

**PENGARUH INFUS ALBUMIN PADA PERUBAHAN KADAR ALBUMIN SERUM, SITOKIN PROINFLAMASI (TNF $\alpha$ , IL1, IL6), CRP, MMP8 DAN EKSPRESI EGFR, ERK1, ERK2, TGF $\beta$ , KOLAGEN, MMP8 JARINGAN, TERHADAP PERCEPATAN PENYEMBUHAN LUKA (Studi eksperimental menggunakan Rats-Sprague Dawley)**

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**ABSTRACT**

**Effect of Albumin Infusion on Changes of Serum Albumin Levels, Proinflammation Cytokines (Tnf $\alpha$ , Il1, Il6), Crp, Mmp8 And Expression of Wound Tissues Egrf, Erk1, Erk2, Tgf $\beta$ , Collagen, Mmp8 on the Acceleration of Wound Healing**

**(Experimental Study on Sprague Dawley Rats)**

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This study aims to explain the mechanism and to prove the role of albumin in the process of wound healing with the administering of preoperative or postoperative albumin infusion or normal protein diet during the state of hypoalbuminemia.

Twenty five Sprague Dawley Rats of known age and weight after being kept in standardized environment and feeding for 7 days, were divided into two groups. In the first group (A) 5 Rats were given normal protein casein 20% and served as control. The other group were fed with casein 2% for 14 days to induce the state of hypoalbuminemia, and then were divided into four groups: group (B) to be given preoperative albumin infusion, group (C) to be given preoperative and postoperative diet casein 20%, group (D) with postoperative albumin infusion and group (E) to continue being fed with casein 2%. Four incisions 2cm each were made on the back of the rat, to expose the muscles and or fasciae. The wound were then kept in sterile dressing. Scheduled evaluation were made on day 1, 3, 5, 7. Elisa method was used to measure plasma TNF $\alpha$ , IL1, IL6, CRP and MMP8. Immunohistochemistry was used to measure wound tissue EGFR, ERK1, ERK2, TGF $\beta$ , Collagen and MMP8. Statistic analysis used ANOVA and MANOVA methods accordingly.

Significant decrease of TNF $\alpha$ , IL-1, CRP were demonstrated as the result of the administration of albumin infusion or feeding with casein 20% ( $p < 0.05$ ). detailed microscopy analyses on the wound tissue healing process of all groups show the significant effect of albumin infusion and casein 20% feeding on the increase of EGFR, ERK1, ERK2, TGF $\beta$ , and Collagen ( $p < 0.05$ ) and the decrease of MMP8 expressions

( $p < 0.05$ ) when compared to the hypoalbumin group. This may indicate an increase in the signaling activity of transduction and transcription of NF $\kappa$ B.

Conclusion: the administration of albumin infusion and normal protein diet are important factors to accelerate wound healing process by correcting hypoalbuminemic state show by the positive changes in the abovementioned indicators.

**Keyword: hypolabuminemia, albumin infusion, nutrition, wound healing**

## SUMMARY

### **Effect of Albumin Infusion on Changes of Serum Albumin Levels, Proinflammation Cytokines (Tnf $\alpha$ , Il1, Il6), Crp, Mmp8 And Expression of Wound Tissues Egfr, Erk1, Erk2, Tgf $\beta$ , Collagen, Mmp8 on the Acceleration of Wound Healing**

#### **(Experimental Study on Sprague Dawley Rats)**

Albumin is dominant protein in the plasma, and is often used a clinical parameter to show the presence of protein deficiency. A low preoperative albumin level has a significant relationship with longevity of the elective post operative wound healing. The data collected prove that albumin level is closely related with prognosis. This ensures experts to improve the hypoalbumin state by administering albumin infusion yet, the use of this is still open to debate as the results of studies which have been published shown differences.

As there are not studies whose results demonstrate the advantages of giving albumin infusion to decrease the patients morbidity and mortality, the frequent inaccurate use of albumin infusion as therapy, and the unclear the controversy in using albumin infusion in the healing process, this experiment or research is intended. To know and to prove the role of albumin in the process of wound healing by administering preoperative albumin infusion and postoperative albumin infusion during the state of hypoalbuminemia, which is related to probability of the effect of transduction and transcription.

This experiment is a pure experiment using 25 Sprague Dawley mice, standardized environment and feed for 7 days, then divided into 2 groups randomly: The first group (A) 5 rats were control group given normal protein casein 20%. The second groups were fed with casein 2% for 14 days until hypoalbuminemia, then they were divided into four groups: group (B) given preoperative albumin infusion, group (C) given preoperative normal protein casein 20% diet, group (D) given postoperative albumin infusion, group (E) given low protein casein 2% diet. To observe the systemic activities of TNF $\alpha$ , IL1, IL6, CRP and MMP8 in plasma, ELISA examination was used, while Immunohistochemistry of wounded tissue was used to observe the EGFR, ERK1, ERK2, TGF $\beta$ , MMP8 expression and collagen in all groups. The ANOVA and MANOVA methods is used for the statistic checking.

Before surgery, the level of albumin serum,  $\text{TNF}\alpha$ , IL1, IL6, MMP8 and CRP were checked, and there is evidence of a significant difference between the test animals, given normal protein diet (casein 20%) and those that are given low protein diet (casein 2%). This is in accordance with the previous research or study that a decrease in the mRNA albumin level in decreased protein diet in rats is caused by the partly decreased albumin gene transcription, and another result demonstrating that low protein diet on experiment animals causes an increase in the level of  $\text{TNF}\alpha$ , IL1, IL6, CRP in the phase which is significantly or markedly higher than that of the experiment animals given normal protein diet.

Previous researches suggest that whenever there is inflammation, CRP, TNF, IL1, IL6 increase as a response to acute phase. This can predict the occurrence of hypoalbumin and malnutrition, which increase the morbidity and mortality. This result confirms the opinion that transcription factor may have an important role in the state of hypoalbumin as a result of protein inadequacy.

In this experiment, preoperative and postoperative albumin infusions and normal protein diet are given to hypoalbumin mice. The result is the significant decrease ( $p < 0,05$ ) of  $\text{TNF}\alpha$ , IL1, IL6, CRP compared to those that were still hypoalbumin. Statistically there is no marked difference between those given albumin infusion and those given normal protein diet. However, when histogram was used, those that are given normal nutrition diet had a greater decrease than those that were given albumin infusion. The same thing was also observed in tissue expression. Meanwhile the difference between preoperative albumin infusion and postoperative albumin infusion is not significant ( $p > 0,05$ ).

Through Immunohistochemical, it is demonstrated that the use of albumin infusion and normal protein diet increase the EGFR, ERK1, ERK2 expressions that indicate an increase in the signal in activating the transcription and the transcription factors of NF $\kappa$ B to express the protein needed in the healing of wound process. It was also found that TGF $\beta$  and collagen increased and that MMP8 decreased. In hypoalbumin the MMP8 increases significantly in comparison to the other group. It can be assumed that MMP8 is needed to degrade collagen in the healing of wounded tissue. The mechanism, however, is not clear yet. It was also found that the area of the wound in the hypoalbumin group was significantly bigger than that of the other groups. Starting from the third day of the healing process. Those that are given normal nutrition diet had a greater decrease than those that were given albumin infusion.

## RINGKASAN

### **Pengaruh Infus Albumin Pada Perubahan Kadar Albumin Serum, Sitokin Proinflamasi (Tnfa, Il1, Il6), Crp, Mmp8 Dan Ekspresi Egfr, Erk1, Erk2, Tgff, Kolagen, Mmp8 Jaringan, Terhadap Percepatan Penyembuhan Luka**

**(Studi eksperimental menggunakan Rats-Sparague Dawley)**

Albumin merupakan protein dominan dalam plasma sering digunakan sebagai salah satu parameter klinik yang menjadi acuan adanya gangguan defisiensi protein. Penelitian kadar albumin prabedah yang rendah mempunyai hubungan secara signifikan dengan lamanya penyembuhan luka pasca operasi elektif. Banyak data membuktikan kadar albumin berkaitan dengan prognosis membuat para ahli berkeyakinan untuk memperbaiki keadaan hipoalbumin dengan infus albumin. Tetapi penggunaan infus albumin sebagai terapi albumin tidak terlepas dari pro dan kontra. Hal ini timbul akibat penelitian yang telah dipublikasikan memberi hasil yang berbeda beda.

Belum adanya hasil penelitian yang mengemukakan keuntungan pemberian infus albumin yang dapat mempengaruhi morbiditas dan mortalitas penderita, akibat banyaknya penggunaan terapi infus albumin yang tidak tepat dan belum jelas atau masih ada kontroversi penggunaan pada proses penyembuhan luka, maka penelitian kami bertujuan untuk mengetahui dan membuktikan adanya peran dan mekanisme albumin pada proses penyembuhan luka yang diberikan berupa infus albumin prabedah ataupun pascabedah pada keadaan hipoalbuminemia dihubungkan dengan kemungkinan adanya faktor transduksi dan transkripsi yang ikut mempengaruhi.

Penelitian ini adalah penelitian Eksperimental murni menggunakan 25 tikus Sprague Dawley, dilakukan standarisasi kondisi lingkungan dan makanan selama 7 hari, kemudian dirandom dibagi 2 : kelompok I, kelompok kontrol A, 5 tikus (diberi pakan normal protein casein 20%). Kelompok II, 20 tikus diberi pakan rendah protein casein 2% selama 14 hari sampai keadaan hipoalbumin, kemudian dibagi 4 antara lain: kelompok B (hipoalbumin diberi infus albumin prabedah), kelompok C (hipoalbumin diberi pakan normal protein casein 20% prabedah), kelompok D (hipoalbumin diberi infus albumin pascabedah), kelompok E (hipoalbumin dengan diet rendah protein casein 2%). Digunakan pemeriksaan ELISA untuk melihat aktifitas sistemik TNF $\alpha$ , IL1, IL6, CRP dan MMP8 plasma serta pemeriksaan Imunohistokimia jaringan luka untuk melihat ekspresi EGFR, ERK1, ERK2, TGF $\beta$ , MMP8 dan Kolagen pada semua kelompok perlakuan. Uji statistik menggunakan metoda ANOVA.

Digunakan pemeriksaan Elisa untuk memeriksa kadar serum albumin, TNF $\alpha$ , IL1, IL6, MMP8, dan CRP sebelum perlakuan pembedahan, terbukti ada perbedaan bermakna antara kelompok hewan coba yang diberi diet normal protein (casein 20%) dibandingkan dengan yang diberi diet rendah protein (casein 2%), hal ini sesuai dengan penelitian sebelumnya bahwa penurunan level mRNA albumin pada pengurangan diet protein tikus dikarenakan penurunan sebagian dari transkripsi gen albumin, hasil lain didapatkan pada hewan coba pemberian diet rendah protein ternyata menyebabkan kenaikan kadar protein positif fase akut TNF $\alpha$ , IL1, CRP, IL6 secara bermakna dibandingkan dengan diet protein normal pada hewan coba. Peneliti terdahulu mengemukakan bahwa pada keadaan

inflamasi terjadi peningkatan CRP, TNF, IL1 dan IL6 yang merupakan respon fase akut, hal ini dapat meramalkan terjadinya hipoalbumin dan malnutrisi yang dapat meningkatkan morbiditas dan mortalitas Hasil ini memperkuat pendapat bahwa faktor transkripsi mungkin berperan penting pada keadaan hipoalbumin akibat kurangnya protein.

Pada penelitian dilakukan pemberian infus albumin prabedah , infus albumin pascabedah dan pemberian pakan normalprotein pada tikus hipoalbumin, didapatkan hasil TNF $\alpha$ , IL1, IL6 dan CRP menurun secara signifikan ( $p < 0.05$ ) dibandingkan dengan yang tetap hipoalbumin. Perbandingan antara pemberian infus albumin dan diet nutrisi secara statistik tidak berbeda bermakna walaupun secara gambaran histogram tampak perbedaan yang diberi diet nutrisi menurun lebih besar dari pada yang diberi infus albumin, demikian juga pada ekspresi jaringan. Sedangkan pemberian infus albumin prabedah dibandingkan dengan pascabedah didapatkan perbedaan yang tidak bermakna ( $p > 0.05$ ).

Pada pemeriksaan imunohistokimia pada kelompok tikus yang mendapat infus albumin atau diet nutrisi menunjukkan peningkatan ekspresi terhadap EGFR, ERK1 dan ERK2 mengindikasikan adanya peningkatan sinyal dalam mengaktifkan faktor transduksi dan transkripsi dari NF $\kappa$ B dalam mengekspresikan protein yang dibutuhkan pada proses penyembuhan luka. Didapatkan juga peningkatan TGF $\beta$ , Kolagen dan penurunan MMP8. Sedangkan pada keadaan hipoalbumin MMP8 meningkat secara bermakna dibandingkan kelompok lain, hal ini diperkirakan MMP8 diperlukan untuk mendegradasi kolagen dalam perbaikan jaringan luka, mekanisme dalam hal ini belum diketahui secara jelas.

Didapatkan luas luka berbeda bermakna antara kelompok hipoalbumin dengan kelompok lain mulai hari ketiga proses penyembuhan luka. Pada kelompok hipoalbumin tampak luas luka lebih lebar, hal ini sesuai dengan peneliti sebelumnya mengenai resiko dan lamanya fase penyembuhan luka pada penderita hipoalbumin. Sedangkan pada kelompok pakan normal protein didapatkan luka lebih cepat menyembuh dibandingkan kelompok lain.