Comparison of molecular subtypes between primary breast cancer and its metastatic auxiliary lymph node(s)

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Abstract

Background
Breast carcinoma primary tumors and synchronous axillaries lymph node(s) metastases may represent different malignant clones, although the daily clinical practice depends only on the molecular subtypes of primary tumor to select the adjuvant systemic treatment. In some instances the absence of primary tumor or presence of technical difficulties that prevent the identification of exact tumor subtypes in the primary mass mandate seeking for alternative to test. Aim: this paper aims to compare of molecular subtypes between primary tumor &metastatic lymph node(s). Method In this prospective cohort study, 50 patients with node positive breast carcinoma included. Immunohistochemical analysis by using Envision method for detection of biomarkers: ER, PR, Her2/neu and Ki-67 to determine the four molecular subtype then comparison between the primary tumor and its metastatic lymph node(s). Results: In this study there was 22 (44%) cases with luminal-A molecular subtype in both mass and lymph node, 21 (42%) cases with luminal-B in primary mass in comparison to 18 (36%) cases in their metastatic node, 3 (6%) cases with Her2/neu enriched in the primary mass in comparison to 6(12%) cases in its metastatic node. There was 4 (8%) cases with triple negative molecular subtype which had no difference between them.. In Conclusions: There is no statistically significant difference between primary tumor and its metastatic lymph node(s) this concordance regarding the molecular subtypes may answer the question that pathologist can depends on the result of molecular subtypes on lymph node with good certainty if cannot do it on the primary tumor.

Keywords: Molecular subtypes, breast cancer, immunohistochemistry, comparison, metastatic lymph node.


Introduction
The breast cancer is the commonest female cancer &it is the second most common cancer worldwide, it compromise 2% of all malignancies [1] the incidence is increasing by 1% per year, the rate of increment is increasing especially in low-risk populations [2], the lifetime risk is 1 in 10, since up to 170,000 new cases diagnosed while approximately 40,000 patients die each year in the U.S, despite that majority of cases are curable if caught early [3]. Invasive breast carcinoma is a heterogeneous group of tumors with variable morphological growth patterns that associated with the clinical behavior and hence the prognosis [4]. The St. Gallen - intrinsic subtypes attempt to classify molecular subtypes of breast cancer into four distinct groups, surrogate definitions according to the biomarkers: ER & /or PR positivity, proliferation rate &/or expression of (HER2),these are the luminal A, luminal B, Erb-B2 over expression (HER-2 enriched) and Basal-like [5]. Primary tumor (PT) and synchronous auxiliary lymph node (sALN) metastases may represent different malignant clones, although the daily clinical practice depends only on the molecular subtypes of the primary tumor to select the proper adjuvant systemic treatment [6]. In some instances the absence of primary tumor (e.g. after lumpectomy or lobotomy in case of breast conserving surgery) or presence of technical difficulties that prevent the identification of exact molecular sub typing in primary tumor biopsy or surgical specimens so it become necessary to test it on alternative such as synchronous metastatic lymph node(s) [7]. The controversial results of many studies regarding the similarity of receptors expressions and subsequent molecular sub typing, makes it so difficult to address the concordance between the primary tumor and synchronous lymph node metastases [8-9].

Aims of the study

- Comparison of molecular subtypes between the primary breast cancer and synchronous lymph node metastasis among breast cancer patients with positive auxiliary lymph node(s).
- Definition of the incidence rate of molecular subtypes in primary breast cancer and synchronous lymph node(s) metastasis among breast cancer patient with positive auxiliary lymph node(s).

Review of literature

Breast cancer expected to represent 20-26% of all new cancer cases among women, it run as the first cause of death at age 29 to 59 [10], it represent a major health problem in the developed and developing world, since one in eight women affected during their lifetime [11].

Tumor Markers in Breast Carcinoma

Tumor markers are substances that could be found in abnormal quantities in blood, urine and tissues of some patients with cancer [12]. Several tumor markers can be detected in breast carcinoma; we include 4 tissue tumor markers as in the table 2.1

Table (2.1): Biomarkers for breast carcinoma [12]

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Role in Breast Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Are used for prognosis and patient selection for ant hormonal therapy</td>
</tr>
<tr>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>HER-2/neu</td>
<td>A marker indicating a poor prognosis , it may be an important predictor of patient survival and tumour recurrence and metastasis</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Is a cell proliferation marker and its high expression indicate a poor prognosis</td>
</tr>
</tbody>
</table>

Immunohistochemistry

Immunohistochemistry (IHC) refers to the process of localizing surface or nuclear proteins in the cells of a tissue sections depending on the antibodies binding specifically to the antigens in the biological tissues. It is widely used in the categorization and in the treatment of malignancy because the specific molecular tumor markers are characteristic of particular cancer types [13].

The Principles

The fundamental concept behind (IHC) is the detection of the antigens (Ag) in the tissue sections by specific antibodies (Ab), as soon as Ag-Ab binding occurs; it is detected with colored immunohistochemical reactions [14].

The detection methods [15]

Direct

Primarily used for (Ig) deposits, although it regarded as easy and fast, is insensitive method and there is only few available Ab can be used, the primary Ab is usually labeled with fluorochromes, e.g.: EPOS (Enhanced Polymer One Step).

Indirect

Involves the application of secondary Ab, it includes several techniques:

- APAAP: It is done as a three step techniques by incubation with the primary (Ab), application of the secondary (Ab), then application of APAAP complex.
- PAP: It is also consist of three steps: in the final step there is application of PAP complex.
- ABC: It is a three steps technique involve the application of primary (Ab), then the biotin labeled secondary (Ab), then Avidin-Biotin-Peroxidase complex, this technique give much more superior results when compared with unlabeled antibody techniques.
- LSAB+: It is a three step technique includes the use of the glycoprotein Streptavidin which is produced by Streptomycin avidities; it is a sensitive and robust method.
- Envision system (Polymeric labeling two-step method): It is easy, fast, and sensitive method it composed of multiple molecules of enzymes and secondary Ab Wich is attached to a compact polymer, the disadvantage of this method is that it is usually more cost than the other methods.

Molecular classification:

As Perou & colleagues in the year 2000 segregate breast cancers into distinct subtypes depending on the similarities in the gene expression profiles and using the microarray platform [16]. The grouping has somehow evolved into a molecular classification of breast cancer. The subtypes of breast cancers as recognized by their gene signature include: luminal (A and B), HER2/neu, basal-like breast cancer. (17) Since the different subgroups of breast cancers have a specific characteristics, they would more likely to benefit from different approaches of therapy as in the table 2.2[18].
Table (2.2): Usage of IHC as a surrogate marker for the molecular sub typing of breast cancer [18]

<table>
<thead>
<tr>
<th>Molecular Sub typing</th>
<th>bioprofile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lominal A</td>
<td>ER, PR</td>
</tr>
<tr>
<td>Lominal B</td>
<td>ER &amp; PR+</td>
</tr>
<tr>
<td>HER-2 positive</td>
<td>ER- &amp; PR-</td>
</tr>
<tr>
<td>Bazal-like</td>
<td>ER- &amp; PR-</td>
</tr>
</tbody>
</table>

| HER2/neu & others   | HER2/neu - Ki-67 (<14%) (Low) | HER2/neu + or HER2/neu - Ki-67 =/>14% | HER2/neu + | HER2- CK5/6 &/or EGFR + |

Material and method
This study was done at the laboratory of Al-Sader Medical Teaching City hospital in Najaf during the period from March 2018 to March 2019 after approval of research ethics committee. 50 cases of female patients with breast carcinoma and positive ipsilateral lymph node(s) had been included in this study; the age is range from 27 to 79 years, with a mean age 49.9 years.
This study was prospective cohort study with signed patient approval and specific illegibility criteria which had been include:
- Either positive or negative HER-2/neu cases were included in this study; the equivocal cases had been excluded from it.
- Specimens should be obtained from modified radical mastectomy (Presence of both primary tumor & synchronous metastatic lymph node(s)).
- Specimens should be handled, dissected, processed & stained with same IHC staining under same environment & circumstances.
The immunohistochemical biomarker (ER, PR, Her2/neu and ki-67) were allocated to certain molecular subtype then the relation between those parameters in both primary tumor & synchronous LN were assessed, both positive control and negative control slides are used with each run.

Materials and Equipment
1- Primary antibody
   A- Estrogen antibody
      It is monoclonal mouse anti-human ER α, 0.2ml/1ml, Code No. M7047, Dako cytometry Dinmark A/S, Produktionsvej 42, DK-2600 Glostrup, Dinmark was used as a primary antibody for the detection of estrogen receptor [19].
   B- Progesterone
      It is a monoclonal mouse antihuman PR, 0.2ml/1ml, Code No. M3569, Dako cytometry Dinmark and used as a primary antibody for the detection of progesterone receptor.
   C- Her2/neu
      It is a polyclonal Rabbit Anti- Human c-erbB-2 Oncoproteinhave, 0.2 ml, Code No. A0485, Dako cytation Denmark A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark and used as primary antibody for the detection of HER -2 /neu protein.
   D- Ki-67:
      Ki67 Monoclonal Mouse Anti-Human Ki67 antigen, 11 ml, Ready-To-Use, DAKO, Clone MIB-1, Code M7240, LOT 00005848, Dako, Denmark
2- Antibody Diluents
   With Background Reducing Components (BRC): 125ml, Code S3022, LOT 00002288, Dako North America, Inc. 6392 Via Real Carpentaria, CA 93013 USA used for the ER and PR dilution range is 1:60, for Her2 /neu dilution range is 1:200 and for Ki-67 is 1:75.
3- Antigen Retrieval
   Target Retrieval Solution, 500 ml, PH 9, Code S2368, LOT 00026677, Dako Denmark A/S Produktionsvej 42 DK-2600 Glostrup Denmark.
4- Buffer Solution:
   Tris buffered Saline with Tween 20.10× Concentrate Code S3306 are used for ER, PR , Her2/neu and Ki-67, Dako cytation Dinmark A/S Produktionsve 42 DK-2600 Glostrup Dinmark, Dako, Inc 6392 via Real Carpentaria, CA 93013 USA.
5- Staining Kit
   Dako cytation, Code K 0679 it includes:
   - Peroxidase Block3% hydrogen peroxide in water
   - Biotinylated Link
   - Streptavidin-HRP
   - DAB substrate buffer PH 7.5
   - DAB chromogen

6-Equipment: Microwave oven, humidity chamber, Water bath, positively charged slides, cover slides, staining jars, hot plate, micropipettes with tips, pastures, timer, gloves, cotton swabs, tissue papers, pap pen, callipered cylinders, calibrated test tube, buffer solution, xylene, hematoxylin, distilled water, ethanol of different concentrations and light microscope.

Scoring System
Positive immunoreactions are (light- dark brown) precipitate in the cell nucleus for estrogen, progesterone and dark brown for Ki-67; and at the cell membrane for Her2/neu.

Estrogen and Progesterone Receptors Scoring System
Scoring is basically based on the examination of all of the tumor cells on the slide, by counting the percentage of all the positive cells in 100 malignant cells at objective 40 total magnifications, then the score assessed according to Allred scoring system [20].

Her-2/neu scoring system [21]
Depend on the intensity and completeness of membranous staining of the tumor cells and it scored into: 0, 1, 2 and 3
Score 0: negative in which there is no staining at all, or faint or incomplete staining in ≤ 10% of tumor cells.
Score 1+: negative in which there is incomplete or faint staining in > 10% of tumor cells
Score 2+: equivocal in which there is cecumferntial membrane staining with incomplete and/ or weak/moderate staining in > 10% of tumor cells or complete and cecumferntial and intense but in < 10% of tumor cells, this score should be evaluated by SISH study to determine positivity or negativity.
Score 3+: positive in which there is cecumferntial membrane staining with complete, intense and >10% of tumor cells

Ki-67 scoring system [22]
Diffuse or dots like brown nuclear staining, the tumor is regarded mitotic ally active when >14% of tumor cells revealing positive staining.

Statistics of the study
It was performed by the help of PQStat software statistical package (version 1.6.2) using McNemar-Bowker test of symmetry for molecular subtype comparison, Kappa-Cohen measure of agreement (level of similarity 0.8-1), and P value level of significance < 0.05.

Results
Fifty cases of breast cancer with positive epsilateral lymph node(s) were included in this study, they were classified according to their molecular subtypes into: (Luminal-A, Luminal-B, Her2/neu enriched and basal-like); and immunohistochemical biomarkers (ER, PR, and Her2/neu& Ki-67) were studied in the primary breast cancer and its ipsilateral metastatic lymph nodes.

1- The biological profile
Luminal A
In this study there was 22 (44%) cases with luminal a molecular subtype (Hormone positive and Ki-67 expression in less than 14% and Her2/neu negative) it was the same in both the primary masses and their ipsilateral metastatic lymph nodes, table (4.1) and figure (4.1).

Luminal B
There was 21 (42%) cases with luminal B in the primary masses in comparison to 18 (36%) cases in their ipsilateral metastatic lymph nodes (Hormone positive and Ki-67 over expressed; whether Her2/neu positive or negative), table (4.1) and figure (4.1).

Her2/neu enriched
There were only 3 (6%) cases with positive HER-2 in the primary masses in comparison to 6 (12%) cases in their ipsilateral metastatic lymph nodes (Hormones negative with Her2/neu positive regardless Ki-67 expression status which is usually high), table (4.1) and figure 4.1).

Triple negative
There were 4 (8%) cases with triple negative (i.e. ER, PR and HER2/neu all are -ve) which had no difference between the primary tumor mass and its metastatic lymph node(s), table (4.1) and figure (4.1).

Table (4.1): Comparison of the molecular subtypes between the primary mass and the ipsilateral metastatic lymph nodes

<table>
<thead>
<tr>
<th>Molecular subtypes</th>
<th>Lymph node</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Her2 enriched</th>
<th>Triple -ve</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>Luminal A</td>
<td>22 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>22 (44)</td>
</tr>
<tr>
<td></td>
<td>Luminal B</td>
<td>0(0)</td>
<td>18 (85.7)</td>
<td>3 (14.3)</td>
<td>0 (0)</td>
<td>21 (42)</td>
</tr>
<tr>
<td></td>
<td>Her2enrich</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>3 (6)</td>
</tr>
<tr>
<td></td>
<td>Triple -ve</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (100)</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>22(44)</td>
<td>18(36)</td>
<td>6 (12)</td>
<td>4 (8)</td>
<td>50 (100)</td>
</tr>
</tbody>
</table>

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2- Immunohistochemical Parameters

The immunohistochemical biomarkers expression

In the 50 cases that were studied, ER expression was +ve in 39 (78%) cases primary breast masses in comparison to 37 (74%) ipsilateral metastatic lymph nodes, PR expression was positive in 39 (78%) primary breast masses in comparison to 36 (72%) cases ipsilateral metastatic lymph nodes, while HER2 expression was +ve in 13 (26%) cases of both the masses and lymph nodes lastly ki-67 over expression was in 28 (56%) cases in the primary breast masses and its ipsilateral metastatic lymph nodes, figure (4.2).

The ER Expression

For the total 39 cases with positive ER expression in the primary mass there was 19 (48.7%) cases Luminal-A in comparison to 20(54.1%) cases out of total 37 cases in the ipsilateral metastatic lymph nodes; and there was 20(51.3%) cases Luminal-B in the primary massing comparison to 17(45.9%) cases in the ipsilateral metastatic lymph nodes, with insignificant statistical difference between the two variables (mass & lymph node) for both luminal A & luminal B as it was evident by Mc-Nemar t-test, table (4.2)

<table>
<thead>
<tr>
<th>Molecular subtypes</th>
<th>ER Positive</th>
<th>ER Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>Lymph node</td>
<td>Mass</td>
</tr>
<tr>
<td>Luminal A</td>
<td>19 (48.7)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>20 (51.3)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Her2 enriched</td>
<td>0 (0)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>TNBC</td>
<td>0 (0)</td>
<td>4 (63.3)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (78)</td>
<td>37 (74)</td>
</tr>
</tbody>
</table>

The PR Expression

For the total 39 cases with positive PR expression in the primary mass there was 18 (46.2%) cases Luminal-A in comparison to 18 (50%) cases out of total 37 cases in the ipsilateral metastatic lymph nodes; and there was 21 (53.8%) cases Luminal-B molecular subtype in the primary massing comparison to 18 (50%) cases in the ipsilateral metastatic lymph nodes with insignificant statistical difference between the two variables (mass & lymph node) for both luminal A & luminal B as it was evident by Mc-Nemar t-test.

<table>
<thead>
<tr>
<th>Biological profile</th>
<th>PR Positive</th>
<th>PR Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>Lymph node</td>
<td>Mass</td>
</tr>
<tr>
<td>Luminal A</td>
<td>18 (46.2)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>21 (53.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Her2 enriched</td>
<td>0 (0)</td>
<td>3 (27.3)</td>
</tr>
<tr>
<td>TNBC</td>
<td>0 (0)</td>
<td>4 (36.3)</td>
</tr>
<tr>
<td>Total</td>
<td>39 (78)</td>
<td>36 (72)</td>
</tr>
</tbody>
</table>
The Her2/neu Expression

For the total 13 cases with positive Her2/neu expression in the primary mass, there were 10 (76.9%) cases of Luminal-B in comparison to 7 (53.8%) cases in the ipsilateral metastatic lymph nodes; and there were 3 (23.1%) cases of Her2 enriched profile in the primary mass, in comparison to 6 (46.2%) cases in the ipsilateral metastatic lymph nodes. There was no significant statistical difference between the two variables (mass & lymph node) for both luminal A & Her2 enriched, as it was evident by McNemar t-test, table (4.4).

Table (4.4): Her2/neu expression in the four biological profiles

<table>
<thead>
<tr>
<th>Biological profile</th>
<th>Her2/neu Positive</th>
<th>Her2/neu Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass</td>
<td>Lymph node</td>
<td>Mass</td>
</tr>
<tr>
<td>Luminal A</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Luminal B</td>
<td>10</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Her2 enriched</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TNBC</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>13</td>
<td>37</td>
</tr>
</tbody>
</table>

The Ki-67 expressions

For the total 28 cases with Ki-67 over expression, there were 21 (75%) cases in the primary mass, in comparison to 18 (64.3%) cases in the ipsilateral metastatic lymph node with Luminal B profile; while it was 3 (10.7%) cases of Her2 enriched profile in the primary mass. In comparison to 6 (21.4%) cases in the ipsilateral metastatic lymph nodes; finally, 4 (14.3%) cases with Triple negative profile in both the primary mass and its ipsilateral metastatic lymph nodes, table (4.5).

Table (4.5): Ki-67 expression in the four biological profiles

<table>
<thead>
<tr>
<th>Biological profile</th>
<th>Ki-67 over expression</th>
<th>Ki-67 low expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mass</td>
<td>L.N</td>
<td>Mass</td>
</tr>
<tr>
<td>Luminal A</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Luminal B</td>
<td>21</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Her2 enriched</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Triple -ve</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>28</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 4.4 (A): Invasive Ductal Carcinoma, red arrow shows PR Nuclear Staining (Score 6) (IHC Stain for PR, 40X).
Figure 4.4 (B): metastatic Invasive Ductal Carcinoma to its ipsilateral auxiliary lymph node. (Immunohistochemical Stain for PR, 10X).
Discussion
As the study addressed in the introduction combining immunohistochemical marker into biological subtypes showed to add prognostic information which might be of importance for recommendation of systemic therapy.

1- Biological profile
Luminal A
In this study there were 22 (44%) cases with luminal A, this finding is agreed to many literatures where they address the range of 40% -66 % which differ according to race/ethnicity, stage and ages of the studied population [23-24]. There is perfect match (kappa coefficient was 1) between the primary masses and their ipsilateral metastatic lymph nodes this findings disagreed to only available similar project which is Anna-Karin Falck et al., which show discordance in between the primary tumor & its metastatic lymph node(s) in 11% of the patients, where the luminal- A profile is shifted to another profile with a worse prognosis in metastatic lymph nodes in 7 out of 45 cases Luminal A (16%) i.e. luminal B & triple negative. A similar shift had been addressed in Raica M et al., but the researchers did not use the proliferative marker (Ki-67) to differentiate between the two luminal subtypes [25].

The luminal B
It comprise 21(42%), 18(36%) of all cases in the primary masses and their ipsilateral metastatic lymph nodes respectively, this was more than the percentage addressed in other literatures which may be due to the advance stage hence all our studied cases were lymph node positive, which was agreed with results of Zorka Inic et al., in which Luminal-B subtype having the highest percentage of involvement of lymph nodes (54.9%) in comparison to other 4 subtypes, or may be due younger age [26]. Although numerically there were discordance between the primary masses and their ipsilateral metastatic lymph nodes 3(14%) had been shifted from luminal B (ER+, PR+, Her2 +) in the primary tumor mass to Her2 enriched in the metastatic lymph node(s) this was statically non-significant P-value 0.248213 (Mc-Nemar test of similarity). This might be due to sample size error. This

Figure 4.6 (A): Metastatic Invasive Ductal Carcinoma to its ipsilateral auxiliary lymph node shows Her2/neu Membranous Staining (Score 3), and shows the nodal lymphocytes. (Immunohistochemical Stain for Her2/neu, 10X)
Figure 4.6 (B): Metastatic Invasive Ductal Carcinoma to its ipsilateral lymph node, shows Her2/neu Membranous Staining (Score 3), and shows the nodal lymphocytes. (Immunohistochemical Stain for Her2/neu, 40X)

Figure 4.7 (A): Invasive Ductal Carcinoma, shows Ki-67 Nuclear Staining (Immunohistochemical Stain For Ki-67, 40X)
Figure 4.7(B): Invasive Ductal Carcinoma metastasis to the lymph node shows ki-67 Nuclear Staining (Immunohistochemical Stain for Ki-67,40X)
result was similar to finding of Anna-Karin Falck et al., who find in two different papers the perfect match of Luminal B between primary masses and their ipsilateral metastatic lymph nodes [27].

**Her2/neu enriched**

There were only 3 (6%) cases in the primary masses in comparison to 6 (12%) cases in their ipsilateral metastatic lymph nodes this percentage was seen across many studies [28]. Also the numerically discordance between the primary masses and their lymph nodes was statistically non-significant P value 0.08 (McNemar test of similarity), though such shift of biological profile will be important to be reported since it will affect the subsequent treatment options specially it represent the change of the disease toward more aggressive profile [29-30]. Two studies Ieni A et al. &Esmail RS et al., in which they found changes in HER2 status between primary breast cancer and corresponding synchronous LN in (4.72%) & (30%) of cases respectively [31-32].

**Triple negative**

There was 4 (8%) cases with triple negative in both primary mass and the metastatic lymph nodes this was lower than the rate recorded in Nadia Howlader et al., (12%) &William D. Foulkes et al., review (12%-20%) that difference may be attributed to presence of more African American women among the studied samples [33]. Also there were both numerical statistical perfect match (kappa coefficient was 1) between the primary masses and their ipsilateral metastatic lymph nodes 4(8%) cases for each, this findings is agree to Anna-Karin Falck et al., studies.

2- Immunohistochemical Expression

In the 50 cases of breast carcinoma that were studied, ER expression was positive in 39 (78%) cases primary breast masses in comparison to 37 (74%) ipsilateral metastatic lymph nodes, the change in ER status was observed in 2 cases (DR 4%), although the difference was statistically not significant, similar expression lost was also noticed in many studies such as Arapantoni-Dadioti Pet al. [34-35] such loss of ER expression in synchronous metastatic LN had been shown to be associated with poorer outcome [36]. PR expression was positive in 39 (78%) primary breast masses in comparison to 36 (72%) cases ipsilateral metastatic lymph nodes, the lost PR was observed in 3 cases (DR 6%), these changes agreed with many studies that address the issue of receptor discordance between primary mass & synchronous LN. HER2/neu expressed identical positivity in 13 (26%) cases of both the masses and lymph node cases. this agreed with results that shown high Her2 concordance between primary tumors and auxiliary lymph node or distant metastases that demonstrated in many studies but disagreed with results of others so it still controversial despite the critical role of the Anti Her2 therapy in the management. Lastly ki-67 was over expressed in 28 (56%) cases in both the primary breast masses and its ipsilateral metastatic lymph nodes, this concordance disagree with Ibrahim T et al., who find 38.8 % difference rate between the primary breast masses and its ipsilateral metastatic lymph nodes.

**Immunohistochemical Expression in relation to biological profile**

Despite negative statistical significant, the loss of ER, PR expression in metastatic lymph nodes (6%) led to shift the entire molecular profile from Luminal B (Her 2 positive) to Her2 enriched might represent major change that not only affecting prognosis of patient but also may affect subsequent therapeutic choices. The net result agreed to some extent with Falck AK et al., conclusion that suggest the concordance for the biomarkers analyzed in matched pairs of primary tumors and lymph node metastases was high; moreover, survival analyses showed that the expression of biomarkers in lymph node metastases can provide prognostic information when no analysis of the primary tumor can be done, treatment selection based on biomarkers in the lymph node is a topic for further studies [37].

**Conclusions**

The concordance between the primary mass of the breast cancer &its metastatic lymph node(s) in regard to the biological profile may answer the question whether the pathologist can depend on the result of IHC that done on the auxiliary lymph node rather than primary breast mass to determine the biological profile with good certainty if it is unbearable to do it on the primary mass.

**Recommendations**

More investigation of larger sample size in prospective study with longer duration of follow up & study of survival rate among them may provide a better vision and validate the finding of this study. Further advanced studies to discover the molecular basis that lead to loss of expression of hormonal receptors in the three cases with luminal B subtype in the metastatic lymph node converting the subtype into HER-2 enriched.

**References**

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