777	1.369
Genotype	(AA)	(AC + CC)	Comparison	OR	CI (95%)	
Cases (200)	45 (22.5%)	155 (77.5%)	$\chi^2 = 0.74$, $P = 0.389$	0.808	Lower	Upper
Controls (200)	38 (19.0%)	162 (81.0%)			0.497	1.311

2000 controls) could not find association of this polymorphism with BC [34]. Studies on this polymorphism did not find correlation with other cancerous disorders such as cervical cancer and gastric cancer in a Korean population [36,37]. There is no conclusive evidence about the nature of association of the other two polymorphisms with BC. In contrast, a study on Austrian population found −592 C > A to be protective against BC [38]. Another study reported that IL-10 (−1082, −819, −592) GCC/ATA haplotype were significantly higher in the patients group when compared with controls [39]. Significantly lower frequencies of IL-10 (−1082, −819, −592) ACC/ATA haplotype were observed in the patients group in comparison to the controls. This could mean significant

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Table 13
Comparison allele and genotype data of IL-10(−592) variants between cases and controls as per menopausal status.

	PREMENOPAUSE		P	OR (95%CI)	POSTMENOPAUSE		P	OR (95%CI)
	Patients (107)	Controls (200)			Patients (93)	Controls (200)		
Genotype								
A/A	22 (21.0)	38 (19.0)	0.74	0.906	23 (24.73)	38 (19.0)	0.261	0.713
CC + AC	85 (79.0)	162 (81.0)	0	(0.53–1.63)	70 (75.27)	162 (81.0)		(0.39–1.28)
Alleles								
A	84 (39.25)	160 (40.0)	0.862	1.031	73 (39.25)	160 (40)	0.862	1.032
C	130 (60.75)	240 (60.0)		(0.73–1.44)	113 (60.75)	240 (60)		(0.72–1.47)

Table 14
Comparative serum level IL-1β, IL-6 and IL-10 between cases and controls.

	Mean	Std. deviation	95% C.I	*P* value
IL-1β				
Cases (n = 20)	27.3	75.5	2.803–51.72	0.23
Controls (n = 20)	6.22	11.9	−18.24–30.67	
IL-6				
Cases (n = 20)	41.3	115	−19.58–102.3	0.86
Controls (n = 20)	48.7	152	−12.28–109.6	
IL-10				
Cases (n = 20)	9.314	41.03	3.048–42.60	0.0836
Controls (n = 20)	3.776	8.148	3.360–11.22	

association of some haplotypes of IL-10 gene despite lack of association of any of these polymorphisms.

Studies on IL-6 (−174 G > C) polymorphism and serum IL-6 level in healthy men and women in a hospital-based population from London reported that GG homozygotes had circulating IL-6 concentrations approximately twice as high as those homozygous for the C allele. This means the mutant C allele is associated with significantly lower levels of plasma IL-6, whereas the wild type G allele is associated with higher IL-6 serum levels [40]. However, some other reported lack of association of IL-6 (−174 G > C) polymorphism with IL-6 plasma levels [41]. In case of IL-10, the three single-base-exchange polymorphisms in the promoter of the IL-10 gene at positions −1082 (G > A), −819 (C > T) and −592 (C > A) exhibits strong linkage disequilibrium, forming three common haplotypes in Caucasian populations; GCC, ACC and ATA. It is found that increased IL-10 plasma levels were associated with the ATA haplotype of IL-10 in healthy individuals [42]. We could not find any reports on association of IL-1β promoter and exon polymorphisms with level of IL-1β expression in serum. Here, we did not find any significant difference in serum level of any of the three interleukins that we investigated in this study. However, the number of sample size used for serum expression level estimation in our study is considerably low due to exclusion at the stage of serum sampling and analysis of results. We view that analysis with larger size of serum samples would be required for drawing strong conclusion for such association.

5. Conclusions

Our study on common polymorphisms in the interleukin genes has revealed interesting observations. We observed no association of IL-1β −511 C/T polymorphism with BC. Similar to our findings, majority of the studies on IL-1β −511 C/T support no association with BC. The presence of mutant allele and genotype at IL-1β +3954 C/T site associated with increased BC risk in our population, while others have found association with survival and aggressive phenotype of breast carcinoma [22,24]. Taken together, IL-1β +3954 C/T possibly influences risk and progression of BC, and thus could be important in BC. Similarly, different studies have reported IL-6 polymorphism (−174 G > C) to affect cancer risk, its severity and progression; however, the biggest hurdle in making a consensus is different objectives of the studies conducted till date. We observed completely contrasting results for this polymorphism. Higher frequency of the mutant allele of IL-6 (−174 G > C) in the controls, as we observed, has not been reported earlier. Earlier studies on IL-10 polymorphisms have reported both correlation and no correlation with BC, with one study describing protective effect of IL-10 (−592 A > C) polymorphism. We, however, did not observe significant association between any of the three polymorphism of IL-10 and breast cancer. In addition to the analysis of genetic polymorphisms, we did not find any difference in the peripheral level of these cytokines between cases and controls. Ethnicity may have strong influence on the association of genetic polymorphisms with BC [19]. Therefore, more studies on other populations need to be conducted before a consensus could be developed about the interleukin gene polymorphisms as risk factor for BC.

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