Mannose receptor in different grades of breast cancer and ER, BR and Herneu-2 panel

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Abstract

Background: breast cancer is recognized as heterogeneous cancer in female patients, need to the discover a new biomarker by simple technique for early diagnosis. Objectives: Measure serum level of mannose receptor, in newly diagnosis patients and compare with control. Methodology: sixty one patients with more than 30 years old were selected after have been diagnosed with breast cancer depending on clinical evaluation, radiology, and FNA. Healthy subjects (n=61), age- matched subjects were taken as controls. For each study subject, clinical characteristics were recorded and the serum mannose receptor was measured by ELISA. Results: Serum mannose receptor level was significantly higher in breast cancer patients than in control. MR had area under the curve (AUC) of 0.847, a sensitivity of 80.0%, a specificity of 70.0%, and a cut off value between patients and controls is 1.7 pg/ml. MR was significantly higher in age > 50 years subgroup in both GII and GIII subgroup. MR had the same correlation with triple negative or positive breast cancer. Conclusion: The study detection of significant increases MR with both GII and GIII also level of MR had significant correlation with IHC. MR is proved to be useful in diagnosis of breast cancer by using ROC and useful for diagnosis and prognosis of breast cancer by using ROC to compare between patients and control with different grades of patients.

Keywords: breast cancer, MR, Grades, IHC

Introduction

Breast carcinoma is the most common malignant tumor and the second most common causes of carcinoma death in women. (1) Molecular subtypes of breast cancer can be classified according immunohistochemistry into Luminal A (ER+/PR+/HER2-); Luminal B (ER+/PR+/HER2-/+); HER2- overexpression (ER-/PR-/HER2+) and triple negative breast cancers/TNBCs (ER-/PR-/HER2-). (2)

Mannose receptor (MR), CD206, is about 175 kDa in size, an endocytotic membrane protein with multiple carbohydrate recognition domains, originally termed the macrophage mannose receptor, overexpresses MR on the cell surface, mediating active endocytosis of glycoconjugates. (3)

Protein glycosylation is one of the most frequent and well-known posttranslational modifications in protein, which plays important roles in physiopathological processes. Altered glycosylation on the cell surface of cancer cells is hallmark of tumorigenesis, tumor progression and metastasis. (4) The major types occur in cell surface protein glycosylation associated with metastatic behavior of cancer cell include changes in O-glycans and N-glycans, these changes in O- and N-glycans core structures which may occur early and late in cancer progression and metastasis. (5)
Material and methods

Patients were recruited from the oncology hospital of medicine city, whom admitted and verified by clinical examination from oncologist doctor, specialist radiologist and specialist pathologist. From period the July 2019 to February 2020, case control study enrolled 122 females case sample including case of cancer and control (61 patient and 61 control), aged ≤ 50 years and > 50. Thirty one patient (50.8%) with age of ≤ 50 years while thirty (49%) were more than 50 years, control group classify according age, have mean ± SD and p valu 0.08 and 0.06, bearing in mind the matching with patients into the same classification (≤ 50 and > 50).

Diagnosis of newly diagnosis of breast cancer by breast ultrasound, mammogram screening with know the BIRAD (Breast Imaging Reporting And Data system), fin needle aspiration and core biopsy. Clinicopath data were collected from archives includes Age, grade, ER, PR, Her2 profile.

Patients who had the following conditions were excluded: 1- Patients who take chemotherapy radio therapy and hormonal therapy, 2- Who had a previous mastectomy, 3- Who recurrence case and, 4- Patients who is less than 30 years old.

Sixty one apparently healthy subjects were recruited from the staff of female have much enough awareness to check up. Each subject has underwent a full history and physical examination with a recording of: age, not have any type of cancer.

They were age matched to patients and comply to the criteria of exclusion in patients group, not hypertensive and not diabetic. In addition, they underwent mammogram screening to check for any changes which might exist in spite of no clinical features of breast cancer so as to be excluded from the study.

Serum was obtained in studied groups to detected Mannose receptor by using ELISA.

Determination of human mannose receptor: (MR) was measured by (ELISA) kit (MyBioSource /USA).

Statistically Analysis

The data were represented as mean ± SD. To find out differences in the levels of serum MR among study groups, comparisons of their levels was made among related groups and subgroups.

Result

Malignant cases also had been arranged according to degree of differentiation of cancer into three subgroups GI (well differentiated adenocarcinoma), GII (Moderately adenocarcinoma) and GIII (Poorly differentiated adenocarcinoma), however no cases among studied groups including in the first category. According agathere was statistically significant increase correlation in blood level of MR in Age> 50 of patients and control group, while in Age≤ 50 the results MR marker, show decrease in the level but statistically not significant table (1).
Table (1) The association between the MR markers and patients Age groups.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Patient n=42(68.9%) Mean ±SD</th>
<th>Control n=61(100%) Mean ±SD</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>GII</td>
<td>2.53 ±0.910</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
<tr>
<td>GIII</td>
<td>2.31±0.799</td>
<td>1.68±0.51</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Most of cancer cases enrolled in the present study are categorized within Grade II and III including 42(68.9%) and 19(31.1%) respectively.

According to the results of present study, blood levels of MR markers showing significant correlation between GII,GIII groups and control as shown in table (2)

Table (2) The associated of MR with grads of the disease

<table>
<thead>
<tr>
<th>Age ≤ 50</th>
<th>Patient n=31(50.8%) Mean ±SD</th>
<th>Control n=32(52.5%) Mean ±SD</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.10±0.689</td>
<td>1.74±0.568</td>
<td>0.151</td>
</tr>
<tr>
<td>Age &gt; 50</td>
<td>n=30(40.2%)</td>
<td>n=29(47.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.84±0.901</td>
<td>1.62±0.447</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Estrogen receptors (ER) detected by Immunohistochemistry achieved on paraffin embedded blocks of breast cancer for the patients including in the present study showing 47(77%) ER positive while 14 (23%) ER negative. MR blood levels by ELISA showing statistically significant correlation between their level in patients with both ER positive and negative in comparison with control as shown in table (3)

Table (3) The associated of the MR marker with ER of the disease

<table>
<thead>
<tr>
<th>ER</th>
<th>Patient n=14(23%) Mean ±SD</th>
<th>Control n=61(100%) Mean ±SD</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2.11±0.82</td>
<td>1.6823±0.513</td>
<td>0.014</td>
</tr>
<tr>
<td>Positive</td>
<td>n=47(77%)</td>
<td>n=61 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5±0.83</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Regarding progesterone receptor (PR) detected by IHC 40(65.6%) of patients have PR positive while 21 (34.4%) have PR negative.

According to the result of present study MR show significant correlation in patients with negative PR.

In comparison with control group level of MR are statistically significant correlation between positive PR and control as shown in table below.

**Table (4) The associated of MR with PR of the disease**

<table>
<thead>
<tr>
<th>PR</th>
<th>Patient n=21 (34.4%) Mean ±SD</th>
<th>Control n=61 (100%) Mean ±SD</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2.39±0.93</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
<tr>
<td>Positive</td>
<td>2.43±0.80</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

A twenty two of patients enrolled in the study showing positive her2-neu receptors detected by IHC.

Those group showing statistically significant correlation in blood level of MR in comparison with control.

The rest 39(64%) patients show negative her2-neu negative result by IHC and shows statistically significant correlation of blood level of MR in comparison with control as shown in table (5).

**Table (5) The association between of the studied marker and her2-neu**

<table>
<thead>
<tr>
<th>HER2</th>
<th>Patient N=39 (64%) Mean ±SD</th>
<th>Control n=61 (100%) Mean ±SD</th>
<th>P valu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2.48±0.87</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
<tr>
<td>Positive</td>
<td>2.31±0.78</td>
<td>1.68±0.513</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Receiver Operator Characteristic Curve Analysis**

Table 6, figure 1 show the results of ROC – analysis between patients and control that estimated AUC for MR and it was 0.847 (sensitivity 80.0%, specificity 70.0% ).The same table and figure 2 shows the result of ROC – analysis between grade 2 and grade 3 of breast cancer estimated AUC (area under curve) for MR which it were 0.796 (sensitivity 83.0%, specificity 64.0%). The cutoff point for the level of MR that had the best potential for the diagnosis of grades.

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Table 6: The Best Discriminative cut-off values of MR that Best Predicted control versus patients

<table>
<thead>
<tr>
<th>Subgroup, grade II versus grade III</th>
<th>Parameters</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut off value</th>
</tr>
</thead>
<tbody>
<tr>
<td>control versus patients</td>
<td>MR</td>
<td>.847</td>
<td>80.000</td>
<td>70.000</td>
<td>1.7902</td>
</tr>
<tr>
<td>grade II versus grade III</td>
<td>MR</td>
<td>.796</td>
<td>83.000</td>
<td>64.000</td>
<td>1.8850</td>
</tr>
</tbody>
</table>

Figure 1: Receiver operator characteristic (ROC) curves MR for (a) breast cancer patients with controls (b) breast cancer GII VS GIII
Discussion

Breast cancer is a highly heterogeneous disease, encompassing a number of biologically distinct entities with specific pathologic features and biological behaviors. Mannose receptors in different breast cancer subtypes showed that the mannose receptors could be a potential marker for identification of breast cancer.\(^7\)

For the best of our knowledge this is the first study in Iraq, using ELISA detection of MR in breast cancer patients and it is correlation with the clinicopathological characteristics.

According age groups in the present study, serum mannose receptor level was high statistical significant correlation in Age group of more than 50 years, similar result was not proved previously, thus it consider as a good marker for the diagnosis of breast cancer. Although serum mannose receptor found in both normal and disease serum but elevated in disease than normal, this finding is in agreement with previous study \(^8\), however they uses Western blot and membrane type of mannose receptor, for this result, we focus the light on the importance of mannose when related with radiological score as benefit in the early diagnosis for breast cancer compared with control.

Serum mannose receptor was highly significant in GII and GIII as compared with control, and showed the same significant in both grade, which is agreed with Qinying \(^9\) who mentioned high MR in tissues and cell of breast cancer correlation with vascular invasion, while disagreed with Maija \(^10\), who when MR correlation with high tumor grade only, might be due to different study design and different laboratory technique.

The present study revealed that the results of immunhistochemistry (IHC) panel including estrogen ER, progesterone PR and her2n receptor, in each sub group both positive and negative receptors had significant correlation in serum mannose receptor compared with control, this result is agreed with PirasandXi-Le Hu \(^3,11\), although they were used histological laboratory technique.

Jing Fang showed interest about the importance of lower traumatic detection and diagnosis method because most of the diagnosis methods failed reflectance the heterogeneity of breast cancer \(^8\)

The result of ROC – analysis between the patients and controls revealed that MR is a good marker for the detection of breast cancer and the cutoff point for its level was 1.7 pg/mL while estimated AUC is 0.847, showed that this marker is good early in diagnosis of breast cancer.

The result of ROC – analysis between GII and III with estimation AUCis 0.796, had noticed that this marker is good tool for recognize between these grades according cut off value is 1.8 pg/ml.
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