Original paper

Characterisation of *IGF-1* (rs7136446) and *IL-6* (rs1800795) polymorphisms among breast cancer patients in western Algeria

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Breast cancer (BC) is the most frequently diagnosed cancer among women worldwide, including Algeria. Certain single nucleotide polymorphisms (SNPs) have been linked to higher risk of BC. Several studies have been performed on the insulin-like growth factor 1 (*IGF-1*) T > C (rs7136446) and the interleukin 6 (*IL-6*) 174G > C (rs1800795) SNPs to explore their role in BC. The aim of this study is to investigate the association between the 2 SNPs, *IL-6* (rs1800795) and *IGF-1* (rs7136446) with BC in the population of western Algeria.

This study was carried out for the first time among the Algerian population. This case-control study included 109 BC patients and 112 healthy controls. Genomic DNA was extracted from peripheral blood samples. DNA concentration was determined using the Qubit 2.0 fluorometer. The genotyping of selected SNPs was performed by real-time polymerase chain reaction followed by statistical analysis to assess genotypic frequencies and genetic association with BC.

The results showed that *IGF-1* rs7136446 was positively associated with BC ($p = 0.00001$) and that the distribution of genotype frequencies of rs7136446 showed a statistically difference between human epidermal growth factor receptor 2 (HER-2) positive and HER-2 negative BC ($p = 0.04$), but this association did not last after the correction of Bonferroni. No association was found in genotype distribution of the *IL-6* (rs1800795) among controls and BC patients or with clinicopathological parameters including HER-2 status in BC ($p > 0.05$).

In summary, our findings indicate that *IGF-1* rs7136446 is associated with BC in our population of western Algeria.

Key words: breast cancer (BC), interleukin 6 (*IL-6*), insulin-like growth factor 1 (*IGF-1*), human epidermal growth factor receptor 2 (HER-2), single nucleotide polymorphisms (SNPs), western Algeria.

Introduction

Breast cancer (BC) is a heterogeneous disease with numerous subtypes that have different treatment responses and clinical outcomes. Human epidermal growth factor receptor 2 (HER-2) is overexpressed in about 15–25% of BC patients, which results in more aggressive tumour biological activity and worse disease outcomes [1]. It is hypothesised that potential crosstalk exists between the insulin-like growth factor 1 (*IGF-1*) pathway and epidermal growth factor receptor family and it should also be noted that HER-2 overexpression activates the secretion of interleukin 6 (*IL-6*). In HER-2-positive subtype, increased IGF signalling tends to accelerate the progression of BC and promote resistance to established therapies [2], causing decreased BC-specific survival [3] and increasing overall mortality [4]. As demonstrated by Camirand *et al*. co-blocking HER-2 and *IGF-1* receptor (IGF-1R) inhibits the growth of HER-2-overexpressing BC cells [5]. A previous study showed that HER-2 overexpression activates a transcriptional inflammatory profile, which includes the secretion of *IL-6* in many cell types, and it was demonstrated that secreted *IL-6* was important for HER-2 mediated oncogenesis and was mediated by autocrine activation of STAT3 in tumour cell populations, which was enhanced by cellular HER-2 expression and in *in vivo* contexts [6].

In the mammary gland and other tissues, *IGF-1* signalling pathway has been linked to cell proliferation, development, and differentiation [7, 8]. IGF-1 is a single-chain polypeptide encoded by chromosome 12 [9]. The association between IGF1 polymorphisms and clinical outcome has recently been investigated and established in various types of cancers, such as prostate [10, 11], colorectal [12, 13] and breast cancers [11, 14]. This effect is attributed to its pro-survival signalling pathways that potentially promote the proliferation of neoplastic cells [15]. The levels of circulating *IGF-1* are highly influenced by genetic factors [16]. Several polymorphic variants including single nucleotide polymorphisms (SNPs) and microsatellites have been associated with both increased *IGF-1* levels and BC risk [17–20]. Also, elevated circulating levels of *IGF-I* may increase BC risk by increasing breast density [21, 22]. Literature data indicate that the SNPs in the *IGF-1* gene region including the *IGF-1* (rs7136446) were associated with BC risk [19, 20]. Moreover, previous studies have confirmed that an increased *IGF-1* levels were associated with a high risk of BC, especially for premenopausal BC and oestrogen receptor-positive BC [11, 14]. On the other hand, some studies carried out on women with premenopausal and postmenopausal BC did not show an association between *IGF-I* levels or BC risk [23, 24].

Inflammatory processes participate in the initiation, promotion and metastasis of cancer through various mechanisms [25]. An increasing number of studies have shown a correlation between increased levels of certain cytokines and the development and progression of tumours [26]. *IL-6* belongs to the cytokine family and is known to regulate immune reactions and inflammation and promote tumour growth by up-regulating angiogenic and anti-apoptotic proteins [27]. The gene encoding for the *IL-6* cytokine is located on human chromosomes 7p15.3 [28]. The best characterised SNP *IL-6*-174G/C (rs1800795) has been shown to cause *IL-6* overexpression at least in part by enabling recognition by other transcription factors [29]. Current findings demonstrate the role of *IL-6* in BC progression, metastasis, and anti-cancer immunity these findings suggest that the IL-6/JAK/STAT3 signalling pathway is an actionable target with preclinical and clinical studies demonstrating therapeutic potential in both primary and metastatic BC. Notably, inhibiting the IL-6/JAK/STAT3 signalling axis has been investigated through directly targeting IL-6, IL-6Rα, gp130 receptor, JAKs, or STAT3 [30].

The first aim of this case-control study was to investigate the association between the rs7136446 polymorphism of *IGF-1* and 174G/C polymorphism (rs1800795) of *IL-6* with BC in the population of western Algeria. Then to study the distribution of the 2 polymorphisms in the 2 BC subtypes; HER-2 positive and HER-2 negative. Lastly, we aimed to investigate the association of *IL-6* (rs1800795) polymorphism with clinicopathological characteristics. This study was carried out for the first time among the Algerian population.

Material and methods

Study population

The study included 221 women, divided into case (with BC, $n = 109$) and control (without BC, $n = 112$) groups. The patients in our study were all from western Algeria and were recruited from 2 different oncology units . The Establishment Hospital University of Oran and the University Hospital Centre of Oran. All women were diagnosed with histologically confirmed *in situ* and invasive BC. Clinicopathological and sociodemographic data were collected in Table I.

DNA isolation

Peripheral blood samples were collected in ethylenediaminetetraacetic acid tubes for each included patient after written informed consent and stored at –20°C until analysis. DNA was isolated from peripheral white blood cells using 2 different kits of extraction: PureLink™ Genomic DNA Mini Kit (Invitrogen) and MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche), following the manufacturer's

instructions. After isolation, DNA concentration was determined using the Qubit 2.0 fluorometer (ThermoFisher Scientific, 2015).

Single nucleotide polymorphism genotyping

The single nucleotide polymorphisms of the *IL-6* gene and *IGF-1* were selected according to the National Centre for Biotechnology Information SNPs database (https://www.ncbi.nlm.nih.gov/snp/): rs1800795, rs7136446 . Genotyping was performed in the molecular biology unit of the Institut Pasteur Oran, Algeria by Applied Biosystems 7500 real-time polymerase chain reaction (RT-PCR). The reactions were conducted in final volumes of 20 ml *per* patient, containing: 10 ml TaqMan Genotyping Master Mix; 1 ml of primers (forward and reverse) and 0.5 ml of probes (FAM and VIC) primers and probes of each gene variant are summarised in Table II; 4 ml of deionised DNA/RNA-free water and 3 ml of DNA sample *per* patient, these volumes were distributed in 96-well reaction plates (MicroAmp Fast Optical 96-Well Reaction Plate). Amplification was performed by using a Fast RT-PCR System 7500 with FAST (software version 2.0.4) incorporated for SNP genotyping (Applied Biosystems), in the following steps:

- pre-PCR, with a duration of 1 min at 50°,
- pre-incubation of the reaction mixture at 95°C during for 10 min,
- thermocycling at 95°C during 15 s for 40 cycles,
- post-PCR, with a duration of 1 min at 60°.

Fluorescence data were captured during 40 reaction cycles.

Statistical analysis

The chi-square (χ^2) test was used to determine whether the distribution of genotypes was consistent with the Hardy-Weinberg equilibrium. Genotype frequencies were compared between women with BC and women without the disease from a control group using Pearson's χ^2 test. The p values were considered statistically significant when $p \leq 0.05$. The Bonferroni correction threshold for the significance of association at $p \leq 0.025$ was mentioned considering the number of tests performed. The odds ratio (OR) and 95% confidence interval (CI) were calculated using SPSS software version 22.0.

Results

Clinical and demographic characteristics of the study population

The study included 221 women (109 cases and 112 controls), recruited from both oncology units in Oran to obtain a more representative sample. These patients represented different regions from western Algeria. Mean age and standard deviation was

Table I. Demographic and clinical characteristics of patients with breast cancer

Characteristics	Patients, *n* (%)
Age at diagnosis (years), $n = 109$	
≤ 50	51 (47)
> 50	58 (53)
Age at menarche (years), $n = 109$	
≤ 14	89 (82)
> 14	20 (18)
Histological grade (SBR), $n = 108$	
Grade I	02 (02)
Grade II	68 (63)
Grade III	38 (35)
Histological type, $n = 107$	
IDC	
Lobular and others subtypes	99 (93)
Tumour size, $n = 109$	08 (07)
T	
T1	16 (15)
T2–T3	64 (59)
T4	22 (20)
Tx	07 (06)
Distant metastases, $n = 109$	
Negative	58 (53)
Positive	51 (47)
ER receptor status, $n = 107$	
Negative	34 (32)
Positive	73 (68)
PR receptor status, $n = 106$	
Negative	40 (38)
Positive	66 (62)
HER2 status, $n = 105$	
Negative	83 (79)
Positive	22 (21)
Ki-67 expression, $n = 90$	
Negative	03 (03)
Positive	87 (97)
Location of BC, $n = 108$	
Left breast	52 (48.1)
Right breast	49 (45.4)
Bilateral	07 (06.5)

BC – breast cancer, ER – oestrogen receptors, IDC – invasive ductal carcinoma, PR – progesterone receptors, SBR – Scarff-Bloom-Richardson

Table II. Sequences of primers and probes used for genotyping

Gene SNP	Primers 5–3	Probes 5–3
IL-6 (rs1800795)	F : CGACCTAAGCTGCACTTTTCC R:GGGCTGATTGGAAACCTTATTAAGATTG	VIC : CCTTTAGCATGGCAAGAC FAM : CCTTTAGCATCGCAAGAC
IGF-1 (rs7136446)	F : TTGGTTACCTGCTACATTGAAAGC R : GGGTCCCAAATTCCTTGAAGGTT	VIC : AAGTGCTGCGTAGTAT FAM : TAAGTGCCGCGTAGTA

SNP – single nucleotide polymorphisms

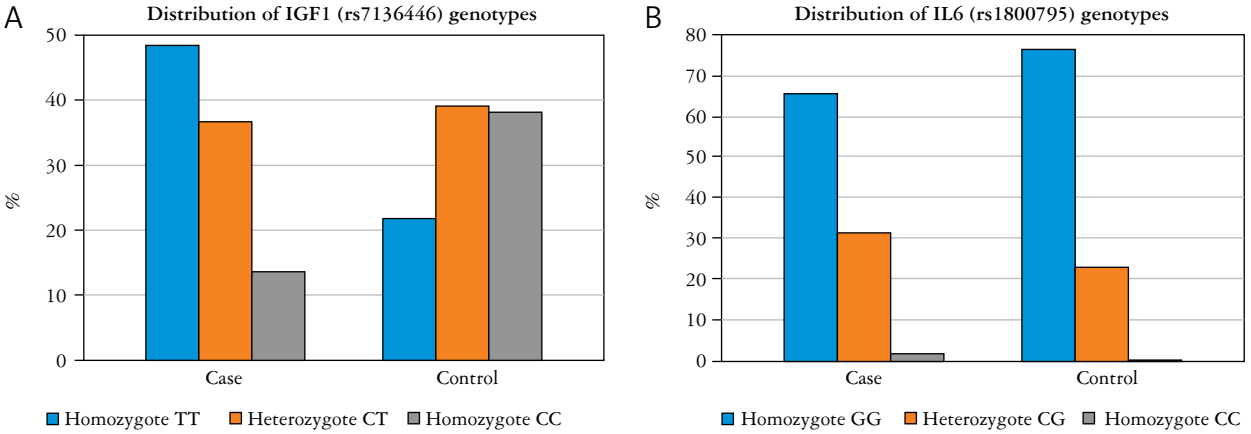

Fig. 1. Distribution of *IGF-1* (rs7136446) and *IL-6* (rs1800795) genotypes among studied groups

51.9 ±11.3 years for cases and 44.7 ±14.2 for controls. Out of the 108 patients 45.4% had cancer in the right breast, (48.1%) in left breast and only (6.5%) had bilateral BC. The majority of patients 64% were presented with histological grade II (SBR), followed by grade III (SBR) 35% and only 1% were found to be in grade I (SBR). Invasive ductal carcinoma was broadly identified in (93%), while lobular and others subtypes represented 7% in 107 patients among 109. Clinical TNM (tumour-nodes-metastasis) stage was evaluated as *per* American Joint Committee on Cancer (AJCC, 8th edition 2017). More than half of the tumours were classified T2–T3 (59%), while T4 and T1 represented (20%) and (15%) respectively. Tx accorded to rare cases of tumours that cannot be evaluated at the initial diagnosis. One hundred and five out of 109 patients were tested for HER-2 status in which it was positive in 22 (21%) and negative in 83 (79%) of the 105 individuals. The expression of oestrogen receptors (ER) was observed in 68% of the 107 from the overall 109 cases, and progesterone receptors (PR) were observed in 62% of the 106 among 109 cases. 90 patients out of 109 were tested for the expression of Ki-67 and 97% of them were positive. Clinical and demographic characteristics of the study population are summarised in Table I.

Genotyping of *IGF-1* (rs rs7136446 T > C) polymorphism

Two hundred and five out of the 221 women (cases $n = 109$ and controls $n = 96$) were genotyped for the *IGF-1* (rs7136446 T > C). Genotype frequencies of the *IGF-1* rs7136446 conformed to the Hardy-Weinberg equilibrium ($x^2 = 3.31$, $p = 0.069$). The CC genotype was found in 15 (14%) cases and in 37 controls (38.5%), while TT genotype was encountered in 53 (49%) cases and in 21 (22%) controls. As for the genotype CT, it was found in 41 (37%) cases and in 38 controls (39.5%) (Fig. 1A). There was difference in genotype distribution among controls and BC patients ($p = 0.00001$). The distribution of IGF1 polymorphism is summarised in Table III. Analysis of the distribution of the allelic frequencies of 96 healthy subjects originating from the west of Algeria revealed that the allele (T) represents the minor allele with a frequency of 0.42 (Table III). In addition to that, the analysis of genotype frequencies of the IGF1 rs7136446 polymorphism between the case and control groups showed a statistically significant difference for the minor (TT) genotype as well as the cumulative CT+CC and CT+GG genotypes (respectively 49% vs. 22%, $p = 10^{-4}$, 86% vs. 61%, $p = 0.00004$ and 51% vs. 78%, $p = 0.00007$) also, the analysis of the distribution of the allelic frequency of the allele T revealed a significant difference between the cases and the controls (67% vs. 42%, $p = 10^{-6}$, OR = 2.88 [1.92–4.30]) (Table III). Furthermore, we studied the correlation between genotypes and the 2 HER-2 positive and HER-2 negative of all 109 cases. A statistical difference was found between the genotype distribution of *IGF-1* polymorphism and BC susceptibility after stratification according to HER-2 positive and HER-2 negative status ($p = 0.04$)

Table III. Distribution of *IGF-1* (rs7136446) and *IL-6* (rs1800795) polymorphisms

Parameters	Cases, *n* (%)	Controls, *n* (%)	MAF (controls)	*p*-value*	Odds ratio [95% CI]
IL-6 (rs1800795)	109	112			
GG	72 (66)	86 (77)			
CG	35 (32)	26 (23)	0.12	0.10	–
CC	02 (02)	00 (00)			
GG vs. CC+CG	37 (34)	26 (23)		0.14	–
CC vs. GG+CG	107 (98)	112 (100)		0.07	–
C allele	39 (18)	26 (12)			
G allele	179 (82)	198 (88)		0.06	–
IGF-1 (rs7136446)	109	96			
TT	53 (49)	21 (22)			
CT	41 (37)	38 (39.5)	0.42	**10-4**	3.32 [1.81–6.10]
CC	15 (14)	37 (38.5)			
CC vs. TT+CT	94 (86)	59 (61)		**0.00004**	0.26 [0.13–0.51]
TT vs. CC+CT	56 (51)	75 (78)		**0.00007**	3.32 [1.81–6.10]
T allele	147 (67)	80 (42)			
C allele	71 (33)	112 (58)		10^{-6}	2.88 [1.92–4.30]

CI –95% confidence, MAF(q) – minor allele frequency, OR – odds ratio, p – significance
** The values are presented as genotypes and alleles number (N) and frequency in percentage (%).*
p-value – threshold value ≤ 0.05, OR – odds ratio with a 95% confidence interval

but this association did not last after the correction of Bonferroni (Table IV). Indeed, we found the frequency of the cumulative CT+CC genotype was statistically significant (27% vs. 54%, $p = 0.02$, OR = 3.0 [1.09–8.18]) this association was confirmed after the correction of Bonferroni (Table IV).

Genotyping of *IL-6*-174 (rs1800795 G > C) polymorphism

All 221 women were genotyped for the *IL-6*-174 (rs1800795 G > C) polymorphism. Genotype frequencies of the *IL-6-174* polymorphism conformed to the Hardy-Weinberg equilibrium ($x^2 = 1.93$; $p = 0.165$). The CC genotype was found in 2 (5.9%) cases and 0 controls, while the GG genotype was encountered in 83 (11.5%) cases and 82 (7.2%) controls. As for the CG genotype, it was found in 25 cases and 26 controls (Fig. 1B). There was no difference in genotype distribution among controls and BC patients ($p = 0.10$). The distribution of *IL-6* polymorphism is summarised in Table II. Analysis of the distribution of the allelic frequencies of 112 healthy subjects originating from the west of Algeria revealed that the allele (C) represents the minor allele with a frequency of 0.12. Also, the analysis of genotype frequencies of the *IL-6* rs1800795 polymorphism between the case and control groups showed no statistically significant difference for the minor (CC) genotype as well as the 2 cumulative genotypes CG+CC and GG+CG ($p > 0.05$) (Table III). Furthermore, we studied the correlation between genotypes and clinicopathological characteristics including the HER-2 positive and HER-2 negative status in the case group. No statistical difference was found between the genotype distribution of *IL-6* polymorphism and BC susceptibility after stratification according to clinicopathological parameters (Table IV) or to HER-2 positive and HER-2 negative (Tables IV, V).

Discussion

In the present case-control study of 221 women, we investigated the association between the IGF1 polymorphism (rs7136446) and BC, and the association between the 174G/C polymorphism (rs1800795) of *IL-6* and BC, in the western Algerian population. Then, we compared the distribution of *IGF-1* and *IL-6* polymorphisms in the 2 HER-2positive and HER-2 negative subgroups among BC patients. Lastly, we focused on studying the association between *IL-6* (rs1800695) polymorphism and clinicopathological characteristics with BC.

First, the distribution of allelic frequencies revealed that the T allele is the minor allele with a frequency of 0.42. No study before has shown a similar result. Second, the analysis of the distribution of *IGF-1* polymorphism showed a significant associ-

Table IV. Distribution of *IGF-1* (rs7136446) and *IL-6* (rs1800795) in HER-2 positive and HER-2 negative breast cancer

Gene SNP	HER2-positive breast cancer, $n = 22$ (%)	HER2-negative breast cancer, $n = 83$ (%)	*p*-value	Odds ratio [95% CI]
IL-6 (rs1800795)				
GG	16 (73)	52 (63)		
CG	06 (27)	29 (35)	0.57	–
CC	00 (00)	02 (02)		
GG vs. CG+CC	06 (27)	31 (37)		–
CC vs. CG+GG	22 (100)	81 (98)		–
C allele	06 (14)	33 (20)		
G allele	38 (86)	133 (80)		–
IGF-1 (rs7136446)				
TT	16 (72)	38 (46)	0.04	3.0 [1.09–8.18]
CT	03 (14)	35 (42)		
CC	03 (14)	10 (12)		
CC vs. CT+TT	19 (86)	73 (46)	0.84	–
TT vs. CT+CC	06 (27)	45 (54)	0.02	3.0 [1.09–8.18]
T allele	35 (80)	111 (67)	0.10	–
C allele	09 (20)	55 (33)		

CI – 95% confidence, OR – odds ratio, p – significance, SNP – single nucleotide polymorphisms
** The values are presented as genotypes and alleles number (N) and frequency in percentage (%).*
p-value – threshold value ≤ 0.05, OR – odds ratio with a 95% confidence interval

Corrected p-value ($p \leq 0.02$) and OR > 1 {CI 95%} after correction of Bonferroni considered significant

ation in genotype distribution among controls and BC patients ($p = 0.00001$). Which indicates that the polymorphism *IGF-1* (rs7136446 T > C) does seem to have an effect on the occurrence of BC in the population of western Algeria. This result needs to be confirmed on a larger sample. In another study, *IGF-1* (rs7136446) was found to be associated with postmenopausal BCs [31]. However, Costa-Silva *et al.*, did not find a significant association with BC in premenopausal or postmenopausal women [23]. The same result was found by Henningson *et al.*, in a Swedish study, in which they revealed a negative association between a set of 9 haplotypes taggins single nucleotide polymorphism (htSNPs) in 3 haplotype blocks including rs7136446 with *IGF-1* levels in this study [32]. In the same context of studying the association between *IGF-1* genomic variants with BC, Al-Zahrani *et al.*, demonstrated that other polymorphisms of the *IGF-1* gene have been associated with BC risk and increased *IGF-1* plasma level [19]. *IGF-1* variant (rs7136446) was positively associated with both subgroups of HER-2 (positive and negative) ($p = 0.04$), but this result did not last after the correction of Bonferroni. To our knowledge, no study has addressed the association between the *IGF-1* (rs7136446) and the 2 groups of HER-2 (positive and negative) with BC before. On the other hand, other genomic variants of the *IGF-1* gene have been associated with clinical outcome of HER-2 positive BC patients [33, 34].

In order to shape the tumour microenvironment and encourage metastasis, cancer cells primarily communicate with the host *via* cytokines, which also aid in tumour dissemination, epithelial-mesenchymal transitions (EMT), motility, and invasion [35]. A pro-inflammatory cytokine *IL-6* is secreted by a variety of cells in the tumour microenvironment, including malignant cells [36]. *IL-6* local and systemic overexpression has been linked to numerous cancer types, including BC [37]. While upregulation of *IL-6* serum levels is generally associated with poor prognosis and low survival rate in patients with BC, downregulation of *IL-6* is related to a better response to treatment [38, 39]. *IL-6* polymorphisms have also been linked to a higher risk of BC. Several studies have been performed on the *IL-6* promoter SNP-174 G > C (rs1800795) collected in meta-analysis [40].

The distribution of allelic frequencies revealed that the C allele is the minor allele with a frequency of 0.12. The frequency observed in our population is similar to those reported in the Tunisian population [41]. Second, the analysis of the distribution

Table V. Frequency distribution of the *IL-6* polymorphism (rs1800795) in the group of cases after stratification by clinicopathological characteristics

Clinicopathological characteristics	IL-6 (rs1800795)				
	CC (%)	CG (%)	GG (%)	*p*-value	Odds ratio [95% CI]
Age at diagnosis (years), $n = 109$					
≤ 50	02	15	41	0.15	–
> 50	00	20	31		
Histological grade (SBR), $n = 108$					
Grade I	00	00	02		
Grade II	01	21	46	0.85	–
Grade III	01	13	24		
Distant metastases, $n = 109$					
Negative	00	18	40	0.28	–
Positive	02	17	32		
ER receptor status, $n = 107$					
Negative	00	13	27	0.52	–
Positive	02	22	42		
PR receptor status, $n = 106$					
Negative	02	29	52	0.57	–
Positive	00	06	16		
Ki-67 expression, $n = 90$					
Negative	00	01	02	0.98	–
Positive	01	27	59		
Location of BC, $n = 101$					
Left breast	01	20	31	0.42	–
Right breast	01	15	40		

** The values are presented as genotypes and alleles number (N) and frequency in percentage (%).*
CI – 95% confidence, OR – odds ratio, p – significance
p-value – threshold value ≤ 0.05, OR – odds ratio with a 95% confidence interval
Corrected p-value (p ≤ 0.02) and OR > 1 {CI 95%} after correction of Bonferroni considered significant

of *IL-6* polymorphism showed no association in genotype distribution among controls and BC patients ($p = 0.10$). Which indicates that the polymorphism *IL-6*-174 (rs1800795 G > C) does not seem to have an effect on the occurrence of BC in the population of western Algeria. This result needs to be confirmed on a larger sample. Similar results were found in previous studies [41, 42]. Also, a study on a French Caucasian population showed no association with BC diagnosis or prognosis [43]. A meta-analysis conducted on 25,703 subjects suggested that this polymorphism was not associated with BC risk [40]. In contrast with our study De-Michele *et al*. [44] found that *IL-6*-174G/C polymorphism is associated with clinical outcome in a cohort of node-positive BC patients who received high-dose adjuvant therapy [44]. Similarly, a study conducted by Iacopetta *et al.* on Australian BC subjects concluded that IL6 174C allele was associated with a more aggressive BC phenotype [45]. Pooja *et al*. were the first to report the protective effect of this polymorphism in BC risk [46].

No significant association was found between *IL-6* (rs1800795) and any clinicopathological characteristics like age, grade, and metastases (respectively, $p = 0.15$, $p = 0.85$, and $p = 0.28$). Our results were in agreement with previous genetic studies [41]. In contrast to our findings, it was shown in a previous study that the CC genotypes and C allele of *IL-6*-174 G > C were higher among BC patients, and they were found to be significantly associated with advanced stage (metastasis) [46]. In another study allelic variations analysis indicated that the risk of developing metastases was higher in patients with the GG genotype at rs1800795 [47]. Metastatic cancer cells undergo 2 phenotype switching processes: mesenchymal-to-epithelial transition and EMT [48]. Inflammation is a well-known factor in the development of EMT

[48, 49]. As a result, it has been shown that *IL-6* takes part in EMT and increases the recruitment of mesenchymal stem cells to human BC cells [50, 51].

According to some research, the mutant GG genotype is a poor prognostic indicator linked to decreased disease-free survival in ER+ patients following chemotherapy [52] as well as a higher risk of metastasis regardless of ER status [53]. The CC genotype, on the other hand, was connected to a more aggressive behaviour and worse overall survival, according to another study [47]. The responsiveness of BC cells to *IL-6* depends heavily on the expression of ER and PR. Hormone-sensitive cells exhibit a higher response to *IL-6* than hormone insensitive cells, which is associated with the intrinsic generation of higher IL-6 in these cells [54, 55]. While the ER-expressing BC cells mostly secrete sIL-6R, ER – cells mainly express mIL-6R [56]. It has also been shown that IL-6 suppresses ER – cells under normal condition in an autocrine manner [57]. In our study, no significant association was found between *IL-6* (rs1800975) and the expression of ER and PR (respectively $p = 0.52$ and $p = 0.5$). In contrast to our result a significant association between the *IL-6* (-174) and both the ER and PR+ was revealed [58].

IL-6 variant (rs1800975) was negatively associated with both groups of HER-2 (positive and negative) ($p = 0.5$). This result was in accordance with another recent Tunisian study, which demonstrated that the distribution of genotype frequencies rs1800795 had no statistical difference between HER-2 positive and HER-2 negative BC [41]. Korobeinikova *et al*. found an association between the *IL-6* (rs1800795) and HER-2 negative status [58]. HER-2 overexpression in BC stem cells has been shown to increase *IL-6* production [6]. Furthermore, the activation of the *IL-6* inflammatory loop induces trastuzumab-resistance in HER-2 positive BC cells indicating that *IL-6*'s pro-inflammatory role mediates BC therapeutic resistance [59]. No significant difference was found between the *IL-6* variant and the 2 clinicopathological characteristics, the expression of Ki-67, and the location (left or right) of BC (respectively, $p = 0.98$ and $p = 0.4$). No study so far has investigated the association between either rs1800795 and Ki-67 expression or between rs1800795 and BC location.

This study is the first report of *IGF-1* rs7136446 and rs1800795 polymorphisms in *IL-6* gene among BC patients from Algeria. However, several limitations to this study should be noted. First, the samples were obtained from a population of women in Algeria, and thus the findings cannot be generalised. Second, a limited number of SNPs in *IGF-1* and *IL-6* genes were chosen for this study. We recommend more SNPs that were not well studied previously be included in further studies. Lastly, the results in this study should be confirmed in more regions in Algeria, not only in the west, and in a larger population.

Conclusions

The results of the current study indicate that BC risks are significantly associated with *IGF-1* rs7136446 and that *IL-6* rs1800795 polymorphism are not associated with BC in the western Algerian population.

Disclosures

1. Institutional review board statement: Not applicable.
2. We want to thank all the medical staff and medical assistants for their help in collecting biological samples and reviewing medical data.
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4. Conflicts of interest: None.

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