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The relationship between vitamins E and C intake with total activity of erythrocytes and breast milk superoxide dismutase in lactating mothers

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ABSTRACT

Background: Human milk contains many components, one of them is superoxide dismutase (SOD). Vitamin E and C, together with SOD, can prevent oxidative stress.

Objective: This study investigated the correlation between vitamin E and vitamin C intake, with total SOD activity, in erythrocyte and breast milk among lactating mothers in Jakarta, Indonesia.

Methods: Sixty lactating mothers aged 20–40 years were recruited in 1–6 months postpartum in Grogol Petamburan and the Cilincing Public Health Centre from March 2019 until April 2019. Vitamins E and C dietary intake were collected using a semi-quantitative food frequency questionnaire. SOD total activity of erythrocyte and breast milk was measured using the Ransod kit 125.

Results: The median value of vitamin E intake was 6.50 mg/day, showing 91.7% of patients do not meet recommended daily intake (RDA) (19 gram/day), and the median of vitamin C intake was 120.05 mg/day with 70% participants fulfilling RDA. SOD total activity in erythrocyte and breastmilk showed a median value of 423.73 U/mL and 58.34 U/mL, respectively. The correlation between vitamin E intake with total SOD activity in erythrocyte ($r = 0.143$ $p > 0,05$) and breast milk ($r = 0.041$, $p > 0,05$) was not significant. Vitamin C intake was also not significantly correlated with SOD total activity in the erythrocyte.

Conclusion: There is no significant correlation between vitamin E and vitamin C intake with the total activity of SOD of erythrocyte and breast milk in lactating mothers.

Keywords: vitamin E, vitamin C, erythrocyte, breast milk, SOD

Introduction

Breast milk contains various bioactive components such as an antioxidant, superoxide dismutase (SOD). This SOD is not be degraded in the baby's digestive tract; therefore it can exert its protection due to oxidative stress [1]. Breast milk contains enzymatic antioxidants including SOD and glutathione peroxidase (GPx), which are carried by the blood circulation to the breast glands or synthesized by breast gland cells [2]. There are three types of SOD, namely ECSOD in extracellular; MnSOD in mitochondria and peroxisomes, and CuZnSOD in cytosols, chloroplasts, and extracellular spaces [2–4]. ECSOD is the most common SOD in plasma and extracellular fluid [5]. Superoxide dismutase, catalase, and GPx are the first-line antioxidants that act to neutralize reactive oxygen species (ROS). Second-line antioxidants, namely vitamins E, C, and GSH, inhibit the initiation of chain reactions by donating electrons to free radicals, converting them to be non-radical compounds [3].

Superoxide dismutase, vitamin E, and vitamin C work together to prevent lipid peroxidation [6]. SOD has a role in converting superoxide anion into hydrogen peroxide, catalase converts hydrogen peroxide into water and oxygen, while GPx converts hydrogen peroxide into water and glutathione disulfide. Vitamin E forms a radical form after donating electrons to free radicals, while vitamin C may help vitamin E form a non-radical structure as a scavenger against free radicals [3,7]. Human milk contains enzymatic antioxidant (SOD) and non-enzymatic antioxidants (vitamin E and C) to prevent oxidative stress. Vitamins E and C collaborate to protect the cell membranes from lipid peroxidation with mechanism stopping the chain reaction caused by superoxide.

Vitamins E and C work together to prevent lipid peroxidation in cell membranes. Meanwhile, SOD and superoxide exit the cell and enter the breast through circulation [8, 9]. Lipid peroxidation may also occur in the breast gland membrane cells, indicated by the presence of malondialdehyde (MDA) in breast milk [10]. Studies of vitamins C and E supplementation for three months increased total erythrocyte SOD in patients with type 2 diabetes mellitus [11]. This study indicated a possible synergistic correlation between vitamin E and vitamin C with SOD total activity in erythrocyte and breast milk that can be beneficial for lactating mothers and the babies. Ramizanipour *et al.* showed a positive correlation between total erythrocyte SOD and vitamin C intake in 30 obese subjects after the intervention [12]. Previous studies have shown SOD total activity in different breast milk during breastfeeding [2,9,13]. This study investigated the correlation between vitamin E and vitamin C intake, with total SOD activity, in erythrocyte and breast milk among lactating mothers in Jakarta, Indonesia. This study will help the researcher uncover the critical area of the loss of antioxidants. Thus, a new theory on this non-enzymatic antioxidant and enzymatic antioxidant combinations, and possibly other combinations in the future, maybe proposed.

Methods

Subjects

This cross-sectional study involved 60 lactating mothers in Grogol Petamburan and the Cilincing Public Health Centre in Jakarta, Indonesia, from March 2019 until April 2019. The Institutional Review Board of Faculty of Medicine University Indonesia approved this study (No.1129/UN2.F1/ETIK/2018, protocol number: 18-10-1242).

Inclusion and exclusion criteria

The recruitment participants were selected using consecutive sampling. Inclusion criteria were exclusively breastfeeding women aged 20 to 40 years in 1–6 months postpartum, having completed 38–40 weeks of pregnancy. They gave written informed consent. Women with a history of preeclampsia/eclampsia, multiple pregnancies from the last gestation, and who had a chronic disease (such as DM, hepatitis, cardiovascular disease, renal failure, and chronic pulmonary disease) were excluded.

Some personal information about the patient was taken with the interview, including maternal age, parity, level of education, smoking status, and physical activity. Anthropometric measures uniformly measured the body weight and height with Seca 703s weight scale and stadiometer.

Assessment of dietary intake

The vitamin E and vitamin C intakes were assessed by a semi-quantitative food frequency questionnaire (FFQ), and 24 hours of food recall for the two non-consecutive days were used to assess energy, protein, and fat intakes. Further information on multivitamin supplementation (vitamin A, C, E) was also gathered in the FFQ.

Determination of SOD total activity

Three mL of blood were collected from the median cubital vein in the morning to measure erythrocyte SOD total activity. Five mL of human milk were collected from one breast after 2 hours of breastfeeding their babies. Lysate from whole blood was stored at -20^{o} C, while human milk was stored at -80^{o} C until measurement was taken. The SOD total activity in erythrocyte and human milk was analyzed using the Ransod kit w125 (Randox Company).

Table 1. Basic characteristics of subjects (n = 60)

Subject's Characteristic	Value
Age (years)	28 ± 4.64†
Infant age (month)	
1–3 months, n (%)	27 (45)
4–6 months, n (%)	33 (55)
Parity	
Primipara, n (%)	22 (36.7)
Multipara, n (%)	38 (63.3)
BMI (kg/m^2)	24.16 ± 4.22†
Nutritional status based on BMI, n (%)	
Underweight	2 (3.3)
Normal weight	27 (45)
Overweight	15 (25)
Obese	16 (26.7)
Education level n (%)	
Low	9 (15)
Moderate	43 (71.7)
High	8 (13.3)
Smoker status	
Active smoker	
Passive smoker	
Yes	47 (78.3)
No	13 (21.7)
Not Smoker	
Multivitamin consumption	
Yes	11(18.3)
No	49 (81.7)
Physical Activity, n (%)	
Light intensity	13 (21.7)
Moderate intensity	41 (68.3)
Vigorous intensity	6 (10)

†: mean ± standard deviation. ‡: median (minimum–maximum).

Data analysis

The normality of data distribution was statistically analyzed using the Kolmogorov-Smirnoff test. Data were presented as either mean and standard or median (minimum–maximum). Correlation between vitamin E and C intake with SOD total activity of erythrocyte and breast milk were analyzed using the Rank Spearman correlation test less than 0.05 ($p < 0.05$).

Results

Characteristic of subjects

Sixty subjects were recruited and analyzed. The average age was 28 ± 4,64 years old, and 27 infants (45%) were in 1–3 months postpartum and 33 infants (55%) were in 3–6 postpartum age. Most of the subjects had multiple pregnancies, moderate levels of education, moderate physical activity, and did not consume multivitamin supplementation. The average body mass index (BMI) was 24.16 ± 4.22 kg/m^2. Most patients were passive smokers (Table 1).

Dietary intake

Table 2 describes the value of dietary intakes, such as energy, protein, and fat intake. They were assessed with 24-hour food recall for two non-consecutive days. Energy and macronutrients were enough if the intake met 80%–100% RDA recommendation, with data being compared to Indonesian Recommended Dietary Allowances (RDA) 2013. Most subjects had low energy intake,

Table 2. Dietary intake of subjects

Dietary intake	Value
Energy (kcal/day)	1853.87 (1055–3375)‡
Low, n (%)	34 (56.7)
Adequate, n (%)	26 (43.3)
Protein (g/day)	70.74 (42–252)‡
Low, n (%)	17 (28.3)
Adequate, n (%)	43 (71.7)
Fat (g/day)	72.32(35–130)‡
Low, n (%)	22 (36.7)
Adequate, n (%)	38 (63,3)
Vitamin E (mg/day)	6.50 (1.20–43.10) ‡
Low, n (%)	55 (91.7)
Adequate, n (%)	5 (8.3)
Vitamin C (mg/day)	120.05 (23–479.2) ‡
Low, n (%)	18 (30)
Adequate, n (%)	42 (70)

†: mean ± standard deviation. ‡: median (minimum–maximum).

Table 3. Correlation between Vitamin E and C intake with SOD total activity in erythrocyte and breast milk

Dietary Intake	Erythrocyte SOD		Breast Milk SOD	
	r	P	r	p
Vitamin E intake	0.143[S]:	0.275[S]:	–0.041[S]:	0.754[S]:
Vitamin C intake	0.019[S]:	0.887[S]:	–0.210[S]:	0.108[S]:

[S]: Spearman correlation test. *: statistically significant.

adequate protein, and fat intake. The majority of vitamin E intake was low, but vitamin C intake was adequate.

Table 3 shows the correlation between vitamins E and C intake with SOD total activity in erythrocyte and breast milk. There was no significant correlation between vitamins E and C intake with SOD total activity in erythrocyte and breast milk.

Discussion

More than half of the participants in this study had low energy intake but adequate protein and fat intake. We assessed them with 24-hour food recall for two non-consecutive days on weekdays and weekends. The study by Maharani *et al.* reported that 47% of lactating mothers in Semarang, Indonesia, have low energy intake [14], which reduced energy intake due to a lack of knowledge about the importance of energy intake for infants' growth and development. Similarly, Fikawati *et al.* also showed that 71.6% of lactating mothers have low energy intake [15]. On the other hand, protein and fat intake in our subjects was adequate. Kurniati *et al.* also showed that 71% of participants in their study had enough protein intake [16]. Protein plays a role in muscle, enzyme, hormone, and antibody formation [17,18]. Lack of protein will affect enzyme function in our body and create an imbalance between oxidant and enzymatic antioxidants [19]. Fat intake in our subjects was adequate, which will help the body to absorb lipid-soluble vitamins from the digestive tract into circulation [18].

Nevertheless, this study showed that almost all subjects' vitamin E intake was low. Some factors could affect vitamin E intakes, such as difficulty in obtaining vitamin E food sources, lack of information about vitamin E food sources, and the economic situation that would affect their choice of foods [20], and this result of low intake was similar to the study of Dini *et al.* that assessed vitamin E intake in DM type II participants [20]. Their research showed that 87.3% of participants' vitamin E intake was low. Seventy percent of the participants had vitamin C levels above the RDA recommendation for vitamin C level intake. According to Madanijah *et al.*, 24 participants in their study showed an increase in vegetable consumption such as sweet leaf or *daun katuk* (*Sauropus androgynus*) and spinach in lactating mothers. The increasing vegetable consumption was believed that it would improve breastmilk quantity and quality [21].

SOD consists of three types, CuZnSOD in the cytoplasm, MnSOD in mitochondria, and ECSOD in extracellular. Erythrocytes have a CuZnSOD in their cytoplasm. As free radicals increase and attack membrane cells, vitamin E is not enough to fight free radicals, resulting in membrane cell damage. Vitamins E and C, SOD, and free radicals such as superoxide in blood circulation, will reach the breast glands. Erythrocyte's SOD total activity in this research was 423.73 U/ml. It was quite different from the study by Saraswati *et al.*, which showed that SOD total activity in osteoarthritis patients was 274.97 U/ml. Both studies used the same reagent kit and measurement, but the study population was different [22].

The breast milk SOD total activity in this research was 58.34 U/ml. L'abbe *et al.* reported that breast milk total activity in their participant was 31 U/ml [13]. The difference may be due to infants' needs changing during breastfeeding, different types of intake during lactation, and any other factors that cause variance in breast milk SOD total activity. However, this study showed no significant correlation between vitamin E and C intake and SOD total activity in erythrocyte and breast milk.

The limitation of the study was that it did not include an analysis of other internal and external factors which may affect the total activity of SOD in breast milk and erythrocytes, such as mother's age, environmental condition, and nutritional status. Further studies are needed to relate other risk factors that may influence SOD total activity in erythrocytes and breast milk.

Conclusion

The study concludes that there is no correlation between vitamin E and vitamin C intake, with total SOD activity, in erythrocyte and breast milk among lactating mothers in Jakarta.

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Author contribution

Conceptualization, Y.M.S., E. Y.; Methodology, Y.M.S.; Investigation: Y.M.S.; Resources, Y. M.S.; Writing – Original Draft, Y.M.S.; Review: Y. M.S., E.Y.; Supervision, EY.

Declaration of interest

The authors declare no conflict of interest.

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