ANALYSIS DIHYDROLIPOAMIDE DEHYDROGENASE (DLD) AND KIDNEY INJURY MOLECULE-1 (KIM-1) mRNA EXSPRESSION IN KIDNEY MODEL OF INITIATION PHASEISCHEMIA - REPERFUSION INJURY(I-RI)

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Abstract - Dihydrolipoamide dehydrogenase (dld) is a complex enzyme subunit of three important enzymes in mitochondria. Kidney injury molecule-1 (KIM-1) is a type I transmembrane molecule in renal proximal tubular epithelial cells. The basic relationship between I-RI is mitochondrial dysfunction caused by ROS burst. This study uses a laboratory experimental method in Wistar rats (RattusNorvegicus). Renal ischemia is performed by clamp the renal a / v using a bulldog clamp. The monitoring samples were 30 and were grouped into 5 groups namely healthy controls (K), Ischemia 30 '(I), reperfusion 30' (IR 30 '). 2 hour reperfusion (2J IR), 24 hour reperfusion (24J IR).Dld and KIM-1 mRNA expressions can use RT-PCR. The results of the measurement of gene expression by normalizing the reference gene using the Livak method are 2-ΔΔCT, comparing the values of KIM1 and DLD expression in several treatment groups (Klp K, I, IR 30 ', IR 2J, IR 24J). Conclusion: KIM-1 expression was higher (1.4 times) in the Ischemia group 30 '(I) compared to the control group. DLD Expression is higher (5 times) compared to control. Conversely, in ischial reperfusion, mLNAdld expression and KIM-1 are lower (down expression) than controls which are interpreted as unexpressed.

Keywords - Dihydrolipoamide dehydrogenase (dld), Kidney injury molecule-1 (KIM-1), ischemia, ischemiareperfusion.

I. INTRODUCTION

Protein dihydrolipoamide dehydrogenase (DLD) is a highly flexible flavin dependent oxidoreductase with a dual important role in energy metabolism and redox balance. Dld is needed for the activity of three major complex enzymes in the mitochondrial matrix, namely Piruvate dehydrogenase (PDH), alpha-ketoglutarate dehydrogenase (AKGDH) and Branch alphaketo acid dehydrogenase (BACK)[1-7]. Proximal tubules in S3 segment are very susceptible to mitochondrial dysfunction due to dependence on mitochondrial dysfunction Aerobic metabolism for oxidative phosphorylation. Injury to the S1 and S2 segments is very reversible; but the S3 segment is irreversible.[9] Kidney injury molecule-1 (KIM-1) is a type I transmembrane molecule in proximal tubular epithelial cells of the kidney.10 In injured kidneys, the KIM-1 gene is activated and the KIM-1 mRNA is quickly duplicated and produces KIM protein -1 is very high in the apical membrane of the proximal tubule.11 KIM-1 plays a role in the removal of apoptotic cells (converting proximal epithelial cells into phagocytes) and necrotic tissue fragments[12.] KIM-1 is also involved in the repair process after injury to renal tubular epithelial cells at the beginning of ischemic reperfusion [13]

II. RESEARCH METHODS

This research is experimental animals using Wistar rat kidney. Renal reperfusion ischemia is performed by clamping the renal a / v with a clumb bulldog. Samples totaled 30 and were grouped into 5 groups namely healthy control (K), Ischemia 30 '(I), reperfusion 30' (IR 30 '), 2 hour reperfusion (IR 2J), 24 hour reperfusion (IR 24J). Measurement of dld and KIM-1 mRNA expression using real time PCR CFX96 touch series machines (Bio-Rad Laboratories, Inc.).

III. RESULTS AND DISCUSSION

From the results of the measurement of gene expression by normalizing the reference gene using the Livak method, 2-ΔΔCT, a comparison of the expression values of the KIM-1 and DLD genes in several treatments was carried out. The conclusion of normalization value of expression 2-ΔΔCT KIM-1 gene is gene expression in Ischemia group 30 '(I) is higher (1.4 times) compared to control group. KIM-1 expression in the reperfusion group 30 minutes, 2 hours and 24 hours is lower than the control (Down expression) which can be interpreted as unexpressed. Analysis of the distribution data shows that the value of the decrease is not significant. But it needs to be compared with statistical analysis and analysis of tubular injury scores on histopathological examination. This means that the 30 minute ischemia injury in this study is reversible. The conclusion of normalization value of expression 2-ΔΔCT DLD gene is that the expression of DLD in the Ischemia group 30 minutes '(I 30') is higher (5 times) compared to control. DLD expression in the reperfusion group 30 minutes, 2 hours and 24 hours is lower than the control (down expression) which can be interpreted

as not expressed. Analysis of the distribution data shows that the value of the decrease is not significant. But it needs to be compared with a statistical analysis.

Tabel DataQuantitatif

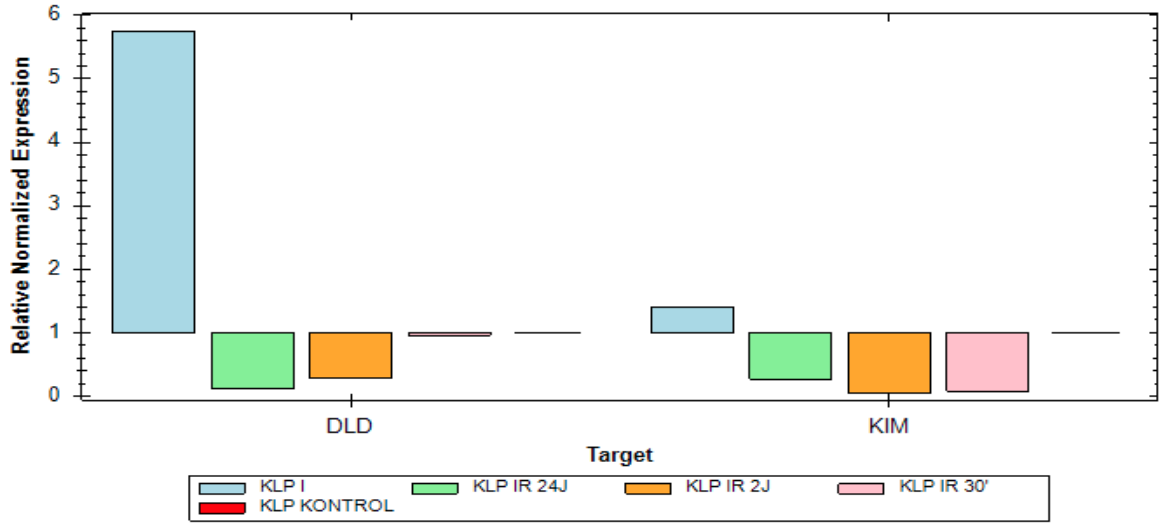
	A	B	C	D	E	F	G
1		Target	Content	Sample	Biological Set Name	Cq	Cq Mean
2		GAPDH	Unkn	KLP KONTROL 1	Kelompok Kontrol	35,82	35,82
3		GAPDH	Unkn	KLP KONTROL 2	Kelompok Kontrol	34,78	34,78
4		GAPDH	Unkn	KLP KONTROL 3	Kelompok Kontrol	31,76	31,76
5		GAPDH	Unkn	KLP KONTROL 4	Kelompok Kontrol	33,20	33,20
6		GAPDH	Unkn	KLP KONTROL 5	Kelompok Kontrol	32,42	32,42
7		GAPDH	Unkn	KLP KONTROL 6	Kelompok Kontrol	36,03	36,03
8		GAPDH	Unkn	KLP I 1	Kelompok I	33,99	33,99
9		GAPDH	Unkn	KLP I 2	Kelompok I	36,51	36,51
10		GAPDH	Unkn	KLP I 3	Kelompok I	39,26	39,26
11		GAPDH	Unkn	KLP I 4	Kelompok I	33,00	33,00
12		GAPDH	Unkn	KLP I 5	Kelompok I	34,61	34,61
13		GAPDH	Unkn	KLP I 6	Kelompok I	34,61	34,61
14		GAPDH	Unkn	KLP IR 30' 1	Kelompok IR 30'	39,62	39,62
15		GAPDH	Unkn	KLP IR 30' 2	Kelompok IR 30'	29,05	29,05
16		GAPDH	Unkn	KLP IR 30' 3	Kelompok IR 30'	29,57	29,57
17		GAPDH	Unkn	KLP IR 30' 4	Kelompok IR 30'	33,70	33,70
18		GAPDH	Unkn	KLP IR 30' 5	Kelompok IR 30'	33,18	33,18
19		GAPDH	Unkn	KLP IR 30' 6	Kelompok IR 30'	39,45	39,45
20		GAPDH	Unkn	KLP IR 2J 1	Kelompok IR 2 jam	35,77	35,77
21		GAPDH	Unkn	KLP IR 2J 2	Kelompok IR 2 jam	34,53	34,53
22		GAPDH	Unkn	KLP IR 2J 3	Kelompok IR 2 jam	30,27	30,27
23		GAPDH	Unkn	KLP IR 2J 4	Kelompok IR 2 jam	32,07	32,07
24		GAPDH	Unkn	KLP IR 2J 5	Kelompok IR 2 jam	26,46	26,46
25		GAPDH	Unkn	KLP IR 2J 6	Kelompok IR 2 jam	33,73	33,73
26		GAPDH	Unkn	KLP IR 24J 1	Kelompok IR 24 jam	28,69	28,69
27		GAPDH	Unkn	KLP IR 24J 2	Kelompok IR 24 jam	36,57	36,57
28		GAPDH	Unkn	KLP IR 24J 3	Kelompok IR 24 jam	30,74	30,74
29		GAPDH	Unkn	KLP IR 24J 4	Kelompok IR 24 jam	32,64	32,64
30		GAPDH	Unkn	KLP IR 24J 5	Kelompok IR 24 jam	33,66	33,66
31		GAPDH	Unkn	KLP IR 24J 6	Kelompok IR 24 jam	33,66	33,66
32		KIM	Unkn	KLP KONTROL 1	Kelompok Kontrol	30,03	30,03
33		KIM	Unkn	KLP KONTROL 2	Kelompok Kontrol	34,25	34,25
34		KIM	Unkn	KLP KONTROL 3	Kelompok Kontrol	36,07	36,07
35		KIM	Unkn	KLP KONTROL 4	Kelompok Kontrol	32,64	32,64
36		KIM	Unkn	KLP KONTROL 5	Kelompok Kontrol	38,63	38,63
37		KIM	Unkn	KLP KONTROL 6	Kelompok Kontrol	26,21	26,21
38		KIM	Unkn	KLP I 1	Kelompok I	34,60	34,60
39		KIM	Unkn	KLP I 2	Kelompok I	34,93	34,93
40		KIM	Unkn	KLP I 3	Kelompok I	31,62	31,62
41		KIM	Unkn	KLP I 4	Kelompok I	38,05	38,05
42		KIM	Unkn	KLP I 5	Kelompok I	32,21	32,21
43		KIM	Unkn	KLP I 6	Kelompok I	32,34	32,34
44		KIM	Unkn	KLP IR 30' 1	Kelompok IR 30'	35,97	35,97
45		KIM	Unkn	KLP IR 30' 2	Kelompok IR 30'	32,27	32,27
46		KIM	Unkn	KLP IR 30' 3	Kelompok IR 30'	36,99	36,99
47		KIM	Unkn	KLP IR 30' 4	Kelompok IR 30'	39,72	39,72
48		KIM	Unkn	KLP IR 30' 5	Kelompok IR 30'	38,46	38,46
49		KIM	Unkn	KLP IR 30' 6	Kelompok IR 30'	38,46	38,46
50		KIM	Unkn	KLP IR 2J 1	Kelompok IR 2 jam	36,30	36,30
51		KIM	Unkn	KLP IR 2J 2	Kelompok IR 2 jam	36,74	36,74

	A	B	C	D	E	F	G
52		KIM	Unkn	KLP IR 2J 3	Kelompok IR 2 jam	36,73	36,73
53		KIM	Unkn	KLP IR 2J 4	Kelompok IR 2 jam	34,48	34,48
54		KIM	Unkn	KLP IR 2J 5	Kelompok IR 2 jam	34,24	34,24
55		KIM	Unkn	KLP IR 2J 6	Kelompok IR 2 jam	34,20	34,20
56		KIM	Unkn	KLP IR 24J 1	Kelompok IR 24 jam	33,96	33,96
57		KIM	Unkn	KLP IR 24J 2	Kelompok IR 24 jam	30,48	30,48
58		KIM	Unkn	KLP IR 24J 3	Kelompok IR 24 jam	35,51	35,51
59		KIM	Unkn	KLP IR 24J 4	Kelompok IR 24 jam	33,12	33,12
60		KIM	Unkn	KLP IR 24J 5	Kelompok IR 24 jam	33,27	33,27
61		KIM	Unkn	KLP IR 24J 6	Kelompok IR 24 jam	33,53	33,53
62		DLD	Unkn	KLP KONTROL 1	Kelompok Kontrol	28,85	28,85
63		DLD	Unkn	KLP KONTROL 2	Kelompok Kontrol	26,73	26,73
64		DLD	Unkn	KLP KONTROL 3	Kelompok Kontrol	35,83	35,83
65		DLD	Unkn	KLP KONTROL 4	Kelompok Kontrol	24,93	24,93
66		DLD	Unkn	KLP KONTROL 5	Kelompok Kontrol	24,39	24,39
67		DLD	Unkn	KLP KONTROL 6	Kelompok Kontrol	26,80	26,80
68		DLD	Unkn	KLP I 1	Kelompok I	25,71	25,71
69		DLD	Unkn	KLP I 2	Kelompok I	25,64	25,64
70		DLD	Unkn	KLP I 3	Kelompok I	27,87	27,87
71		DLD	Unkn	KLP I 4	Kelompok I	27,69	27,69
72		DLD	Unkn	KLP I 5	Kelompok I	27,46	27,46
73		DLD	Unkn	KLP I 6	Kelompok I	27,46	27,46
74		DLD	Unkn	KLP IR 30' 1	Kelompok IR 30'	25,69	25,69
75		DLD	Unkn	KLP IR 30' 2	Kelompok IR 30'	26,37	26,37
76		DLD	Unkn	KLP IR 30' 3	Kelompok IR 30'	28,15	28,15
77		DLD	Unkn	KLP IR 30' 4	Kelompok IR 30'	30,93	30,93
78		DLD	Unkn	KLP IR 30' 5	Kelompok IR 30'	29,69	29,69
79		DLD	Unkn	KLP IR 30' 6	Kelompok IR 30'	27,62	27,62
80		DLD	Unkn	KLP IR 2J 1	Kelompok IR 2 jam	28,59	28,59
81		DLD	Unkn	KLP IR 2J 2	Kelompok IR 2 jam	27,18	27,18
82		DLD	Unkn	KLP IR 2J 3	Kelompok IR 2 jam	26,61	26,61
83		DLD	Unkn	KLP IR 2J 4	Kelompok IR 2 jam	29,20	29,20
84		DLD	Unkn	KLP IR 2J 5	Kelompok IR 2 jam	26,44	26,44
85		DLD	Unkn	KLP IR 2J 6	Kelompok IR 2 jam	29,15	29,15
86		DLD	Unkn	KLP IR 24J 1	Kelompok IR 24 jam	32,59	32,59
87		DLD	Unkn	KLP IR 24J 2	Kelompok IR 24 jam	29,43	29,43
88		DLD	Unkn	KLP IR 24J 3	Kelompok IR 24 jam	28,61	28,61
89		DLD	Unkn	KLP IR 24J 4	Kelompok IR 24 jam	27,78	27,78
90		DLD	Unkn	KLP IR 24J 5	Kelompok IR 24 jam	28,41	28,41
91		DLD	Unkn	KLP IR 24J 6	Kelompok IR 24 jam	29,84	29,84

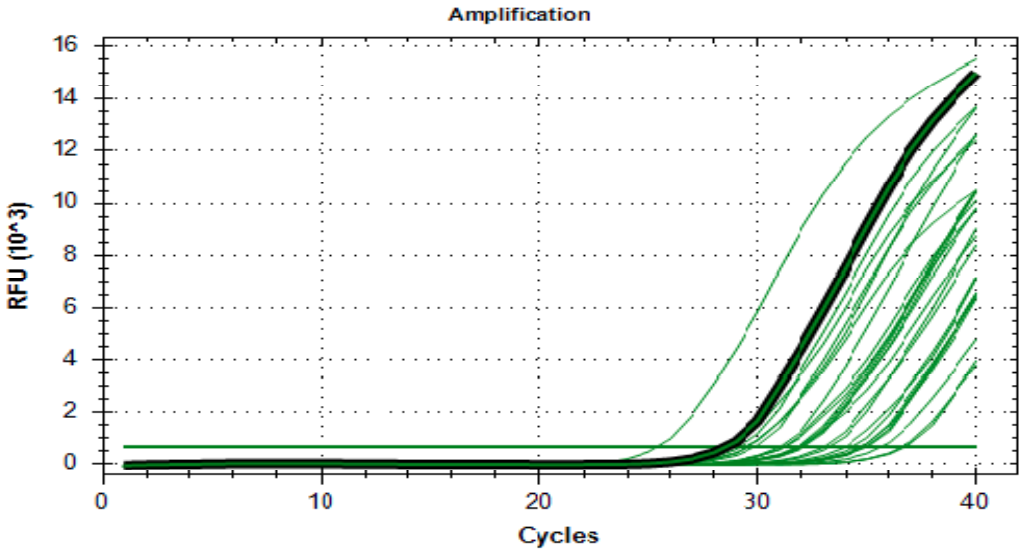
1. Tabel Data Expresi gen
(Perhitunganberdasarkanjenisdanpreferensigrafik yang dipilih)

Target	Biological Group	Control	Expression	Expression 95% CI Low	Expression 95% CI High	P-Value
DLD	KLP I		5.74120	5.74120	5.74120	N/A
DLD	KLP IR 24J		0.11973	0.11973	0.11973	N/A
DLD	KLP IR 2J		0.28630	0.28630	0.28630	N/A
DLD	KLP IR 30'		0.95789	0.95789	0.95789	N/A
DLD	KLP KONTROL	C	1.00000	1.00000	1.00000	N/A
GAPDH	KLP I		N/A	N/A	N/A	N/A
GAPDH	KLP IR 24J		N/A	N/A	N/A	N/A
GAPDH	KLP IR 2J		N/A	N/A	N/A	N/A
GAPDH	KLP IR 30'		N/A	N/A	N/A	N/A
GAPDH	KLP KONTROL	C	N/A	N/A	N/A	N/A
KIM	KLP I		1.40197	1.40197	1.40197	N/A
KIM	KLP IR 24J		0.27135	0.27135	0.27135	N/A
KIM	KLP IR 2J		0.04927	0.04927	0.04927	N/A
KIM	KLP IR 30'		0.08145	0.08145	0.08145	N/A
KIM	KLP KONTROL	C	1.00000	1.00000	1.00000	N/A

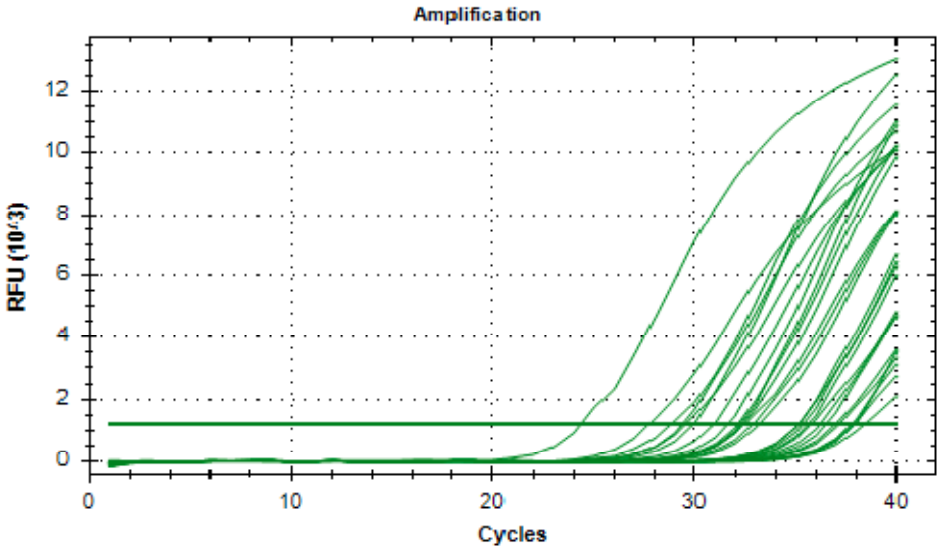
2. Chart bart Expression Gene



3. Kurva amplifikasisampel DLD



4. Kurvaamplifikasisampel KIM-1



IV. CONCLUSION

DLD mRNA expression in ischemia for 30 minutes, an increase of 5 times compared to control. KIM-1 mRNA expression in ischemia for 30 minutes, an increase of 1.4 times compared to controls. In reperfusion-ischemia, expression of DLD and KIM-1 mRNA decreases below the control value which means it is not expressed. The increase in DLD expression causes an increase in KIM-1. An increase and decrease of DLD and KIM-1 expression still needs statistical analysis. The significance of the expression with the severity of the injury still requires analysis of the tubular injury score through a histological examination of renal model.

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