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Neuron Specific Enolase: A New Marker for Diagnosis and Evaluating the Benefit of Specific Therapies in Treatment of Breast Cancer

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ABSTRACT:

Malignant cells multiplying unusually in the breast, ultimately increasing to the rest of the body if untreated. Breast cancer happens practically entirely in women. It is one of the leading causes of cancer related death, when it is the second most common cancer in women after lung, when it constitutes 23% of all cancer cases in women, moreover it presents the first in global mortality (18.6%) of cancer According to the latest statistics, breast cancer ranks the first number (2,088,849 new cases) of recorded cases worldwide, annually. Breast cancer is a significant and common disease that has a negative effect on women health, and deaths, 626,679 cases). Seventy four females have been included in the current study, they were classified into three groups depending on their health and the type of tumor suffered by patients. The first included 25 females with malignant breast tumors, the second group included 24 women who had benign breast tumors, and the last group included 25 women who appeared to be healthy. Sandwich-ELISA technique was followed for detecting of NSE concentration in the serum. Results showed a statistical significant increase of NSE concentration in the samples of malignant breast tumors as compared to those of benign breast tumors ($p = 0.032$).

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The highest levels of NSE were recorded in the samples of advanced malignancy stage (III), moreover; right breast injury had the highest proportion of cases recorded in the current study with higher levels of NSE compared to cases recorded in the left breast. Evaluation of the sensitivity% NSE showed a respectable proportion of the susceptibility of this enzyme (76 %) to the distinction between breast cancer patients and healthy individuals. Present work showed a gradual decrease in the levels of NSE after receiving the chemotherapy sequence. For the purpose of studying the possibility of using NSE as an effective tool in the follow-up response of breast cancer patients to treatment and evaluation of the healing stages on the one hand, on the other hand investigate the possibility of proliferation of cancer or recurrence, for that concentrations of NSE after receiving the last dosage of chemotherapy or (chemotherapy and radiotherapy) "according to the program suggested by the specialist" were compared with their levels in the group of patients with benign tumors and also with the control groups. Fifteen of the total patients (25 cases) who received chemotherapy (60% of all infected samples) showed significantly lower rates at the first diagnosis of the disease and this result enhances the possibility of using NSE as a tool to assess the extent of recovery of breast cancer patients.

KEYWORDS: NSE, breast cancer, tumors, chemotherapy, tumor marker.

INTRODUCTION:

Enolase (2-phospho-D glycerate hydrolyase or phosphopyruvate hydratase; E.C: 4.2.1.11) stands for a multifunctional enzyme chiefly concerned in catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate throughout glycolysis and the inverse reaction

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throughout gluconeogenesis[1,2]. There have been 3 isozymes of enolase: enolase α is omnipresent, enolase β is muscle-specific, besides enolase γ that represents neuron-specific. Enolase occurs as numerous dimeric isoenzymes (αα, αβ, ββ and γγ)[3]. The heterologous αγ besides the homologous γγ (more common) isoenzymes have been identified as Neuron-Specific Enolase (NSE) as these isoenzymes primarily supposed to be entirely existing in neurons and neuroendocrine cells[4].

Generally, NSE (Gamma-enolase, also known as enolase 2) is a dimeric intracytoplasmic glycolytic enzyme, with 45 KDa molecular weight, existing in neurons and in neuroendocrine cells, nevertheless, it has been consequently exposed that neuroendocrine cells and some non-neuronal and non-neuroendocrine cells also have NSE. In the embryonic stage, NSE has been constructed in the neural and lung tissues, while throughout childhood and maturity it has created in neuroendocrine cells as it is existing in blood and cerebrospinal fluid[5]. Firstly, it has been taken in consideration that the gene coding NSE (*ENO2 gene*) has constrained to neurons, and that it has been merely existing in the dominant nervous system. Previously, researches have shown that NSE has existing in exterior and central neuroendocrine cells. As NSE goes on the extracellular compartment and the bloodstream produces physical impairment in neuronal brain cells. In adult and pediatric studies, NSE was considered as a marker of intracranial injury and found in the serum of traumatic brain damage[6]. Moreover; NSE has arisen in cerebrospinal fluid and blood as a result to injury as a result of different diseases and complications, like cardiac arrest, open heart surgery[2], epilepsy[7], and Creutzfeldt-Jakob disease[8], along with liver diseases[9], erythrocytes and benign lung tumor[10]. NSE presents in numerous amine precursor uptake and decarboxylation (APUD) neoplasms with islet tumors of the pancreas[11], VIPomas[12] , medullary carcinoma of the thyroid[13], pheochromocytoma[14], and small-cell carcinoma of the lung (SCLC)[15]. Based on above, NSE has been an important tumor indicator for cancers of neuroendocrine type like neuroblastoma, carcinoid tumors, small-cell

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lung cancer (SCLC), seminoma, Merkel cell carcinoma, melanoma, medulloblastoma or retinoblastoma.

Recently, the level of NSE was measured in a number of cancer cases as well as non-cancerous tumors and compared with levels measured in healthy individuals' samples as well as non-tumor pathological diseases such as ulcers and various immunological and inflammatory infections. The levels were significantly and morally observed in cancerous tumors compared to other tumor and non- tumor and healthy[15,17,18].

MATERIALS AND METHODS:

Subjects:

From the beginning of December 2017 to the late May 2018, 74 female residents of Al Najaf Al Ashraf Governorate were included to participate in the current study. The participator women were classified into three groups depending on their health and the type of tumor suffered by patients. The first included 25 females between the ages of 25 and 73 (47.520±12.613) who were diagnosed as patients with malignant breast tumors. The 2nd group included 24 women who had benign breast tumors between the ages of 12 and 70 years (27.170±16.090), and the last group included 25 women who appeared to be healthy, aged between 25 and 68 years (47.730±11.885).

Twenty-five women who were diagnosed with Breast Cancer had body weights ranged between 47 and 115 Kg, while their length ranged between 142 and 168cm, with a body mass index (BMI) of 30.297±5.391 Kg/m^2. Thirteen of the malignant tumor patients had a tumor location in the right breast and 11 of them had left breast tumor location while the tumor was in both breasts in one of cases only. The stages of the patients

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were divided between the first and the third, where they were divided as follows: 3 females were in the first stage of cancer, 14 of them were in the stage Π, finally the remained cases of them were in the stage ΙΙΙ. All women with cancer who participated in the study, except one patient, were mothers of a number of children, and the number of births ranged between 1-11 times. The study required exclusion of all breast cancer patients who had suffered from renal and cardiovascular diseases, diabetes or hypertension from participating in the current study. Moreover; the study excluded smoker women with breast cancer, as well as, those whose cancer symptoms coincided with taking oral or intravenous contraceptives or who took oral contraceptives for 3 consecutive years before the onset of symptoms. Additionally; the study excluded cancer patients who underwent surgery within 5 years of onset of symptoms. The study included women who took contraceptives during their lifetime but all these women had stopped taking contraceptives at least 3 years before the onset of the symptoms of cancer. The study included women who took contraceptives during their lifetime but all these women had stopped taking contraceptives at least 3 years before the onset of the symptoms of cancer.

The weight of patients with Benign Breast Tumors ranged between 49 and 95Kg, while their length ranged between 152 and 165 cm with BMI of 32.166±8.115 Kg/m^2. Ten of the women with benign breast tumors, the right breast was the location of the tumor and the remaining left breast was the site of the tumor. All patients with benign breast tumors were married and had more than a healthy birth (2-7 children), in addition to that, they were non-smokers as well, all of whom did not take contraceptives during the onset of the tumor. Ten of the women with benign breast tumors, the right breast was the location of the tumor and the remaining left breast was the site of the tumor. All women with diabetes, cardiovascular disease or kidney disease are excluded from the current study in the group of women with benign breast tumors. Finally, some patients in this group underwent Cesarean delivery only as a surgical intervention prior to injury.

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Selection of healthy females as a control group according to some criteria including normal menstrual cycle for as a minimum six succeeding months "for healthy pre-menopausal women". They might at approximated age range with the patients group, with comparable food style, without main clinical or surgical disease in the previous five years, without hospital admissions, without current treatment, and a subjective perception of good health as evaluated based on applied health survey. Average BMI of no smoking healthy group was 22.829±0.752Kg/m^2, their weight ranged between 45 and 88 Kg, while their length were 153-168 cm ranged.

Evaluation of NSE Concentration in Sera Samples of Patients and Control Groups:

Sandwich-ELISA technique was followed for detecting of NSE concentration in the serum.

RESULTS AND DISCUSSION:

Evaluation of NSE Concentration in The Serum Samples of Breast Cancer Patients (at Diagnosis), Benign Breast Tumors' Patients and Healthy Control Groups:

Estimation of the NSE in the divers study groups revealed a statistical significant increase of this enzyme concentration in the samples of malignant breast tumors as compared to those of benign breast tumors (**p = 0.032**). On the other side, the statistical comparisons among malignant and benign breast tumors with healthy individuals (**p=0.306** and **p=0.173**; respectively) groups failed to show the same results, as illustrated in **Table 1**.

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Table 1: Levels of NSE (Mean ± S.D.) Concentration (pg/ml) in Sera of Tumoral Patients and Controls Subjects

Study Groups (n)	**NSE Concentration (pg/ml) Mean ± S.D.**	**Min.-Max. Range**	**p-value**
Malignant Tumors 25	1.314 ± 0.531	0.618 - 2.523 1.905	0.032 MT *vs* BT
Benign Tumors 24	0.897 ±0.409	0.220 - 1.744 1.524	0.306 MT *vs* C
Controls 25	1.157 ± 0.598	0.244 - 2.905 2.661	0.173 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls:

The highest levels of NSE were recorded in the samples of advanced malignancy stage (**III**), moreover; right breast injury had the highest proportion of cases recorded in the current study with higher levels of NSE compared to cases recorded in the left breast. Evaluation of the sensitivity percentage of NSE showed a respectable proportion of the susceptibility of this enzyme (76%) to the distinction between breast cancer patients and healthy individuals; where 19 of the 25 breast cancer patients (according to a histopathologist´s diagnosis) showed elevated NSE levels compared to its average in healthy individuals group. While, the specificity of this parameter was seemed to be unacceptable (54%),

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when only 13 of 24 females with benign breast tumors illustrated level of NSE lower than average of this enzyme at healthy individuals. Normally, the NSE levels are fairly low in normal healthy people and in people with benign diseases, but during cancerous changes and malignant proliferation many components of the cell will increase; NSE is one of these components thus increased body fluids levels of NSE as well as specific tissues[8].

Previously, increase NSE levels than normal value could be value in diagnosis, staging and treatment of related neuroendocrine specifically and several other tumors [19-22]. In addition, NSE was studied in some of the noncancerous diseases[23-25]. In the current study, an elevation of NSE concentration in the malignant breast tumors group could be attributed to the abnormal production in the cellular contents that synchronized with the progress of cancer, especially that present study samples were at the metastasis stage. The results of the current study have been agreed with several previous global studies that indicated that this enzyme was elevated during the abnormal cellular transformation process.

Levels of NSE in Sera Samples of Patients With Breast Cancer after Chemotherapy or Radiotherapy Comparison to The Benign Breast Tumors and Control Individuals:

In order to evaluate the possibility of using NSE as an indicator to assess the efficiency of the treatment provided and the response of the patients, concentrations of this enzyme were estimated; periodically. Figure 1 shows the gradual decrease in the levels of NSE after receiving the chemotherapy sequence. For the purpose of studying the possibility of using NSE as an effective tool in the follow-up response of breast cancer patients to treatment and evaluation of the healing stages on the one hand, on the other hand investigate the possibility of proliferation of cancer or recurrence, for that concentrations of NSE after receiving the last dosage

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of chemotherapy or (chemotherapy and radiotherapy) "according to the program suggested by the specialist" were compared with their levels in the group of patients with benign tumors and also with the control groups.

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Figure 1: Follow-up of NSE Levels During Consecutive Chemotherapy Stages

The current results are compatible with the findings of Feng and his team, in their study of the effect of chemotherapy on lowering the levels of serum NSE in the serotonin of lung cancer patients which was directly correlated with the progression of chemotherapy[25], as well as it agreed with the observations of Xiaofan study that carried out on the group of small cell lung cancer patients receiving first-line platinum-based chemotherapy[27]. While outcomes of the present study were disagreed with other previous studies that had done with other malignant tumor types in same field[26-30].

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Table 2 shows the absence of statistical significance when comparing the levels of the enzyme in the group of breast cancer patients (after treatment with at least four doses of chemotherapy) with both benign breast tumors and controls groups.

Table 2: Concentration (Mean ± S.D.) of NSE (pg / ml) in Sera of Malignant Tumor Patients After Treatment, Benign Breast Tumors and Controls Subjects

Study Groups (n)	**NSE Concentration (pg/ml) Mean ± S.D.**	**Min.-Max. Range**	**p-value**
Malignant Tumors 25	0.997 ± 0.420	0.402 - 1.713 1.311	0.573 MT *vs* BT
Benign Tumors 24	0.897 ±0.409	0.220 - 1.744 1.524	0.256 MT *vs* C
Controls 25	1.157 ± 0.598	0.244 - 2.905 2.661	0.141 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

Comparison of NSE Levels in Sera Samples of Patients with Breast Cancer Before and After treatment with Chemotherapy or Radiotherapy

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Using **Student's *t*-test**, comparison between levels of NSE in the sera samples of breast cancerous patients after treatment with chemotherapy (radiotherapy) and their corresponding samples at the diagnosis was done. **Figure 2** revealed significant decreases in the NSE levels of the malignant breast tumors patients after treatment, regardless stage of cancer.

Figure 2: Comparison Levels of NSE in the Sera Samples of Cancerous Patients Before and After Treatment with Chemotherapy (Radiotherapy)

Fifteen of the total patients (25 cases) who received chemotherapy (60% of all infected samples) showed significantly lower rates at the first diagnosis of the disease and this result enhances the possibility of using NSE as a tool to assess the extent of recovery of breast cancer patients.

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