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Evaluation of tissue 5'-Nucleotidase as tumor marker in breast and brain tumors using receiver operating characteristic curve

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Abstract

The pursuit of the ideal tumor marker has generated many tests for using in the diagnosis and management of cancer. Several of which are now widely available. The objective of the current study was to evaluate 5'-NT activity as a tumor marker for breast & brain cancer. Receiver Operating Characteristic curve (ROC) & other statistical parameters were used to investigate this possibility. The results throughout this study supported the motion that 5'-NT specific activity can be considered as an acceptable marker for breast and brain cancer when the comparison was made with benign group. For the breast cancer, the true-positive rate (sensitivity) of tissue extract 5'-NT specific activity was 85.7% while the false-positive rate (1-specificity) was 11.11%, meanwhile both the predictive value of positive test (pv^+) and the negative predictive value (pv) was 88.9% for breast cancer women and benign group. The true positive rate (sensitivity) of tissue extract's 5'-NT specific activity in the case of brain cancer was (80.4%) with a false-positive rate (1-specificity) equal to (13.6%). The predictive value of positive test (pv^+) when compared malignant brain group with benign group was (88.09%), while the negative predictive values (pv) was found to be (78.04%). The area under ROC curve for tissue extract 5'-NT specific activity were 0.955 (95% CI 0.907–1.0) and 0.922 (95% CI 0.867–0.978) for breast and brain tumor respectively.

Keywords: 5'-Nucleotidase; Receiver Operating Characteristic (ROC) curve; breast cancer; brain cancer; tumor marker

Introduction

A tumor marker is a substance, which is expressed by a neoplasm and can be monitored in diagnosis and management of the tumor, such markers can be intracellular, expressed on cell surfaces, or extracellular, secreted into body fluid [1]. However, the term tumor marker is usually used in more restricted sense to indicate a substance that is synthesized by a tumor in abnormal amounts and, secreted into the circulation where it can be measured [1].

Many cancers are associated with the abnormal production of enzymes, proteins, and hormones, which can be measured in plasma or serum. The potential uses of tumor markers are screening the disease, diagnose a tumor, determine the prognosis, monitor response to treatment and identify the recurrence of tumor [2].

For a measurement of a tumor marker to be clinically useful, the result should clearly separate those patients with, from those without a tumor. Generally, the statistical parameters that define the effectiveness of a tumor marker are its sensitivity, specificity, positive and negative

predictive values, receiver operating characteristics (ROC curve), area under curve (UAC) with confidence intervals (CI) and a cut-off value that effectively distinguishes patients (cases) from healthy individuals (controls) [3].

It was reported that the changes in some tumor markers are sensitive and specific enough to be used as a target in clinical trials, since the sensitivity & specificity levels should be raised in the cases where the tumor is present [4].

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The sensitivity means percentage of the positive results among patients with the disease [2].

$$\text{Sensitivity} = \frac{\text{True positive (TP)}}{\text{True positive (TP)} + \text{False negative (FN)}} \times 100$$

TP = the number of the true positive results among patients with cancer.

FN = the number of false negative results among patients with cancer.

While the specificity means percentage of negative results among people who do not have the disease [2].

$$\text{Specificity} = \frac{\text{True negative (TN)}}{\text{True negative (TN)} + \text{False positive (FP)}} \times 100$$

TN = the number of true negative results among subjects who have no cancer.

FP = the number of false positive results among subjects who have no cancer.

Positive predictive value (PV+): refers to percent of patients with positive test results who are diseased:

$$PV^+ = \frac{TP}{TP + FP} \times 100$$

Negative predictive value (PV-): refers to percent of patients with negative test results who are non-diseased:

$$PV^- = \frac{TN}{TN + FN} \times 100$$

Alteration of 5'-NT levels in a considerable number of diseases had been recognized [5]. During the processes of development and growth, 5'-NT seems to be related in some way to proliferation and contact formation [6]. It is present in many developing tissues or migratory cells; leukocytes, granulocytes, lymphocytes [7-9], maturing cartilage [10], and in numerous tumors like; human astrocytomas [11], lymphatic cells [12], colon [13], bladder [14] and laryngeal tissues [15]. In our previous study, it was reported that a significant alterations in 5'-NT activity in sera and tissues of women with cervix and uterus cancer [16].

The aim of the current work was to check the possibility of using tissue 5'-NT as a biomarker for tumors of human breast and brain tissues.

Materials and methods

Patients

All patients were admitted for surgical operations to either Medical City, Al- Yarmok Hospital, Al-Jadyria private hospital or Al-Karada private hospital. Tissue samples of different types and stages of benign (n=27) and malignant (n=36) of breast tumors, and benign (n=42) and malignant (n=41) brain tumors were used as samples for this work. Any patient known to have other disease (e.g. hypertension, diabetes mellitus), or any infectious diseases were excluded. The diagnosis is confirmed by cytological and histopathological examinations, which were carried out in the laboratories of the above mentioned hospitals.

Tissue extract's preparation

The tissues were washed with cooled saline solution, minced and homogenized in three volumes of veronal buffer (pH=7.73) containing Triton X-100 (1%) using a hand homogenizer for 5min. The homogenate then was sonicated at 10 amplitude for 1.5 min. at intervals of 15 sec. After centrifugation of the homogenate at 800 xg for 30 min., the supernatant was collected and kept at -20°C. All these steps were carried out at 4°C. This homogenate was used for determination of the enzyme activity & the protein.

Determination of protein concentration

Total protein concentration in the tissue extract was determined using modified Lowry's method [17], where bovine serum albumin was used as standard protein.

Determination of 5'-Nucleotidase activity

5'-NT activity was determined using Wood & Williams modification [18], which depends on measurement of the phosphate liberation, from the substrate (AMP), as a result of 5'-NT activity. The specific activity is expressed as units of enzyme activity per milligram of total tissue protein.

Statistical analysis

Data are expressed as mean \pm SD and analyzed for statistical significance by using paired student t-test. Differences between mean values were considered significant at p<0.05. ROC curves were constructed by using IBM SPSS program version 19. All other statistical parameters (sensitivity, specificity, positive and negative predictive value and cut-off values) were calculated traditionally using previously demonstrated formula.

Results

The results presented in table 1 shows the significant changes in activity and the specific activity of 5'-nucleotidase in malignant and benign tissues homogenate of breast and brain patients.

Table 1 Tissue extracted 5'-NT activity and specific activity in total benign and malignant human breast and brain tumors.

Samples Size (n)	Activity (U/L) Mean \pm S.D	Specific activity (U/mg) Mean \pm S.D
Benign n=42 (brain)	41.293 \pm 19.4122*	0.0142 \pm 0.0072*
Malignant n=41 (brain)	121.729 \pm 39.0326	0.0389 \pm 0.0148
Benign n=27 (breast)	75.54 \pm 37.6*	0.0406 \pm 0.0198*
Malignant n=36 (breast)	56.21 \pm 35.92	0.0154 \pm 0.0119

*significant at p<0.05 compared to benign group

From figures 1 & 2, it could be predicted that the cut-off values were (0.028 U/mg) and (0.026 U/mg) for 5'-NT specific activity in brain and breast patients respectively.

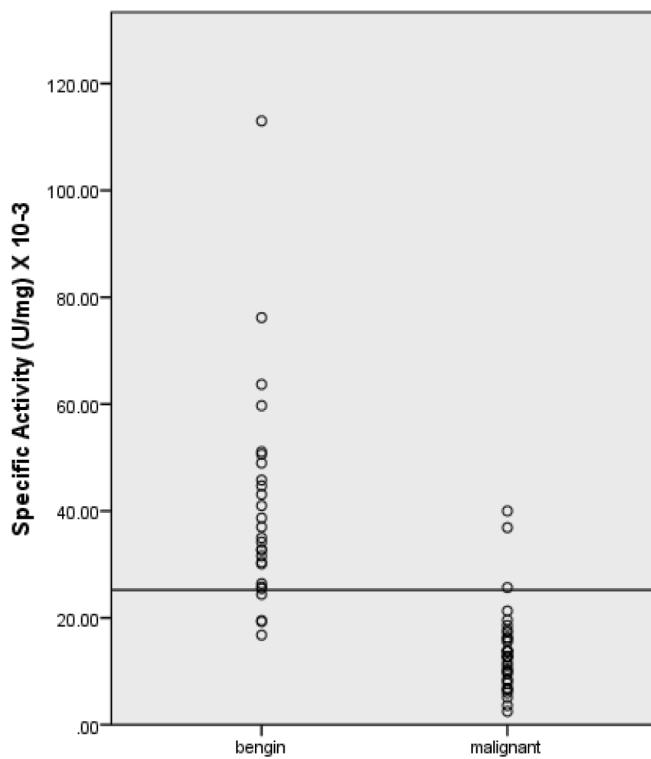


Figure 1 Distribution figure of 5'-NT specific activity for benign & malignant breast tumors.

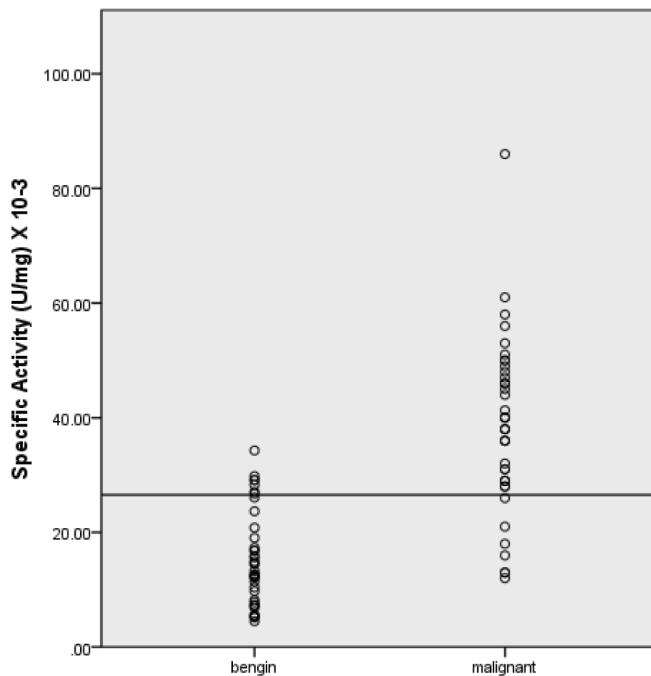


Figure 2 Distribution figure of 5'-NT specific activity for benign & malignant brain tumors.

The true positive rate (sensitivity) of tissue extract of 5'-NT activity in brain and breast were (80.4%) and (85.7%) respectively, and the false-positive rate (1-specificity) were (13.6%) and (11.11%) for brain and breast patients respectively.

Throughout the present study the predictive value of positive test (pv^+) when compared malignant brain and

breast group with benign group were for both (88.09%), and the negative predictive value (pv) were found to be (78.04%) and (88.9%) respectively. The (ROC) curve for 5'-NT specific activity is illustrated for brain cases in figure 3 and for breast once in figure 4. The area under ROC curve for tissue extract's 5'-NT specific activity in breast and brain tumors were found to be 0.955 (95% CI 0.907 – 1.0) and 0.922(95% CI 0.867-0.978) respectively.

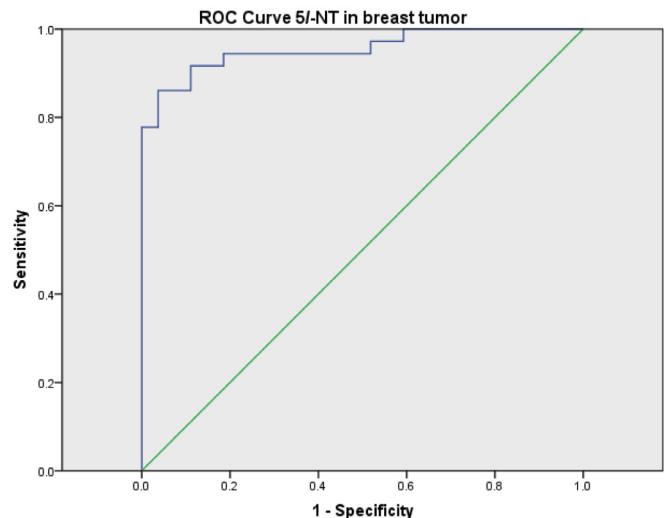


Figure 3 ROC curve of 5'-NT on outcomes of 63 patients (benign & malignant breast tumors). 5'-NT specific activity (AUC=0.955; P<0.05).

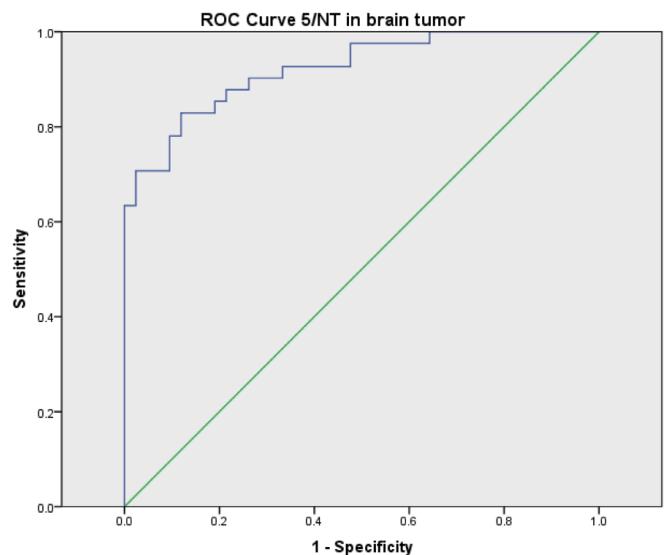


Figure 4 ROC curve of 5'-NT on outcomes of 83 patients ((benign & malignant brain tumors). 5' -NT specific activity (AUC=0.922; P<0.05).

Discussion

5'-NT is considered as a marker of lymphocyte differentiation, since immature B and T cells express less activity than mature cells [19]. However, previously Moss & Rosalk suggested using elevated 5'-NT activity as a marker of liver malignancy [20]. On the other hand, Schoen & Kreutzberg mentioned that 5'-NT activity may serve as a potential specific indicator of plastic synapses or newly formed terminals in the human brain [21]. Furthermore, Cappellari et al. suggested that ecto-5'-NT, was involved

in the glioma cell adhesion and tumor cell-extracellular matrix interactions [22]. And recently, Bavaresco et al. have reported that ecto-5'-NT/CD73 activity, mRNA and protein expression increase during the proliferative process in glioma cell lines, suggesting an important role of this enzyme during brain tumor development [23].

A number of studies have reported that CD73 expression was very variable in different cell types constituting of normal and neoplastic transformation of mammary gland [24, 25]. Supernat et al. showed that elevated CD73 expression in breast cancer can predict a good prognosis of the disease [26]. In addition, Zhi et al. suggested that CD73 may be used as a potential prognostic & therapeutic biomarker for breast cancer [27].

In a previous study, we showed that elevated total superoxide dismutase might reflect a response to oxidative stress, and may predict a state of excess reactive oxidative species in carcinogenesis process, consequently may result in a reduction of 5'-NT activities in tissues of breast cancer women [28]. Our results agree with Durak et al., results which showed a lower adenosine deaminase & 5'-NT activities in cancerous tissues than those in corresponding non-cancerous human laryngeal tissues [29]. Souza et al. suggested that acute stress might be provoked a decrease in nucleotidase activity in rat serum [30].

Since significant changes were observed in 5'-NT activity in the groups included in the present work (Table 1), therefore the sensitivity and specificity of 5'-NT activity were studied in an attempt to evaluate these changes as a tumor marker for breast and brain cancer. In general, (ROC) curve is useful approach that is used to evaluate any parameters as a tumor marker, and it reflects the degree of overlap between the disease and non-diseased population [31]. The (ROC) curve provides an alternative to sensitivity and specificity that often allows the examination of a test's ability to discriminate between two populations regardless of the cut-off level selected. The curve is constructed by graphing the sensitivity on the ordinate as a function of the false-positive rate or 1-specificity, for all possible cut-off values of the diagnostic test [32].

Conclusion

From figures 3 and 4, one can conclude that the tissue extract 5-/NT specific activity (ROC) curve may represent an acceptable marker for breast and brain cancer when the comparison was made with their corresponding benign group (good clinical performance of a test is characterized by a high true positive rate, and a low false positive rate, this is reflected in an (ROC) plot by the curve being close to the upper left corner) [33].

Conflict of interest

The authors wish to express that they have no conflict of interest.

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