

## EXPRESSION IMUNOHISTOCHEMICAL CHROMOGRANIN A AND SYNAPTOPHYSIN AS MARKERS OF NEUROENDOCRINE TUMOUR (NET) IN HISTOPATHOLOGICAL PREPARATION OF APPENDICITIS

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### Abstract

**Background:** Neuroendocrine tumor (NET) is neoplasm of epithelial cells that differentiates based on neuroendocrine cells. Appendix NET is the third most common gastrointestinal neuroendocrine tumor after the small intestine and rectum one. NET in the appendix is often found incidentally on histopathological examination of appendectomy tissues. Immunohistochemistry of chromogranin A and synaptophysin are the most tumor markers in NET.

**Objective:** To know expression immunohistochemistry chromogranin A and synaptophysin as markers of neuroendocrine tumor (NET) in histopathological preparations of appendicitis.

**Materials and methods:** This is a descriptive study with a cross sectional approach on 52 samples of histopathological appendicitis preparations stained with hematoxylin and eosin (H&E) and immunohistochemistry chromogranin A and synaptophysin as markers of NET

**Results:** NET in the appendix is often found incidentally in histopathological preparations of appendicitis. The suspect of NET in the appendix can be confirmed by immunohistochemical chromogranin A and synaptophysin as markers of neuroendocrine tumors.

**Keywords:** neuroendocrine tumor, appendix, appendicitis, chromogranin A, synaptophysin

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### 1. Introduction

Neuroendocrine tumor (NET) in the appendix is mostly found incidentally in adult and children, during or after appendectomy. Appendix NET comprises 43-67% primary tumor of the appendix and is responsible for 0.16-23% of all tumors of the appendix.<sup>1</sup> Appendix NET arises from subepithelial neuroendocrine cells located in the lamina propria of the mucosa and the submucosal layer of the appendix wall. Histopathological diagnosis of appendix NET includes immunohistochemical profiling with respect chromogranin A and synaptophysin of which these two immunohistochemical are the most commonly used tumor markers.<sup>1,2,3</sup>

NET is common and often found incidentally on histopathological examination of appendectomy tissues. This tumor does not cause clinical symptoms on 80% of cases found incidentally after surgery for indication of appendicitis. NET in the appendix is generally found at the distal end of the appendix. In some cases, it occurs in the other part of the appendix causing obstruction of the lumen and leading symptoms that resemble appendicitis. In size, NET is less than 1 cm so that the detection of this tumor also depends on the accuracy of the histopathological examination of the appendix tissues. It is considered uncommon and may be no specific in appearance, so that there is often a delayed diagnosis and the NET itself has many metastases.<sup>4</sup>

## 2. Material and method

This is descriptive study with a cross sectional approach which aims to determine immunohistochemical appearance of chromogranin A and synaptophysin as markers of neuroendocrine tumor (NET) in the histopathological preparation of appendicitis. This research was conducted in the Anatomical Pathology Laboratory at a private hospital in the city of Medan. This research was conducted from October 2021 to March 2022, after obtaining the ethical clearance from Health Research Ethics Committee in the Medical Faculty of Universitas Sumatera Utara. The samples of this study were embedded paraffin of 52 appendicitis tissues, and they were diagnosed as appendicitis which have fulfilled the inclusion and exclusion criteria. Samples were obtained through consecutive sampling method. The criteria of inclusion were that all paraffin blocks were sufficient with their medical records which consist of age, sex, main complaint, clinical diagnosis and histopathological diagnosis. The criteria of exclusion for this study were insufficient or damaged paraffin blocks which cannot be incised.

The microscope slide was examined by the author and two anatomical pathology specialists to diagnoses the appendicitis with suspect NET and the ones without NET. The next step is the immunohistochemical examination of chromogranin A and synaptophysin. If both immunohistochemistries show positive results then the diagnosis indicates the occurrence of a neuroendocrine tumor (NET).

Data collected in this research were processed by using statistical software of statistical package for the social science (SPSS 26) version and the result is presented in the table.

## 3. Result

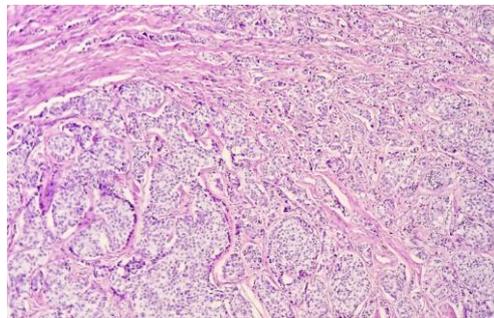
In this study, 52 samples appendicitis which have fulfilled the criteria of inclusion were examined to check the immunohistochemical chromogranin A and synaptophysin. The distribution of the characteristic and immunohistochemical of the samples in this study can be seen in the table below.

**Table.** Frequency distribution of characteristics of the patients with appendicitis based on age, gender, main complaint, clinical diagnosis, histopathological diagnosis and immunohistochemical chromogranin A and synaptophysin.

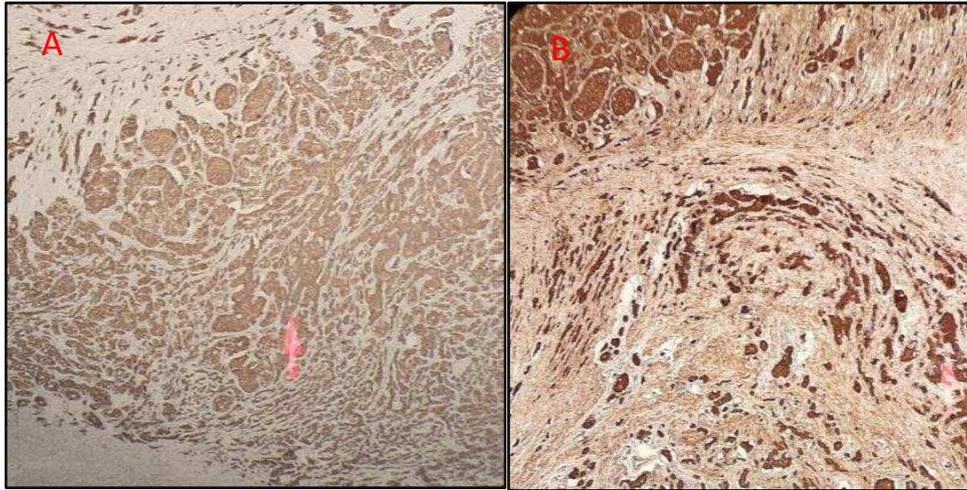
Characteristic	N	(%)
Age, average $\pm$ SD (Years old)	Average age 32.05 $\pm$ SD 14.64 (min-max: 7-60 years old)	
Sex		
Male	28	53,8
Female	24	46,2
Main complaint		
- Lower right abdominal pain	39	75,0
- Fever	6	11,5
- Nausea and vomiting	2	3,8
- etc.	5	9,6
Clinical diagnosis		
- Acute appendicitis	38	73,1

- Acute exacerbation of chronic appendicitis	3	5,8
- Perforated appendicitis	7	13,5
- etc.	4	7,7
<b>Histopathological</b>		
- Acute appendicitis + suspect NET	0	0
- Non Specific chronic appendicitis + suspect NET	4	7,7
- Chronic appendicitis and perforate + suspect NET	0	0
- Appendicitis with abscess + suspect NET	0	0
- Acute appendicitis without NET	0	0
- Non Specific chronic appendicitis without NET	29	55,8
- Chronic appendicitis with perforated without NET	4	7,7
- Apendicitis with abscess without NET	15	28,8
<b>Immunohistochemical</b>		
- Chromogranin A	1	1,9
- Synaptophysin	1	1,9

From the table we can see that average age of the patients is 32,05 years old with dominant patients of 28 male samples, main complaint is lower right stomach pain with clinical diagnosis majority as acute appendicitis, diagnosis histopathological as chronic non Specific appendicitis without NET is 29 samples, examination of immunohistochemical chromogranin A obtained positive 1 sample and synaptophysin obtained positive 1 sample.



**Figure 1.** Overview of Neuroendocrine tumor (NET) from research sample



**Figure 2.** Overview of immunohistochemical.  
 Figure A: Chromogranin A positive. Figure B: Synaptophysin positive

#### 4. Discussion

The number of samples diagnosed histopathologically as appendicitis at the Anatomical Pathology Laboratory at a private hospital in the city of Medan was 52 samples. In this study, 2 immunohistochemical stains were used as markers of neuroendocrine which aim to determine NET in histopathological preparations diagnosed as appendicitis.

The average age of patients with appendicitis in this study was at the age of 32,05 years in line with research conducted by Hartawan et al., (2020) finding 28 patients in the 17-25 year, Pujawan et al., (2019) finding patients in 32-43 year. This situation is caused by the development of lymphoid in the appendix that is reaching its maximum point in adolescence so that there can be a risk of lumen obstruction which can lead to the incidence of appendicitis. Appendicitis and NET occur almost at the same age as it is supported by the previous researches by Pujawan et al., (2019) reported on average 32-43 years and Egin et al., (2019) reported on average 40-50 years.<sup>4,5,6</sup>

In this study, as many as 28 cases of appendicitis were suffered by men, the results of this study are in line with the research conducted by Shahmoradi et al., (2021) and Hartawan et al., (2020). The high ratio of men to women is due to the percentage of lymphoid tissues in women is less than in men. However the occurrence of NET in the appendix are more common in women than in men. Abreu et al., (2018) reported appendectomy is more often performed in women than men.<sup>4,5</sup>

The main complaint of patients with appendicitis was lower right abdominal pain based on the findings of as many as 39 patients. This is because appendicitis is an inflammation of the vermiform appendix. Anatomically the appendix is a hollow organ located at the tip of the caecum, usually in the right lower quadrant of the abdomen. NET in the appendix is generally asymptomatic, about 80% of cases are found incidentally at surgery for appendicitis.<sup>7,8</sup>

Based on the clinical diagnosis, it is found that patients with the most clinical diagnosis suffered from acute appendicitis. In general from various sources, appendicitis is an acute inflammation of vermiform appendix which usually appears acutely within 24 hours after onset but can also appear in a more chronic condition. NET in the appendix is often found after the

patient undergo appendectomy with clinical indications of acute appendicitis in 80% of cases.<sup>9,10</sup>

From distribution of histopathological diagnosis, it was found that the most non-specific chronic appendicitis without NET is 29 patients and non-specific chronic appendicitis with suspect NET is 4 patients. This is in line with the research conducted by Hartawan et al., (2018) and Omotoso et al., (2018) where patients with appendicitis have pathological changes that are dominated by acute changes to chronic appendicitis, abscesses and perforation.<sup>9,11</sup>

From the table, it is known that the immunohistochemical display of chromogranin A was shown (positive) in 1 sample and synaptophysin was displayed (positive) in 1 sample. Immunohistochemical appearance with chromogranin A and synaptophysin positive was diagnosed as NET. Based on histopathological examination and two immunohistochemical examinations the diagnosis was made as NET. This is in accordance with the literature for establishing the diagnosis of NET if the immunohistochemical display shows a positive result with two used-antibodies-markers.<sup>12</sup>

## 5. Conclusion

Neuroendocrine tumor (NET) in the appendix was found in 1 histopathological sample which was diagnosed as appendicitis.

## 6. Competing interests

The author has had no financial interest with product or companies mentioned in the article.

## 7. Acknowledgment

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## 8. Ethical Approval

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study.

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