Chitinase-3-Like Protein1 (YKL-40) as Biomarker in Serum of Breast Cancer Females: A Hospital Based Study

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Abstract

Background: Chitinase-3-like protein1 (YKL–40) is a human glycoprotein related in amino acid sequence to the chitinase protein family, but has no chitinase activity, its expression was shown to be highly expressed in breast cancer.

Aim: To detect level of YKL-40 in metastatic and non-metastatic breast cancer patients and to compare the results with healthy females.

Patients and Methods: 109 female patients were enrolled in this study; 43 non-metastatic & 36 metastatic breast cancer patients. Thirty healthy females were also included as controls. YKL-40 level was detected in serum of studied groups using ELISA kit provided from Quantikine R&D systems, Minneapolis, USA.

Results: The median level of YKL-40 was statistically significant higher in both non-metastatic and metastatic cases when compared to the healthy controls (p<0.001). Comparing the levels of YKL-40 at different nodal affection showed that there was statistical significant difference between N1, N2 and N3 in non-metastatic group (with the highest median in patients with N3) as well as between N2 and N3 (with higher median in patients with N3) in metastatic group (p<0.001). Receiver operator characteristic analysis suggested that a serum YKL-40 level cut-off value of 92.696 may have predictive value for breast cancer.

Conclusion: We concluded that there is significantly elevated serum YKL-40 level in breast cancer compared to healthy controls. YKL-40 may discriminate between various stages and between metastatic and non-metastatic cases.

Key Words: Biomarker – Breast cancer – YKL-40.

Introduction

BREAST cancer is the top cancer in women both in the developed and the developing world. Despite being the most common cancer, 5-year relative survival rate of breast carcinoma is still over 80% when they are detected in early phase [1]. The incidence of breast cancer varies greatly around the world, being comparatively lower in less-developed countries than in the more-developed countries [2].

Like other carcinomas, breast cancer occurs based on an interaction between genetic heterogeneity and the environment. It has been reported that an accumulation of genetic variants is involved in the process of breast carcinogenesis [3]. Among these genetic variants, many of them play roles in apoptosis or cellular proliferation, since the balance between the two decides which direction to go; normal mammary development or carcino genesis of the mammary gland [4].

YKL-40 is a new biomarker, which represents a heparin-binding and chitin-binding glycoprotein. It belongs to a group of mammalian proteins with an amino acid sequence similar to that of 18 glucosyl hydrolases, a group of bacterial chitinases [5]. Although YKL-40 does not have a chitinase activity, it is possible that YKL-40 has a role in the process of angiogenesis, stimulating the endothelial cells, and contributes to the formation of branching tubules, it is also well established that YKL-40 is a potent growth factor inducing the proliferation of chondrocytes and fibroblasts [6].

YKL-40 may have a role in the proliferation and differentiation of malignant cells; may protect tumor cells from apoptosis, stimulates angiogenesis; participates in the extracellular tissue remodeling; stimulates fibroblasts around the tumor; but this hypothesis still has to be confirmed in vivo [7].

Aim of the work:

In this study we aim to detect the level of YKL-40 in metastatic and non-metastatic breast cancer patients and to compare the results with healthy
females. Also to compare YKL-40 level at different T stages, and N stages in breast cancer group.

Patients and Methods

After screening 580 females patients with breast cancer at our National Cancer Institute during the period from August 2011 to February 2012, the present study included 109 Egyptian women with age ranged from (30-65 years). All diagnosed breast carcinoma patients who fulfilled our inclusion criteria during this period were included in the study, and those having any exclusion criteria have been dismissed from the study. Diagnosis was based on history taking, clinical examination and confirmed by mammography and surgical biopsies. C, who were proven to be healthy with no family history of breast cancer were also included in this study. The studied subjects were divided into three groups as follows:

Group I : (n=30) healthy females as a control group.

Group II: (n=43) patients with non-metastatic breast carcinoma, they were classified according to TNM grading system into 56% (24 cases) with T2 and 44% (19 cases) with T3, also 12% (5 cases) with N1, 56% (24 cases) with N2 and 32% (14 cases) with N3.

Group III: (n=36) patients with metastatic breast carcinoma, they were classified according to TNM grading system into 78% (28 cases) with T2 and 22% (8 cases) with T3, also, 56% (20 cases) with N2 and 44% (16 cases) with N3.

Inclusion criteria:

Adult females, age ranged from (30-65) years with no previous treatment with chemotherapy.

Exclusion criteria:

Age below 30 or above 65 years, inflammatory breast cancer, or recurrence of the disease, and any condition known to increase the YKL-40 serum level such as liver disease, arthritis, or other cancers.

Written consent forms were signed by all participants in this study including controls. All cases were subjected to estimation of YKL-40 level in serum. The carcinoma biopsies were examined histopathologically.

Methods:

Detailed history was taken. The following information was particularly stressed upon: Course of illness, age of onset of the disease, mode of presentation, positive family history. Routine preoperative investigations were done including: Complete blood count (CBC), fasting blood glucose (FBG), liver function tests (ALT & AST), kidney function tests (urea and creatinine) and PT&PC.

Quantitation of Chitinase 3-like 1(YKL-40) by using ELISA kit provided from Quantikine R&D systems, Minneapolis, USA (Catalog Number DC3L10) was done on all sera. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for YKL-40 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any YKL-40 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for YKL-40 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of YKL-40 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical analysis:

Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows. The results of Kolmogorov-Smirnov test indicated that data was not normally distributed (non parametric data) so non parametric tests were used for comparisons. Comparison between non parametric quantitative variables was carried out by Mann-Whitney U when comparing two groups. Kruskal-wallis test was used when comparing between more than two groups of independent quantitative variables. Qualitative data was compared using chi-square test. Binary correlation was carried out by Spearman correlation test. Receiver operator characteristic (ROC) curve was done to suggest a serum YKL-40 level cut-off value in breast cancer patients.

Results

The present study was conducted on 109 female subjects. They were classified into three groups: Group I included 30 healthy control females with no family history of breast cancer. Group II included 43 female patients with non-metastatic breast cancer patients with nodal affection but without distal metastasis (bone, liver, brain, and lung). Group III included 36 female patients with metastatic breast cancer patients with nodal affection and distal metastasis (bone, liver, brain, lung).

As regards the median age of the studied groups, it was 48 years in the control group, 50 years and 51 years in non-metastatic breast cancer and metastatic breast cancer respectively. There was no
statistical significant difference between the three groups regarding their age \( (p=0.816) \) using Mann-Whitney U test analysis.

Table (1) also showed that the non-metastatic breast cancer patients and the metastatic breast cancer patients with positive family history were 26 out of 43 \( (60.55\%) \) and 22 out of 36 \( (61\%) \) respectively. We found no statistical significant difference between both groups \( (p=0.248) \) regarding the presence of positive family history.

In non-metastatic group, patients \( (43 \) females) were either Invasive duct carcinoma \( (ii, \ iii) 74.4\%, 18.6\% \), respectively or Invasive lobular carcinoma \( (ii, \ iii) 4.7\% \) and 2.3\% respectively (Table 2). Regarding the grades of the tumor in non-metastatic group, 24/43 \( (55.8\%) \) were grade T2 and 19/43 \( (44.2\%) \) were grade T3 (Table 2). Regarding the nodal affection distribution among the patient group, it was 11.6\%, 55.8\% and 32.6\% in N1, N2, and N3 respectively (Table 2).

In metastatic breast cancer, 77.8\% had Invasive duct carcinoma \( (iii) \) and 22.2\% had Invasive lobular \( (iii) \) (Table 3). Regarding the grades of the tumor in metastatic group, 28/38 \( (77.8\%) \) were grade T2 and 8/36 \( (22.2\%) \) were grade T3 (Table 3). Meanwhile, the nodal affection distribution among the group was 55.5\% and 44.5\% in N2 and N3 respectively (Table 2).

Table (3) revealed a statistical significant difference in the median level of YKL-40 when comparing the three studied groups together using the Kruskal-wallis test analysis, and that the median level of YKL-40 was statistically significant higher in breast cancer cases (both non-metastatic and metastatic cases) when compared to the healthy controls \( (p<0.001) \) using Mann-Whitney U test analysis. No statistical significant difference was found between the non-metastatic breast cancer versus metastatic breast cancer cases \( (p=0.899) \) using Mann-Whitney U test analysis.

On comparing the results of the levels of YKL-40 at different tumor stages among non-metastatic and metastatic groups, there was no statistical difference between T2 and T3 in non-metastatic as well as metastatic group (Table 4).

Meanwhile, when comparing the results of the median levels of YKL-40 at different nodal affection among non-metastatic and metastatic groups, there was statistical significant difference between N1, N2 and N3 in non-metastatic group (with the highest median in patients with N3 using Mann-Whitney U test analysis) as well as between N2 and N3 (with higher median in patients with N3 using Mann-Whitney U test analysis) in metastatic group \( (p<0.001) \) (Table 4). Comparing the median levels of YKL-40 at different N stage to each other among non-metastatic patient group revealed a statistical significant difference between each group to the other one \( (p<0.001) \) using Mann-Whitney U test analysis.

The ROC curve for serum levels of YKL-40 in 79 patients with breast cancer and in 30 healthy controls is shown in Fig. (1). ROC analysis suggested that a serum YKL-40 level cut-off value of 92.696 may have predictive value (Area under curve: 0.774; sensitivity: 70.6\%; specificity: 93.1\%) for breast cancer \( (p<0.001; 95\% \) CI: 0.641 to 0.770).

YKL-40 was correlated to other variables (age, tumor stage and nodal affection) among the whole groups and showed statistical significance correlations between YKL-40 and both age \( (r=0.561) \) and nodal affection \( (r=0.527) \) \( (p<0.001) \).

Table (1): Range and median of age, and family history of all studied groups.

<table>
<thead>
<tr>
<th>Age/years</th>
<th>Controls ((n=30))</th>
<th>Non-metastatic breast cancer ((n=43))</th>
<th>Metastatic breast cancer ((n=36))</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range, Median</td>
<td>30-65 (48)</td>
<td>40-65 (50)</td>
<td>40-65 (51)</td>
<td>0.816</td>
</tr>
<tr>
<td>Family history:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (60.55)</td>
<td>22 (61%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30 (100)</td>
<td>17 (39.5)</td>
<td>14 (39%)</td>
<td></td>
</tr>
<tr>
<td>P-value**</td>
<td></td>
<td></td>
<td>0.248</td>
<td></td>
</tr>
</tbody>
</table>

*p>0.05 is non-significant \( (p\)-value showed non-significant difference in the age distribution between the three studied groups using Kruskal-wallis test)

**p>0.05 is non-significant \( (p\)-value showed non-significant difference in the family history association between both the non-metastatic and the metastatic breast patients groups using chi-square test).

Table (2): Pathological classification, grading and nodal affection of non-metastatic breast cancer and metastatic breast cancer.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Non-metastatic breast cancer ((n=43))</th>
<th>Metastatic breast cancer ((n=36))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive duct:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii</td>
<td>32</td>
<td>74.4</td>
</tr>
<tr>
<td>iii</td>
<td>8</td>
<td>18.6</td>
</tr>
<tr>
<td>Invasive lobular:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii</td>
<td>2</td>
<td>4.7</td>
</tr>
<tr>
<td>iii</td>
<td>1</td>
<td>2.3</td>
</tr>
<tr>
<td>Grades:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>24</td>
<td>55.8</td>
</tr>
<tr>
<td>T3</td>
<td>19</td>
<td>44.2</td>
</tr>
<tr>
<td>Nodal affection:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>5</td>
<td>11.6</td>
</tr>
<tr>
<td>N2</td>
<td>24</td>
<td>55.8</td>
</tr>
<tr>
<td>N3</td>
<td>14</td>
<td>32.6</td>
</tr>
</tbody>
</table>
Table (3): Range and median serum level of YKL-40 in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=30)</th>
<th>Non-metastatic breast cancer (n=43)</th>
<th>Metastatic breast cancer (n=36)</th>
<th>( p )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YKL40 (ng/mL)</td>
<td>20-70 (30)</td>
<td>90-250 (150)</td>
<td>100-280 (180)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>( p )-value**</td>
<td></td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td></td>
</tr>
<tr>
<td>( p )-value***</td>
<td></td>
<td></td>
<td>0.899</td>
<td></td>
</tr>
</tbody>
</table>

** \( p <0.05 \) is significant (comparing the three groups together using Kruskal-wallis test).
*** \( p >0.05 \) is non significant (comparing both non-metastatic versus metastatic breast cancer groups using Mann-Whitney \( U \) test).

Table (4): Median levels of YKL-40 at different tumor stages and at different nodal affection among non-metastatic and metastatic breast cancer groups.

<table>
<thead>
<tr>
<th>Tumor stage</th>
<th>Non-metastatic breast cancer group</th>
<th>Metastatic breast cancer group</th>
<th>( p )-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2 (n=24)</td>
<td>T3 (n=19)</td>
<td></td>
</tr>
<tr>
<td>YKL-40 (ng/mL)</td>
<td>130</td>
<td>142</td>
<td>0.575</td>
</tr>
<tr>
<td>Nodal affection</td>
<td>N1 (n=5)</td>
<td>N2 (n=24)</td>
<td></td>
</tr>
<tr>
<td>YKL-40 (ng/mL)</td>
<td>59</td>
<td>98</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

** \( p <0.05 \) is significant.
*** \( p <0.05 \) is significant (comparing between 3 groups using Kruskal-wallis test).

Discussion

According to the Centers for Disease Control, 1 in every 4 deaths in the United States is due to cancer. Many of these deaths could be averted by improved early cancer detection, since existing therapies, especially surgery, are much more effective in early cancer stages as compared to later stages [8].

Breast cancer is the commonest cancer among women, contributing for 30% of all female cancers. It affects one in 14 women during their life time, almost one every three affected women will die of the disease. Breast cancer constitutes 33% of all female cancers at the National Cancer Institute, Cairo University [9].

To the best of our knowledge this is the first work that studies the association between the serum YKL-40 and female breast cancer in the Middle East. The present study demonstrated that the median age was 50 and 51 years in non-metastatic breast cancer and metastatic breast cancer respectively. These results are coincided with another study [10] who concluded that the incidence of breast cancer increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically. At the same time another study [11], confirmed that the risk of getting breast cancer increases with age. A woman is more than 100 times more likely to develop breast cancer in her 60s than in her 20s. Another study [12] stated...
that the strongest risk factor for breast cancer (after gender) is age: The older the woman, the higher her risk. These data are coincided with our results.

Family history of breast cancer is an important established risk factor of the disease. In the present study, positive family history was present in 60.55% (n=26) of the non-metastatic breast cancer patients and in 61% (n=22) of the metastatic breast cancer patients. Previous study [13] confirmed our results as it found that a positive family history of breast cancer seemed to increase risk similarly for ER+ and ER- tumors and similarly for all ER/PR subtypes.

Pathological classification of our non-metastatic breast cancer patients, were either Invasive duct carcinoma (ii, iii) 74.4%, 18.6%, respectively or Invasive lobular carcinoma (ii, iii) 4.7% and 2.3% respectively, and our metastatic breast cancer patients, were either Invasive duct carcinoma (iii) 77.8% or Invasive lobular carcinoma (iii) 22.2%. These results are in agreement with a study [14] who stated that the most common tumor for breast cancer in Egypt was invasive duct carcinoma (83.4%). They stated that carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 29% of National Cancer Institute cases. Median age at diagnosis is one decade younger than in countries of Europe and North America and most patients are premenopausal. Tumors are relatively advanced at presentation. The majority of tumors are invasive duct subtype and the profile of hormone receptors is positive for estrogen receptors and/or progesterone receptors in less than half of cases.

The present study demonstrated that the grades of the tumor in non-metastatic group was (55.8%) of grade T2 and (44.2%) of grade T3. These results coincided with that of King et al. [15] who studied 346 patients with invasive breast cancer. They found the majority of the patients (58.3%) had T2 and (28.5%) had T3.

Biological function of YKL-40 in cancer is not yet known. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells. It protects the cancer cells form undergoing apoptosis, stimulates angiogenesis, and has an effect on extracellular tissue remodeling. It stimulates fibroblasts surrounding the tumor, although in vivo proof of these hypotheses is yet to be obtained [16].

It could not be excluded that YKL-40 might act as a growth factor for cancer cells, too. Another possibility is that YKL-40 protects cancer cells from undergoing apoptosis. It is also called “breast regression protein” (Brp), because it induces mammary involution in mice a few days after weaning. YKL-40 facilitates cell attachment and migration of vascular endothelial cells, which is an indication that the protein may function in angiogenesis [17].

Obviously, our study showed that serum YKL-40 level in breast carcinoma is significantly higher than the healthy women (p<0.001). No statistical significant difference was found between the non-metastatic breast cancer versus metastatic breast cancer cases. It is believed that increased expression of YKL-40 may improve the identification of women at increased breast cancer risk.

The present work was coincided with the clinical studies [18,19] which revealed that serum levels of YKL-40 were elevated in patients with a series of carcinomas including breast, colorectal, ovary, prostate, brain and blood. They concluded that these increased levels were correlated with poorer survival of cancer patients suggesting that serum levels of YKL-40 serve as a prognostic cancer biomarker.

Our results were similar to Jensen et al. [20] who surveyed 78 age-matched healthy females and 100 breast cancer patients with local regional metastasis and distant metastasis including bone, lung and liver tumor and found that serum levels of cancer patients were significantly higher than those observed in healthy subjects. Patients with distant metastasis like liver metastasis demonstrated the highest serum levels of YKL-40 (an average of 230ng/ml).

Also, our results go with hand with a study done by Yamak et al. [21] who demonstrated that the median serum YKL-40 concentration in patients with locally advanced breast cancer was 149.5ng/ml and it was higher than levels observed in healthy female controls. They suggested that YKL-40 may be a useful prognostic indicator of outcome for patients with locally advanced breast cancer.

An in vitro study has shown that ectopic expression of YKL-40 in breast cancer cells led to tumor formation with an extensive angiogenic phenotype and that recombinant YKL-40 protein promoted vascular endothelial cell angiogenesis both in vitro and in vivo [22]. Therefore, the occurrence of high YKL-40 levels in highly differentiated and advanced cancers and recurrent cancer states could be explained by the role of YKL-40 in both angiogenesis and fibrogenesis, since highly differentiated tumors are characterized by high vascu-
larization and a high turnover of extracellular matrix.

On comparing our results of the levels of YKL-40 at different tumor stages among non-metastatic and metastatic groups, there was no statistical difference between T2 and T3 in non-metastatic as well as metastatic group. Yamac et al. [21] found in their study the Serum YKL-40 levels were also higher in patients with tumor size >2cm and node-positive disease but the differences were not significant (p>0.05).

In the present study, YKL-40 was correlated to other variables among the whole groups and showed statistical significant correlations between YKL-40 and both age and nodal affection (p<0.001). These results were similar in part to that of Yamac et al. [21]. They did multivariate analysis including tumor size, lymph node status, estrogen and progesterone receptor status, tumor grade, and serum YKL-40 levels indicated that serum YKL-40 levels were an independent prognostic variable for overall survival (hazard ratio, 1.004; 95% confidence intervals: 1.00, 1.07; p=0.027). Tumor size, lymph node status and estrogen receptor status were also independent prognostic variables for overall survival (p<0.05).

We concluded that there is significantly elevated serum YKL-40 level in breast carcinoma compared to healthy women and prospective investigations are aimed at evaluation of YKL-40 as a reliable biomarker and an appropriate target for development of anticancer therapy.

**Recommendations:**

Future focused translational researches combining basic and clinical research are needed in a joint effort to answer the questions:

Is plasma YKL-40 a useful clinical biomarker in patients with cancer?

Is YKL-40 a target for development of new cancer therapeutics? with close collaborations between multidisciplinary teams including surgeons, oncologists, pathologists, biochemists, tumor biologists, molecular biologists, biotech companies and the pharmaceutical industry. Without such collaboration it is unlikely that these two questions will ever be answered.

**References**


