

RINGKASAN

A. Pendahuluan

Sindrom nefrotik (SN) dihubungkan dengan risiko penyakit kardiovaskuler (PKV) terutama aterosklerosis karena adanya hipoalbuminemia, hiperlipidemia, hiperoksidatif stres, hiperkoagulasi, inflamasi dan pemakaian steroid jangka panjang.^{1,2}

Hiperlipidemia pada SN ditandai dengan peningkatan kadar kolesterol total, kolesterol *low density lipoprotein* (K_LDL), trigliserida, atau kolesterol *high density lipoprotein* (K_HDL) normal atau meningkat. Hiperlipidemia merangsang timbulnya spesies oksigen reaktif (SOR) yang dapat menimbulkan kerusakan pada lipid, karbohidrat dan DNA.³

SOR memicu reaksi oksidasi LDL, disfungsi endotel dan inflamasi yang mengawali terjadinya aterosklerosis.⁴ Terjadinya aterosklerosis pada anak SN terbukti melalui studi kohort, otopsi, hasil nekropsi dan laporan kasus. Penelitian kohort selama 16 tahun di Kalifornia Utara menemukan 11/142 anak SN (7,7%) infark miokard dengan risiko relatif (RR) 5,5 (IK 95%: 1,6 – 18,3) dan risiko kematian akibat penyakit koroner 2,8 kali (KI 95% 0,7–11,3).⁵ Prevalensi *advanced vascular age* pada SN lebih tinggi secara signifikan dibanding kontrol (29,4% vs 10,7%).⁶ Keadaan yang sering kambuh dan persistensi lipid pada 40–50% kasus SN menyebabkan paparan terus menerus faktor risiko PKV yang akan menyebabkan disfungsi endotel sehingga terjadi aterosklerosis dini.^{7,8}

Pencegahan aterosklerosis sebaiknya dimulai pada anak sejak dini, karena pada masa ini proses aterosklerosis telah dimulai. Restriksi diet, memberikan hasil yang kurang memuaskan, dan penggunaan obat penurun lipid mengganggu pertumbuhan dan menyebabkan miopati berat, sehingga membahayakan bagi anak.⁹

Konsensus di Bagian Ilmu Kesehatan Anak (IKA) melalui unit kerja koordinasi (UKK) nefrologi anak belum memberikan rekomendasi nyata untuk mengatasi hiperlipidemia yang berkaitan dengan pencegahan aterosklerosis secara dini karena terbatasnya penelitian. Tanpa ada penanganan yang serius akan meningkatkan mortalitas, morbiditas dan progresivitas penyakit glomerulosklerosis dan aterosklerosis.^{10,11}

Penelitian pemberian anti oksidan α -tokoferol, β -karoten dan asam askorbat yang dilakukan pada hewan coba tikus Sprague-Dawley hiperlipidemia, memberi efek menurunkan indeks aterogenik pada kelompok dengan asam askorbat.¹² Penelitian pada orang dewasa dengan risiko aterosklerosis dan yang sudah mengalami aterosklerosis memberi hasil yang bervariasi.¹³ Penelitian ini bertujuan membuktikan apakah pemberian kombinasi

dl- α -tokoferol dan asam askorbat dengan dosis 10–15 mg/kgBB/hari selama 12 minggu pada anak SN usia >1–<15 tahun berpengaruh terhadap stres oksidatif yang diukur dengan kadar LDL-ox, ekspresi reseptor penyapu makrofag dalam serum yang diukur melalui kadar sCD36), dan marker disfungsi endotel yang diukur dengan kadar sVCAM-1 dan NO sebagai petanda dini aterosklerosis.¹⁴⁻¹⁷ (Hubungan antar variabel dijelaskan dalam kerangka teori terlampir).

B. Metode penelitian

Penelitian ini menggunakan rancangan *Randomized Pretest-posttest Control Group Design*. Subyek penelitian adalah penderita sindrom nefrotik yang mengalami hiperlipidemia berumur >1–<15 tahun, dirawat di RSUP dr.Kariadi Semarang/ RSUD/RS swasta sekitarnya yang bersedia kontrol di RSUP dr. Kariadi Semarang serta memenuhi kriteria inklusi-eksklusi. Randomisasi dilakukan dengan cara *simple block random sampling*.¹⁸ Kelompok perlakuan mendapat terapi standar (steroid) dan kombinasi dl- α -tokoferol dan asam askorbat dengan dosis sama 10–15 mg/kgBB/hari dibagi dua dosis diberikan secara oral bersama makan selama 12 minggu. Kelompok kontrol mendapat terapi standar dan plasebo. Variabel bebas: dl- α -tokoferol dan asam askorbat. Variabel perantara: kadar LDL-ox. Variabel tergantung: sVCAM-1, sCD36, dan NO. Sampel subyek diambil dari serum darah puasa dan diperiksa dengan cara ELISA kecuali kadar NO dengan kolorimetri. Variabel perancu: asupan makanan (lipid dan vitamin), kadar albumin, hipertensi, steroid.

C. Hasil penelitian

Sebanyak 42 dari 46 anak SN memenuhi kriteria inklusi dan eksklusi. Selama 12 minggu perlakuan 36 subyek (85,71%) dapat menyelesaikan penelitian masing-masing kelompok 18 subyek. Sembilan belas subyek (52,7%) mengalami remisi di akhir perlakuan, dengan 10 subyek dari kelompok perlakuan.

Berikut adalah tabel karakteristik klinik penderita sebelum perlakuan..

Tabel 1. Karakteristik klinik subyek sebelum intervensi

Parameter klinik	Kelompok (N:42)				p
	Perlakuan (N:21)		Kontrol (N:21)		
	Rerata	SB	Rerata	SB	
Usia(bulan)	87,8	45,10	80,5	42,24	0,458*
Jenis kelamin L	13		12		0,753**
P	8		9		
IMT/umur	0.37	2,3	0,37	1,32	0,99***
Pendidikan Ibu (N,%)					1**
SD	5 (23,8%)		6 (28,6%)		
SLTP	3 (14,3%)		4 (19,0%)		
SLTA	11 (52,4%)		8 (38,1%)		
D2/D3/S1	2(9,5%)		3(14,3%)		
Tekanan Darah (N,%)					0,753**
-Normotensi	9 (42,9)		8 (38,1)		
-Pra - Ht stage II	12 (57,1)		13 (61,9)		
Onset sakit (tahun)	5,28	3,50	4,91	3,17	0,830*
Lama sakit(tahun)	1,72	1,84	1,86	2,31	0,862*
Diagnosis masuk(N,%)					0,123**
-SN serangan pertama & kambuh jarang	8(38,1)		13(61,9)		
-SN kambuh sering & resisten steroid	13(61,9)		8(38,1)		

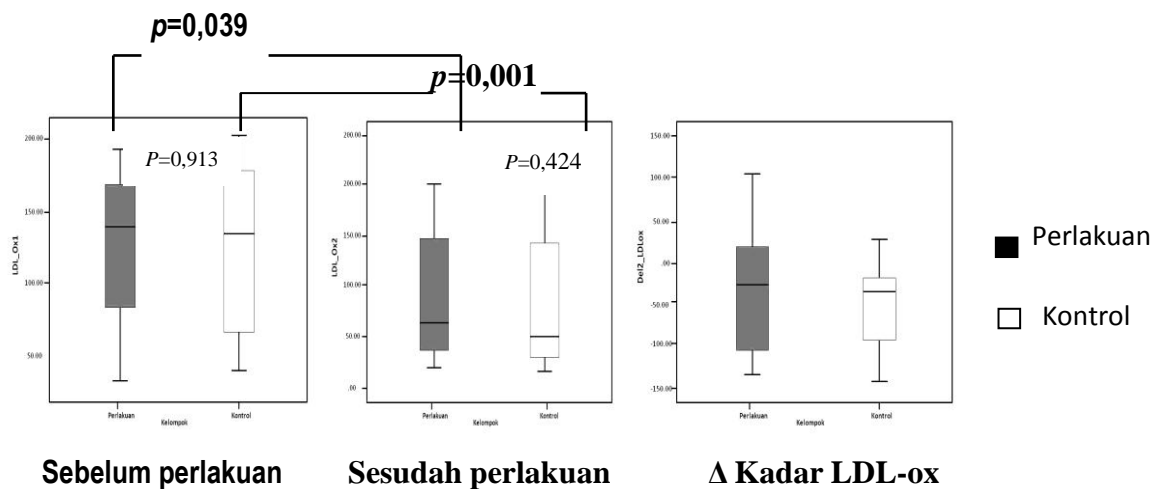
Keterangan: IMT/umur: indeks masa tubuh/umur; Ht: hipertensi; *= Mann-Whitney U, **= uji *Chi square*; ***= uji *t* ; *) Uji *Kolmogorov-Smirnov*; SB: Simpang Baku; TD: Tekanan Darah; Ht: hipertensi

Tabel 2. Gambaran klinik subyek sebelum dan sesudah intervensi

Gambaran klinik	Kelompok (N:36)				Nilai p
	Perlakuan (N:18)		Kontrol (N:18)		
	Sebelum	Sesudah	Sebelum	Sesudah	
Subyek remisi (N,%)	-	10 (55,6%)	-	9 (50%)	0,738**
Tekanan Darah (mmHg)					
-Normotensi (N,%)	8(44,4)	15(83,3)	8(44,4)	9(50)	0,075*
-PraHt-Ht stage II (N,%)	10(55,6)	3(16,7)	10(55,6)	9(50)	
Morbiditas*	-	3 ^a	-	2 ^b	
Total subyek akhir	18	18	18	18	

Keterangan: *): Uji *Fisher exact* ; Morbiditas:^aSatu anak cacar air, satu anak infeksi saluran kencing¹⁹, satu anak kambuh saat perlakuan. :^b Kambuh karena infeksi saluran pernafasan akut (ISPA). Persentase yang ditampilkan per kelompok

Hasil *pre test-post test* kelompok perlakuan: kadar LDL-ox:127,2 menjadi 89,5 U/L; sCD36:72,5 menjadi 123,5 ng/mL; sVCAM-1:929,4 menjadi 868,4 ng/mL; NO: 8,9 menjadi 8,5 μ M/L. Kelompok kontrol: LDL-ox:125,2 menjadi 76,7U/L; sCD36:56,9 menjadi 61,7 ng/mL; sVCAM-1: 1231,2 menjadi 1008,0 ng/mL ; NO: 8,2 menjadi 7,9 μ M. Uji *Wilcoxon* kelompok perlakuan dan kontrol berturut-turut untuk kadar LDL-ox $p=0,039$ dan $p=0,001$ (Gambar 1). Kadar sCD36 $p=0,163$ & $p=0,088$, kadar sVCAM-1 $p=0,306$ & $0,122$; Kadar NO $p=0,983$ & $p=0,760$. Kadar Δ diantara dua kelompok perlakuan dan kontrol berturut-turut Δ LDL-ox -37,7vs - 48.6U / L ; Δ sCD36: 51,1vs 17,2 ng /mL; Δ sVCAM-1: -61,0 vs – 223ng /mL ; Δ NO -0.4 vs- 0,6 mg/dL. Uji *t tidak berpasangan* Δ LDL-ox, Δ sVCAM-1 $p=0,594$ dan $0,327$, dan uji *Mann Whitney U* untuk Δ sCD36 dan Δ NO p berturut-turut =0,883 dan 0,864.



Gambar 1. Diagram *box plot* kadar LDL-ox, sebelum, sesudah intervensi dan Δ kelompok perlakuan dan kontrol

Tabel 3. Uji beda rerata dan Δ kadar LDL-ox, sCD36, sVCAM-1, NO berdasarkan status remisi kelompok perlakuan

Variabel	Remisi (N:10)				Tidak remisi (N:8)				p
	Rerata \pm SB	Median	Min	Maks	Rerata \pm SB	Median	Min	Maks	
LDL-ox (U/L)	58,3 \pm 50,36	40,05	19,2	181,9	128,4 \pm 59,3	130,9	37,1	202,6	0,016*
sCD36 (ng/mL)	153,7 \pm 205,2	33,7	18,1	652,1	85,8 \pm 150,0	19,8	12,3	451,6	0,274*
sVCAM-1 (ng/mL)	714,8\pm 109,8	735,5	507,8	843,5	1060,3\pm 199,2	1024,9	861,4	1474,4	0,000#
NO (μ M)	8,7 \pm 6,4	6,6	2,5	19,4	8,2 \pm 5,6	7,4	2,8	20,7	1,000*
Δ LDL-ox	-66,6\pm 67,6	-81,0	-136,0	49,2	-1,6\pm 58,0	-1,4	-84,2	106,5	0,047#
Δ sCD36	75,5 \pm 111,3	5,4	-8,8	255,2	20,3 \pm 102,1	0,44	-126,2	241,2	0,696*
Δ sVCAM-1	-129,9 \pm 207,9	-65,8	-549,7	48,5	25,1 \pm 267,6	88,3	-430,3	343,3	203*
Δ NO	0,11 \pm 4,4	0,1	-7,0	7,4	-1,05 \pm 10,2	0,3	-22,4	14,4	0,748**

Keterangan: * Uji *Mann-Whitney U* ; # Uji *t* tidak berpasangan;

Berdasarkan status remisi maka uji beda kadar LDL-ox *post test*, Δ kadar LDL-ox dan kadar sVCAM-1 *post test* pada kelompok perlakuan berbeda bermakna dengan berturut-turut $p=0,016$; $0,047$ dan $0,000$ (Tabel 3)

D. Bahasan

1. Pengaruh dl- α -tokoferol dan asam askorbat terhadap kadar LDL-ox.

Penelitian ini menemukan bahwa kadar LDL-ox pada kedua kelompok perlakuan dan kontrol menurun setelah intervensi, namun tidak berbeda bermakna di antara dua kelompok. Hasil berbeda bermakna ditemukan pada Δ kadar LDL-ox kelompok perlakuan berdasarkan status remisi, yang tidak ditemukan pada kelompok kontrol. Hal ini menunjukkan bahwa pemberian dl- α -tokoferol dan asam askorbat memperkuat kerja prednison. Prednison menurunkan SOR melalui hambatan pembentukan radikal superoksida sehingga menurunkan peroksidasi lipid. Penelitian ini dibuktikan oleh Marumo, dkk melalui isolasi sel otot polos aorta yang diinduksi dengan deksametason.¹⁹ Fase remisi akibat respon steroid juga memberi peluang untuk menurunkan kadar LDL-ox karena peningkatan serum albumin sebagai pengikat ion Cu^{2+} akan mengurangi ion metal bebas yang dapat memicu reaksi oksidasi.

Albumin mengangkut ion Cu^{2+} bebas $\pm 15\%$, dimana pengangkut ion Cu^{2+} lainnya diperankan terutama oleh seruloplasmin (70%) dan sisanya diangkut oleh macroglobulin (15%). Kadar seruloplasmin ditemukan menurun pada sindrom nefrotik saat kambuh.²⁰

Penelitian Engler menunjukkan pemberian dl- α - tokoferol (400 IU/hari) dan asam askorbat (500mg/hari) pada 15 anak hiperlipidemia familial tidak berefek pada biomarker stress oksidatif (LDL-ox, dll) tetapi memperbaiki disfungsi endotel yang diukur dengan *flow mediated dilatation* arteri brakialis (FMA).²¹ Devaraj, dkk memberikan 1200 IU α -tokoferol selama 2 tahun untuk penderita penyakit arteri koroner stabil usia 40–70 tahun memberikan hasil bermakna.²² Penelitian oleh Boushehri, dkk dilakukan pada orang laki-laki dewasa dengan faktor risiko PKV. Pemberian 400 IU Vitamin E, 500 mg Vitamin C, 15 mg β -karoten atau kombinasi ketiganya selama 12 minggu menunjukkan penurunan kadar LDL-ox secara bermakna.²³

Penambahan anti oksidan kurang memberikan pengaruh terhadap penurunan LDL-ox pada kelompok perlakuan yang tidak remisi. Beberapa kemungkinan yang bisa menjadi faktor penyebab adalah: subyek tidak respon terhadap steroid menyebabkan stress oksidatif terus berlangsung, variasi absorpsi α -tokoferol cukup besar antara 20–60%, hipoalbuminemia mengurangi jumlah ion Cu^{2+} yang terikat, proteinuria masif menyebabkan berkurangnya pembentukan protein dan α -tokoferol transfer protein (α -TTP).²⁴

Penelitian Sato, dkk menyebutkan bahwa pemakaian glukokortikoid dapat menurunkan aktifitas antioksidan enzimatik seperti *superoxide dismutase* (SOD), katalase (Kat), *glutathione peroxidase* (GPx). Penurunan anti oksidan enzimatik menyebabkan terganggunya pembentukan asam askorbat. Asam askorbat dapat mengubah bentuk radikal tokoferoksil menjadi α -tokoferol secara langsung dan anti oksidan thiol seperti glutation dan asam lipoat dapat menghasilkan α -tokoferol secara tak langsung melalui pembentukan asam askorbat.²⁵

2. Pengaruh dl- α -tokoferol dan asam askorbat terhadap kadar sCD 36.

Rerata kadar sCD36 sebelum intervensi pada 40 subyek adalah $62,6 \pm 81,5$ dengan median 28,6 (13,1- 396,9) ng/mL (Rujukan normal menurut kit pemeriksaan antara 3,12-200 ng/mL, rujukan untuk anak dewasa muda 18 – 25 tahun: 32,3 ng/mL (16,8 – 102,4 ng/mL). Hasil penelitian ini menunjukkan bahwa kadar sCD36 cenderung meningkat pada kedua kelompok, meskipun berbeda tidak bermakna ($p > 0,05$). Penelitian ini berbeda dengan hasil penelitian Devaraj, dkk yang memberikan alfa tokoferol 50 μ M (2,3 mg) dan 100 μ M (4,5 mg) pada makrofag derivat monosit *volunteer* normal ternyata dapat menurunkan ekspresi CD36 sebanyak 47% dan 77%.²⁶

Kemungkinan peningkatan ini berkaitan dengan mikropartikel yang berasal dari trombosit, leukosit dan sel endotel. Mikropartikel ini ditemukan pada kasus resistensi insulin dan obesitas dengan diabetes mellitus tipe-2 karena adanya inflamasi derajat rendah.²⁷

Suryohudoyo mengatakan bahwa sekali reseptor penyapu sCD36 terangsang, maka tak akan mengalami *down regulation* sehingga makrofag terus menerus memproduksi sCD36.²⁸

Kadar sCD36 tinggi dapat ditemukan pada penderita dengan aterosklerosis yang simtomatik, hiperinsulinisme, DM tipe-2, obesitas dan polikistik ovarii dan perlemakan hati.¹¹ Analisis regresi menunjukkan bahwa kadar sCD36 dipengaruhi oleh K_HDL, IMT/umur, dan berat badan sebanyak 30%. Hasil penelitian ini menunjukkan kadar HDL yang tinggi pada kelompok perlakuan (70mg/dL) dibanding kontrol (58,7mg/dL).

3. Pengaruh dl- α -tokoferol dan asam askorbat terhadap kadar sVCAM-1

Penurunan kadar rerata dan Δ kadar sVCAM-1 di akhir intervensi tidak berbeda bermakna. Hal ini bisa disebabkan oleh pengaruh steroid yang mungkin lebih dominan sebagai anti inflamasi yang kuat.

Glukokortikoid memiliki efek menurunkan gen transkripsi sitokin (interleukin, TNF- α), kemokin (antara lain *macrophage inflammatory protein-1*(MIP-1) α , *monocyte chemoattracted protein-1*(MCP-1) dan 2), enzim (*inducible nitric oxide synthase* (iNOS), siklooksigenase2, Cytoplasmic phospholipase A), dan molekul adhesi (ICAM-1 dan VCAM-1). Penelitian yang dilakukan oleh Upritchard, dkk pada orang dewasa dengan DM tipe II menunjukkan bahwa suplementasi vitamin E 800 IU/hari dan vitamin C 500 mg/hari dan jus tomat 500 ml/hari selama 4 minggu ternyata tidak memberi perubahan bermakna pada kadar sVCAM-1 maupun sICAM-1.²⁹

Hasil analisis regresi menyebutkan bahwa prediktor sVCAM-1 yaitu HDL memberikan kontribusi 16,1%, sedangkan faktor-faktor lain masih banyak berpengaruh diantaranya adanya endotoksinemia, atau respon inflamasi. Pengaruh infeksi sudah disingkirkan dengan pemeriksaan CRP. Respon inflamasi masih mungkin terjadi pada penderita SN terutama yang tidak remisi, oleh karena adanya hiperlipidemia, hipertensi dan hipoalbuminemia. Kadar sVCAM-1 sangat tinggi (2664,3;2480,6;2258,9 ng/mL) memiliki kadar HDL yang sangat rendah yaitu 18,27 dan 36 mg/dL ditemukan pada kelompok kontrol.

Rendahnya kadar NO akibat hiperlipidemia dan hipertensi (55,6% kasus) pada penelitian ini mendukung untuk terjadinya peningkatan aktivitas molekul adhesi sVCAM-1.

Berdasarkan status remisi di akhir perlakuan, kadar sVCAM-1 menurun secara bermakna pada kelompok intervensi. Perbedaan bermakna ini tidak ditemukan pada kelompok kontrol, sehingga perubahan yang terjadi pada kadar sVCAM-1 kemungkinan besar oleh adanya pengaruh steroid dan kombinasi dl- α -tokoferol serta asam askorbat. Efektivitas alfa tokoferol dan asam askorbat terjadi pada saat remisi kemungkinan karena kadar albumin yang sudah membaik sehingga tercukupinya *α -tocopherol transfer protein* (α -TTP) yang mengangkut α -tokoferol menuju lipoprotein plasma (HDL & LDL) agar terlindung dari proses oksidasi.³⁰

4. Pengaruh pemberian dl- α tokoferol dan asam askorbat terhadap kadar NO

Kadar NO yang rendah saat awal intervensi kemungkinan dipengaruhi oleh hiperlipidemia, peningkatan kadar LDL-ox (72% kasus) dan hipertensi (55,6% kasus). Δ kadar NO mengalami penurunan yaitu 0,35 μ M pada kelompok perlakuan dan 0,39 μ M pada kelompok kontrol.

Pemberian glukokortikoid (prednisone/metil prednisolon) menghambat pembentukan ion superoksida, seharusnya dapat meningkatkan kadar NO. Stres oksidatif dan peroksinitrit (ONOO-) yang berlebihan karena terjadinya reaksi NO dengan superoksida (O₂-) menyebabkan timbulnya perubahan fisiologis dan serologis. Perubahan ini berkaitan dengan peningkatan tekanan darah, proteinuria, disfungsi trombosit, peningkatan tromboksan (TXA) dan endotelin serta penurunan prostasiklin (PGI₂).³¹

Penelitian Engler menyebutkan bahwa pemberian vitamin E dan C memperbaiki fungsi endotel yang ditunjukkan dengan pemeriksaan dilatasi arteri brakialis (*flow mediated dilatation*, FMD), namun tidak mempengaruhi petanda stres oksidatif.²¹

Penelitian oleh Shouman menunjukkan bahwa penderita SN kelainan minimal (SNKM) mampu nyai kadar nitrit plasma sebesar 45,87 \pm 20,85 μ mol/L dan nitrat 45,58 \pm 19,7 μ mol/L sedangkan pada anak normal sebesar 12,33 \pm 7 μ mol/L.³²

Penyebab lain penurunan produksi NO adalah menurunnya kadar L-arginin yang diinduksi oleh peningkatan lipoprotein dan rendahnya L-arginin menyebabkan produksi yang berlebihan dari superoksida. Kadar NO juga dipengaruhi oleh kadar kalsium, dan asupan makanan yang kaya akan NO seperti daun seledri, bit, sayur-sayuran berdaun seperti bayam, dan *lettuce*.³³

Asam askorbat juga mengurangi aktivitas NAD(P)H oksidase dan produksi superoksida secara *in vitro* dan *in vivo*. NAD(P)H oksidase adalah sumber utama dari superoksida endotel yang bertanggung jawab untuk *uncoupling* eNOS dini, yang dapat diinaktivasi oleh α -tokoferol dan asam askorbat.¹⁷

Penelitian Heller menyebutkan bahwa pemberian α -tokoferol meningkatkan pembentukan NO dan diperkuat oleh adanya asam askorbat. Penelitian ini dilakukan melalui isolasi sel endotel vena umbilikalis manusia.³⁴

Temuan yang menarik dari penelitian ini adalah didapatkan perbaikan secara klinis tekanan darah pada 40 % kasus (7/18 subyek) dari kelompok perlakuan. Hal ini membuktikan bahwa terdapat peran kombinasi dl- α -tokoferol yang diperkuat oleh asam askorbat dalam meningkatkan kadar NO sebagai vasodilator vaskuler, meskipun belum terdapat bukti peningkatan kadar NO dan tidak bermakna secara statistik. Kadar NO tetap rendah kemungkinan terjadinya peningkatan pemakaian NO dalam jaringan, atau diperlukan waktu tertentu untuk meningkatkan kadar NO dalam darah sebagai proses adaptasi dalam tubuh, sehingga perlu penelitian lebih lanjut. Penelitian ini menunjukkan efek saling memperkuat antara dl- α -tokoferol dan asam askorbat memperbaiki fungsi vaskuler baik di membran sel maupun dalam sel.

E. Simpulan dan Saran

Simpulan : Pemberian kombinasi dl- α -tokoferol dan asam askorbat dengan dosis masing-masing 10-15 mg/kgBB/hari yang diberikan selama 12 minggu pada anak SN :

1. Menurunkan kadar LDL-ox secara tidak bermakna. Kadar LDL-ox, Δ LDL-ox menurun secara bermakna pada kelompok perlakuan yang mengalami remisi dibanding tidak remisi.
2. Meningkatkan kadar sCD36 secara tidak bermakna.
3. Menurunkan kadar sVCAM-1 secara tidak bermakna. Kadar sVCAM-1 menurun secara bermakna pada kelompok perlakuan yang mengalami remisi dibanding tidak remisi
4. Menurunkan kadar NO secara tidak bermakna.

Saran:

1. Perlu penelitian lebih lanjut untuk memisahkan subyek penelitian SN tanpa hipertensi atau dengan hipertensi.
2. Perlu penelitian pemantauan kadar *biomarker* secara serial selama penelitian untuk melihat kecenderungan peningkatan atau penurunan selama remisi.
3. Pemberian kombinasi dl- α -tokoferol dan asam askorbat lebih lama minimal 6 bulan untuk melihat respon obat terhadap *biomarker* stress oksidatif dan disfungsi endotel selama remisi

SUMMARY

A. Introduction

Nephrotic syndrome (NS) in children is associated with a risk of cardiovascular disease (CVD) especially atherosclerosis due to hypoalbuminemia, hyperlipidemia, hyperoxidative stress, hypercoagulation, inflammation and long-term steroid use.^{1,2}

Hyperlipidemia in NS is characterized by elevated levels of total lipids (cholesterol, triglycerides), and enormous increase in cholesterol in the low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL)-cholesterol fraction, whereas the concentration of high-density lipoprotein (HDL)-cholesterol is normal or even decreased. Hyperlipidemia induce reactive species oxygen (ROS) and cause damage of lipid, carbohydrate and deoxyribo nucleic acid (DNA).³

Reactive oxygen species lead to oxidized LDL, endothelial dysfunction and inflammation that initiate atherosclerosis.⁴ Atherosclerosis in NS was proven through cohort studies, autopsy, necropsy and case reports. One of the studies from North California was a 16 years cohort study. Eleven of 142 children (7.7%) with NS were found to have myocardial infarction with a relative risk (RR) 5.5 (95% CI:1.6-18.3) and coronary mortality risk 2.8 (95%CI:0.7 –11.3).⁵ Prevalence of advance vascular age in NS was higher significantly compared with control (29.4% vs 10.7%).⁶ Continuous exposure because of the risk of recurrence and persistence of lipids (40-50 % of NS) increases the risk of these events.^{7,8}

Prevention of atherosclerosis should begin early in childhood because this process has already begun. Diet restriction gives less real results, and use of lipid-lowering drugs have the risk of impaired growth and development and severe myopathy, so it is harmful to children.⁹

There has been no agreement or consensus in Child Health Department through Pediatric Nephrology Working Group to manage hyperlipidemia associated with early atherosclerosis prevention. Without any serious treatment, this would increase mortality, morbidity, glomerulosclerosis and atherosclerosis progressivity.^{10,11}

Previous studies showed that the use of anti-oxidant α -tocopherol, β -carotene and ascorbic acid in hyperlipidemic Sprague Dawley rats reduced the atherogenic index in the group with ascorbic acid.¹² Studies in adults with the risk and who had experienced of

atherosclerosis gave variable results.¹³ This study aimed to prove that the oral combination of 10-15mg/kgBW/day dl- α -tocopherol and ascorbic acid in children with NS aged >1- <15 years had any effect on biomarkers of oxidative stress (oxidized low density lipoprotein, ox-LDL), scavenger receptor of macrophage (soluble CD36, sCD36), disfunction of endothelial (soluble vascular cell adhesion molecule, sVCAM-1) and nitric oxide (NO).¹⁴⁻¹⁷

B. Method

A Randomized Pretest-Posttest Control Group Design was conducted. Subjects who fulfilled inclusion and exclusion criteria were enrolled in the study. Subject with idiopathic nephrotic syndrome (INS) who had hyperlipidemia aged >1 - <15 years and were treated in the outpatient or inpatient clinic in the Department of Child Health/Dr.Kariadi Hospital Semarang or other hospitals willing to be followed in Dr. Kariadi Hospital were included in the study. Randomization was done by simple block random sampling.¹⁸ The treatment group received standard steroid therapy and combination of oral dl- α -tocopherol and ascorbic acid of 10-15 mg / kg/day divided into two doses daily, orally and the control group received standard therapy and placebo. Independent variables were dl- α -tocopherol and ascorbic acid, intermediate variables was ox- LDL and dependent variables were sVCAM-1, sCD36, NO. The levels of oxLDL, sVCAM-1, and sCD36 were measured by enzyme linked immunosorbent assay (ELISA), NO was measured by colorimetric assay. Confounding variables were food intake that measured by 3 days food recall & record (lipids and vitamins), and albumin serum, hypertension, and steroid treatment.

C. Results

Forty two of 46 children were included. During the 12-week treatment, 36 subjects (85.71%) completed the study (18 subjects of the each group). A total of 19 subjects in remission (52.8%) at the end of treatment (10 subjects from the treatment group).

The mean of pretest and posttest level of ox-LDL serum in treatment group were 127.2 to 89.5 U/L; sCD36:72.5 to 123.5ng/mL; sVCAM-1:929.4 to 868.4ng/mL; NO: 8.9 to 8.5 μ M/L. In the control group: ox-LDL:125.2-76.7U/L ; sCD36:56.9-61.7ng/mL; sVCAM-1: 1231.2–1008.0 ng/mL; NO: 8.2-7.9 μ M. The Wilcoxon test of ox-LDL levels were showed significant difference in each group by *p value* =0.039 in the treatment group and *p*=0.001 in the control group. There were no significant difference of sCD36 levels, sVCAM-1 levels and NO levels. In treatment and control group sCD36 levels showed *p value* = 0.163 and 0.088; sVCAM-1 levels *p value* = 0.306 dan 0.122 and NO levels were *p value* =0.983 and

0.760. The Δ level of ox-LDL on treatment group compared to control was -37.7 vs -48.6U/L ; Δ sCD36: 51.1vs 17.2ng/mL; Δ sVCAM-1: -61.0 vs -223ng/mL ; Δ NO -0.4 vs -0,6 mg/dL. Independent t test of Δ ox-LDL, Δ sVCAM-1 and Mann Whitney U test of Δ sCD36 and Δ NO were not significantly different with *P value of* 0.594, 0.327, 0.883, 0.864, respectively. According to remission state at the end of the study the level of ox-LDL, Δ ox-LDL and level of sVCAM-1 in treatment group were significantly different $p=0,016,0,047$ and 0,000 respectively.

D. Discussion

1. The effect of dl- α -tocopherol and ascorbic acid on ox-LDL

In this study we found that ox-LDL tend to decrease in both groups, although not significantly difference ($P>0.05$). There was a reduction on ox-LDL level at remission state on both group. The remission of treatment group showed a significantly different in reduced Δ ox-LDL. This fact should be taken into special consideration. This result was likely caused by the effect of combined dl- α -tocopherol and ascorbic acid in synergism with steroid to induce remission and lowering the lipid peroxidation. Prednisone was known to lower reactive oxygen species (ROS) via inhibition of superoxide radical generation and the consequences of the reduction on lipid peroxidation. The study was done by Marumo, et al in dexamethasone-induced isolated aortic smooth muscle.¹⁹ The remission phase on steroid therapy were also considered to lead the reduction in ox-LDL level in relation with the albumin's role as the Cu^{2+} binding agent which would reduce the free metallic ion capacity to trigger oxidation process. Albumin bind to 15% free Cu^{2+} while the rest will be bound to both macroglobulin and ceruloplasmin. The last is a major copper-binding protein in human plasma (70%), while albumin bind the other 15% and the rest is bound to macroglobulin. Ceruloplasmin level will be decreased in relaps nephrotic syndrome.²⁰

Previous studies in 15 children with familial hypercholesterolemia and familial combined hyperlipidemia, treatment by vitamin C (500 mg/day) and E (400 IU/day) for 6 weeks did not have an effect on biomarkers of oxidative stress (autoantibodies to epitopes of oxidized LDL, F2-isoprostanes, 8-hydroxy-2'-deoxyguanosine), but improve the brachial artery dilatation.²¹ Two other studies among adult revealed a significant results. A study conducted by Devaraj , et al which administered 1200 IU α -tocopherol for 2 years to subject with stable coronary artery disease aged 40–70,²² and other study was conducted by Boushehri, et al in men with risk factor of cardio vascular disease given 12-week

supplementation of 400 IU Vitamin E, 500 mg Vitamin C, 15 mg β -carotene as a combination or isolated treatment were showed a significant result in ox-LDL reduction.²³

Antioxidant addition not effectively induced the ox-LDL reduction in non-remission treatment group. There are several possibilities which likely to be the etiologic factors: unresponsiveness to steroid, wide absorption spectrum dose of α -tocopherol (20–60%), elevation of oxidative stress level in the non-remission period, hypoalbuminemia reduces the Cu^{2+} bound, reduction of α -tocopherol transfer protein (α -TTP).²⁴

Study conducted by Sato, et al revealed that glucocorticoid administration reduced enzymatic antioxidant activity such as superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPx). This would lead to reduced ascorbic acid production which directly converts tocopheroxyl radical into α -tocopherol. While thiolic compound-antioxidant like glutathione and lipoic acid have the capacity of producing α -tocopherol indirectly from ascorbic acid production pathway.²⁵

2. The effect of dl- α -tocopherol and ascorbic acid on sCD 36 level

The mean of serum level of sCD36 of 40 subjects was 62.6 ± 81.5 ng/mL, the median was 28.6 (13.1- 396.9) ng/mL. Normal reference for young adult 18 – 25 years was 32.3 (16.8 – 1024)ng/mL. The result of this study showed that sCD36 tend to increase in both groups, although it was not significantly different. The combination of dl- α -tocopherol and ascorbic acid administration were unable to lower the sCD36 expression. Devaraj et al showed a different declined where 50 and 100 μM α -tocopherol caused the 47% and 77% sCD36 expression in monocyte-derived macrophage isolation of normal volunteer.²⁶ Elevation on sCD36 was more likely related to microparticle of platelets, leukocytes, and endothelial either stimulation or apoptosis.²⁷

According to Suryohudoyo, once the sCD36 scavenger receptor is being induced, it never be down-regulated so the macrophages keep producing the sCD36.²⁸ High level of sCD36 is found in symptomatic atherosclerosis, hyperinsulinism, type-2 Diabetes Mellitus (DM), obesity, polycystic ovaries, and fatty liver.¹¹

Regression analysis showed that 30% serum level of sCD36 was influence by HDL cholesterol, basal metabolism index for age (BMI/age) and body weight. The serum level of HDL cholesterol in treatment group (70 mg/dL) was higher than control group (58 mg/dL).

3. The effect of dl- α -tocopherol and ascorbic acid on sVCAM-1 level

Combined regiment of dl- α -tocopherol and ascorbic acid had no effect on the mean and Δ sVCAM-1 post treatment. This might be due to the strong anti-inflammation property of

steroid more superior than dl- α -tocopherol and ascorbic acid, which reduced the sVCAM-1 level.

While glucocorticoid showed the lowering effect on cytokine transcription gene (interleukin, TNF- α), chemokines (MIP-1 α , MCP-1 and 2), enzymes (iNOS, cyclooxygenase-2, Cytoplasmic phospholipase A), adhesion molecules (ICAM-1 and VCAM-1), and receptors (IL-2 receptor, tachykinin 1(NK-1) receptor). Upritchard, et al study revealed supplementation of Vitamin E, Vitamin C, and tomato juice with a dose 800 IU, 500 mg, 500 ml given daily for 4 weeks among type-2 DM subjects had no effect on sVCAM-1 and sICAM-1 level.²⁹

Regression analysis was used to determine the sVCAM-1 predictor, and HDL contributed 16,1%, meanwhile other contributing factors were endotoxemia and inflammatory responses. Infection were excluded by CRP examination. There might be a sustaining inflammation especially among the non remission nephrotic syndrome patients, hyperlipidemia, hypertension and other factors stimulating adhesion molecules expression.

Low NO level in this study supports the increase in activity of adhesion molecule sVCAM-1.

Based on the remission status at the end of treatment, there was a remarkable decline in sVCAM-1 level among the treatment group. On the other hand, there was no difference in sVCAM-1 level among the control, so this fact showed that combination supplementation dl- α -tocopherol and ascorbic acid strengthen steroid activity in the remission state. This was a possibility which likely increasing albumin at remission provide α -tocopherol transfer protein (α -TTP). α -TTP will pick α -tocopherol up to cholesterol LDL and HDL in order to prevent oxidation process.³⁰

4. The effect of dl- α tocopherol and ascorbic acid on NO

In this study, there was no increase in NO level as expected after the administration of dl- α tocopherol and ascorbic acid. Low level of serum NO before intervention maybe caused by hyperlipidemia, high level of ox-LDL serum (72% of cases) and hypertension (55.6% of cases). Moreover, the Δ NO level was decreasing 0,35 μ M and 0,39 μ M in the treatment and control group respectively. Among the treatment group, the Δ NO level was increasing 0,11 during remission period and decreasing to the value of -1,05 among the non remission sub group.

Glucocorticoid administration blocked the superoxide production, so there must be an increase in NO level. Oxidative stress and accumulation of nitrite peroxide (ONOO-) as a

result of NO and superoxide (O_2^-) reaction cause changes in physiological and serological environment. These changes include an increase blood pressure, proteinuria, platelet dysfunction, increase thromboxane (TXA) and endotheline, and also a decrease in prostacycline (PGI₂) level.³¹

A decrease in NO production was likely caused by reduction in L-arginine level induced by increased lipoprotein. Moreover, the low L-arginine level lead a superoxide over production. NO level is also affected by calcium, NO-rich food intake such as celery, bit, leavy vegetables like spinach and lettuce.³²

Engler stated that vitamin E and C administration improved endothelial function, determined by brachialis artery dilatation evaluation (*flow mediated dilatation arteria brachialis*, FMA), with no effect on oxidative stress markers.²¹

Shouman revealed a different result where plasma nitrite level was found high in minimal change nephrotic syndrome patient $45,87 \pm 20,85 \mu\text{mol/L}$ and nitrate level $45,58 \pm 19,7 \mu\text{mol/L}$ whereas normal value is $12,33 \pm 7 \mu\text{mol/L}$.³³

Ascorbic acid assists NO synthesis by supporting redox system and stabilizing tetrahydrobiopterin (BH₄) level. It also reduces NAD(P)H oxidase activity and superoxide production *in vitro* and *in vivo*. NAD(P)H oxidase plays as a major superoxide endothelial responsible for early eNOS uncoupling, inactivated by α -tocopherol and ascorbic acid.¹⁷

Heller revealed that α -tocopherol improved NO production and being intensified by ascorbic acid. This study was conducted in isolated human endothelial umbilical vein.³⁴

The importance clinical finding in this study was improvement of blood pressure on 40 % cases (7/18 subjects) of treatment group. This proved that combined supplementation of dl- α -tocopherol and ascorbic acid had synergistic action increasing vascular dilatation, although the serum level of NO did not increase and it was not significantly different between two groups. The serum level of NO remained low because increasing level consumption of NO in cellular or needed time to adapt as physiological condition and then reach optimal level of NO. This study showed sinergistic effect between dl- α -tocopherol and ascorbic acid to improve vascular function in membrane and inside of the cell.

E. Conclusion and Suggestion

Conclusion: Combined supplementation of dl - α -tocopherol and ascorbic acid with a dose of 10-15 mg kg/ day administered for 12 weeks in children with NS:

1. Decreased the serum levels of ox-LDL , but it was not significantly different. Mean levels of ox-LDL, delta of ox- LDL level decreased significantly in the treatment group on remission state.
2. Increased the serum levels of sCD36, but it was not significantly different .
3. Decreased the serum levels of sVCAM-1, but it was not significantly different. SVCAM –1 decreased significantly only in the treatment group on remission state.
4. Decreased the levels of NO, but it was not significantly different.

Suggestions:

1. Further research was needed to separate subject of NS with hypertension.
2. Research with serial examination to confirm trend of biomarker.
3. Administration of dl - α -tocopherol and ascorbic acid to overcome oxidative stress minimally 6 months to maintain of remission state.

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4. Fellowship Training Pediatric Nephrology by International Pediatric Nephrology Association (IPNA) 2012- 2013 (supervisor. Prof. Kazumoto Iijima, MD, PhD) di Kobe University Graduate School of Medicine, Kobe, Jepang

Nasional:

- 2007 -Kursus Continuous Ambulatory Peritoneal Dialysis(CAPD), Solo, Pernefri
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-International seminar and Workshop on Modern Biology and It's application focusing on stem cell and Human Genetic
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E. Kegiatan Ilmiah:

Presentasi Poster/oral

- 1 **Omega Mellyana**, Sutaryo, Ida Savitri, Haris Marta Saputra: *Mud Aspiration: an experience at Cut Nyak Dhien Hospital (case report)* Konika Bandung, 2005
- 2 **Omega Mellyana**, Tantri, Haris Marta Saputra: *Psychological Reactions in refugees children in Meulaboh post earthquake and* Konika Bandung, 2005

- tsunami*
- 3 Ida Savitri, **Omega Mellyana**, Haris Marta Saputra: *Increasing Malaria patient in Cut Nyak Dhien Hospital post tsunami* Konika Bandung, 2005
 - 4 Chrisna H, **Omega Mellyana**, Uropati Obstruktif (*poster*) Proceeding KONIKA XIV, 2008 Surabaya
 - 5 Heru Muryawan, **Omega Mellyana**, Fenty K : *Pola Kuman Penyebab infeksi saluran kemih dan uji kepekaan kuman terhadap antibiotika tahun 2001-2005 RSUP dr. Kariadi Semarang,* Proceeding KONIKA XIV, 2008
 - 6 **Omega Mellyana**, Herumuryawan, Rochmanadji Widajat: *Hemodialysis and Peritoneal dialysis in acute renal failure* Konika Menado, 2011
 - 7 Agus Fitrianto, **Omega Mellyana**, Herumuryawan, Rochmanadji Widajat: *Hemodialysis in acute on chronic kidney disease: 9 cases report* Konika Menado, 2011
 - 8 **Omega Mellyana**, Herumuryawan, Rochmanadji Widajat: *Hemodialysis in Children at dr. Kariadi Hospital (oral)* Konker Pernefri 2012
 - 9 Adi Hijaz, M Supriatna, **Omega Mellyana**: *Risk Factor of acute kidney injury in pediatric intensive care unit's patients* PIT Bandung, 2012
 - 10 Christine Tampubolon, **Omega Mellyana**, Herumuryawan: *Clinical description of edema incidence, albumin and calcium serum level in children with nephrotic syndrome* PIT Bandung, 2012
 - 11 Taufan Prasetya, **Omega Mellyana**: Hubungan antara respon terapi induksi pada serangan awal sindrom nefrotik dengan kekambuhan (oral) PIT Bandung, 2012
 - 12 Timtri Dilli M, **Omega Mellyana**, M Herumuryawan, Rochmanadji W: *Association between clinically response of steroid and atherogenic index in nephrotic syndrome (poster)* PIT Bandung, 2012
 - 13 Sondang Panjaitan, **Omega Mellyana**: Keluaran Terapi metilprednisolon pada anak dengan sindrom nefrotik resisten steroid di RS dr. Kariadi Semarang (poster) PIT Bandung, 2012
 - 14 **Omega Mellyana**, Kar-Hui Ng, Mya-Than, Lourdes Paula Resontoc, Noor Haziha Hussain, Caresse Jeng, Perry Yew-Weng Lau, Isaac Liu, Wee Song Yeo, Hui Kim Yap: *Dialysis duration is the most important predictor of growth failure in children on peritoneal dialysis (poster)* The 16th Congress of the IPNA, Shanghai, 2013
 - 15 **Omega Mellyana**, Hiroshi Kaito, Natsuki Matsunoshita, Shingo Ishimori, Takeshi Ninchoji, Norishige Yoshikawa, Kazumoto Iijima: *Prevention of Focal Segmental Glomerulosclerosis (FSGS) recurrence by* The 16th Congress of the IPNA, Shanghai, 2013

combination of pretransplant bilateral nephrectomy, rituximab and plasma exchange, two cases reports

- 16 **Omega Mellyana**, Heru Muryawan, Rochmanadji Widajat, Edi Dharmana: *Effect of dl- α -tocopherol supplementation on lipid profile, oxidized LDL level, sVCAM-1 and sICAM-1 in nephrotic syndrome* The 12th Asian Congress of Pediatric Nephrology Association, 2014
- 17 Arlita Putri, **Omega Mellyana**, Susilo Adi: *Ankle Brachial Indeks among school-aged children* Konika Palembang, 2014
- 18 Arlita Putri, Herumuryawan, Mutya Dyah, Ririn S, **Omega Mellyana**: *Remission rate of cyclophosphamide therapy in children with steroid resistant nephrotic syndrome.* Konika Palembang, 2014

Fasilitator/Pembicara Workshop

- Kegawatan Nefrologi Anak, Manajemen hipertensi pada anak ,PKB Semarang, 2008
- Hemodialisis pada anak, Workshop PKB, 2010
- Inkontinensia pada anak, Workshop PKB Semarang 2014
- Biopsi Ginjal pada anak, Sinas Manado, 2014

Publikasi artikel ilmiah:

1	Omega Mellyana , JC Susanto: Impact of Six Month Oil Supplementation on Growth in the Weaning Period (research), Media Medika Indonesia, 2007
2	Mervin Tri hadianto, Omega Mellyana : Hemolytic Uremic Syndrome following dengue hemorrhagic fever, <i>Pediatrica Indonesiana</i> , 2011
3	Kurnia Dwi Astuti, M Herumuryawan, Omega Mellyana : Correlation between lipid profile and C-reactive protein (CRP) in children with nephrotic syndrome (submitted and review)
4	Omega Mellyana , Edi Dharmana, Hardhono Susanto, Nanan Sekarwana: The effect of combined vitamin C and E on the ox-LDL, sCD36, sVCAM-1 and NO level in idiopathic pediatric nephrotic syndrome (Submitted and review)

Kontributor Buku:

- Herumuryawan, Rochmanadji Widajat, **Omega Mellyana**, Transplantasi Ginjal: Buku Kompendium Nefrologi Anak, 2011

Publikasi populer

1. Meningkatkan ikatan Kasih Sayang antara Ibu dan Anak melalui ASI, Dalam rangka Hari ASI sedunia, Suara Merdeka, Agustus 2007
2. Jika Anak terkena sakit ginjal, petunjuk praktis bagi orang tua, Suara Merdeka, Maret 2014
3. Waspada dibawah 5 tahun jika anak masih mengompol, Suara Merdeka, April

2014

F. Organisasi:

- Anggota IDI, 1996 – sekarang
- Anggota IDAI, 2004 – sekarang (bagian Pembinaan dan Kesejahteraan Anggota, 2015)
- Anggota InaSN (Indonesia Society of Nephrology)/Pernefri :2007- sekarang
- Anggota ASPNA (Asian Pediatric Nephrology Association) dan IPNA (International Pediatric Nephrology Association) (2007-sekarang)
- Anggota Rotary Club Semarang Indraprasta Rotary International District 3420, 2008 - sekarang
- Anggota Tim Ginjal RSUP dr. Kariadi Semarang (2011-sekarang)
- Anggota Tim Paliatif RSUP dr. Kariadi (2012- sekarang)

G. Pengabdian:

- Anggota Tim Medis Pengobatan Gratis dalam rangka hari sakit sedunia, Gua Kereb, Ambarawa (2010, 2011)
- Pemeriksaan Kesehatan Proyek Pengembangan Anak Mugas tiap tahun (2011-sekarang)

H. Penghargaan

- Poster terbaik dalam rangka Risbiniptekdok 2011