RELATIONSHIP OF SEROTONIN TO NEURON SPECIFIC ENOLASE IN SERUM SAMPLES OF PATIENTS WITH ADVANCED STAGES OF CANCER

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Cancer is heterogeneous disorders characterized by cellular different genetic alterations and diverse clinical behaviours results from uncontrolled division leads to loss of control of the cells growth. Neuron Specific Enolase(NSE) is considered as a marker of many diseases such as brain damage (traumatic brain injury), stroke and anoxic encephalopathy after cardiac arrest. Generally, in adults NSE accepted as a marker protein in the brain. Serotonin exhibits a growth stimulatory effect on several types of carcinoma, carcinoid and other tumor cells. In contrast, a few data are available on serotonin involvement in cancer cell migration and metastatic processes. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma. 201 patients with malignant tumors, 74 patients with different benign tumors and, 83 healthy individuals were enrolled in the present study. Our results show a significant increase (p = 0.011, and 0.043) of serum serotonin levels in malignant tumors group when compared with those of benign tumors (as a pathological control) group, and healthy individuals groups; respectively. No such results were shown when the two control (benign tumors and healthy individuals) groups were compared together. While evaluation of the NSE concentrations revealed a significant decrease in patients with malignant tumors when compared with those of benign tumors (p = 0.028), and healthy individuals (p< 0.000). According to ANOVA test, Same variations (p < 0.000) were obtained when benign tumors and healthy controls groups were compared together. Results shows a significant decrease in the serotonin concentration (p < 0.05) as well as the NSE (p < 0.01) levels, correlation between the levels of the two examined parameters has been negative and statically acceptable (r = - 0.723 at p<0.05) at malignant tumor patients group after treatment by chemotherapy or radiotherapy. Before treatment with chemotherapy or radiotherapy, negatively significant correlation (r = - 0.792 at p < 0.001) was observed for the concentrations of Serotonin to the NSE in the sera of malignant tumor patients group, while no such correlations were noted at this relation examined in the benign tumor patients. Serotonin and NSE correlations together can be used as primary diagnostic tools for distinguishing between cancerous and benign tumors, this correlation increases the sensitivity of the two biomarkers together comparing to measure everyone alone.

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Introduction

Cancer is a genetic disorder consequent uncontrolled and abnormal genetic changes,[1] The prime cancerous cells formed by growth resemble the parent, but as cancer progresses they lose the exterior and function of the parent cell, this dysfunction cells will become life menacing if they left unchecked.[2] Cancer is the main cause of death in economically developed countries and the second leading cause of death in developing countries. The heaviness of cancer is increasing in economically developing countries in consequence of growth and population aging also increasingly, an adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and type of diets.[3]

Neuron Specific Enolase (NSE)is the glycolytic enzyme when it converts 2-phosphoglycerate into phosphoenol-pyruvate (2-phospho-D-glycerate hydrolase) "**E.C: 4.2.1.11**". It comprises three distant subunits α, β, and γ, these subunits might organize spatial as γ-γ and α-γ dimeric forms of enolase. The number of the amino acid is 433 residues and sizes of monomer and dimer differ in molecular weight 39,45, 47, and that of the dimer 7,78, 8, 95 and 96 kDa.[4-9] During the embryonic stage NSE is manufactured in the neural and lung tissue,[10] while, in healthy adults, it is synthesized in neuroendocrine cells.[11] Normally, it is found in blood and cerebrospinal fluid.[10,12] It was first considered that the gene coding for NSE was restricted to neurons and that it was only present in the central nervous system. In 1978 researches have shown that NSE is present in peripheral and central neuroendocrine cells, named APUD (amine precursor uptake and decarboxylation) cells. Moreover, studies have extended to involve immune-histochemical and extraction techniques.[13-15] When NSE passes into the extracellular compartment and the bloodstream causes structural damage in neuronal brain cells. In adult and pediatric study, NSE was considered as a marker of intracranial injury and found in the serum of traumatic brain damage.[16] NSE was shown to be increased in cerebrospinal fluid and blood as a result of injury due to reasons, such as cardiac arrest, open heart surgery, tonic-clonic seizures, epilepsy, and Creutzfeldt-Jakob disease.[17] In addition to that, in the liver diseases, erythrocytes, and benign lung tumor; the concentration of NSE increased (grow up to 20 μg L^{-1}).[10] NSE presents in a wide variety of APUD neoplasms or APUDomas including islet tumors of the pancreas, gastronomes, VIPomas, medullary carcinoma of the thyroid, pheochromocytoma, and small-cell carcinoma of the lung (SCLC).[15] According to that, NSE

was shown to be a valuable tumor marker for cancers of a neuroendocrine type such as small-cell lung cancer (SCLC), neuroblastoma, carcinoid tumors, melanoma, seminoma, Merkel cell carcinoma, medulloblastoma or retinoblastoma.[17] NSE is considered as a marker of many diseases such as brain damage (traumatic brain injury), stroke and anoxic encephalopathy after cardiac arrest. Generally, in adults NSE accepted as a marker protein in the brain.[18] It is found in neurons and neuroendocrine tissues as a neuronal form of the glycolytic enzyme enolase.[13]

In the late of 1940 serotonin (5-hydroxytryptamin "5-HT") as a neurotransmitter was discovered in the central nervous system(CNS) of animals,[19] after that; exactly at 1950 heterogeneous serotonin receptors in rat brain was discovered.[20,21] In mammals serotonin produced principally by enterochromaffin cells. It is found in the gut and stored within blood platelets. In the brain, serotonin is produced within axon terminals. Serotonin is released in response to an action potential and then diffuses across the synapse to activate postsynaptic receptors. They are specialized groups of cell bodies known as the raphe nuclei, located in the brainstem reticular formation.[22] Serotonin has an effect on the number of physiologic and behavioural function, It plays a number of very important roles in normal brain functions, which include modulation of mood states, memory, emotion, anxiety, endocrine effects appetite, hunger, aggression, cognition, gastrointestinal function, emesis, endocrine function, motor function, perception, neurotrophic, sensory function, sex, sleep and vascular function, and many others.[19,23] Serotonin exhibits a growth stimulatory effect on several types of carcinoma, carcinoid and other tumor cells. In contrast, a few data are available on serotonin involvement in cancer cell migration and metastatic processes. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma.[24] Serotonin used in oncology as a tumor marker of gastrointestinal carcinoid, hepatic and ovarian carcinoid.[24] In addition to, serotonin can be used as a specific tumor marker for gastrointestinal tumors of the pancreatic islet cells and intestinal tract.[23,25] Serotonin is synthesized by conversion L-tryptophan into 5-hydroxytryptamine in the body by using two catalyze factors: they are tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase.[19,26] The chemical structure of serotonin have comprised of a basic amino group separated from an aromatic nucleus by a two carbon aliphatic chain. In mammals, serotonin is biosynthetically derived by two enzymatic steps: (1) ring hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase, the rate-limiting step, and (2) side chain decarboxylation by aromatic amino acid decarboxylase.[24,22,23] These processes occur in a number of systems body such as immune system cells and gastrointestinal tract (GIT), central and peripheral nervous system.[27-29] GI contains 90 % of serotonin and it is synthesized basically in enterochromaffin cells and enteric neurons of submucous and myenteric plexus layer.[29] When the serotonin levels fall in the brain leads to a large number of the emergence of foul bad.[30] While serotonin function increases in humans to strengthen the qualities of the positive behaviour and the desired, whenever dropped the serotonin levels increased the emergence of aggressive behaviour.[31,32]

Materials and Methods

During the period from the beginning of March 2016 to the end of September 2016; 358 individuals were enrolled in the present study and classified into three groups. The first group involved 201 patients with different malignant tumors, while the second group included 74 patients underwent benign tumors were used as a pathological control, and the last group included 83 healthy individuals. The enrolled patients (malignant and benign tumors), were collected from several public and private hospitals in addition to centers in Al-Najaf Al-Ashraf governorate; involved: Al-Sadder Medical City, Al-Zahra Teaching Hospital, Al- Ameer Privet Hospital, Al-Ghadeer Hospital, Middle Euphrates Cancer Center, and Daily Specialized Najaf Clinic. Patients with malignant tumors were the basic group of the present study. The cancerous patient's group were classified into six general subgroups (Breast, Lung, Brain, Bladder, Lymphoma, and Acute Lymphocytic Leukemia (ALL)) according to the cases that have been followed during treatment with chemotherapy or radiotherapy.

Five milliliters of venous blood samples were collected from the patients and healthy individuals, after fasting period more than eight hours. Samples were allowed to clot at lab temperature, centrifuged at 5000 g for 5 minutes. Sera were collected and divided into two parts: the first was used for evaluating oxidative stress parameters, and the second part was used for tests of the immunoassay, then these parts were stored at -18˚C until used. Sandwich-Enzyme-Linked Immune Sorbent Assay (Sandwich-ELISA) method was applied to evaluate Serotonin and Neuron Specific Enolase (NSE) concentration in the serum.

Results and Discussion

Levels of serum Serotonin Concentration were measured in the three study groups; malignant and benign tumors' patients as well as healthy control individuals, at diagnosis and before treatment with chemotherapy or radiotherapy or the two types together. Table 1shows a significant increase (p=0.011, and 0.043) of serum serotonin levels in malignant tumors group when compared with those of benign tumors (as a pathological control) group, and healthy individuals groups; respectively. No such results were shown when the two control (benign tumors and healthy individuals) groups were compared together.

Table 1. Levels of serotonin concentration (ng mL^{-1}) in sera of tumoral patients and controls subjects (mean ± S.D.)

Groups (n)	Serotonin, ng mL^{-1}, mean±S.D.	Range	*P*
Malignant (201)	1.315±0.622	0.203-2.721	0.011, malignant vs benign
Benign (74)	0.856±0.594	0.152-2.496	0.043, malignant vs healthy
Healthy (83)	0.895±0.606	0.031-2.113	0.858, benign vs healthy

Measurement of the Neuron Specific Enolasein the various study groups revealed a significant decrease in patients with malignant tumors when compared with those of benign tumors (**p = 0.028**), and healthy individuals (*p* <0.000). Same variations (**p < 0.000**) were obtained when benign tumors and healthy controls groups were compared together (Table 2).

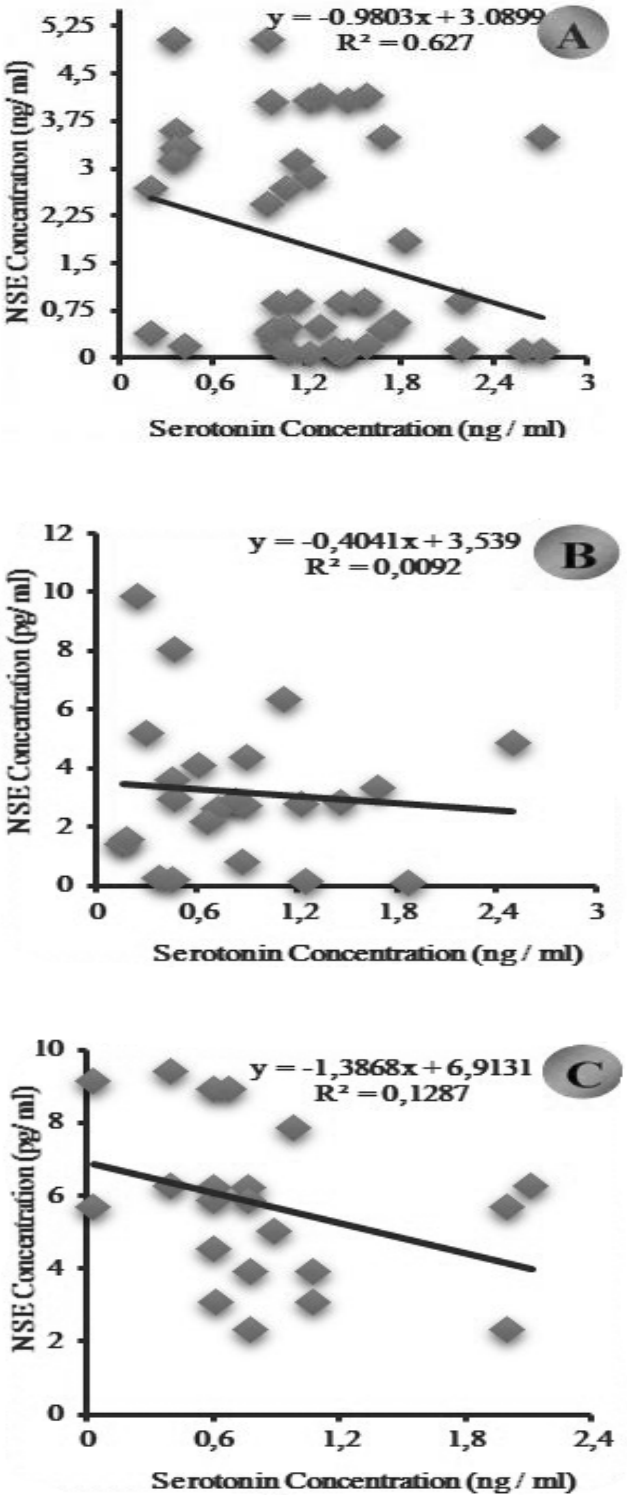

Figure 1. Relationship of serotonin concentration to Neuron Specific Enolase in the sera samples of (A) malignant tumor patients, (B) benign tumor patients, and (C) healthy control individuals

Table 2. Levels of neuron specific enolase concentration (ng mL^{-1}) in sera of tumoral patients and controls subjects (mean±S.D.)

Groups (*n*)	NSE conc. pg mL^{-1}, mean±S.D.	Range	p
Malignant (201)	1.801±1.623	0.060-5.020	0.028, malignant vs benign
Benign (74)	3.193±2.500	0.114-9.884	0.000, malignant vs healthy
Healthy (83)	6.237±2.445	2.310-9.408	0.000, benign vs healthy

Linear Regression Analysis "Pearson's Correlation" was used to analyze the results of Serotonin and Neuron Specific Enolase.

Before treatment with chemotherapy or radiotherapy, negatively significant correlation (r = - 0.792 at p < 0.001) was observed for the concentrations of Serotonin to the Neuron Specific Enolase in the sera of malignant tumor patients group as shown in Figure 1A, while no such correlations were noted at this relation examined in the benign tumor patients (Figure 1B, as well as; healthy controls as shown in Figure 1C.

Despite the recorded significant decrease in the serotonin concentration (p<0.05) as shown in Figure 3.2 as well as the Neuron Specific Enolase (p<0.01) as shown in Figure 3.4 levels, correlation between the levels of the two examined parameters has been negative and statically acceptable (r=-0.723 at p<0.05) at malignant tumor patients group after treatment by chemotherapy or radiotherapy, as illustrated in Figure 2.

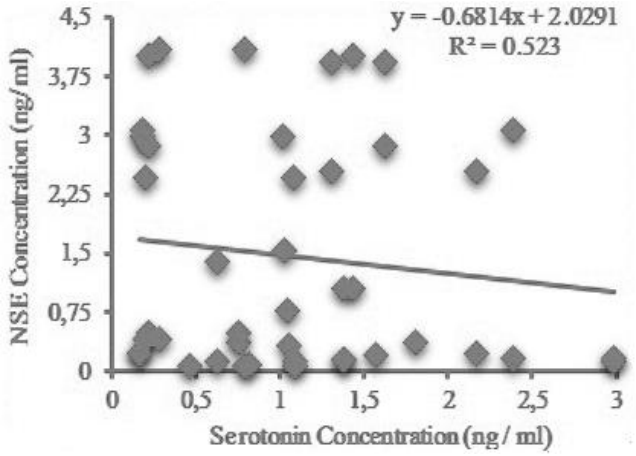

Figure 2. Relationship of Serotonin Concentration to Neuron Specific Enolase in The Sera Samples of Malignant Tumor Patients after at Least two Dosages of Treatment

According to the present results (**Figures 1** and **2**), correlation of Serotonin and Neuron Specific Enolase together increases the possibility of using them together as primary diagnostic tools for distinguishing between cancerous and benign tumors. This correlation increases the sensitivity of the two biomarkers together comparing to measure everyone alone. Moreover, due to the relationship

of serotonin and Neuron Specific Enolase is remained statistically significant in the samples of malignant tumors which are subjected to chemotherapy or radiotherapy, so testing of the two parameters together as follow-up tools became possible for noting the cellular changes occurring during and after treatment. Literatures survey didn't provide real help in tracing a relationship that links serotonin and neuron-specific enolase in cases of cancers.

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