

Original Article

Determining The Diagnostic Value Of Neuron Specific Enolase Staining Of The Mucosal-submucosal Rectal Biopsies Obtained From Patients Suspected Of Hirschsprung's Disease

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ABSTRACT

Background and Objective: Diagnosis of Hirschsprung's disease (HD) as the most common cause of neonatal intestinal obstruction is based on the presence of aganglionosis from seromuscular or full thickness biopsy. Due to the complication of full thickness or seromuscular rectal biopsy, mucosal-sub mucosal biopsy is more intended. However, interpretation of these biopsies stained with hematoxylin and eosin (H&E) and even using immunohistochemical (IHC) methods such as acetylcholine esterase is often problematic. Although neuron-specific enolase staining (NSE) is an available and easy method to perform for diagnosis of HD, however, our knowledge on its specificity is not adequate. Therefore, this study was aimed to determine the diagnostic value of NSE on the mucosal-sub mucosal rectal biopsy for the diagnosis of HD and the allied disorders deficit.

Materials and Methods: This study was conducted on 65 mucosal-submucosal and 65 seromuscular rectal biopsies (standard) obtained from the patients suspected of HD and allied disorders referred to the Avicena and Shafa hospitals (Sari, Iran) from April 2003 to September 2004. Two biopsies were taken from each patient: the mucosal-submucosal biopsy was stained by NSE and H&E staining was used for seromuscular samples. The prepared slides were observed and evaluated at double blind condition and the results were compared.

Results: Sensitivity, specificity, efficiency, positive and negative predictive values in the diagnosis of HD in NSE method were 100%, 84.2%, 89.1%, 81.8%, and 100% respectively ($p < 0.05$). On evaluation of hypoganglionosis, there were one false-negative and nine false-positive.

Conclusion: In NSE staining, finding ganglion cell definitely rules out HD, but lack of ganglion cell confirms 81.8% of H.D cases. Thus, NSE staining on mucosal- submucosal specimens is possibly adequate for establishing the presence or absence of ganglion cells.

Key words: Hirschsprung's disease, Allied disorders, Neuron-specific enolase, Immunohistochemistry

Received: 7 May 2006

Accepted: 22 July 2006

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Introduction

Hirschsprung's disease is a common congenital disorder (1) and has been known as the most prevalent etiological cause of neonatal intestinal obstruction (2). The definite diagnosis of this disorder is based on the demonstration of aganglionosis in seromuscular or full thickness rectal biopsies and excluding allied disorders (Hyperganglionosis and hypoganglionosis) must be excluded (1, 3, 4). After clarifying aganglionosis surface at two submucosal and intra muscular regions, mucosal-sub mucosal rectal biopsy is preferred to surgical procedures and their related complications (perforation, bleeding, and stricture) and application of general anesthesia. The former procedure is relatively easy, economically cost effective, requires less hospitalization, possible to perform in the clinic on condition of using rectal suction tool (1, 3, 5) with less complications. Meanwhile, interpretation of mucosal-submucosal biopsy is more difficult than seromuscular biopsy. Submucosal plexus and ganglionic cells are smaller with irregular spreading as compared to the myenteric plexus. In staining with H&E method, ganglion cells may be mistaken with endothelial cells, fibroblasts, histocytes, and lymphocytes (3). Acetylcholine esterase staining has been the first used IHC marker for rectal mucosal submucosal biopsy (6) but has had some limitations as fresh specimen is required and due to submucosal bleeding its interpretation is difficult (5). In addition, it may be followed by false-negative and false-positive results (3, 7, 8). Therefore, although studies on many immunohistochemical markers have been undertaken, but the pathologists still do not agree with any particular marker (9-13). Thus, attention should be paid to NSE and in this respect some studies have been done, but there are contradictions between the results and no proper report on its specificity and accuracy exists. Although Lampert and Hall (14), Vinore et al (13), Frykberg et al (15), and Machenzie et al (16) reported usefulness of NSE in the diagnosis of HD, but they did not provide any information on its diagnostic value. In the report given by Ana Margarida et al (17), no priority was given for NSE and H&E in the study

of submucosal specimen. In addition, Talebi et al in their study on rectal biopsy reported the diagnostic significance of cathepsin D, CD 56, and NSE (12). Since NSE is relatively easy and cheap, possible to do on paraffin block, and can demonstrate ganglion cells in addition to nerve fibers, and also lack of information on its diagnostic value on mucosal-sub mucosal rectal biopsy in patients suspected of HD and allied disorders, this study was conducted to determine the diagnostic value of this marker on such biopsies for this disorder.

Materials and Methods

This prospective study was undertaken on the mucosal-submucosal rectal specimens collected from 67 patients suspected of having HD and allied disorders, referred to Boalicina and Shafa hospitals (Sari, Iran) from April 2003 to September 2004. Two rectal biopsies (one mucosal-submucosal and the other full thickness or seromuscular) two centimeters or more in length above the anal valve were taken. All specimens were fixed with 10% formalin solution and fixed for 12-18 hours, paraffin blocks were then prepared, 4 µm thick sections were prepared from each sample, 5 to 10 of them stained with H&E and two with NSE (Polyclonal Rabbit Anti-NSE) according to the manufacturer recommendation (DAKO, Denmark). The prepared slides were studied double-blind for presence and number of ganglion cells and each sample was compared with its standard. Data were analyzed using SPSS software (version 11.0). Sensitivity, specificity, and positive and negative predictive values were also calculated for the NSE method.

Histopathologic diagnosis criteria

a. Hirschsprung's disease: Lack of ganglion cell in neural plexus (1), b. Hypoganglionosis: Presence of 1-2 ganglion cells in each neural plexus or intramural ganglia (10, 17), and c. Intestinal neural dysplasia (IND): Presence of hypertrophic neural ganglia containing normal (3-5) or proliferated large ganglion cell with large perikaryon and bizarre nucleus, and occasionally isolated ganglion cell in submucosa (1, 17, 18).

Results

Out of 67 collected samples, 65 met the inclusion criteria (47 boys and 18 girls). Their age ranged from one day to 10 years and in this respect their mean, median, and mode were 1.7 year, 60, and 1 day respectively. Meanwhile, 63 patients had complained of constipation and two out of them had imperforated anus. Based on the routine method (standard), the patients were categorized as 27 negative cases and 38 positive cases regarding the presence of ganglion cells. In addition, out of these 38 patients, 33 cases were normal and 5 cases were hypoganglionic. No hyperganglionosis was also observed in this study. The obtained results in this study using NSE method as compared with standard one showed no false-negative and false-positive cases were 6 (9.2%). The diagnostic values of NSE method are shown in Table 1.

Table 1. The results of NSE diagnostic value in detecting HD

Specificity	84.2 %
Sensitivity	100 %
Positive predictive value (PPV)	81.8%
Negative predictive value (NPV)	100%
Efficiency	89.1 %
P value	< 0.05

$p < 0.05$ indicates that the difference between this test method and the standard one is statistically significant and the diagnostic efficiency of these two methods is different. In this study, 38 GC-positive cases were differentiated into normal (33) and hypoganglionosis (5) using H&E method. In this regard, 33 normal GC-positive cases were further differentiated into normal (19), hypoganglionosis (9), and HD (5) based on the NSE method. The 5 hypoganglionosis cases as diagnosed by H&E method were differentiated into hypoganglionosis (4) and HD (1).

Discussion

Currently, only few IHC markers including NSE have been reported for identification of the ganglion cells (1, 19), and for ruling out of HD,

observation of such cells is necessary for HD diagnosis based on its definition. In the study of mucosal-submucosal biopsies by NSE, the neural plexus becomes brownish in a colorless background and is easily observed even by a low power field. Therefore, finding ganglion cells will be facilitated. The cytoplasm of ganglion cells is brownish and their nucleolus is a colorless (figure 1).

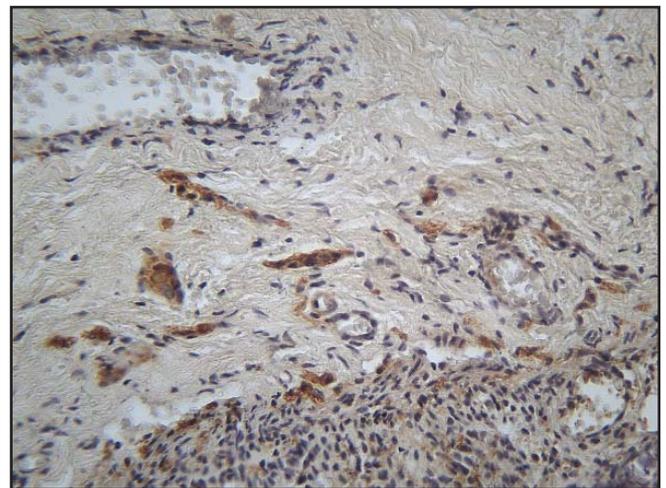
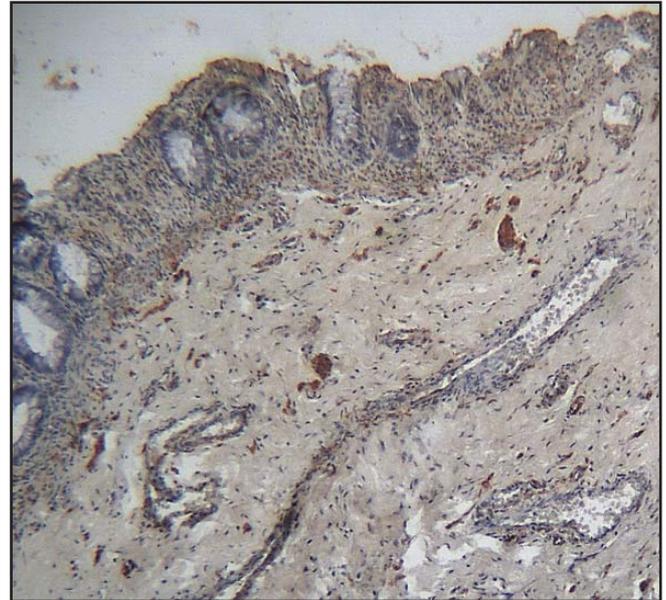


Figure 1 : The Cytoplasm of Ganglion Cells is Brownish (Positive) and Nuclei are Colorless

The inflammatory cells may show weak reaction, but considering ganglion cells position in the neural plexus and knowing its cytology makes it possible not to mistake it with other cells. In this study, in 27 of patients with HD, no false-negative was observed using NSE method, hence, a sensitivity of 100% is achieved by this marker in the diagnosis of HD.

Athow et al (1990) studied 60 HD suspected patients using acetylcholine esterase on mucosal-submucosal biopsies and 44% false-negative and 2 false-positive cases were reported from 51 studied ones (7). In addition, 44% false-negative as compared to null false-negative in NSE method (as reported in our study) weakens the diagnostic validity of acetylcholine esterase method. In the present study, there were 6 false-positive, which can be due to heterogeneous distribution of NSE in ganglion cells, particularly immature ganglion cells. Nevertheless, a negative informative value of about 100% is extremely important, so through using mucosal-submucosal rectal biopsy, HD definitely can be ruled out. Christopher et al in California (1985) studied 11 known HD cases and 16 non-HD cases by NSE method. They showed that the results obtained from NSE method on mucosal-submucosal biopsies corresponded the standard method and even was better for 2 cases (one HD patient and one hypoganglionosis one) (19). Of course, it seems that because of low number of cases under study and lack of blindness, the results can not be generalized. Meanwhile, Hall et al in USA (1985) used NSE method on 27 mucosal-sub mucosal rectal biopsies suspected of HD with a sensitivity of 100% and found that this technique facilitates identification of immature and small ganglion cells (14). However, studies on more cases are required in order to generalize the results. Furthermore, Sternberg (2004) successfully used NSE immunohistochemistry tests on pathologic samples (20). Meanwhile, Talebi et al in Isfahan (2004) used 10 markers on each 47 rectal biopsies and showed that the diagnostic evaluation of each marker based on the quality of staining and differentiation efficiency is in the order of cathepsin D, CD 56, and NSE (12).

This study was done on 38 ganglion cell patients by differentiating normal and hypoganglionosis and no correspondent result was observed with the standard method. Though, practically and clinically, differentiation between normal, hypoganglionosis, and aganglionosis is important, it seems that this lack of relationship between the results of NSE and standard method is due to the lower number of submucosal ganglion cells as compared to the

intramuscular area. In addition, the diagnosis of normal vs. submucosal allied disorders with existing criteria and using NSE method as well as all other markers which show the ganglion cells in this region is difficult while there is no separate criteria for diagnosis of submucosal samples in the literature. In order to solve this problem, further investigations are required for finding submucosal criteria for the differentiation of normal from allied disorders.

In this study no hyperganglionosis was observed. There is controversy about its diagnostic criteria and some authors believe that this test must be done when there are significant pathologic findings (20). Vinore et al (1985) used NSE for submucosal rectal biopsies and by referring to the results obtained from surgery concluded that NSE facilitates the diagnosis of hypoganglionic from aganglionic (13). It seems that in order to generalize the obtained results, all the samples of the study should be mucosal-sub mucosal biopsies. The results of this study showed that finding of ganglion cell in NSE method definitely rejects HD and presence of ganglion cells in 81.8% of the cases indicates HD.

Conclusion

Using NSE method on mucosal-submucosal rectal biopsies is suitable for ruling out HD. Meanwhile, high sensitivity of NSE can be used along with another high specific marker for the diagnosis of HD.

Acknowledgements

The authors would like to thank Vice-chancellor for Research of Mazandaran Univ. Med. Sci. for financial support of this project. We also would like to extend our gratitude to Dr. Enayati and Mr Siamian for their editorial assistance.

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