Comparison of some salivary biomarkers between luminal and triple negative subtypes after surgical removal of invasive ductal carcinoma

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Abstract:

Luminal (L) and triple negative (TN) subtypes of breast cancer (BC) are recorded in Iraqi women which have different incidence, treatment, progression, and metastases. This study is aimed to compare some risk factors and salivary biomarkers between them. Only premenopausal Iraqi women with invasive ductal carcinoma (IDC) are involved in this study. Based on histopathologic and immunohistochemistry (IHC) report carried out by hospital itself, the age, Subtype and grade of tumor were recorded, while waist/hip ratio, secretory status, and salivary levels of Ca 15-3, total protein (TP), and PH were determined 2-3 weeks after surgery and before starting therapy. Results revealed that luminal is the predominant subtype of IDC and constitute about 80.5% and the rest (19.5%) is TN subtype, however HER-2 subtype didn’t recorded. Also women with TN are presented at age 40.7 year which is significantly (P < 0.05) lower than that in (L) subtype (47.1 year). All TN cases are non-secretor and presented at grade 3 and their saliva is more acidic and containing higher levels of Ca 15-3, and TP than those in (L) cases. Our results suggested that saliva can be used as an alternative diagnostic sample in differentiation between various subtypes of IDC, and secretory status of patients may be contribute in determining the subtype of BC.

Key words: Breast cancer, triple negative, Ca 15-3, saliva, secretory status, luminal subtype.

Introduction:

Breast cancer (BC) has been classified into three major subtypes namely; luminal, HER2, and triple negative with different incidence, treatment, progression, and metastases (1, 2). The risk of getting BC in women is increased with advanced age and delayed menopause but it is more aggressive in younger ones (3-5). Furthermore, obesity increases risk of BC in post-menopausal women, but reduces its risk pre-menopausal women (6). This heterogeneity in the acting of obesity as BC risk factor may vary based on the menopausal status or ER, and PR status (7). Most case-control studies have found an association between waist/hip ratio (WHR) and BC in postmenopausal patients (8), but not in premenopausal women (9). Recently, it was reported that non-secretors as a non-preventable risk factor significantly increases the risk for getting BC (10). Concerning with biomarkers of BC, Ca 15-3 is still the most important one and can give important information about responsiveness of tumor cells to certain therapy and their biological behavior during disease monitoring (11). Recently, it was found that Ca 15-3 can be detected in the saliva of women with malignant breast cancer in comparison with benign and normal breast (12). Since some of biomarker molecules can pass from blood to saliva (13), about 2,340 salivary protein were identified, 20% of them are derived from plasma (14). Also another 57 salivary metabolites were identified in saliva of patients with oral, breast, and pancreatic cancer and periodontal diseases (15). Recently, about 3,200 - 8,000 gene transcripts were found in human saliva, the majority of them are mRNA (16). More recently, 31 salivary biomarker that diffused from circulation has been described in BC patients (17). Therefore, saliva become an attractive and non-invasive fluid for assessment disorders in different sites of body rather than oral cavity (18). Since BC is more aggressive in premenopausal women also there are few studies that interested in evaluation of their biomarkers in saliva after surgical removal of tumor mass, thus this study attempt to find an additional risk factors (e.g. secretory status) and biomarkers (e.g. salivary PH and total protein) that may be explain the different behaviour of IDC subtypes.

Materials & Methods:

More than one hundred BC women in different public and private hospitals in Baghdad after 2-3 weeks
according to the results obtaining from histopathological investigation, only 41 cases of premenopausal patients are recorded as invasive ductal carcinoma (Figure-1).

The cellular staining pattern for ER and PR is nuclear, thus a positive result is defined as nuclear staining in ≥ 1% of tumor cells which is consistent with ASCO/CAP’s recommended cut-off of ≥ 1% positive tumor cells for positive assessment (23) as shown in figure-2.

However, the scoring method for HER2 expression is based on the cell membrane staining pattern and Allred scoring system was used to interpret the staining result based on the proportion and the intensity of staining tumor cell (Figure-3): Positive HER2 expression (+++ ) with uniform intense membrane staining of more than 30% of invasive tumor cells, Equivocal HER2 expression (++) with complete membrane staining that is either non-uniform or weak in intensity but has circumferential distribution in at least 10% of cells, and finally Negative HER2 expression (0 or +) with non-completely membrane staining in less than 10% of cells (24).

Results:

According to the results obtaining from histopathological investigation, only 41 cases of premenopausal patients are recorded as invasive ductal carcinoma (Figure-1).
Among all premenopausal Patients, 33 (80.5%) of IDC are presented as luminal carcinoma subtype and only 8 (19.5%) of them having triple negative (TN) subtype (Figure-4).

The age of TN women is (40.7 ± 2.3 years) which significantly (P < 0.05) lower than that of luminal women (47.1 ± 0.5 years), however, W/H ratio showed non-significant difference between luminal (0.89 ± 0.02) and TN subtype (0.88 ± 0.04) (Table-1).

### Table-1: Differences in age and W/H ratio between Luminal and TN of IDC premenopausal women

<table>
<thead>
<tr>
<th>Character</th>
<th>IDC subtype (N=41)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luminal</td>
<td>TN</td>
</tr>
<tr>
<td>Age (years) (M ± SE)</td>
<td>47.1 ± 0.5</td>
<td>40.7 ± 2.3</td>
</tr>
<tr>
<td>W/H ratio (M ± SE)</td>
<td>0.89 ± 0.02</td>
<td>0.88 ± 0.04</td>
</tr>
</tbody>
</table>

[S]: significant difference; [NS]: non-significant difference at P < 0.05

Tumor grade shows significant association (P < 0.05) with different subtypes of IDC (Figure-5), in which all TN patients (100%) are presented in grade 3, while only 21.2% of luminal subtype patients are at grade 3 and the remainder are either grade 1 (24.3%) or grade 2 (54.5%).
Similarly, significant association was shown between secretory status and different subtypes of IDC (Figure-6), in which all patients (100%) of TN subtype are non-secretors in comparison with only 63.6% of luminal subtype.

Concerning with the levels of biomarkers in the saliva of IDC patients, results in Table-2 shows that saliva of TN patients is significantly more acidic (PH = 5.68 ± 0.05) than that in luminal patients (PH = 6.06 ± 0.07). Furthermore, salivary level of Ca 15-3 in TN (12.1 ± 0.32 U/ml) is significantly higher than that in luminal subtype (8.8 ± 0.4 U/ml), also TP level in saliva of TN (1.05 ± 0.05 g/dl) is significantly higher than that in luminal subtype (0.78 ± 0.02 g/dl).

**Table-2: Salivary levels of Biomarkers in different subtypes of IDC women**

<table>
<thead>
<tr>
<th>biomarker</th>
<th>IDC subtype</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Luminal (n=33)</td>
<td>TN (n=8)</td>
</tr>
<tr>
<td>Ca 15-3 (U/ml)</td>
<td>8.8 ± 0.40</td>
<td>12.1 ± 0.32</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>0.78 ± 0.02</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>Saliva PH</td>
<td>6.06 ± 0.07</td>
<td>5.68 ± 0.05</td>
</tr>
</tbody>
</table>

[S]: significant difference at P < 0.05
Discussion:

Results of this study recorded that luminal carcinoma subtype is the most predominant subtype of IDC among premenopausal patients who are presented at age of 47.1 years with different grades of cancer cell differentiation, while the minority is triple negative subtype at average age of 40.7 years and all cases are presented at third grade of differentiation (Figure-4 & 5, and Table-1). Consistent with our results, most of the previous studies stated that IDC is the prominent histopathological type of BC and it is a disease of older women, its incidence increases with age, but rare below 20 years (25-28). Although TN subtype constitute about 15–20% of all BC cases, it is highly frequent among premenopausal women and characterized by poor prognosis, early recurrence, and distant metastasis when compared to other subtypes of BC (29-32).

Concerning with WHR as a measure of central obesity, our result found that premenopausal BC women are presented with high WHR (0.89, and 0.88) whether they have luminal or TN subtype respectively (Table-1). However, previous studies reported that WHR above 0.85 for females increases risk for getting BC only in postmenopausal women (7, 33-35), but not in premenopausal ones (36, 37). Differences in these associations possibly due to different roles of fat and fat distribution among pre- and postmenopausal women as well as across ethnic groups on metabolism particularly insulin signalling on endogenous production of androgen and estrogen (38-41). Furthermore, higher BMI is associated with higher risk for ER+/PR+ tumors in postmenopausal women but has a protective effect in premenopausal women and with no associations in ER−PR− tumors (7). However, recent study suggest that body fat distribution may be associated with an increased risk for ER− tumor among premenopausal women (40). In contrast, elevated BMI in younger women (≤50 years) was associated with the risk of only ER+ or PR+ BC but triple-negative BC may has distinct etiology because it was found that high BMI and high parity have been associated with increased risk for TN, instead of low parity in other subtypes of breast cancer (42-45).

In respect to secretory status as a risk factor, our result showed significant association between secretory status and different subtypes of IDC (Figure-6), in which all patients (100%) of TN subtype are non-secretors in comparison with 63.6% of luminal subtype. Secretor phenotype is defined by the functioning fucosyltransferase FUT2 enzyme that allows the secretion of ABO antigens into body fluids, however non-functional FUT2 occur in 20% of non-secretor individuals groups (46). Since non-secretor phenotype is associated with metastasis, it can be used as a predictive value for BC susceptibility and lymph metastasis (47). Few data are available about this genetic feature and its relation with the aggressive behaviour in TN subtype of BC and need further investigation.

Concerning with salivary biomarkers, our results shows that saliva of TN patients is significantly more acidic and contain higher levels of Ca 15-3 and TP than those in luminal patients (Table-2). Presence of such markers in saliva facilitate easy detection without even minimal surgical procedure and will also be candidates for population based screening (48). In clinical practice, ER, PR, and HER2, are still the routinely biomarkers for in determination of different subtypes of BC (49), although new markers are needed to more accurately approximate the basal-like subtype (32), thus BC detection and management remains dependent on invasive procedures (50), and CA 15-3 is still the most commonly used serum marker that is helpful in diagnosing and monitoring the breast cancer disease (51, 52). Our results were in accordance with previous studies that found a significant association between tumor size and negative ER/PR status (53-55). Furthermore, it was reported that the level of CA 15-3 is significantly increased in positive hormonal receptor cases and contributes to E-2 mediated growth of cancer cells (56). Also this protein has a high potential for immune intervention via decreasing immunoendothelial markers in nipple aspirate fluid (NAF) and found that EGF and TGF-β1 levels in NAF of premenopausal women were significantly higher than postmenopausal women (62). More recent study demonstrated a relationship between salivary levels of estrogen, progesterone, and percent mammographic density in premenopausal women (63).

Since defining molecular abnormalities in BC is an important strategy for early detection, assessment of prognosis, and treatment selection, thus the present findings clearly indicated two facts; the first one is the possibility to use saliva as a good alternative sample for differential diagnosis between the different subtypes of BC; and secondly, salivary biomarkers particularly those with protein nature are detectable and can constitute the backbone of etiology that is often linked in receptor expression status.

References:


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مقارنة بعض المؤشرات الحيوية اللعابية مابين النمط اللمعي والنمط السلبي الثلاثي
للسرطان القنوي الإجتياحي بعد الأزالة الجراحية

خالد مهدي صالح
الجامعة المستنصرية/ كلية العلوم/ قسم علوم الحياة

الخلاصة:

إن التصنيفات (L) والسلبي الثلاثي (TN) للسرطان الذي تم تسجيلهما لدى النساء العراقيات، وأنهما يختلفان من حيث نسبة حدوثهما والعلاج وشدة تطور المرض وانتشاره. تهدف هذه الدراسة لمقارنة بعض عوامل الخطورة ومؤشرات الحياة الدينية للрак الذي يتألف من هذه النصفيتين. علاج الدراسة قطع نساء عراقيات دون سن اليأس ومصابات بسرطان الثدي القنوي الإجتياحي، عند وضع تقرير الفحص المرضي، وكشف كيمياوية المناعة وفحص CA15-3 والبروتينات التي تتألف من كل الناتج المبكر TN والأس الهيدروجيني TP والبروتين الكلي، حيث تم تحديدها في لعاب المرضى بعد 2-3 أسابيع من عملية استئصال الورم وقبل البدء بالعلاج. أظهرت النتائج بأن النمط TN هو الأكثر شيوعاً ما بين النساء المصابات بسرطان الثدي القنوي الإجتياحي حيث بلغت نسبة حوالي 80.5%، والنمط TN لمدة كانت حوالي 19.5% كانت للنمط L في حين لم تسجل أي حالة من النمط TN في حالة HER-2. كما أن معدل عمر النساء المصابات بالنمط TN كان حوالي 40.7 سنة وهو أقل بشكل ملحوظ عن P < 0.05 من متوسط عمر المصابات بالنمط L وهو كان حوالي 47.1 سنة. إن جميع حالات النمط TN كانت غير عازلة ومن المريضة الثالثة وكان لها أكثر فارز مكان TN وحمولة مع النمط TN والبروتينات Ca15-3 والبروتين TN، حيث كانت عينة من المستضدات TN، يمكن استخدامها كعينة تشخيصية بدلاً من الأحماض المختلفة للрак الذي القنوي الإجتياحي، وأن حالة الإفراز عند المرضى قد تكون مساهمة في تحديد النمط النثوي للسرطان الذي.

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The summary:

The luminal (L) and triple negative (TN) breast cancer tumors were recorded among Iraqi females, and they differ in terms of incidence, treatment severity, and disease progression. This study aimed to compare some of the risk factors and biological indicators between these two types. The study included only Iraqi women without menopause and with breast cancer metastatic disease. Based on the histopathological report and the histopathological examination conducted by the hospital, the determination of the patient's age and tumor type, and hereditary status, as well as the tumor grade, were considered. The results showed that the TN type was more common among breast cancer metastatic patients, with a percentage of around 80.5%, while the TN type was 19.5% for the L type. The age of women with the TN type was around 40.7 years old, which was significantly less than P < 0.05 of the average age of women with the L type, which was around 47.1 years old. All cases of the TN type were non-secretory and the third party, and the TN type had a higher level of the tumor antigen and TN proteins, Ca15-3, and the TN protein, where it was found in the sample of the different breast cancers. This can be used as a diagnostic sample for the luminal breast cancer tumors, and the effusion case among patients may be a contribution in determining the secondary breast cancer type.