Serum Transferrin Receptors of Iron Deficiency Anemic Rats That Feeding Tempe Fortification Combination Iron and Vitamin A

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Abstract. This research investigated tempe fortified with *iron and vitamin A on* serum transferrin receptors. *Experimental research* R*andomized* P*re* T*est-Post Test* C*ontrol* Group D*esign* was *conducted on 30 Sprague*-Dawley *rats with iron deficiency anemia. The rats* divided into 6 groups randomly, was *treated for 6 weeks with a standard feed supplemented* by *soybean* tempe *fortified* with *iron and vitamin A.* Group 1 control group was given standard feed AIN-93G (SF), group 2 SF+T0, group 3 SF+T1, group 4 SF+T2, group 5 SF+T2+V15, (6) SF+T2+V50. T0 was tempe without fortification. T1 and T2 was tempe that was fortified with 230 ppm and 271 ppm of iron ($FeSO_47H_20$, respectively. V15 and V50 was tempe that was fortified with 15 ppm and 50 ppm of vitamin A (retinyl acetate, respectively. *Statistical test* was *used Kruskal Wallis test*. The result showed that before intervention, there was no significantly different on mean of serum transferrin receptors (sTfR) (p=0,280). After intervention, sTfR became 13,0±4,0; 8,7±2,8; 8,5±2,6; 7,4±3,4; 7,2±1,7 and 1,9±0,4 µg/mL, respectively. Tempe fortification with iron and vitamin A significantly decreased sTfR (p=0,004). Average of sTfR lowest in the treatment of SF+T2+V50 (standard feed+tempe was fortified with 271 ppm of iron + 50 ppm vitamin A).

INTRODUCTION

Nutritional anemia is one of the most common nutritional problem in the world, including in Indonesia. The findings of several studies in Indonesia show that the anemia prevalence of adolescent remained high (26,1%-42,6%). In nutritional anemia, iron deficiency anemia was considered as the most common cause. Among various solutions to improve nutrition, food fortification is one effort to do. Tempe-based soybean meal as an alternative to allow fortified with iron.

Fortification in this research with added the iron and vitamin A because various studies suggest a role of vitamin A in hematopoiesis. The relationship between vitamin A deficiency and anemia has been studied for several year. The results of the research in Indonesia, the baby and mother with serum retinol <0,7 µmol/L have 2,4 times the risk of becoming iron deficiency anemia (Dijkhuizen et al., 2001). Other studies have shown that children who received iron fortified soup and vitamin C to increase levels of serum iron and transferrin saturation higher when serum retinol levels> 40 mg/dL compared to <20 mg/dL, thus stated that vitamin A status has the effect of mobilizing iron stores (Stuijvenberg et al., 1997; Zimmermann, 2007).

Over recent years, serum concentrations of soluble transferrin receptor have been investigated as a marker of iron status. The transferrin receptor is a transmembrane glycoprotein, made up of two identical subunits connected by a pair of disulphide bridges, forming a molecule of 190 kDa (Feelders et al., 1999; Seligman et al., 1979;

Trowbridge et al., 1984). The role of the transferrin receptor is to insert iron into a cell center by joining transferrin molecules in the blood (Bali et al., 1991; Feelders et al., 1999).

MATERIAL AND METHODS

This study was conducted with Randomized Pre Test-Post Test Control Group Design. A total of 30 Sprague-Dawley rats underwent depletion period for 2 weeks (Naruki et al., 2010) with standard fed (AIN-93G) of free-Fe so iron deficiency anemia rats. Samples were divided into 6 groups randomly, and then the rats were treated for 6 weeks. The treatment ware as follows: 1) Standard feed (SF) (Reeves et al., 1993), 2) SF+TWF, 3) SF+T1, 4) SF+T2, 5) SF+T2+VA15, 6) SF+T2+VA50, which TWF was tempe without fortification, T1 and T2 tempe was fortified with iron 230 ppm and 271 ppm, respectively and VA15 was 15 ppm vitamin A and VA50 was 50 ppm vitamin A.

Iron used ferrosulfat heptahydrate (FeSO47H2O) and vitamin A, retinyl acetate. Iron levels refer Tawali research (2000) and Astuti et al. (2012). Standard feed was prepared referring to the principle of iso-caloric and iso protein. Amount of 36,5% casein protein of standard feed was substituted by flour of tempe.

Blood sampling in rats conducted at the orbital sinus and the maintenance of experimental animals at the Center for Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. Ethical clearance was obtained from the Health Research Ethics Committee, Faculty of Medicine, Diponegoro University.

Sprague-Dawley rats aged 4 weeks, were weighed 100-175 gram adaptated for 7 days with standard feed AIN 93G (Reeves et al., 1993). The body weight was measured weekly. At 7 a.m. every day, rats were fed with 10 grams pellet. Residual feed were weighed every day. The next stage was depletion period which the rats were fed AIN-93G free-iron for 14 days, and then hemoglobin level was measured until was ≤ 6.0 g/dL (Naruki et al., 2010). Before and after the intervention was measured levels of serum transferrin receptor (sTfR). During the study none of the rats died.

Transferrin receptor levels in serum were measured by enzyme-linked immunosorbent assay (ELISA) using a Model Elx 800 ELISA reader (Universal Micro-plate Reader) Bio-tek Instruments Inc. with a wavelength of 450 nm and units of mg/mL. Kruskal Wallis test was used to analyze the difference of serum transferrin receptors (sTfR) level between groups. Normality test of biomarker data was conducted by Kolmogorov Smirnov test. The differences were considered significant at $p < 0,05$.

RESULTS AND DISCUSSION

Serum transferrin receptors (sTfR) levels

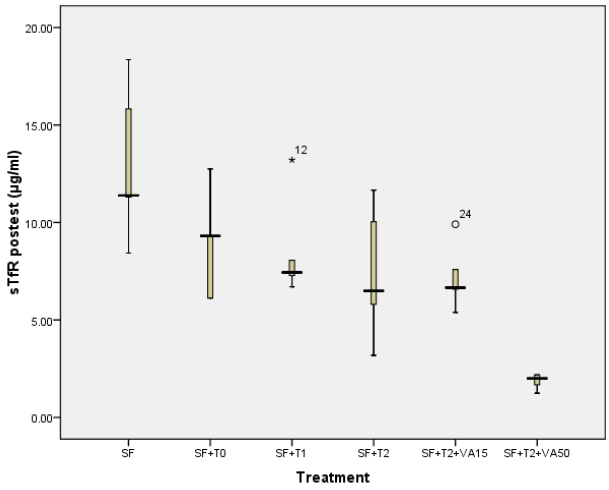
Mean of serum transferrin receptors (sTfR) levels at baseline, after a period of depletion was 46,4±23,8 µg/mL, and there was no significant difference in sTfR before intervention based on treatment (p=0,280).

Table 1. Mean of sTfR level before and after treatment

Treatment	sTfR level before treatment (µg/mL)	sTfR level after treatment (µg/mL)
SF	54,4 ± 42,3	13,0 ± 4,0[a]
SF+T0	30,3 ± 16,3	8,7 ± 2,8[a]
SF+T1	63,7 ± 27,3	8,5 ± 2,6[a]
SF+T2	47,0 ± 13,5	7.4 ± 3,4[a]
SF+T2+VA15	39,4 ± 8,0	7,2 ± 1,7[b]
SF+T2+VA50	41,2 ± 7,8	1,9 ± 0,4[c]
p-value	0,280	0,004**

1) **There were significantly difference (Kruskal-Wallis test)

2) On each collom *superscript* that different showed there were significantly difference based on posterior Mann Whitney test



Figur 1. Boxplot of sTfR level based on treatment

After intervention sTfR in the control rats (SF) were still high is 13,1 ± 4,0 µg/mL; rats fed soybean tempe fortification only iron, mean of sTfR is 8,5±2,6 µg/mL and 7,4±3,4 µg/mL, whereas rats fed soybean tempe fortification iron and vitamin A combination (SF+T2+VA15 and SF+T2+VA50), mean sTfR levels more decrease is 7,2±1,7 µg/mL and 1,9±0,4 µg/mL. The test results showed that administration of soybean tempe fortified iron and vitamin A combination may decrease levels of sTfR was significantly (p=0,004). Mean of sTfR levels highest in the treatment 271 ppm of iron + 50 ppm of vitamin A. The combination of iron and vitamin A significantly decreases sTfR levels.

DISCUSSION

1. sTfR in rats under conditions of iron deficiency anemia (before treatment) and after treatment

Average levels of serum transferrin receptors (sTfR) in rats under conditions of iron deficiency anemia was 46,4 ± 23,8 µg/mL, where the condition of the rat hemoglobin ranged from 5,2 to 6,0 g/dL. In line with the research Beguin et al., 1988 that in male rats aged 8-12 weeks were iron deficiency an average of transferrin receptors was 32,6±8,6 µg/mL. Normal rats in Beguin study was 5,7±0,7 µg/mL, whereas in this study, the mean sTfR after treatment was 7,9±5,0 µg/mL. Beguin (2003) in other studies also showed serum sTfR levels average 5,0±1,0 µg/mL in normal rats but the various commercial assays give different values because of the lack of international standards. The most important determinant of the level of sTfR was marrow erythropoietic activity which can cause variations up to 8 times below and up to 20 times the average value normal.

This study showed that in the status of iron deficiency anemia, the sTfR levels are high. The mechanism can be explained that in the intestine, the amount of iron absorbed is set according to the needs of the body by altering levels of DMT-1 and ferroportin levels according to the iron status of the villous enterocytes of duodenal crypts. Iron into the crypts enterocytes from transferrin plasma binds to transferrin receptors on the surface of basal cells (Hoffbrand et al., 2005). In rats with iron deficiency, iron deposits in the form of hemosiderin and ferritin decreased progressively and not sufficient to meet the requirement to be a normal turnover (WHO, 2001). In these circumstances, supply of iron to apotransferrin transport protein causes decreased transferrin saturation and increased transferrin receptor on the cell surface circulation and including eritron. Iron deficiency conditions in the crypts cells will lead to increased expression of DMT-1, where the ability of iron regulatory protein (IRP) to bind to the iron response element (IRE) increases, so that the mechanism of transferrin receptor is increased in iron deficiency (Hoffbrand et al., 2005). Transferrin receptor is a parameter that is intended to measure the activity of erythropoiesis (WHO, CDC, 2007; Gropper et al., 2009). Beguin et al., 2003 suggests that sTfR is a quantitative

assay of marrow erythropoietic activity and markers of tissue iron deficiency. This test is useful for identifying iron deficiency in patients with concurrent inflammation because ferritin values were then generally normal. Higher levels of sTfR also describes the characteristics of functional iron deficiency, which is the state that is defined by a network of iron deficiency despite adequate iron stores. WHO (2001) noted the advantages measurement of serum transferrin receptors (sTfR) measurement was that this fact is not significantly affected by infection or inflammatory process and not too varied according to age, sex or pregnancy. In this study, no significant correlation was obtained sTfR with infection status.

Serum transferrin and transferrin receptors undergo changes when there is a decrease iron stores. In the first phase of iron deficiency conditions, namely "*early negative iron balance*", the iron stores in the liver, spleen and bone marrow begins to decline, serum transferrin receptors is still stable. Iron deficiency entered the second phase of "*iron depletion*", more decreased iron stores, the recent increase in serum transferrin receptors. In the third phase, namely "*iron deficient erythropoiesis*", is the stage of iron deficiency on erythropoiesis activity with an increased state of serum transferrin receptors is also high. As well as the fourth phase of "*iron deficiency anemia*", is the stage of iron deficiency anemia with more severe state with higher serum transferrin receptors and ferritin decline heavier and Hb level is below the normal range (Gropper et al., 2009).

2. The relationship between iron status and vitamin A

The results of this study showed that combination of iron and vitamin A may decrease sTfR levels. The relationship between iron status and vitamin A has been widely studied. The influence is due to the presence of vitamin A roles in hematopoiesis (Fishman et al., 2000; Gropper et al., 2009). Several mechanisms may explain the influence of vitamin A deficiency upon the status of anemia: 1) the decrease of iron mobilization from iron deposit to bone marrow (Zimmermann, 2007; Gropper et al., 2009); 2) lower resistance to infection which may increase the status of anemia due to infection (Semba and Bloem, 2002; Zimmermann, 2007); 3) the influence of iron absorption or metabolism; and 4) direct modulation or stimulation of erythropoiesis (Semba and Bloem, 2002; Zimmermann, 2007; Gropper et al, 2009).

Vitamin A in relation to iron absorption associated with the results of studies in humans that vitamin A and beta-carotene can form complexes with soluble iron in the intestinal lumen, and then prevent the inhibitory effect of phytate and polyphenols on the absorption of Fe (Garcia-Casal et al., 1998). The results of the research on school children is in the children deficiency of vitamin A and iron, suplementation of vitamin A mobilizes iron from iron store thus increase erythropoiesis, and the effect is mediated by increased circulating EPO (erythropoietin). Erythropoietin is a hormone made in the kidneys that stimulates erythropoiesis. Vitamin A, in particular retinoic acid responds element binding in the erythropoietin gene and stimulates the formation of red blood cells. When vitamin A is inadequate, erythropoietin gene could not be transcripted so the synthesis of red blood cells decreased (Gropper et al., 2009).

The role of vitamin A in the gene expression mechanism through the retinoic acid affects cell differentiation. A series of events retinoic acid moves into the nucleus, which subsequently interact with DNA. Firstly, all-trans retinoic acid or 9-cis retinoic acid are transported into the nucleus, then it binding to the CRABP (Cellular retinol-binding protein) (Gropper et al., 2009). In the cell nucleus, all-trans retinoic acid and 9-cis retinoic acid binds to retinoic acid receptors (RAR) and retinoic X receptors (RXR) (Noy N, 2010; Theodosiou et al., 2010). RAR and RXR regulate gene transcription by binding to retinoic acid response element (RARE) in the target gene (Tang and Gudas, 2011). In general, the absence of ligand, RAR/RXR suppress gene transcription because the RAR/RXR interact more with the co-repressor, whereas the presence of ligand RAR/RXR interacts with co-activators (Noy N, 2010). Each retinoic acid receptors are divided into α, β, and γ whose isoforms; each isoform encoded by a different gene (Tang and Gudas, 2011). RXR regulates gene transcription as homodimers or heterodimers with other nuclear receptors, including RAR, peroxisome proliferator activated receptor that is, vitamin D receptor, and the hormone receptor tiroid (Tang and Gudas, 2011). Homodimer is formed when two similar receptors interact as RAR-RXR-RAR or RXR. Heterodimer formed between two or more different receptors such as RAR or RXR-VDR (Vitamin D Receptor)-RXR (Gropper et al., 2009). Changes in mRNA transcription will lead to changes in protein synthesis.

CONCLUSION

This research suggests that before intervention with tempe fortification combination iron and vitamin A was not significantly different on mean of serum transferrin receptors (sTfR) level (p=0,280). After intervention, sTfR levels

decreased significantly (p=0,004). The lowest average sTfR levels in the treatment is tempe fortification 271 ppm of iron ($FeSO_4 7H_20$) + 50 ppm of vitamin A.

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