

Study of the Potential Role of Serum Neutrophil Gelatinase-Associated Lipocalin (NGAL) Levels in the Diagnosis and Prognosis of Breast Cancer in Egyptian Females.

ABSTRACT:

Background: breast cancer (BC) is the most common cause of malignancy in females all over the. Continuous scientific research for discovery of new markers helping is a corner stone for early disease detection and proper management. Aim of the study: this study aimed to evaluate the role of Neutrophil gelatinase-associated lipocalin (NGAL) as prognostic markers for breast cancer in Egyptian females population. Patients and methods: 120 BC patients and 30 health controls are the subjects of the study, serum NGAL levels were investigated and correlated with the clinicopathologic characteristics of the BC patients. Results: our study showed that NGAL is significantly differing between healthy controls and BC patients, and it revealed gradual increase with disease severity. Conclusion: our findings suggested that NGAL could be diagnostic marker for early cases detection, and is shown to be associated with breast cancer prognosis, supporting its role as prognostic biomarker.

KEY WORDS:

Breast cancer, NGAL, diagnosis, prognosis.

INTRODUCTION:

Recently, variable tumor markers levels in the serum have been approved as a diagnostic utility the tumor activity detection. Tumor markers are considered as minimal invasive low cost indicators for follow up of the disease, its prognosis, and decision of treatment planning. Attention should be paid to the test benefits and limitations for achieving the best interpretation of results. The little diagnosis sensitivity of breast cancer at its early stage elicits questionable role of tumor markers [1].

For diagnostic, prognostic and predictive issues, the use of recently discovered BC biomarkers has been widely applied, such as Neutrophil gelatinase-associated lipocalin (NGAL).

NGAL (lipocalin 2) is a protein that was described in human neutrophil, evolved in bone marrow during the maturation of granulocyte [2]. Recently, it has been assessed in several physiological and pathological conditions as acute renal injury and variable types of human cancer as GIT, liver, lung and thyroid cancers. Few studies investigated its relation to BC [3].

Considering these facts, this study aimed to evaluate the role of NAGL as a biomarker in Egyptian females with BC and to evaluate the optimal cutoff values determining disease prognosis.

PATIENTS AND METHODS

Patients

This is study was performed in Zagazig university hospital, Zagazig, Egypt, from January 2019 to January 2020. This study was approved by Zagazig university ethical committee.

The study groups:

- Group 1 (control): 30 healthy female subjects, age and BMI matching to the patient group.
- Group 2 (patients): 120 breast cancer patients that were, with recently pathologically proved breast cancer, of different disease stages (I, II, III and IV).

Methods

Study subjects serum samples, that were -20 were assayed for serum CEA and CA 15-3 levels according to the manufacturer's instructions (Biovendor Inc, Brno, Czech Republic). Patients data were achieved from the medical records.

Statistical analysis

MedCal_version 17.9.7 software was used for the analysis of the (MedCalc Software bib, Ostend, Belgium). Quantitative data were expressed as mean and standard deviation, while qualitative data were expressed as frequency and percentage. Nottingham prognostic index (NPI) values of the patients were calculated and interpreted [4]. Pearson tests were carried out for correlation of the serum marker with the clinic-pathological data of the patients. ROC curve analysis was done to estimate cutoff point for differentiation between healthy subjects and breast cancer patients.

RESULTS:

Age and BMI (table 1)

Table 1: Mean \pm SD of women age and BMI among studied groups.

| Parameter | Control Group | Breast Cancer Group | | | |
|-------------|--------------------|-----------------------------|------------------------------|-------------------------------|------------------------------|
| | (Group I) n =30 | (Group IIa) Stage I n=30 | (Group IIb) Stage II n=30 | (Group IIc) Stage III n=30 | (Group IId) Stage IV n=30 |
| Age (years) | 48.3 + 9.7 | 50.1+ 12.4 | 49.3+9.9 | 48.9 +10.2 | 50.3+11.1 |
| P | | >0.05 | >0.05 | >0.05 | >0.05 |
| BMI (kg/m2) | 31.5 + 6.1 | 30.4+7.6 | 30.8+6.6 | 31.3 + 6.4 | 29.2+ 11.4 |
| P | | >0.05 | >0.05 | >0.05 | >0.05 |

Histopathological type and tumor grade

The most prevalent histopathological type of BC (99 cases; 82.5%) was invasive ductal carcinoma (IDC). 9 cases (7.5 %) were invasive lobular carcinoma (ILC), 4 cases (3.34%) were mucinous carcinoma; 3 cases (2.5%) were medullary carcinoma, 3 cases (2.5%) were malignant phyllodes tumor, and 2 cases (1.66%) were poorly differentiated carcinoma. Regarding to the tumor grade, 12 patients were of grade I (10%), 79 patients were of grade II (65.8%) and 29 patients were of grade III (24.2%).

NPI (table 2)

Table 2: The breast cancer patients prognosis according to the NPI values.

| Patients prognosis according to NPI | n | Percentage |
|-------------------------------------|-----|------------|
| - Excellent prognosis. | 2 | 1.67% |
| - Good prognosis. | 19 | 15.83 % |
| - Moderate prognosis. | 75 | 62.5% |
| - Poor prognosis. | 24 | 20% |
| * Total | 120 | 100% |

Serum NGAL levels

Step rise increase in the serum omentin-1 levels as the patients stage progress is evident in table 3, with high significant difference ($p < 0.01$) could be noted between control subjects and breast cancer group and between the stage III and stage IV patients.

Table 3: the mean values of serum NGAL in the groups of the study.

| Mean±SD | C | Stage I | Stage II | Stage III | Stage IV |
|--------------|------------|----------|----------|------------|-------------|
| NGAL (ng/mL) | 117.4±15.7 | 332±60.6 | 325±43.6 | 335.3±62.6 | 410.6±112.7 |
| p | | <0.01 | >0.05 | >0.05 | <0.01 |

Pearson correlation testing of the serum NGAL with the clinicopathological characteristics of the patients is shown in table 4 and figures 1,2, 3 & 4 which revealed that omentin-1 levels were showing non-significant correlation with the age, while significant correlation was noted with the patient NPI values and high significant correlation was noted with tumor size, nodes status and histopathological grade.

Table 4: Correlations between the serum NGAL levels and different clinicopathological parameters in the breast cancer patients.

| Variables | NGAL | |
|-------------|------|-------|
| | r | p |
| Age | 0.1 | >0.05 |
| Tumor size | 0.42 | <0.01 |
| Node status | 0.45 | <0.01 |
| Tumor grade | 0.35 | <0.01 |
| NPI values | 0.4 | <0.05 |

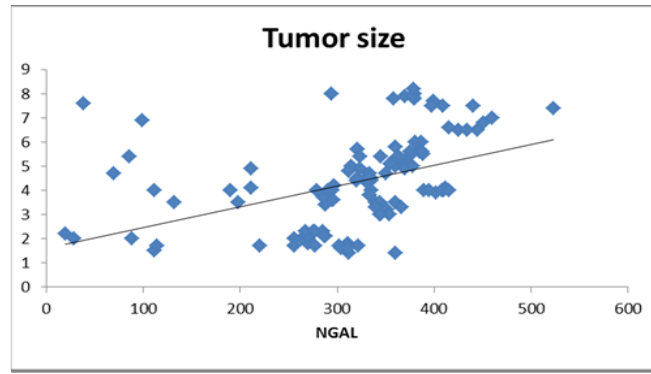


Figure 1: Correlation between Serum NGAL level and tumor size.

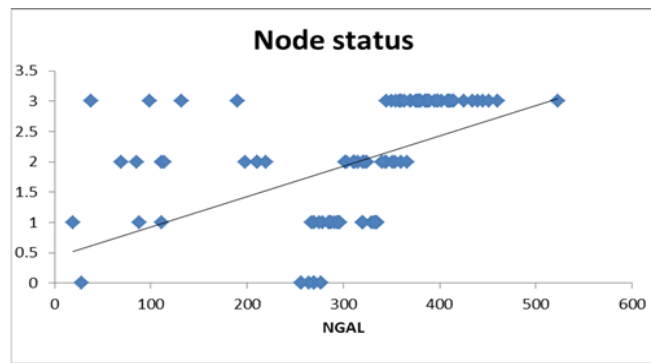


Figure 2: Correlation between Serum NGAL level and node status.

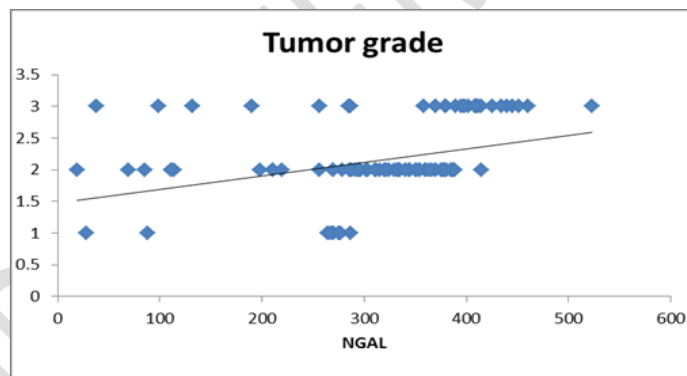


Figure 3: Correlation between Serum NGAL level and tumor grade.

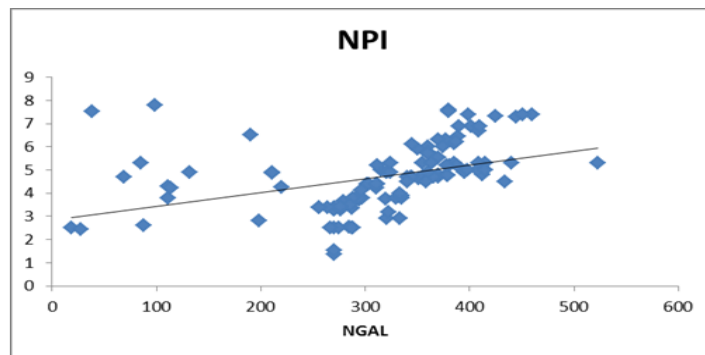


Figure 4: Correlation between Serum NGAL level and NPI.

In this study, the serum NGAL cutoff value to differentiate healthy controls from breast cancer patients was 277.9 ng/mL, the calculated sensitivity and specificity were 83% and 100% respectively.

DISCUSSION:

Breast cancer is highly heterogeneous in terms of its etiology and pathological characteristics [5], some cases are showing slow growth with excellent prognosis, whereas other cases are taking a highly aggressive clinical course. Much effort is made on the scientific, economical, and organizational levels for better understanding of the eliciting factors, the molecular motivations for progression and the best effective, least hazardous intervention lines [6]. Serum levels of NGAL in breast cancer patients are recently considered as predictive and prognostic indicators for the disease [7, 8]. This study is a case control study and included 150 subjects, of which 30 are healthy controls and 120 are BC patients who were admitted to oncology department of Zagazig University Hospital, Egypt. This study aimed to evaluate the diagnostic and prognostic role of NAGL biomarkers in Egyptian female patients with BC.

The glycoprotein NGAL/ Lipocalin 2 has been originally proposed for early pick up of acute renal injury states [9]. However, some of its use limitations have been reported [10].

NGAL role in oncological process has been growingly evidenced. Interestingly, NGAL shows both up and down regulation depending on the type of the malignancy [11]. Scientists have begun focusing on NGAL biomarker assessment as a novel simple non-complicated, easily accessible non-invasive method for cancer diagnosis and prognosis, owing to its availability of being detected in both urine and blood [11].

NGAL is proved to be related to the regulation of epithelial mesenchymal transition (EMT), which is known to be incorporated in BC progression [12, 13]. Some studies have proposed that NGAL leads to apoptosis and suppression of the proliferation process [14, 15]. Meanwhile, others have concluded that the NGAL stimulate tumor proliferation and invasion [16, 17]. In this study, the NGAL levels in serum were investigated. The levels were differing significantly between control subjects and BC patients and between stage III and stage IV BC patients. The results of this study revealed that serum NGAL levels were showing no significant correlation with patients' age, while showing and significant/high significant correlation with tumor size, node status, tumor grade and accordingly the NPI status. In this study, the serum NGAL cutoff value to differentiate healthy controls from breast cancer patients was 277.9 ng/mL, the calculated sensitivity and specificity were 83% and 100% respectively.

One previous systematic review revealed the overall NGAL diagnostic and prognostic value in breast cancer. As with our study, in that study, a relation was proposed between the higher NGAL levels and BC poor prognosis [18]. Many other studies also showed similar results [19, 20, 21].

A previous study included females with pathologically proved non-palpable breast carcinomas and 30 healthy females acting as controls. Notably, the NGAL showed significantly higher levels in BC patients when comparing with control subjects [19]. Another study reported positive correlation of NGAL with tumor grade and N stage [22]. Li et al study has concluded the association between NGAL levels and BC patients poor prognosis [23].

CONCLUSION

Our findings suggested that NGAL could be diagnostic marker for early cases detection, and it revealed association with the BC prognosis, as they are shown to have step rise increase as the disease stage get worse, ensuring its value as prognostic biomarkers.

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