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Inflammatory breast cancer in North Africa: Comparison of clinical and molecular epidemiologic characteristics of patients from Egypt, Tunisia, and Morocco

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Abstract

Understanding molecular characteristics that distinguish inflammatory breast cancer (IBC) from non-IBC is crucial for elucidating breast cancer etiology and management. We included 3 sets of patients from Egypt (48 IBC and 64 non-IBC), Tunisia (24 IBC and 40 non-IBC), and Morocco (42 IBC and 41 non-IBC). Egyptian IBC patients had the highest combined erythema, edema, peau d'orange, and metastasis among the 3 IBC groups. Egyptian IBC tumors had the highest RhoC expression than Tunisians and Moroccan IBCs (87% vs. 50%, vs. 38.1, for the 3 countries, respectively). Tumor emboli were more frequent in Egyptian IBC than non-IBC (Mean $\pm$ SD: 14.1 $\pm$ 14.0 vs. 7.0 $\pm$ 12.9, respectively) ($P < 0.001$) and Tunisians (Mean $\pm$ SD: 3.4 $\pm$ 2.5 vs. 1.9 $\pm$ 2.0, respectively) ($P < 0.01$). There was no difference of emboli in Moroccan tumors (1.7 $\pm$ 1.2 vs. 1.8 $\pm$ 1.2 for IBC and non-IBC, respectively) ($P = 0.66$). This study illustrates that RhoC overexpression and tumor emboli are more frequent in IBC relative to non-IBC from Egypt and

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Conflict of interest

None.

Tunisia. Tumors of Moroccans were significantly different from Egyptian and Tunisian tumors for RhoC expression and emboli. Future studies should focus on relating epidemiologic factors and clinical pictures to molecular features of IBC in these and other populations.

Keywords

Inflammatory breast cancer; tumor emboli; RhoC; Egypt; Tunisia; Morocco; North Africa

1. Introduction

Inflammatory breast cancer (IBC) is a rare but the most lethal form of locally advanced breast cancer. Compared to other types of breast cancer, IBC affects younger women, progresses rapidly, and tends to be more advanced at the time of diagnosis [6,33]. IBC is characterized by a greater metastatic potential and significantly shorter survival than other forms of breast cancer [11,23,34].

The diagnosis of IBC is primarily based on clinical not histopathologic characteristics [23]. Clinical manifestations, which remain the most reliable method of diagnosis include: peau d'orange, erythema, edema, bruising, diffuse enlargement, ulceration, nipple retraction, and breast warmth [23]. IBC is often characterized by an increased number of dermal tumor lymphatic emboli that block the drainage of lymph fluids and lead to (erythema and edema) that have given IBC its name [10,14,16,30].

Despite its poor prognosis and advanced stage at presentation, little is known about the etiology of IBC, and the diagnostic criteria of IBC have not been standardized [2]. Using clinical criteria only and lack of standard disease definition complicates the diagnosis of IBC, especially in unclear and clinically-confusing cases that might be difficult to differentiate from locally advanced breast cancer (LABC). The American Joint Committee on Cancer (AJCC) diagnostic criteria of IBC require that erythema must involve at least 50% of the breast although some IBC do not fulfill this criterion. Furthermore, erythema might occur in LABC. While ulceration can occur in rare IBC cases, it is considered more indicative of neglected LABC and it is critical to accurately distinguish between these two diseases to ensure appropriate treatment [32]. However, given the difficulty in distinguishing neglected LABC from IBC, the distinction is often difficult to make [8].

This question of distinguishing IBC from neglected LABC becomes more critical in North Africa, where the proportion of IBC among all breast cancers has been reported higher than elsewhere in the world [5,18,27]. In both Tunisia and Egypt, research has suggested that IBC accounts for 6–10% of all breast cancer cases, respectively [5,21,24,27]. This estimate is substantially higher than the proportion of IBC in the United States, where IBC accounts for 1–2% of all breast cancers [2, 25]. Without a globally-accepted standardized case definition and specific criteria, the identification of IBC is largely subject to the diagnosing physicians, as evidenced in various imprecise estimates. Therefore, we explored the clinical and molecular characteristics of IBC and non-IBC cases diagnosed in 3 North African countries Egypt, Tunisia and Morocco. Our aim was to compare and contrast the clinical

characteristics of patients and the molecular epidemiologic features of tumors across the 3 countries with variable but high proportion of IBC.

2. Methods

2.1. Study population

The study included patients and their tumor tissues from Egypt, Tunisia, and Morocco.

The Egyptian patients and their tumor characteristics are included in our previous publications [19,20]. Briefly, 41 IBC patients from the National Cancer Institute of Cairo University (NCI-Cairo), and 7 patients from the Gharbiah Cancer Registry in Tanta, were recruited during the period of September, 2005 to December, 2006. The diagnosis of IBC was confirmed clinically and pathologically by 5 co-authors (SO, HMK, MSE, CGK, and SDM). Bilateral breast involvement was found in 3 patients. The majority of IBC tumors from Egypt was invasive ductal carcinoma (IDC), accounting for 41 (89.1%) patients, lobular carcinoma (2 patients, 4.4%), and other histological types included mixed IDC and lobular carcinoma (3 patients, 6.5%). The non-IBC cases were obtained by a thorough review of the medical records and pathology reports to ensure including a representative non-biased sample of non-IBC patients whose biopsies contained skin to specifically examine the presence of dermal lymphatics in this group. The 64 non-IBC patients were all the non-IBC patients that fulfilled the inclusion criteria of tumors 5 cm or larger (T3) stage II or III tumors (with no distant metastasis or M0). These criteria were determined before beginning the study and before reviewing the pathology reports or finding the paraffin blocks, so in the process of selection, there was no other source of bias introduced at all. We decided on the criteria that would allow us to answer the scientific question of whether there are significant differences between IBC and non-IBC with regards to the variables studied and included all of the patients who fulfilled these criteria [20].

The Tunisian cases included 24 IBC patients and 40 non-IBC patients. The IBC cases were identified from the department of pathology of Saleh Azaiz Cancer Institute in Tunis from all T4D patients with diagnostic tissue biopsies of patients diagnosed as IBC from the surgical and medical oncology departments at the institute during the period of 2007–2008. The Non-IBC patients were all the non-IBC patients that fulfilled the inclusion criteria of tumors, 5 cm or larger (T3) stage II or III tumors. Medical records of the patients were retrieved and demographic and demographic data were abstracted.

In Morocco, women diagnosed and/or treated as IBC at Ibn Rochd Oncology Center in Casablanca, Morocco between 2005–2007 were selected. All breast cancer cases were initially identified using the Ibn Rochd Oncology Center's Hospital-based Registry, which contained the following information: Tumor characteristics, node involvement, metastasis, TNM, age, histopathological type, and tumor grade. The categorization of tumor size (T of TNM) as T4D indicated a diagnosis of IBC; thus, the registry was used as a launching point to identify diagnosed cases of IBC among all breast cancers. Of the total 2004 documented breast cancer cases, the registry reported sufficient TNM information to assess T4D status for 1568 cases, of which 112 were classified as T4D (IBC). Of all 112 T4D cases, a medical

abstraction form was applied to 96 records; the remaining 16 cases were either unable to be located (11 cases) or were deemed insufficient for abstraction (5 cases).

2.2. Data collection

In the 3 countries, we accessed medical records and pathology reports to obtain information on age, menopausal status, parity, disease duration, symptoms, and pathological characteristics of the identified patients. Duration of disease was the self-reported time period between the onset of IBC-related symptoms and the first visit to a hospital for diagnosis. Exhibition of IBC-related symptoms, including the 3 core signs (i.e. erythema, edema, and peau d'orange), and presence of palpable mass and ulceration at the time of diagnosis were expected to elicit, along with information on pathological factors such as histology, the diameter of the tumor, grade, lymph node involvement and the expression status of estrogen receptor (ER) and progesterone receptor (PR). The diagnosis of IBC in the study hospitals is primarily based on the degree of erythema (at least one-third of the size of the breast involved), edema, and presence of peau d'orange. Less than 6-month duration of redness is highly suggestive of IBC. However, in some cases the typical clinical picture of IBC was encountered in patients with duration of erythema of more than 6 months.

This study was approved by the institutional review boards of the participating institutions in North Africa and the University of Michigan.

2.3. Tissue specimens

For IBC patients, information on erythema, edema, and peau d'orange at diagnosis was collected and confirmed in by oncologists at the local institutions (SO and HMK) in Egypt, (FB) in Tunisia, and (AB) in Morocco. The pathological diagnoses of cancer were confirmed by pathologists (MSE) in Egypt, (KM) in Tunisia and (MK) in Morocco. All tumors were examined again by (CGK) in the U.S. *Tumor Specimens and Immunohistochemistry (IHC)*

Paraffin-embedded tumor tissues were obtained from all three participating institutions. We then stained the slides with hematoxylin and eosin (H&E) and the polyclonal antibody specific to RhoC [12]. All the H&E histology slides of IBC and non-IBC patients were reviewed by at least 2 of co-authors (AL, EDK, EJM, KT, QX, and MSE) and confirmed by CGK who were blinded to the other results. The H&E slides were examined to determine the number of tumor emboli in each slide as reported in our recent study [19,20]. Because the H&E slides from non-IBC patients do not usually exhibit epidermis, we were able to calculate the average number of tumor emboli in the breast tissue that did not include epidermis.

Details of the IHC staining and scoring of RhoC GTPase expression were reported in our previous studies [12,13,19,20]. To ensure reproducibility and eliminate observation bias, all slides were analyzed by 2 coauthors for each country (AL and KT) for Egypt, (EJM and MSE) for Tunisia, and (EDK and KT) for Morocco. The slides were analyzed a third time blindly where the identifying tag was covered and therefore one could not discriminate between IBC and non-IBC slides. Each slide was RhoC-scored 3 times to ensure

reproducibility. In the few instances where a discrepancy existed between scores, the score from the blind scoring trial performed by (CGK) was used to help eliminate observation bias. We defined the moderate to strong cytoplasmic stain adherence as indicative of overexpression of RhoC (scores 3 and 4) while no staining or faint staining reflected low levels of expression of the RhoC gene (scores 1 and 2) [12,13,19,20].

2.4. Statistical methods and analysis

Differences in frequencies of descriptive variables and molecular factors between the IBC and non-IBC patients were evaluated by Mantel-Haenszel chi-square tests and Fisher's exact tests. BMI was categorized according to the WHO classification of BMI in adulthood [26]. The Mann-Whitney U-test was used to evaluate differences in age and number of tumor emboli between IBC and non-IBC patients. To evaluate the correlation between RhoC expression level and number of tumor emboli, we also computed the non-parametric one-way ANOVA tests for both IBC and non-IBC groups. All *P*-values were two-sided. Variables were statistically analyzed using SAS version 9.1.

2.5. Results

Table 1 depicts demographic and pathological characteristics of IBC and non-IBC patients from Egypt, Tunisia and Morocco and compares these within and between countries. We observed a significant difference in age between IBC and non-IBC patients from Morocco only ($P < 0.001$). IBC cases from Egypt were also more likely to be younger than Moroccan IBC cases ($P = 0.04$). IBC cases from Morocco were also more likely to be postmenopausal ($P < 0.0001$) with significant differences observed in the menopausal status of Egyptian and Moroccan IBC ($P = 0.002$) and non-IBC ($P = 0.02$) with more cases being premenopausal in Egypt. Similar significant differences were also observed in menopausal status between Tunisian and Moroccan IBC cases ($P = 0.03$) with more premenopausal women observed among IBC cases of Tunisia (55% vs. 25% in Tunisia and Morocco, respectively) (Table 1). Egyptian IBC cases were more likely to have fewer children in the IBC than the non-IBC group ($P = 0.005$) and the significant difference was not observed among patients from Tunisia or Morocco. Significant differences were also observed for ER/PR status between IBC and non-IBC cases in Tunisia ($P = 0.03$) with higher proportion of non-IBC cases being ER/PR- than in Egypt (83.3% vs. 46.7%, for Tunisia and Egypt, $P = 0.03$ respectively). Similar significant differences were observed for non-IBC comparisons of Tunisian ER/PR- with Egyptian ($P = 0.0007$ and Moroccan tumors $P = 0.02$). Lymph node involvement was observed in over 90% of the IBC tumors from Egypt and Tunisia and 83.3% of the Moroccan IBC tumors (Table 1).

Duration of symptoms was longer in Moroccan IBC patients compared to the duration in Egyptian patients ($P = 0.0002$) and Tunisian patients ($P = 0.0014$) (Table 2). Erythema was less common among IBC patients in Tunisia compared to patients in Egypt ($P < 0.0001$) and Morocco ($P < 0.0001$). Both edema and peau d'orange were common among IBC patients in Egypt compared to patients in Morocco ($P < 0.0001$) and Tunisia ($P < 0.0001$). Overall, all three clinical symptoms of IBC – erythema, edema and peau d'orange were more common among Egyptian IBC cases compared to the other two countries ($P < 0.0001$). Ulceration was absent in Tunisia and was seen in 1/3 of IBC patients in Morocco compared to 11.9% of

IBC patients in Egypt ($P = 0.01$, for the comparison of Egypt and Morocco) and ($P = 0.0006$ for the comparison of Tunisia and Morocco) (Table 2).

Table 3, illustrates the comparison of molecular characteristics of IBC and non-IBC cases in the three North African countries. Tumor emboli among IBC cases were significantly higher than non-IBC cases in Egypt ($P < 0.0001$) and Tunisia ($P = 0.01$). Tumor emboli among both Egyptian IBC (mean $\pm$ SD = 14.1 ± 14.0) and non-IBC cases (mean $\pm$ SD = 7.0 ± 12.9) were significantly more frequent than those seen for in tumors from Tunisia and Morocco. The number of tumor emboli among Tunisian IBC cases was significantly higher than Moroccan IBC cases ($P = 0.0003$) (Table 3). In the tumors from Egypt, RhoC expression was significantly higher among IBC tumors compared to non-IBC tumors ($P < 0.0001$) (Fig. 1). Egyptian IBC cases had significantly higher RhoC expression compared to Tunisia ($P = 0.001$) and Morocco ($P < 0.0001$) while Egyptian non-IBC tumors had significantly lower RhoC expression compared to tumors from Tunisia ($P < 0.0001$) and Morocco ($P = 0.001$). Tunisian non-IBC tumors had significantly higher RhoC expression compared to non-IBC tumors from Morocco (72.2% vs. 46.3%; $P = 0.02$). E-cadherin expression was significantly higher in both IBC and non-IBC cases of Tunisia compared to Morocco ($P < 0.0001$ for both) (Table 3). Our previous results from Egypt that is included in this study did not include results on E-cadherin expression [19,20]. Additional analysis of RhoC and E-cadherin expression with relevant clinical characteristics showed no significant correlation (data not shown).

Patients from Morocco had the longest duration of symptoms. Because the Moroccan IBC patients were the only group with duration of symptoms of more than one year, we added additional analysis of this group based on the duration of symptoms. Table 4 illustrates our results from the analysis of comparing Moroccan IBC and non-IBC cases classified by duration of symptoms. There were very few differences between IBC and non-IBC cases classified by duration except for the more frequent lymph node involvement ($P < 0.0001$), erythema ($P < 0.0001$) and peau d'orange ($P = 0.007$) among IBC cases with duration of symptoms ≥ 6 months. Higher lymph node involvement was also observed among IBC cases compared to non-IBC cases ($P = 0.04$) in patients with symptoms of more than 6 months but less than or equal one year (Table 4). On comparing only IBC cases by duration of symptoms, we observed differences in erythema ($P = 0.005$), peau d'orange ($P = 0.05$), ulceration ($P = 0.002$) and RhoC expression ($P = 0.03$). The percent of patients with erythema and peau d'orange decreased among the IBC cases with increasing duration while the likelihood of ulceration increased with duration. RhoC expression was higher for IBC cases with duration less than 1 year compared to cases with duration more than 1 year (Table 4).

3. Discussion

Following our previous finding of the significantly higher level of RhoC expression and the more frequent tumor emboli in IBC patients in Egypt compared to the U.S. [20] and the higher levels of RhoC and frequent emboli in IBC than non-IBC tumors in Egypt [19], this study served to determine if these molecular characteristics differed between IBC and non-IBC between patients from Egypt, Tunisia, and Morocco.

This study has several interesting observations. First, the study showed variations in the age of IBC patients between the 3 countries with the oldest patients in Morocco. The variation of time since onset of symptoms was also noted between patients from the 3 countries with longest duration in Moroccan patients. Second, the 3 symptoms of IBC (edema, erythema, and peau d'orange) were most common in patients from Egypt than Tunisian and Moroccan patients. Ulceration was absent in Tunisian patients and most common in IBC patients from Morocco.

Third, Egyptian and Tunisian IBC tumors had significantly higher number of tumor emboli compared to non-IBC tumors in each respective country. Egyptian IBC tumors had the highest number of tumor emboli among IBC tumors of the 3 countries. Tunisian tumors had higher number of tumor emboli than Moroccan tumors. Fourth, Egyptian IBC tumors had the highest RhoC expression among IBC tumors from the 3 countries. Tunisian IBC tumors had higher RhoC expression than Moroccan IBC tumors. Fifth, Moroccan IBC patients had the longest duration of symptoms. A declining proportion of patients with erythema and peau d'orange and increasing ulceration were observed with increasing duration of symptoms. In addition, number of tumor emboli did not differ significantly between the 3 groups of Moroccan IBC tumors based on duration of symptoms. Furthermore, RhoC overexpression did not differ between IBC and non-IBC Moroccan tumors for patients under one year of symptoms. Even though RhoC overexpression was not high in Moroccan IBC tumors, the lowest expression was observed in tumors of IBC patients with duration of symptoms of more than one year.

Our observation of higher overexpression of RhoC and high number of tumor emboli in IBC tumors than in non-IBC tumors in Egyptian patients is in agreement with previous research [13,19,20,31]. The combined results from our previous and current studies suggest that IBC tumors from Egyptian patients exhibit higher levels of RhoC overexpression and tumor emboli than IBC tumors from U.S. patients or non-IBC tumors from either Egyptian or U.S. patients. The Egyptian IBC tumors had the highest RhoC overexpression and tumor emboli than other IBC groups from Tunisia or Morocco although the Tunisian tumors had slightly higher RhoC expression than the Moroccan IBC tumors but lower than the Egyptian IBC in this study and lower than a recent study from Tunisia [22]. The Moroccan IBC tumors had the lowest RhoC overexpression compared to the other 2 countries and the lowest overexpression was noted in IBC tumors of patients with duration of symptoms of more than one year. Currently, no set of histopathologic criteria are suitable for the diagnosis of IBC and it is routinely diagnosed only by suggestive clinical criteria (erythema, edema, and peau d'orange) [9]. The ability to detect the level of RhoC expression in patient tissue biopsies may prove to be important tools that improve the diagnosis of IBC. Only the collection of data and specimen with defined guidelines and strict verifiable criteria as explained above would help clarify the diagnosis of IBC. The Moroccan IBC patients included in the study were diagnosed clinically by the treating oncologists in Morocco although some of them had duration of illness of more than one year. Exclusion of the Moroccan IBC group of patients with more than one year of duration of symptoms from the analysis did not change the results of RhoC overexpression.

In addition to molecular factors, we observed significant differences between the groups with youngest age in Egyptian IBC patients compared to other IBC patients. The 3 symptoms of erythema, edema, and peau d'orange were most common in the Egyptian IBC patients. It is interesting to note that ulceration was found in about 11% of IBC patients from Morocco with duration of symptoms of less than 6 months and 12% of Egyptian IBC patients but in 37% of Moroccan patients with duration of symptoms of more than 6 months but less than one year and 56% of IBC patients with symptoms of more than one year. Ulceration was not present in any of the Tunisian IBC patients.

Most of the descriptive data included in the present study showed no statistically significant difference between the two groups. Both the IBC and non-IBC patients had no significant differences in previous family history of any type of breast cancer, similar to other studies in the U.S., France, and Tunisia [7,15]. Although BMI was not significantly different between IBC and non-IBC patients in this study between each countries or between the 3 countries, it is important to study it further in future studies because of the increasing incidence of overweight and obesity among women in North Africa [1,3,4].

The variations in the proportion of IBC among all breast cancers between countries in North Africa with about 10% in Egypt and about 6-7% in Tunisia and Morocco [5,24,27] and the differences in clinical presentations and molecular characteristics reported in this study raise important questions regarding the disease etiology carcinogenesis in this region. Our previous research on non-IBC patients in Egypt suggests that women without a history of lactation had a significantly higher level of serum dichlorodiphenyldichloroethylene (DDE) than women who breast fed ($P = 0.002$) [29]. Younger age of onset and older age at first childbirth were also associated with higher levels of serum DDE in premenopausal women [29]. Egyptian women living in urban areas, with infertility, and a higher level of oxidative DNA damage, presented with 7,8-dihydro-8-oxo-2'-deoxyguanine (8-oxo-dG), showed a significantly higher risk of breast cancer than other Egyptian women [28]. Although the differences were not statistically significant, we found more rural residents in the IBC group than in the non-IBC group in the current study, suggesting a potentially high likelihood of farming-related exposures among IBC patients in Egypt [19]. Levine et al., 2004 reported the detection of MMTV-like env gene sequence with a significantly higher percentage of positive patients with breast carcinoma in Tunisia (74%) compared with patients with breast carcinoma in the United States (36%), Italy (38%), Australia (42%), Argentina (31%), and Vietnam (0.8%) [17]. The clinical presentation of IBC in Morocco with duration of symptoms of more than one year and the lack of significant differences in molecular characteristics and symptoms by duration of illness warrant further investigation. It is possible that patients with longer duration of symptoms have pre-existing conditions such as chronic mastitis. The reporting of the long duration of symptoms in those patients could a reporting of a chronic mastitis that might have preceded IBC diagnosis. On the other hand, patients with longest duration of illness in Morocco might not be IBC cases but rather neglected locally advanced cases. However, tumor emboli and RhoC overexpression of this group did not differ significantly from the other Moroccan groups of tumors of patients with duration of symptoms of less than one year. Furthermore, exclusion of this group of tumors

of patients with symptoms of more than one year did not result in further differences between molecular characteristics of tumors of Egyptian or Tunisian patients.

This study has several strengths. Although a few studies have been published by our group and others on the clinical and/or molecular characteristics of IBC, this is the first study to include clinical, epidemiological, and molecular characteristics of IBC and non-IBC patients from the 3 North African countries. Another strength that should be noted for this study is the reproducibility of the data. Both the IBC and non-IBC slides from each of the 3 countries were examined multiple times and by multiple researchers to determine RhoC scores and the number of tumor emboli. In the majority of the samples, all evaluations yielded the same results for each specimen. Additional strength of this study was that RhoC scoring was done with the researcher blinded to the IBC status of the slide. This helped to remove any potential observation bias and ensured reproducibility. Additionally, this study included a relatively large number of patients with IBC, which is a rare condition, from 3 North African countries with reported high incidence of IBC. Study participants were selected from a large pool of non-IBC patients who were then matched with IBC patients on histopathologic type to eliminate any variables that could have possibly mediated or confounded the comparisons between the groups.

The study also has limitations. The lack of reproductive data is a limitation. Data on reproductive factors were abstracted from the medical records and clinical reports, wherever possible, but this information was often not recorded. We aimed at collecting samples with epidermis for all non-IBC patients by including mastectomies of non-IBC tumors. However, a few of the specimens did not contain full thickness of skin, including epidermis, so we were reluctant to review those few cases for tumor emboli counts, due to concerns that the counts might be inaccurate.

The results of this study set the stage for important questions to be explored in future clinical, epidemiologic, and molecular investigations. Possibilities include comparisons of molecular components of IBC in different racial and ethnic groups. Additional genetic, lifestyle, and environmental factors should be carefully examined in future large-scale studies in diverse populations to better clarify the relationship between RhoC expression, number of tumor emboli, and stem cells in the etiology and pathogenesis of IBC. Given that the diagnostic criteria for IBC have not been standardized worldwide, international research will shed more light on IBC etiology and aid in developing a more accurate definition of IBC to be used in diagnosis of the disease based on different etiologies in diverse populations.

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Fig. 1. Immunohistochemistry Images of IBC and non-IBC tissue slides for RhoC and Tumor Emboli from Egypt. A: H &E slide showing frequent tumor emboli in an IBC case. B: Scoring system for RhoC expression. C: High expression of the slide shown in A. D. Invasive ductal carcinoma extending into the adipose tissue of non-IBC. E. Cancer cells forming nests and are pleomorphic and hyperchromatic, with occasional prominent nucleoli. (Colours are visible in the online version of the article; <http://dx.doi.org/10.3233/BD-2012-0337>)

Table 1

Characteristics of IBC study population from Egypt, Tunisia and Morocco

Variables	Egypt			Tunisia			Morocco			Inter-country Comparison P-values				
	IBC cases (n = 48) No. (%)	Non-IBC cases (n = 64) No. (%)	P-value	IBC cases (n = 24) No. (%)	Non-IBC cases (n = 40) No. (%)	P-value	IBC cases (n = 42) No. (%)	Non-IBC cases (n = 41) No. (%)	P-value	Egypt-Tunisia		Egypt-Morocco		
										IBC case	Non-IBC case	IBC case	Non-IBC case	
Age														
Median (Min–Max)	43 (28–70)	49 (32–79)		46 (30–74)	46 (32–78)		54 (31–87)	46 (30–75)						
Mean ± SD	46.9 ± 11	50.2 ± 11.3	0.13 ^a	48.1 ± 13.4	49.7 ± 13.0	0.77 ^a	54.2 ± 12.8	45.8 ± 8.9	< 0.001 ^a	0.7 ^a	0.84 ^a	0.0005 ^a	0.07 ^a	0.13 ^a
Residence														
Urban	28 (62)	41 (79)		14 (70)	18(52.9)		28 (66.7)	25 (69.4)						
Rural	17 (38)	11 (21)	0.72 ^b	6 (30)	16(47.1)	0.22 ^b	14 (33.3)	11 (30.6)	0.56 ^b	0.55 ^b	0.01 ^b	0.67 ^b	0.32 ^b	0.16 ^b
Menopausal status														
Pre–	30 (62.5)	43 (67.2)		11 (55)	20 (61)		8 (25)	19 (79.2)						
Post–	18 (37.5)	21 (32.8)	0.61 ^b	9 (45)	13 (39)	0.69 ^b	24 (75)	5 (20.8)	< 0.0001 ^b	0.68 ^b	0.4 ^b	0.002 ^b	0.03 ^b	0.14 ^b
Parity														
Median (Min–Max)	3 (0–7)	4 (0–9)		1.5 (0–8)	4 (0–9)		3 (0–12)	3 (0–9)						
Mean ± SD	2.6 ± 1.8	3.8 ± 1.8	0.0008 ^a	2.5 ± 2.8	3.5 ± 2.3	0.18 ^a	3.6 ± 3.1	3 ± 2.2	0.38 ^a	0.9 ^a	0.43 ^a	0.07 ^a	0.19 ^a	0.43 ^a
Tumor Grade														
1	0	0		2 (8.3)	5 (14.3)		0	1 (4.6)						
2	38 (83.0)	46 (80.7)		3 (12.5)	12 (34.3)		2 (50)	9 (40.9)						
3	8 (17.0)	11 (19.3)	0.04 ^c	19 (71.2)	18 (51.4)	0.01 ^c	2 (50)	12 (54.6)	0.33 ^c	< 0.0001 ^c	< 0.0001 ^c	0.11 ^c	0.18 ^c	0.50 ^c
ER/PR														
Negative	14 (38.9)	22 (36.1)		5 (45.5)	15 (83.3)		7 (46.7)	14 (48.3)						
Positive	22 (61.1)	39 (76.5)	0.31 ^b	6 (54.6)	3 (16.7)	0.03 ^c	8 (53.3)	15 (51.7)	0.92 ^b	0.27 ^b	0.0007 ^c	0.24 ^b	0.95 ^b	0.02 ^c
Lymph node involvement														
Negative	2 (5.3)	4 (7)		2 (10)	3(8.8)		7 (16.7)	37 (90.2)						
Positive	36 (94.7)	56 (93)	0.33	18 (90)	31 (91.2)	0.89	35 (83.3)	4 (9.76)	< 0.0001 ^b	0.32 ^c	0.29 ^c	0.08 ^c	0.49 ^c	< 0.0001 ^c

^aIndependent t test
^bMantel-Haenszel chi-square test
^cFisher's exact test.

Table 2

Clinical symptoms among IBC patients from Egypt, Tunisia and Morocco

Variables	Egypt IBC cases (n = 48) No. (%)	Tunisia IBC cases (n = 24) No. (%)	Morocco IBC cases (n = 42) No. (%)	Inter-country Comparison P-values			
				Egypt-Tunisia		Tunisia-Morocco	
				Egypt-Tunisia	Egypt-Morocco	Tunisia-Morocco	
IBC cases							
IBC case							
IBC case							
Duration of symptoms (months)							
Median (Min-Max)	4.0 (0.5-18.7)	3.00 (0.04-12.00)	12 (1-96)				
Mean ± SD	8.3 ± 10.6	4.38 ± 4.41	18.3 ± 18.9	0.0874 ^a	0.0002 ^a		0.0014 ^a
Erythema							
Yes	45 (93.8)	3 (14.29)	35 (83.3)				
No	3 (6.2)	18 (85.71)	7 (16.6)	<0.0001 ^c	0.08 ^c		< 0.0001 ^c
Edema							
Yes	46 (95.8)	3 (14.29)	6 (7.3)				
No	2 (4.2)	18 (85.71)	36 (92.7)	< 0.0001 ^c	< 0.0001 ^c		0.30 ^c
Peau D'orange							
Yes	39 (92.9)	8 (38.10)	12 (28.6)				
No	3 (7.1)	13 (61.90)	30 (71.4)	<0.0001 ^b	< 0.0001 ^b		0.44 ^b
Clinical symptoms ^d							
Group 1	38 (90.5)	3 (14.29)	1 (2.4)				
Group 2	4 (9.5)	18 (85.71)	41 (97.6)	< 0.0001 ^c	< 0.0001 ^c		0.09 ^c
Ulceration							
Yes	5 (11.9)	0	14 (33.3)				
No	37 (88.1)	22 (100.0)	28 (66.7)	0.11 ^c	0.01 ^b		0.0006 ^b

^aIndependent t test

^bMantel-Haenszel chi-square test

^cFisher's exact test

^dGroup 1 consists of all cases having all the three clinical signs of erythema, edema and peau d'orange. Group 2 cases have at least one of those three signs absent.

Table 3
Number of tumor emboli, and RhoC and E-Cadherin expression in IBC tumors from Egypt, Tunisia and Morocco

Variables	Egypt		Tunisia		Morocco		Inter-country Comparison P-values			
	IBC cases (n = 46) No. (%)	Non-IBC cases (n = 64) No. (%)	P-value	IBC cases (n = 24) No. (%)	Non-IBC cases (n = 40) No. (%)	P-value	IBC cases	Non-IBC case	Egypt-Tunisia	Tunisia-Morocco
Tumor Emboli										
Median (Min – Max)	9.5 (1–62.2)	1.5 (0–57)		3.1 (0–9.4)	1.2 (0–9)		1.5 (0.2–4.2)	1.6 (0.2–5.0)		
Mean ± SD	14.1 ± 14.0	7.0 ± 12.9	< 0.0001 ^a	3.4 ± 2.5	1.9 ± 2.0	0.01 ^a	1.7 ± 1.2	1.8 ± 1.2	0.66 ^a	0.02 ^a
RhoC expression level										
Low	6 (13.0)	53 (82.8)		12 (50)	10 (27.8)		26 (61.9)	22 (53.7)		
High	40 (87.0)	11 (17.2)	< 0.0001 ^b	12 (50)	26 (72.2)	0.08 ^b	16 (38.1)	19 (46.3)	0.45 ^b	0.001 ^b
E–Cadherin										
Positive	–	–	–	21 (87.5)	33 (84.6)		16 (38.1)	11 (26.8)		
Negative	–	–	–	3 (12.5)	6 (15.4)	0.28 ^c	26 (61.9)	30 (73.2)	0.27 ^b	–

^aIndependent t test
^bMantel–Haenszel chi–square test
^cFisher's exact test

Table 4

Characteristics of IBC and non-IBC cases of Morocco by duration of symptoms

Variables	6 months		P- value	1 year		P-value	> 1 year		P-value	Comparison of IBC cases by duration P-value	Comparison of IBC cases vs. > 6 months to 1 year P-value	Comparison of IBC cases > 1 year vs. value
	IBC cases (n = 18) No. (%)	Non-IBC cases (n = 24) No. (%)		IBC cases (n = 8) No. (%)	Non-IBC cases (n = 9) No. (%)		IBC cases (n = 16) No. (%)	Non-IBC cases (n = 2) No. (%)				
ER/PR												
Negative	5 (62.5)	11 (52.4)	0.29 ^c	1 (33.3)	2 (33.3)	0.54 ^c	1 (75)	1 (100)	0.4 ^c	0.1 ^c		
Positive	3 (37.5)	10 (47.6)		2 (66.7)	4 (66.7)		3 (25)	0			0.36	0.29
Lymph node involvement												
Negative	2 (8.3)	22 (91.7)	<0.0001 ^c	2 (25)	7 (77.8)	0.04 ^c	3 (18.8)	2 (100)	0.07 ^c	0.09 ^c		0.31
Positive	16 (88.9)	2 (8.3)		6 (75)	2 (22.2)		13 (81.3)	0			0.29	
Erythema												
Yes	18 (100)	1 (4.2)	<0.0001 ^c	6 (75)	0	0.0023 ^c	11 (68.8)	0	0.13 ^c	0.005 ^c		0.05
No	0	23 (95.8)		2 (25)	9 (100)		5 (31.3)	2 (100)			0.09	
Edema												
Yes	3 (16.7)	1 (4.2)	0.18 ^c	1 (12.5)	0	0.47 ^c	2 (12.5)	0	0.78 ^c	0.15 ^c		0.34
No	15 (83.3)	23 (95.8)		7 (87.5)	9 (100)		14 (87.5)	2 (100)			0.44	
Peau D'orange												
Yes	7 (38.9)	1 (4.2)	0.007 ^c	2 (25)	1 (11.1)	0.37 ^c	3 (18.8)	0	0.68 ^c	0.05 ^c		0.16
No	11 (61.1)	23 (95.8)		6 (75)	8 (88.9)		13 (81.3)	2 (100)			0.29	
Clinical symptoms ^d												
Group 1	1 (5.6)	0	0.43 ^c	0	0	-	0	0	-	0.43 ^c		
Group 2	17 (94.4)	24 (100)		8 (100)	9 (100)		16 (100)	2 (100)			0.69	0.62
Ulceration												
Yes	2 (11.1)	0	0.18 ^c	3 (37.5)	0	0.08 ^c	9 (56.3)	0	0.24 ^c	0.002 ^c		0.02
No	16 (88.9)	24 (100)		5 (62.5)	9 (100)		7 (43.8)	2 (100)			0.13	
Tumor Emboli												
Median (Min-Max)	1.4 (0.2-4.2)	1.4 (0.2-4)		1.4 (0.4-4)	1.8 (0.2-2.6)		1.6 (0.2-3.4)	1.3 (0.2-2.4)				

Variables	6 months		P- value	> 6 months and 1 year		P-value	> 1 year		P-value of IBC cases by duration P-value	Comparison of IBC cases 6 months vs. > 6 months to 1 year P-value	Comparison of IBC cases 1 year vs. > 1 year P-value
	IBC cases (n = 18) No. (%)	Non-IBC cases (n = 24) No. (%)		IBC cases (n = 8) No. (%)	Non-IBC cases (n = 9) No. (%)		IBC cases (n = 16) No. (%)	Non-IBC cases 2) No. (%)			
Mean ± SD	1.6 ± 1.3	1.7 ± 1.1	0.75 ^a	1.8 ± 1.2	1.6 ± 0.8	0.72 ^a	1.7 ± 1.1	1.3 ± 1.6	0.67 ^a	0.82	0.85
RhoC expression level											
Low	10 (55.6)	14 (58.3)	0.86 ^b	4 (50)	4 (44.4)	0.36 ^c	12 (75)	2 (100)	0.6 ^c	0.03 ^c	
High	8 (44.4)	10 (41.7)		4 (50)	5 (55.6)		4 (25)	0		0.69	0.62
E-Cadherin											
Positive	7 (38.9)	9 (37.5)	0.93 ^b	4 (50)	1 (11.1)	0.1 ^c	5 (31.3)	0	0.51 ^c	0.06 ^c	
Negative	11 (61.1)	15 (62.5)		4 (50)	8 (88.9)		11 (68.7)	2 (100)		0.29	0.20

^aIndependent t test

^bMantel-Haenszel chi-square test

^cFisher's exact test

^dANOVA. Group 1 consists of all cases having all the three clinical signs of erythema, edema and peau d'orange. Group 2 cases have at least one of those three signs absent.