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High-resolution anorectal manometry in newborns: normative values and diagnostic utility in Hirschsprung disease

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Key Messages

- Lack of normal reference data limited the application of HRAM in newborn patients. In this paper, 180 asymptomatic newborns and 16 newborns with clinical manifestations suspicious of HD were enrolled. Normative values in asymptomatic preterm and term newborns were assessed and we demonstrate that the anal sphincter progressively matures in the early days and weeks of life, establishing stable resting pressure after the first month. We also demonstrate that presence of the rectoanal inhibitory reflex is universal in newborns, and absence of this reflex has high sensitivity and specificity for diagnosis of Hirschsprung disease when compared with rectal suction biopsy. We establish the higher diagnostic accuracy of HR-ARM compared with barium enema in the diagnosis of Hirschsprung disease in newborns. HR-ARM is an effective and safe method that complements the diagnosis of HD in newborns.

Abstract

Background *Conventional methods of screening for Hirschsprung disease (HD) in newborns (barium enema, BE; anorectal manometry, ARM; rectal suction biopsy, RSB) have limitations and/or are invasive. High-resolution anorectal manometry (HR-ARM) is a minimally invasive technique that has potential to overcome most of these limitations, but normative data and performance characteristics have not been reported in newborns. The aims of our study were to assess anorectal sphincter metrics including resting pressure (RP), anal canal length (ACL), and rectoanal inhibitory reflex (RAIR) in healthy and asymptomatic newborns, and to explore the role of HR-ARM in the diagnosis of HD using these normal parameters.* ***Methods*** *All procedures were performed using solid state HR-ARM equipment (Medical Measurement Systems, Enchede, The Netherland) by a single operator. In the first phase, 180 asymptomatic newborns (term newborns 95, preterm newborns 85) were studied, and anal RP, ACL, and RAIR were measured. In the second phase, 16 newborns with clinical manifestations of HD were studied (9 of whom had histopathologic confirmation), and parameters compared to asymptomatic newborns.* ***Key Results*** *Normative RP values were higher in term newborns compared with preterm newborns ($p < 0.05$), and correlated with age. Progressive maturation of the anal sphincter was evident with chrono-*

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logic age, both in preterm and term newborns. RAIR was present in all normal subjects. Using absent RAIR as indicative of HD, HR-ARM had a sensitivity 89% and specificity of 83% compared to RSB; these performance characteristics were better than BE (sensitivity 78%, specificity 17%), with significantly higher diagnostic accuracy (80% vs 53%, respectively, p = 0.009). ***Conclusions & Inferences*** *Anorectal sphincter pressure progressively matures with incremental increase in RP during the first months of life. HR-ARM is an effective and safe method that complements the diagnosis of HD in newborns.*

Keywords *high-resolution anorectal manometry, Hirschsprung disease, newborns.*

INTRODUCTION

Hirschsprung disease (HD) is a common congenital disorder worldwide, with an estimated incidence of 1 in 5000 in live births,[1] and with ~80% presenting in the first few months of life.[2] With early diagnosis and surgery, as many as 81% can restore bowel function to normal.[3] Delayed passage of meconium can be an important early symptom that raises suspicion for HD.[4–6] Diagnosis of HD is based on a combination of clinical features, barium enema (BE), anorectal manometry (ARM), and rectal suction biopsy (RSB). BE has a reported sensitivity of 76% and specificity of 97% in diagnosing HD.[7,8] However, early HD without colonic dilation can be missed,[9] Therefore, the main use of BE should be to help determine the extent of the aganglionic bowel when planning corrective surgery.[10,11] RSB, although considered gold standard for HD diagnosis,[7,12] is an invasive procedure with potential for complications such as bleeding, perforation, and infection, and can be cumbersome for HD screening in newborns.[13] Conventional ARM provides good diagnostic sensitivity and specificity of 83% and 93%, respectively,[7,13] but ARM reference values are not standardized, and precise sensor location is needed for accurate diagnosis. Further, infants need to be tested while calm, as crying can affect anorectal sphincter assessments.

High-resolution anorectal manometry (HR-ARM) is a new technique of assessing motor function in the anorectum, with potential advantages over ARM. Solid state catheters are used with multiple pressure sensors, computerized data acquisition and display-utilized color-coded amplitude depiction, and better visual and analytic assessment of sphincter function, while remaining non-invasive similar to ARM. [14] In particular, movement of the catheter within the sphincter does not confound sphincter pressure measurements because of the increased number of pressure sensors. Similar systems have been utilized by others in assessing anorectal motor function in children.[15] Thus, we hypothesized that HR-ARM may have high diagnostic yields matching or surpassing those of ARM, and could be a safe method for screening and diagnosis of HD.

The objectives of this study were twofold: (i) to assess anorectal sphincter metrics including resting pressure (RP), anal canal length (ACL), and rectoanal inhibitory reflex (RAIR) in healthy and asymptomatic newborns, with the purpose of obtaining normative HR-ARM parameters, and (ii) to explore the role of HR-ARM in the diagnosis of HD using these normal parameters.

SUBJECTS AND METHODS

Subjects

In the first phase of the study, asymptomatic newborns presented to Ba Yi Children's Hospital, the General Hospital of Beijing Military Command, from April 2011 to September 2012 were enrolled as normal controls. All newborns who passed meconium within 24 h and had stable vital signs, flat and soft abdomen and without distension were eligible for inclusion in this study. Both vaginal and cesarean section births were included. Exclusion criteria consisted of gastrointestinal symptoms suggesting altered bowel transit (such as constipation or a defecation disorder), vomiting and obstructive features, evidence of birth trauma, requirement for surgery of any sort, family history of genetic and infectious diseases, and need for gastrointestinal medications including those that could affect gastrointestinal motility. Studies on infants who were upset or crying during the study were also excluded. In the second phase, 16 newborns with clinical features suspicious for HD, including delayed passage of meconium, vomiting, and/or abdominal distention, undergoing testing during the same study period were identified. This study protocol was approved by the Ethics Research Committee of General Hospital of Beijing Military Command, and written informed consent was obtained from parents or legal guardians before each study.

Manometric techniques

High-resolution anorectal manometry was performed with an extruded silicone catheter (4.42 mm outer diameter) containing 20 individual channels with side openings spirally at intervals of 0.2 cm, suitable for use in infants.[16] A balloon with 400 mL maximum capacity was located between tip and the first side channel on the catheter (Fig. 1). All side holes were perfused with distilled, degassed water at a rate of 0.25 mL/min using a pneumatic perfusion pump. The HR-ARM unit further consisted of pressure transducers that converted recorded pressures into digital data, and displayed as color plots using the Solar GI HRM Compact Pole system (Medical Measurement Systems). In this system, warm colors (green–orange–red) depict higher pressures, and cold colors (blue spectrum) indicate lower pressures (Fig. 2A).

Protocol

High-resolution anorectal manometry was performed as previously described by Jones *et al.*,[17] with minor modification. Briefly,

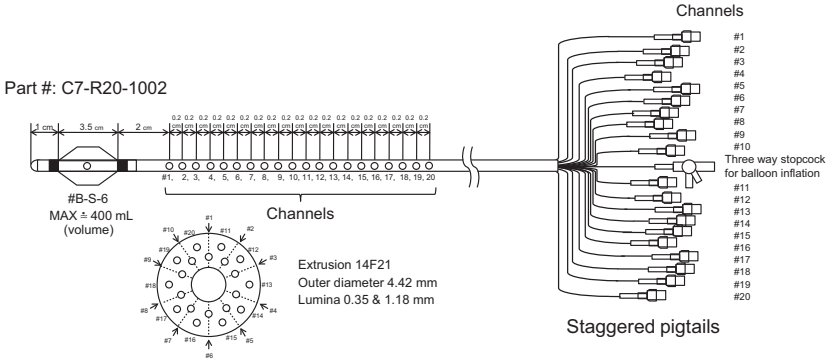

Figure 1 Anorectal manometry catheter (Medical Management Systems, Enschede, Holland) used for high-resolution anorectal manometry (HD-ARM) in this study. The length of the catheter was 10.3 cm, with 0.2 cm between each of 20 spiral perfusion side holes. A 3.5-cm, balloon with maximum capacity of 400-mL volume was located between the first side hole and the tip of the catheter. A cross-section of the catheter is also shown, demonstrating the locations of the perfusion channels within the extruded catheter.

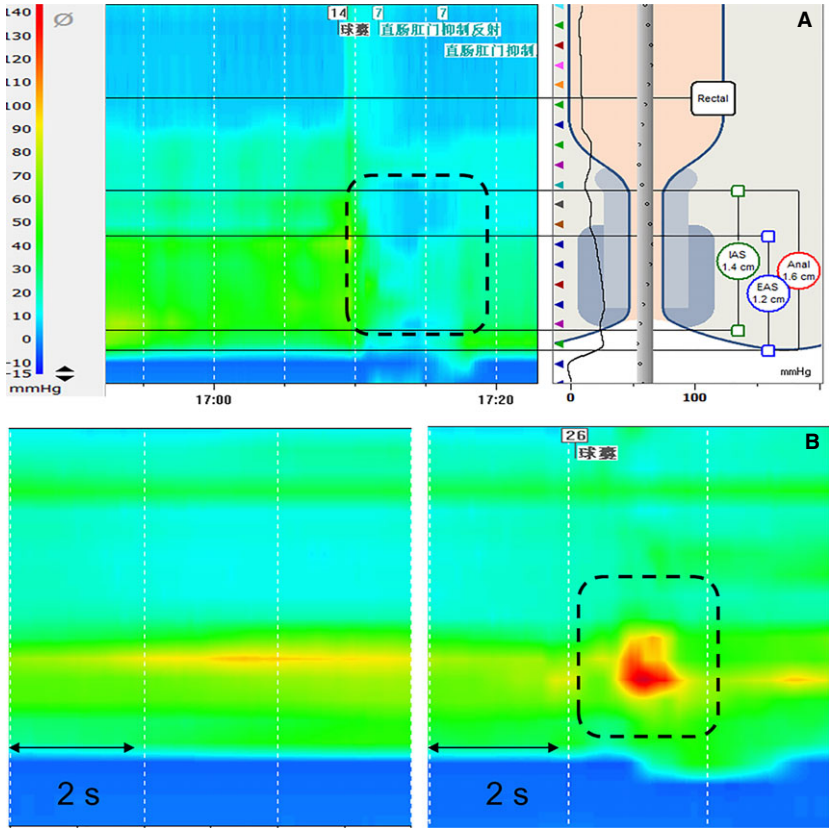

Figure 2 Isobaric contour plots obtained using HD-ARM. (A) The band of pressure depicted by the green color identifies the anal sphincter. The length of the sphincter is identified by change in color from cold to warm colors as depicted in the line diagram. This image also demonstrates a normal rectoanal inhibitory reflex (RAIR), where sphincter pressure decreases during intrarectal balloon inflation (box). (B) HD-ARM contour plot from a patient with Hirschsprung disease (HD). The left panel demonstrates resting pressure. The right panel demonstrates lack of sphincter relaxation with rectal balloon inflation (box) establishing the diagnosis of HD. IAS: internal anal sphincter; EAS: external anal sphincter.

newborns were placed in the left knee lateral position, and were studied without anesthesia or sedation. Bowel preparation was not routinely performed. The catheter was calibrated before each study according to manufacturer's recommendations. The anorectal catheter was lubricated with tetracaine hydrochloride and gently inserted through the anus to an approximate distance of 10 cm to ensure that the balloon could be inflated adequately within the rectum. Data acquisition was performed with the infants in a quiet state without crying.

Resting pressure Resting pressure was measured at the beginning of the manometric procedure. The catheter was kept in the anal canal until topographic plots stabilized and uniform color plots were visualized, and RPs were measured over at least 10 s. The maneuver was repeated 3 times, and mean RPs from the 3 contour plots generated were recorded as the RP.

Anal canal length The vertical length of the warm color band of pressure depicting the anorectal sphincter (high-pressure zone) at the resting state was recorded as the ACL (Fig. 2A).

Rectal anal inhibitory reflex The initial balloon volume used for eliciting RAIR was 5 mL. If the color contour plots changed from warm to cold colors indicating that the RAIR was elicited (Fig. 2), the maneuver was repeated by injecting air from 1 mL to 5 mL at 1-mL aliquots with intervals of at least 30 s between inflations until RAIR was elicited,[9–11] and this balloon volume was considered as threshold volume for RAIR. If the RAIR was not elicited at 5 mL, balloon volume was further increased stepwise in 1-mL aliquots from 5-mL until the RAIR elicited; this was similarly recorded as the threshold volume for RAIR. Unchanged anal sphincter pressures despite balloon volumes of 20 mL constituted a negative RAIR.

Barium enema Barium enema was performed using standard technique by pediatric radiologists.[18] Briefly, diluted barium was infused rectally using a catheter, and x-ray images were obtained. All BE images were reviewed by the same pediatric radiologist. Classic findings of a dilated distal colon with a transition zone and a spastic segment at the anorectum were considered consistent with HD.[19]

Rectal suction biopsy Biopsy specimens were obtained by suction biopsy using standard technique. These were fixed, cut, and stained. The presence or absence of ganglion cells was evaluated by an experienced histopathologist with specific interest and expertise in HD. A deficiency of ganglion cells was considered diagnostic of HD.

Statistical analysis

Data are expressed as mean ± SD unless otherwise indicated. Grouped data were compared using the unpaired Student's *t*-test. Multivariate logistic regression analysis evaluated the relationship between HR-ARM metrics and demographic data (including age, gestational age, height, weight, and birth weight). Fisher's exact test was used for the comparison of binomial data between normal and patient groups, and to determine performance characteristics of HR-ARM in the diagnosis of HD. In all instances, $p < 0.05$ was required for statistical significance. All statistical analysis was performed using SPSS13.0 software (Chicago, IL, USA).

RESULTS

In the first phase of the study, 180 asymptomatic newborns were enrolled during the 1.5-year study period, evenly divided between preterm ($n = 85$, 32.9% female, mean gestational age 34.5 weeks) and term ($n = 95$, 45.3% female, mean gestational age 39.2 weeks). All the healthy newborns passed meconium within 24 h. HR-ARM studies were performed at age ranging from 0.5 to 87 days from birth in preterm newborns, and 1–67 days in term newborns, with a mean age of 14 days in preterm and 17 days in term newborns. The baseline characteristics of the asymptomatic newborns are depicted in Table 1. In the second phase, 16 newborns (mean age 30.5 days, 43.7% female) with suspected HD were identified during the same study period.

Table 1 Baseline characteristics of asymptomatic newborns

	Preterm		Term	
Characters	Mean ± SD	Range	Mean ± sd	Range
Age (days)	14.0 ± 16.2	0.5–85	17.8 ± 16.2	1–67
Weight (kg)	2.3 ± 0.5	1.5–4.5	3.4 ± 0.8	1.6–5.5
Height (cm)	46.1 ± 3.7	31–54	41.8 ± 4.0	41–63
Gestational age (w)	34.5 ± 1.5	28–36	39.2 ± 1.6	37–42
Birth weight (kg)	2.3 ± 0.5	1.4–3.7	3.2 ± 0.6	1.7–4.5

HR-ARM data in normal controls

For the entire normal cohort, mean RP was 29.7 ± 9.9 mmHg, and ACL 1.9 ± 0.5 cms. However, there was significant variation in RP between preterm and term newborns, and between infants of varying chronologic age (Fig. 3). Resting sphincter pressures were significantly lower in preterm newborns compared with term newborns regardless of age at testing. In both preterm and term newborns, there was an initial rapid phase of increase in RP in the first month, following which RP appeared to stabilize (Fig. 3C). ACL did not vary by gestational age. RAIR was elicited in all healthy newborns. Volumes required for RAIR were lower in preterm newborns when tested during the first month, but not thereafter. There were no gender differences in both preterm and term newborns in all the parameters evaluated ($p > 0.05$, data not shown). We therefore report normative data for RP, ACL, and volume required for total RAIR based on chronologic age, and segregated by preterm *vs* term (Table 2).

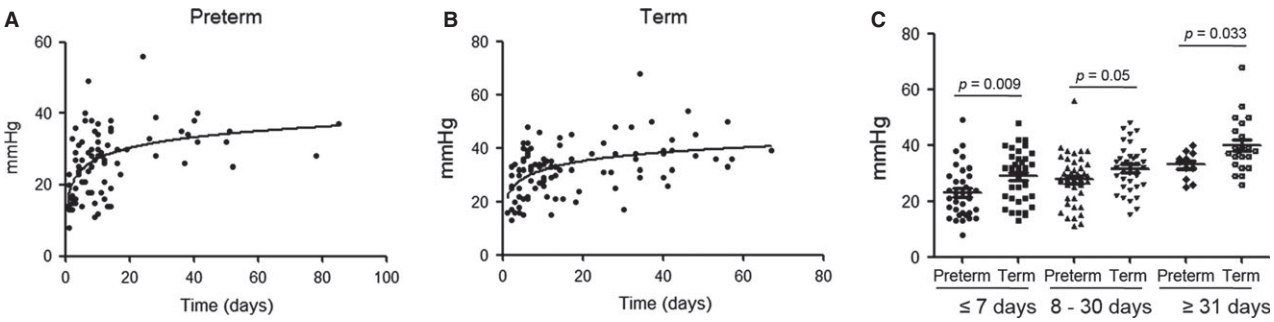

Figure 3 Relationship between RP and chronologic age in preterm and term newborns. (A and B) Demonstrate progressive maturation of sphincter pressures with increasing age. Trend lines demonstrate stable sphincter pressures after the first month, with sharper increments in sphincter pressure in preterm newborns in the first days of life compared with term newborns. (C) Demonstrates higher resting pressures in term newborns compared with preterm newborns, stratified by chronologic age.

Table 2 Normative HR-ARM values in healthy full-term and preterm newborns

	Mean (5th–95th percentile values)		
Age (day)	Preterm	Term	*p*-value
RP (mmHg)			
≤7	23.1 (19.9–26.2)	28.9 (25.8–32.0)	0.009
8–30	27.7 (24.8–30.6)	31.6 (28.9–34.3)	0.05
≥31	32.9 (29.6–36.2)	39.9 (35.6–44.1)	0.033
ACL (cm)			
≤7	1.8 (1.7–2.0)	1.9 (1.7–2.1)	0.628
8–30	1.9 (1.7–2.0)	2.0 (1.9–2.1)	0.285
≥31	2.0 (1.7–2.3)	2.3 (2.1–2.4)	0.09
Threshold RAIR volume			
≤7	1.6 (1.4–1.9)	2.8 (2.3–3.3)	0.000
8–30	2.2 (1.7–2.7)	3.5 (2.9–4.0)	0.001
≥31	3.7 (2.8–4.7)	4.5 (3.9–5.0)	0.122

In a multivariate logistic regression model, RP independently associated with chronologic age ($p = 0.024$) and gestational age ($p = 0.035$) in term newborns, but only with age in preterm newborns ($p < 0.001$) (Table 3). No correlation was found between ACL, threshold RAIR volume on one hand and age, gestational age, and body length on the other (data not shown).

HR-ARM in detection of HD

In the second phase of the study, 16 newborns (9 male, 7 female) with symptoms and signs suggestive of HD were enrolled (2 with vomiting, 9 with abdominal distension, 5 with both vomiting and abdominal distension, and 15 with delayed passage of meconium). Of these, two were preterm and 14 full-term. Within this cohort, 15 underwent BE and RSB, and all 16 were investigated with HR-ARM; 15 underwent all three investigations. A continuous pressure band at the anorectal sphincter with absence of RAIR was considered suggestive of HD (Fig. 2B), and was seen in 9 newborns; RAIR could be elicited in the remainder. There was no difference in RP between HD and normal controls. Newborns with HD also had shorter ACL compared with the entire normal cohort, driven mainly by differences in the 1 week to 1 month chronologic age cohort (Table 4). Corrective surgery was undertaken in 15 of the infants, and a conclusive histopathologic diagnosis of HD was made in 9 of the infants by a trained pathologist. Of note, RAIR could be demonstrated in two newborns following corrective surgery for HD.

Table 3 Multivariate logistic regression analysis evaluating independent predictors of resting sphincter pressure in healthy preterm and term newborns

	Preterm newborns		Term newborns	
	Standard coefficient	*p*-value	Standard coefficient	*p*-value
Gender	0.151	0.139	0.074	0.456
Age at testing	0.670	0.000	0.381	0.024
Gestational age	0.220	0.117	0.215	0.035
Length	0.026	0.836	−0.239	0.134
Weight at testing	−0.179	0.429	0.319	0.215
Birth weight	0.361	0.119	−0.063	0.724

To evaluate performance characteristics of HR-ARM in the diagnosis of HD, we compared HR-ARM and BE with RSB, the gold standard for diagnosis of HD. The sensitivity and specificity of HR-ARM were 89% and 83%, respectively; positive and negative predictive values were 89% and 83%. Comparatively, with the same RSB gold standard, sensitivity of BE was substantially lower at 78%, with specificity of 17%, positive predictive value 58%, and negative predictive value 33%. Therefore, the diagnostic accuracy of HR-ARM in this context was 80%, compared to 53% with BE ($p = 0.009$).

DISCUSSION

In this study evaluating anorectal sphincteric function in newborns ranging in age from 0.5 day to 3 months of age, we report normative HR-ARM values for RP, ACL, and threshold volume for eliciting RAIR in both preterm and term newborns, and stratify the normal values by chronologic age. We demonstrate that the anorectal sphincter progressively matures in the first month of life with increasing RP values, following which RP stabilizes. However, despite lower RP values in preterm and young newborns, RAIR is uniformly present at birth in all healthy newborns. Consequently, we report that demonstration of absent RAIR with HR-ARM is an efficient method of diagnosis of HD, with performance characteristics better than BE, and similar to RSB. These data establish the value of HR-ARM as a non-invasive tool in screening for HD when clinical features suspicious for HD are encountered in both term and preterm newborns.

Anorectal manometry has been effective as a non-invasive, simple, and safe method for characterizing anorectal sphincter dynamics and function since the 1960s.[20] ARM is also utilized as part of the diagnosis and classification of functional constipation.[19, 21] However, conventional ARM methods were cumbersome, and required placement of the recording sensors precisely within the anal sphincter for accurate diagnosis, as catheter movement with anorectal maneuvers could confound the values. Therefore, conventional ARM is a test only useful in experienced centers. The advent of solid state sensors allowed development of

Table 4 Comparison of RP and ACL in HD and healthy newborns

	RP (mmHg)			ACL (cm)		
Age (days)	HD	Controls	*p*-value	HD	Controls	*p*-value
≤7	36.7 ± 12.9	26.1 ± 9.6	0.067	1.4 ± 0.4	1.8 ± 0.5	0.133
8–30	32.5 ± 8.2	29.6 ± 8.8	0.519	1.4 ± 0.3	1.9 ± 0.5	0.013
≥31	42.5 ± 27.6	37.5 ± 8.9	0.842	2.0 ± 0.8	2.2 ± 0.4	0.827
All	36.1 ± 13.4	29.7 ± 9.9	0.064	1.5 ± 0.5	1.9 ± 0.5	0.007

catheter systems with multiple recording sites. With computerized data acquisition and display systems first utilized in esophageal high-resolution manometry,[22] topographic contour plots of pressure data could be displayed with better recognition of anorectal pressure events. However, lack of normative values continues to impact precise interpretations of anorectal metrics. With our large cohort of 180 healthy newborns, we now provide normal reference values for important anorectal pressure metrics, of which RP is an essential parameter in the diagnosis and classification of anorectal disorders in newborns.[21] The normative RP values in term and preterm newborns may facilitate further application of HR-ARM in the diagnosis of anorectal motor dysfunction.

As part of its role in evaluating anorectal motor disorders, ARM is considered an important technique for the diagnosis of HD since first reports in 1967.[20] We now report the added utility of HR-ARM in this regard. While having higher start-up costs, HR-ARM has several advantages over conventional ARM. Data collection is more precise, as multiple sensors are utilized with short intervals between sensors on the anorectal motility catheter. Computerized display systems with color-coded contour plots allow intuitive visual interpretation of motor events. Finally, high-resolution methods lead to shorter procedure times, and higher accuracy in diagnosis of motor disease has been demonstrated in the esophagus compared with traditional manometry.[23]

In our report, we modified the RAIR balloon distension maneuver, where the volume of air used to distend the rectal balloon was reduced to 1-mL aliquots rather than the reported 5-mL aliquots.[16,24] This allowed determination of an accurate threshold volume for eliciting the RAIR. Although reports in the literature suggest that RAIR cannot be elicited in newborns less than 1 week old because of an immature anorectum,[25,26] we could demonstrate RAIR in newborns less than 7 days old and with gestational age as low as 28 weeks, similar to de Lorijn *et al.*, who reported that 81% premature infants older than 26 weeks of postmenstrual age have normal RAIR. [26] Our results may reflect an advantage of HR-ARM over conventional 4 channel ARM systems utilized in older reports. Our results also provide a minimally invasive avenue for early diagnosis of HD in neonates and even preterm newborns, as absence of RAIR constitutes an important feature of this diagnosis. This value of early HR-ARM providing clues to a diagnosis of HD is well represented in the case example presented in the Results section.

Hirschsprung disease, also called congenital aganglionic megacolon, is characterized by ganglion cell deficiency in the myenteric and submucosal nerve plexuses in the distal colon and rectum. Because of the absence of ganglion cells, the internal anal sphincter does not relax in response to the presence of stool in the rectum, resulting in constipation, dilation of the antecedent colon, abdominal distension, and delayed meconium production, which may alert health-care providers to the possibility of HD. Accordingly, RAIR cannot be elicited during ARM, and is considered a diagnostic feature for HD. The performance characteristics of HR-ARM in our study (sensitivity 89%, specificity 83%) had significant improvements over BE in our cohort, further confirming the diagnostic value of HR-ARM. We suggest that absence of RAIR on HR-ARM should prompt RSB for diagnostic confirmation, and/or proceeding to corrective surgery.

Our study has a few limitations. Our HD cohort was rather small, in comparison to our normal healthy newborn cohort; however, HD is a relatively rare condition, and longer study periods would have been necessary to collect higher numbers. The small sample size may be one of the reasons for our lower performance characteristics of HD-ARM (sensitivity 89%, specificity 83%) compared with reports using conventional ARM (sensitivity 91%, specificity 94% in one report).[24] Symptoms could not be standardized in HD subjects, which is part of the challenge in evaluating newborns. Special stains for neural tissue were not performed in RSB specimens; however, biopsies were examined by expert pathologists well versed in the diagnosis of HD. Finally, our data is obtained in Chinese newborns, and universal applicability of our

normative values will need to be further validated by studies in newborns of other ethnicities.

In conclusion, we report normative HR-ARM values for prominent anorectal parameters including RP, ACL, and threshold RAIR volume in a large cohort of term and preterm newborns. Further, we demonstrate the diagnostic value of HR-ARM in evaluating newborns with clinical features suspicious for HD.

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DISCLOSURE

We have no financial relationships relevant to this article to disclose.

AUTHOR CONTRIBUTION

TYF, AHJ, DZF, HLM, FRY, LSJ, WJH, YJW performed the research; TYF, YXY, HY, and SJQ designed the research study; YL, JP, CJG, GCP analyzed the data; TYF, GCP, SJQ wrote and revised the paper.

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