MAKROFAG PENGEKSPRESI IL-1β SERTA RESPONS INFLAMASI SISTEMIK PADA FIKSASI INTERNA DINI FRAKTUR FEMUR TERTUTUP LEBIH RENDAH DIBANDINGKAN DENGAN YANG TERTUNDA

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Abstrak

Fiksasi interna merupakan salah satu modalitas terapi dalam penanganan fraktur. Fiksasi interna dini dan tertunda masih menjadi suatu perdebatan karena adanya perbedaan komplikasi yang ditimbulkan, terutama yang berhubungan dengan respons inflamasi sistemik. Tujuan penelitian ini adalah untuk mengetahui perbedaan makrofag pengekspresi IL-1β (dengan pengecatan imuno histokimia) antara saat tindakan fiksasi interna dini dan tertunda serta untuk mengetahui perbedaan respons inflamasi sistemik (IL-6 sebagai marker) pascafiksasi interna dini dan tertunda pada fraktur femur tertutup.

Penelitian ini merupakan penelitian quasi eksperimental *" post test only control group design"*. Penelitian ini dilakukan di Bagian Bedah RSUP Sanglah Denpasar dengan besar sampel dihitung dengan rumus Pocock. Kemudian dilakukan uji normalitas K-S, t test, t-paired test dan uji korelasi.

Dari penelitian ini diperoleh hasil bahwa (1) terdapat perbedaan bermakna antara makrofag pengekspresi IL-1β pada saat perlakuan **(**hari I) dan makrofag saat kontrol (hari III-V) (2.37±2.98 % *vs* 4.99±4.89 %, $p<0.05$) dan (2) terdapat perbedaan yang bermakna kadar IL-6 serum sesudah fiksasi interna antara kelompok fiksasi interna dini dan tertunda (51,17± 23,19 pg/ml vs 95,39± 80 pg/ml, $p<0.05$).

Dari penelitian ini dapat dibuat suatu kesimpulan, yaitu (1) reaksi inflamasi sekitar lokasi fraktur lebih rendah pada fiksasi interna dini daripada pada fiksasi interna tertunda dan (2) kadar IL-6 pasca fiksasi interna dini lebih rendah dari pada yang tertunda, dan kadar IL-6 serum sebelum fiksasi interna dapat dipakai sebagai prediktor kadar IL-6 pascafiksasi interna.

Kata kunci : Fiksasi interna dini, fiksasi interna tertunda, makrofag pengekspresi IL-1β, respons inflamasi sistemik

Pendahuluan

Tindakan fiksasi interna dini dan tertunda saat ini masih menjadi sebuah perdebatan, khususnya mengenai *early total care* (tindakan dini), *damage control* dan *delayed total care* (tindakan tertunda) pada trauma multiple. Johnson (1985)

melaporkan bahwa fiksasi interna pada *major fracture* dengan penundaan lebih dari 24 jam menyebabkan peningkatan 5 kali terjadinya komplikasi ARDS (*Adult Respiratory Response Syndrome*). Pada *isolated femoral fracture,* terjadi 10% *fat embolism syndrome* jika tindakan fiksasi dilakukan setelah 10 jam dan 0% jika dikerjakan sebelum 10 jam (Pinney, 1998). Fakta ini disebabkan oleh terjadinya aktivasi *innate immunity* (Heitbrink, 2006). Namun, sampai saat ini perbedaan inflamasi lokal pada saat fiksasi interna dan respons inflamasi sistemik akibat tindakan fiksasi interna dini dan tertunda pada fraktur belum diketahui.

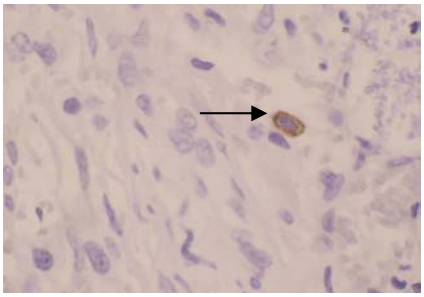
Makrofag merupakan sel imun utama dijaringan dan pada trauma hebat makrofag sering mengalami gangguan respons imun berupa gangguan imunitas seluler (Franke,2006). Demikian juga kerusakan jaringan karena pembedahan akan memicu makrofag yang telah teraktivasi sebelumnya untuk mengekspresikan mediator inflamasi sehingga mempengaruhi respons inflamasi baik lokal maupun sistemik. Untuk mengurangi komplikasi pascafiksasi interna, jenis tindakan (cara fiksasi) dan *timing* (waktu kapan tindakan dilakukan) dapat dipertimbangkan sebagai cara pencegahan.

Materi dan Pembahasan

Penelitian ini merupakan penelitian quasi eksperimental dengan rancangan *" post test only control group design"* pada fiksasi interna dini dan tertunda fraktur femur tertutup (Suryabrata,2000).

Makrofag pengekspresi IL-1β

Dari hasil penelitian ini diperoleh makrofag pengekspresi IL-1β pada fiksasi interna dini reratanya 2,37±2,98 %, sedangkan pada fiksasi interna tertunda reratanya 4,99±4,89 %. Dengan menggunakan uji t persentase makrofag pengekspresi IL-1β saat fiksasi interna dini berbeda bermakna dengan persentase makrofag pengekspresi IL-1β saat fiksasi tertunda ($p<0,05$).



Gambar 1 : Makrofag pengekspresi **IL-1β**

Penelitian ini menunjukkan adanya aktivitas makrofag yang lebih tinggi pada hari III-V (kelompok kontrol). Aktivasi makrofag bargantung pada lingkungan mikro jaringan. Hal ini ada kesesuaian dengan penelitian Einhorn (1995) yang menggunakan hewan coba tikus Sprague-Dawley. Ogura (1999) melaporkan bahwa puncak *priming index* terjadi pada hari II-V.

Pada respons imun seluler, makrofag merupakan sel yang sangat penting dan akan mengalami aktivasi karena adanya *danger signal*. Aktivasi makrofag secara klasik memerlukan signal berupa INF-γ melalui INF-γ-R untuk mengekspresikan mediator proinflamasi (Mosser, 2003). Pada trauma bedah jantung terjadi penurunan IFN-γ pada hari-hari pertama sehingga tidak banyak terjadi aktivasi makrofag melalui jalur klasik dan akan normal kembali pada hari III-V (Franke,2006). Sehingga makrofag aktif klasik lebih tinggi pada hari III-V.

Dalam proses inflamasi, jika keadaan homeostasis tercapai, maka PMN akan mengalami apoptosis dan berkurang jumlahnya pada hari III-V dan digantikan fungsinya oleh makrofag (Kumar, 2005). Dari penelitian Daley (2005) yang menggunakan hewan coba tikus yang mengalami netropenia didapatkan bahwa PMN pada luka 100 kali lebih rendah dari kontrol, dan *supernatant* dari cairan luka dianalisis dan diketahui bahwa terbentuknya sitokin proinflamasi lebih tinggi daripada kontrol tetapi tidak terdapat perbedaan terbentuknya IL-10. Ia menyimpulkan bahwa produk PMN seperti PGE2 akan menekan terbentuknya

proinflamasi mediator tetapi tidak menekan IL-10. Pada awal trauma, PMN sangat banyak disekitar jaringan yang cedera dan produknya seperti PGE2 meningkatkan IL-10 dan menekan IL-12 sehingga secara tak langsung akan menurunkan proinflamasi sitokin (Harizi, 2002; Daley, 2005; Franke,2006).

Dari penelitian tersebut disimpulkan bahwa adanya hambatan produksi sitokin proinflamasi oleh produk PMN menyebabkan aktivasi makrofag terhambat, sehingga makrofag pengekspresi IL-1β (reaksi proinflamasi dijaringan) lebih rendah pada hari I.

Kadar IL-6 dalam serum 6 jam pascafiksasi interna

IL-6 dalam serum 6 jam pascafiksasi interna kelompok perlakuan sebesar 51,17± 23,19 pg/ml, dan kadar IL-6 dalam serum 6 jam pascafiksasi interna kelompok kontrol sebesar 95,39± 80,29. Dengan uji t test terdapat perbedaan yang bermakna antar kelompok ($p<0,05$).

Besarnya respons inflamasi pascapembedahan diperkirakan bergantung pada interaksi yang kompleks antara molekul mediator proinflamasi dan antiinflamasi (Warltier *et al*., 2002). Kerusakan jaringan lokal pada fraktur akan memicu makrofag dan respons inflamasinya akan memicu respons sistemik yang awalnya merupakan pelindung tubuh agar kerusakan tidak meluas (Munford dan Pugin, 2001).

IL-6 sebelum fiksasi interna mempunyai pengaruh positif terhadap IL-6 pascafiksasi interna ($p<0,05$). Keadaan ini dapat diartikan bahwa kadar IL-6 serum sebelum fiksasi interna dapat meramalkan kadar IL-6 pascafiksasi interna. Dengan analisis *univariate of Variance (Univariate General Linear Model)*, pada penelitian ini didapatkan bahwa adanya perbedaan kelompok (dalam hal ini menunjukkan adanya perbedaan waktu fiksasi interna) menyebabkan terjadinya perbedaan secara bermakna ($p<0,05$) IL-6 serum pascafiksasi interna pada kedua kelompok. Hal ini karena aktivitas makrofag yang tinggi pada hari III fraktur (Einhorn,1995). Puncak

priming index terjadi pada hari II-V (Ogura,1999). Daley (2005) dalam percobaannya dengan tikus neutropenia mengatakan bahwa produk PMN menghambat terbentuknya sitokin proinflamasi.

Jadi, dalam penelitian ini diperoleh hasil bahwa adanya makrofag pengekspresi IL-1β lebih tinggi pada kelompok kontrol merupakan petanda adanya proses inflamasi yang lebih aktif. Keadaan tersebut disertai dengan adanya *priming* yang maksimal pada hari III-V dan fiksasi interna yang merupakan tindakan bedah akan menambah kerusakan jaringan sehingga dapat memicu IL-6 serum lebih banyak pada kelompok kontrol.

Kesimpulan

1. Reaksi inflamasi sekitar lokasi fraktur lebih rendah pada fiksasi interna dini daripada fiksasi interna tertunda.
2. Kadar IL-6 serum pasca fiksasi interna dini lebih rendah daripada yang tertunda dan kadar IL-6 serum sebelum fiksasi interna dapat dipakai sebagai prediktor kadar IL-6 serum pascafiksasi interna.

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IL-1β - EXPRESSING MACROPHAGE AND SYSTEMIC INFLAMMATORY RESPONSE IN EARLY INTERNAL FIXATION OF CLOSED FEMORAL FRACTURE LOWER THAN DELAYED ONE

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Abstract

Internal fixation is one of the treatment modalities in fracture management. Recently, the early and delayed internal fixations still become debatable topics. It is caused by complication distinction especially related with systemic inflammatory response. The research aims are: (1) to know the difference between IL-1β-expressing macrophage before early and delayed internal fixation procedure, and (2) to know the difference between the systemic inflammatory response after early internal fixation and delayed internal fixation of closed femoral fracture.

The design of the study is quasi experimental *"post test only control group design"*. All study activities were carried out at Central Hospital Denpasar. Sample size was estimated by Pocock formula. Then, K-S normality test, t-test group, t-paired test, and correlation test were conducted.

The result shows that there is a significant lower of IL-1β -expressing macrophage at first day **(**day I) than at third –fifth day (day III-V) (2.37±2.98 %, vs 4.99±4.89 %, $p<0.05$). Another result shows that serum IL-6 after internal fixation at first day (early) significantly lower than that third-fifth day (delayed) (51,17± 23,19 pg/ml vs 95,39± 80 pg/ml, $p<0.05$).

From this study, it can be concluded that: (1) there is lower local inflammatory reaction at early internal fixation than delayed one and (2) the increase of serum IL-6 before and after internal fixation at first day (early) significantly lower than that third-fifth day (delayed), and the serum IL-6 before internal fixation can be used as a predictor of serum IL-6 after internal fixation.

Key words : Early internal fixation, delayed internal fixation, macrophage activity, systemic inflammatory response.

Background

Internal fixation is one of modalities in fracture treatment. Recently, the procedure is still under debate especially concerning early total care, damage control and delayed total care.

Johnson (1985) reported that an internal fixation on a major fracture with a delay more than 24 hours would cause a five times increase in occurrence of ARDS (Adult Respiratory Response Syndrome) as a complication. On an isolated femoral fracture, 10% incidence of fat embolism syndrome will occur if the fixation is delayed after 10 hours and 0% if it is done before that (Pinney, 1998). These facts are due to the innate immunity activation which is in synergy with the losing of tissue barrier function (Heitbrink, 2006). However, till recently the difference between local inflammation in internal fixation and systemic inflammatory response due to early internal fixation and delayed one are not adequately understood.

Macrophage is a primary immune cell which produces local cytokine in tissue and in severe trauma macrophage often suffers alteration in cellular immune response (Franke,2006). Surgeon, while doing internal fixation will manipulate tissue, therefore creating more tissue damage. This condition will induce the pre-activated macrophage to express inflammatory mediators which give influences on either local or systemic inflammatory response. To reduce post-internal fixation's complications, types of procedure (fixation methods) and timing of procedure can be considered as preventive measures.

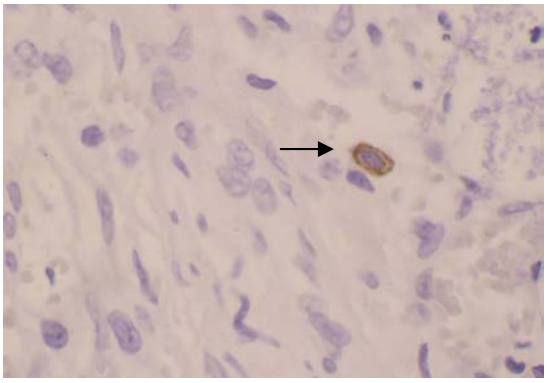
Materials and Discussion

This is a quasi experimental study with *"post-test only control group design"* on early internal fixation and delayed internal fixation of closed femoral fracture (Suryabrata,2000).

IL-1β - expressing macrophage

From this study we found that the mean for IL-1β - expressing macrophage at intervention group **(**day I) was 2,37±2,98 %, however in control group (day III-V) was 4,99±4,89 %. Using t-test, the percentage of IL-1β - expressing

macrophage in early internal fixation was significantly different with the delayed one ($p<0,05$).



Picture 1: **IL-1β - expressing macrophage**

This study showed that the activity of the macrophage was higher at day III-V (control group). Macrophage activation depends on tissue micro environemnt. The similar result was also shown by Einhorn (1995) who used Sprague-Dawley mouse whose legs made broken. Ogura (1999) reported that the peak for priming index occured at day II-V.

In the cellular immune response, macrophage is a very important cell and it will be activated by the danger signal. Macrophage activation classically need signal as INF-γ through INF-γ-R to express pro-inflammatory mediators (Mosser, 2003). In cardiac surgery IFN-γ decreases at first days so there is not much macrophage activation through classic pathway and backs to normal again at day III-V (Franke,2006). Therefore active macrophage is classically higher at day III-V.

At inflammatory process if the homeostasis is achieved, the PMN will disapear through apoptosis process and its number will decrease at day III-V and its function is replaced by macrophage (Kumar, 2005). Daley (2005) who used neutropenic mouse found that PMN in the wound was 100 times lower than

control, and he analyzed the supernatant from wound oozing fluid and found that the pro-inflammatory cytokine was higher than the control, but not for IL-10. He concluded that the product of PMN such as PGE2 will suppress the production of pro-inflammatory mediators. Because of PGE2 will increase IL-10 and suppress IL-12 so indirectly will reduce pro-inflammatory cytokine (Harizi, 2002; Daley, 2005), thus the macrophage activation at day III-V is higher than day I which is shown by the higher percentage of macrophage which expresses cytokine. The result of this study and statistical analysis prove that IL-1β - expressing macrophage on intervention group is lower than control group.

IL-6 serum concentration at 6 hours post-internal fixation

IL-6 serum concentration at 6 hours post-internal fixation in intervention and control groups were 51,17± 23,19 pg/ml and 95,39± 80,29 pg/ml, respectively. And t-test showed a significant difference between groups ($p<0,05$).

Instead of surgical procedure itself (secondary insult), the magnitude of inflammatory response assumed depends on complex interaction between pro-inflammatory and anti-inflammatory mediators (Warltier *et al*., 2002). This local tissue damage will induce macrophage and its inflammatory response will induce systemic response which in the beginning is for body's protection so the damage tissue is localized (Munford and Pugin, 2001).

IL-6 pre-internal fixation had positive correlation to IL-6 serum concentration post-internal fixation ($p<0,05$). This finding can be interpreted IL-6 serum concentration pre-internal fixation may predict IL-6 serum concentration post-internal fixation. Using univariate of variance analysis (Univariate General Linear Model), in this study we found that the difference between groups (the difference in internal fixation timing) will cause a significant difference ($p<0,05$) in IL-6 serum concentration post-internal fixation. It means that IL-6 serum concentration post-internal fixation in intervention group is different with the

control group. Maybe it is caused by higher macrophage activity at day III of fracture that has been studied by Einhorn (1995). Besides that Ogura in 1999 reported that the peak of priming index occured at day II-V. And Daley (2005) in his experiment using neutropenic mice found that PMN product inhibited the production of pro-inflammatory cytokine.

Conclusion

Therefore from the study result and supported by discussion, we conclude that:

1. Inflammatory reactions surround fracture location is lower in early internal fixation compare to delayed one.
2. IL-6 serum concentration post-early internal fixation is lower than the delayed one and IL-6 serum concentration before internal fixation can be used as predictor for IL-6 serum concentration after internal fixation.

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