

## RESEARCH ARTICLE

**Dynamic Pattern of Interleukin-1 $\beta$  and -10 in Cerebrospinal Fluid following Aneurysmal Subarachnoid Hemorrhage**Yesaya Yunus<sup>1</sup>, Julius July<sup>1,\*</sup>, Andi Asadul Islam<sup>2</sup>, Eka Julianta Wahjoepramono<sup>1</sup>, Irawan Yusuf<sup>3,4</sup><sup>1</sup>Department of Neurosurgery, Faculty of Medicine, Universitas Pelita Harapan / Neuroscience Centre, Siloam Hospital, Lippo Village, Jl. Siloam No.6, Tangerang, Banten, Indonesia<sup>2</sup>Department of Neurosurgery, Faculty of Medicine, Hasanuddin University / Dr. Wahidin Sudirohusodo Hospital, Jalan Perintis Kemerdekaan Km.11, Makassar, Indonesia<sup>3</sup>Department of Physiology, Faculty of Medicine, Hasanuddin University Jl. Perintis Kemerdekaan Km.10, Makassar, Indonesia  
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## Abstract

**BACKGROUND:** Inflammation following aneurysmal subarachnoid hemorrhage (SAH) is associated with patient outcome. This study aimed to analyze the dynamics pattern of the pro-inflammatory interleukin (IL)-1 $\beta$  and anti-inflammatory cytokine IL-10 in the cerebrospinal fluid (CSF) following aneurysmal SAH.

**METHODS:** This is a prospective observational study. The CSF was collected prior to surgery, and on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> days after surgery. The CSF was then analyzed for IL-1 $\beta$  and -10 using enzyme-linked immunosorbent assay. Delayed Ischemic Neurological Deficits (DIND) was determined based on new neurological deficits within the first week after surgery. Data was analysed with T test or Wilcoxon Rank-Sum test to evaluate the pattern of biomarkers between DIND and non-DIND groups.

**RESULTS:** There were 33 patients enrolled in this study, 16 patients (48.48%) who experienced DIND and 17 patients (51.52%) were non-DIND. There were significant increase dynamic levels of IL-10 and -1 $\beta$  in DIND patients ( $p < 0.05$ ).

**CONCLUSION:** Significant increase levels of IL-10 and -1 $\beta$  in CSF after aneurysmal SAH are associated with DIND.

**KEYWORDS:** IL-1 $\beta$ , IL-10, DIND

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## Abstrak

**LATAR BELAKANG:** Inflamasi setelah perdarahan subaraknoid karena aneurysma (PSA) berhubungan dengan keluaran pasien. Penelitian ini hendak melihat pola dinamika sitokin proinflamasi interleukin (IL)-1 $\beta$  dan sitokin anti-inflamasi IL-10 dalam cairan serebrospinal (CSS) setelah PSA.

**METODE:** Penelitian ini merupakan penelitian observasional prospektif. CSS diambil sebelum operasi dan pada hari ke-2, -4, dan -6 setelah operasi. Kemudian dilakukan pemeriksaan kadar IL-1 $\beta$  dan -10 dalam CSS menggunakan metode *enzyme-linked immunosorbent assay*. Kejadian *Delayed Ischemic Neurological Deficits* (DIND) didasarkan pada defisit neurologis baru dalam minggu pertama setelah operasi. Data dianalisa menggunakan *T test* atau *Wilcoxon Rank-Sum test* untuk menilai pola biomarker antara kelompok DIND dan non-DIND.

**HASIL:** Terdapat 33 pasien yang memenuhi kriteria penelitian, terdiri dari 16 pasien (48.48%) yang mengalami DIND dan 17 pasien (51.52%) tidak mengalami DIND. Terdapat peningkatan signifikan level IL-10 dan -1 $\beta$  pada pasien-pasien DIND ( $P < 0.05$ ).

**KESIMPULAN:** Peningkatan kadar yang signifikan pada IL-10 dan -1 $\beta$  dalam CSS setelah PSA karena aneurysma, berhubungan dengan kejadian DIND.

**KATA KUNCI:** IL-1 $\beta$ , IL-10, DIND

## Introduction

Inflammation has an important role in the Delayed Ischemic Neurological Deficits (DIND) after aneurysmal subarachnoid hemorrhage (aneurysmal SAH). Interleukin (IL)-1 $\beta$  is a pro-inflammatory cytokine that produced within the first 24 hours after the aneurysmal SAH.(1) IL-1 $\beta$  itself will stimulate anti-inflammatory cytokines, such as IL-10 through activation of beta adrenoceptor and cyclic adenosine monophosphate (cAMP) pathway.(2) IL-10 could then counter the action of pro-inflammatory cytokines (3), such as the activity of IL-1 $\beta$ , thus its role is important to reduce the brain injury after aneurysmal SAH.(4)

This study aimed to analyze the dynamics pattern of the proinflammatory IL-1 $\beta$  and anti-inflammatory cytokine IL-10 in the cerebrospinal fluid (CSF) following aneurysmal subarachnoid hemorrhage.

## Methods

This research was conducted as an observational study using the prospective study approach. This study has been reviewed and approved by Ethical Committee of Hasanuddin University, no. 1014/H4.8.4.5.31/PP36-KOMETIK/2011. Sample size was calculated based on  $Z\beta=1.96$  (with significance level was set at  $p=0.05$ ) and the power of 90% ( $Z\alpha=1.28$ ), total sample of each group is 16, totally 32 for both groups. The consecutive aneurysmal SAH patients (age >18 years old) those underwent clipping surgery were enrolled in this study. All the cases were operated within the first three days after aneurysm ruptured. Patients with heart diseases, unstable hemodynamic state, liver and kidney diseases, pregnancy, current use of steroid, and patient with sepsis or infection were all excluded. CSF was drawn for the first time through lumbar drain that was inserted during preparation of craniotomy. Then post operatively were drawn through the lumbar drain at day 2,4, and 6.

### Enzyme-linked Immunosorbent Assay (ELISA) for IL-1 $\beta$ and -10

Two point five ml of CSF was collected for each test. The CSF was inserted into a blood clot tube, then put in the refrigerator of 4 $^{\circ}$ C for 30 minutes, then centrifuged with 1,500 rounds per minute (RPM) for 20 minutes. The supernatant of the CSF was then aliquoted and kept in the -80 $^{\circ}$ C freezer. After required number of subjects was achieved, all samples were analyzed using ELISA kits for IL-1 $\beta$  (Cat No. EL10028, Abcam, Cambridge, UK) and IL-

10 (Cat. No. PD1000B, R&D Systems, Minneapolis, MN, USA).

### DIND

DIND were recorded when it happened, based on clinical assessment, by investigator and team. It is determined as a new deficit compare to pre-operative state, and it was monitored for the first 7 days after surgery. The T test (normal data distribution) or Wilcoxon Rank-Sum (abnormal data distribution) was used to analyze the mean difference between groups.

## Results

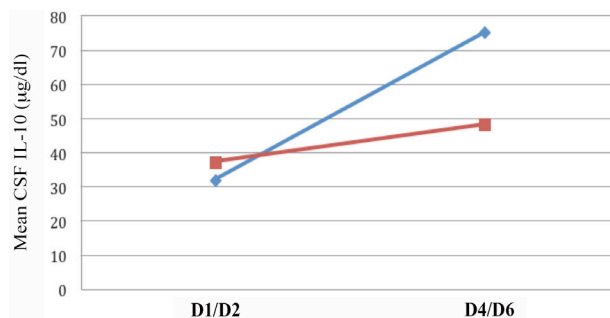
Thirty-three patients enrolled in this study, consisted of 16 males (48.48%) and 17 females (51.52%). The average age of patients was 53.91 years old ( $53.91 \pm 12.27$ ) with the range from 18 to 84 years old. There were 16 patients (48.48%) who experienced DIND and 17 patients (51.52%) did not experience DIND.

The mean level of IL-10 and -1 $\beta$  were grouped in D1/D2 and D4/D6 to follow the natural course of DIND. If it is happened, it usually starts at the first three day, then escalating during the first week. The D4/D6 level represents the escalating level of the IL-10 and -1 $\beta$ . The

**Table 1. Dynamic pattern of IL-10 in CSF between DIND and non-DIND.** D1: 1<sup>st</sup> day, prior to surgery; D2: 2<sup>nd</sup> day after therapy, D4: 4<sup>th</sup> day after therapy, D6: 6<sup>th</sup> day after therapy.

Group	The mean concentration of IL-10 ( $\mu$ g/dL)			Wilcoxon test
	D1/D2	D4/D6	$\Delta$	
DIND (n=16)	32.04 $\pm$ 27.30	75.22 $\pm$ 46.1	43.18	$p=0.008$
Non-DIND (n=17)	37.25 $\pm$ 35.77	48.23 $\pm$ 43.16	10.97	$p=0.239$

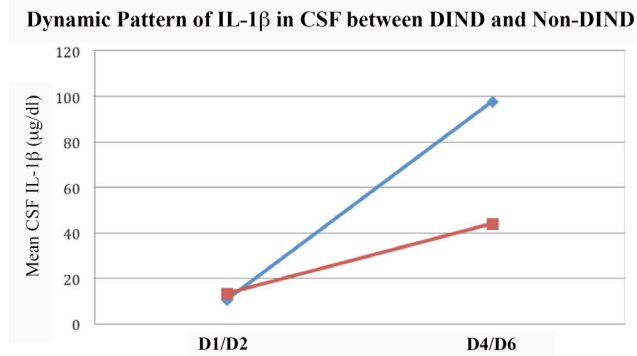
**Dynamic Pattern of IL-10 in CSF between DIND and Non-DIND**



**Figure 1. Dynamic pattern of IL-10 between DIND (blue line) and Non-DIND (red line) groups.** D1: 1<sup>st</sup> day, prior to surgery; D2: 2<sup>nd</sup> day after therapy, D4: 4<sup>th</sup> day after therapy, D6: 6<sup>th</sup> day after therapy.

**Table 2. Dynamic pattern of IL-1 $\beta$  in CSF between DIND and non-DIND.** D1: 1<sup>st</sup> day, prior to surgery; D2: 2<sup>nd</sup> day after therapy, D4: 4<sup>th</sup> day after therapy, D6: 6<sup>th</sup> day after therapy.

Group	The mean concentration of IL-1 $\beta$ ( $\mu$ g/dL)			Wilcoxon test
	D1/D2	D4/D6	$\Delta$	
DIND (n=16)	10.59 $\pm$ 34.15	97.79 $\pm$ 108.57	87.2	$p=0.005$
Non-DIND (n=17)	13.31 $\pm$ 30.18	44.21 $\pm$ 86.84	30.9	$p=0.158$



**Figure 2. Dynamic pattern of IL-1 $\beta$  between DIND (blue line) and Non-DIND (red line) groups.** D1: 1<sup>st</sup> day, prior to surgery; D2: 2<sup>nd</sup> day after therapy, D4: 4<sup>th</sup> day after therapy, D6: 6<sup>th</sup> day after therapy.

mean difference during the time course of D1/D2 to D4/D6 is simple presented as the value of  $\Delta$ . The details for IL-10 is presented in Table 1 and Figure 1, and the details for IL-1 $\beta$  is presented in Table 2 and Figure 2. There were significant increase dynamic levels of IL-10 and -1 $\beta$  (Figure 1 and 2) in DIND patients ( $p<0.05$ ).

## Discussion

This study shows there was no age or gender predilection between DIND and non-DIND group ( $p=0.0282$ ). This result is similar with the previous study done by July *et al.* in 2012 and Polin *et al.* in 1998.(5,6) There were 16 patients (48.48%) suffered DIND within 6 days after rupture. Majority happened on day 4 after ruptur (8 patients). This was accordance with the DIND theory which start on day 3-4 after rupture, the peak on day 7-10, and resolved after day 14.(7)

Based on Figure 1, dynamic changes of IL-10 were more dominant for DIND patients. On day 4/6, level of IL-10 in CSF was also higher in patients with DIND. The DIND group shows that the IL-10 was lower on day D1/D2 compare to Non-DIND group, but it was continuously increase and on day D4/D6, it became significantly higher ( $p=0.008$ ). We also could see, the level of IL-10 increased

in the non-DIND group, but it's not significant. Mellegard in 2011 had mentioned that IL-10 level will keep increasing from day 2 to day 5 after the aneurysmal SAH.(8)

In term of the dynamic pattern of IL-1 $\beta$  on day 4/6, it also shows higher level on the patients with DIND. In Table 2, on day D4/D6, IL-1 $\beta$  increased significantly ( $p=0.005$ ) for DIND group, but the non-DIND group, the increase is not significant. The IL-1 $\beta$  tend to stable during the first day after rupture, then increase on day 4 to 6. The increase level in the CSF during day 4 to 10 is associated with unfavourable condition of patients. It's been mentioned in the previous study that, persistent increase of IL-1 $\beta$  in the CSF after aneurysmal SAH is associated with DIND.(9,10) It's probably related with more severe vasospasm and ischemic brain injury. Clinically those conditions are recognized as DIND.

## Conclusion

Significant increase level of IL-10 and -1 $\beta$  in CSF after the aneurysmal SAH are associated with DIND.

## References

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