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Comparison of neuron-specific enolase (NSE) serum levels in patients with medullary thyroid carcinoma versus healthy people: a case-control study

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Abstract

Background: Medullary thyroid cancer (MTC) is a rare and aggressive form of cancer that can be fatal. Therefore, it is crucial to promptly diagnose and treat this disease. Biomarkers are secreted in response to the presence of a tumor rather than being produced by the tumor itself in many kinds of cancer. In this study, the neuron-specific enolase (NSE) biomarker, which is predominantly found in neuroendocrine tissues, was chosen for analysis.

Objectives: The main objective of this case-control study was to measure and compare the levels of NSE serum biomarkers in patients with MTC and healthy individuals.

Methods: For this case-control study, patients with MTC who had not yet received any treatment were included in the case group, while healthy individuals served as the control group. Demographic and anthropometric data, such as age, gender, marital status, smoking history, medical history, drug use, and BMI, were recorded for both groups. A blood sample of five milliliters was collected from all participants to measure the levels of NSE biomarkers using an ELISA kit. All statistical analyses were conducted using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). A significance level of "P value" less than 0.05 was considered statistically significant.

Results: The case group consisted of ninety patients with MTC, while the control group included ninety healthy subjects. Demographic and anthropometric data were carefully matched between the two groups ($P < 0.05$). Within the MTC group, there were 39 men (43.3%) and 51 women (56.7%), with an average age of 29.7±12.8 years. The healthy group was comprised of 42 men (46.7%) and 48 women (53.3%), with an average age of 30.5±11.2 years. The ELISA test results revealed that the mean serum level of NSE in MTC patients was 23.91±2.1 µg/L, whereas in healthy subjects it was 5.11±0.38 µg/L. There were significant differences in the serum concentration of NSE between the control and MTC groups ($p = 0.001$).

Conclusion: This study shows a significant rise in serum NSE levels in MTC patients when compared to healthy individuals. These preliminary results indicate a possible link between NSE and MTC, necessitating further research.

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

Introduction

Thyroid cancer, the most common endocrine malignancy, makes up around one percent of all cancer cases. The exact cause of this cancer remains uncertain; however, scientific evidence suggests that factors such as exposure to ultraviolet radiation, family history, aging, and imbalances in iodine intake may contribute to an increased risk of developing the disease. Statistical studies indicate that the incidence of thyroid cancer in women is more than three times higher than in men, potentially linked to the production and elevation of estrogen receptors during tumorigenesis. The highest occurrence of this disease is

observed in individuals in their thirties and forties.[1-5]

Thyroid cancer can be categorized into three types: differentiated thyroid carcinoma, medullary thyroid carcinoma (MTC), and anaplastic thyroid carcinoma. MTC represents about 10% of all thyroid tumors.[4,6]

Jaquet identified MTC as an uncommon malignant tumor in a 1906 German paper titled "Malignant goiter with amyloid." It develops from parafollicular C cells originating from the sternum. About 25% of MTC cases are inherited, with the remaining 75% being sporadic. The inherited form of MTC follows an autosomal dominant pattern with age-dependent traits. This particular form of the disease may affect organs other than the thyroid gland, such as the parathyroid and adrenal glands.[7,8] MTC is becoming more prevalent despite its rarity.[9] Studies show that this cancer's incidence has gradually increased over the last 20 years. The risk of metastasis in MTC patients rises with age, making early detection crucial for effective treatment and recovery. MTC can be treated if caught before metastasis, but the chances of recovery decrease significantly once it spreads. Although rare, MTC has a high mortality rate if left untreated, making prompt diagnosis and treatment essential.[10]

Enolase is an enzyme involved in glycolysis that aids in the conversion of 2-phosphoglycerate to phosphoenol pyruvate. It has different isoforms, including alpha, beta, and gamma, which can form homodimers and heterodimers. Gamma enolase, also known as enolase 2 or neuron-specific enolase (NSE), is a phosphopyrroate hydratase enzyme in humans that is encoded by the ENO2 gene. This specific isoform is present in adult neurons, neuroendocrine tissue, and the origin of neuronal cells. It is not found in tissues other than erythrocytes, which makes it a potential marker for certain diseases. With a molecular weight of 27 kDa, gamma enolase has a half-life of 24 hours in body fluids. Elevated levels of serum NSE have been observed in lung cancer cells, neuroblastoma, and neuroendocrine-derived cancers, making it a useful tumor marker in these cases. However, there is limited and conflicting evidence regarding its measurement in patients with MTC.[11,12]

MTC, an invasive thyroid carcinoma, heavily depends on early detection for successful treatment.[13] Currently, diagnoses mostly involve molecular and genetic testing, which can be costly and time-consuming.[14–17] As a result, researchers are exploring non-genetic biomarkers in addition to genetic testing for cancer. The goal is to find a new biochemical biomarker with improved diagnostic capabilities, faster results, and lower costs to help identify MTC patients. Biomarkers are released in reaction to the presence of the tumor in many cancer forms, rather than being secreted directly by the tumor. Therefore, the NSE biomarker was selected for investigation in this study.

Objectives

The purpose of this study was to assess the potential of NSE serum levels as a biochemical biomarker for diagnosing MTC in patients.

Methods

This case-control study was conducted in collaboration with the Center for Cellular and Molecular Research, Endocrinology, and Metabolism Research Institute of Shaid Beheshti University of Medical Sciences, after obtaining approval from the Ethics Committee of Hamadan University of Medical Sciences. As the case group, patients with MTC who had not yet received therapy and were verified by tomography, CT scan, and pathology results were selected from the Endocrine Sciences Research Institute clinic at Shaid Beheshti University of Medical Sciences. Written and informed consent was obtained from all participants. Healthy individuals were also invited to participate as the control group, and written and informed consent was obtained from them as well.

The inclusion criteria for the case group were a confirmed diagnosis of medullary thyroid carcinoma based on pathological results. The exclusion criteria were non-confirmation of pathological findings of medullary thyroid carcinoma. The inclusion criteria for the control group were the absence of pathological confirmation for the presence of medullary thyroid carcinoma or any history of thyroid disease. The exclusion criteria for the control group were the presence of thyroid disease based on clinical symptoms or abnormal T3, T4, or TSH tests.

The participants' demographic and anthropometric characteristics, such as age, sex, marital status, smoking habits, history of disease, and drug use, as well as height

and weight, were documented. To mitigate the potential influence of confounding variables (age, sex, marital status, smoking habits, history of disease and drug use, height, and weight), group matching was conducted between the case and control groups in this study. Subsequently, a 5 ml blood sample was collected from all participants to assess the serum level of NSE. Finally, the gathered data was compared using suitable statistical methods to analyze the differences between the two groups.

Serum level of NSE

The NSE serum level was determined by utilizing an assay kit obtained from ZellBio GmbH, a German company (Cat.No. ZB-0937-H9648). This kit was designed based on the ELISA sandwich method, which is an enzyme-linked immunosorbent assay. The measurement process followed the instructions provided by the company. An ELISA reader was employed to measure the serum level of NSE at a wavelength of 450 nm.

The NSE-specific monoclonal antibodies are applied to coat the plate.

Following that, standards and specimens are placed in each microplate well and treated with biotinized NSE antibodies and avidin-HRP-conjugated avidin. The wells are then washed with a wash buffer, and polyclonal antibodies specific for NSE are added to detect nonspecific bindings and NSE. After washing with the wash buffer, a nonspecific polyclonal antibody called HRP-conjugated immunoglobulin G antibody is added to the wells. Finally, peroxidase activity is determined using chromogenic substrates A and B. Only wells containing NSE, biotinized antibodies, and enzymatically conjugated avidin exhibit discoloration. By adding the sulfuric acid solution, the enzymatic substrate reaction is stopped, and the color change is monitored spectrophotometrically at 455 nm. The NSE concentration in the samples is determined by comparing the OD of the samples with the standard curve.

The sensitivity of the minimum detectable amount of human NSE is 0.05 ng/ml, while the diagnostic range falls between 0.1 and 40 ng/ml. The final absorbance was measured at 450 nm, and a unit conversion was conducted.

Statistical analysis

For statistical analysis, a paired t-test was employed to compare quantitative variables, while a McNemar test was utilized to compare qualitative variables. All statistical analyses were conducted at a 95% confidence level using Stata software version 11.

Ethical considerations

In terms of ethical considerations, this study ensured that the objectives and research process were clearly explained to the voluntary participants. Additionally, written and informed consent was obtained from all individuals, and measures were taken to maintain the confidentiality of the information gathered.

Results

In this case-control study, a total of 90 individuals diagnosed with medullary thyroid carcinoma were included in the case group, while 90 healthy individuals were included in the control group, based on the predefined inclusion and exclusion criteria. Among the patients with medullary thyroid carcinoma, 39 (43.3%) were men, whereas in the control group, 42 (46.7%) were men. Statistical analysis revealed no significant difference between the two groups in terms of gender distribution [Table 1]. The average age of patients with medullary thyroid carcinoma was 29.7±12.8 years, while in the control group, it was 30.5±11.2 years. Importantly, both groups were well-matched in terms of age [Table 2]. Furthermore, when considering other variables such as marital status, smoking habits, medical history, drug use, height, and weight, the case and control groups were also found to be well-matched, although the specific data is not presented in this report.

NSE Serum level: The results of an independent t-test revealed that the average NSE serum level in patients was 23.91±2.10 µg/l, while in healthy individuals it was 5.11±0.38 µg/l. The serum concentrations of NSE were significantly different between the control group and the group with thyroid cancer [Figure 1] (P = 0.001).

Table 1. Gender in two groups: 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	

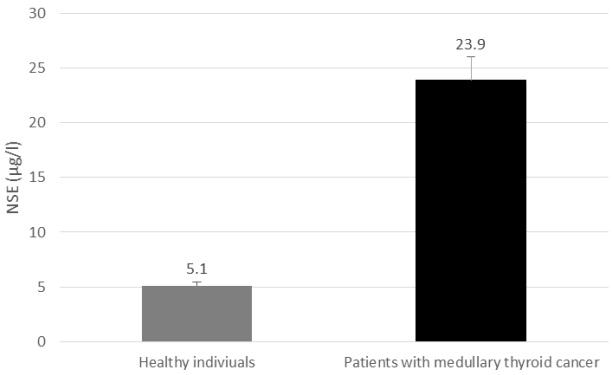

Figure 1. Comparison of serum NSE concentrations in control group (n=90) and patients (n=90) with medullary thyroid cancer by ELISA sandwich (P=0.001).

Discussion

The findings of the current investigation indicate a significant rise in serum NSE among patients diagnosed with medullary thyroid carcinoma in comparison to healthy individuals. Consequently, the measurement of NSE biomarkers in the serum of individuals can be a useful tool in the diagnosis, confirmation, or recurrence of medullary thyroid carcinoma. The exact reason behind the elevation of serum NSE levels in medullary thyroid carcinoma and some other cancers mentioned earlier remains unclear, and further research is required to shed light on this matter.

Pacini et al. conducted a study to measure serum NSE levels in patients with MTC. The study followed 25 patients for approximately 45 months, during which NSE concentration was within the normal range in five patients before each treatment intervention. After a complete thyroidectomy, patients with high calcitonin and metastasis exhibited abnormally high NSE levels. The extended follow-up period revealed that NSE levels were higher in patients with larger tumor volumes and typically followed 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 metastasis, although it is a poor prognosis for the tumor.[12]

Grauer et al. evaluated the levels of NSE (neuron-specific enolase) in 32 patients with medullary thyroid carcinoma in a study. It was found that all of these patients had elevated levels of calcitonin. Furthermore, positive immunocytochemical results for both NSE and calcitonin were observed in the C cells of all patients. However, only 5 out of the 32 patients exhibited elevated serum NSE levels, and no correlation was found between NSE and calcitonin concentrations. The researchers also conducted a long-term follow-up, and once again, no relationship was observed between serum NSE and serum calcitonin levels. Based on these findings, the researchers concluded that, while NSE immunocytochemistry results may be useful in some cases, they cannot be used as a serum tumor marker for medullary thyroid carcinoma.[11] This conclusion contradicts the findings of the present study.

As previously mentioned, the early diagnosis of medullary thyroid carcinoma is crucial for effective treatment and surgery. Calcitonin, which is released by C cells, is a specific and diagnostic marker for detecting this condition in the majority of patients.[8] Furthermore, biochemical screening based on serum calcitonin elevation is used to identify patients at risk for hereditary medullary thyroid cancer. Unfortunately, this screening method may yield false results, leading to the failure to identify individuals with mutated genes.[18] Consequently, ongoing research aims to identify and introduce biomarkers with high sensitivity and specificity in order to reduce the cost associated with diagnosis and treatment.[20,19]

The current study had certain limitations, specifically related to the small sample size in the study groups. In order to obtain more accurate and reliable results regarding the use of the NSE biomarker as an indicator for the diagnosis and prognosis of medullary thyroid carcinoma, it is advisable to conduct future studies with a larger sample size. Although the number of participants in this study was determined using the appropriate formula, it is important to note that this population may not fully represent the entire population of individuals with

medullary thyroid carcinoma. Therefore, it is recommended to include a larger and more diverse population in future research. Furthermore, given the observed rise in serum NSE levels in patients with medullary thyroid carcinoma compared to the control group, further research into the mechanism behind this elevation in this specific kind of cancer is highly advised. Additionally, it would be beneficial to measure other biochemical parameters in these patients to gain a comprehensive understanding of the disease.

Conclusions

In the present investigation, it was observed that patients diagnosed with medullary thyroid carcinoma exhibited elevated levels of serum NSE. These initial results propose a potential association between NSE and medullary thyroid carcinoma, indicating a possible role of NSE in the development of this disease. However, more extensive research is needed to determine the diagnostic utility of this biomarker in medullary thyroid carcinoma as well as its predictive significance in assessing the chance of cancer development.

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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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