SERUM FETUIN-A AND RANKL LEVELS IN PATIENTS WITH EARLY STAGE BREAST CANCER

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Summary

Background: Breast cancer (BC) is the primary cause of mortality due to cancer in females around the world. Fetuin-A is known to increase metastases over signals and peroxisomes related with growing. Receptor activator of nuclear factor-κB ligand (RANKL) takes part in cell adhesion, and RANKL inhibition is used in the management of cancer. We aimed to examine the relationship between serum fetuin-A, RANKL levels, other laboratory parameters and clinical findings in women diagnosed with early stage BC, in our population.

Methods: Women having early stage BC (n=117) met our study inclusion criteria as they had no any anti-cancer therapy before. Thirty-seven healthy women controls were also confirmed with breast examination and ultrasonography and/or mammography according to their ages. Serum samples were stored at -80 °C and analysed via ELISA.

Results: Median age of the patients was 53 (range: 57–86) while it was 47 (range: 23–74) in the healthy group.
Patients had lower high-density lipoprotein levels (p=0.002) and higher neutrophil counts (p=0.014). Fetuin-A and RANKL levels did not differ between the groups (p=0.116 and p=0.439, respectively) but RANKL levels were found to be lower in the favorable histological subtypes (p=0.04).

Conclusions: In this study, we found no correlation between serum fetuin-A levels and clinical findings in patients diagnosed with early stage BC. However, RANKL levels are found to be lower in subgroups with favorable histopathologic subtypes such as tubular, papillary and mucinous BC and there was statistically significant difference.

Keywords: breast cancer, fetuin-A, RANKL, serum

Introduction

Breast cancer (BC) is the second most frequently diagnosed malignancy just behind lung cancer and also the primary cause of mortality due to cancer in female around the world. Over 1.5 million women (25% of all women with cancer) are diagnosed with BC every year throughout the world (1). Today, the total number of BC patients have increased in response to exposure to several risk factors such as abnormal levels of estrogen, smoking, alcohol and obesity (2). Nevertheless, we know that early BC detection could reduce BC death rates significantly in the long-term (3).

Therefore, specific screening methods with the use of the correct biomarkers are important in the detection of early stage BC (4). Specially, blood, saliva, and urine were considered as ideal origin in which to assign the presence of cancer biomarkers such as annexin, peroxiredoxin and calreticulin (5).

Fetuin-A, also called Alpha 2-Heremans Schmid Glycoprotein (AHSG), is a serum glycoprotein synthesized by the liver and secreted into the blood stream (6). Its founded principal role is the inhibition of ectopic calcification, but mounting evidence suggests that it is a multifunctional protein capable of modulating a number of critical signaling pathways and it has roles in disease processes such as diabetes mellitus and kidney disease (7). In particular, high fetuin-A concentrations are found to be associated with atherogenic lipid profile and metabolic syndrome, low fetuin-A levels are related to vascular calcifications and inflammation (8). In addition, there are few suggesting increased RANKL levels may be a new serum biomarker in early BC (9).

Receptor activator of nuclear factor-kappa B ligand (RANKL) appertain to tumour necrosis factor superfamily which is a group of proteins that act as bidirectional signalling molecules. RANKL with osteocyte origin induces bone destruction by stimulating osteoclasts, while RANKL released from osteoblasts functions in the reverse effect (10). At the same time, available evidence suggests that the RANKL signalling system is associated with in almost all steps in BC development, from primary oncogenesis to the establishment of secondary tumors in the bone (10).

Materials and Methods

Demographic features of patients

A total of 117 female patients with early stage BC between the ages of 27–86 years (median: 53) were included in the study. Our control group was 37 healthy women between the ages of 23–74 (median: 47) years. BC was diagnosed according to the ultrasonography and histopathologic findings of the patients. The patients were asked for their medical history (type 2 diabetes, hypertension, smoking and medictions) and measured for their glomerular filtration rate (GFR) and other biochemical parameters. Exclusion criteria included trauma history, major surgical history, chronic kidney disease with a glomerular filtration rate (GFR) and other biochemical parameters. Exclusion criteria included trauma history, major surgical history, chronic kidney disease with a glomerular filtration rate (GFR) and other biochemical parameters.

Serum preparation

Blood was drawn after 12 hours of fasting in the morning. Serum was obtained after at least 30 minutes of clotting by centrifugation at 2500xg for 15 minutes. Serum was stored at -80 °C until assayed. All icteric or haemolytic blood samples were discarded. All parameters were analyzed in all samples together in a single batch at the termination of the experimental protocol (control and patient samples were analysed in the same batch).
Measurement of serum fetuin-A and RANKL levels

Serum fetuin-A and RANKL levels of patients with BC and of the control group were measured in the venous blood. A commercial kit (Assaypro, USA, cat no: EG 63501-1), based on a quantitative sandwich ELISA, was used and results were determined with ELX 800 UV version ELISA reader and calculated in grams per liter. Mean intra-assay and inter-assay coefficients of variation were less than 4.9% (n:10) and 6.7% (n:10).

Measurement of other biochemical parameters

Levels of serum glucose, urea, creatinine, total cholesterol, HDL, LDL, AST, ALT, GGT, LDH, ALP, total protein, albumin, parathyroid hormone (PTH), calcium, phosphorus, magnesium, CRP, complete blood count (CBC), CEA, CA 15.3 and erythrocyte sedimentation rate (ESR) were measured in the patient and control groups using the same biochemistry laboratory in our hospital.

Statistical analysis

Statistical analyses (Mann–Whitney U-test, Student t test) were performed with SPSS 19 (Statistical Package for Social Sciences). The difference in various parameters were analyzed by the Chi-square test. Pearson correlation test was used for correlating fetuin-A, RANKL and the different biochemical parameters. Multivariate logistic regression model was performed to determine the effect of independent risk factors for BC. P-values < 0.05 were considered significant.

Results

Median age of the patients was 53 (range: 57–86) while it was 47 (range: 23–74) in the healthy group. Patients were 56% postmenopausal, 40% premenopausal and 4% perimenopausal. Twenty-four (20.5%) of the patients had cerbb2 3 positive or cerb2 2 positive and SISH/FISH positive disease. Grade 2 disease was found in 60 (52.6%) patients and 47 patients (41.2%) had grade 3 disease. Seventy-four (63.2%) patients had invasive ductal carcinoma (IDC), 15 (12.8%) had invasive lobular carcinoma (ILC), 6 (5.1%) had mixed (IDC+ILC) carcinoma, 1 had metastatic cancer and 21 (17.9%) had other favorable (tubular, apocrine, papillary and mucinous) types. Twenty-nine patients (24.7%) had stage I, thirty (25.6%) had stage II and fifty-eight (49.5%) patients had stage III BC.

There was no statistically significant difference between ER status, PR status, cerbb2 status, grade, lymphovascular invasion, perineural invasion, stage, menopausal status and serum parameters. Patients had lower high-density lipoprotein levels (p=0.002) and higher neutrophil counts (p=0.014) rather than the control group (IA and IB). Fetuin-A and RANKL levels did not differ between the patients and control groups (p=0.116 and p=0.439, respectively) (Table II). There was no statistically significant difference in fetuin-A levels according to various clinical/laboratory

| Table IA | Laboratory values, cell adhesion markers and age of the patients’ and control group. |
|-----------------|----------------------------------|---------------------------------|-------------------|-------------------|---------|---------|
| **Age** | **N** | **Mean** | **Std. Dev.** | **Min** | **Max** | **p** | **units** |
| Control | 37 | 47.65 | 11.106 | 23 | 74 | .014 | g/L |
| BC | 117 | 53.21 | 12.026 | 27 | 86 | | |
| Total | 154 | 51.88 | 12.015 | 23 | 86 | | |
| Fetuin-A (g/L) | Control | 37 | .35137 | .096804 | .215 | .674 | .116 | g/L |
| BC | 117 | .38076 | .099044 | .224 | .743 | | |
| Total | 154 | .37370 | .099000 | .215 | .743 | | |
| RANKL (pM) | Control | 37 | 382.895 | 326.0093 | 82.9 | 1763.7 | .439 | pM |
| BC | 117 | 447.340 | 470.5775 | 61.1 | 2486.6 | | |
| Total | 154 | 431.856 | 440.0707 | 61.1 | 2486.6 | | |
| Glucose (mmol/L) | Control | 37 | 5.8492 | 0.7293 | 4.2185 | 7.7154 | .324 | mmol/L |
| BC | 117 | 6.2067 | 2.1565 | 3.8854 | 21.7584 | | |
| Total | 154 | 6.1207 | 1.9169 | 3.8854 | 21.7584 | | |
| Urea (mmol/L) | Control | 37 | 23.08 | 9.8388 | 8.1585 | 49.1175 | .247 | mmol/L |
| BC | 117 | 26.1372 | 14.9833 | 6.4935 | 83.4166 | | |
| Total | 154 | 25.4029 | 13.9537 | 6.4935 | 83.4166 | | |
| Creatinine (mmol/L) | Control | 37 | 55.85445 | 11.2394 | 39.7808 | 84.8656 | .279 | mmol/L |
| BC | 117 | 58.354 | 12.5857 | 34.4767 | 101.662 | | |
| Total | 154 | 57.744 | 12.287 | 34.4767 | 101.662 | | |
**Table IB** Laboratory values, cell adhesion markers and age of the patients’ and control group.

|                        | Control (37) | BC (117) | Total (154) |    | mmol/L |    | U/L |    | mmol/L |    | mmol/L |    | mmol/L |
|------------------------|--------------|----------|-------------|----|--------|----|-----|----|--------|----|--------|----|--------|
| **Total cholesterol (mmol/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 5.4354       | 5.3865   | 5.3984      |    | 0.9677 | 1.1391 | 1.0975 | 3.1553 | 8.1469 | 0.814 | 0.3415 | 0.7242 | 2.276 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **HDL-Chol (mmol/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 1.475        | 1.2784   | 1.3255      |    | 0.341  | 0.3279 | 0.3416 | 0.6466 | 2.1208 | 0.002 | 0.3287 | 0.6466 | 2.276 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **LDL-Chol (mmol/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 3.2435       | 3.2323   | 3.3041      |    | 0.7428 | 0.9931 | 0.9374 | 0.6052 | 6.7272 | 0.654 | 0.7283 | 0.9374 | 6.6727 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **AST (U/L)**          |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 21.78        | 20.58    | 20.87       |    | 6.872  | 5.339  | 5.744  | 11     | 54     | 0.268 | 3.788  | 5.339  | 54     |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **ALT (U/L)**          |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 21.24        | 19.02    | 19.55       |    | 15.673 | 8.484  | 10.643 | 5      | 56     | 0.269 | 20.047 | 8.484  | 56     |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **GGT (U/L)**          |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 21.03        | 23.53    | 22.93       |    | 14.544 | 16.180 | 15.793 | 6      | 104    | 0.403 | 17.127 | 16.180 | 104    |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **LDH (U/L)**          |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 178.59       | 180.79   | 180.27      |    | 33.815 | 42.218 | 40.265 | 20     | 263    | 0.773 | 112.64 | 42.218 | 296    |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **ALP (U/L)**          |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 78.08        | 80.79    | 80.15       |    | 23.285 | 24.051 | 23.823 | 40     | 143    | 0.553 | 166.73 | 24.051 | 105    |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **Total protein (g/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 74.435       | 72.614   | 73.054      |    | 6.4162 | 4.5792 | 5.1213 | 60.7   | 107.0  | 0.059 | 52.8    | 4.5792 | 81.6   |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **Albumin (g/L)**      |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 44.089       | 43.708   | 43.799      |    | 2.2737 | 2.8838 | 2.7474 | 35.8   | 52.3   | 0.463 | 25.27  | 2.8838 | 52.3   |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **Calcium (mmol/L)**  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 2.3851       | 2.3582   | 2.3646      |    | 0.0822 | 0.1191 | 0.1117 | 2.2456 | 2.5201 | 0.201 | 0.1563 | 0.1191 | 2.1955 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **Phosphorus (mmol/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 1.0448       | 1.0445   | 1.0446      |    | 0.1542 | 0.1788 | 0.1726 | 0.6161 | 1.5839 | 0.994 | 0.1563 | 0.1788 | 1.5839 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
| **Magnesium (mmol/L)** |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Control                | 0.7791       | 0.7951   | 0.7912      |    | 0.0669 | 0.0718 | 0.0707 | 0.6213 | 0.9298 | 0.232 | 0.1563 | 0.0718 | 0.9545 |
| BC                     |              |          |             |    |        |    |     |    |        |    |        |    |        |
| Total                  |              |          |             |    |        |    |     |    |        |    |        |    |        |
### Table IC: Laboratory values, cell adhesion markers and age of the patients’ and control group.

|                         | Control | BC | Total | Mean          | Standard deviation | Minimum | Maximum | p  |
|-------------------------|---------|----|-------|---------------|--------------------|---------|---------|----|
| **PTH (ng/L)**          |         |    |       | 37  | 58.981    | 24.830 |  22.8  | 121.9 | 0.571 |
|                         | 117     | 62.193 | 31.394 | 11.9 | 213.5    |        |         |      |       |
|                         | 154     | 61.421 | 29.904 | 11.9 | 213.5    |        |         |      |       |
| **Sedimentation (mm/h)**| 37      | 22.84 | 14.299 | 1    | 72       |        |         |      | 0.914 |
|                         | 110     | 23.11 | 12.883 | 4    | 76       |        |         |      |       |
|                         | 147     | 23.04 | 13.204 | 1    | 76       |        |         |      |       |
| **CRP (nmol/L)**        | 37      | 36.7514 | 36.1676 | 4.957 | 148.957 |        |         |      | 0.319 |
|                         | 117     | 47.3524 | 61.0316 | 2.095 | 434.286 |        |         |      |       |
|                         | 154     | 44.7924 | 56.1467 | 2.095 | 434.286 |        |         |      |       |
| **WBC (10^9/L)**        | 37      | 6.7614 | 1.1087 | 4.43 | 9.45     |        |         |      | 0.086 |
|                         | 117     | 7.3162 | 1.8484 | 3.63 | 13.12    |        |         |      |       |
|                         | 154     | 7.1829 | 1.7135 | 3.63 | 13.12    |        |         |      |       |
| **Neutrophil (10^9/L)** | 37      | 3.9443 | 1.0057 | 1.90 | 5.82     |        |         |      | 0.014 |
|                         | 117     | 4.6630 | 1.6626 | 2.00 | 10.53    |        |         |      |       |
|                         | 154     | 4.4903 | 1.5584 | 1.90 | 10.53    |        |         |      |       |
| **Lymphocyte (10^9/L)** | 37      | 2.1519 | .5033  | 1.25 | 3.41     |        |         |      | 0.328 |
|                         | 117     | 2.0429 | .6129  | 1.05 | 4.41     |        |         |      |       |
|                         | 154     | 2.0691 | .5887  | 1.05 | 4.41     |        |         |      |       |
| **PLT (10^9/L)**        | 37      | 264.32 | 49.311 | 160  | 356      |        |         |      | 0.895 |
|                         | 117     | 265.88 | 65.808 | 123  | 524      |        |         |      |       |
|                         | 154     | 265.51 | 62.097 | 123  | 524      |        |         |      |       |
| **MPV (Fl)**            | 37      | 10.408 | .8958  | 8.7  | 13.8     |        |         |      | 0.708 |
|                         | 115     | 10.481 | 1.0618 | 8.4  | 14.2     |        |         |      |       |
|                         | 152     | 10.463 | 1.0215 | 8.4  | 14.2     |        |         |      |       |
| **Ca 15.3 (U/mL)**      | 5       | 15.940 | 5.6145 | 10.7 | 24.1     |        |         |      | 0.733 |
|                         | 104     | 22.933 | 45.8327 | 3.0  | 392.6    |        |         |      |       |
|                         | 109     | 22.670 | 44.7968 | 3.0  | 392.6    |        |         |      |       |
| **CEA (µg/L)**          | 4       | 3.117  | 4.6920 | .3   | 10.1     |        |         |      | 0.854 |
|                         | 103     | 3.864  | 8.0352 | .3   | 56.4     |        |         |      |       |
|                         | 107     | 3.836  | 7.9228 | .3   | 56.4     |        |         |      |       |

### Table II: Serum fetuin-A and RANKL levels in the patients’ and the control group.

|                         | N  | Mean       | Standard deviation | Minimum | Maximum | p  |
|-------------------------|----|------------|--------------------|---------|---------|----|
| **Fetuin-A (g/L)**     |    | 0.35137    | 0.096804           | 0.215   | 0.674   | 0.116 |
|                         | Control 37 | 0.35137       | 0.096804      | 0.215   | 0.674   | 0.116 |
|                         | BC 117  | 0.38076 | 0.099044        | 0.224   | 0.743   | 0.116 |
|                         | Total 154 | 0.37370       | 0.099000      | 0.215   | 0.743   | 0.116 |
| **RANKL (pM)**         |    | 382.895    | 326.0093           | 82.9    | 1763.7  | 0.439 |
|                         | Control 37 | 382.895       | 326.0093      | 82.9    | 1763.7  | 0.439 |
|                         | BC 117  | 447.340 | 470.5775        | 61.1    | 2486.6  | 0.439 |
|                         | Total 154 | 431.856       | 440.0707      | 61.1    | 2486.6  | 0.439 |
| Variables | Fetuin Median (Range) | n | P   |
|-----------|----------------------|---|-----|
| **Ki-67** |                      |   |     |
| ≤ 30      | 0.345 (0.235–0.522)  | 43 | 0.09|
| > 30      | 0.389 (0.224–0.743)  | 71 |     |
| **ER**    |                      |   |     |
| Negative  | 0.398 (0.264–0.692)  | 25 | 0.38|
| Positive  | 0.375 (0.224–0.743)  | 92 |     |
| **PR**    |                      |   |     |
| Negative  | 0.399 (0.247–0.692)  | 30 | 0.38|
| Positive  | 0.373 (0.224–0.743)  | 87 |     |
| **HER2**  |                      |   |     |
| Negative  | 0.362 (0.224–0.743)  | 90 | 0.71|
| Positive  | 0.369 (0.249–0.692)  | 24 |     |
| **Classification** |          |   |     |
| Luminal   | 0.358 (0.224–0.743)  | 92 | 0.39|
| Others    | 0.392 (0.264–0.692)  | 25 |     |
| **Classification** |          |   |     |
| Triple positive | 0.345 (0.249–0.497) | 13 | 0.40|
| Others    | 0.368 (0.224–0.743)  | 100|     |
| **Classification** |          |   |     |
| Triple negative | 0.336 (0.267–0.591) | 11 | 0.63|
| Others    | 0.362 (0.224–0.743)  | 106|     |
| **Classification** |          |   |     |
| HER2 enriched | 0.369 (0.249–0.692) | 24 | 0.71|
| Others    | 0.362 (0.224–0.743)  | 90 |     |
| **Histological subgroups** |      |   |     |
| Favorable groups* | 0.355 (0.242–0.526) | 24 | 0.51|
| Unfavorable groups** | 0.361 (0.224–0.743) | 93 |     |
| **Stage** |                      |   |     |
| Early stage | 0.360 (0.224–0.650)  | 60 | 0.55|
| Local advanced stage | 0.370 (0.235–0.743) | 54 |     |

Significant p values (less than 0.05) are highlighted in bold. * Apocrine, papillary, mucinous, metaplastic, neuroendocrine, tubular. ** Invasive ductal carcinoma, invasive lobular carcinoma, mixed type.
However, patients with favorable histopathologies such as tubular, apocrine, papillary and mucinous subtypes (n=24) had lower RANKL values and it was found to be significant (p=0.04) (Table IV).

### Table IV Comparisons of RANKL levels according to various clinical/laboratory parameters.

| Variables          | RANKL Median (Range) | n  | P     |
|--------------------|----------------------|----|-------|
| Ki-67              |                      |    |       |
| ≤ 30               | 203.500 (61.100–1577.500) | 43 | 0.16  |
| > 30               | 273.300 (101.800–2486.600) | 71 |       |
| ER                 |                      |    |       |
| Negative           | 490.888 (101.800–2486.600) | 25 | 0.54  |
| Positive           | 434.228 (61.100–1890.500) | 92 |       |
| PR                 |                      |    |       |
| Negative           | 441.337 (101.800–2486.600) | 30 | 0.66  |
| Positive           | 448.059 (61.100–1890.500) | 87 |       |
| HER2               |                      |    |       |
| Negative           | 240.950 (61.100–1973.600) | 90 | 0.61  |
| Positive           | 276.600 (101.800–2486.600) | 24 |       |
| Classification     |                      |    |       |
| Luminal            | 250.250 (61.100–1890.500) | 92 | 0.54  |
| Others             | 294.500 (101.800–2486.600) | 25 |       |
| Classification     |                      |    |       |
| Triple positive    | 314.600 (121.700–956.700) | 13100 | 0.22 |
| Others             | 239.250 (61.100–2486.600) |       |       |
| Classification     |                      |    |       |
| Triple negative    | 372.900 (113.200–1973.600) | 11  | 0.50  |
| Others             | 250.250 (61.100–2486.600) | 106 |       |
| Classification     |                      |    |       |
| HER2 enriched      | 276.600 (101.800–2486.600) | 24  | 0.61  |
| Others             | 240.950 (61.100–1973.600) | 90  |       |
| Histological subgroups |                  |    |       |
| Favorable groups*  | 184.500 (80.500–638.300) | 24  | 0.04  |
| Unfavorable groups**| 269.100 (61.100–2486.600) | 93  |       |
| Stage              |                      |    |       |
| I and II           | 254.600 (61.100–1973.600) | 60  | 0.62  |
| III                | 261.450 (101.800–2486.600) | 54  |       |

Significant p values (less than 0.05) are highlighted in bold. * Apocrine, papillary, mucinous, metaplastic, neuroendocrine, tubular. ** Invasive ductal carcinoma, invasive lobular carcinoma, mixed type.

Discussion

For BC, different serum markers were evaluated up to now and some of them are found to be prognostic, some are diagnostic and/or predictive (11). Fetuin-A was originally discovered to be an inhibitor of vascular calcification. Furthermore it is demonstrated that it plays an important role in free fatty acid induced insulin resistance in the liver (12, 13). Increased fetuin-A had been also been linked to increased occurrence of non-alcoholic fatty liver disease and cardiovascular events, believed to be due to its proinflammatory effects. Thus, in contrast it has some anti-inflammatory properties. It is a negative acute-phase reactant in sepsis, promotes wound healing, and is neuroprotective (14). The potential role of fetuin-A in tumor progression stemmed from earlier...
studies that suggested that it was the cell attachment factor in serum (15). In head and neck squamous cell carcinoma (HNSCC), there was an increased expression of a higher molecular weight fetuin-A (16). There is ectopic synthesis of fetuin-A by divergent cancer cell lines (17). Patients with high ectopic expression of fetuin-A in lung cancer and gastric cancer tend to have lower survival (18, 19). Fetuin-A is an important marker in the tumor microenvironment, for cancer stem cells and for matrix metalloproteinases (20, 21).

Fetuin-A is found to be a serum biomarker for colorectal cancer patients (22). It is found to be increased in malignant pleural effusion of lung cancer patients (23). Furthermore, in a study done in Mexican BC population, the presence of serum autoantibodies against fetuin-A protein found to be useful as serum biomarkers for early-stage BC screening (24). Fetuin-A seems to be a serum chemo-attractant protein that also promotes invasion of BC tumor cells (25).

In our study, we found no association of serum fetuin-A levels for BC patients with other laboratory parameters and with control subjects. This may be a result of exploring only early staged patients. In the Mexican BC population (24), there were 36 patients (30 with ductal and 6 lobular carcinoma) but they used an immune proteomic approach, combining two-dimensional (2D) electrophoresis, Western blot, and matrix-associated laser desorption/ionization-mass spectrometry (MALDI-MS) methods. We used one method which was the ELISA method for the detection of fetuin-A levels. We performed this study in 117 patients with invasive ductal, lobular, tubular, papillary and mucinous cancers. However, in our study, there was a trend to be lower for fetuin-A levels for more favorable histologic subtypes. It is very well known that BC has different histologic subtypes as well as its diffrerenet molecular characteristics. In the Mexican study (24), there is no data about the tumors’ molecular characteristics such as ER, PR and cerbb2 status. In our study, there are older patients than the other study. Taken together all these discrepancies, in our study which was done in Turkish BC patients, fetuin-A levels did not differ.

RANKL/RANK system is seen as a downstream mediator of progesterone-driven mammary epithelial cells proliferation, BC initiation and progression. Expression of RANKL, RANK has been detected in BC cell lines and in human primary BCs. To date, dysregulation of RANKL/RANK at the skeletal level has been widely documented in the context of metastatic bone disease (26). The interference with the RANK/RANKL system could therefore serve as a potential target for prevention and treatment of BC (27, 28). For metastatic BC patients, specifically for patients with bone metastasis, RANKL levels were found to be diagnostic and somewhat predictive for therapy (27). In our study; for early staged BC patients, RANKL levels were found to be lower in the favorable histological subtypes of BC. This is a new topic for early stage BC patients.

Conclusion
We found a correlation between serum RANKL levels and favorable histological subtypes of BC. However, there was no significance between fetuin-A levels and other clinical/laboratory parameters. Further and detailed studies can enlighten the role of these cell adhesion markers better for BC patients.

Conflict of interest statement
All the authors declare that they have no conflict of interest in this work.

References
1. Coleman MP, Quaresma M, Berrino F, Lutz JM, Angelis RD, Capocaccia R, et al. Cancer survival in five continents: a worldwide population-based study (CONCORD). Lancet Oncol 2008; 9(8): 730–56.
2. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci 2017; 13(11): 1387–97.
3. Migowski A. Early detection of breast cancer and the interpretation of results of survival studies. Cienc Saude Coletiva 2015; 20: 1309.
4. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. J Clin Oncol 1996; 14: 2843–77.
5. Yi JK, Chang JW, Han W, Lee JW, Ko E, Kim DH, et al. Cancer Epidemiol Biomarkers Prev 2009: 18; 1357–64.
6. Dabrowska AM, Tarach JS, Wojtysiak-Duma B, Duma D. Fetuin-A (AHSG) and its usefulness in clinical practice. Review of the literature. Biomed Pap Med Fac Univ Palacky 2015: 159; 352–9.
7. Ochieng J, Nangami G, Sakwe A, Moye C, Alvarez J, Whalen D, et al. Impact of Fetuin-A (AHSG) on Tumor Progression and Type 2 Diabetes. Int J Mol Sci 2018; 19: 2211.
8. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Kettele M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation 2006; 113: 1760–7.
9. Fernández-Grijalva AL, Aguilar-Lemarroy A, Jave-Suarez LF, Gutiérrez-Ortega A, Godinez-Melgoza PA, Herrera-Rodríguez SE, et al. Alpha 2HS-glycoprotein, a tumor-associated antigen (TAA) detected in Mexican patients with early-stage breast cancer. J Proteomics 2015; 112: 301–12.

10. Ono T, Hayashi M, Sasaki F, Nakashima T. RANKL biology: bone metabolism, the immune system, and beyond. Inflamm Regen 2020; 40: 2.

11. Aglan SA, Elsammak M, Elsammak O, El-Bakoury EA, Elsheredy HG, Ahmed YS, Sultan MH, Awad AM. Evaluation of serum Nestin and HOXAIR rs12826786 C>T polymorphism as screening tools for breast cancer in Egyptian women. J Med Biochem 2021; 40 (1): 17–25.

12. Trepanowski JF, J Mey J, Varady KA. Fetuin-A: A Novel Link Between Obesity and Related Complications. Int J Obes (Lond) 2015; 39(5): 734–41.

13. Ochieng J, Korolkova OY, Li G, Jin R, Chen Z, Matusik RJ, et al. Fetuin-A Promotes 3-Dimensional Growth in LNCaP Prostate Cancer Cells by Sequestering Extracellular Vesicles to Their Surfaces to Act as Signaling Platforms. Int J Mol Sci 2022; 23(7): 4031.

14. Mukhopadhyay S, Mondal SA, Kumar M, Dutta D. Proinflammatory and antiinflammatory attributes of fetuin-a: a novel hepatokine modulating cardiovascular and glycemic outcomes in metabolic syndrome. Endocr Pract 2014; 20(12): 1345–51.

15. Fisher HW, Puck TT, Sato G. Molecular growth requirements of single mammalian cells: The action of fetuin in promoting cell attachment to glass. Proc Natl Acad Sci 1958; 44: 4–10.

16. Arnaud P, Mirbel L, Emerson DL. α2-HS glycoprotein. Methods Enzymol 1988; 163: 431–41.

17. Mintz PJ, Rietz AC, Cardo-Vila M, Ozawa MG, Dondossola E, Do KA, et al. Discovery and horizontal follow-up of an autoantibody signature in human prostate cancer using multidimensional protein identification technology. J Proteome Res 2011; 10: 4671–82.

18. Gyorffy B, Surowiak P, Budczies J, Lanczky A. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. PLoS ONE 2013; 8: e82241.

19. Szasz AM, Lanczky A, Nagy A, Forster S, Hark K, Green JE, et al. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. Oncotarget 2016; 7: 49522–33.

20. Ochieng J, Nangami G, Sakwe A, Moyo C, Alvarez J, Whalen D, et al. Impact of Fetuin-A (AHSG) on Tumor Progression and Type 2 Diabetes. Int J Mol Sci 2018; 19(8): E2211.

21. Dong Y, Ding D, Gu J, Chen M, Li S. Alpha-2 Heremans Schmid Glycoprotein (AHSG) promotes the proliferation of bladder cancer cells by regulating the TGF-β signalling pathway. Bioengineered 2022; 13(6): 14282–98.

22. Fan NJ, Kang R, Ge XY, Li M, Liu Y, Chen HM, et al. Identification α2-HS glycoprotein precursor and tubulin β-chain as serology diagnosis biomarker of colorectal cancer. Diagn Pathol 2014; 9: 53.

23. Yu CJ, Wang CL, Wang CI, Chen CD, Dan YM, Wu CC, et al. Comprehensive proteome analysis of malignant pleural effusion for lung cancer biomarker discovery by using multidimensional protein identification technology. J Proteome Res 2011; 10: 4671–82.

24. Fernández-Grijalva AL, Aguilar-Lemarroy A, Jave-Suarez LF, Gutiérrez-Ortega A, Godinez-Melgoza PA, Herrera-Rodríguez SE, et al. Alpha 2HS-glycoprotein, a tumor-associated antigen (TAA) detected in Mexican patients with early-stage breast cancer. J Proteomics 2015; 112: 301–12.

25. Nangami GN, Watson K, Parker-Johnson K, Okereke KO, Sakwe A, Thompson P, et al. Fetuin-A (α2HS-glycoprotein) is a serum chemo-attractant that also promotes invasion of tumor cells through Matrigel. Biochem Biophys Res Commun 2013; 438(4): 660–5.

26. Infante M, Fabi A, Cognetti F, Gorini S, Caprio M, Fabbri A. RANKL/RANKL pathway in breast cancer. Maturitas 2016; 86: 10–6.

27. Kiesel L, Kohl A. Role of the RANK/RANKL pathway in breast cancer. Maturitas 2016; 86: 10–6.

28. Mularczyk M, Bourebaba Y, Kowalczyk A, Marycz K, Bourebaba L. Probiotics-rich emulsion improves insulin signalling in Palmitate/Oleate-challenged human hepatocarcinoma cells through the modulation of Fetuin-A/TLR4-JNK-NF-κB pathway. Biomed Pharmacother 2021; 139: 111560.

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