Original Article

Molecular breast cancer subtypes in Sudan and their impact on management; with emphasis on Luminal sub-type A

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The objective of the current study was to study the association between luminal A molecular subtype with clinical, pathological parameters and to discuss the expected impact on management of this type of cancer in Sudan.

Patients & Methods
This study was a cross sectional descriptive analytical correlation study designed primarily to evaluate the molecular subtypes of breast cancer in Sudanese female. The

Abstract

Introduction
Breast cancer accounts for about one-fifth of all the cancers treated in Sudan. Breast cancer is a group of heterogeneous diseases, encompassing a number of distinct biological entities. Gene microarrays has demonstrated that breast cancers cluster into luminal A, luminal B, normal breast-like, HER2, and basal-like. Studies using selected immunohistochemical stains have achieved similar stratifications of tumors. There is strong evidence in several studies that there is no benefit of adding chemotherapy to endocrine therapy in adjuvant therapy of luminal A.

The objective of the current study was to study the association between luminal A molecular subtype with clinical, pathological parameters and to discuss the expected impact on management of this type of cancer in Sudan.
A study was conducted in Gezira and Khartoum states during the period from April 2012 to April 2013. Demographic data, clinical and pathological characteristics were collected for each patient. Histopathological specimens were obtained, reviewed and immunohistochemistry to determine molecular subtypes was performed.

**Results**

A total number of 165 cases of invasive breast cancer were collected, 141 of them had adequate tumor blocks for review and immunohistochemical studies. Of the 141 cases studied, 130 (92.2%) had both adequate tumor and interpretable immunohistochemical result for ER, PR, and ki67; 129 cases (91.4%) for HER2neu and 121 cases (85%) for ck 5/6. The majority of the cases were classified as Luminal A (34.6%). The Clinicopathological features for this subtype were found to be indolent and indicate a favorable biology.

**Conclusion**

Immunohistochemical studies to define molecular breast cancer subtypes in Sudan could positively impact on adjuvant management of patients. Identifying patients with low risk Luminal A subtypes could avoid unnecessary prescription of cytotoxic agents.

**Keywords:** Breast cancer, molecular subtypes, Sudan

**Introduction**

Breast cancer is the commonest site specific malignancy affecting women and the most common cause of cancer mortality in women worldwide and constitutes a major public health issue globally. The global incidence increased from 641,000 cases in 1980 to 1,643,000 cases in 2010, an annual rate of increase of 3.1%. In developing countries, breast cancer killed 425,000 women in 2010 of whom 68,000 were aged 15-49 years. (1, 2,3,4,5) Breast cancer accounts for about one-fifth of all the cancers treated in Sudan and is the most frequent site-specific malignancy seen at both RICK (20%, i.e.2,084/10,410 recorded cancer cases during the period 1967-1984) and National Cancer Institute of the University of Gezira (NCI-UG) (19%, i.e. 1,009/5,236 recorded cancer cases during the period 1999-2008) (6,7,8).

Breast cancer is a group of heterogeneous diseases, encompassing a number of distinct biological entities with specific pathologic features and biological behavior. The last WHO tumor classification ‘blue’ book published in 2008 reported 18 varieties of breast cancer. It is debatable whether all the described variants are biologically significant or are the result of pathologists’ active imagination, nonetheless, they do illustrate what pathologists have highlighted for a very long time, that breast cancers are heterogeneous group of disorders and not a single disease. While this may seem obvious today, it has not always been articulated by the medical and scientific community (9). With the advent of high throughput molecular techniques such as gene expression profiling, the idea of heterogeneity became embedded in the community. A new taxonomy of breast cancer classification is being developed, and in the excitement, there is a fear amongst surgeons and oncologists that if standard histopathological analysis is all they are being given, they are missing some data vital to their management of patients. In fact, they wonder, is it time to ditch the old-fashioned morphological classification and go straight to the microarray chip? (9) Currently, there are several well validated clinical, histopathological and molecular factors that are used routinely as prognostic and predictive markers for breast cancer. These include patient age, tumor grade, tumor size, lymph node status and presence of metastasis, histological type and hormone receptor status (estrogen receptor (ER) and progesterone receptor (PR)). More recently, human
epidermal growth factor receptor 2 (HER2/neu) status and vascular peritumoural invasion (VPI) have been included as routine markers. Historically, breast cancers were divided into hormone receptor positive and negative tumors. This helped guide patient management in the use of endocrine therapies that directly or indirectly targeted the hormone receptor, such as selective estrogen receptor modulators (SERMs), e.g. tamoxifen, raloxifene, aromatase inhibitors, and ‘pure’ oestradiol analogues like fulvestrant. Widespread use of these agents in the adjuvant setting has significantly prolonged disease-free and overall survival and reduced the incidence of contralateral breast cancer. Despite this, up to half of all hormone receptor positive breast cancers do not respond to endocrine treatment at initial presentation (intrinsic resistance) or there is inevitable development of resistance over time (acquired resistance). A major challenge in current clinical practice is separating patients into groups who do or do not derive benefit from adjuvant systemic therapy.

Expression profiling analysis using gene microarrays has demonstrated that breast cancers cluster into distinct subsets according to their gene expression patterns. These initially reported groups are: luminal A, luminal B, normal breast-like, HER2, and basal-like. This classification has been highly consistent in independent studies using different array platforms, tumor sets, and statistical analyses. Studies using selected immunohistochemical stains have achieved similar stratifications of tumors according to clinical outcomes, suggesting that this molecular classification is robust. These subgroups correspond reasonably well to clinical characterization on the basis of ER and HER2 status, and proliferation markers and histologic grade. Follow-up studies have shown these subtypes to be conserved across diverse patient series and array platforms, and have shown that different gene expression based predictors are likely tracking a similar, common set of biologic subtypes, with significant agreement in predicting patient outcome.

The objective of the current study was to study the association between breast cancer molecular sub-type A with clinical, pathological parameters and to discuss the expected impact on management of this type of cancer in Sudan.

Patients & Methods
This study was a cross sectional descriptive analytical correlation study designed primarily to evaluate the molecular subtypes of breast cancer in Sudanese female. The study was conducted in Gezira and Khartoum states. The samples were collected from the medical laboratory, Faculty of Medicine- university of Gezira (FOM-UOG), and RICK in Khartoum during the period from April 2012 to April 2013. The study included all female patients who were diagnosed with histological confirmed in-situ or invasive breast carcinoma in the period of the study. Patients who received neo-adjuvant chemotherapy were excluded. A total coverage was achieved in University of Gezira in the period of the study. In Khartoum specimens were randomly selected according to availability of samples and patients' data. Demographic data, clinical and pathological characteristics were collected for each patient. Histopathological specimens were obtained, reviewed and immunohistochemistry to determine molecular subtypes was performed. Consistent with criteria developed from peer reviewed publications, the combinations of IHC markers used to define breast cancer molecular subtypes were as follows: luminal A (ER+ and/or PR+, HER2-, ki 67 <14% and any ck 5/6), luminal B (ER+ and/or PR+, HER2- or +, ki 67 >14% and any ck 5/6), basal-like (ER-,PR-, HER2-, CK5/6+ and any ki 67), HER2-overexpressed (HER2+, ER-, PR- and any ki 67), and unclassified (negative for all four markers).
The statistical analysis was implemented in the SPSS Statistical Software. Morphologic lesions were expressed with descriptive statistics. Immunohistochemical data were analyzed with nonparametric methods on semi-quantitative IHC scores using same statistical software. Statistical significance was determined at P value less than 0.05 and confidence interval of 95% adjusting for main clinic-pathologic variables. Approval was obtained from Ethical and Research Committee, Faculty of Medicine, University of Gezira. Verbal consent was taken from the patient after explaining the objectives of the study as per current practice in those institutes.

**Results**

A total number of 165 cases of invasive breast cancer were collected, 141 cases had adequate included 37 cases (26.2%) from Khartoum (Radiation and Isotope Centre) selected tumour blocks for review and immunohistochemical studies. These cases according to the availability of the data and 104 cases (73.7%) from Medani (medical laboratory, Faculty of Medicine treated at National Cancer Institute University of Gezira). 24 cases excluded from the study because they did not have adequate tumour tissue. Successful contact was obtained in 78 (55.3%) cases and the rest of study population data was reached from their medical records from both National Cancer Institute (NCI) and RICK.

Of the 141 cases studied, 130 (92.2%) had both adequate tumour and interpretable immunohistochemical result for ER, PR, and ki67; 129 cases (91.4%) for HER2neu and 121 cases (85%) for ck 5/6 (Table 1).

**Table 1: Immunohistochemistry of the study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>positive</th>
<th>negative</th>
<th>over expressed</th>
<th>Low &lt;14%</th>
<th>High &gt;14%</th>
<th>Difficult to assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>81(57%)</td>
<td>60(42.6%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PR</td>
<td>68(48.2%)</td>
<td>73(51.8%)</td>
<td>20(14.2%)</td>
<td>-</td>
<td>-</td>
<td>12(8.5%)</td>
</tr>
<tr>
<td>Her2</td>
<td>24(17%)</td>
<td>85(60.3%)</td>
<td>20(14.2%)</td>
<td>-</td>
<td>-</td>
<td>12(8.5%)</td>
</tr>
<tr>
<td>CK5/6</td>
<td>32(22.7%)</td>
<td>75(53.2%)</td>
<td>-</td>
<td>-</td>
<td>11(7.8%)</td>
<td>-</td>
</tr>
<tr>
<td>Ki67</td>
<td>-</td>
<td>-</td>
<td>66(46.8%)</td>
<td>64(45.4)</td>
<td>-</td>
<td>11(7.8%)</td>
</tr>
</tbody>
</table>

**Discussion**

Breast cancer is a heterogeneous group of lesions that differ in their clinical presentation, radiologic appearance and behavior\(^{17,18}\). This has been clearly illustrated among the current study population where one hundred and thirty one consecutive breast cancer patients were evaluated for different molecular subtypes of breast cancer using immunohistochemical feature as a surrogate for genetic assessment (Table 1). This study demonstrated that the majority of cases were classified as Luminal A (34.6%) (Table 2).

**Table 2: Clinicopathological characteristics of molecular breast cancer subtypes**

<table>
<thead>
<tr>
<th>Variables</th>
<th>All cases n = 141</th>
<th>Luminal A n = 45 (34.6%)</th>
<th>Luminal B n = 36 (27.7%)</th>
<th>Basal-like n = 22 (16.9%)</th>
<th>HER2-overexpression n= 20 (15.4%)</th>
<th>Unclassified n = 7 (5.4%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-specific group n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-30</td>
<td>15 (10.8%)</td>
<td>1 (2.2%)</td>
<td>3 (8.3%)</td>
<td>8 (36.3%)</td>
<td>2 (10%)</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>31-45</td>
<td>53 (38.4%)</td>
<td>17 (37.8%)</td>
<td>11 (30.5%)</td>
<td>11 (50%)</td>
<td>7 (35%)</td>
<td>4 (57.3%)</td>
</tr>
<tr>
<td>46-65</td>
<td>49 (35.5%)</td>
<td>19 (42.3%)</td>
<td>17 (47.2%)</td>
<td>3 (13.6%)</td>
<td>10 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;65</td>
<td>21 (15.2%)</td>
<td>8 (18.8%)</td>
<td>5 (13.9%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Premenopausal age &lt;50 n (%)</td>
<td>50 (35.4%)</td>
<td>22 (52%)</td>
<td>14 (49.2%)</td>
<td>6 (27.3%)</td>
<td>6 (49.8%)</td>
<td>2 (20.6%)</td>
</tr>
<tr>
<td>Postmenopausal age &gt;51 n (%)</td>
<td>62 (43.9%)</td>
<td>20 (47.6%)</td>
<td>15 (51.7%)</td>
<td>16(72.7%)</td>
<td>6 (50.9%)</td>
<td>5 (78.4%)</td>
</tr>
</tbody>
</table>

**Table 2: Clinicopathological characteristics of molecular breast cancer subtypes**
This subtype offers the best prognosis of all the subtypes. Luminal A breast cancers were predominant in Asian, white, and postmenopausal African American populations (all > 50%), approximately 40% in premenopausal African Americans, and only 27% in indigenous Africans. Interestingly, Luminal A was also predominant in people of Egyptian and Tunisian descent (19,20). The rest of the subtypes and their distribution are shown in the same table. This study focused on the clinicopathological features of luminal A subtype and discusses the impact of diagnosing this subtype on general management of breast cancer in Sudan. The authors believe that by ordering an extra test (Ki67) to the already existed immunohistochemistry tests that are currently on routine use in Sudan (ER, PR, HER2) a major change in the adjuvant treatment could be considered for the majority of breast cancer patients in Sudan.

By reviewing the clinicopathological features of luminal A subtypes (Table 2) it is evident that the majority of patients are diagnosed at ages above 30 and most of them above 45 years. Interestingly it is the only subtype that has more patients who are above 65 years of age. Although this has not been reflected on the menopausal status where almost equal numbers of patients are distributed between premenopausal (50%) and postmenopausal (50%). The majority did not have positive...
family history. The majority has a number of children ranging between one to more than 5. T1 (<2 cm) and T2 (>2cm) tumour sizes comprised the majority of tumour sizes at presentation. Only 20% of those who had evaluable lymph nodes status had positive lymph nodes. It is also evident that the number of patients who had distant metastases at presentation was low (9.1%). Invasive ductal carcinoma was the most common histological type reported among this group (75%). Tumour grade 1 and 2 were the most common grades reported. The disease was uni-focal in the majority of those who were evaluable for this criterion (55.6%) and 46.7% had no vascular invasion. The existence of bilateral disease was reported in only one patient. For the majority of patients there was no synchronous in situ component. Looking at the above mentioned clinicopathological features for this subtype- which are similar to those reported for luminal A in international literature\(^{21}\), it clearly reflects a more indolent and favorable biology of this disease in comparison with the other hormone positive luminal subtype which is associated with higher grade, increased proliferation rate, and overall poorer prognosis. Other genetic molecular subtyping, can be expensive and not readily available (e.g. Gene microarray, Recurrence Score Oncotype Dx and mammaprint), especially in low income countries as the case for Sudan. It may be possible to differentiate between these two subgroups using immunohistochemical assessment of the proliferative marker Ki67, as reported by Cheang et al\(^{22}\), where a cut off of Ki67 14% was used. This method of assessment is readily available in many laboratories in Sudan and demonstrated utility in providing prognostic information, although there are known limitations and difficulties in measuring Ki67 even in the most respected laboratories. In this regard it has to be mentioned that the false positive and false negative rates were around 25%, suggesting there is significant room for improvement within such a method\(^{21}\). The differentiation between these two subtypes has a significant impact on the way clinicians tailored postoperative adjuvant therapy. The benefit of prescribing cytotoxic chemotherapy in the adjuvant setting in almost all early breast cancer patients is evident in terms of delaying relapse and increasing overall survival. The guidelines from the 2000 National Institutes of Health Consensus development conference stated that “because adjuvant polychemotherapy improves survival, it should be recommended to the majority of women with localized breast cancer regardless of lymph node, menopausal, or hormone receptor status”. This statement took no consideration of the heterogeneous nature of breast cancer that has been reported later in many researches. The subtypes that have been identified through these studies differ in terms of epidemiology, natural history and response to various therapies\(^{23, 24}\). The 2011 St Gallen Consensus conference panel of experts accepted the principle of using intrinsic tumor subtypes as a basis for identifying patients for whom each type of therapy is most likely to be beneficial and, conversely, those for whom a particular treatment may be futile. The panel also accepted using immunohistochemistry as reasonable tool in the current practice\(^{25}\). The question of can we identify a group of patients who are appropriately managed without the need for chemotherapy has been looked at in several well designed trials although no published studies have used luminal A subtype as an eligibility or stratification criterion. However, high expression of estrogen receptor, low proliferation, and no amplification or over-expression of Her2 oncogene are well documented and accepted by St Gallen 2011 panel as adequately defining the subtype for clinical use. There is strong evidence in these studies that there is no benefit of adding
chemotherapy to endocrine therapy compared with endocrine therapy alone for a group resembling luminal A in these studies. The International Breast Cancer Study Group (IBCSG) trial IX in postmenopausal women with node negative disease as well as trial VIII in premenopausal women with high expression of estrogen receptors, or low proliferation as measured by Ki67 labeling index, both failed to indicate benefit of chemotherapy in these setting\textsuperscript{(26,27)}. In premenopausal women the efficacy of chemotherapy is complicated by its direct effect on the ovaries resulting in chemotherapy induced ovarian failure which is associated with superior outcome\textsuperscript{(28, 29, 30, 31, 32)}.

More recently at the 13th St Gallen International Breast Cancer Conference in 2013, an international expert panel reviewed the clinicopathological surrogate definitions of breast cancer subtypes. On the basis particularly of results of the Prat et al. study, the panel advised that the clarity of the distinction between “luminal A–like” and “luminal B–like” tumors could be improved from the previous consensus guidelines by including a requirement for substantial progesterone receptor (PgR) positivity ($\geq$20%) in the definition of “luminal A–like” disease\textsuperscript{14,18,33}. The updated pathological definition of intrinsic molecular subtypes may maximize the number of patients classified as having the luminal A intrinsic subtype of breast cancer and for whom the use of cytotoxic drugs could mostly be avoided. The Panel also was of the strong opinion that patients with Luminal A-like disease were ‘less responsive to chemotherapy’, but this treatment could be added to endocrine therapy based on the large tumor volume, assessment of risk or patient preference. The Panel did not select a specific chemotherapy regimen for these patients and expressed the view that any of the standard regimens, including the first and second generation regimens (CMF, AC, TC), could be considered\textsuperscript{33}.

Using immunohistochemical studies to define molecular breast cancer subtypes in Sudan could positively impact on the current adjuvant management of patients. Identifying patients with low risk Luminal A subtype could avoid unnecessary prescription of cytotoxic agents. Larger prospective and well-designed studies are required to further explore this aspect of breast cancer.

References
Original Article

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