RESEARCH ARTICLE

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Investigating the association of Angiotensin II Type I Receptor A1166C Polymorphism with Breast Cancer Risk in the Pakistani Population

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Abstract

The polymorphisms of the Renin-Angiotensin System are related to many disorders like diabetes, cardiovascular disease, and different types of cancer. Among all the polymorphisms related to *AGTR1*, A1166C has been associated with several disorders, including cardiovascular diseases and breast cancer. This study was conducted to discover the association of *AGTR1* polymorphism (A1166C) Renin-Angiotensin and its effect on the development and progression of breast cancer in the Pakistani population. One hundred forty participants, including seventy diagnosed breast cancer patients and seventy healthy individuals, were included in this study and genotyped with an allele-specific polymerase chain reaction. The most frequent genotype in healthy participants and breast cancer patients was CC. An insignificant (p value>0.05) risk of breast cancer was found with A1166C polymorphism in codominant (CC vs. AA OR=1.200 [0.256-5.631] and AC vs. AA 0.941 [OR=0.223-3.976]), dominant (OR=1.00 [0.240-4.167]), recessive (OR=1.230 [0.593-2.552]) and additive models (OR=1.028 [0.533-1.983]) of general population genotypes. Nonetheless, when the AA genotype was considered a reference group, a significant association was found between AC and CC genotypes and invasive ductal and ductal carcinoma development in breast cancer patients. In conclusion, this study demonstrated no significant association between *AGTR1* (A1166C) polymorphism and breast cancer risk.

Keywords: Breast cancer- renin-angiotensin system- angiotensin II type I receptor- A1166C polymorphism

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Introduction

Breast cancer is a heterogeneous disease characterized by multiple tumor entities with different clinical behavior, biological features, and distinctive histological patterns [1]. About a million breast cancer cases are reported annually in women worldwide. According to the World Health Organization (WHO) report in 2020, the incidence rate of breast cancer was 34.4 % worldwide, the highest among all the top ten cancers among females. The incidence rate of breast cancer in Pakistani females was 28.7% in the same report. Thus, the increased risk of breast cancer incidence and related mortality invokes the need to inspect the other associated risk factors.

Rennin angiotensin system (RAS) is a hormonal circulating system involved in systemic cardiovascular homeostasis, blood pressure regulation, neovascularization, inflammation, cell proliferation, and cell-cell adhesion. RAS has been investigated in different research studies as a prognostic biomarker and risk factor against different cancers, including prostate [2] and breast cancer [3]. In most of the neoplastic stages, overexpression of RAS components such as angiotensin II type I receptor (*AGTR1*) and angiotensin-converting enzyme (ACE) has been reported [4]. ACE (17q23) spans ~21kbps in humans, further encoding 25 introns and 25 exons. Zn-dependent dipeptidyl carboxypeptidase cleaves angiotensinogen (synthesized by the liver) into angiotensin I (inactive decapeptide); upon low blood pressure or plasma sodium levels reduction, it is released into the bloodstream. Angiotensin-I-converting enzyme (ACE) further acts on angiotensin I to convert it into active angiotensin II (octapeptide) [5]. Angiotensin II increases blood pressure by promoting vasoconstriction. It also increases plasma aldosterone and retains water and electrolytes like sodium [6]. Angiotensin II mediates its effect by two G-protein coupled receptor family receptors, *AGTR1* and angiotensin II type II receptor (AGTR2). Due to its site of origin and the location of its receptors, Angiotensin II has an important role in the cell proliferation and apoptotic stages of the breast cell cycle [7, 8].

The AGTR1 is present on chromosome 3 (3q21-q25) and covers >55kbps region comprising 5 exons. Sharkawy et al. and Ostrosky et al. suggested that *AGTR1* (A1166C) single nucleotide polymorphism (SNP) is associated with breast cancer [5, 6]. This polymorphism is present in the

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3' untranslated region and is considered functionally insignificant. However, it was found to be associated with T810A transversion present in the gene's promoter region. Thus, T810A influences transcriptional factor binding and subsequent transcription of mRNA. Moreover, a study observed an elevated *AGTR1* mRNA transcription rate in breast cancer patients compared to normal individuals [9]. Generally, the *AGTR1* signaling pathway involves the induction of cell proliferation, inflammation, and angiogenesis and the inhibition of apoptosis [10, 11]. *AGTR1* receptors are seen to be over-expressed in breast cancer cases, and this increase is about 100 folds more than in normal breast tissues. Additionally, overexpression of *AGTR1* due to genetic mutations leads to cellular invasion and tumorigenesis in breast cancer patients [12].

Several studies worldwide have investigated the association of A1166C SNP with the incidence of breast cancer. A study of *AGTR1* (A1166C) polymorphism in the Indian population has described the association of AC and CC genotypes and C alleles with the high risk of Breast cancer [3]. Moreover, they linked it with severe disease leading to progressive staging and large tumor size. Conversely, in another study conducted on Brazilian women, A1166C polymorphism was not associated with breast cancer risk [13]. Similarly, in other populations association of A1166C polymorphism has been investigated with breast cancer risk and its progression. Such as the AC genotype was a risk-associated factor against the AA genotype in Iranian women with higher tumor node metastasis [14]. Although many studies have investigated the association of A1166C single nucleotide polymorphism of *AGTR1* with the risk of developing disease, the outcomes remain contradictory in different populations. Considering these results, it can be inferred that breast cancer risk association with A1166C polymorphism can be a population-specific phenomenon. Thus, this study aimed to find out the association of *AGTR1* (A1166C) polymorphism with breast cancer in the Pakistani population as the proper understanding of this association in Pakistani women could be used as biomarkers for early diagnosis and screening of breast cancer.

Materials and Methods

Sample Collection

The Institutional Review Board of Kinnaird College approved this case-control analytical study for women in Lahore. In this study, 140 individuals were enrolled, including 70 with breast cancer and 70 healthy women without disease and any other disease. The breast cancer case subjects with other chronic diseases like cardiovascular disorder, cancer (apart from breast cancer), diabetes, kidney disease, and hypertension were excluded from the study. All the samples were procured from INMOL Cancer Hospital, Lahore, while the genotypic analysis was performed at the Biochemistry Laboratory of Kinnaird College for Women, Lahore. The present study was performed according to the principles of the Declaration of Helsinki, and ethical approval was obtained from the institutional board committee of Kinnaird College for Women, Lahore (KC/ORIC/ERC/2015/002). Informed consent was obtained from all participants of the study. A structured questionnaire was used to obtain data from each participant regarding the demographics (age and body mass index) and clinical and pathological details. The data include participant age, body mass index, family history of the disease, menstrual cycle status, tumor type, and location, etc. 140 blood samples were collected, 70 from healthy Pakistani women (control group) and 70 from Pakistani women diagnosed with breast cancer (case group).

DNA Extraction

Each participant's blood sample of 2-3ml was collected in EDTA vacutainers (Fisher Scientific, Franklin Lakes, NJ, USA). 750µl of peripheral blood of each participant was used to extract DNA by standard phenol-chloroform method with the two-day protocol (Sambrook and Russell, 2006). The quality of DNA was checked by running the samples on 1% agarose gel (Thermo Fisher Scientific, Baltic, UAB).

Genotyping

The genotype of the samples was determined using allele-specific polymerase chain reaction (PCR). Two reaction mixtures were prepared for each sample (Ugozzoli and Wallace, 1992). Two PCRs and 3 primers amplified the *AGTR1* polymorphic sequence. For the first PCR following primers were used: forward allele specific A primer 5'-GCACTTCACTACCAAATGAGCA-3' (sense) and normal reverse primer 5'-AGGGAGATTGCATTTCTGTCAGT-3' (antisense). For 20µl reaction mixture of PCR contains 2.5U Taq polymerase (Thermo Fisher Scientific, Baltic, Lithuania), 25ng of genomic DNA, 50pmol of each primer, 2mM MgCl2 (Thermo Fisher Scientific, Baltic, Lithuania), 0.2mM dNTPs (Thermo Scientific, Foster City, California, USA), 1X PCR buffer (Thermo Fisher Scientific, Baltic, Lithuania). The second reaction for the same sample contains all the reagents mentioned above but different primers, i.e., 50pmol of allele-specific C primer 5'-GCACTTCACTACCAAATGAGCC-3' (sense) and normal reverse primer 5'-AGGGAGATTGCATTTCTGTCAGT-3' (antisense). PCR was run through a thermocycler (Bio-Rad Laboratories, Singapore) at conditions as follows: an initial denaturation at 94ºC for 4mins, followed by 30 cycles of denaturation at 94ºC for 30sec, annealing at 67ºC for 30 sec, extension at 72ºC for 30 sec with a final extension at 72ºC for 5 mins. The results of the PCR were analyzed using 1.5% agarose gel.

Statistical Analysis

Data was analyzed using SPSS version 26.0 for Microsoft Windows (SPSS, Chicago, IL, USA). The data for the continuous variables such as age and BMI was presented mean±standard deviation, while the categorical data was presented as frequencies (percentages). The data was categorized into different groups for each variable, and their risk factor was analyzed through logistic regression. The p-value less than 0.05 was considered significant.

The association of *AGTR1* polymorphisms (A1166C) genotype/allele with the risk of breast cancer development was calculated by odds ratio (OR) at 95% confidence interval (95% CI) using codominant (AC vs AA and CC vs AA), dominant (AC+CC vs AA), recessive (CC vs AC+AA), and heterozygous (AC vs AA+CC) models. The risk analysis of breast tumor type and location against genotypic data of breast cancer patients was also recorded as OR [95% CI]. The baseline characteristics of both case and control groups were analyzed against *AGTR1* A1166C polymorphisms through regression analysis.

Results

Demographic and Clinical Characteristics of Study Participants

140 (70 healthy individuals and 70 breast cancer patients) participated in this study. The data regarding the patient's physical fitness, family history, and history related to cancer was obtained and analyzed by determining the p-value for each variable, thus defining the risk factors for breast cancer. Table 1 summarizes the demographic and clinical characteristics of the study population groups (controls and cases). The mean ages of controls and breast cancer patients were calculated as 40.14±10.93 and 46.49±15.72 (p-value 0.006), respectively. In the subgroup analysis of variables, including age, BMI, marital status, number of children, history of menstrual irregularities, and breast cancer, a significant difference (p-value<0.05) was found in the data distribution amid the case and control population. An insignificant difference was found in the distribution of data for miscarriages between both groups (p-value 0.052). According to risk analysis, patients aged between 31-45 years (OR=14.00 [4.145-47.291]) and greater than 46 years (OR=9.333 [2.925-29.785]) showed higher risk for developing breast cancer about 16-30 years. Likewise, overweight patients (OR=3.022 [1.379-6.626]) have more chances of developing breast cancer than normal subjects. Married women (OR=1.296 [1.141-1.473]) were also likely to develop breast cancer 1.3 times more than unmarried women having 1-3 (OR=9.913 [2.634-37.306]) and 4-6 (OR=8.636 [2.270-32.856]) children. Moreover, it was found that there were more than 3.5 times the chances of women getting breast cancer who has gone through menopause as compared to women with oligomenorrhea, amenorrhea, or with no irregularities at all.

Association of AGTR1 Polymorphism with Breast Cancer

The *AGTR1* (A1166C) polymorphism genotypes/alleles association with breast cancer was found using logistic regression analysis and chi-square test (Table 2). As it had been shown in the table, frequencies of AA, AC, and CC were 4 (5.7%), 15 (21.45%), and 51 (72.85%) in controls and 4 (5.7%), 18 (25.72%), and 48 (68.58%) in cases, respectively. Nonetheless, no association was found between the genotypes and the risk of breast cancer. Considering this, the combined effect of genotypes was assessed for risk analysis as dominant (AC+CC vs AA), recessive (CC vs AC+AA), and heterozygous (AC vs CC+AA) models. Yet, all the results were insignificant and showed no association with the disease. Furthermore, alleles A/C were also compared between control and case groups but then again, the insignificant results were obtained OR=1.028 [0.533-1.983] (p-value 0.933).

Association of AGTR1 Polymorphism with Tumor Type and Location

The genotypes were then compared with three tumor types (Invasive ductal carcinoma, Lobular carcinoma, and Ductal carcinoma) and tumor locations (left, right, and both) (Table 3). By considering the AA genotype as a reference group, there was a significant association found between the CC genotype with invasive ductal carcinoma (OR=11.400 [1.068-121.70]) (p-value 0.04) and ductal carcinoma (OR=0.116 [0.013-1.016]) (p-value 0.052). The AC genotype was also found to be associated with the significant risk of breast tumors occurring as invasive ductal carcinoma (OR=15.00 [1.136-198.039]) (p-value 0.04) and ductal carcinoma (OR=0.059 [0.004-0.979]) (p-value 0.048). Lastly, no association was found between the location of breast tumor and lobular carcinoma (type of tumor) (p-value>0.05).

Association of AGTR1 Polymorphism with Baseline Characteristics

Moreover, the association of baseline characteristics of the study participants was compared with the genotypic and allelic frequencies. No significant association was found in genotypes and alleles with age groups, BMI groups, number of children, miscarriages, marital status, family history, and menstrual irregularities (Figure 1).

Discussion

Breast cancer constitutes a major health problem in women, with over a million cases diagnosed annually. There is increasing evidence that the local renin-angiotensin system may impact angiogenesis, apoptosis, cell proliferation, and inflammation [15]. Consequently, it may provide an adjuvant therapy by the blockade of RAS that can be implemented as an alternative to previous ones for managing breast tumors. Polymorphism in RAS is involved in the proliferation of cells during different kinds of malignancies [6]. If Angiotensin II binds with *AGTR1*, it facilitates the proliferation of cells. The A1166C polymorphism of *AGTR1* is the most studied polymorphism for various diseases, such as cardiovascular diseases and multiple cancers, including breast cancer.

The results of this study have shown that the mean age of the control group was 40.14±15.72 while the mean age of cases was 46.49±10.93. Similarly, Body Mass Index was also observed; the mean BMI of controls was 24.33±4.47, and that of cases was 25.58±4.76. An Independent t-test was applied to check the difference in the means of BMI and ages of controls and patients suffering from breast cancer. The p-value showed no difference between the mean BMI and the ages of controls and patients suffering from breast cancer, which is important for examining the other risk factors. These results were supported by previous studies by different previous studies [16, 17], while marital status did not seem to correlate with the

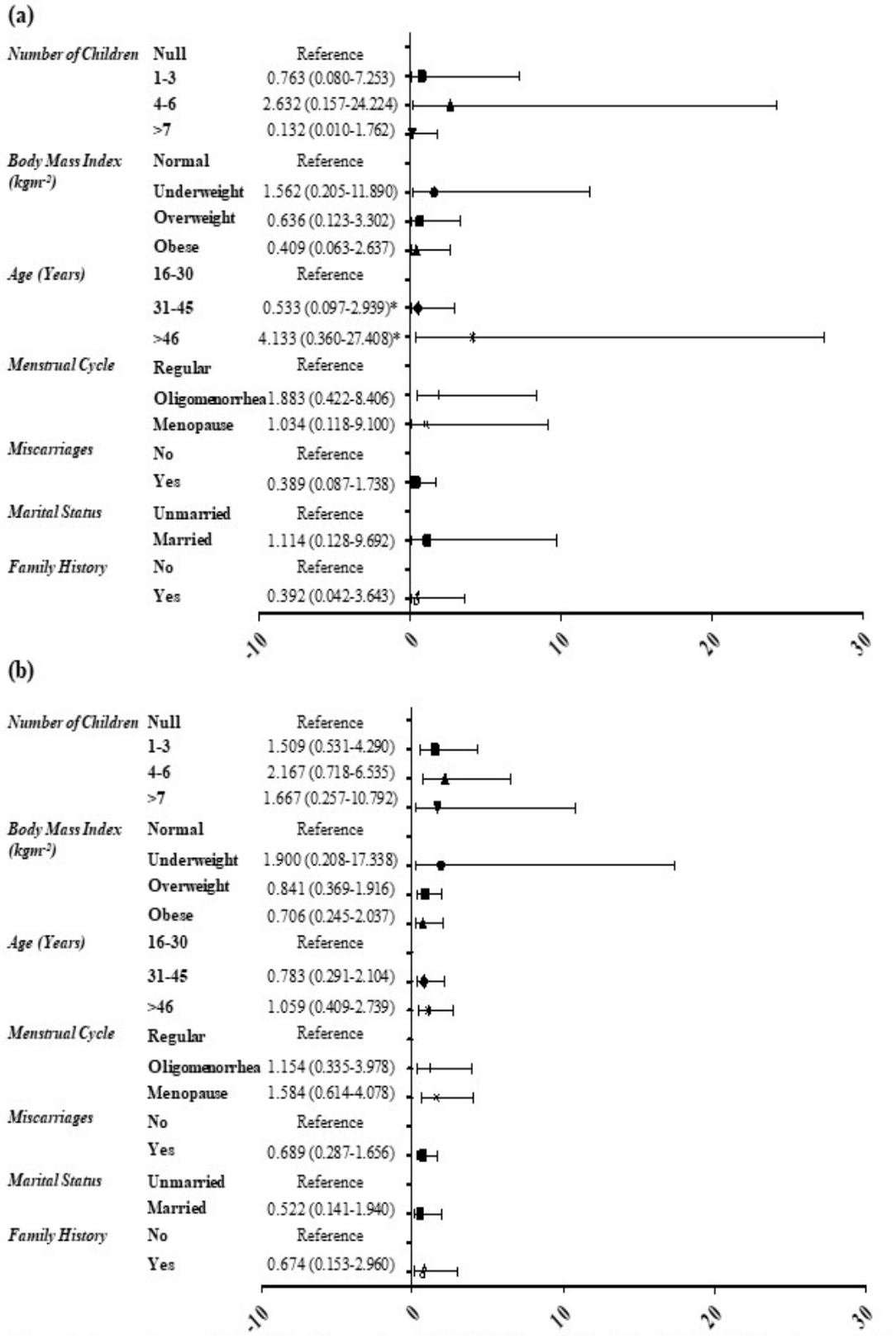

Figure 1. Regression Analysis of the (a) genotype (AA/AC+CC) and (b) alleles (A/C) with breast cancer risk and associated baseline characteristics of participants

occurrence of breast cancer as reported in a study [18].

AGTR1 (A1166C) polymorphism genotypic results were evaluated in different modes to assess its association with breast cancer. This study found no significant association between breast cancer cases and genotypes CC and AC concerning AA. The same results were observed in a study on Brazilian women diagnosed with breast cancer (p-value 0.114) [13]. However, another study conducted in the North Indian population presented contradictory results. A significant association was found between AC

Table 1. Demographic and Clinical Data Distribution along with Their Risk Analysis between Controls and Cases

Variable	Categories	Controls N (%)	Cases N (%)	p-value	OR [95% CI]	p-value	Variable	Categories	Controls N (%)	Cases N (%)	p-value	OR [95% CI]	p-value
Age	Mean age ±SD	40.14±15.7	46.49±10.9	0.006*	-	-	Age	Mean age ±SD	40.14±15.8	46.49±10.10	0.006*	-	-
	16–30 years n (%)	28 (40)	4(5.7)	0.000*	Ref.			16–30 years n (%)	29 (40)	4(5.7)	0.000*	Ref.	
	31–45 years n (%)	15(22)	30(42.8)		14.00 (4.145-47.291)	0.000*		31–45 years n (%)	15(22)	30(42.8)		14.00 (4.145-47.291)	0.000*
	Older than 46 years n (%)	27(38)	36(51.4)		9.333 (2.925-29.785)	0.000*		Older than 46 years n (%)	27(38)	36(51.4)		9.333 (2.925-29.785)	0.000*
BMI	Mean BMI±SD	24.33±4.47	25.58±4.76	0.111	-	-	BMI	Mean BMI±SD	24.33±4.48	25.58±4.77	1.111	-	-
	Normal (18.5-24.9 kg/m2) n (%)	41(58.5)	26(37)	0.032*	Ref.			Normal (18.5-24.9 kg/m2) n (%)	41(58.5)	26(37)	0.032*	Ref.	
	Under weight (< 18.5 kg/m2) n (%)	2(2.8)	5(7.1)		2.340 (0.600-9.128)	0.221		Under weight (< 18.5 kg/m2) n (%)	2(2.8)	5(7.1)		2.340 (0.600-9.128)	1.221
	Overweight (25-29.9 kg/m2) n (%)	16(22.8)	30(42.8)		3.022 (1.379-6.626)	0.006*		Overweight (25-29.9 kg/m2) n (%)	16(22.8)	30(42.8)		3.022 (1.379-6.626)	0.006*
	Obese (Over 30 kg/m2) n (%)	11(15.7)	9(12.8)		1.135 (0.401-3.210)	0.812		Obese (Over 30 kg/m2) n (%)	11(15.7)	9(12.8)		1.135 (0.401-3.210)	1.812
Marital status	Unmarried n (%)	16(23)	0(0)	0.000*	Ref.		Marital status	Unmarried n (%)	16(23)	0(0)	0.000*	Ref.	
	Married n (%)	54(77)	70(100)		1.296 (1.141-1.473)	0.033*		Married n (%)	54(77)	70(100)		1.296 (1.141-1.473)	0.033*
No. of children	Null n (%)	19(27)	3(4.2)	0.000*	Ref.		No. of children	Null n (%)	19(27)	3(4.2)	0.000*	Ref.	
	1-3 n (%)	23(33)	36(51.4)		9.913 (2.634-37.306)	0.001*		1-3 n (%)	23(33)	36(51.4)		9.913 (2.634-37.306)	0.001*
	4-6 n (%)	22(31.4)	30(42.8)		8.636 (2.270-32.856)	0.002*		4-6 n (%)	22(31.4)	30(42.8)		8.636 (2.270-32.856)	0.002*
	More than 7	6(8.5)	1(1.4)		1.056 (0.092-12.137)	0.956		More than 8	6(8.5)	1(1.4)		1.056 (0.092-12.137)	0.956
Miscarriage	Yes n (%)	60(85.7)	52(74.2)	0.052	Ref.		Miscarriage	Yes n (%)	60(85.7)	52(74.2)	1.052	Ref.	
	No n (%)	10(14.2)	18(25.7)		0.426 (0.177-1.029)	0.058		No n (%)	10(14.2)	18(25.7)		0.426 (0.177-1.029)	0.058
Family history	Yes n (%)	1(1.4)	7(10)	0.021*	Ref.		Family history	Yes n (%)	1(1.4)	7(10)	0.021*	Ref.	
	No n (%)	69(98.5)	63(90)		0.130 (0.016-1.090)	0.06		No n (%)	69(98.5)	63(90)		0.130 (0.016-1.090)	0.06
Irregularities in the menstrual cycle	No irregularities n (%)	56(80)	39(55.7)	0.007*	Ref.		Irregularities in the menstrual cycle	No irregularities n (%)	56(80)	39(55.7)	0.007*	Ref.	
	Oligomenorrhea n (%)	5(7.1)	9(12.8)		2.585 (0.804-8.304)	0.111		Oligomenorrhea n (%)	5(7.1)	9(12.8)		2.585 (0.804-8.304)	0.111
	Menopause n (%)	9(12.8)	22(31.4)		3.510 (1.461-8.434)	0.005*		Menopause n (%)	9(12.8)	22(31.4)		3.510 (1.461-8.434)	0.005*

*, The p-value <0.05 was considered statistically significant; N, number; %, frequency; Ref, reference; OR 95% CI, Odd Ratios at 95% confidence interval

Table 2. Risk Analysis of A1166C Polymorphism of *AGTR1* Gene as a Function of the Inheritance Model in Controls and Breast Cancer Patients

Inheritance model	Genotype	Control N (%)	Cases N (%)	χ^2	p-value	OR 95% CI	p-value
Co-dominant	AA	4 (5.7)	4 (5.7)	-	-	Ref.	
	AC	15 (21.45)	18 (25.72)	0.357	0.55	1.200 (0.256-5.631)	0.817
	CC	51 (72.85)	48 (68.58)	0.31	0.577	0.941 (0.223-3.976)	0.934
Recessive	AC + AA	19 (27.15)	22 (31.42)	-	-	Ref.	
	CC	51 (72.85)	48 (68.58)	0.311	0.577	1.230 (0.593-2.552)	0.578
Dominant	AA	4 (5.7)	4 (5.7)	-	-	Ref.	
	AC + CC	66 (94.3)	66 (94.3)	0	1	1.00 (0.240-4.167)	1
Heterozygous	AA + CC	55 (78.55)	52 (74.28)	-	-	Ref.	
	AC	15 (21.45)	18 (25.72)	0.623	0.43	0.732 (0.337-1.592)	0.431
Additive	A	23 (15)	26 (18.58)	-	-	Ref.	
	C	117 (85)	114 (81.42)	0.007	0.933	1.028 (0.533-1.983)	0.933

*The p-value <0.05 was considered statistically significant, N, number; %, frequency; Ref, reference; χ^2, Chi-square analysis; OR 95% CI, Odd Ratios at 95% confidence interval

Table 3. Risk Analysis of *AGTR1* Polymorphism (A1166C) in Relation to Breast Tumor Type and Location among Breast Cancer Patients

Genotype		AA	AC	CC	AA	AC	CC
		N (%)	N (%)	N (%)		OR [95% CI]; p-value	
Breast Tumor type	Invasive ductal carcinoma N = 54	1 (25)	15 (83)	38 (79)	Ref	15.00 [1.136-198.039]; 0.04*	11.400 [1.068-121.70]; 0.044*
	Lobular carcinoma N = 8	1 (25)	2 (11)	5 (10.5)	Ref	0.375 [0.025-5.572]; 0.476	0.349 [0.030-4.024]; 0.399
	Ductal carcinoma N = 8	2 (50)	1 (6)	5 (10.5)	Ref	0.059 [0.004-0.979]; 0.048*	0.116 [0.013-1.016]; 0.052*
Breast Tumor Location	Left breast N = 36	2 (50)	9 (50)	25 (52)	Ref	1.125 [0.127-9.943]; 0.916	0.800 [0.103-6.191]; 0.831
	Right breast N = 30	2 (50)	8 (44)	20 (42)	Ref	0.889 [0.104-7.856]; 0.916	1.250 [0.162-9.674]; 0.831
	Both breasts N = 4	0 (0)	1 (6)	3 (6)	Ref	-	-

*The p-value <0.05 was considered statistically significant, N, number; %, frequency; Ref, reference; χ^2, Chi-square analysis; OR 95% CI, Odd Ratios at 95%

and CC genotype/C allele and the risk of developing a more aggressive disease with large tumor size at advanced stages.

In this study, CC genotype frequency was higher in the case group than the AC genotype. CC homozygous and AC heterozygous genotype was found to be significantly associated with a higher risk of breast cancer patients developing invasive ductal carcinoma than ductal carcinoma in comparison to AA homozygous. These results aligned with a study on the Iranian population where a stronger significant association was found between the homogenous groups of 61 breast cancer patients with A1166G *AGTR1* polymorphism [14]. Although, they didn't find any specific association between gene polymorphism and breast cancer occurrence risk.

Due to the availability of less data on the expression of *AGTR1* concerning the effect of *AGTR1* (A1166C) polymorphism, additional studies are suggested to assess the risk of (A1166C) polymorphism with breast cancer. An insignificant association of genotypes may be due to its relatively small sample size. If a large sample of data is obtained, it can be easier to highlight the risk association of polymorphism with breast cancer. Moreover, the main reason for the significant association of invasive ductal and ductal carcinoma with (A1166C) polymorphism can be identified, possibly due to overexpression of *AGTR1*. As elucidated in a research study, A1166C polymorphism can lead to renin-angiotensin system activation and overexpression of *AGTR1* [19]. As mentioned, *AGTR1* mediates tumorigenic actions such as inflammation, angiogenesis, and cell proliferation. These findings can lead to proper diagnosis and prognosis of breast cancer [20].

In conclusion, the present study found that *AGTR1* (A1166C) polymorphism was not associated with the risk of breast cancer in the Pakistani population. Further analysis of a large population should be performed to validate the results of this study.

Author Contribution Statement

All the authors contributed to the study's conception and design. MS, ZK, RT, and HY prepared materials, data collection, and curation. KF performed the statistical analysis. MS has written the first draft of the manuscript; all the other authors commented on it, and subsequent changes were made. All the authors have approved the final manuscript.

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consented to the present study.

Conflict of Interest

The authors declare that there was no conflict of interest.

Ethical Declaration:

The present study was performed according to the principles of the Declaration of Helsinki, and ethical approval was obtained from the institutional board committee of Kinnaird College for Women, Lahore (KC/ORIC/ERC/2015/002).

Availability of Data

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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