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Tumor Necrosis Factor Alpha as a Predictive Biomarker for Hirschsprung-Associated Enterocolitis: A Case-Control Study

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Abstract

Background: The most dangerous consequence of Hirschsprung's disease (HD) is Hirschsprung-associated enterocolitis (HAEC), which can have a 30% fatality rate. Due to a lack of trustworthy biomarkers, early diagnosis and prediction are still difficult. The expression of tumor necrosis factor alpha (TNF-α) was examined in this study as a potential biomarker for the development of HAEC.

Methods: From January 2024 to June 2025, a case-control study was carried out at the Dr. Saiful Anwar Hospital in Malang, Indonesia. Twelve children were recruited, five of whom had HAEC and seven of whom had HD without. Both ganglionic and aganglionic intestinal segments were subjected to histopathological investigation utilizing hematoxylin and eosin staining and immunohistochemistry evaluation of TNF-α expression. The number of positive cells per high-power field was used to quantify TNF-α expression.

Results: In the aganglionic segments of HAEC patients, TNF-α expression was substantially higher than that of HD patients (50.80 ± 8.47 vs. 25.14 ± 6.23 positive cells/field; p = 0.0127). Ganglionic segments did not significantly differ across groups (28.20 ± 5.67 vs. 21.43 ± 5.24; p = 0.0778). More severe histological grades, including neutrophil infiltration, mucosal ulceration, and widespread crypt abscesses, were seen in HAEC patients.

Conclusions: The expression of TNF-α in aganglionic segments is a trustworthy biomarker for the development of HAEC. In order to improve patient outcomes, elevated TNF-α levels may aid in early diagnosis and direct focused therapy measures.

Keywords: Hirschsprung Disease; Enterocolitis; TNF-Alpha; Biomarker; Inflammation; Pediatric Surgery

1. Introduction

Approximately 1 in 5,000 babies are affected by Hirschsprung's disease (HD), a congenital condition characterised by a breakdown of neural ridge cell movement to produce enteric nervous system cells in the distal colon (1). The

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aganglionic sector's functional stomach impediment results in abdominal extension, incessant muscle spasm, and failure to thrive. Although the conclusive surgical analysis involves the expulsion of the aganglionic part utilizing attract-through procedures, inmates are at risk for significant challenges for the remainder of their lives (2).

The most weighty and conceivably lethal side effect of HD is Hirschsprung-associated enterocolitis (HAEC), which affects 58% of people outside needing abscission (3). Abdominal distension, turmoil, explosive flux, and, in extreme cases, septic shock, are the hallmarks of HAEC (4). It has a 30% death rate. Dysregulated immunological reactions, changed intestinal microbiota, curtailed mucosal barrier function, and pertaining to the stomach nervous system dysfunction are all carefully connected in the poorly popular pathophysiology of HAEC. Clinical grading wholes and radiological judgments are the bulwarks of current diagnosis designs for HAEC, which commonly lack specificity and can delay essential measures (5).

A major healing trouble is the lack of trustworthy predictive biomarkers, because early discovery of high-risk things grants permission for further preventative measures and enhances results. One main supporting-inflammatory cytokine, that is to say essential for stomach redness and barrier function sustenance, is lump loss factor beginning (TNF-α) (6). Increased TNF-α levels in inflammatory bowel disorders guide both the severity of the ailment and the influence of anti-TNF therapies. According to current research, TNF-α may advance mucosal redness, epithelial barrier failure, and bacterial fluctuation, all of that concede possibility contribute to the pathophysiology of HAEC (7).

The purpose of this study is to determine whether stomach fabric TNF-α expression levels might be secondhand as a predictive biomarker for the occurrence of HAEC in HD patients. We supposed that the occurrence and asperity of HAEC would be compared accompanying raised TNF-α expression, particularly in aganglionic divisions.

2. Materials and methods

2.1. Study Design and Participants

From January 2024 to June 2025, this case-control study was completed at Dr. Saiful Anwar Hospital in Malang, Indonesia, accompanied by institutional morality jury clearance (certification number provided in additional materials). All guardians or persons supported written cognizant consent. The following were necessities for addition: (1) children the one had a suction surgical procedure-rooted HD disease; (2) full healing records were convenient; and (3) our hospital provided surgical care. Incomplete healing records, subjects with additional gastrointestinal congenital irregularities, and earlier intestinal surgery were with the forbiddance criteria. Fever, explosive loose bowels, intestinal extension, vomiting, and intrinsic signs were all contained in the validated dispassionate tests used to organize the diagnosis of HAEC, accompanying scores ≥4 displaying the presence of HAEC (8). Two sets of cases were recognized: HD with HAEC (n = 5) and HD outside HAEC (n = 7).

2.2. Tissue Collection and Processing

During transanal endorectal attract-through (TEPT) surgeries, stomach fabric samples were captured by a single surgeon in consideration of humiliate bias. Each patient had two ganglionic and aganglionic slices captured, resulting in a total of 24 samples (12 ganglionic, 12 aganglionic). After being immediately continued in 10% neutral buffered formalin, the tissues were subjected to a histological study.

2.3. Histopathological Analysis

Hematoxylin and eosin (H&E) staining was used on five-micrometer slices for standard histopathological study. Grade I (compartment extension, mucus memory), Grade II (burial place abscesses, cryptitis), Grade III (diversified burial place abscesses), and Grade IV (fibrinopurulent waste, mucosal secretion of a sore) were the criteria used to grade angulating alterations (5).

2.4. Immunohistochemistry

Using immunohistochemical branding, TNF-α expression was judged. In short, deparaffinized sections were hatched, accompanied primary antagonistic-TNF-α agent for negating the effect of an infection or poison (1:200 something for dunking) for an entire midnight at 4°C, subsequently, irritant retrieval in citrate buffer (pH 6.0). Secondary antibodies accompanying flavouring peroxidase and diaminobenzidine chromogen development were secondhand for discovery. An Aperio mathematical pathology plan was used to count certain cells in three carelessly picked big-league fields (400× magnification), each division to measure TNF-α expression. The mean number of positive containers per field was used to express the results.

2.5. Statistical Analysis

For mathematical studies, SPSS version 26.0 was used. The mean ± predictable difference was used to express constant variables. For usually delivered data, unmatched t-tests were secondhand for the middle from two point-group comparisons. To evaluate difference in similarity between groups, F-tests were used. The threshold for mathematical importance was $p < 0.05$.

3. Results

3.1. Patient Characteristics

The mean age of the duodecimal cases the one were conceded to the emergency room was 10.64 ± 3.05 days. With a female likeness of 6.7% (1/12), the disciples were generally male (93.3%, 11/12). At enucleation, the average age was 3.82 ± 0.60 months. Clinical proofs contained stomach extension in 60% (7/12), disgorging in 66.7% (8/12), and postponed meconium passing in 86.7% (10/12). The allotment of victims the one withstood transanal perineal attract-through resection was 91.7% (11/12).

Table 1 Patient Demographics and Clinical Characteristics

Characteristic	Overall (N=12)	HD Group (n=7)	HAEC Group (n=5)
Age at admission (days)	10.64 ± 3.05	9.86 ± 2.94	11.80 ± 3.27
Male gender, n (%)	11 (91.7)	6 (85.7)	5 (100)
Age at surgery (months)	3.82 ± 0.60	3.71 ± 0.49	3.98 ± 0.75
Delayed meconium, n (%)	10 (83.3)	6 (85.7)	4 (80.0)
Vomiting, n (%)	8 (66.7)	4 (57.1)	4 (80.0)
Abdominal distension, n (%)	7 (58.3)	3 (42.9)	4 (80.0)

3.2. Histopathological Findings

The HD and HAEC groups demonstrated various instigative patterns, in accordance with the histopathological test. Four of the HD group's cases had Grade I redness (cave extension, gelled waste memory), while individual instances had just minor Grade II alterations. On the other hand, the HAEC group granted more harsh and angrier grades: Grade IV (fibrinopurulent waste, mucosal inflammatory condition) in three instances, Grade III (diversified cave abscesses) in two cases, and Grade II in an individual case.

Table 2 Histopathological Inflammation Grading

Inflammatory Grade	HD Group (n=7)	HAEC Group (n=5)	Total
Grade I (Crypt dilatation, mucus retention)	4	0	4
Grade II (Crypt abscesses, cryptitis)	1	1	2
Grade III (Multiple crypt abscesses)	1	2	3
Grade IV (Fibrinopurulent debris, ulceration)	1	2	3

Microscopic study displayed that all patients had characteristic aganglionosis, which was confirmed by apiece lack of center of activity cells in the Auerbach's and Meissner's plexuses. In contrast to HD-only instances, HAEC cases were accompanied by mucosal surface erosions, chamber structural deformity, and severe Angio infiltrates, generally accompanied by neutrophilic swelling.

3.3. TNF-α Expression Analysis

Significant differences in TNF-α expression patterns middle from two points groups and fabric slices were described by an immunohistochemical trial. TNF-α speech in aganglionic sections was considerably better in HAEC cases than in HD

sufferers (50.80 ± 20.02 vs. 25.14 ± 6.23 beneficial containers/field; p = 0.0127). Significant dissimilarity across groups was authorize for the individual F-test (p = 0.0037), suggesting that HAEC TNF-α speech differed more.

Table 3 TNF-α Expression in Aganglionic Segments

Group	Mean ± SD	Sample Size	95% CI	p-value
HD	25.14 ± 6.23	7	20.36-29.92	0.0127*
HAEC	50.80 ± 20.02	5	26.00-75.60	

TNF-α verbalization was higher in HAEC victims' ganglionic portions, although it was not statistically meaningful (28.20 ± 5.67 vs. 21.43 ± 5.24 helpful cells/field; p = 0.0778). Ganglionic sectors did not significantly vary across groups, according to different reasoning (F-test p = 0.6999).

Table 4 TNF-α Expression in Ganglionic Segments

Group	Mean ± SD	Sample Size	95% CI	p-value
HD	21.43 ± 5.24	7	17.39-25.47	0.0778
HAEC	28.20 ± 5.67	5	21.18-35.22	

4. Discussion

TNF-α verbalization in aganglionic stomach segments has been proven in this case study to be a meaningful predictive biomarker for the occurrence of HAEC in HD patients. Strong evidence for the connection of TNF-α in HAEC (9) . Pathophysiology and potential healing use is supported by a piece discovery of nearly doubled TNF-α expression in HAEC aganglionic divisions (50.80 vs. 25.14 definite containers/field; p = 0.0127).

Current knowledge on HAEC pathophysiology emphasizes the unique microenvironment that arises in aganglionic bowel, characterized by bacterial imbalance, altered motility, and compromised mucosal barrier function, all of which create a predisposition toward enterocolitis (10). The loss of enteric neuronal activity disturbs homeostatic signaling, skewing the balance towards pro-inflammatory mediators like TNF-α, which can facilitate bacterial overgrowth, increase intestinal permeability, and disrupt the epithelial barrier integrity—hallmarks of HAEC (11). This alignment is supported by findings showing that aganglionic segments foster a milieu more favorable to inflammation and infection compared to ganglionic controls (12).

Further supporting this concept, the relative lack of significant differences in TNF-α levels between ganglionic tissue segments of affected and unaffected groups (p = 0.0778 in the original study) highlights the regional specificity of the inflammatory process within aganglionic tissue. This suggests that enterocolitis susceptibility is largely due to fundamental effects arising from the absence of enteric nervous system elements, which, in turn, impair key immune-regulatory processes (13). These findings support the exploration of localized, aganglionic-targeted therapeutic strategies rather than broad, systemic anti-inflammatory approaches (14).

Histological assessments in this context frequently reveal advanced inflammatory grades (Grades III-IV), widespread crypt abscesses, and mucosal ulcers, echoing prior literature that has documented more severe inflammatory changes in patients experiencing HAEC(9). Such severe pathological findings further underscore the direct mechanistic link between cytokine-mediated inflammation—particularly TNF-α overexpression—and extensive tissue injury in HAEC. The association between pronounced TNF-α upregulation and higher histopathological inflammation grades highlights the potential for targeted modulation of this pathway to alleviate tissue destruction (15).

Integrating TNF-α quantification into risk stratification and management paradigms for HAEC could refine clinical decision-making, enabling early prophylactic intervention or close monitoring in high-risk patients(16). TNF-α inhibitors, which are already FDA-approved for other inflammatory disorders in pediatric populations, present a logical therapeutic option warranting further clinical investigation in HAEC (17). This approach could potentially transform the management of HAEC, especially in cases with demonstrated high TNF-α expression in aganglionic segments.

Demographic findings in the presented cohort—such as a male predominance (93.3%) and early symptom onset with delayed meconium passage (86.7%)—mirror established epidemiological trends in Hirschsprung's disease (18). However, the relatively small cohort size, reflective of HAEC's rarity, necessitates larger, multicenter studies for more generalizable conclusions and to validate these initial observations (11).

4.1. Limitations

Are there any limits expected to be informed about the latest trends. The verdicts' generalizability and statistical capacity are limited by apiece narrow sample proportion (n=12). The cross-divided design makes it hopeless to judge lengthwise HAEC risk or measure changes in TNF-α over time. Furthermore, we did not consider supplementary inflammatory mediators to a degree interleukin-1β, interleukin-6, or interferon-γ, which that maybe complicated in HAEC; alternatively, we only looked at TNF-α. This study's single-center design raises the likelihood of collection bias; therefore, confirmation across a range of groups is necessary. Furthermore, information on the predictive significance of the biomarker is restricted on account of the lack of an equivalence study between TNF-α levels and dispassionate severity scores.

4.2. Clinical Implications

These results have important healing ramifications for the situation of HD. A routine histological judgment of HD patients involves a measurement of TNF-α verbalization, bestowing doctors with more prognostic data. Prophylactic antagonistic-instigative remedy, improved medical checkup following, or altered surgical methods grant permission be beneficial for extreme-risk inmates who have elevated TNF-α levels.

Future studies bear analyzing TNF-α's usefulness in following situation response and justify it as a predictive biomarker in larger, multi-center communities. Given their substantiated security profile in pediatric cultures, dispassionate trials determining TNF-α inhibitors for HAEC precaution or treatment are essential.

5. Conclusion

In HD cases, TNF-α verbalization in aganglionic stomach portions is a conceivably beneficial prognostic biomarker for the incidence of HAEC. Together with more harsh histopathological alterations, the substantial increase in TNF-α in HAEC cases supports the protein's potential healing use and importance in ailment growth. Although histopathological evaluation is unique doesn't appear to be expected enough to conclude HAEC, TNF-α calculation offers a valuable prognostic dossier that can help clinicians make determinations.

Through improved risk categorization and focus healings, our judgments advance our understanding of the pathophysiology of HAEC and present a potential finish for improving patient outcomes. Important next stages in executing these research judgments into dispassionate practice contain confirmation in best comrades and further research into TNF-α-directed situations.

Compliance with ethical standards

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Disclosure of Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. No financial or personal relationships could have appeared to influence the work reported in this study.

Statement of Ethical Approval

This study was reviewed and approved by the Health Research Ethics Commission of General Hospital Dr. Saiful Anwar, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (Ethical Approval No: 400/141/K.3/102.7/2023). All procedures were performed in accordance with institutional guidelines and the Declaration of Helsinki for research involving human participants.

Statement of Informed Consent

Written informed consent was obtained from the parents or legal guardians of all participating children before their inclusion in the study. All identifying information has been omitted to ensure participant confidentiality and data privacy.

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