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Comparison of Neuron-specific Enolase (NSE) Serum Level in Patients with Medullary Thyroid Carcinoma and Healthy individuals: Case-Control Study

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Abstract

Background: Medullary thyroid cancer (MTC) is rare but invasive and fatal; therefore, rapid diagnosis and treatment are important in the management of this disease. In many types of cancer, biomarkers are secreted in response to the presence of the tumor, i.e. they are not necessarily secreted by the tumor itself, so the Neuron-specific enolase (NSE) biomarker, which is mostly present in neuroendocrine tissues, was selected for assay in the present study.
Objectives: The aim of this case-control study was to measure and compare NSE serum biomarker levels in patients with MTC and healthy individuals.
Methods: In the current case-control study, patients with MTC, for whom no treatment has yet been performed, were included in the case group as well as healthy individuals as a control group. Demographic and anthropometric data including age, sex, marital status, smoking, history of disease and drug use, BMI of patients and healthy individuals were recorded. Five ml blood was collected from all participants to measure serum NSE levels by using an ELISA kit. All statistical analyses were performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A "P value" less than 0.05 was considered significant.
Results: Ninety patients with MTC in the case group and 90 healthy subjects in the control group were evaluated. Demographic and anthropometric data were matched between the case and control groups (P <0.05). The MTC group consisted of 39 men (43.3%) and 51 women (56.7%) with a mean age of 29.7±12.8 years, and the healthy group included 42 (46.7%) men and 48 (53.3%) women with a mean age of 30.5±11.2 years. The results of ELISA test showed that the mean serum level of NSE in patients with MTC was 23.91±2.1 µg/L and in the healthy subjects was 5.11±0.38 µg/L. Significant differences were observed between the serum concentration of NSE in the control and MTC groups (p=0.001).
Conclusion: In the present study, the serum levels of NSE had significantly increased in patients with MTC compared to the healthy subjects. These preliminary findings suggest that NSE can be associated with MTC, and further studies are needed.

Keywords: Medullary Thyroid Carcinoma, Neuron-specific Enolase (NSE), Biomarker.

Introduction

Thyroid cancer is the most common endocrine malignancy, accounting for about one percent of all cancers. The main cause of this cancer is not exactly known, but scientific reports suggest that exposure to ultraviolet radiation, family history, aging, and too little or too much iodine in the diet can increase the risk of developing the disease. According to statistical studies, the malignancy of this cancer in women is more than three times that of men, which is probably related to the production and increase of estrogen receptors in the process of tumorigenesis. The incidence of this disease reaches a maximum in the third and fourth decades of life.[1-5]

There are three different types of thyroid cancer based on histological features, including Differentiated Thyroid Carcinoma, Medullary Thyroid Carcinoma (MTC), and Anaplastic Thyroid Carcinoma. In general, MTC is

responsible for approximately one-tenth of all thyroid tumors.[4,6]

MTC is a rare malignant tumor that was first described by Jaquet in a German article entitled "Malignant goiter with amyloid" in 1906. MTC is a malignant tumor originating from parafollicular C cells derived from a sternum. About 25% of cases of medullary thyroid carcinoma are inherited and 75% are sporadic. The inherited type of medullary thyroid carcinoma has an autosomal dominant trait with age-dependency. In this form of the disease, in addition to the thyroid gland, some other organs such as the parathyroid and adrenal glands are also involved.[7,8]

Although MTC is a relatively rare neoplasm, its prevalence is increasing.[9] Epidemiological studies also report a gradual increase in this cancer over the last twenty years. The rate of metastasis in patients with MTC increases with age. Therefore, early diagnosis of the disease in its early stages is very important in the process of treatment and recovery of patients. There is also the possibility of treating MTC if they are diagnosed before metastasis, but if metastasis has occurred, the chances of recovery are greatly reduced. Although medullary thyroid cancer is rare, if it is not treated, its mortality rate is high, so prompt diagnosis and treatment are important in the management of this disease.[10]

Enolase is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenol pyruvate. Enolase exists in the form of several tissue-specific isoforms, consisting of homo and heterodimers of three monomeric isoforms of alpha, beta, and gamma. Gamma enolase, also known as enolase 2 or neuron-specific enolase (NSE), is a phosphopyrroate hydratase enzyme encoded in humans by the ENO2 gene. It is one of the three isoenzymes found in mammals. This isoenzyme is a homodimer found in adult neurons and the origin of neuronal cells and neuroendocrine tissue and is not present in tissues other than erythrocytes, and this tissue specificity has made it a potential marker for some diseases. Gamma enolase is 27 kDa and its half-life in body fluids is 24 hours. Elevated serum NSE levels have been observed in lung cancer cells and patients with neuroblastoma and neuroendocrine-derived cancers, which is used as a tumor marker in these patients, however, there are limited and contradictory studies to measure it in patients with MTC.[11,12]

In fact, given that MTC is an invasive thyroid carcinoma, its recovery depends to a large extent on its early diagnosis.[13] Today's diagnoses are mostly molecular and genetic, and doing so is costly and time-consuming. Therefore, in addition to genetic testing of cancers, non-genetic biomarkers are being studied today,[14-17] so in the present study, we are looking to study and find a new biochemical biomarker, perhaps with more diagnostic power and in a shorter time and at a lower cost to help identify patients with MTC. In many types of cancer, biomarkers are secreted in response to the presence of the tumor, ie they are not necessarily secreted by the tumor itself, so the NSE biomarker was selected in the present study.

Objectives

Therefore, in the present study, we sought to measure the serum level of NSE as a biochemical biomarker for the diagnosis of MTC in patients.

Methods

This case-control study was approved by the Ethics Committee of Hamadan University of Medical Sciences, then performed in collaboration with the Center for Cellular and Molecular Research, Endocrinology and Metabolism Research Institute of Shahid Beheshti University of Medical Sciences. In this study, patients with MTC referred to the clinic of the Endocrine Sciences Research Institute of Shahid Beheshti University of Medical Sciences, whose cancer was confirmed by tomography, CT scan and pathology findings and had not yet undergone treatment, entered the study as a case group after obtaining written and informed consent. Then, healthy individuals were invited to cooperate and written and informed consent was obtained from them and entered the study as a control group.

Inclusion criteria: The patient was diagnosed with medullary thyroid carcinoma based on pathological results.

Exclusion criteria: Based on non-confirmation of pathological findings of medullary thyroid carcinoma.

Inclusion criteria of healthy individuals: no pathological confirmation for the presence of medullary thyroid carcinoma or no history of thyroid disease.

Exclusion criteria for healthy people: the presence of thyroid disease based on clinical symptoms or abnormal T3, T4, TSH tests.

Demographic and anthropometric characteristics of participants including age, sex, marital status, smoking, history of disease and drug use, height and weight were recorded.

In this study, in order to eliminate the possible effects of confounding variables (age, sex, marital status, smoking, history of disease and drug use, height and weight), group matching was performed between case and control groups.

Then, 5 ml of blood was taken from all participants to measure the serum level of NSE.

In the end, the collected information was compared with appropriate statistical methods between the two groups.

Serum level of NSE

To measure the serum level of NSE, the assay kit was prepared from the German company ZellBio (ZellBio GmbH (Germany), Cat.No: ZB-0937-H9648) which was based on the ELISA Sandwich (Enzyme Linked-Immunosorbent Assay) method. The measurement was done according to the company's instructions, by using the ELISA reader at a wavelength of 450 nm as follows:

The plate is coated with NSE-specific monoclonal antibodies. Standards and specimens are added to each microplate well and incubated with special biotinized antibodies to NSE and avidin-HRP conjugated. After washing by a wash buffer, polyclonal antibodies specific for NSE are added to the wells to detect nonspecific bindings and detect NSE. After washing with the wash buffer, a nonspecific polyclonal antibody called HRP-conjugated immunoglobulin G antibody is added to the wells. After final washing, peroxidase activity is determined using substrates A and B, which are chromogenic. Only wells containing NSE, biotinized antibody, and enzymatically conjugated avidin showed discoloration. The reaction of the enzymatic substrate is terminated by adding the sulfuric acid solution and the color change is measured spectrophotometrically at 455 nm. The NSE concentration in the samples is determined by comparing the OD of the samples with the standard curve.

The minimum detectable amount of human NSE (sensitivity) is 0.05 ng/ml and the diagnostic range was 0.1-40 ng/ml and the final absorbance was read at 450 nm and unit conversion was performed.

Statistical analysis

Paired t-test was used to compare quantitative variables and McNemar test was used to compare qualitative variables. All statistical analyzes were used at 95% confidence level using Stata software version 11.

Ethical considerations

In this study, explaining the objectives and research process to the voluntary participants, obtaining written and informed consent from all individuals, and keeping information confidential was observed.

Results

In the present case-control study, according to the inclusion and exclusion criteria, 90 individuals with medullary thyroid carcinoma were included in the case group and 90 healthy individuals in the control group.

The group of patients with medullary thyroid carcinoma included 39 men (43.3%) and the control group (healthy) included 42 men (46.7%) and there was no significant difference between the two groups (Table 1). The mean age of patients with medullary thyroid carcinoma was 29.7±12.8 years and in the control group (healthy) was 30.5±11.2 years, both groups were matched in terms of age (Table -2).

Regarding other variables such as marital status, smoking, history of disease and drug use, height, and weight, the two groups of case and control were matched (data not shown).

NSE Serum level: Independent t-test showed that the mean serum level of NSE in patients was 23.91±2.10 µg/l and in healthy individuals was 5.11±0.38 µg/l. There was a statistically significant difference between the serum concentrations of NSE in the control group and the group with thyroid cancer (Figure-1) (P=0.001).

Table 1. Gender in two groups of control (healthy) and patients with medullary thyroid carcinoma

	Control (n=90)	**Case** (n=90)	**P value**
Male	42 (46.7 %)	39 (43.3 %)	0.65
Female	48 (53.3 %)	51 (56.7 %)	

Table 2. Age in two groups of control (healthy) and patients with medullary thyroid carcinoma

	Control (n=90)	**Case** (n=90)	**P value**
Age (years)	30.5±11.2	29.7±12.8	0.65
Min	11	12	
Max	60	59	

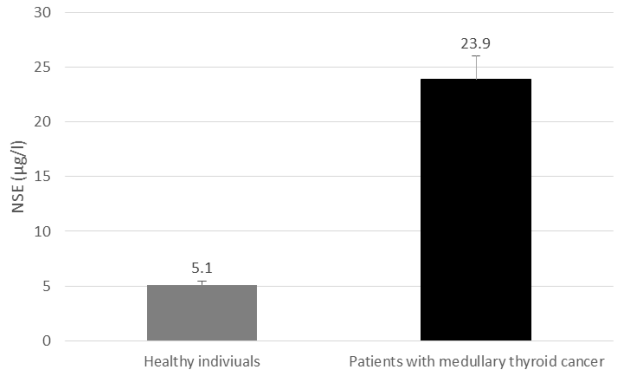

Figure 1. Comparison of serum NSE concentrations in control group (n=90) and patients (n=90) with medullary thyroid cancer by ELISA sandwich (P=0.001).

Discussion

The results of the present study showed a significant increase in serum NSE in patients with medullary thyroid carcinoma compared with healthy individuals. Therefore, measuring the NSE biomarker in the serum of individuals seems to be helpful in the diagnosis, confirmation or recurrence of medullary thyroid carcinoma. It is not clear exactly why serum NSE levels increase in medullary thyroid carcinoma and some other previously mentioned cancers, and further studies are needed.

In a study by Pacini et al., serum NSE levels were measured in patients with MTC. In this study, 25 patients were followed for about 45 months. In 5 patients, NSE concentration was in the normal range before each treatment intervention. After complete thyroidectomy, an abnormally high NSE level was observed in patients with high calcitonin and metastasis. The long follow-up period showed that NSE levels were higher in patients with larger tumor volumes and also usually had a pattern similar to calcitonin levels. Therefore, effective treatment can be aimed at reducing serum NSE levels. Thus, serum NSE can serve as a hormonal marker for medullary thyroid cancer, and its increased level is associated with metastatic, although it is a poor prognosis for the tumor.[12]

In a study by Grauer et al., NSE levels were assessed in 32 patients with medullary thyroid cancer. Calcitonin levels were elevated in all of these patients. Positive immunocytochemical results for NSE and calcitonin were recorded in C cells in all patients. Elevated serum NSE levels were observed in only 5 out of 32 patients and no association was found between NSE and calcitonin concentrations. Long-term follow-up was also performed in this study and again no relationship was observed between serum NSE and serum calcitonin. The researchers concluded that although the results of NSE immunocytochemistry could be useful, but could not be a suitable serum tumor marker for medullary thyroid cancer,[11] which contradicts the findings of the present study.

As mentioned earlier, the effective treatment and surgery of patients with medullary thyroid carcinoma depends to a large extent on its early diagnosis. In almost all cases of medullary thyroid carcinoma, calcitonin secreted by C cells, as a specific and sensitive marker, plays an important role in the diagnosis of this disease.[8] Biochemical screening based on the serum calcitonin-enhancement scheme is also used to diagnose patients at risk for hereditary medullary thyroid carcinoma. Unfortunately, false results in biochemical screening lead to some people with mutated genes not being identified.[18] Therefore, studies are running to find and present biomarkers with high sensitivity and specificity to reduce the cost of diagnosis and treatment.[20,19]

The present study had some limitations that can be referred to the small sample size in the study groups. For better results regarding the use of NSE biomarker, as an indicator in the diagnosis and/or confirmation of medullary thyroid carcinoma as well as the prognosis of cancer, it is better to examine a larger sample size.

Although the number of populations studied was determined using the relevant formula, however, this population may not represent the total population of people with medullary thyroid carcinoma, and it is better to test more populations in future studies.

Finally, due to the increase in serum NSE levels in patients with medullary thyroid carcinoma compared to the control group, it is recommended that the exact mechanism of this increase in this type of cancer be investigated and other biochemical parameters are measured in these patients.

Conclusions

In this study, serum NSE levels were increased in patients with medullary thyroid carcinoma. These preliminary findings suggest that NSE may be associated with medullary thyroid carcinoma or increase the risk of developing the disease. Further studies are needed to investigate more closely so that this biomarker may be used to diagnose or confirm the diagnosis of medullary thyroid cancer and to predict the occurrence of cancer.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Medullary Thyroid Carcinoma: MTC;

Neuron-specific Enolase: NSE;

Thyroid Stimulating Hormone: TSH;

Triiodothyronine: T3;

Thyroxine: T4;

Enzyme-linked immunosorbent assay: ELISA.

Authors' contributions

MH and NS were responsible for study concept and design. RA wrote the first draft. SAE contributed to the writing of the second and third draft. MH and NS provided comments on initial drafts and coordinated the final draft. All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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