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Original Article

The Effectiveness of Rectal Suction Biopsy in the Diagnosis of Hirschsprung's Disease

Hirschsprung Hastalığı Tanısında Rektal Aspirasyon Biyopsisinin Etkinliği

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ABSTRACT

Objective: In order to analyse the adequacy, sensitivity, and specificity of samples obtained with rectal suction biopsy (RSB) as the standard histopathological diagnostic method in patients with suspected Hirschsprung's disease (HD).

Method: This study was carried out between November 2016 and March 2018 with 24 suspected HD patients aged 0-3 years. After calculating rectosigmoid indexes (RSIs) according to barium enema images obtained in contrast-enhanced colon graphies, patients with RSI <1 underwent RSB. Clinical features of patients, treatment options, complications, laboratory test results and radiological findings were recorded for further analyses. An expert pathologist evaluated biopsy specimens, and observations were recorded.

Results: There were no ganglion cells in RSB specimens in 10 (41.6%) patients who received the diagnosis of HD later on. Ganglion cells were detected in specimens of 5 (20.8%) patients; thus, the diagnosis of HD was excluded. Inadequate or suspicious biopsy specimens for histopathological evaluation were observed in 9 (37.5%) patients. If the biopsy specimen volume was more voluminous than 4 mm^3, its diagnostic sensitivity and specificity for HD were 80% and 66.67%, respectively (area under the curve=0.789); If the submucosa/mucosa ratio was greater than 0.75 or the submucosa/total specimen ratio was greater than 0.42, then the diagnostic sensitivity and the specificity of the specimen for HD were 86.67% and 66.67%, respectively.

Conclusion: In this prospective cross-sectional study, we demonstrated that RSB in diagnosing HD is a feasible, safe method with high sensitivity and specificity and low complication rates.

Keywords: Hirschsprung's disease, ganglion, rectal aspiration biopsy, rectal biopsy

ÖZ

Amaç: Hirschpsung hastalığı (HH) şüphesi olan hastalarda histopatolojik tanı için standart tanı olarak rektal aspirasyon biyopsi (RAB) örneklerinin yeterliliğini, duyarlılığını ve özgüllüğünü araştırmak.

Yöntem: Çalışma Kasım 2016-Mart 2018 tarihleri arasında prospektif olarak gerçekleştirildi. Çalışmaya yaşları 0-3 arasında değişen HH şüphesi olan 24 hasta dahil edildi. Kontrastlı kolon grafisine göre rektosigmoid indeks (RSI) hesaplandıktan sonra RSI <1 olan hastalara RAB yapıldı. Hastaların klinik özellikleri, tedavi seçenekleri, komplikasyonlar, laboratuvar sonuçları ve radyolojik bulguları ileri analizler için kaydedildi. Biyopsi örnekleri uzman patolog tarafından değerlendirildi ve bulgular kaydedildi.

Bulgular: HH tanısı alan 10 (%41,6) hastanın RAB örneklerinde ganglion yoktu. Beş (%20,8) hastada ganglion hücresi saptandı ve HH tanısı dışlandı. Dokuz (%37,5) hastada histopatolojik değerlendirme için yetersiz veya şüpheli biyopsi örnekleri gözlendi. Biyopsi hacmi 4 mm^3'ten büyükse HH tanısında duyarlılık %80, özgüllük %66,67 (eğrinin altındaki alan=0,789); submukoza/mukoza oranı 0,75'ten büyük veya spesmendeki submukoza oranı 0,42'den büyük ise duyarlılık ve özgüllük oranları sırasıyla %86,67 ve %66,67 idi.

Sonuç: Bu prospektif kesitsel çalışmada, HH tanısında RABnin kolay uygulanabilir, güvenli, duyarlılığı ve özgüllüğü daha yüksek ve komplikasyon oranlarının düşük olduğunu gösterdik.

Anahtar kelimeler: Hirschsprung hastalığı, gangliyon, rektal aspirasyon biyopsisi, rektal biyopsi

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INTRODUCTION

Hirschsprung's disease (HD) occurs in the newborn and childhood period and manifests with signs of intestinal obstruction and constipation[1]. The primary underlying pathology of the disease is the migration deficiency of neural crest cells during the development of the bowels in the fetal period. Functional colonic obstruction in the HD occurs due to a lack of relaxation of an aganglionic colon segment[2-5].

HD occurs in approximately 1 in 5,000 live births, and male to female ratio ranges from 3: 1 to 4: 1[6-8]. Most patients are diagnosed during the newborn period[9]. Sometimes, in infants with short-segment HD, the disease may progress with mild symptoms so that diagnosis may be delayed until childhood. Only up to 10% of patients are diagnosed after age three[10,11].

The diagnosis is made by demonstrating the complete absence of enteric ganglion cells in the submucosal (SM) and myenteric plexuses of the distal colon in pathology preparations. Rectal biopsy could be performed by two methods: full-thickness rectal biopsy (FTRB) and rectal suction biopsy (RSB)[12]. Biopsies must be taken 2 cm above the dentate line to prevent misdiagnosis because there is a physiological aganglionic region below of dentate line[13].

Previous studies reported that anal dilatation, rectal irrigation and barium enema used in diagnosing and treating HD might also cause inflammation and edema in the anorectal mucosa, which may prevent retrieval of sufficient biopsy material required for an accurate diagnosis. However, these confounding factors could not be eliminated due to the retrospective design of these studies[14].

Our study aims to determine the diagnostic accuracy of RSB, the sufficient amount of RSB samples required for histopathological diagnosis, and to estimate the sensitivity and specificity of RSB.

MATERIALS and METHODS

The main findings of this study were obtained between November 15, 2016 and March 15, 2018. After the İzmir Katip Çelebi University Clinical Research Ethics Committee (decision number: 16, approval date: 02.09.2017) was obtained, the data of 24 patients aged 0-3 years with an initial diagnosis of HD who were hospitalized in our clinic for advanced examination and treatment were analyzed prospectively.

Laboratory test results and plain abdominal radiograms of the patients were evaluated. Then contrast-enhanced colon graphies and 24th hour control X-ray graphies (retention graphy) were examined and recorded (Figure 1). Rectosigmoid index (RSI) was calculated for all patients. In the RSI evaluation, the presence of the transition zone and the barium in the intestines on the radiograms obtained after 24 hours were evaluated and recorded. The RSI was calculated by proportioning the diameter of the widest part of the rectum to the diameter of the sigmoid colon on the barium enema graphy. RSI <1 was considered in favour of HD (Figure 2)[15].

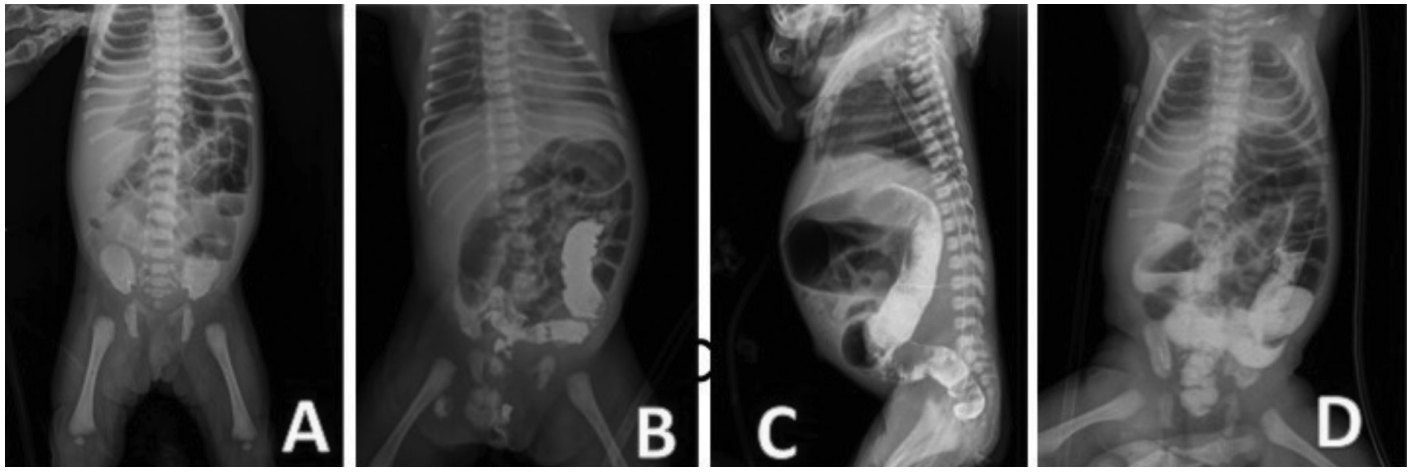

Figure 1. X-ray imaging of the patient with suspected Hirschsprung disease (**A:** Broad-based air-fluid levels in the abdominal radiography of a patient with abdominal distension, **B:** Anterior/posterior barium enema graphy, **C:** Lateral barium enema graphy, **D:** Control X-ray radiography 24 hours after application of barium enema)

After the complaints of these patients, such as abdominal distention and vomiting, were relieved by nasogastric decompression; RSB was performed at least 48 hours after anal dilatation or rectal irrigation procedures. The biopsy was performed at the bedside for the patients younger than 1-year-old without anaesthesia and in the operating room with sedation (0.01 mg/kg IV midazolam) for patients older than 1-year-old.

Rectal suction biopsies were performed with Rbi2 biopsy kit (Aus systems Pty Ltd, Allenby Gardens, Australia). For histopathological diagnosis, two samples were obtained, one from the posterior wall and one from the lateral wall of the affected colonic segment at a level of 2 cm proximal of the anocutaneous line. Negative pressure during the procedure was adjusted to 150 mm H_2O by creating a vacuum of 5 cc in a 10 cc injector[14]. The fresh samples were taken to the pathology laboratory and fixed in 10% formaldehyde solution, and then their paraffin blocks were prepared. Very thin (4-5 µ) sections of paraffin blocks were stained with hematoxylin-eosin and examined under a light microscope at a magnification of 100 - 400 x (Figure 3). Ret-oncoprotein (Figure 3) and neuron-specific enolase staining was performed by immune-histochemical method on samples whose ganglion cells and nerve plexuses could not be discerned during a routine examination. In addition, all sections were evaluated by measuring the mucosal (M) and SM areas in NIS-Elements ver 4.30 digital imaging program of Nikon Eclipse Ni microscope (Figure 3).

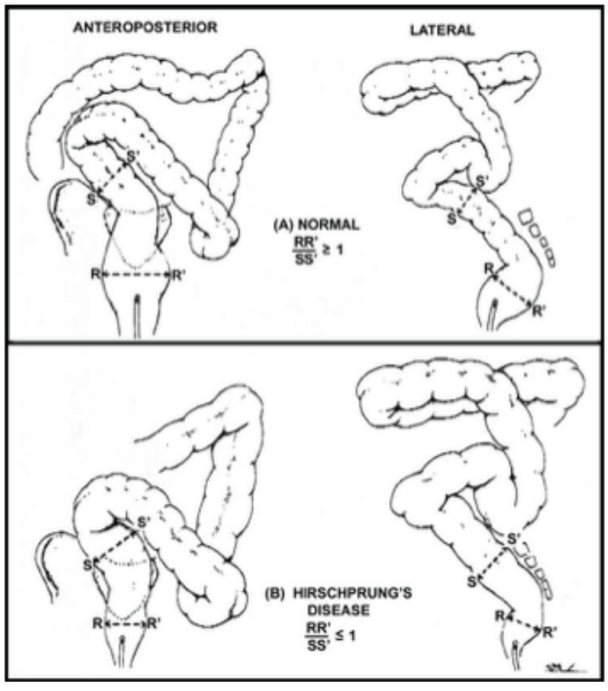

Figure 2. A: Normal RSI measurement. The ratio of the widest diameter (RR') of the rectum to the widest diameter (SS') of the sigmoid colon is greater than or equal to 1. **B:** Abnormal RSI: Hirschsprung's disease: RSI <1

RSI: Rectosigmoid index

Statistical Analysis

The data were evaluated in the IBM SPSS Statistics 25.0 statistical package program. We used numerical values, percentages (%), and mean ± standard deviation for the descriptive statistics. The distribution of numerical variables was evaluated with the Shapiro-Wilk test of normality and Q-Q graphs. Comparisons of the two groups for the normally distributed variables were made with an independent two-sample t-test. The relationship between categorical variables was examined with the exact method of the Pearson chi-square test. The volume of the biopsy specimen resected the SM/mucosa (M) ratio and the percentage of SM (SM%) tissue in the postoperatively resected intestinal segment were determined by receiver operating characteristic (ROC) curve analysis. A p-value of <0.05 was considered statistically significant.

Results

The study population (n=24) consisted of 14 (58%) female, and 10 (48%) male patients. Fifteen (62.5%)

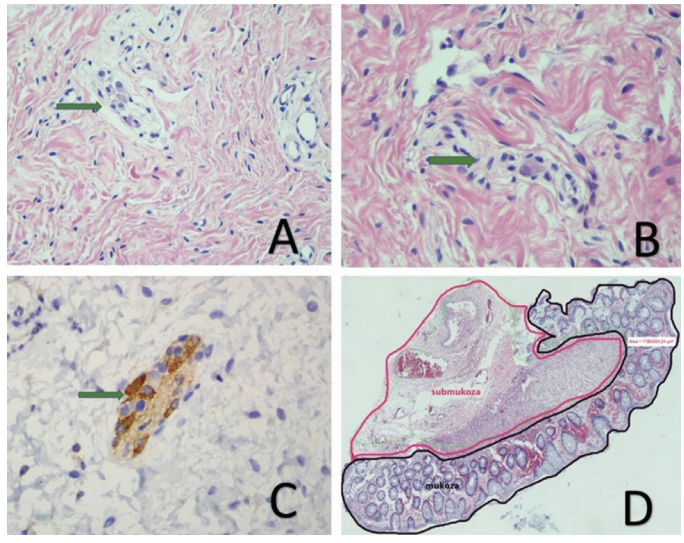

Figure 3. A: Ganglion cells in nerve plexuses (HE × 100), **B:** Ganglion cell (HE × 400), **C:** Ganglion cell stained with ret-oncoprotein (DAB × 400), **D:** Measurement of mucosal/submucosal areas

patients were newborns (0-28 days), and 9 (37.5%) patients were children aged between 1 and 36 months. Meconium output was not observed in 16 (66.7%) patients within the first 24 hours after birth, and 10 (62.5%) of these 16 patients were diagnosed with HD after further examinations and evaluations. RSI was calculated as <1 in 7 (29.1%) and ≥1 in 9 (37.5%) patients. Adequate radiography could not be obtained or evaluated due to previous ileostomy or colostomy in 8 (33.3%) patients. Seven (29.1%) patients with RSI less than one were diagnosed with HD after subsequent examinations.

During the study, none of the patients developed rectal bleeding, intestinal perforation, sepsis or similar complications after RSB at the time of diagnosis.

No ganglion cells were found in the samples of 10 (41.6%) patients in the histopathological evaluation and diagnosed as HD. Ganglion cells were observed in the histopathological examination of the samples in 5 patients (20.8%). Biopsy samples of 9 (37.5%) patients were evaluated and reported as insufficient or suspicious samples for the pathological examination.

FTRB specimens obtained from 4 (16.6%) patients who were evaluated as inadequate and suspicious RSB, and HD diagnosis was excluded in these patients due to the presence of ganglion cells detected during the histopathological examination of the samples. The other 5 (20.8%) patients with insufficient results were discharged after their complaints regressed; thus, the diagnosis of HD was excluded. Afterwards, patients were monitored for six months during periodic follow-up controls in outpatient clinics. During the follow-up period, no findings suggestive of HD were detected, so their families were informed, and these patients were excluded from routine follow-up controls. In addition, all patients were followed up with outpatient clinic controls after discharge for at least six months. Based on the RSB results obtained from 24 patients, the volume of the biopsy specimens, the SM/mucosa (M) ratio and the percentage of SM tissue in the preparation (SM%) were calculated. The results are summarized in Table 1.

When the preoperative RSB and postoperative resection materials were compared, 10 (41.6%) patients with preoperative ganglion-negative RSB results also had aganglionosis in postoperative resection materials. In the histopathological evaluation of samples in 5 (20.8%) patients, ganglion cells in BRS materials were reported. According to these results of 5 patients; due to the disappearance of symptoms in favour of HD during clinical observation, their families were informed after 6 months of outpatient clinic follow-up, and the patients were excluded from the follow-up.

When ROC analysis was performed to compare RSB samples in terms of histopathology and clinical examination results, it was calculated that the volume of the biopsy material greater than 4 mm^3 had 80% sensitivity and 66.67% specificity in the diagnosis of HD [area under the curve (AUC)=0.789]. While the SM/M ratio greater than 0.75 or the SM greater than 0.42 had diagnostic sensitivity, and specificity of 86.67% and 66.67%, respectively (AUC=0.748, and 0.752, respectively) (Table 2).

When the results of RSB were evaluated, samples of 15 (62.5%) patients were sufficient, and 9 (37.5%) patients were insufficient or suspicious. The mean volume of adequate biopsies (8.4 mm^3), SM/M ratio (1.22), and percentage of SM (0.52%) were estimated as indicated.

Table 1. Histopathological evaluation results of biopsy specimens

	Volume (mm^3)	SM/M	SM%
Number of specimens (mean ± SD)	24 (7.04±4.63)	24 (1.05±0.55)	24 (0.47±0.12)
Minimum	3.00	0.36	0.26
Maximum	24.00	2.41	0.70

SD: Standard deviation, SM/M: Submucosa/mucosa

Table 2. ROC analysis results for histopathological evaluations

	Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)	+PV	-PV	AUC	p-value
SM%	>0.42	86.67 (59.5-98.0)	66.67 (30.1-92.1)	81.3	75.0	0.752	0.011
SM/M	>0.75	86.67 (59.5-98.0)	66.67 (30.1-92.1)	81.3	75.0	0.748	0.013
Volume (cc)	>4	80.00 (51.9-95.4)	66.67 (30.1-92.1)	80.0	66.7	0.789	0.001

+PV: Positive predictive value, -PV: Negative predictive value, AUC: Area under the curve, SM/M: Submucosa/mucosa, CI: Confidence interval

The mean volume of inadequate biopsy specimens (4.7 mm^3), SM/M ratio (0.77), and percentage of SM (0.40) were also determined. In terms of SM% of patients, the difference between groups was statistically significant (p=0.033) (Table 3).

We estimated the diagnostic adequacy rate of RSB as 73.3% in newborns and 44.4% in children aged between one month and three years (Table 4).

DISCUSSION

In this study, we aimed to measure the diagnostic adequacy, sensitivity and specificity of the RSB method applied to obtain rectal biopsy samples, the gold standard diagnostic method for HD. The RSB results obtained for HD were found to be sufficient for diagnosis. Most (62.5%) of the samples obtained using the RSB method had sufficient, and 37.5% had insufficient or suspicious diagnostic accuracy. Different studies have presented variable and insufficient sampling rates when the literature is reviewed. Apart from the discussions on this subject, insufficient sampling rates between 11-50%[16-22] were stated when the studies were considered. What makes these studies different from each other is that the biopsy sampling process was carried out using variable methods. In the literature, in most of the studies with low insufficient diagnostic material rates, 3 or 4 samples were taken in each biopsy procedure, and the biopsy was repeated if the SM tissue was deficient. When the studies were examined in general, it was stated that 1-4 biopsy samples should be taken during biopsy performing process. In many studies, at least three samples were taken[17-22]. Based on our study, two samples were taken from each patient, so our insufficient sampling rate is at an acceptable level. However, this rate was higher compared to other studies in the literature because only two samples in each procedure were obtained, and the patients did not undergo RSB for the second time according to the recently applied protocol in our department. In addition, the biopsy material of our two patients that were reported as suspicious but considered insufficient may have increased this rate.

In our study, when samples containing sufficient and insufficient tissue samples were examined to confirm or exclude the disease in the histopathological examinations of biopsy materials, the average volumes of sufficient and insufficient samples obtained to establish diagnosis were 8.4 mm^3 and 4.7 mm^3, respectively. Although these values were not statistically significant, it is noteworthy that sufficiently higher volumes of samples are required to make an accurate diagnosis. There are limited studies in the literature regarding the volume of samples that should be retrieved for diagnostic purposes. In a different study, the average volume of RSB samples obtained was reported as 14.8±7.8 mm^3[14]. Several studies have reported that an adequate biopsy sample should be at least 3.5 mm in diameter[17,23,24]. The increase in the volume of the samples contributes to the decision-making process, but the depth of the biopsy is also another important diagnostic criterion. In the histopathological examinations of the samples in our study, the average percentages of sufficient and insufficient SM tissue specimens were found to be 52%,

Table 3. Assessment of histopathological adequacy of rectal suction biopsy

	Histopathological adequacy of biopsy	Mean	Standard deviation	p-value
Volume (mm^3)	Insufficient (n=9)	4.77	1.56	0.62
	Sufficient (n=15)	8.40	5.35	
SM/M	Insufficient (n=9)	0.77	0.46	0.55
	Sufficient (n=15)	1.22	0.54	
SM%	Insufficient (n=9)	0.40	0.11	0.33
	Sufficient (n=15)	0.52	0.11	

SM/M: Submucosa/mucosa; SM%: Percentage of submucosal tissue

Table 4. Assessment of histopathological adequacy of rectal suction biopsy according to age groups

			Age groups			
			Newborn	1 month-3 years	Total	p-value
Biopsy adequacy	Insufficient	Number (%)	4 (26.7)	5 (55.6)	9 (37.5)	0.212
	Sufficient	Number (%)	11 (73.3)	4 (44.4)	15 (62.5)	
Total		Number (%)	15 (100)	9 (100)	24 (100)	

and 40%, respectively, with a statistically significant difference between them (p=0.033). Although there are few prospective studies on this issue in the literature, remarkably retrospective studies reported that the rate of SM in the biopsy tissue material should not be less than 35-50% as the inclusion criteria for the samples[14,16,17]. In our study, similar rates were reported with these studies in the literature in terms of both the volume of RSB samples and the relative percentage of SM tissue.

In this study, we found that 73.3% of the biopsy materials obtained from the newborn age group were sufficient, while biopsy specimens retrieved from 44.4% of the patients aged between one month and three years were considered sufficient for histopathological examination. Although this difference is not statistically significant, tissue adequacy rates in RSB were higher in newborns than in older children in our study. When the studies in the literature are examined, different results draw attention when the diagnostic features of RSB according to age groups are evaluated. While some studies reported no difference in the diagnostic yield of RSB between age groups, several studies reported that the insufficiency rate was higher in infants younger than 1.5 months than in infants older than 1.5 months[5,22]. In another study, higher tissue adequacy rates were reported in babies older than a year[25]. According to some studies, it has been stated that the tissue adequacy ratio of RSB decreases after 3 years of age[20,26,27]. When it was desired to evaluate the literature, it was seen that different results prevented the formation of a consensus on this issue. Hypertrophic nerve fibres in the pathological evaluation of the tissue obtained is a positive finding favouring the diagnosis of HD. Previous attacks of enterocolitis and thicker intestinal mucosa could be considered a negative factor and may play a role in obtaining different results in each study. Although the immaturity of the ganglion cells in the neonatal period and the lack of hypertrophic nerve fibres are factors that challenge the pathologist, an experienced pathologist can make a definitive diagnosis with a sample that contains a sufficient amount of SM tissue. It is well-known that the FTRB procedure is more complex, especially in the newborn period, compared to older ages. In our study, a higher material adequacy rate in the newborn period could be achieved with the RSB method, and low complication rates were observed in patients of all ages. These results have shown that the BRS technique is an easily applicable and safe method in the neonatal period.

In patients with an initial diagnosis of HD, methods of anal dilatation and rectal irrigation are often recommended in terms of diagnosis and treatment during the decision-making process for biopsy[4]. We planned a prospective study by eliminating these pre-biopsy procedures for the first time in the literature, with the suspicion that both procedures may have an oedema-forming effect on the rectum wall and may prevent retrieval of sufficient SM tissue during the subsequent RSB. We concluded that our insufficient material rates were parallel to similarly designed previous studies in the literature. As a result, we have revealed that a 48-hour period without anal intervention before RSB does not provide any benefit, and it is possible to perform a biopsy whenever desired. When nine biopsy materials obtained from 24 patients considered insufficient for accurate diagnosis were not included in the evaluation, the diagnostic specificity and sensitivity of RSB performed in 15 patients were found to be 100%. Diagnostic sensitivity and specificity of RSB in HD have been reported as 88-93% and 95-99%, respectively[22,23,28-30]. Similar to our study, Sharp et al.[28] did not report any false negative or positive RSB results.

Compared to previous studies, our study yielded 100% diagnostic accuracy without any margin of error caused by the low number of cases. We consider that excluding suspicious RSB results from the evaluation and working with an experienced pathologist to establish the diagnosis of HD increased the accuracy of the study results. In further prospective randomized, double-blind studies including large-patient populations, it may be expected that our study's sensitivity and specificity rate, which was 100%, will decrease slightly. Therefore, this high rate supports the opinion that the RSB technique should be preferred over FTRB.

CONCLUSION

This study showed that diagnosing HD using the RSB provides a significant advantage over FTRB. It could be performed safely and without anaesthesia due to the very low rate of serious complications in newborn and older children. In addition, if the problem of insufficient amount of biopsy material required for accurate diagnosis is left aside, RSBs sensitivity and specificity rates were very high, leading to the prompt diagnosis of HD. We suggest RSB is the gold standard for diagnosing HD accurately.

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Ethics

Ethics Committee Approval: Approval was obtained from İzmir Katip Çelebi University Clinical Research Ethics Committee (decision number: 16, approval date: 02.09.2017).

Informed Consent: Prospective study.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: C.B., M.O.Ö., T.Ö., G.K., Concept: C.B., M.O.Ö., G.K., Design: C.B., M.O.Ö., G.D., T.Ö., A.S., G.K., Data Collection and Processing: C.B., G.D., Analysis and Interpretation: M.O.Ö., Literature Search: C.B., M.O.Ö., G.K., Writing: C.B., M.O.Ö., G.D., A.S., G.K.

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