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ORIGINAL ARTICLE

Clinicopathological and Molecular Study of Triple-Negative Breast Cancer in Algerian Patients

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Abstract Triple-negative breast cancer (TNBC) is associated with aggressive tumor behavior, poor prognosis and *BRCA1* mutations. There are limited data regarding TNBC among Algerian women. In this study, we sought to determine clinical and tumor characteristics associated with TNBC. We also screened for the prevalence of *BRCA1* mutations in unselected cohort of TNBC patients. Clinical and tumor characteristics data of 877 breast cancer patients diagnosed between 2011 and 2015, were collected from cancer registry of public hospital of Rouiba. Patients were divided in two groups: those with TNBC and those with other breast cancer subtypes. Differences between the two groups with regard to clinical and tumor characteristics were compared using Fisher's exact test. *BRCA1* mutations analysis was performed in unselected cohort of 103 women with TNBC, including all exons where a mutation was previously found in Algerian population (exons 2, 3, 5, 11). The median age at diagnosis for TNBC and non-TNBC patients was 47.4 years and 49.4 years, respectively. The proportion of TNBC was 19.95%. Our data showed significant differences in menopausal status, TNM stage, histological type, tumor histological grade, Ki67 expression and family history of breast cancer between TNBC and non-TNBC patients. Four distinct deleterious mutations in *BRCA1* gene were detected in eight young TNBC patients. TNBC is associated with young age, poor histopathological characteristics and family history of breast cancer. *BRCA1* mutations have been detected in young TNBC patients. TNBC phenotype should be added as criterion to screen for *BRCA1* mutations in Algerian women.

Keywords Algerian women · Breast cancer · Triple negative · Clinicopathological characteristics · *BRCA1* mutations · Genetic testing

Abbreviations

Array–CGH	Array Comparative Genomic Hybridisation
ER	Estrogen Receptor
HER2	Human Epidermal growth factor Receptor 2,
Ki67	Antigen Ki67
IDC	Invasive Ductal Carcinoma
IHC	Immunohistochemistry
ILC	Invasive Lobular Carcinoma
IPC	Infiltrating Papillary Carcinoma
LGR	Large Genomic Rearrangement
MC	Mixed Carcinoma (invasive ductal and invasive lobular)
MLPA	Multiplex Ligation Probe Amplification
NGS	Next Generation Sequencing
PR	Progesterone Receptor

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Introduction

Breast cancer is currently the leading cause of cancer deaths among Algerian women. To date, there has been an increase in breast cancer incidence in Algeria, over the past two decades [1, 2]. Triple-negative breast cancer (TNBC), defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, accounts for 10 to 17% of all breast cancers in Caucasian women [3–5]. However, several studies have reported that African-American women, Sub-Saharan women, West African women, and premenopausal women have higher incidence of TNBC [6–11]. The prevalence of TNBC differs between races and it is associated with early recurrence of disease and poor outcome [12–15]. TNBC is characterized by a lack of therapeutic target in contrast with hormonal receptor positive (ER+ and PR+) and HER2+ breast cancers [16]. Interestingly, women with a history of breast cancer family have a significantly increased risk of triple-negative breast cancer [17]. TNBC shows substantial overlap with basal-type and BRCA1-related breast cancers [18, 19]. TNBC accounts for 70% of breast tumors arising in *BRCA1* germline mutations carriers and 16 to 23% of breast tumors in *BRCA2* carriers [20]. Studies have reported that triple-negative cancers which harbor a dysfunctional *BRCA1* pathway may be sensitive to platinum-based therapy and to inhibitors of PARPs (such Olaparib) that selectively target cells deficient in homologous DNA repair [21–23]. In the present study, we aimed to determine the differences in clinicopathological features between TNBC and non-TNBC patients. We also screened for *BRCA1* germline mutations in unselected cohort of 103 women with TNBC, including all exons where a mutation was previously found in Algerian population (exons 2, 3, 5, 11) [24].

Materials and Methods

Study Population

A total of 1317 breast cancer patients were diagnosed between 2011 and 2015 in the academic medical oncology service of public hospital of Rouiba. Among these cases, 432 did not have sufficient medicals records and eight male breast cancers. Therefore, 877 female breast cancer patients with sufficient clinical information were included in the present study. Patients with triple-negative breast cancer were categorized into TNBC and those with the other breast cancer subtypes were categorized non-TNBC.

We analyzed breast cancer patients' ≥18 years from the cancer registry of public hospital of Rouiba. This cancer registry covered an area of 30 provinces among 48 of Algeria (Fig. 1). Patient and tumor information included: age at diagnosis, age at first menarche, breast feeding, oral contraception, marital status, parity, menopausal status, receptor status, Ki67 index, TNM stage, histological type, tumor histological grade and family history of breast cancer. The clinical stage of breast cancer was determined according to the 6th edition of the TNM manual [25]: T1N0M0, T2-T3N0M0, T4anyNM0 or AnyTN3M0 and AnyTNM1.

TNBC patients tested for *BRCA1* germline mutations signed written informed consent. The study was approved by the institutional review boards and ethical approval was obtained from appropriate institutions.

Immunohistochemistry

Tumor expression for hormone receptors (ER and PR), HER2 and Ki67 index was evaluated by breast cancer pathologists of the main Algerian health public quality- controlled laboratories. Immunohistochemistry staining of hormone receptors (ER and PR) was performed by using the Kit Envision+™ (Dako). HER2 expression was tested by immunohistochemistry by using HercepTest™ kit (code K5204, Dako). Ki67 expression was tested by immunohistochemistry by using EnVision™ FLEX kit (K8000, Dako). The ER assay clone used was 1D5, the PR assay clone was PgR636 and the detection system was a polymer. The Ki67 assay clone was MIB-1. Hormone receptors (ER and PR) were positive when at least 10% of tumor cell nuclei were stained. HER2 was considered positive if graded 3+ on immunohistochemistry performed according to ASCO guidelines [26]. All other grades (0 to 2+) were considered negative unless chromogenic in situ hybridization (CISH) of 2+ cases confirmed increased gene copy number (Dako DuoCISH ™, code SK108). Ki67 expression was classified as low (<20%) or high (≥ 20%).

Breast Cancer Subtypes Definitions

We classified breast cancers into five subtypes based on hormone receptor status, HER2 status and Ki67 index as follow [27, 28]: luminal A (ER+ and/or PR+, HER2-, low Ki67); luminal B-HER2 negative (ER+ and/or PR+, HER2-, high Ki67); luminal B-HER2 positive (ER+ and or/PR+, HER2+, any Ki67), HER2+ (enriched) (ER-, PR-, HER2+, any Ki67), triple-negative (ER-, PR-, HER2-, any Ki67). In this study, combined negative ER, PR, and HER2 status was classified as triple-negative breast cancer, and any positive receptor status was considered non–triple-negative breast cancer.

DNA Isolation

Genomic DNA was extracted from peripheral blood lymphocytes using Promega Wizard Genomic DNA Purification Kit (Promega, Madison, MI, USA) (Cat. # A1120) and in accordance with the manufacturer's protocols.

Mutation Analysis

BRCA1 gene was screened by PCR-direct sequencing in unselected cohort of 103 women with TNBC, including all

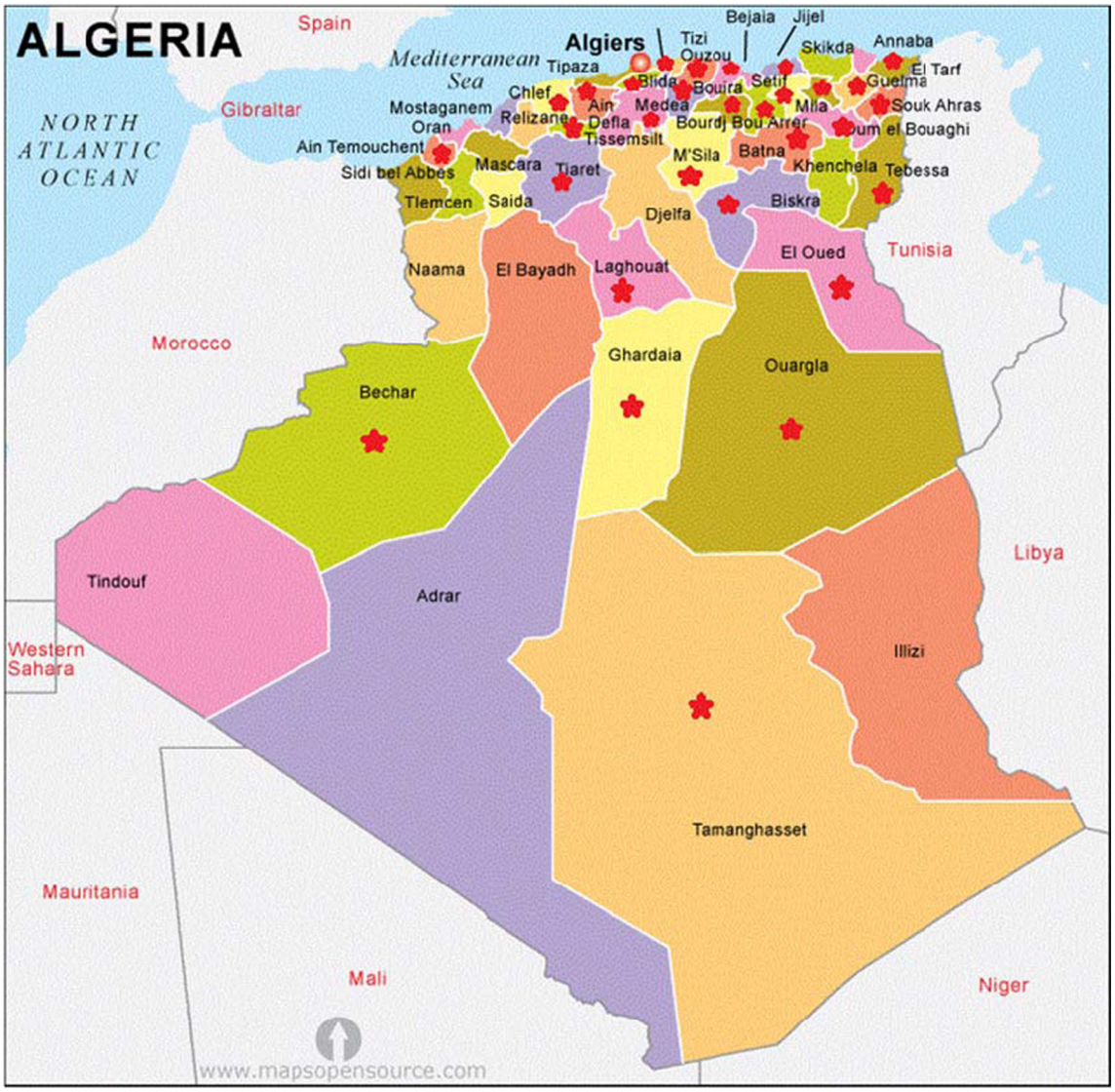

Fig. 1 Map showing the 30 Algerian provinces (indicated by red star symbol) covered by the cancer registry of public hospital of Rouiba where the patients included in our study were diagnosed and treated

exons where a mutation was previously found in Algerian population (exons 2, 3, 5, 11). PCR and Sanger sequencing were performed as described elsewhere [29].

Nomenclature and Variant Analysis

All nucleotide numbers refer to the wild-type cDNA human sequence of *BRCA1* (accession no. U14680; version U14680.1 GI: 555,931) as reported in the GenBank database. The description of nucleotide sequence variants is in accordance with HGVS (Human Genome Variation Society) nomenclature (www.hgvs.org/mutnomen). The HGVS approved systematic nomenclature follows the rule where the nucleotide +1 is the A of the ATG translation initiation codon.

Statistical Analysis

Differences between TNBC and non-TNBC with regard to clinico-pathological parameters were compared using Fisher's exact test. A P value of <0.05 was considered as statistical significance. IBM SPSS statistics V20 was used for data analysis.

Results

Comparison of Clinicopathological Features of TNBC and Non-TNBC

A total of 877 female breast cancer patients were included in the present study. They were categorized into TNBC and those

with the other breast cancer subtypes were categorized non-TNBC. The median age at diagnosis of TNBC patients and non-TNBC patients was 47.4 years and 49.4 years (age ranged from 22 to 84 years). Tables 1 and 2, Figs. 2, 3, 4 and 5 summarize the results. TNBC had 175 patients (19.95%). TNBC and non-TNBC were significantly different by premenopausal and postmenopausal status ($P = 0.0345$). We noticed a higher proportion of TNBC patients with premenopausal status than in non-TNBC patients (68% VS 58.31%) (Fig. 2). The ratio of non-TNBC patients with postmenopausal status was higher compared to TNBC patients (40.56% VS 32%) (Fig. 2). A significant difference in the distribution of the clinical stage of tumor between TNBC and non-TNBC was found ($P = 0.000$). 70.29% of TNBC patients were diagnosed at stage T4 anyNM0 compared to 54.08% in non-TNBC patients. The histologic grade of TNBC and non-TNBC was compared. The Fisher's exact test showed a significant difference in the distribution of histologic grade of the diagnosed TNBC and non-TNBC. In TNBC cases, the proportion of grade 3 tumors was 52.57% compared to 22.96% in non –TNBC patients (Fig. 3). Our results showed a significant difference in the distribution of histological tumor type between TNBC and non-TNBC ($P = 0.004$). Invasive ductal carcinoma (IDC) was the commonest histological type in the two groups (85.71% VS 80%). Comparison of Ki67 expression between TNBC and non-TNBC revealed a significant difference ($P = 0.001$). We found 91.43% of TNBC with high Ki67 expression (≥ 20) compared to 52.40% in non-TNBC. 47.60% of non-TNBC had low Ki67 expression (<20%) compared to 8.57% in TNBC (Fig. 4). We also compared the family history of breast cancer in TNBC and non-TNBC patient. Among TNBC patients, 65 (37.15%) of them had a family history of breast cancer (Fig. 5). In the group of non-TNBC patients, 123 (17.32%) of them had a family history of breast cancer (Fig. 5). The statistical analysis showed a significantly high prevalence of breast cancer in the family members of TNBC patients compared to non-TNBC patients ($P = 0.000$). Comparison of age at menarche, parity, oral contraception and breast feeding between TNBC and non-TNBC patients didn't show any significant differences.

Table 1 Clinicopathological features of breast cancer patients included in our study

Characteristics	Patients N (%) =877
Overall mean age	48.88 year ($T = 42,870$)
Age	
< 40y	138 (15.73%)
40–49	372 (42.42%)
50–59	224 (25.54%)
60–69	111 (12.66%)
≥ 70	32 (3.65%)
Menopausal status	
Premenopausal	533 (60.78%)
Postmenopausal	344 (39.22%)
TNM stage	
T1N0M0	95 (10.83%)
T2-T3N0M0	174 (19.84%)
T4anyNM0 or AnyTN3M0	505 (57.58%)
AnyTNM1	87 (9.92%)
Unclassified	16 (1.83%)
Histological grade	
I	74 (8.44%)
II	551 (62.83%)
III	250 (28.50%)
unknown	2 (0.23%)
Histological type	
IDC	712 (81.19%)
ILC	86 (9.81%)
MC	31 (3.53%)
Others	48 (5.47%)
Ki −67	
< 20%	347 (39.57%)
≥ 20%	530 (60.43%)
Luminal A	279 (31.81%)
Luminal B-HER2 negative	212 (24.17%)
Luminal B-HER2 positive	159 (18.13%)
HER2+ (enriched)	52 (5.93%)
Triple negative	175 (19.95%)
Family history	
Yes	185 (21.10%)
No	679 (77.42%)
unknown	13 (1.48%)
Age of menarche (years)	
(All cases 850)	13.13 y
Marital status	
Married	746 (85.06%)
Single	124 (14.14%)
unknown	7 (0.80%)
Oral contraception	
Yes	465 (53.02%)
No	386 (44.01%)
unknown	26 (2.97%)
Breastfeeding (All cases 746)	
Yes	631 (84.58%)
No	96 (12.87%)
unknown	19 (2.55%)
Parity(All cases 746)	
Yes	689 (92.36%)
No	50 (6.70%)
unknown	7 (0.94%)

BRCA1 Mutations Analysis in TNBC Patients

The analysis of DNA samples of unselected cohort of 103 women with TNBC revealed that eight (08) patients carried pathogenic germline mutations in *BRCA1* gene (7.8%). Tables 3 and 4 summarize the results. The *BRCA1* deleterious mutations have been detected in patients with TNBC diagnosed at age ≤ 45 years. In addition, most of TNBC patients with mutations had family history of breast and/or ovarian cancer, high grade tumors and high Ki67 expression (Table 4). Four (04) distinct pathogenic mutations: c.83_84delTG, c.181T>G, c.798_799delTT and c.2125_2126insA were identified in this study (Table. 4). The recurrent *BRCA1* mutation c.83_84delTG has been identified in this study in 3 young TNBC patients with a frequency of 2.91%. The c.181T>G/p.Cys61Gly mutation has been

Table 2 Clinical and tumor characteristics of patients with TNBC and non-TNBC

Characteristics	TNBC N (%) $N = 175$ (19.95%)	Non TNBC N (%) $N = 702$ (80.04%)	P value*
Mean age	47.40y ($T = 8295$)	49.25 y ($T = 34,575$)	
Age at diagnosis			
< 40	37 (21.14%)	101 (14.39%)	0.078
40–49	78 (44.57%)	294 (41. 88%)	
50–59	36 (20.57%)	188 (26.78%)	
60–69	17 (9.72%)	94 (13.39%)	
≥ 70	7 (4.00%)	25 (3.56%)	
Menopausal status			
Premenopausal	119 (68%)	414 (58.97%)	0.0345
Postmenopausal	56 (32%)	288 (41.03%)	
TNM stage			
T1N0M0	6 (3.43%)	89 (12.68%)	0.000
T2-T3N0M0	24 (13.71%)	150 (21.37%)	
T4anyNM0 or AnyTN3M0	123 (70.29%)	382 (54.42%)	
AnyTNM1	16 (9.14%)	71 (10.11%)	
Unclassified	6 (3.43%)	10 (1.42%)	
Histological grade			
I	6 (3.43%)	68 (9.69%)	0.000
II	77 (44.00%)	474 (67.52%)	
III	92 (52.57%)	158 (22.51%)	
Unknown	0 (0%)	2 (0.28%)	
Histological type			
IDC	150 (85.71%)	562 (80.06%)	0.004
ILC	10 (5.72%)	76 (10.83%)	
MC	1 (0.57%)	30 (4.27%)	
Others	14 (8.00%)	34 (4.84%)	
Ki-67			
< 20	15 (8.57%)	332 (47.30%)	0.001
≥ 20	160 (91.43%)	370 (52.70%)	
Family history			
Yes	65 (37.15%)	120 (17.10%)	0.000
No	107 (61.14%)	572 (81.48%)	
Unknown	3 (1.71%)	10 (1.42%)	
Age of menarche (years)	13.05	13.13	
Marital status			
Married	142 (81.14%)	604 (86.18%)	0.162
Single	32 (18.29%)	91 (12.96%)	
Unknown	1 (0.57%)	6 (0.86%)	
Oral contraception			
Yes	92 (52.57%)	373 (53.13%)	0.262
No	81 (46.29%)	305 (43.45%)	
Unknown	2 (1.14%)	24 (3.42%)	
Breastfeeding(All cases 746)			
Yes	122 (85.91%)	509 (84.27%)	0.317
No	19 (13.38%)	77 (12.75%)	
Unknown	1 (0.70%)	18 (2.98%)	
Parity (All cases 746)			
Yes	131 (92.25%)	558 (92.38%)	0.3251
No	11 (7.75%)	39 (6.46%)	
Unknown	0 (0%)	7 (1.16%)	

IDC: infiltrating ductal carcinoma; IMC: infiltrating metaplastic carcinoma; MC: Mixed Carcinoma (invasive ductal and invasive lobular). *Fisher's exact test

detected here in a young TNBC patient (diagnosed at age 36 years). The *BRCA1* mutation c.798_799delTT was identified in this study in a young TNBC patient with a family history of breast cancer. Interestingly, the *BRCA1* c.2125_2126insA deleterious mutation has been detected here for the first time in three Algerian young TNBC patients with a family history of breast cancer and/or prostate cancer.

Discussion

TNBC is a subtype of breast cancer associated with poor prognosis, aggressive tumor phenotype (s), *BRCA1* mutations and lack of targeted therapies. To date, there are limited data regarding TNBC in Algerian women. We hereby provide a study which aims to determine the proportion of TNBC, its

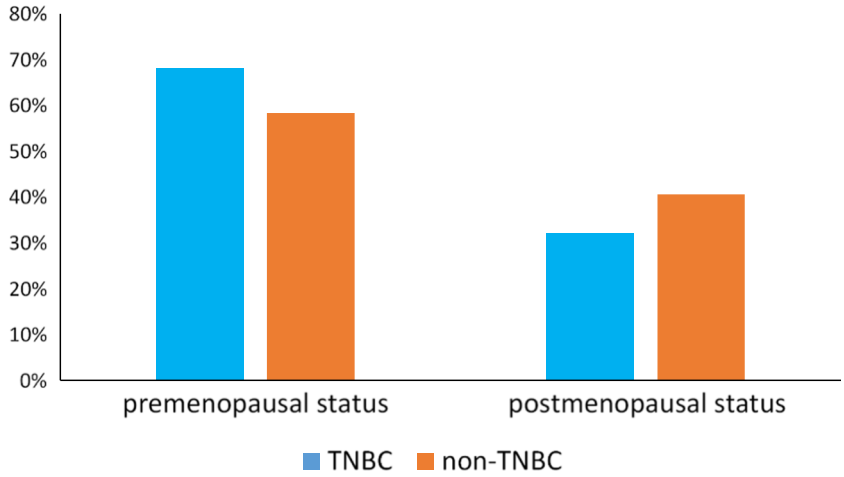

Fig. 2 Proportions of TNBC and non-TNBC patients associated with premenopausal and postmenopausal status. ($P = 0.0345$). P value from Fisher's exact test

clinical, histopathological and molecular characteristics among Algerian women. In our study population, the mean age at diagnosis of TNBC was 47.40 years less than the median onset age of non-TNBC which was 49.40 years. To date, the average of age at onset of breast cancer in North Africa is 10 years below of that in Western countries [2]. This finding is in accordance with previous studies in TNBC patients from Tunisia and Morocco [15, 30] and in African-American Women and Sub-Saharan women [6, 11]. In our study, TNBC and non-TNBC were significantly different by premenopausal and postmenopausal status. We have found that 68% of the TNBC patients were premenopausal women compared to 58.31% in non-TNBC patients. The results coincide with epidemiological studies that have shown that TNBC tends to occur in pre-menopausal women in young African-American women, Sub-Saharan women and West African women [6–11]. The proportion of TNBC in our study was 19.77%. Interestingly, this proportion is higher compared to TNBC patients (Caucasian women) in Europe, America and in Chinese women. Reports from Europe, America and China have shown that proportion of TNBC subtype varies from 11.39 to 16% [6, 12, 14, 31]. We noted that the proportion of TNBC subtype in Algerian women is similar to that in African-American patients (20 to 26.4%) [9, 14, 32], in South African Black women 20.4% [33] and in Tunisia (22.5%) [15]. The proportion of TNBC in patients from Mali, Senegal and Nigeria is much higher (45% to 55%) than in Algerian patients [8, 11].). The high proportion of TNBC subtype in Algerian women compared to Caucasian women with European ancestry could be linked to environmental factors and to Sub-Saharan African genetic elements of Algerian population [24]. The present study has shown that TNBC has distinct clinicopathological features compared to non-TNBC. The results showed also significant differences in clinical stage, histological grade, histological type and Ki67 expression between TNBC and non-TNBC. TNBC is associated with higher clinical stage, higher percentage of invasive ductal carcinoma, higher histological grade, higher Ki67 expression than that in the non-TNBC. These findings were consistent with previous studies in TNBC patients from various populations [5, 33–36]. In this study, interestingly, the results showed that the rate of individuals who had the family history of breast cancer in TNBC group was statistically significant higher than that in the non-TNBC group. These findings are similar with

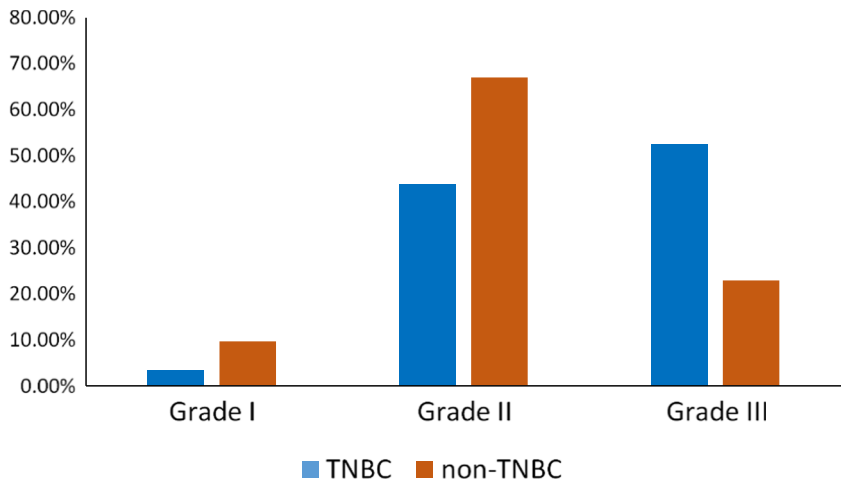

Fig. 3 Proportions of TNBC and non-TNBC patients associated with histological tumor grade. ($P = 0.000$). P value from Fisher's exact test

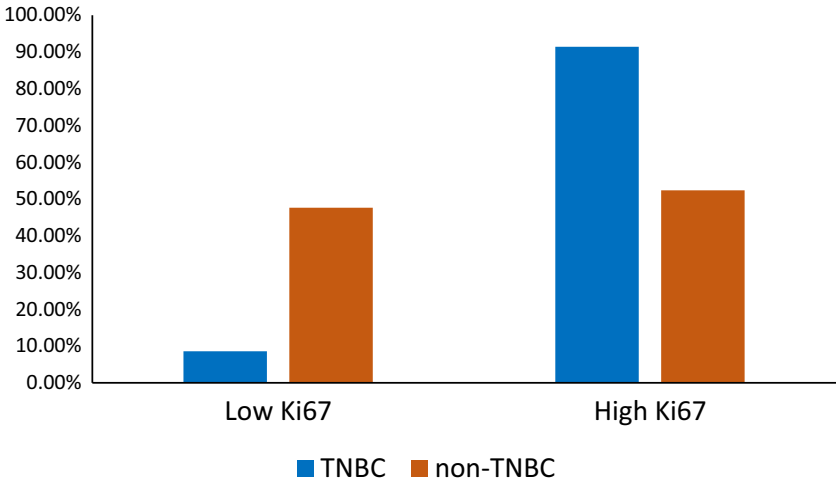

Fig. 4 Proportions of TNBC and non-TNBC patients associated with Ki67 expression. ($P = 0.001$). P value from Fisher's exact test. Ki67 cut-off: low (<20%) or high (≥ 20%)

studies in TNBC patients from UK and China [19, 35, 36]. Our results have shown no significant differences in age at menarche, oral contraception, parity and breastfeeding between TNBC and non-TNBC patients. However, in term breastfeeding, many studies have reported that breastfeeding is inversely associated with overall risk of breast cancer [37–39]. Interestingly, this association may differ in breast cancer subtypes defined by receptor status [39]. High parity and the absence or short duration of breastfeeding were independently associated with triple-negative breast cancer in young African American and Hispanic patients [40]. The absence of association of parity and breastfeeding Algerian breast cancer patients with TNBC compared with young African American and Hispanic TNBC patients, might be due to genetic and environmental factors.

Interestingly, we identified in the present study ten (10) Triple negative cancer (TNC) in invasive lobular carcinoma (ILC) (11.62%) in 86 ILC cases (Table. 2). This result is very unusual. These 10 TNC in ILC showed higher incidence of high histologic grade (II and III), advanced stage and high expression of Ki67 compared to non-TNC in ILC. Usually, ILC is more likely to occur in older patients, it's larger in size, it's estrogen receptor (ER) and progesterone receptor (PgR) positive (Luminal A subtype) and has low to absent human epidermal growth factor receptor-2 (HER2) expression [41]. To date, Koo and Jung (2011) [42] reported 6.8% TNC in ILC in a cohort of 117 ILC cases from South Korea. TNC in ILC showed distinct clinicopathologic and IHC characteristics such as higher histologic grade and increased expression of galectin-3, compared to non-TNC in ILC [42]. As histological types and IHC analysis of ER, PR, HER2 receptors of our breast cancer patients have been determined in several clinical pathology laboratories around all over Algeria. We could not rule out some bias in the high proportion of TNC in ILC found in our present study.

To our knowledge, this is the first study to analyze *BRCA1*germline mutations in unselected cohort of 103 Algerian women with TNBC phenotype, including all exons where a mutation was previously found in Algerian population (exons 2, 3, 5, 11) [24]. This current study has identified four (04) *BRCA1* distinct deleterious mutations in eight (08) TNBC patients. These mutations are recurrent in Algerian

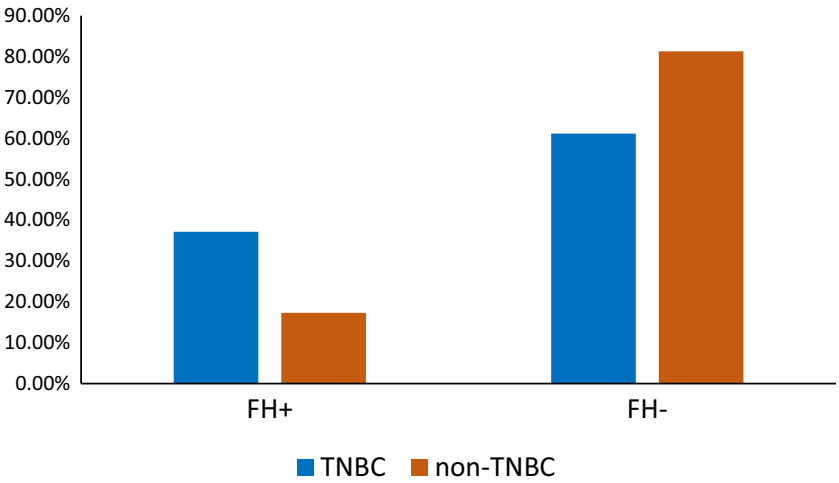

Fig. 5 Proportions of TNBC and non-TNBC patients associated with family history of breast cancer. ($P = 0.000$). P value from Fisher's exact test. FH+: family history. FH-: no family history

Table 3 Clinical characteristics of TNBC patients screened for *BRCA1* mutations (103 patients)

Characteristics	Levels	Number of cases
Mean age	43 years	103
Age at diagnosis	<40	35 (33.98%)
	40–49	46 (44.66%)
	50–59	20 (19.42%)
	60–69	2 (1.94%)
Menopausal status	Premenopausal	81 (78.64%)
	Postmenopausal	22 (21.36%)
TNM stage ($T = 102$)	T1N0M0	5 (4.90%)
	T2-T3N0M0	13 (12.75%)
	T4anyNM0 or AnyTN3MO	73 (71.57%)
	AnyTNM1	7 (6.86%)
	Unclassified	4 (3.92%)
Histological grade	I	2 (1.94%)
	II	44 (42.72%)
	III	57 (55.34%)
Histological type	IDC	92 (89.32%)
	ILC	4 (3.88%)
	MC	1 (0.97%)
	Others	6 (5.83%)
Bilateral breast cancer	contralateral cancer	3 (2.91%)
Ki-67 status	<20	2 (1.94%)
	≥20	42 (40.78%)
	Unknown	59 (57.28%)
Family history with any cancer	Yes	75 (72.82%)
	No	28 (27.18%)
Family history with breast ovarian cancer	Yes	43 (41.75%)
	No	60 (58.25%)
Age of menarche	(years)	13.42 ($T = 1383$)
Marital status	Married	79 (76.70%)
	Single	24 (23.30%)
Oral contraception	Yes	51 (49.51%)
	No	52 (50.49%)
Breast feeding (all cases 99)	Yes	61 (77.22%)
	No	18 (22.78%)
Parity (all cases 79)	Yes	70 (88.61%)
	No	9 (11.39%)

population [24, 29]. In this study, TNBC patients with *BRCA1* mutations were diagnosed at an earlier age, had high-grade tumors and high Ki67 expression. Interestingly, we found that *BRCA1* mutation has been detected in 62% of TNBC patients that reported family history of breast cancer, this finding is in agreement with previous studies [19, 20]. Several studies have reported that within *BRCA1* mutations carriers, TNBC status was associated with younger age at diagnosis and higher tumor grade in patients from various ethnicities [43–45]. The frequency of deleterious *BRCA1* mutations carriers was 7.8% (8/103) in our cohort. Interestingly, studies including patient populations with TNBC unselected for family history of breast cancer reported mutation frequencies ranged between 6 and 15% [21, 44–49].

There are potential limitations of our present study which should be considered. Sample size of the included study population is small. Firstly, we cannot rule out the fact that some differences not found significant between TNBC and non-TNBC in the current study may have reached statistical significance with a larger population. One limitation is under diagnosis and delayed diagnosis are effective in Algerian women living in rural areas of Algeria. Despite recent

Table 4 Clinicopathological characteristics of TNBC patients with deleterious *BRCA1* mutations

Index case ID	*BRCA1*	Nucleotide change	Amino acid change	Mutation type	Dx	Y	Histological type	Grade	TNM	Ki67	Family history
161398	Exon 3	c.83 _84delTG	p.Leu28Argfsx12	FS	30	31	IDC	III	$T_4N_1M_0$	70%	-Father PC dx: 74 -Uncle (M) SC dx:50
1613100	Exon 3	c.83 _84delTG	p.Leu28 Argfsx12	FS	45	46	IDC	II	$T_3N_1M_0$	NA	-Father CSU dx:72 -Cousin (P) CSU dx:65
0912421	Exon 3	c.83 _84delTG	p.Leu28 Argfsx12	FS	41	41	IDC	III	NA	NA	- Cousin (P) OC dx:61 dd:62 - Cousin (P) BC dx:55 dd:62 - Cousin (P) BC? dx:? dd:18
161372	Exon 5	c. 181T>G	p.Cys61Gly	MS	36	37	IPC	III	$T_4N_1M_0$	60%	-Father PC and metastatic LC dx73 -Aunt (P) BC dd 65
O161433	Exon 11	c.798_799delTT	p.Ser267Lysfsx19	FS	34	35	IDC	II	T_2NM	20%	Cousin (M) BC dx45y - Aunt (M)colon cancer, dx 50, dcd -brother: larynx cancer dx 35y, - uncle (P): pancreas cancer dx 60y - Cousin (P) LC
1613770	Exon 11	c.2125_2126insA	p.Phe709Tyrfsx3	FS	33	33	IMC	III	$T_4N_1M_0$	20%	-Sister, BC dx: 24 -Aunt (P) B BC dx 56 -Cousin (P) nodule in breast and ovarian cysts dx: 23 -Uncle (P) PC dd: 66 -Cousin (P) BC -Cousin (P) CSU dx 30 -Cousin (P) CSU dd:40
161348	Exon 11	c.2125_2126insA	p.Phe709Tyrfsx3	FS	34	35	IDC	II	$T_2N_2M_0$	55%	-G M (M) CC dd 79 -Aunt (M), BCC dx 42 -Sister, BCC dx 21 -Cousin (P) BC dd:42
161335	Exon 11	c.2125_2126insA	p.Phe709Tyrfsx3	FS	38	41	IDC	III	$T_1N_1M_0$	NA	NA

BC: breast cancer; BBC: bilateral breast cancer; BCC: breast cancer cysts; CC: colon cancer, CSU: cancer site unknown; FS: frameshift; dx: age et diagnosis; KC: kidney cancer; IDC: infiltrating ductal carcinoma; IMC:infiltrating metaplastic carcinoma; IPC infiltrating papillary carcinoma; M: Maternal; MS: missense; NA: not available, OC: ovarian cancer; P: Paternal; PC: prostate cancer; LC: liver cancer; SC: stomach cancer; y: age.

advances, a launch of national anti-cancer program (2015–2019) and the improvement of investment diagnosis and treatment capabilities to help strengthen the government fight against breast cancer, Algerian women with aggressive breast cancer like TNBC or HER2+ in rural areas could die before to be referred to medical oncology services. A bias could be generated in the proportions and distribution of TNBC and non-TNBC subtypes. We should point out another study limitation is that a proportion (32,8%) of cases information for assigning Ki67 index, histological grade, histological type … were not available in the clinical records. However, this range of unavailable data in this study is in line with the proportion of missing data reported in other population based-studies [31]. Another limitation of this study is we have not estimated the 5 year overall survival (OS) and disease free survival (DFS) rate in TNBC and non-TNBC patients. Further studies in larger population are needed to estimate the overall/disease free survival for TNBC and non-TNBC subtypes, to assess subtype-risk factors and breast cancer subtype-specific survival among Algerian women. Preliminary epidemiological data collected in the public hospital of Rouiba showed that TNBC and HER2+ were associated with poor outcome compared to Luminal A subtypes (unpublished results).

Although, we screened for *BRCA1* recurrent or founder mutations which are population-specific and occur with high frequency in breast cancer patients with a family history of cancer, a limitation of our current study is several exons of *BRCA1* gene have not been sequenced, that means the frequency of *BRCA1* mutations in our TNBC patients is underestimated. In addition, *BRCA1* mutations analysis was performed only by PCR- Sanger sequencing and we did not screen for LGR (large genomic rearrangement, large exonic deletions or duplications) by using MLPA or Array-CGH. Screening of all coding exons of *BRCA1* including flanking intronic regions in a large series of TNBC patients by using NGS technologies will allow the assessing of the prevalence of *BRCA1* mutations. This study was the first to compare differences of clinicopathological features between TNBC and non-TNBC in Algerian population and to estimate the frequency of *BRCA1* mutations in Algerian women with TNBC. To date, Algeria has a socialized health system that covers the entire population, before primary chemotherapy, ER, PR, and HER2 status of breast cancer patients were routinely measured in quality-controlled laboratories since 2008. The results of these tests are recorded in cancer registries allowing for a population-based research study. This study was also the first that used Ki67 status in association with ER, PR and HER2+ receptor status for breast cancer subtypes classification and distribution in Algerian breast cancer patients, according to the recommendations of St Gallen international panel [27, 28]. To date, since the year 2012, Algerian medical oncology services are progressively including the Ki67 labeling index for classifying breast cancer subtypes in breast cancer patients.

The present study can contribute to provide information in order to arrive in the near future to a comprehensive picture of the etiology of breast cancer in Algerian population. The current study is likely to be representative of breast cancers in the national public health care system of Algeria.

Conclusions

This first study comparing TNBC and non-TNBC patients in Algerian population, showed the TNBC subtype patients are younger, have poorer histopathological characteristics, have family history of breast cancer, compared to breast cancer patients with positive receptor status. The proportion of TNBC in Algerian women was higher compared with Caucasian women of European descent. *BRCA1* mutations have been detected in TNBC patients diagnosed at earlier age. TNBC immunophenotype should be considered as an additional criterion for genetic counseling and testing in Algerian women with early onset breast cancer.

Further prospective studies should reaffirm our findings, screen for *BRCA1* and *BRCA2* germline mutations, and perform gene expression profiles in large series of TNBC patients. These studies will help to find targeted therapies to improve the outcome of TNBC patients in Algeria.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare non conflict of interest.

Ethical Approval All TNBC patients tested for *BRCA1* germline mutations signed written informed consent. The study was approved by the institutional review boards and ethical approval was obtained from appropriate institutions (USTHB, EPH Rouiba, FNRSDT and CNEPRU Project N° D01N01UN160420130007, 103 participants, start date: 3/07/2013, end date: 12/16/2015).

Informed Consent Informed consent was obtained from all individual participants included in the study.

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