

Role of Erythrocyte Acetylcholinesterase in the Diagnosis of Hirschsprung's Disease

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ABSTRACT

BACKGROUND: Serological tests have not been validated in the diagnosis of Hirschsprung's disease. The aim of the present study was to evaluate the role of erythrocyte acetylcholinesterase in the diagnosis of Hirschsprung's disease.

METHODS: This study was a prospective study conducted on 30 patients clinically suspected to have Hirschsprung's disease who were admitted in a tertiary care referral center in Srinagar, India. All patients had erythrocyte acetylcholinesterase measured and underwent a full thickness rectal biopsy.

RESULTS: In our study, the mean (\pm standard deviation) age at presentation was 3.51 ± 0.4 years. Most patients were males (76.7%). Rectal biopsy was considered as the gold standard for the diagnosis of Hirschsprung's disease with absent ganglion

cells in 22 (73.3%) and absent ganglion cells with nerve hypertrophy in other 4 (13.3%) patients. Preoperative erythrocyte acetylcholinesterase had a diagnostic accuracy of 66.7% and a sensitivity of 84.6% with a specificity of 75%, positive predictive value (PPV) of 95.6% and negative predictive value (NPV) of 42.9%. Mean postoperative acetylcholinesterase levels were 11.5 KU/L, significantly lower than the mean preoperative levels of 17.6 KU/L ($p < 0.05$).

CONCLUSION: Erythrocyte acetylcholinesterase can be used as an initial screening test in clinically suspected cases of Hirschsprung's disease due to its high sensitivity. It confers an additional advantage in that it is comparatively non-invasive and does not require any preparation.

Key Words: Hirschsprung's Disease; Erythrocyte Acetylcholinesterase; Rectal Biopsy; Aganglionosis

INTRODUCTION

Hirschsprung's disease is a congenital disorder of the bowel motility characterized by absence of ganglion cells in the myenteric and submucosal plexuses in distal colon. The incidence is about 1 in 5000 live births [1]. The embryologic defect in Hirschsprung's disease is the failure of craniocaudal migration of neural crest cells in the distal part of colon [2]. Mutations in the Ret gene and endothelin-B receptor gene have been associated with it [3, 4]. The most frequent clinical features of Hirschsprung's disease are constipation, abdominal distension, vomiting and failure to thrive [1]. In the absence of these ganglion cells, an abundance of acetylcholine is

present in the gut wall and correspondingly an excess of the enzyme acetylcholinesterase. This excess of acetylcholinesterase in submucosa of the gut is thought to be the source of increased acetylcholinesterase levels in serum and erythrocytes. A full thickness rectal biopsy remains the gold standard for the diagnosis of Hirschsprung's disease [5]. The aim of present study was to study the role of erythrocyte cholinesterase in the diagnosis of Hirschsprung's disease.

METHODS AND MATERIALS

This study was conducted in a tertiary care referral centre in Srinagar, India from June 2007

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to November 2009. Thirty patients with clinically suspected Hirschsprung's disease presenting to the surgical emergency or outpatient department (OPD) were enrolled in the study. Neonates were excluded from the study because of the lack of facilities for postoperative monitoring in our hospital. Other causes of constipation were ruled out before recruiting the patients for the study. A detailed history regarding the presence of symptoms, gestational history of mother, milestones, history of consanguinity, family history, and history of drug intake was taken. Besides general physical and systemic examination, local examination of anorectal region for congenital malformations was performed on all patients enrolled in the study. Routine investigations including complete blood count, kidney function test, liver function test, thyroid function test, electrolytes, blood glucose, abdominal radiographic films, barium enema and rectal biopsy were performed on all the patients. A full thickness rectal biopsy was obtained at least 2.5 cm above the dentate line. Absence of ganglion cells and hypertrophy of nerve trunks was taken as diagnostic of Hirschsprung's disease.

For the estimation of erythrocyte acetylcholinesterase, 2 mL of blood was drawn from the patient and was immediately heparinized. The samples were kept on ice and assayed the same day for cholinesterase activity. Blood was assayed with the Epoch Microplate Spectrophotometer [Biotek Inc., Winooski, VT, USA] according to the standard Ellman procedure [5]. 220 μ L of pH 8 phosphate buffer and 10 μ L DTNB (Dithiobisnitrobenzoic acid) were added to each well, followed by 30 μ L of sample. The samples were incubated for 5 minutes and then 30 μ L of acetylthiocholine were added. The substrate, pH, and chromogen concentrations used for the commercial kit assay were as specified in the package insert for the BMC kit for manual acetylcholinesterase assays (Boehringer-Mannheim Corporation, Indianapolis, Indiana). The erythrocyte acetylcholinesterase was calculated by the following formula:

Erythrocyte acetylcholinesterase (KU/L red cells)

$$= \frac{\Delta A_{412} / \text{min}}{\text{PCV}} \times 114 \quad \text{where PCV is}$$

packed cell volume and

ΔA is change of absorbance per millimeter.

The change in absorbance was assayed at 412 nm spectrophotometric wavelength. The normal

range of erythrocyte acetylcholinesterase was taken from 8 to 13 KU/L. Statistical analysis was done by *Graphpad Instat Version 3.10* for Windows [Graphpad Software Inc., San Diego, California, USA]. To calculate the p value, "Fisher's exact test" or "unpaired t-test" was used, as and when needed. The cut off value for level of significance was taken as p value < 0.05. This study was approved by the institutional review board.

RESULTS

The mean (\pm standard deviation) age of the 30 patients at presentation was 3.51 ± 0.4 years. Majority of patients (53.3%) presented between 2 years to 5 years. The socio-demographic profile of our study population is given in Table 1. Rectal biopsy was diagnostic of Hirschsprung's disease in 26 (86.7%) of these suspected cases with 22 of these patients showing absent ganglion cells and the other 4 showing absent ganglion cells with nerve hypertrophy. The preoperative erythrocyte acetylcholinesterase ranged from 8 KU/L to 25 KU/L. In the majority of patients (63.3%), the levels ranged from 13 KU/L to 23 KU/L. Mean (\pm standard deviation) value of erythrocyte acetylcholinesterase in our study was 17.62 ± 1.17 KU/L. Erythrocyte acetylcholinesterase was elevated (>13 KU/L) in 22 (84.6%) patients with biopsy documented Hirschsprung's disease and was normal in other 4 (15.4%). In the other 4 patients who were clinically suspected to have Hirschsprung's disease, but had negative rectal biopsy, erythrocyte acetylcholinesterase was high in 1 (25%).

The calculated sensitivity, specificity, positive predictive value and negative predictive value of erythrocyte cholinesterase in the diagnosis of Hirschsprung's disease in this carefully selected group of patients was 84.6%, 75%, 95.6% and 42.9% respectively giving a diagnostic accuracy of 66.7%. The mean (\pm standard deviation) levels of erythrocyte cholinesterase obtained at 2 weeks postoperatively of Duhamel procedure were 11.47 ± 0.89 KU/L which were significantly decreased as compared to the mean (\pm standard deviation) preoperative levels of 17.62 ± 1.17 KU/L (p < 0.05), suggesting that most likely the source of increased cholinesterase activity was the aganglionic segment of colon.

DISCUSSION

In this study of patients with clinically suspected

Table 1: Socio-demographic characteristics of the study population

Feature	Male	Female
Number	23	7
Age (Mean \pm Standard Deviation)	3.56 \pm 0.5 years	3.40 \pm 0.5 years
Abdominal Distention	15	5
Growth Retardation	6	3
Fecaloma	5	3
Anemia	4	2

Hirschsprung's disease, we found that testing for erythrocyte cholinesterase has high sensitivity and positive predictive value making this test a potential tool in the diagnosis of this disease. Hirschsprung's disease is commonly encountered by a pediatric surgeon in his/her clinical experience. Traditionally, the diagnosis of Hirschsprung's disease has been based on rectal biopsy which is considered the gold standard for its diagnosis [6, 7]. The present study was aimed to find a suitable non-invasive investigation which could improve the diagnosis of Hirschsprung's disease before a patient is subjected to rectal biopsy. Erythrocyte acetylcholinesterase promises to be such a marker.

In our study, 30 patients with clinical suspicion of Hirschsprung's disease were evaluated with rectal biopsy and erythrocyte cholinesterase level. Most patients in our study were below 5 years. Yoshida et al reported that Hirschsprung's disease is mostly manifested within the first several weeks of life, and it is diagnosed in persons aged 5 years or younger [8]. Because we have excluded neonates from our study, we had very few patients below the age of 2.

Sibling history of the disease was present in 2 cases (6.7%). Reding R et al observed a sibling history of this disease in 7% of his 59 patients [9]. Sibling history was observed in patients with long segment disease. Twenty-three (76.7%) of our patients were males giving a male to female ratio of 3.28:1. Jung et al reported a male to female ratio of 3.6:1, which is consistent with our findings [10].

Rectal biopsy was diagnostic of Hirschsprung's disease in 26 (86.66%) patients with 22 without any ganglion cells and the other 4 with absent ganglion cells and nerve hypertrophy [Figure 1]. Similar to our study, Swenson et al considered rectal biopsy as a gold standard for the diagnosis of Hirschsprung's disease [6]. Campbell and Noblett justified rectal biopsy to be very accurate and diagnostic, provided the specimen

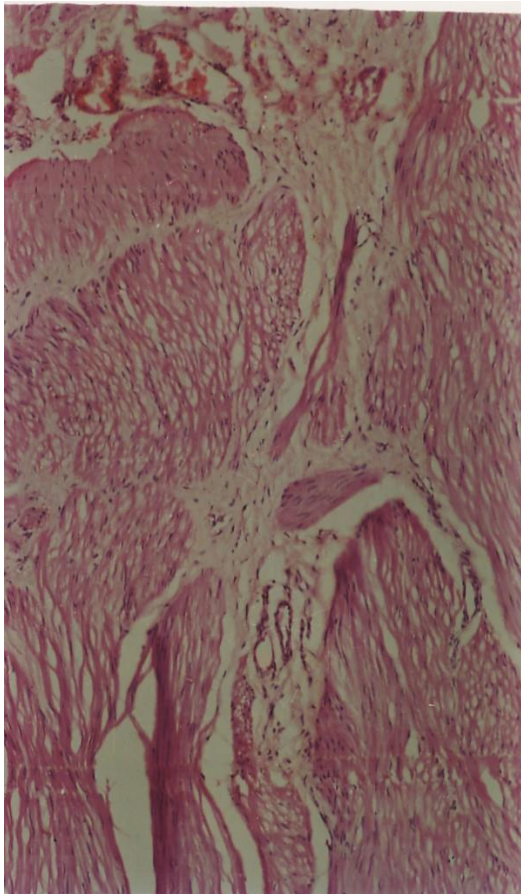
has been taken above the hypoganglionic zone, 3 cm cranial to the pectinate line [7]. Only these twenty six patients were operated for Hirschsprung's disease, the other four patients were followed up as outpatients.

The calculated sensitivity, specificity, positive predictive value and negative predictive value of erythrocyte cholinesterase in the diagnosis of Hirschsprung's disease was 84.62%, 75%, 95.65% and 42.86% respectively, giving a diagnostic accuracy of 66.67%. She YX et al (1984) reported that the mean value of preoperative erythrocyte acetylcholinesterase in patients of Hirschsprung's disease was significantly higher than that of normal children [11]. Yanagihara J et al (1983) in their study of 13 patients with Hirschsprung's disease found that preoperative erythrocyte cholinesterase activity correlated well with acetylcholinesterase activity in rectal biopsy specimens [12]. Boston VE et al (1978) in their study of 22 patients reported that in 12 patients of biopsy documented Hirschsprung's disease the value of erythrocyte acetylcholinesterase was significantly higher than the 10 patients in whom the diagnosis was excluded [13]. The calculated sensitivity (84.62%) of erythrocyte acetylcholinesterase may potentially make it a potential screening tool. Most studies on the subject point to the use of the assay as an effective screening test for the diagnosis of Hirschsprung's disease [12].

As the part of the gut with increased levels of acetylcholinesterase is removed during surgery, the levels of this enzyme should decrease in the postoperative period. In our study the postoperative erythrocyte acetylcholinesterase was done at 2 weeks after surgery and was found to be significantly lower than the preoperative levels in 23(88.5%) patients while the levels did not change significantly in 3 (11.5%) patients in the postoperative period ($p < 0.0001$). The mean levels of erythrocyte cholinesterase obtained at 2 weeks postoperatively were also significantly decreased than the mean preoperative levels ($p < 0.05$). In a study conducted by She YX et al, the erythrocyte acetylcholinesterase showed a tendency to decline with time in the postoperative period [11].

Our study has several potential limitations. First, the study population was not from general population but was from the patients referred to the pediatric surgery service with a suspicion of Hirschsprung's disease, thus limiting the generalizability of our findings. We also excluded neonates from the study sample due to logistic issues. Second, the use of erythrocyte

Figure 1: Rectal biopsy showing aganglionosis (hematoxylin and eosin staining)



acetylcholinesterase test as we propose can be used once patients have been selected based on their clinical characteristics and there is suspicion of Hirschsprung's disease; it cannot be used in the general population as the prevalence of the disease is so low that the most of the positive results will be false positives and may result in increased anxiety in patient's parents and increased use of rectal biopsies. We have a relatively small sample size; however, considering the rarity of the condition, this sample size is a significant number from one center.

CONCLUSION

From our study we conclude that erythrocyte acetylcholinesterase has a potential to be used as a screening test for the diagnosis of Hirschsprung's disease due to its high sensitivity in a carefully selected population. It confers an additional advantage in that it is comparatively non-invasive and does not require any preparation. Future studies should examine this

test as a screening tool and identify proper cut-off values to decrease the chances of misclassification.

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