

The serum acetylcholinesterase (AChE) levels as an alternative diagnostic tool in pre-operative Hirschsprung's disease



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ABSTRACT

Background: The golden standard of Hirschsprung's disease diagnosis is through a histopathological examination of the aganglionic site. The significance of serum acetylcholinesterase (AChE) immunohistochemistry for diagnosing Hirschsprung's disease has been widely accepted. However, the study of serum AChE levels in Hirschsprung's disease patients is still scarce. We aimed to examine the serum levels of AChE in individuals with Hirschsprung's disease.

Methods: An analytical observational study with a cross sectional design was conducted on 29 patients with Hirschsprung's disease. We divided the patients into two groups: 14 in Hirschsprung's disease group and 15 in the control group. Serum AChE level was measured using the enzyme-linked immunosorbent assay (ELISA) method. The optimal cut-off value for detecting Hirschsprung's disease was determined using the ROC analysis.

Results: The prevalence of Hirschsprung was higher in males than in females. The average AChE level in the Hirschsprung group was 95.89 ± 51.11 Units/mL, while the average level of AChE in the control group was 44.45 ± 33.40 Units/mL. The optimal cut-off value was 46.615 Units/mL. We found that this study's sensitivity, specificity, positive predictive value, negative predictive value, and accuracy values were 83.3%, 70.6%, 66.7%, 85.7%, and 75.9%, respectively. There was a significant relationship between serum AChE levels and Hirschsprung's disease ($p=0.004$).

Conclusion: The AChE levels in blood serum can be used as an alternative diagnostic parameter for Hirschsprung's disease. Patients with Hirschsprung's disease had higher serum AChE levels than patients without Hirschsprung's disease.

Keywords: Acetylcholinesterase, diagnostic, Hirschsprung's disease.

Cite This Article: Wasito, D.C., Ali, I., Kusumowidagdo, F., Hariastawa, I.G.B.A. 2022. The serum acetylcholinesterase (AChE) levels as an alternative diagnostic tool in pre-operative Hirschsprung's disease. *Bali Medical Journal* 11(3): 1543-1547. DOI: 10.15562/bmj.v11i3.3610

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Received: 2022-08-01

Accepted: 2022-10-04

Published: 2022-11-13

INTRODUCTION

Hirschsprung's disease is one of the most prevalent congenital conditions affecting newborns' defecation. Hirschsprung's disease affects approximately 1 in 5000 live births.^{1,2} The absence of parasympathetic ganglion cells in the myenteric and submucosal plexus of the distal colon is a hallmark of Hirschsprung's disease. The common, typical clinical symptom of Hirschsprung disease is problems with passing the stool, which results in intestinal obstruction, abdominal distension, green vomiting, and failure to defecate.³ Hirschsprung disease also may cause another complication, such as Hirschsprung-associated enterocolitis (HAEC), which may cause dysregulation of gut microbiota in the intestine and, subsequently, a disturbance in the gut-

brain axis.^{4,5} The golden standard of Hirschsprung's disease diagnosis is through a full-thickness biopsy of the affected site, and the only effective treatment for this problem is surgery.^{6,8}

The purpose of a full-thickness biopsy is to confirm the existence of ganglion cells in the constricted area of the colon. If the proper specimens are collected by skilled pathologists using suitable infrastructure, the results will be more accurate. Invasive surgery is needed to get representative material from a full-thickness biopsy, which also runs the risk of providing a false-positive diagnosis of Hirschsprung hypoganglionosis disease.⁹⁻¹¹

Numerous investigations have been conducted to identify diagnostic alternatives that are minimally invasive and may be performed in primary health care centers because Hirschsprung's

disease demands invasive techniques as the gold standard diagnostic. According to several studies, acetylcholinesterase (AChE) blood levels have been found to rise in people with Hirschsprung's disease. Acetylcholine concentration rises in the affected colon due to the loss of ganglion cells, increasing the AChE activity expressed in the blood and nerve plexus. In erythrocytes, concentrated as a transmembrane protein, AChE enzymes are distributed throughout the blood with a 4:1 ratio between erythrocytes and plasma. The excessive direct release of AChE from the aganglionic intestinal segment and the increased production of AChE in the liver as a result of the release of acetylcholine into the bloodstream in the absence of adequate hydrolytic processes in the aganglionic intestinal segment are the two main contributors to

the increase AChE in the blood.^{10,12,13}

The significance of elevated blood AChE levels for the diagnosis of Hirschsprung's disease has been reported in various studies. Previous studies demonstrated that AChE was a biomarker with good performance in diagnosing Hirschsprung disease.^{9,13} However, no study examined the AChE levels in patients with Hirschsprung's disease in Indonesia. Considering the potential use of AChE as a diagnostic modality for Hirschsprung's disease, it is important to conduct this study in Indonesia. In this study, we aimed to examine the serum levels of AChE in individuals with Hirschsprung's disease.

MATERIALS AND METHODS

Study design and participants

We conducted an analytical observational study with a cross sectional design at Dr. Soetomo General Hospital, Surabaya, Indonesia. Study samples consisted of 2 groups: the Hirschsprung's disease group and the control group. The experimental group included patients diagnosed with Hirschsprung disease based on histopathologic examination. The control group included patients without gastrointestinal symptoms, such as the delayed passage of meconium, constipation, diarrhea, anemia, and growth retardation. The inclusion criteria in this study were patients diagnosed with Hirschsprung's disease by histopathological examination, aged 3 months – 17 years old, and willing to participate. We excluded patients aged less than 3 months with no complete medical record history, including the result of histopathology examination. Each parent of the study samples had signed an informed consent form and agreed to participate in the study. Patients whose parents refused to participate in the study were excluded from the sample.

Blood acetylcholinesterase level measurement

Acetylcholinesterase (AChE) is the main neurotransmitter in the enteral nervous system. AChE levels examined in this study were AChE levels concentrated in the serum. We measured the level of AChE in the serum sample using an enzyme-linked immunosorbent assay (ELISA).

Following the manufacturer's instruction, we measured the level of AChE using the commercially available kit (Human Acetylcholinesterase ELISA kit, Cat. No.: E-EL-H6031; Elabscience). The AChE was measured in IU/mL units.

Data collection

The parents of patients with Hirschsprung's disease, who were going to undergo definitive surgery and met the inclusion and exclusion criteria of the study, were explained the purpose and benefits of the examination. They were asked for approval to participate in the study by signing the informed consent and informed consent forms. Additionally, general information on study samples was gathered, including name, age, gender, residence, and phone number. Before having the last procedure, the study samples had their blood drawn to be examined for AChE level.

Statistical analysis

The research data were analyzed using SPSS version 26.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The chi-square test was used to analyze the variables for statistical comparison. A p-value of less than 0.05 was considered statistically significant. The best cut-off value was determined using receiver operating characteristics (ROC) analysis. In this study, the best cut-off was the one that yielded the highest accuracy value. The optimal cut-off value was obtained in the ROC plane study by using the point closest to the (0,1) corner. This cut-off was then utilized to convert the AChE ratio scale into a nominal scale of high and low AChE levels. This information was added to the Materials and techniques section. AChE level performance to diagnose Hirschsprung's disease was assessed by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy.

RESULTS

The subjects' demographic data

A total of 29 patients were included in this study. We divided the subjects into two groups; the first group was the Hirschsprung group, which consisted of 12 patients who were confirmed to have Hirschsprung's disease through the

suction biopsy results. In comparison, the second group is the control group. In this study, there were 20 males (69.0%) and 9 females (31.0%). From 20 male patients, there were 9 patients with Hirschsprung's disease (45.0%) and 11 without Hirschsprung's disease (55.0%). Meanwhile, most female patients were included in the control group (66.7%). The mean age of patients in the Hirschsprung group was 50.33 ± 62.18 months, while the control group was 47.12 ± 23.23 months. The age range of the study subjects in the group with Hirschsprung's disease was 3 – 216 months, and the control group was 6 – 104 months. The median age of the Hirschsprung group was 27.5 months, while the control group was 46 months. The demographic data of the subjects are presented in [Table 1](#).

Acetylcholinesterase level of the research subjects

We analyzed the serum AChE profile in the Hirschsprung's disease group; we found the mean level of AChE was 95.89 ± 51.11 Units/mL. The median value in this group was 87.93 Units/mL. The range of AChE levels in the group with Hirschsprung's disease was 27.43–204.93 Units/mL. Meanwhile, in the control group, we found the average level of AChE was 44.45 ± 33.40 Unit/mL. The median value in this group was 29.75 Units/mL. The range of AChE levels in the group with Hirschsprung's disease was 10.46–97.57 Units/mL. Statistical analysis showed a significant difference between the distribution of AChE levels in the Hirschsprung group and the control group ($p=0.003$). The distribution of AChE levels in the Hirschsprung group was higher than AChE levels in the control group. The profiles of AChE levels are shown in [Table 2](#).

The determination of optimal cut-off value for acetylcholinesterase level

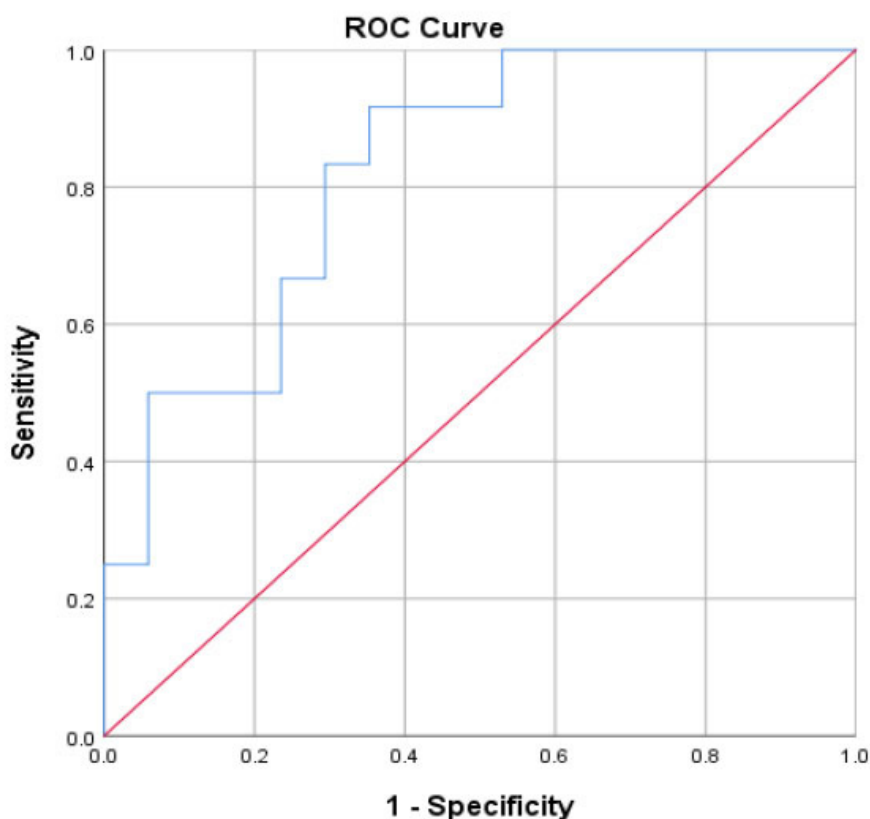
Determination of the optimal cut-off point to determine the diagnosis of Hirschsprung using ROC analysis. The ROC curve is obtained using ROC analysis with an area under the curve (AUC) value of 0.824 (95%CI=0.674 – 0.973) and a P value of 0.003. From the curve, it is found that the optimal cut-off value is 46.615

Table 1. Demographic data of the study subject.

Characteristic	Group		p-value
	Hirschsprung	Control	
Sex			
Male (n = 20)	9 (45.0%)	11 (55.0%)	0.555
Female (n = 9)	3 (33.3%)	6 (66.7%)	
Age			
Minimum	3 months	6 months	0.231
Maximum	216 months	104 months	
Average	50.33 months	47.12 months	
Median	27.5 months	46 months	
SD	62.18 months	23.23 months	

Table 2. Profiles of the acetylcholinesterase levels.

Serum AChE level (IU/mL)	Group	
	Hirschsprung	Control
Minimum	27.43	10.46
Maximum	204.93	97.57
Average	95.89	44.45
Median	87.93	29.75
SD	51.11	33.40

**Figure 1.** Receiver operating characteristic (ROC) analysis for the determination of the cut-off value.

Units/mL. The ROC curve is shown in Figure 1.

The association between AChE levels and Hirschsprung disease

In this analysis, research subjects were divided into two groups: high serum AChE levels and low serum AChE levels. The group was determined based on the optimal cut-off value obtained by the previous ROC analysis. With a cut-off value of 46.615 Units/mL, we found that there were 14 patients with low serum AChE levels (14/29; 48.3%) and 15 patients with high serum AChE levels (15/29; 51.7%). In the group of patients with high serum AChE levels, 10 had Hirschsprung's disease (10/15; 66.7%), and 5 were included in the control group (5/15; 33.3%). In contrast, the group of patients with low TNF- levels showed a lower percentage in the group with Hirschsprung's disease than in the control group (Hirschsprung: 2/14; 14.3% vs. control: 12/14; 85.7%). Statistical analysis showed a significant relationship between serum AChE levels and Hirschsprung's disease ($p = 0.004$) (Table 3).

Furthermore, we analyzed the performance of serum AChE levels as a diagnostic modality as indicated by several diagnostic parameters, namely sensitivity, specificity, positive predictive value, negative predictive value, and accuracy. We found that this study's sensitivity, specificity, positive predictive value, negative predictive value, and accuracy values were 83.3%, 70.6%, 66.7%, 85.7%, and 75.9%, respectively.

DISCUSSION

This study aimed to analyze the relationship between serum AChE levels and the incidence of Hirschsprung's disease. Hirschsprung's disease is a congenital disorder characterized by the presence of an aganglionic segment of the intestine. The examination that becomes the gold standard for diagnosing Hirschsprung's disease is the histopathological examination, where evidence of aganglionic bowel segments must be obtained.^{14,15} Histopathological examination can be performed on

Table 3. Association between AChE levels and Hirschsprung disease.

AChE group	N=29	Group		P
		Hirschsprung (N=12)	Control (N=17)	
High, n (%)	14	2 (14.3)	12 (85.7)	0.004*
Low, n (%)	15	10 (66.7)	5 (33.3)	

*Chi-Square: statistically significant if p-value less than 0.05.

bowel specimens obtained from medical procedures that tend to be invasive, such as full-thickness biopsy and rectal suction biopsy.^{15,16} Although classified as minimally invasive, suction biopsies performed on pediatric patients require an anesthetic procedure. Therefore, the process followed to obtain histopathological specimens is not simple and still carries the risk of medical procedures.

The use of acetylcholinesterase as a diagnostic parameter in Hirschsprung's disease is based on the fact that there are prominent cholinergic nerve fibers in Hirschsprung's patients and these nerve fibers contain an increased amount of AChE expression.¹⁷ The presence of AChE can be identified by staining using the immunohistochemical method.¹⁸ However, various studies around the world have also shown that an increase in AChE levels is not only found in aganglionic intestinal specimens but also in blood, both in serum and erythrocytes.¹⁹ Therefore, we are interested in examining the AChE levels in the serum of patients with Hirschsprung's disease in Indonesia. This is supported by the fact that the research related to measuring AChE levels in Hirschsprung patients in Indonesia is still scarce.

In this study, we collected samples from patients with confirmed Hirschsprung's disease using a suction biopsy and samples from patients without Hirschsprung's disease who served as the control group. We used ROC analysis to determine the most optimal cut-off value for the best accuracy. We found that serum AChE level is a potential diagnostic method to diagnose Hirschsprung's disease, characterized by a relatively good AUC value. This cut-off value is the most optimal in the population we studied, so in our next analysis, this cut-off value will be used. After dividing the sample into two groups using the cut-off value, we found a statistically significant association

between the AChE levels and the incidence of Hirschsprung's disease. This indicates that serum AChE levels can be used as a diagnostic modality to differentiate between patients with Hirschsprung's disease and normal patients with relatively high accuracy. The results obtained in this study are in line with previous studies, which also reported that AChE levels in the serum of Hirschsprung patients were higher than AChE levels in subjects who were not diagnosed with Hirschsprung.²⁰ Another study showed that measuring AChE levels in patient serum samples was an easy, relatively inexpensive, and non-invasive test. This supports using serum AChE levels as a potential Hirschsprung diagnostic parameter, especially in health centers with limited facilities.

Based on age, we found that the prevalence of Hirschsprung was higher in males than in females, although there was no significant association between sex and Hirschsprung's disease found in this study. Our result was in concordance with previous study results. Previous studies revealed that males had a higher prevalence of Hirschsprung's disease. Hirschsprung disease is reported to affect males 3 – 4 times higher than females.^{21,22}

Age is one of the important factors that must be taken into account in this study. In this study, we excluded patients under 3 months of age. Patients under 3 months of age can potentially get false positive results. AChE is a very important substance for infants in early life to promote cell adhesion and neurogenesis in the developing enteric nervous system. Therefore, early in life, high AChE levels are common. This is why the use of AChE levels in the blood as a diagnostic parameter of Hirschsprung's disease must be carried out carefully and consider the patient's age. In this study, it was also found that there was no significant difference in the age distribution between the group of patients with Hirschsprung's disease

and the control group. This indicates that age has no relationship with the incidence of Hirschsprung's disease. This is not surprising because Hirschsprung's disease is an inherited disorder, so age at diagnosis is not a variable related to the pathophysiology of Hirschsprung's disease.²³

We realized that there were limitations in this study. First, the number of research samples is relatively small. Further research with a larger sample size is needed to provide more accurate data. Second, this study was conducted in one health center, so the results of this study may not be used to represent the situation in the entire population in Indonesia in general. It is necessary to conduct further research involving various health centers throughout Indonesia so that the results obtained can provide a more accurate picture of the diagnostic application of AChE for patients with Hirschsprung's disease in Indonesia. Third, in this study, we only measured AChE levels in serum but did not measure AChE levels in tissues. So in this study, there was no comparison for the results of measuring AChE levels in research subjects. In addition, we did not measure the length of the aganglionic segment of the patients with Hirschsprung disease. Further study might be needed to confirm the association between the aganglionic segment length and the serum AChE level.

CONCLUSION

We can conclude that AChE levels in blood serum can be used as an alternative diagnostic parameter for Hirschsprung's disease. Patients with Hirschsprung's disease had higher serum AChE levels than those without Hirschsprung's disease.

CONFLICTS OF INTEREST

No competing interests were declared.

ETHICAL CLEARANCE

This study was reviewed and approved by the Medical Ethical Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (No. 0418/KEPK/V/2022), following the guidelines of the Declaration of Helsinki.

AUTHOR CONTRIBUTION

Conceived the study: SK. Designed the study: DCW, IA, and IGBAH. Analyzed the data: DCW, IA, FK, and IGBAH. Wrote the manuscript: DCW, FK, and IA. Review the manuscript: FK, IA and IGBAH.

FUNDING

The author(s) received no financial support for this article's research, authorship, and publication.

REFERENCES

- Poerwosusanta EE, Hariastawa IGBA, Setiawan A, Sulistiawati S, Ary Wibowo A. Correlation between the Initial-Surgery Age with The Hirschsprung's Disease Patient Stooling Patterns in Ulin Hospital Banjarmasin, South Kalimantan, Indonesia In 2012-2015. *Maj Biomorfologi*. 2021;31(2):66-69.
- Fury ER, Athiyyah AF, Darma A. Diagnostic Accuracy of Recto-Sigmoid Index Evaluation on Barium Enema in Hirschsprung's Disease. *Indian J Forensic Med Toxicol*. 2020;15(1):1045-1051.
- Barmadisatrio B, Hariyono H. Case Report: Secondary Laparoscopic Assisted Transanal Endorectal Pull-Through. A Pre-Eliminary Study. *Folia Medica Indonesia*. 2017 ;52(1):74-79.
- Neuvonen MI, Korpela K, Kyrklund K, Salonen A, de Vos W, Rintala RJ, et al. Intestinal Microbiota in Hirschsprung Disease. *J Pediatr Gastroenterol Nutr*. 2018;67(5):594-600.
- Ranuh R, Athiyyah A, Darma A, Risky V, Riawan W, Surono IS, et al. Effect of the probiotic *Lactobacillus plantarum* IS-10506 on BDNF and 5HT stimulation: role of intestinal microbiota on the gut-brain axis. *Iran J Microbiol*. 2019;11(2):145-150.
- Russell MB, Russell CA, Niebuhr E. An epidemiological study of Hirschsprung's disease and additional anomalies. *Acta Paediatr Int J Paediatr*. 1994;83(1):68-71.
- Chia ST, Chen SCC, Lu CL, Sheu SM, Kuo HC. Epidemiology of Hirschsprung's Disease in Taiwanese Children: A 13-year Nationwide Population-based Study. *Pediatr Neonatol*. 2016;57(3):201-206.
- Kessmann J. Hirschsprung's disease: diagnosis and management. *Am Fam Physician*. 2006;74(8):1319-1322.
- Yanagihara J, Iwai N, Tsuto T, Majima S. Acetylcholinesterase activity in rectal mucosa and blood in the diagnosis of Hirschsprung's disease. *Jpn J Surg*. 1983;13(1):42-48.
- Kuvelkar N, D'Souza S, Vidhyashree K, Shankar G, Bukel M, Noronha S, et al. Estimation of plasma and RBC acetylcholinesterase in children: An evaluation tool for Hirschsprung's disease? *Indian J Pathol Microbiol*. 2021;64(2):266-176.
- Haricharan RN, Georgeson KE. Hirschsprung disease. *Semin Pediatr Surg*. 2008;17(4):266-275.
- Okasora T, Okamoto E, Kuwata K, Toyosaka A, Ohashi S, Ueki S. Serum and Erythrocyte Acetylcholine Esterase in Hirschsprung's Disease. 1983;(1):298-300.
- Chalkoo MA, Rashid A, Kakroo SM, Razvi SA, Wani AA. Role of Erythrocyte Acetylcholinesterase in the Diagnosis of Hirschsprung's Disease. 2013;3(2):79-82.
- Szylberg L, Marszałek A. Diagnosis of Hirschsprung's disease with particular emphasis on histopathology. A systematic review of current literature. *Prz Gastroenterol*. 2014;9(5):264-269.
- Ambartsumyan L, Smith C, Kapur RP. Diagnosis of Hirschsprung Disease. *Pediatr Dev Pathol*. 2019;2;23(1):8-22.
- Muise ED, Hardee S, Morotti RA, Cowles RA. A comparison of suction and full-thickness rectal biopsy in children. *J Surg Res*. 2016;201(1):149-155.
- Agrawal RK, Kakkar N, Vasishta RK, Kumari V, Samujh R, Rao KLN. Acetylcholinesterase histochemistry (AChE)--A helpful technique in the diagnosis and in aiding the operative procedures of Hirschsprung disease. *Diagn Pathol*. 2015;10:208.
- Moore SW, Johnson G. Acetylcholinesterase in Hirschsprung's disease. *Pediatr Surg Int*. 2005;21(4):255-263.
- Boston VE, Cywes S, Davies MR. Serum and erythrocyte acetylcholinesterase activity in Hirschsprung's disease. *J Pediatr Surg*. 1978;13(4):407-410.
- She YX, Shi CR, Chen JZ, Wu YL. Observation on erythrocyte acetylcholinesterase (AChE) in infants and children with Hirschsprung's disease. *J Pediatr Surg*. 1984;19(3):281-284.
- Jannot A-S, Amiel J, Pelet A, Lantieri F, Fernandez RM, Verheij JBG, et al. Male and female differential reproductive rate could explain parental transmission asymmetry of mutation origin in Hirschsprung disease. *Eur J Hum Genet*. 2012;20(9):917-920.
- Granéli C, Dahlin E, Börjesson A, Arnbjörnsson E, Stenström P. Diagnosis, Symptoms, and Outcomes of Hirschsprung's Disease from the Perspective of Gender. *Surg Res Pract*. 2017;2017:1-8.
- Nugraha, HG, Miranti PI. Comparison of the sacral ratio between children with Hirschsprung disease and non-Hirschsprung. *Bali Medical Journal*. 2021;10(3):864-866.



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