F2α-isoprostane, Na^+-K^+ ATPase and membrane fluidity of placental syncytiotrophoblast cell in preeclamptic women with vitamin E supplementation

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Abstrak

Latar belakang: *Penelitian ini bertujuan untuk menganalisis kadar F2α-isoprostan, aktivitas enzim Na^+-K^+ ATPase dan fluiditas membran sel sinsitiotrofoblas plasenta penderita pre-eklampsia yang diberi vitamin E.*

Metode: *Penelitian dilakukan pada bulan September 2003 – Februari 2005 di Rumah Sakit Bersalin Budi Kemuliaan, Jakarta Pusat. Sampel penelitian adalah 6 wanita pre-eklampsia yang mendapatkan vitamin E, 6 wanita pre-eklampsia yang tidak mendapat vitamin E dan 6 wanita hamil normal. F2α-isoprostan diukur dengan ELISA Reader pada λ = 450 nm. Fluiditas diukur dengan membandingkan rasio molar kolesterol total dan kadar fosfolipid membran sel. Kolesterol diukur menggunakan Modular C800 dengan reagen Roch. Fosfolipid diukur menggunakan spektrofluorometer Shimadzu RF5301PC dengan filter eksitasi 267 nm dan emisi 307 nm. Aktivitas Na^+-K^+ ATPase dihambat dengan ouabain. Produksi Pi diukur dengan metode Fiske dan Subbarow menggunakan spektrofotometer pada λ = 660 nm. Data dianalisis menggunakan uji F melalui ANOVA 1 arah.*

Hasil: *Pemberian vitamin E pada penderita pre-eklampsia menurunkan stres oksidatif dengan indikasi turunnya F2α-isoprostan secara bermakna (26,72 ± 11,21 vs 41,85 ± 7,09 ng/mL, p = 0.017). Vitamin E mampu menangkal radikal bebas sehingga peroksidasi fosfolipid dapat dihambat dan fluiditas membran sel dapat dipertahankan pada 0,39 ± 0,08 dibandingkan tanpa pemberian vitamin E yaitu 0,53 ± 0,14 (p = 0,024). Aktivitas enzim Na^+-K^+ ATPase membran sel sinsitiotrofoblas tidak dipengaruhi oleh vitamin E (p = 0,915).*

Kesimpulan: *Suplementasi vitamin E pada wanita pre-eklampsia menurunkan kadar F2α-isoprostan, mempertahankan fluiditas membran sel, namun tidak meningkatkan aktivitas enzim Na^+-K^+ ATPase sel sinsitiotrofoblas.* ***(Med J Indones. 2012;21:225-9)***

Abstract

Background: The aim of our study was to analyze F2α-isoprostane level, Na^+-K^+ ATPase activity and placental syncytiotrophoblast cell membrane fluidity in preeclamptic women who received vitamin E supplementation.

Methods: The study was conducted between September 2003 and February 2005 at Budi Kemuliaan Maternity Hospital, Central Jakarta. Samples were 6 preeclamptic women with vitamin E supplementation, 6 preeclamptic women without vitamin E supplementation and 6 normal pregnant women. The dose of vitamin E was 200 mg daily. F2α-isoprostane was measured with ELISA reader at λ of 450 nm. Cell membrane fluidity was measured by comparing the molar ratio of total cholesterol and cell membrane phospholipid concentration. The cholesterol was measured by Modular C800 using Roche reagent. Phospholipid was measured by Shimadzu RF5301PC spectrofluorometer (excitation 267 nm, emission 307 nm). Na^+-K^+ ATPase activity was inhibited by ouabain. Pi production was measured with Fiske and Subbarow method using spectrophotometer at λ of 660 nm. Data was analyzed using F test with one-way ANOVA.

Results: Vitamin E supplementation in preeclamptic women decreased the oxidative stress, indicated by significantly lower level of F2α-isoprostane compared to those without vitamin E (26.72 ± 11.21 vs 41.85 ± 7.09 ng/mL, respectively, p = 0.017). Membrane fluidity in syncytiotrophoblast cell of preeclampsia with vitamin E group was maintained at 0.39 ± 0.08 while in those without vitamin E was 0.53 ± 0.14 (p = 0.04). Na^+-K^+ ATPase activity in syncytiotrophoblast cell membrane was not affected by vitamin E (p = 0.915).

Conclusion: Vitamin E supplementation in preeclamptic women decreases F2α-isoprostane level and maintains cell membrane fluidity of syncytiotrophoblast cells; however, it does not increase Na^+-K^+ ATPase enzyme activity. ***(Med J Indones. 2012;21:225-9)***

Keywords: *F2α-isoprostane, membrane fluidity, Na^+-K^+ ATPase, preeclampsia, vitamin E*

Preeclampsia is a disorder in pregnancy which marked by increased blood pressure and proteinuria, decreased uterus-placental blood perfusion, increase of trophoblast cell death and is one of the important etiologies of maternal and fetal mortality and morbidity.[1-3] One of the theories describing the etiology of preeclampsia is oxidative stress including increased free radicals and reduced antioxidant. Free radicals can disrupt endothelial cell and prevent trophoblastic cell invasion into spiral artery in decidua.[3]

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Low oxygen partial pressure as a result of spiral artery vasoconstriction in decidua has negative effect on trophoblast development in placental villi. Hypoxic condition also increases the formation of radical oxygen species through hypoxanthine reoxygenation pathway with the aid of xanthine oxidase enzyme.[4]

Free radicals exert a destructive effect to the cells. The destructive effects can be observed from the products of bio-macromolecular reaction, such as $F_2\alpha$-isoprostane.[5] The reaction between nitric oxide (NO) and oxygen radicals (O_2) results in peroxynitrite, which causes the oxidation of arachidonic acid product, $F_2\alpha$-isoprostane.[5] Increased level of $F_2\alpha$-isoprostane is a characteristic of oxidative stress that disrupts cell membrane.[6]

Increased $F_2\alpha$-isoprostane level is assumed to induce vasoconstriction of placental spiral arteries. This will cause entrapment of syncytiotrophoblast cell growing in decidua due to vasoconstriction.[7] This condition may cause syncytiotrophoblast cell destruction and necrosis in fetal artery and increased apoptosis of trophoblast.[8] Destruction of placental syncytiotrophoblast cells may also bring negative effects on fetal development, because it is the epithelial cell responsible for exchange of nutrients and substances between mother and fetus.

Free radicals can attack cell membrane protein such as Na^+-K^+ ATPase enzyme, which is a Na^+ and K^+ ion pump. Free radicals can also attack cell membrane phospholipid, which can disrupt cell membrane fluidity. If Na^+-K^+ ATPase enzyme activity and fluidity in syncytiotrophoblast cell membrane were disrupted, it will destruct the cell and causes apoptosis.

One of modalities to overcome the effects of free radicals is by vitamin E supplementation such as alpha tocopherol. Vitamin E supplementation in 36 weeks of pregnancy will decrease plasma lipid peroxide, thus negative effect of free radicals to the cells can be prevented.[9]

This study was aimed to recognize the protecting effect of vitamin E on syncytiotrophoblast cell in preeclamptic women by measuring $F_2\alpha$-isoprostane level, Na^+-K^+ ATPase enzyme activity, and fetal placental syncytiotrophoblast cell membrane fluidity.

METHODS

The study was conducted between September 2003 and February 2005 at Budi Kemuliaan Maternity Hospital, Central Jakarta. Ethical clearance from Ethical Research Committee of Medical Faculty Universitas Indonesia had been obtained. It is an experimental study with total-random sampling of 6 preeclamptic women given 200 mg/day of vitamin E from 30 weeks of pregnancy until delivery on top of routine medication, six preeclamptic women without vitamin E supplementation, and 6 normal pregnant women.[10]

Isolation of syncytiotrophoblast cell was performed based on modified methods developed by Smith et al,[11] Rand et al,[12] and Subakir.[13] Immediately after delivery, placenta was cut ± 10 g, and submerged into buffer solution of 0.05 Mol/L Tris-HCl, 0.1 Mol/L NaCl, 1 mMol/L $CaCl_2$, pH 7.4 (Tris-buffer saline solution/ TSS) with 4°C temperature.

Chorionic plate and the surface of decidua were removed, washed with TSS solution to remove maternal blood. Tissue was crushed with TSS solution and filtered with gauze. Then, tissue pellet was centrifuged with 2000 rpm for 10 minutes in 4°C temperature. Erythrocyte-lysing solution was added; incubated for 10 minutes in 37°C temperature. Afterward, it was centrifuged and syncytiotrophoblasts were isolated. Erythrocyte-lysing solution contained 2.0727 g of NH_4Cl, 0.25 g of $NaHCO_3$, 0.009275 g EDTA and 250 mL of 131.3329 osmolality distilled water.[13]

Cytosol and syncytiotrophoblast cell membrane preparation

Syncytiotrophoblast cells were weighted 500 mg, and then crushed with Wheaton voter. Subsequently, the cells were centrifuged at 3,000 G for 15 minutes in 4°C. Supernatant was added and centrifuged with 22,000 G for another 15 minutes in 4°C. Pellet were discarded and supernatant was centrifuged with 100,000 G for 30 minutes. If after this centrifugation no more pellet were found, then the whole part were taken and 20 mL TSS was added as cytosol and cell membrane sample and kept at -80°C until measurement.

Parameter measurement

$F_2\alpha$-isoprostane was isolated with chromatography and measured with $F_2\alpha$-isoprostane kit from Oxford Biomedical Research using ELISA Reader at 450 nm wavelength.

Na^+-K^+ ATPase enzyme activity was inhibited using ouabain and inorganic phosphate (Pi) production was measured with Fiske and Subbarow methods using spectrophotometer at 660 nm wavelength.[14] Protein level was measured at 280 nm wavelength.

Cell membrane fluidity was measured by comparing molar ratio of total cholesterol and cell membrane

phospholipid. Cholesterol was measured by C800 Modular with Roch reagent and Phospholipid was measured with RF5301PC Shimadzu spectrofluometer (excitation: 267 nm, emission 307 nm) in 37°C. 1,6-diphenyl-1,3,5-hexatrin (DPH) probe were dissolved in tetrahydrofuran until 0.6 µg/mL concentration.

Data analysis

Data normality was analyzed with Kolmogorov-Smirnov, and one-way ANOVA was used for comparisons of mean values of each parameter. P value of < 0.05 was taken as the limit of statistical significance.

RESULTS

Data of mean blood pressure, proteinuria, maternal age, weight and length of the baby were shown in Table 1.

Data of mean $F_2\alpha$-isoprostane, Na^+-K^+ ATPase enzyme activity and placental syncytiotrophoblast cell membrane fluidity were shown in Table 2.

DISCUSSION

In this study, vitamin E supplementation has no effect on the weight and length of the newborn. Mean diastolic pressure in preeclamptic women with vitamin E supplementation were decreased, but still in higher range, as also with the proteinuria. However, diastolic pressures of preeclamptic women with vitamin E supplementation at the end of the study were lower, although it was still higher than normal pregnant woman. Similar result has also been reported by Bastani et al who found that supplementation of 400 IU vitamin E started from 14 weeks of pregnancy has no effects on fetal weight and preeclampsia incidence.[15] Wangkhemayum also reported that 400 IU vitamin E supplementation daily is not positively correlated with blood pressure.[16]

In preeclampsia, there is oxidative stress marked with increase of $F_2\alpha$-isoprostane or 8-isoprostane.[17] In our study, 200 mg daily vitamin E supplementation started from 30 weeks of pregnancy in preeclamptic women has significantly lowered $F_2\alpha$-isoprostane level of syncytiotrophoblast cell than those preeclamptic women without vitamin E ($p = 0.017$). Lower $F_2\alpha$-isoprostane indicates that vitamin E supplementation can reduce oxidative stress since it functions as a chain breaking antioxidant which neutralizes free radicals effects.[7,18] Vitamin E is hydrophobic and lipid soluble,[16] thus enables it to enter cell membrane and deposited between fatty acids. Therefore, vitamin E is capable to

Table 1. Mean blood pressure, proteinuria, maternal age, newborn weight and length

Parameters		Normal	PE	PE + Vit E
Sample size (n)		6	6	6
Systolic blood pressure (mmHg)	Baseline	120 ± 0.00	146 ± 8.16	150 ± 7.53
	End	110 ± 5.77	144 ± 12.25	150 ± 14.14
Diastolic blood pressure (mmHg)	Baseline	80 ± 0.00	96.67 ± 8.16	98.30 ± 7.53
	End	73.33 ± 4.71	100 ± 10.95	96.67 ± 8.16
Proteinuria	Baseline	-	++/+	++/+
	End	-	+/-	+/-
Gestational age (weeks)		39 ± 0.82	37.33 ± 0.52	37.50 ± 0.84
Newborn weight (g)		2.937 ± 221.11	2.775 ± 216.02	2.760± 216.02
Newborn length (cm)		45.50 ± 1.67	47.00 ± 0.63	45.00 ± 1.26
Maternal age (years)		30 ± 1.63	32 ± 1.90	33 ± 1.67

PE = preeclampsia

Table 2. Mean F2α-isoprostane level, Na^+-K^+ ATPase enzyme activity, and syncytiotrophoblast cell membrane fluidity

Parameters	Normal	PE	PE + Vit E	p
Sample size (n)	6	6	6	
$F_2\alpha$ Isoprostane (ng/mL)	24.80 ± 10.66	41.85 ± 7.09	26.72 ± 11.21	0.017
Na^+-K^+ ATPase activity (µmol Pi/mg protein/hour)	0.28 ± 0.13	0.25 ± 0.14	0.27 ± 0.13	0.915
Membrane fluidity*	0.36 ± 0.08	0.53 ± 0.14	0.39 ± 0.08	0.024

Membrane fluidity = total cholesterol/phospholipid ratio

protect cell membrane fatty acid against free radicals attack, by inhibiting oxidation of arachidonic acid into $F_2\alpha$-isoprostane. This result is consistent with the study of Poston et al who found that vitamin C and E supplementation could decrease the concentration of 8-epi and $F_2\alpha$-isoprostane.[19]

In preeclampsia, trophoblast cells fail to invade spiral artery.[8] This can be shown by an increase of mannose-binding lectin (MBL) which inhibits trophoblastic and endothelial cell interaction.[8] Failure of syncytiotrophoblast invasion into maternal spiral arteries leads to inability of the spiral arteries to transform, hence caused reduced oxygen transportation and hypoxia. This condition will induce oxidative stress. Vitamin E supplementation reduces oxidative stress thus decrease the producion of $F_2\alpha$-isoprostane. Therefore, it is expected to induce syncytiotrophoblast growth and invasion to spiral artery. Transformation of spiral arteries to cone-shaped can reduced hypoxia effect, and formation of free $F_2\alpha$-isoprostane through hypoxanthine pathway can be reduced.

By decreasing oxidative stress in placenta, it is expected that syncytiotrophoblast cell destruction, necrosis, and apoptosis in fetal placenta trophoblast can be prevented. It means that vitamin E supplementation in preeclamptic women will prevent ischemic damage in syncytiotrophoblast cell which may bring negative effects on the fetus. It is important because placental syncytiotrophoblast is the epithelial cell responsible for substances transportation between mother and fetus.

Vitamin E supplementation in preeclamptic women has shown no effect on Na^+-K^+ ATPase enzyme activity in syncytiotrophoblast cell membrane. Total enzyme activity was measured with and without ouabain to cease the Na^+-K^+ ATPase activity (sensitive to ouabain). Data analysis showed that vitamin E did not increase Na^+-K^+ ATPase enzyme activity ($p = 0.915$).

In oxidative stress, free radical can destruct cells through many pathways, such as oxidation of enzyme protein. One of the enzymes in cell membrane is Na^+-K^+ ATPase, which pump $3Na^+$ out and $2K^+$ into the cell.[20] This pump maintains ion charges balance in syncytiotrophoblast cell. Decreased activity of Na^+-K^+ ATPase enzyme will disrupt Na^+ and K^+ ion transport. It could be fatal as cell membrane might have a malfunction with transmembrane potential leading to accumulation of intracellular Na^+ and it may cause apoptosis.[20]

It has been assumed that increased oxidative stress may disrupt Na^+-K^+ ATPase, thus decreasing its activity. In our study, vitamin E supplementation did not increase Na^+-K^+ ATPase enzyme activity in syncytiotrophoblast cell membrane. Presumably, there is a specific mechanism to regulate Na^+-K^+ ATPase, i.e. subunit α of the enzyme, which function is controlled directly by subunit β.[7] Subunit α and β protein are peripheral membrane cell protein and hydrophilic in nature.[20] Vitamin E is lipid soluble and hydrophobic, therefore it is less likely to protect the hydrophilic subunit α and β protein. Therefore, Na^+-K^+ ATPase enzyme activity is not affected by vitamin E supplementation.

Meanwhile, vitamin E supplementation in preeclamptic women is capable to maintain fluidity of syncytiotrophoblast cell membrane significantly. Membrane fluidity is a ratio of cholesterol and phospholipid concentration. Membrane is more flexible with higher phospholipid, which shown by low ratio of cholesterol and phospholipid. Free radicals might cause oxidation of cell membrane phospholipid, thus decreasing phospholipid level, and make the cell membrane more rigid. Fiore and Capasso also suggested that vitamin E and C supplementation alone or in combination can protect the cell from reactive oxygen species (ROS) effects.[3]

Peroxidation of lipid cell membrane by free radicals affects membrane fluidity and permeability, which leads to cell membrane destruction.[21,22] In preeclampsia, increased cholesterol and phospholipid ratio indicates that membrane fluidity is decreased.

In preeclamptic women who received vitamin E supplementation, cholesterol and phospholipid ratio were lower. It indicates that vitamin E supplementation is able to maintain cell membrane fluidity. Vitamin E maintain membrane fluidity by: (1) insertion of vitamin E between phospholipid preventing free radicals to attack phospholipid, (2) vitamin E as an antioxidant can neutralize free radicals effect against phospholipid oxidation.[23] Therefore, vitamin E is able to protect phospholipid, hence maintaining the fluidity of cell membrane. Cell membrane fluidity is important to maintain membrane integrity, thus preventing membrane destruction.

In conclusion, vitamin E supplementation of 200 mg daily is beneficial to provide protection against oxidative stress as indicated by reduced level of $F_2\alpha$-isoprostane. Vitamin E can overcome free radicals effect and inhibit phospholipid peroxidation, thus maintain cell membrane fluidity. However, Na^+-K^+ ATPase enzyme activity of fetal placental syncytiotrophoblast cell in preeclampsia does not increase. This is due to the hydrophilic nature of vitamin E; while Na^+-K^+ ATPase enzyme activity was

regulated by peripheral protein, subunit α and β, which are hydrophilic in nature. Further studies regarding Na^+-K^+ ATPase enzyme physiology through subunit α and β protein, with water soluble antioxidant such as vitamin C are needed.

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