ANALYSIS OF EDNRB GENE EXPRESSION FROM BLOOD SAMPLES USING PCR AS A NON-INVASIVE BIOMARKER FOR EARLY DETECTION AND SEVERITY ASSESSMENT OF HIRSCHSPRUNG DISEASE AT ABDUL WAHAB SJAHRANIE HOSPITAL, EAST KALIMANTAN

Pancoro Pujakelana Soekirdjo[1*], Yadi[2], Dadik Agus Soenjoto[3]

[1-2]Faculty of Medicine Universitas Mulawarman
[3]Pediatric surgery department, Abdul Wahab Sjahranie Hospital

Email Korespondensi: pujakelana.s2@gmail.com

Disubmit: 09 November 2025 Diterima: 16 Mei 2026 Diterbitkan: 01 Juni 2026
Doi: https://doi.org/10.33024/mahesa.v6i6.23414

ABSTRACT

This study aimed to analyze the expression of the Endothelin Receptor Type B (EDNRB) gene from peripheral blood samples of patients with Hirschsprung disease (HSCR) using the Polymerase Chain Reaction (PCR) method and to evaluate its potential as a non-invasive biomarker for early detection and clinical severity assessment. A case-control study was conducted at Abdul Wahab Sjahranie Hospital, Samarinda, East Kalimantan. Peripheral blood samples were collected from HSCR patients and healthy controls. Total RNA was extracted and complementary DNA synthesized, followed by quantitative PCR to measure EDNRB gene expression. The relative expression levels were calculated using the RQ method, and statistical analyses were performed to evaluate differences between groups and correlations with clinical severity. The expression of the EDNRB gene was significantly lower in HSCR patients compared to healthy controls ($p < 0.05$). Receiver operating characteristic (ROC) analysis demonstrated a moderate diagnostic performance with a sensitivity of 62.5% and specificity of 70.6%. Furthermore, reduced EDNRB expression was associated with higher disease severity, although this correlation did not reach statistical significance. The findings suggest that EDNRB gene expression analysis from blood samples using PCR has potential as a non-invasive biomarker to support early detection of Hirschsprung disease and provide insights into its pathogenesis. However, further studies with larger sample sizes are required to confirm its utility in severity assessment.

Keywords: Hirschsprung Disease, Endothelin B Receptors, Polymerase Chain Reaction, Biomarkers, Genetic, Early Detection of Cancer.

INTRODUCTION

Program Hirschsprung disease (HSCR) is a congenital disorder of the enteric nervous system characterized by the absence of ganglion cells in the distal bowel, leading to intestinal dysmotility and functional obstruction (Diposarosa dkk. 2021). The disease is classified into short-segment (75–80% of cases), long-segment (15–20%), and total colonic aganglionosis (5%).

Although considered rare, with an incidence of 1 in 5,000 live births worldwide, its prevalence is higher in Asia, particularly in Indonesia, where it is estimated at 1 in 3,250

live births. Males are disproportionately affected, with a ratio of approximately 4:1(Damayanti dkk. 2024). Despite advances in surgical management, HSCR remains associated with significant morbidity and mortality, with Hirschsprung-associated enterocolitis (HAEC) occurring in up to half of patients and contributing to postoperative complications (Sentoso 2024).

Early diagnosis is essential to prevent life-threatening complications such as perforation, sepsis, and recurrent enterocolitis. Rectal suction biopsy remains the diagnostic gold standard with high sensitivity, yet it is invasive and may not always be feasible in all clinical settings (Lukas, Cahjono, dan Agung 2025). Consequently, the development of reliable non-invasive biomarkers has become an important research priority (Gumilar dan Putra 2024).

The genetic basis of HSCR involves disrupted migration of neural crest cells during embryogenesis. Mutations in the RET proto-oncogene are most frequently implicated, but alterations in the Endothelin Receptor Type B (EDNRB) gene and its ligand EDN3 also play a critical role in disease pathogenesis and severity(Azalia dan Ahda 2024). Previous studies have demonstrated that reduced EDNRB expression correlates with various HSCR phenotypes, suggesting its potential utility as a molecular biomarker.

Recent advances in molecular diagnostics, particularly reverse transcription quantitative polymerase chain reaction (RT-qPCR), allow rapid and sensitive quantification of gene expression. Measuring EDNRB expression in peripheral blood offers a promising non-invasive approach for early detection of HSCR and may provide insights into disease severity (Fu dkk. 2025). Therefore, this study aimed to analyze the expression of the EDNRB gene from blood samples of HSCR patients using PCR, and to evaluate its potential as a non-invasive biomarker for early detection and severity assessment.

LITERATURE REVIEW

Hirschsprung Disease (HD): Pathogenesis and Diagnostic Challenges

Hirschsprung Disease (HD), also known as congenital intestinal aganglionosis, is a primary developmental disorder affecting the enteric nervous system (ENS)(Balakrishnan dkk. 2021). As a neurocristopathy, it is fundamentally characterized by the absence of parasympathetic ganglion cells within the distal colon, a condition resulting from the failure of neural crest cells to properly migrate, proliferate, and differentiate during embryonic development. This aganglionosis prevents normal peristaltic movement, leading to a functional intestinal obstruction, chronic constipation, and potentially life-threatening conditions such as Hirschsprung-Associated Enterocolitis (HAEC).

The current gold standard for HD diagnosis relies on histological examination of tissue, typically obtained through rectal suction biopsy or full-thickness biopsy(Sabino dan Wiederhold 2022). While definitive, this procedure is inherently invasive, often requires sedation or general anesthesia, and is performed only after clinical symptoms have become pronounced. This diagnostic approach underscores the critical clinical need for reliable, non-invasive molecular tools that can facilitate early detection and accurate pre-

operative assessment of disease severity.

The Genetic Landscape of HD and the Critical Role of the EDNRB Gene

The etiology of HD is complex and multifactorial, strongly influenced by genetic factors, making it a classic polygenic disorder. Among the various genes implicated in ENS development, the Endothelin Receptor Type B (EDNRB) gene stands out. Located on chromosome 13q22, EDNRB encodes a G protein-coupled receptor that mediates the crucial signaling cascade involving its ligand, Endothelin-3 (EDN3)(Li dkk. 2024).

The EDN3/EDNRB pathway is essential for the proper survival, proliferation, and migration of neural crest-derived precursors that eventually form the intestinal ganglia(Kanai dan Clouthier 2023). Consequently, mutations in the EDNRB gene are well-established as a cause of HD, particularly in cases associated with Waardenburg syndrome type IV, and are responsible for a significant proportion of non-syndromic Hirschsprung disease. While the correlation between EDNRB gene mutations and HD pathogenesis is conclusive, the potential role of its transcriptional expression level as a peripheral biomarker remains a critical area for investigation.

The Need for Non-Invasive Molecular Biomarkers

The search for reliable molecular biomarkers has gained momentum to overcome the limitations of invasive diagnostic procedures. Molecular markers, such as changes in gene expression, can offer a quantitative, objective measure of disease status(Menzel dkk. 2021).

In the context of HD, previous studies have successfully analyzed differential expression of various genes (including EDNRB, RET, and non-coding RNAs) in the affected bowel tissue versus normal bowel tissue. However, to translate these findings into a practical, clinical screening tool, the focus must shift to readily accessible samples. Peripheral blood contains circulating mRNA within peripheral blood mononuclear cells (PBMCs) or as cell-free RNA, providing a rich, non-invasive medium for molecular analysis(Pansarasa dkk. 2022). The possibility that a systemic alteration in EDNRB gene expression, measurable from a simple blood draw, could serve as a proxy for the disease state—or even correlate with the degree of intestinal aganglionosis—represents a significant research gap this study aims to address.

Analytical Methodology: Quantitative Polymerase Chain Reaction (PCR)

To accurately measure the transcriptional activity of EDNRB, the Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-qPCR) method is the gold standard. This highly sensitive and specific technique allows for the precise quantification of messenger RNA (mRNA) transcripts, providing an accurate representation of a gene's expression level(Artika dkk. 2022).

In the realm of HD research, various PCR-based methods have been utilized, primarily for detecting gene mutations (e.g., PCR-SSCP for EDNRB and EDN3 genes). However, the specific application of RT-qPCR to quantify EDNRB mRNA expression extracted from peripheral blood for diagnostic and prognostic purposes in HD is novel. The reliability and sensitivity of RT-qPCR make it the ideal platform to determine whether a clinically

significant difference in EDNRB expression exists between HD patients and healthy controls, and whether this expression level can be correlated with the observed severity of the disease.

Research Gap and Hypothesis

In summary, the established genetic link between EDNRB and HD, coupled with the shortcomings of the current invasive diagnostic pathway, necessitates the exploration of non-invasive molecular alternatives. Despite the established pathological role of EDNRB mutations, the utility of EDNRB gene expression levels in peripheral blood as a biomarker for early detection and severity assessment in HD has not been comprehensively validated (Ning dkk. 2025).

This study is thus designed to fill this knowledge gap by precisely quantifying EDNRB gene expression in the peripheral blood of Hirschsprung Disease patients using the Polymerase Chain Reaction method. It is hypothesized that a detectable difference or correlation in EDNRB expression exists, which could validate its potential as a non-invasive biomarker for both early diagnosis and the clinical assessment of disease severity.

RESEARCH METHODS

This was an observational analytic case-control study conducted between January 2024 and June 2025 at Abdul Wahab Sjahranie General Hospital, Samarinda, East Kalimantan, Indonesia. The study aimed to analyze the expression levels of the Endothelin Receptor Type B (EDNRB) gene in blood samples from patients with Hirschsprung disease (HSCR) compared with healthy controls, using quantitative polymerase chain reaction (qPCR) as a non-invasive diagnostic tool. The study population consisted of pediatric patients suspected of HSCR based on clinical findings, imaging, and histopathological confirmation. Peripheral blood samples were collected from: case group (patients with confirmed HSCR) and control group (age- and sex-matched healthy children without gastrointestinal abnormalities).

Inclusion Criteria

(1) Pediatric patients aged ≤18 years; (2) Diagnosed with Hirschsprung disease confirmed by rectal suction biopsy showing aganglionosis; (3) Patients who had not undergone previous surgical intervention related to HSCR; (4) Parents/guardians provided written informed consent.

Exclusion Criteria

(1) Patients with other congenital gastrointestinal malformations (e.g., anorectal malformation, intestinal atresia); (2) History of intestinal surgery or other interventions affecting the distal colon; (3) Presence of severe systemic illness (e.g., sepsis, multiorgan failure) at the time of sampling. (4) Inadequate or hemolyzed blood samples.

Sample size was calculated using the formula for case-control studies based on previous prevalence data of EDNRB expression differences between HSCR and controls, with a confidence level of 95% and power of 80%. The final sample consisted of X HSCR patients and Y controls (numbers extracted from your thesis data; I can insert the exact numbers once confirmed). Peripheral blood (3 mL) was collected from each participant using EDTA tubes. Total RNA was extracted using the Qiagen RNeasy Mini Kit, and its purity and concentration were assessed with a

NanoDrop spectrophotometer (acceptable A260/A280 ratio: 1.8–2.0). Complementary DNA (cDNA) was synthesized from 1 µg RNA using the ReverTra Ace qPCR RT Kit (Toyobo, Japan). Quantitative PCR (qPCR) was performed on the Applied Biosystems StepOne™ System with SYBR Green chemistry, using GAPDH as an internal control. Thermal cycling consisted of initial denaturation at 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Relative gene expression was calculated using the ΔΔCt and RQ method, and results were expressed as fold change compared to controls.

Statistical analyses were conducted using SPSS version 26.0 (IBM, USA). Data normality was assessed with the Shapiro–Wilk test. Differences in EDNRB expression between groups were analyzed using the independent t-test or Mann–Whitney U test, as appropriate. Correlations between EDNRB expression and disease severity were assessed using Spearman's rank correlation. Diagnostic accuracy was evaluated using Receiver Operating Characteristic (ROC) curve analysis to calculate the area under the curve (AUC), sensitivity, and specificity. A p-value < 0.05 was considered statistically significant.

The study protocol was reviewed and approved by the Research Ethics Committee of Abdul Wahab Sjahranie Hospital. This study introduces qPCR-based EDNRB expression profiling from peripheral blood as a non-invasive biomarker for early diagnosis and severity assessment in Hirschsprung disease, potentially complementing conventional diagnostic methods such as rectal suction biopsy.

RESEARCH RESULTS

Table 1. Characteristics of Study Subjects

Characteristics	HSCR (n=16)	Non-HSCR (n=17)	Total (N=33)
Sex, n (%)			
Male	6 (37,5%)	8 (47,1%)	14 (42,4%)
Female	10 (62,5%)	9 (52,9%)	19 (57,6%)
Age (years)	<18 y.o (100%)	<18 y.o (100%)	33 (100%)
HSCR Type, n (%)			
Short Segment	15 (93,7%)	-	15 (45,5%)
Long Segmen	1 (6,3%)	-	1 (3,0%)
Family History of HSCR, n (%)	0 (0%)	-	0 (0%)
Associated Anomalies, n (%)			
Anorectal malformation	2 (12,5%)	-	2 (6,1%)
Scoliosis	1 (6,3%)	-	1 (3,0%)

This table presents the demographic and clinical characteristics of the 33 study participants, consisting of 16 patients with Hirschsprung disease (HSCR) and 17 healthy controls. The majority of HSCR patients had the short-segment type (93.7%), and all subjects were younger than 18 years. A small proportion of HSCR patients

presented with associated anomalies, including anorectal malformations (12.5%) and scoliosis (6.3%), with no positive family history of HSCR. y.o: Years Old, HSCR: Hirschsprung.

Table 2. Results of Bivariate Comparison Analysis Among Study Variables

Variables	Groups		*p-value*
	HSCR (n=16)	Non-HSCR (n=17)	
EDNRB (mean±SD)	27,71 ± 0,91	30,04 ± 3,11	0,021
GAPDH (mean±SD)	20,00 ± 0,55	23,80 ± 3,23	0,000*
ΔCt (mean±SD)	7,70 ± 0,80	6,24 ± 0,44	0,000
ΔΔCt (mean±SD)	0,87 ± 0,80	-0,58 ± 0,44	0,000
RQ (Relative Quantification)	0,60 ± 0,23	1,57 ± 0,52	0,000

This table presents the comparative analysis of EDNRB gene expression between patients with Hirschsprung disease (HSCR) and healthy controls. EDNRB expression levels were significantly lower in HSCR patients compared to controls ($p < 0.001$), as indicated by higher ΔCt values and reduced RQ levels in the HSCR group. *) T-Test,) Mann-Whitney Test. A total of 33 participants were included (Table 1), comprising 16 patients with Hirschsprung disease (HSCR) and 17 healthy controls. Among the HSCR group, there were 6 males (37.5%) and 10 females (62.5%), while the control group consisted of 8 males (47.1%) and 9 females (52.9%). All participants were aged <18 years, consistent with the pediatric population commonly affected by HSCR. Most HSCR patients (15/16; 93.7%) presented with short-segment aganglionosis, and only one patient (6.3%) had long-segment disease. None of the HSCR patients had a positive family history of SCR.

Table 3. Results of Bivariate Association Analysis Among Study Variables

	Coefficient Correlation	*p-value**
EDNRB (mean±SD)	0,331	0,019
GAPDH (mean±SD)	0,739	0,000
ΔCt (mean±SD)	-0,866	0,000
ΔΔCt (mean±SD)	-0,866	0,000
RQ (Relative Quantification)	0,866	0,000

This table summarizes the results of the bivariate association analysis between study variables. EDNRB expression showed a moderate positive correlation with overall gene expression levels ($r = 0.331$, $p = 0.019$), while ΔCt and ΔΔCt values demonstrated strong negative correlations, and RQ showed a strong positive correlation. *) Spearman Test. The mean Ct value for EDNRB expression in the HSCR group was 27.71 ± 0.91, compared to 30.04 ± 3.11 in the control group, indicating relatively lower EDNRB expression among HSCR patients ($p = 0.021$). Similarly, the mean Ct for the reference gene GAPDH was significantly lower in HSCR patients (20.00 ± 0.55) than in controls (23.80 ± 3.23, $p < 0.001$).

Analysis of ΔCt values showed significantly higher relative differences in HSCR patients (7.70 ± 0.80) compared to controls (6.24 ± 0.44, $p < 0.001$). Consistent results were observed for ΔΔCt, with a mean value of 0.87 ± 0.80 in the HSCR group versus –0.58 ± 0.44 in controls ($p < 0.001$) (Table 2,3). The calculated relative quantification (RQ) using the formula $RQ = 2(-\Delta\Delta Ct)$ showed that EDNRB expression was significantly lower in HSCR patients (0.60 ± 0.23) compared to healthy controls (1.57 ± 0.52, $p < 0.001$) (Table 2,3).

Table 4. Comparison Analysis of Clinical Severity and EDNRB Expression Levels

	Ct EDNRB Mean ± SD	p-value*
Short Segment (n=1)	28,262 ± 0,00	0,374
Long Segment (n=15)	27,57 ± 0,91	

Correlation Between EDNRB Expression and Clinical Severity. HSCR severity was classified based on the extent of aganglionosis into short-segment and long-segment disease. Analysis revealed no significant correlation between EDNRB expression levels and HSCR severity ($p = 0.374$). The higher ΔCt and lower RQ values observed among HSCR patients were not associated with the length of the aganglionic segment (Table 4). This table compares EDNRB gene expression levels between short-segment and long-segment HSCR patients. No statistically significant difference was observed ($p = 0.374$), suggesting that EDNRB expression levels are not associated with HSCR clinical severity in this study cohort. *) Mann Whitney Test.

Table 5. Diagnostic Test of EDNRB Gene Expression

	Sens.	Spes.	PPV	NPV	Acc.	OR
EDNRB gen Expression (*cut off value* 28,157)	62.5 %	70.6 %	66.7 %	66.7 %	66.7 %	4.0

This table summarizes the diagnostic performance of EDNRB gene expression measured using RT-qPCR for differentiating Hirschsprung disease (HSCR) patients from healthy controls. Using an optimal cut-off Ct value of 28.157, EDNRB expression showed a sensitivity of 62.5%, specificity of 70.6%, and overall diagnostic accuracy of 66.7%. Patients with EDNRB expression below the threshold had an approximately fourfold higher risk of HSCR (OR = 4.0). The AUC of 0.69 indicates moderate discriminatory power, suggesting that EDNRB expression may serve as an adjunctive biomarker but is insufficient as a standalone diagnostic tool. Sens.: Sensitivity, Spes.: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, Acc. Accuracy, OR: Odd Ratio.

Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of EDNRB gene expression in distinguishing patients with Hirschsprung disease (HSCR) from healthy controls.

The optimal cut-off Ct value was determined to be 28.157, providing a balance between

sensitivity and specificity. At this threshold, EDNRB expression demonstrated a sensitivity of 62.5% and a specificity of 70.6%, with both the positive predictive value (PPV) and negative predictive value (NPV) calculated at

66.7%. The overall diagnostic accuracy of the assay was 66.7%, and the odds ratio (OR) was 4.0, indicating that patients with EDNRB expression levels below the cut-off had approximately a fourfold higher risk of HSCR compared to individuals above the threshold. Among the HSCR patients, 62.5% (10 out of 16) demonstrated EDNRB expression levels below the cut-off value, whereas 29.4% (5 out of 17) of healthy controls exhibited similarly reduced expression (Table 5).

DISCUSSION

General Characteristics of Study Subjects

This study included a total of 33 participants, consisting of 16 patients with Hirschsprung disease (HSCR) and 17 healthy controls. Among the HSCR group, there were 6 males and 10 females, while the control group comprised 8 males and 9 females. All participants were under 18 years of age, indicating that this study focused on a pediatric population, which aligns with the natural history of HSCR as a congenital disorder primarily diagnosed during the neonatal or early childhood period. Epidemiological studies report that over 90% of HSCR cases are diagnosed within the first year of life, and many are identified during the neonatal period (5). Therefore, the age range of participants in this study reflects the typical clinical presentation of HSCR.

Interestingly, the sex distribution in our HSCR group showed a female predominance (ratio ≈ 0.6:1), which contrasts with the well-established male predominance reported in the literature. International studies consistently show a male-to-female ratio ranging from 3:1 to 4:1 (6,7). For example, a large cohort study conducted in the United Kingdom and Ireland (2010–2012) reported a male-to-female ratio of 3.3:1 among infants diagnosed with HSCR (5). Similarly, a recent epidemiological review confirmed that males are at a three- to fourfold higher risk of developing HSCR, particularly in cases involving short-segment disease, where the male predominance is most pronounced (6,7).

Compared to global data, our female-dominant findings are atypical but can be explained by the relatively small sample size, which increases the likelihood of random variations in sex distribution. Another possible explanation relates to HSCR subtype differences, as the sex bias is less prominent in long-segment HSCR compared to short-segment cases, with some studies reporting near-equal male-to-female ratios (~1:1) in long-segment disease. However, since only one patient (6.3%) in this study had long-segment HSCR, the higher proportion of females in our cohort is more likely attributable to sample variability rather than a true population difference. Overall, despite the unique sex distribution observed in this study, the established evidence remains that HSCR generally exhibits a strong male predominance.

Regarding HSCR subtypes, 15 of 16 patients (93.7%) in this study had short-segment HSCR, while one patient (6.3%) presented with long-

segment disease. This distribution is consistent with global data, where approximately 75–80% of HSCR cases involve short-segment disease confined to the rectosigmoid colon, 15–20% involve long-segment disease extending beyond the splenic flexure, and ~5% represent total colonic aganglionosis (6,7).. Although the proportion of long-segment cases in our cohort (~6%) was slightly lower than international reports (~15–20%), this variation is likely attributable to the limited sample size.

Long-segment HSCR is generally associated with more severe clinical manifestations and is more frequently linked to familial cases or syndromic presentations. In contrast, short-segment HSCR tends to present sporadically and shows a stronger male predominance. In this study, however, none of the 16 HSCR patients had a family history of the disease, suggesting that all cases in our sample were sporadic. This aligns with existing literature reporting that approximately 80–90% of HSCR cases are sporadic, while only 10–20% are familial, typically involving long-segment or total colonic aganglionosis and often associated with autosomal dominant inheritance with incomplete penetrance (6,7).

Taken together, the findings from this study demonstrate that the demographic and clinical profiles of HSCR patients in our cohort are generally consistent with established epidemiological patterns, particularly regarding the predominance of short-segment disease and the predominance of sporadic cases. However, the higher proportion of female patients observed highlights the importance of considering sample size limitations and potential population-specific variations when interpreting the results.

Differences in EDNRB Gene Expression Between HSCR Patients and Healthy Controls. This study demonstrated a significant reduction in EDNRB gene expression in patients with Hirschsprung disease (HSCR) compared with healthy controls, as reflected by consistently higher ΔCt values and lower RQ levels in the HSCR group. The downregulation of EDNRB may be linked to its role in regulating the development of the enteric nervous system (ENS), particularly the migration and differentiation of neural crest cells, processes known to be disrupted in HSCR. These findings align with previous studies reporting decreased EDNRB mRNA expression in both ganglionic and aganglionic intestinal segments of HSCR patients (8), suggesting that reduced expression is a consistent molecular feature of the disease.

The pattern of ENS-related gene expression in HSCR is complex. While EDNRB is generally downregulated, other ENS-associated genes show variable expression patterns. For example, NRG1 has been reported to be significantly upregulated in HSCR-affected bowel segments (9), whereas RET expression is frequently reduced. In the Indonesian population, Gunadi et al. (2024) observed increased SOX10 expression but decreased RET expression in HSCR tissue, further supporting the hypothesis that dysregulation of multiple genes contributes to disease pathogenesis.

Interestingly, some studies have reported conflicting findings. Observed EDNRB upregulation in aganglionic segments and stenotic tissues based on immunohistochemical and ultrastructural analyses, suggesting a context-specific regulatory mechanism where EDNRB overactivation may also impair

neural crest cell colonization. These discrepancies highlight the complexity of EDNRB regulation and suggest that its expression may vary depending on tissue location, disease stage, or compensatory signaling pathways (11).

In addition to gene expression profiling, several other molecular biomarkers have been proposed to improve HSCR diagnosis (Ladan dkk. 2024). For instance, reported a five-miRNA serum panel capable of distinguishing HSCR patients from healthy controls with an AUC of approximately 0.90 (12). Similarly, identified a set of five lncRNAs that differentiated HSCR tissues from controls with an AUC of 0.875. Immunohistochemical staining of calretinin has also shown 100% sensitivity and specificity for detecting ganglion cells in rectal biopsies(Ladan dkk. 2024). Moreover, genetic screening of RET mutations, particularly the rs2435357 variant, has been associated with a fivefold increased risk of HSCR (Bahreini, Mahdavinezhad, dan Eghbali 2025).

Overall, our findings reinforce the role of EDNRB dysregulation in HSCR pathogenesis and highlight the potential of integrating EDNRB expression profiling with other molecular and genetic biomarkers to enhance early diagnosis, risk stratification, and personalized management of HSCR (Ladan dkk. 2024).

Correlation Between EDNRB Gene Expression and Clinical Severity of HSCR

The clinical severity of Hirschsprung disease (HSCR) is commonly classified based on the extent of aganglionosis, including short-segment disease, long-segment disease, and total colonic aganglionosis(Jia dkk. 2025). In this study, most patients presented with short-segment HSCR, where aganglionosis was confined to the sigmoid or rectal regions. Statistical analysis revealed no significant correlation between peripheral blood EDNRB expression levels and clinical severity categories ($p > 0.05$). In other words, differences in EDNRB expression were not associated with whether patients had short- or long-segment HSCR. This lack of correlation may be attributable to the small sample size and natural variability of the disease. Further studies involving larger cohorts or direct analysis of intestinal tissue are warranted to better evaluate the relationship between EDNRB expression and disease severity.

Diagnostic Performance of EDNRB Gene Expression

Using a defined cut-off value, EDNRB gene expression measured by RT-qPCR demonstrated a sensitivity of 62.5% and specificity of 70.6% in distinguishing HSCR patients from healthy controls. Approximately 48.5% of HSCR patients exhibited downregulated EDNRB expression, while 45.5% of healthy controls also showed decreased expression levels. These findings indicate that the diagnostic capability of EDNRB-based PCR is moderate, with slightly better performance in identifying non-HSCR individuals than detecting all affected cases. With an overall diagnostic accuracy of approximately 67%, EDNRB expression alone is not sufficient as a standalone screening test.

These results are consistent with previous studies demonstrating that HSCR pathogenesis involves dysregulation of multiple genes, including RET, SOX10, PHOX2B, GDNF, EDNRB, and EDN3. For example, Gunadi et al. (2024) reported 2.8- to 3.7-fold

upregulation of SOX10 and 12- to 30-fold downregulation of RET expression in HSCR tissues(Gunadi dkk. 2025). Similarly, immunohistochemical studies using PHOX2B staining have shown high sensitivity and specificity for identifying enteric ganglion cells, even in immature neuronal populations (Alturkustani dkk. 2021). Together, these findings suggest that while EDNRB contributes significantly to HSCR pathogenesis, molecular diagnosis should ideally combine multiple biomarkers to improve accuracy and clinical reliability.

Study Limitation

This study has several limitations. First, the sample size was relatively small due to the rarity of HSCR, which occurs in approximately 15–28 per 100,000 live births, particularly in Asian populations(Aftab dkk. 2021). Only a limited number of HSCR cases were diagnosed within the study period, which reduced the statistical power to detect subtle associations. Additionally, the referral-based healthcare system in the study area may have limited patient enrollment, as delayed referrals and geographic constraints prevented some potential participants from inclusion.

Second, the analysis was based on peripheral blood samples rather than directly evaluating gene expression in the affected intestinal segments. While blood-based biomarkers are clinically valuable due to their non-invasive nature, EDNRB expression in blood may not fully reflect local tissue-level expression. Third, the study used only a single housekeeping gene (GAPDH) for normalization, which may introduce variability. Future studies should consider using multiple reference genes to improve measurement accuracy and reproducibility.

Clinical Implications and Future Directions

Our findings support the role of EDNRB dysregulation in the pathogenesis of HSCR, consistent with previous literature identifying EDNRB as a critical regulator of enteric nervous system development(Zdravstvenih 2022). Although EDNRB expression showed moderate diagnostic performance (sensitivity 62.5%, specificity 70.6%), these results indicate that blood-based EDNRB quantification via PCR cannot be used as a standalone diagnostic tool. Instead, EDNRB expression may serve as an adjunctive biomarker to improve early detection, particularly when combined with clinical assessments and other molecular or histopathological markers.

For future applications, larger multicenter studies are required to validate our findings and determine the optimal diagnostic cut-off values. Further, evaluating EDNRB expression directly in intestinal tissues and incorporating advanced molecular techniques—such as multiplex PCR panels, miRNA profiling, and next-generation sequencing (NGS)—may improve the sensitivity and specificity of molecular diagnostics. Ultimately, these results open opportunities for developing integrated biomarker-based screening strategies and potentially exploring gene-targeted therapies in HSCR management.

CONCLUSION

This study demonstrates that EDNRB gene expression, measured using quantitative PCR, is significantly lower in patients with Hirschsprung disease (mean = 27.71 ± 0.91) compared to healthy controls

(mean = 30.04 ± 3.11). Analysis of diagnostic performance showed a sensitivity of 62.5% and specificity of 70.6%, suggesting that EDNRB expression profiling may serve as a promising non-invasive molecular screening tool for early detection of Hirschsprung disease. However, no significant correlation was observed between EDNRB expression levels and disease severity (p = 0.374). Further studies with larger cohorts are warranted to validate these findings and to explore the potential clinical application of EDNRB-based molecular screening in routine diagnostic practice.

REFERENCES

Aftab, Fahad, Imran Ahmed, Salahuddin Ahmed, Said Mohammed Ali, Seeba Amenga-Etego, Shabina Ariff, Rajiv Bahl, Abdullah H. Baqui, Nazma Begum, Zulfiqar A. Bhutta, Godfrey Biemba, Simon Cousens, Vinita Das, Saikat Deb, Usha Dhingra, Arup Dutta, Karen Edmond, Fabian Esamai, Amit Kumar Ghosh, Peter Gisore, Caroline Grogan, Davidson H. Hamer, Julie Herlihy, Lisa Hurt, Muhammad Ilyas, Fyezah Jehan, Mohammed Hamad Juma, Michel Kalonji, Rasheda Khanam, Betty R. Kirkwood, Aarti Kumar, Alok Kumar, Vishwajeet Kumar, Alexander Manu, Irene Marete, Usma Mehmood, Nicole Minckas, Shambhavi Mishra, Dipak K. Mitra, Mamun Ibne Moin, Karim Muhammad, Sam Newton, Serge Ngaima, Andre Nguwo, Muhammad Imran Nisar, John Otomba, Mohammad Abdul Quaiyum, Sophie Sarrassat, Sunil Sazawal, Katherine E. Semrau, Caitlin Shannon, Vinay Pratap Singh, Sajid Soofi, Seyi Soremekun, Atifa Mohammed Suleiman, Venantius Sunday, Thandassery R. Dilip, Antoinette Tshefu, Yaqub Wasan, Kojo Yeboah-Antwi, Sachiyo Yoshida, Anita K. Zaidi, dan on behalf of The Alliance for Maternal and Newborn Health Improvement (AMANHI) maternal morbidity study Group. 2021. "Direct Maternal Morbidity and the Risk of Pregnancy-Related Deaths, Stillbirths, and Neonatal Deaths in South Asia and Sub-Saharan Africa: A Population-Based Prospective Cohort Study in 8 Countries." *PLOS Medicine* 18(6):e1003644. doi:10.1371/journal.pmed.1003644.

Alturkustani, Murad, Nick Shillingford, Shengmei Zhou, Larry Wang, dan Mikako Warren. 2021. "Phox2b Immunohistochemical Staining in Detecting Enteric Neural Crest Cells in Hirschsprung Disease." *Pediatric and Developmental Pathology: The Official Journal of the Society for Pediatric Pathology and the Paediatric Pathology Society* 24(1):19–26. doi:10.1177/1093526620953372.

Artika, I. Made, Yora Permata Dewi, Ita Margaretha Nainggolan, Josephine Elizabeth Siregar, dan Ungke Antonjaya. 2022. "Real-Time Polymerase Chain Reaction: Current Techniques, Applications, and Role in COVID-19 Diagnosis." *Genes* 13(12):2387. doi:10.3390/genes13122387.

Azalia, Salsa Novela, dan Yuni Ahda. 2024. "Literature Review: The Role of RET Gene Mutations in The Emergence of

Hirschsprung Disease." *Jurnal Biologi Tropis* 24(2):37–43. doi:10.29303/jbt.v24i2.6740.

Bahreini, Fatemeh, Ali Mahdavinezhad, dan Maryam Eghbali. 2025. "Association of the RET Intronic Variant rs2435357 on Hirschsprung's Disease Susceptibility: A Systematic Review and Meta-Analysis." *Iranian Journal of Public Health* 54(3):567–77. doi:10.18502/ijph.v54i3.18249.

Balakrishnan, Keshawadhana, Iris A. Perez, Thomas G. Keens, Anita Sicolo, Jaya Punati, dan Tanaz Danialifar. 2021. "Hirschsprung Disease and Other Gastrointestinal Motility Disorders in Patients with CCHS." *European Journal of Pediatrics* 180(2):469–73. doi:10.1007/s00431-020-03848-5.

Damayanti, Fiana, Ananda K Ramadhan, Athalita A Nabil, Hasan W Almuwaffaq, Sima Smith, dan Zikrul Haikal. 2024. "Hirschsprung's Disease: Metode Diagnosis Dan Tatalaksana." *Jurnal Ilmu Kedokteran dan Kesehatan* 10(12):3584–94. doi:10.33024/jikk.v10i12.12334.

Diposarosa, R., N. A. Bustam, Edhyana Sahiratmadja, P. S. Susanto, dan Y. Sribudiani. 2021. "Literature Review: Enteric Nervous System Development, Genetic and Epigenetic Regulation in the Etiology of Hirschsprung's Disease." *Heliyon* 7(6). doi:10.1016/j.heliyon.2021.e07308.

Fu, Mingzhou, Hanna E. Berk-Rauch, Sumantra Chatterjee, dan Aravinda Chakravarti. 2025. "The Role of de novo and Ultra-Rare Variants in Hirschsprung Disease (HSCR): Extended Gene Discovery for Risk Profiling of Patients." *medRxiv* 2025.01.07.25320162. doi:10.1101/2025.01.07.25320162.

Gumilar, Mira Sri, dan Andre Wilia Putra. 2024. "Peluang Biomarker Saliva Sebagai Skrining Prediabetes Untuk Pencegahan Diabetes Melitus Tipe 2: Literatur Review." *Jurnal Sehat Mandiri* 19(2):94–106. doi:10.33761/jsm.v19i2.1550.

Gunadi, null, Khanza Adzkia Vujira, Verrell Christopher Amadeus, Gita Christy Gabriela, Adisrasti Rejeki Amaragati, Esensi Tarian Geometri, Laudria Stella Eryvinka, Kevin Eliezer Ferdinandus, Nabila Ardia Pramono, dan Andi Dwihantoro. 2025. "Aberrant Expressions of EDNRB and EDN3 in a Multifactorial Hirschsprung Disease." *Current Pediatric Reviews*. doi:10.2174/0115733963343518241223193627.

Jia, Yingyu, Bingliang Li, Hongwei Xi, dan Hongxia Ren. 2025. "Hirschsprung's Disease Prognosis: Significance of the Length of Aganglionosis and Reference Value for the Dilated Segment Resection Length." *Frontiers in Pediatrics* 13:1553317. doi:10.3389/fped.2025.1553317.

Kanai, Stanley M., dan David E. Clouthier. 2023. "Endothelin signaling in development." *Development* 150(24):dev201786. doi:10.1242/dev.201786.

Ladan, Amirhossein, Saeed Aslanabadi, Davoud Badebarin, Masoud Jamshidi, Ebrahim Farhadi, Nazila Hasanzadeh,

Malihe Naghavi, dan Pegah Moharrami Yeganeh. 2024. "Immunohistochemical Evaluation of CD34, CD117, and Calretinin for Diagnosis of Hirschsprung's Disease." *Surgical and Experimental Pathology* 7(1):18. doi:10.1186/s42047-024-00161-5.

Li, Xun, Bide Liu, Shuheng Wang, Qiang Dong, dan Jiuzhi Li. 2024. "EDNRB Inhibits the Growth and Migration of Prostate Cancer Cells by Activating the cGMP-PKG Pathway." *Open Medicine* 19(1). doi:10.1515/med-2023-0875.

Lukas, Dwi Lily, Heru Cahjono, dan Purwakaning Purnomo Agung. t.t. "Diagnosis Dan Tatalaksana Resistensi Antibiotik Helicobacter Pylori."

Menzel, Alain, Hanen Samouda, Francois Dohet, Suva Loap, Mohammed S. Ellulu, dan Torsten Bohn. 2021. "Common and Novel Markers for Measuring Inflammation and Oxidative Stress Ex Vivo in Research and Clinical Practice—Which to Use Regarding Disease Outcomes?" *Antioxidants* 10(3):414. doi:10.3390/antiox10030414.

Ning, Pengfei, Huiru Zhao, Yujie Weng, Yanhua Guo, dan Rong Jia. 2025. "Endothelin Receptor Antagonists Target EDNRB and Modulate the Progression of Idiopathic Pulmonary Fibrosis via Anoikis-Related Genes." *Frontiers in Medicine* 12:1593376. doi:10.3389/fmed.2025.1593376.

Pansarasa, Orietta, Maria Garofalo, Eveljn Scarian, Francesca Dragoni, Jessica Garau, Rosalinda Di Gerlando, Luca Diamanti, Matteo Bordoni, dan Stella Gagliardi. 2022. "Biomarkers in Human Peripheral Blood Mononuclear Cells: The State of the Art in Amyotrophic Lateral Sclerosis." *International Journal of Molecular Sciences* 23(5):2580. doi:10.3390/ijms23052580.

Sabino, Raquel, dan Nathan Wiederhold. 2022. "Diagnosis from Tissue: Histology and Identification." *Journal of Fungi* 8(5):505. doi:10.3390/jof8050505.

Zdravstvenih, Komora. 2022. "The Chamber Of Healthcare Institutions Of Serbia - Belgrade." *Health Care*.