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Research Article
IL23 mRNA Expression in Hirschsprung-Associated Enterocolitis

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Abstract

Background and Objective: Hirschsprung-associated enterocolitis (HAEC) is the cause of morbidity as well as mortality in Hirschsprung patients. Interleukin-23 (IL-23) is a cytokine with pro-inflammatory properties which has potential for autoimmune inflammatory responses in HAEC. Aim of this study was to investigate the IL-23 mRNA expression in patients with HAEC. **Materials and Methods:** Hirschsprung patients' colon were assessed on HAEC degree based on Teitelbaum, then IL-23 mRNA expression was assessed through real time PCR examination. **Result:** IL-23 mRNA expression was obtained on average of 11,954 and SD of 0.772. There was a relationship IL-23 mRNA expression and HAEC degrees (p-value = 0.039, $p<0.05$). **Conclusion:** The higher expression of IL-23 mRNA, the higher the HAEC degree will be. The high expression of IL-23 mRNA in HAEC patients indicates that HAEC is an autoimmune disease.

Key words: Hirschsprung, enterocolitis, mortality, Interleukin-23 mRNA, autoimmune disease

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hirschsprung disease (HD) which is also known as congenital megacolon, is an innate abnormality in the form of the absence of intramural ganglion cell and the presence of nerve stem hypertrophy in distal intestine causing functional obstruction[1]. Genetics which play a role[2] in HD are RET, GDNF, NTN, GFRα, EDNRB, EDN-3, ECE-1, SOX 10, PHOX2B, PAX3 and SIP1.

Hirschsprung-associated enterocolitis (HAEC) is an inflammatory bowel condition, that has clinical characteristics such as fever, abdominal distension, diarrhea, foul-smelling stools and sepsis. Although many etiologies have emerged, biological mechanisms underlying HAEC are still poorly understood[3]. Incidence of HAEC around the world ranges from 6-58%, whereas the mortality rate of HAEC is still quite high at between 6-30%. Non-specific clinical manifestations of HAEC cause frequent diagnosis of gastroenteritis, therefore diagnosis of HAEC is missed or delayed[4].

Interleukin-23 (IL-23) is a newly discovered cytokine which its structure and receptor are very similar to Interleukin-12 (IL-12). Both are heterodimer cytokines that have the same subunit, p40, while another subunit which is specific to IL-23 is p19. IL-23 is an important part of inflammatory response to infection. Interleukin-23 (IL-23) is a member of IL-12, a cytokine with pro-inflammatory properties, which is potential for increasing type 17 T helper (Th17) cells and responsible for autoimmune inflammatory responses[5].

Prior to the discovery of Interleukin-23 (IL-23), Interleukin-12 (IL-12) had been proposed to represent a key mediator of inflammation in mouse models of inflammation[3]. IL-23 is an important part of inflammatory response against infection. It promotes upregulation of the Matrix Metalloprotease 9 (MMP9), increasing angiogenesis and reducing CD8+T-cell infiltration into tumours. IL-23 mediates its effects on both innate and adaptive arms of the immune system that express IL-23 receptor. T-helper 17 (Th17) cells represent the most prominent T cell subset that responds to IL-23, although, IL-23 has been implicated in inhibiting the development of regulatory T cell development in the intestine. Th17 cells produce Interleukin-17 (IL-17), a proinflammatory cytokine that enhances T cell priming and stimulates the production of other proinflammatory molecules such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-alpha), Nitric Oxide Synthase 2 (NOS-2) and chemokines resulting in inflammation. The expression of Interleukin-23A (IL-23A) is decreased after Aryl Hydrocarbon Receptor (AHR) knockdown in THP-1 cells and primary mouse macrophages[6].

The aim of this study was to identify relationship of Interleukin-23 (IL-23) mRNA expression to Hirschsprung-associated enterocolitis (HAEC) degrees. Therefore, providing a reference for future research of HAEC therapy by giving monoclonal antibodies to the p19 subunit from IL-23 mRNA.

MATERIALS AND METHODS

Study design: The design of this study was a cross sectional study with simple random sampling in hirschsprung disease (HD) patients at the Pediatric Surgery Division of Wahidin Sudirohusodo Hospital Makassar-Indonesia from January, 2018-2019.

Subject: Thirty colonic preparations of HD patients were extracted using guanidinium thiocyanate (GuSCn)[7,8] and assessed by anatomical pathology based on Hirschsprung-associated enterocolitis (HAEC) severity of the Teitelbaum classification. IL-23 mRNA expression was examined by Reverse transcription polymerase chain reaction (RT-PCR), used Syber Green PCR Master Mix (Applied Biosystems, USA). This protocol was optimized for the CFX Connect system, Bio-Rad Laboratories instrument. The protocol was synced using instrument by changing coloring dilution according to the manual and following the producer company which was recommended for RT-PCR cycle program[8-10]. The primer set for detection IL23 genes mRNA with realtime PCR were IL-23 forward: GTTCCCCATATCCAGTGTGG and IL23 reverse: 5'-GGATCCTTTGCAAGCAGAAC-3'. Beta actin was used as housekeeping gene with primer β-actin forward: 5'-AGCACTGTCTTGGCGTACAG-3' and β-actin reverse: 5'-GGACTTCGAGCAAGAGATGG-3'. PCR amplification was performed in 35 cycles using the following sequence: 95°C for 5 min initially and 95°C for 15 sec, 55°C for 30 sec and repeated 35 times. Data were normalized based on beta actin expression as housekeeping gene. Reaction was centrifuged shortly and placed into instrument and PCR. Program was ready to go by using Realtime PCR machine (CFX Connect system, Bio-Rad Laboratories, Realtime PCR 96 well 0.1 mL, USA)[11-13].

Ethical clearance: This study was conducted by approval of the ethical clearance of the Health Research Ethics Committee of Medical Faculty of Hasanuddin University, Wahidin Sudirohusodo Hospital, Makassar-Indonesia, the Health Research Ethics Committee no 1115/H4.8.4.5.31/PP36- KOMETIK/2017.

Data analysis: Statistical analysis of the data were utilized by spearman rank test, conclusion for statistical analysis test was based on significance of p-value, if p-value<0.05 then the research hypothesis was accepted. The researchers used the SPSS version 23 computer program for windows.

RESULTS

Total of thirty patients with Hirschsprung-associated enterocolitis (HAEC) were included in this study. Based on sex types reported there were 25 (83.3%) male patients and five (16.7%) female patients (Table 1).

Based on age, mostly 14 (46.63%) patients were ≤1 year old and at least 3 (10.0%) patients were 4-5 years old and more then 5 years old. There were ten patients (33.33%) who were 2-3 years old (Table 1).

According to HAEC Degrees, reported mostly patients were in second and forth degrees (cryptitis or abscess two crypts also fibrin purulent debris and mucous ulceration) who were seven (23.11%) patients and at least two patients were in fifth degree (6.7%) (trans luminal or perforated necrosis) as presented in Table 2. Besides, there were two patients (6.7%) in 0 degree which no abnormality and lastly 6 patients (20%) in first and third degrees which were found crypt dilatation also mucin retention and multiple crypt abscess, respectively (Table 2).

Table 1: Characteristic of HAEC patients according to sex and age

	Sex			
	Male		Female	
Age	Number	Percentage	Number	Percentage
≤1 year	4	13.3	10	33.33
2-3 years	0	00.0	10	33.33
4-5 years	0	00.0	03	10.00
>5 years	1	03.3	02	06.07
Total	5	16.7	25	83.33

Table 2: Description of HAEC degree frequency distribution

Degree	HAEC	Frequency	Percentage
0	There is no abnormality	002	6.70
1	Crypt dilatation and mucin retention	006	20.00
2	Cryptitis or abscess two crypts	007	23.11
3	Multiple crypt abscess	006	20.00
4	Fibrinopurulent debris and mucous ulceration	007	23.11
5	Transluminal or perforated necrosis	002	6.70
Total		30	100.00

Table 3: Overview of frequency distribution of HAEC patients based on IL-23 mRNA expression

IL-23 mRNA expression	Frequency	Percentage
Low*	12	40.0
High**	18	60.0
Total	30	100

*IL-23 mRNA expression below of 11.954, **IL-23 mRNA expression over of 11.954

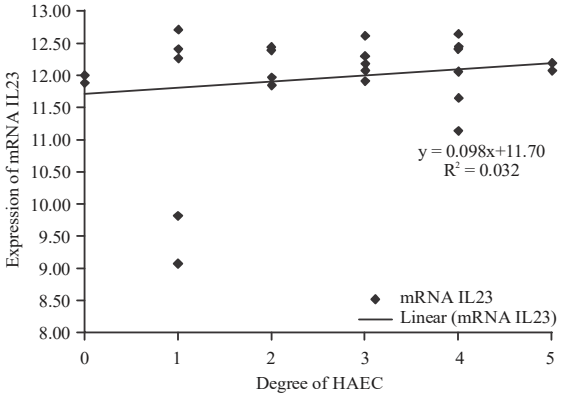

Fig. 1: Relationship between IL-23 mRNA expression and HAEC degree correlations

IL-23 mRNA expression was obtained on average of 11.954 and SD of 0.772, there were 18 patients (60.0%) with high IL-23 mRNA expression and 12 patients (40.0%) with low IL-23 mRNA expression (Table 3). There was a relationship between IL-23 mRNA expression and HAEC incidences (p-value = 0.039, p<0.05 (Fig. 1), it concluded that the higher IL-23 mRNA expression would increase HAEC incidences.

DISCUSSION

The findings exhibit that there were more male patients than female: 25 (83.3%) compared to 5 (16.7%) patients. Similarly to the study from Frykman *et al.*[14], reported in his study that there were 116 Hirschsprung-associated enterocolitis (HAEC) patients, 99 (85.34%) were male and the remaining, 17 (14.66%) were female. Likewise, from the research also conducted[15]. it was identified that out of 110 patients, comparison of male to female ratio was 3.6-1.

From description of the age of Hirschsprung-associated enterocolitis (HAEC) patients obtained in this study, majority was under one year old by 13 children (43.3%). Similarly, Surana *et al.*[16] also reported that occurrence of HAEC in infants was higher in the first week of life (24%) compared to those diagnosed more than first week of life (11%). In addition, delay of meconium also affects occurrence of HAEC (53 h: 44 h), therefore, delayed diagnosis of hirschsprung disease (HD) also affects the occurrence of HAEC in children (16, 6: 4, 6 days). Frykman *et al.*[14] reported that failure to recognize HD in initial perinatal period could make children at greater risk of HAEC.

The results of this study found that the highest degree of hirschsprung-associated enterocolitis (HAEC) was

histopathological second and forth degrees (cryptitis or abscesses of two crypts and fibrinpurulent debris and mucosal ulceration) of seven patients (23.3%) of each degrees. Similarly, Teitelbaum *et al.*[17] reported there were 88% of patients in third degree and above of HAEC and 83% without HAEC in second grade and below. Teitelbaum *et al.*[17] used the degree of Enterocolitis in HD patients to predict the development of HAEC after definitive pull through surgery for third degree and above. Cheng *et al.*[18] reported, compared to normal intestine in the transitional segment, the third degree and above in HAEC patients area increased HAEC incidence by 4.75-fold.

This study found that mean IL-23 mRNA expression in Hirschsprung-associated enterocolitis (HAEC) patients was 11,954 and SD was 0.772. There were 18 children (60.0%) with high IL-23 mRNA expression and remaining 12 children (40.0%) were patients with low IL-23 mRNA expression. It obtained there was a relationship between IL-23 mRNA expression with the occurrence of HAEC (p-value = 0.039<0.05). The higher IL-23 mRNA expression, the higher incidence of HAEC. The higher histopathological degree of HAEC, the higher incidence of HAEC.

Excessive IL-23 expression from this study shows that Hirschsprung-associated enterocolitis (HAEC) in patients with Hirschsprung Disease (HD) is an autoimmune disease[19]. The presence of functional obstruction due to genetic abnormalities in the absence of ganglion cells in the Auerbach plexus and Meissner in patients with HD causes the growth of excess pathogenic bacteria. An infection of the tissue can trigger a local natural immune response which will cause an increase in production of co-stimulators and cytokines by Antigen Presenting Cell (APC)[20]. The APC activated tissue can stimulate autoreactive T cells that meet tissue autoantigen. Inflammatory processes in HAEC stimulate releasing of proinflammatory cytokines, receptors for cytokines IL-23, which promote the development of pro-inflammatory Th17 cells that make autoimmunity response[21].

Immune system that attacks layer of the intestine causes chronic inflammation in the digestive tract[5]. Repeated intestinal inflammation causes damage to the intestinal mucosal immunity system, immature intestinal barrier defences and microbiota dysbiosis, which results in diarrhea, blood stools and foul smelling, abdominal pain, fever, weight loss and even shock[22].

Expression of IL-23p19 is significantly increased in the mucosa of inflamed crohn disease compared to ulcerative colitis and healthy controls. Multiple staining confirms that cells IL-23p19 (+) are mostly CD68 (+) macrophages/DC[23]. IL-23R (+) cells increases significantly in peripheral T cells and NK blood and lamina propria -CD4 (+) and -CD8 (+)[23]. IL-23 significantly promotes the activation and cytotoxicity of IEL and NK IBD cells and triggers peripheral blood inflammatory bowel disease (IBD) cells and lamina propria-T to secrete IFN-γ, TNF, IL-2 and IL-17A which are much higher than controls. Most importantly, IL-23 promotes peripheral blood IBD T cells or lamina propria-CD4 (+) to differentiate into Th17 cells, which is characterized by the increased of IL-17A and RORC expression[24]. To applicate monoclonal antibody therapy to the p19 subunit from IL-23 mRNA to Hirschsprung Disease (HD) therefore, it can prevent and treat Hirschsprung-associated enterocolitis (HAEC).

CONCLUSION

The results of this study confirm statistically correlated between IL-23 mRNA expression and Hirschsprung-associated enterocolitis (HAEC) incidens. IL-23 mRNA expression were higher in HAEC patients and related to degrees of HAEC. Finally, IL-23 mRNA level may become elevated as higher degrees in HAEC patients, potentially indicates that HAEC is an autoimmune disease. Further studies are necessary to con rm these results.

SIGNIFICANCE STATEMENT

This study discovers the possible association of IL-23 mRNA expression to Hirschsprung-associated enterocolitis (HAEC) degree that can be beneficial for HAEC patients. This study will help the researcher to uncover the critical areas of immunological mechanisms in HAEC that many researchers were not able to explore. Thus, a new theory on these immunological mechanisms and possibly other mechanisms may be arrived at.

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REFERENCES

1. Cheng, S., J. Wang, W. Pan, W. Yan and J. Shi *et al.*, 2017. Pathologically assessed grade of Hirschsprung-associated enterocolitis in resected colon in children with Hirschsprung's disease predicts postoperative bowel function. J. Pediatr. Surg., 52: 1776-1781.

2. Amiel, J., E. Sproat-Emison, M. Garcia-Barcelo, F. Lantieri and G. Burzynski *et al*., 2008. Hirschsprung disease, associated syndromes and genetics: A review. J. Med. Genet., 45: 1-14.
3. Austin, K.M., 2012. The pathogenesis of Hirschsprung's disease-associated enterocolitis. Semin. Pediatr. Surg., 21: 319-327.
4. Frykman, P.K. and S.S. Short, 2012. Hirschsprung-associated enterocolitis: Prevention and therapy. Semin. Pediatr. Surg., 21: 328-335.
5. Abraham, C. and J.H. Cho, 2009. IL-23 and autoimmunity: New insights into the pathogenesis of inflammatory bowel disease. Annu. Rev. Med., 60: 97-110.
6. Moore, S.W., D. Sidler and M.G. Zaahl, 2008. The *ITGB2* immunomodulatory gene (CD18), enterocolitis and Hirschsprung's disease. J. Pediatr. Surg., 43: 1439-1444.
7. Dwiyanti, R., M. Hatta, R. Natzir, S. Pratiwi and M. Sabir *et al*., 2017. Association of typhoid fever severity with polymorphisms NOD2, VDR and NRAMP1 genes in endemic area, Indonesia. J. Med. Sci., 17: 133-139.
8. Tambaip, T., M.B. Karo, M. Hatta, R. Dwiyanti and R. Natzir *et al*., 2018. Immunomodulatory effect of orally red fruit (*Pandanus conoideus*) extract on the expression of CC chemokine receptor 5 mRNA in HIV patients with antiretroviral therapy. Res. J. Immunol., 11: 15-21.
9. Surachmanto, E.E., M. Hatta, A.A. Islam and S. Wahid, 2018. Association between asthma control and Interleukin-17F expression levels in adult patients with atopic asthma. Saudi Med. J., 39: 662-667.
10. Hatta, M., E.S. Eko, A.I. Andi and W. Syarifuddin, 2017. Expression of mRNA IL-17F and sIL-17F in atopic asthma patients. BMC Res. Notes, Vol. 10. 10.1186/s13104-017-2517-9.
11. Khodadadi, A., M. Razmkhah, A.R. Eskandari, A. Hosseini, M. Habibagahi, A. Ghaderi and M. Jaberipour, 2014. IL-23/IL-27 ratio in peripheral blood of patients with breast cancer. Iran. J. Med. Sci., 39: 350-356.
12. Yajima, T., A. Yagihashi, H. Kameshima, D. Kobayashi, D. Furuya, K. Hirata and N. Watanabe, 1998. Quantitative reverse transcription-PCR assay of the RNA component of human telomerase using the TaqMan fluorogenic detection system. Clin. Chem., 44: 2441-2445.
13. Simanjuntak, T.P., M. Hatta, S. Rauf, I. Yusuf and M. Tahir, 2018. Forkhead box P3 messenger-RNA expression after *Curcuma longa* extract intervention in early pregnant mice with toxoplasmosis. Res. J. Immunol., 11: 1-6.
14. Frykman, P.K., S. Kim, T. Wester, A. Nordenskjold and A. Kawaguchi *et al*., 2018. Critical evaluation of the Hirschsprung-associated enterocolitis (HAEC) score: A multicenter study of 116 children with Hirschsprung disease. J. Pediatr. Surg., 53: 708-717.
15. Mabula, J.B., N.M. Kayange, M. Manyama, A.B. Chandika, P.F. Rambau and P.L. Chalya, 2014. Hirschsprung's disease in children: A five year experience at a University teaching hospital in Northwestern Tanzania. BMC Res. Notes, Vol. 7. 10.1186/1756-0500-7-410
16. Surana, R., F.M.J. Quinn and P. Puri, 1994. Evaluation of risk factors in the development of enterocolitis complicating Hirschsprung's disease. Pediatr. Surg. Int., 9: 234-236.
17. Teitelbaum, D.H., D.A. Caniano and S.J. Qualman, 1989. The pathophysiology of Hirschsprung's-associated enterocolitis: Importance of histologic correlates. J. Pediatr. Surg., 24: 1271-1277.
18. Cheng, Z., D. Dhall, L. Zhao, H.L. Wang, T.M. Doherty, C. Bresee and P.K. Frykman, 2010. Murine model of hirschsprung-associated enterocolitis. I: Phenotypic characterization with development of a histopathologic grading system. J. Pediatr. Surg., 45: 475-482.
19. Teng, M.W., E.P. Bowman, J.J. McElwee, M.J. Smyth, J.L. Casanova, A.M. Cooper and D.J. Cua, 2015. IL-12 and IL-23 cytokines: From discovery to targeted therapies for immune-mediated inflammatory diseases. Nat. Med., 21: 719-729.
20. Maloy, K.J. and M.C. Kullberg, 2008. IL-23 and Th17 cytokines in intestinal homeostasis. Mucosal Immunol., 1: 339-349.
21. Strober, W., I. Fuss and P. Mannon, 2007. The fundamental basis of inflammatory bowel disease. J. Clin. Invest., 117: 514-521.
22. Catana, C.S., I.B. Neagoe, V. Cozma, C. Magdas, F. Tabaran and D.L. Dumitrascu, 2015. Contribution of the IL-17/IL-23 axis to the pathogenesis of inflammatory bowel disease. World J. Gastroenterol., 21: 5823-5830.
23. Kobayashi, T., S. Okamoto, T. Hisamatsu, N. Kamada and H. Chinen *et al*., 2008. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. Gut, 57: 1682-1689.
24. Liu, Z., P.K. Yadav, X. Xu, J. Su and C. Chen *et al*., 2011. The increased expression of IL-23 in inflammatory bowel disease promotes intraepithelial and lamina propria lymphocyte inflammatory responses and cytotoxicity. J. Leukocyte Biol., 89: 597-606.