ORIGINAL ARTICLE

Comparison of Interleukin-6 (IL-6) as An Inflammations Marker in One-Step and Multistep Surgery in Hirschsprung's Disease Patients

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ABSTRACT

Introduction: Hirschsprung's disease (HD) is a congenital disease with incidence of 1: 10.000 births. HD is signed by part of the intestine that does not have a ganglion causing obstruction symptoms. Definitive management of HD is by performing surgery on the aganglionic bowel. Several decades ago, the management of HD required multistep surgery. However, lately one-step surgery have been developed that allow minimal intervention. The purpose of this study was to compare Interleukin-6 (IL-6) in one-step and multistep postoperative patients in HD. **Methods:** Patients sample in total of 7 people (4 one-step surgery, 3 multistep surgery) taken from May to October 2022 at the Arifin Ahmad Hospital which were then examined for IL-6 at the LONTAR biomedical integrated laboratory, Faculty of Medicine Riau University. Plasma IL-6 levels were measured from postoperative venous blood samples examined using the sandwich ELISA method and the results were processed by statistical T-test to assess the difference IL-6 levels in the two types of surgery methods. **Results:** The average IL-6 level in patients using one-step surgery was 0,14 pg/mL – 47,92 pg/mL and the average IL-6 level in patients using multistep surgery was 0,98 pg/mL – 28,00 pg/mL. This study showed that the level of IL-6 with one-step surgery was higher than multistep surgery but there was no significant difference in terms of inflamatory marker's especially Interleukin-6. **Conclusion:** Interleukin-6 levels as a mark of inflammatory factors in one-step or multistep Hirschprung's disease surgery were not statistically significant.
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Keywords: Interleukin-6 (IL-6), One-step Surgery, Multistep Surgery, Hirschsprung's Disease

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INTRODUCTION

Hirschsprung disease (HD) is a congenital disease with an incidence of 1:10.000 births, with the highest prevalence occurs in males than females (1). HD is characterized by the presence of intestine without ganglion in plexus myenteric (aurbach) and submucosal (Meissner), causing obstructive symptoms (2). The definitive treatment of HD is to perform surgery on the aganglionic part of the intestine. Several decades ago, the management of HD required two step of sugery, the first operation to make colostomy in proximal bowel of the aganglionic segment, followed by a definitive reconstructive procedure several months later. However, in recent years, many single-step surgical prosedures have been developed that allow minimal intervention (3).

From several center who have tried the new procedure, the result of the operation in clinical outcome by looking at defecation pattern after one-step surgery have the same quality as well, even more than the multistep surgical technique (3,4). The intestinal tract is one of the largest immune organ in the body. Interleukin-6 (IL-6) is one of immune systems which is a pro-inflammatory cytokine that mediator of inflammation. IL-6 receptors are widely distributed throughout the intestinal tract, so that is one of the triggers for inflammation in the respone to various stimuli (5).

IL-6 expression was found in enterocytes and colocytes in inflammatory bowel disease and control, that indicates the role of IL-6 in intestinal tract that needs further investigation (6). Recent data suggest that IL-6 also plays role in intestinal epithelial proliferation when there is injury (7). One-step surgery, with minimal trauma, results in lower interleukin-6 levels compared to multistep surgery. The purpose of this study is to analyze the differences IL-6 level in post operative HD patients with one-step surgery and multistep surgery.

MATERIALS AND METHODS

Design, population and study sample

This study used an analytic observasional design with an approach cross sectional which aims to analyze IL-6 levels in patients post operation HD by two different surgery methods. This study took place from May to October 2022. The patients ages ranged from 18 days to 12 years, with 5 male patients. The research sample was 7 patients with Hirschsprung disease who operated with one-step surgery or Transanal pulltrough (4 patients) and multistep surgery or Endorectal pulltrough (3 patients) at Arifin Ahmad Hospital. Inclusion criteria of this study were all patien HD who underwent pulltrouhgh surgery. Exclusion criteria of this study were finding focus of infection before surgery, and patients with additional congenital abnormalities.

Data collection

Plasma IL-6 levels were measured by taking 3 mL post operative venous blood sample (less than 1 hour for one-step surgery and 1.5-2 hours for multistep surgery) which was put into a vacutainer tube containing EDTA. Blood samples are transported as soon as possible using coolbag to the LONTAR FK UNRI Integreted Biomedical Laboratory. Then the sample preparation was carried out by centrifuging the sample for 20 minutes, the temperature was 4°C with speed 3000 rpm. Then separate the supernatant into a microcentrifuge tube each containing 250 µL then store at -80°C before examined. After the samples were collected, plasma IL-6 levels were examined using sandwich ELISA (®Merck) method.

Statistical analysis

Statistical analysis was carried out to see the comparison of IL-6 in the two surgery methods using the T-test on SPSS v.22 on the Windows.

Ethical clearance

The research was approved by Research Ethics Committee, Faculty of Medicine Riau University No. B/155/UN19.5.1.1.8/UEPKK/2022.

RESULTS

From May to October 2022, there were 7 patients according to the inclusion criteria. Four of them were patients who were operated with one-step surgery and three patients were operated with multistep surgery. From the examination of blood plasma samples using the ELISA technique, IL-6 levels were obtained in one-step surgery is higher than multistep surgery (Figure 1).

The highest IL-6 level was obtained from HD patients with one-step surgery is 47.92 pg/mL and the lowest level is 0.14 pg/mL. While the highest IL-6 levels in HD patients operated by multistep surgery is 28.00 pg/mL and the lowest level is 0.98 pg/mL. The results of the

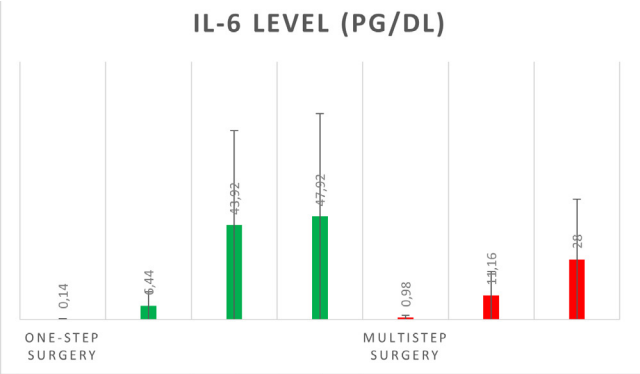

Figure 1: IL-6 level (pg/dl) in One-step surgery and Multistep surgery

T-test statistical test showed p> 0.05, which means that there was no significant difference in IL-6 levels between one-step and multistep surgery.

DISCUSSION

Definitive treatment of HD is to remove the aganglionic segment of the bowel and connect the proximal segment to the gut with ganglion (8). This procedure initially required two step operation or multistep, first by making colostomy in the proximal intestine of the aganglionic segment, after several months and healing of the intestine is good, a definitive reconstruction procedure was carried out known as the Sweson, Duhamel and Soeve method (endorectal pullthrogh) (9). Currently, surgery with minimal injury is becoming popular, considering the healing time, complications, and costs incurred are better than previous traditional surgery methods. So the previous method is modified by using only one-step surgery (10).

Cytokines are one of the mediators of immune system that are dissolved in plasma. Intercellular communication mediated by cytokines. Interleukins are cytokines that work primarily on leukocytes. Interleukin 6 (IL-6) is a pleiotropic cytokine that influences immune responses and inflammatory reactions (5). Higher IL-6 levels in one-step surgery is caused by mucoserectomy surgery, which separates the mucosa from muscular stratum of colon which can increase tissue injury, while in multistep method, mucoserectomy is not performed (11).

When tissue injury occurs, IL-6 will be synthesized and contributes to defense and stimulates the acute phase immune reaction and hematopoiesis. The production of IL-6 will be stopped when tissue homeostasis returns. The immediate and transient expression of IL-6 when tissue is injured or infected is a contribution of IL-6 to host defense. When the source of tissue injury or stress is stopped, there will be a negative regulatory mechanism that terminates the IL-6 transduction cascade, resulting in a decrease in the value of acute phase proteins such as CRP, and at the same time IL-6 synthesis also decreases (12).

In this study, it was also found that the range of IL-6 levels was quite far from patients who were operated on with the same surgery method. In patients with one-step surgery, the lowest level of IL-6 was 0.14 pg/mL and the highest was 47.92 pg/mL, whereas patients with multistep surgery, the lowest IL-6 level was 0.98 pg/ mL and the highest was 28.00 pg/mL. This happen due to previous research by Haeruddin et al. demonstrated that after trauma or tissue injury, IL-6 concentrations can be detected within 60 minutes, peaks at 4-6 hours. After 24-48 hours post-trauma, IL-6 levels gradually decrease but still be detected up to 10 days post-trauma (13). Elevated IL-6 concentrations have been found to correlate with the severity of organ dysfunction and adverse outcomes in various medical conditions. IL-6, or interleukin-6, is a pro-inflammatory cytokine that plays a crucial role in the body's immune response. Increased levels of IL-6 are often observed in patients with severe infections, sepsis, autoimmune diseases, and other inflammatory conditions (14). In this study, blood samples of patients were taken post operative with duration of one-step surgery less than 1 hour and multistep surgery is 1.5-2 hours. The duration of surgery does not provide more severe than mucoserectomy (tissue injury) in stimulating IL-6 synthesis as a sign of acute phase immune reactions and hematopoiesis (11). The results of T-test statistical showed p> 0.05, which means there was no significant difference in IL-6 levels between one-step method and multistep method. From the results of experimental study by Ka Khun et al regarding the role of IL-6 in proliferation and repair of intestinal epithelial tissue after injury, it was found that IL-6 plays role in systemic inflammation that occurs, but does not describe injury from the intestine (7). A study involving neonates with Hirschsprung's disease showed that this transanal pulltrough procedure is effective but can cause inflammatory complications, including elevated IL-6 levels (15).

This study is limited by the number of samples analyzed. Including a larger sample size could potentially reveal additional trends or nuanced findings. Additionally, the research focused solely on the one-step mucocerectomy technique. Future studies investigating alternative surgical approaches for mucocerectomy, such as multi-stage procedures or minimally invasive techniques, could provide valuable insights into the effectiveness and potential advantages of different methods.

CONCLUSION

IL-6 levels by operation one-step surgery higher than multistep surgery , this research is still being investigated to assess how the difference in IL-6 levels in the two surgery methods of HD surgery with even more samples.

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