

## Serum and urinary neutrophil gelatinase-associated lipocalin as a predictor of rat kidney histopathology in an early ischemia-reperfusion model

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### Abstrak

Latar belakang: Tingkat keparahan cedera ginjal iskemia-reperfusi (I/R) berhubungan erat dengan tingginya angka kesakitan dan kematian. Hasil penelitian terdahulu pada manusia dan hewan telah membuktikan bahwa Neutrophil Gelatinase Associated Lipocalin (NGAL) dapat mendeteksi dan memprediksi terjadinya cedera ginjal I/R dini. Tujuan penelitian ini adalah untuk membuktikan bahwa peningkatan kadar NGAL serum dan urin berhubungan dengan kerusakan epitel tubuli ginjal pada tikus yang mengalami iskemia reperfusi dini.

Metode: Penelitian ini menggunakan 28 ekor tikus Sprague-Dawley jantan sebagai hewan model, dikelompokkan dalam 4 kelompok: sham 4 jam (Sham 4), sham 8 jam (Sham 8), iskemia 10 menit reperfusi 4 jam (I/R 4), dan iskemia 10 menit reperfusi 8 jam (I/R 8). Analisis kadar kreatinin serum diperiksa dengan metode Jaffe, sedangkan NGAL serum dan urin menggunakan metode ELISA Direct Sandwich. Evaluasi tingkat kerusakan jaringan ginjal dilakukan secara semi kuantitatif pada sediaan histologi dengan pulasan HE. Deskripsi kelainan tingkat seluler ginjal diperjelas melalui evaluasi menggunakan mikroskop elektron dan Imunohistokimia (IHK).

Hasil: Kadar NGAL serum berkorelasi bermakna dengan tingkat kerusakan ginjal (&#961;Spearman NGAL serum = 0,701,  $p < 0,001$ ), juga kadar NGAL urin berkorelasi bermakna dengan tingkat kerusakan ginjal (&#961;Spearman = 0,689,  $p < 0,001$ ). Tingkat ekspresi NGAL lebih tinggi pada kelompok I/R dibanding sham (t-test,  $t = -26635,046$ ,  $p < 0,001$ ), juga tingkat kerusakan ginjal tikus (t-test,  $t = -5,028$ ,  $p < 0,001$ ), dan kadar NGAL serum dan urin pada kelompok I/R berbeda nyata dibanding sham (Mann-Whitney,  $U = 0$ ,  $p < 0,001$ ). Pada cutoff point 136,95 ng/mL dan 58,69 ng/mL berturut-turut untuk NGAL serum dan urin diperoleh sensitivitas = 1, spesifisitas = 1.

Kesimpulan: Peningkatan kadar NGAL serum dan urin berkorelasi dengan kerusakan epitel tubuli ginjal pada tikus yang mengalami cedera ginjal iskemia reperfusi dini.

*Background:* The severity of ischemia-reperfusion (I/R) kidney injury is highly correlated with mortality and morbidity rate. Research on human and animal prove that NGAL predicts kidney injury at early phase. The objective of this study is to prove that the increase in serum and urinary NGAL are correlated with kidney tubular epithelial damage, and this increase has occurred in initiation phase, indicated by rat kidney histopathology in an early I/R model.

*Methods:* Twenty eight male Sprague-Dawley rats were divided into 4 groups: 4 hour sham (Sham 4), 8 hour sham (Sham 8), 10 minute ischemia 4 hour reperfusion (I/R 4) and 10 minute ischemia 8 hour reperfusion (I/R 8). Blood, urine and kidney samples were collected. Serum creatinine level was analyzed with Jaffe method, while serum and urinary NGAL level were analyzed with direct sandwich ELISA

method. Evaluation of kidney damage were measured semi quantitatively in tissue stained with HE. Further evaluation to confirm cellular changes on kidney was performed by electron microscope and immunohistochemistry.

Results: Serum NGAL was found significantly correlated with degree of kidney tissue damage (Spearman NGAL serum = 0.701,  $p < 0.001$ ), also urinary NGAL (Spearman = 0.689,  $p < 0.001$ ). NGAL expression differs significantly between I/R group and sham (t-test,  $t = -26635.056$ ,  $p < 0.001$ ), also kidney damage (t-test,  $t = -5.028$ ,  $p < 0.001$ ), and serum and urinary NGAL levels (Mann-Whitney,  $U = 0$ ,  $p < 0.001$ ). With cutoff points of 136.95 ng/mL and 58.69 ng/mL subsequently for serum and urinary NGAL , it is found that sensitivity = 1, specificity = 1.

Conclusion: Elevation of serum and urinary NGAL are significantly correlated with epithelial tubular kidney damage on rat undergoing early ischaemia reperfusion.