

Brief Report

Running Title: Chromogranin Level in NETs

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Serum Chromogranin Level in Gastrointestinal, Pancreas, and Liver Neuroendocrine Tumors, A Brief report

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Abstract

Background: Chromogranin is a marker that can be detected in the tissue of the neuroendocrine tumors by immunohistochemistry and as a biomarker for the diagnosis and follow-up of NET. In this study, we evaluated the correlation of prognostic characteristics of NETs (Ki67, location, and size) with the chromogranin level.

Method: In this case-control study, we measured the serum level of chromogranin in 50 cases of NETs from different locations of the gastrointestinal tract, liver, and pancreas as well as 30 healthy individuals for one year (2016). The correlation of this level was evaluated with Ki67, size, and location of the tumors (main prognostic predictors of NETs).

Results: The level of chromogranin in the above-mentioned 80 tumoral and healthy cases was 37 to 2585 ng/ml (242.3±439.4). The level of chromogranin in NETs and normal cases was 326.3±525.3 and 51.5±16.7, respectively. This level showed a statistically significant correlation with the Ki67 percentage and the tumor grade (P -value <0.05). There was no correlation between size and chromogranin level, but the highest level was detected in liver NETs. The cut-off level of 61.2 ng/ml correlated with the presence of NET with a sensitivity of 80% and specificity of 70%.

Conclusion: Chromogranin level can be used as a prognostic biomarker that is correlated with the grade of NETs and very high levels of this marker can be indicative of liver involvement. The cut-off level of 61.2 ng/ml can be considered as one of the predictors of the NET in the gastrointestinal, liver, or pancreas.

Keywords: Chromogranin, Biomarker, Neuroendocrine tumor

Introduction

Chromogranin A (CgA) is a soluble (hydrophilic) acidic glycoprotein with a molecular weight of 49 kilodaltons and 439 amino acids that is encoded by the CgA gene on chromosome 14. This protein is found in the neurosecretory granules of the endocrine system. The serum level of CgA correlates with the number of dense-core granules in neuroendocrine cells.¹ Because of this correlation, there have been some studies about the potential of CgA as a diagnostic and prognostic biomarker in the liver, pancreas, and gastrointestinal neuroendocrine tumors (NET).² It has been claimed by some studies that the CgA level is useful for the evaluation of therapeutic response as well.³

There are many characteristics in NETs that determine the response to therapy, prognosis, and survival. The most documented factors that directly correlate with prognosis and survival are the location, size, and proliferative activity of the tumor. Proliferative activity is determined by immunohistochemistry for Ki67 and counting the number of positive nuclei.⁴ In this study, we have tried to find out the correlation of this biomarker with different clinicopathologic characteristics of gastrointestinal, liver, and pancreatic NET (such as patients' age, sex, and location or size of the tumor as well as Ki67 activity) in 50 resected tumors in our center as the largest referral center in the south of Iran. In the meantime, considering the level of CgA in normal controls, we also tried to define a cut-off level for CgA in the diagnosis of NETs.

Patients and Methods

Patients

In this case-control study, there were 50 cases of NETs from different parts of gastrointestinal tract, liver, and pancreas during a one-year period (2016). Thirty

normal volunteer individuals were also included. There were 30(60%) female and 20(40%) male patients with NET during this period with an age range of 12-82 (47 ± 16.7) years. Renal function tests were normal in all the included cases. In all the cases, the patients underwent surgery or endoscopy for the resection of the NETs. All patients gave informed consent to participate in the study. The project was approved by the Ethics Committee (Islamic Azad University, Fars branch, Code Number: 16330520941021).

Different characteristics of the NETs are summarized in tables 1 to 3. There were also 30 age- and sex-matched normal controls consisting of 20 females and 10 males, with an age range of 21-43 years (31.7 ± 7.2).

Immunohistochemistry

After the initial diagnosis of NET based on the Hematoxylin-Eosin-stained sections, immunohistochemistry was performed in all of the resected tumors by the antibodies against chromogranin and synaptophysin from DAKO Company, to confirm the diagnosis (reactive either for chromogranin, or synaptophysin) and also Ki67 to determine the proliferative activity and tumor grade ($\leq 3\%$: G1, 3-20% G2, $\geq 20\%$: G3) (5). The antibodies were prediluted in all three markers (Chromogranin, synaptophysin, and Ki-67), and antigen retrieval was done by heat.

Immunoassay

The level of chromogranin was determined in a serum sample driven during the first week after the operation or endoscopic resection by ELISA (Enzyme-linked immunosorbent assay) from Eastbiopharm Company (USA) by a double antibody sandwich assay where samples and peroxidase-conjugated antibody to CgA were incubated together in micro wells coated with antibody against CgA. The chromogenic substrate was then added after a washing step. Oxidation of the substrate through an enzyme-catalyzed reaction

caused the development of color. The antibody source was a rabbit against the C-terminal fragment of human CgA (ng/ml).

Statistical analysis

The cut-off level of CgA for the diagnosis of NETs was characterized by the receiver-operating characteristic (ROC) curve. The area under the ROC curve (AUC) was determined to evaluate the reliability of serum CgA to discriminate between NETs and controls. The relationships between CgA and clinicopathologic variables were further specified. The comparison of values between NETs and control group or within NETs in different locations and different characteristics was performed using the Mann-Whitney U test or Fisher's exact test. A *P*-value of < 0.05 was statistically significant. Statistical analysis was conducted using the IBM SPSS Statistics version 19.0 (SPSS Inc, Chicago, IL, USA).

Results

In this study, the level of chromogranin was between 37 and 2585 ng/ml (242.3 ± 439.4) in 50 cases of NET, and 30 normal volunteer individuals. The level of chromogranin in 50 NET cases was 326.3 ± 525.3 ng/ml. The level of CgA in normal healthy volunteers was also 51.5 ± 16.7 ng/ml.

The level of chromogranin showed a statistically significant correlation with proliferative activity, meaning Ki-67 percentage (*P*-value < 0.01). With every 1% increase in the Ki67 (1-50%, $5.74\% \pm 10.1\%$), the chromogranin level showed an increasing level of 37.6 ng/ml.

The level of chromogranin did not show a statistically significant correlation with the size of the tumor (0.1-10 cm, 1.96 ± 0.2). (*P* = 0.069)

The highest levels of chromogranin were detected in the NETs located in the liver. Table 4 shows the level of chromogranin according to the location of the NETs.

According to our results, a cut-off level of 61.2 ng/ml can predict the presence of a NET with a sensitivity of 80% and a specificity of 70%. Chromogranin levels above 201.2 ng/ml correlated with grades 2 and 3 (Ki67 above 3%) with a sensitivity of 100% and a specificity of 80%.

Discussion

In this study, our results showed that CgA can be a good biomarker in the diagnosis of NET, especially with liver metastasis. Moreover, the CgA level can be a useful marker for predicting grade of NET and its proliferative activity (Ki67 index).

We also found the cut-off level of 61.2 ng/ml as a useful level in the diagnosis of NETs.

According to the current literature, CgA is widely used as an immunohistochemical marker for the confirmation and definite diagnosis of NETs in tissue. The level of CgA has also been measurable in serum and plasma.⁶ With the development of a tumor in neuroendocrine tissue, the main source of CgA is NET which is found in the blood of 85% to 90% of the patients with NETs. In various studies, CgA has been shown to correlate with disease severity, tumor volume, tumor burden, and overall prognosis.⁷ There are controversial reports about the sensitivity and specificity of circulating CgA levels in NETs ranging from 27% to 81%.^{8,9} There are very few reports from Asian countries and no reports from Iran. Additionally, there is not enough research about the cut-off level of CgA serum and its correlation with the main prognostic markers (such as size and Ki67).^{10,11}

In this study, we found a statistically significant correlation between this biomarker and the proliferative activity of the NETs as the most important characteristic used for grading and predicting metastasis. This is in line with the previous studies which reported a positive

connection between grades of NETs determined by Ki67 percentage.^{10, 11}

One of the main diagnostic challenges in the practical application of CgA in diagnosis and follow-up of CgA in NETs is the absence of an accurate cut-off level in NETs.¹² In this study, we determined that a cut-off level of 61.2 ng/ml can predict the presence of a NET with a sensitivity of 80% and specificity of 70%. There have been few studies with reported cut-off levels ranging from 53 to 99 ng/ml.^{12, 15, 16} Chromogranin levels above 201.2ng/ml correlated with grades 2 and 3 with a sensitivity of 100% and a specificity of 80%. In the previous reports, a cut-off level of 142 ng/ml was determined to differentiate between NETs with and without liver metastasis.¹³ Besides, after resection of the NETs, decreasing and normalizing levels of the CgA were also shown in some other studies.¹⁷

In our study, the highest level of CgA was shown in liver NETs. This high level can be partly due to impaired liver function secondary to tumoral involvement.¹⁷ Primary NETs of the liver are very rare and most of the NETs in the liver are metastatic, with undetected unknown primary source; therefore, another explanation for the high levels of liver NETs can be their metastatic nature.⁴

The low number of our patients and lack of follow-up were the two main limitations of our study.

Conclusion

In conclusion, CgA level is a good, accurate, and noninvasive predictor of the grade of NETs. The cut-off level of 61.2ng/ml can be used to diagnose NETs and levels above 201.2 ng/ml is indicative of higher grades of the NETs in the gastrointestinal tract, pancreas, and liver.

Conflict of Interest

None declared.

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Table 1. Number of the NET cases according to the location in this study

Location	Numbers
Stomach	5
Small Intestine	13
Large Intestine	10
Appendix	8
Liver	9
Pancreas	5
Total	50

Table 2. Number of NET cases according to proliferative activity (percent of Ki-67)

Ki-67	Numbers
<3% (G1)	38
3-20% (G2)	6
≥20% (G3)	6

Table 3. Number of NET cases according to the size (cm)

Size	Numbers
<2cm	39
2-10 cm	11
≥10 cm	0

Table 4. The chromogranin level according to the location of NET

	Number	Mean ±Standard Deviation (ng/ml)	Range(ng/ml)
Stomach	5	107.7±69.5	49.6-222.8
Small Intestine	13	326.6±427.1	37-1421.4
Appendix	8	142.2±104.3	62.4-361.8
Large Intestine	10	298.7±407.4	49-1279.2
Liver	9	949.6±824.5	64.2-2584
Pancreas	5	72.0±40.9	37.6-142.2
Total	50	326.3±525.3	37-2584