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Original Research Article

Description of Reticulocyte-Hemoglobin, Immature Reticulocyte Fraction and Reticulocyte Production Index on the Student 12-15 Years Who Get Local Fortification Rice

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ABSTRACT

Anemia as a crucial problem in many parts of the world, is no exception in Indonesia, with a prevalence of 21.7%, of which 18.4% among the young men, ranging from mild to severe anemia, (Riskesdas-2013). The objective: (1) Knowing the concentration description of Reticulocyte Hemoglobin (RET-He) on the students, (2) Know the concentration description of Immature Reticulocyte Fraction (IRF) on the students, 3. Know the concentration description of Reticulocyte Production Index (RPI) to the students. The Methods was experimental Study has be done in the 2 groups, the treatment group and control, in Pesantren Annihayah, Karawang. Giving the treatment conducted during 6 months (first 3 months or midline and the second 3 months or endline), but the data retrieval of Ret. He, IRF and RPI on endline only. Total sample counted 36 rounded 40 people / groups, but at the end of the study to 32 treated group and 32 controls. Treatment by the provision of fortified rice 6 micronutrients. Given through rice lunch and dinner, as much as 200 grams / times of administration. Taking and examination of blood by laboratory personnel of "Prodia Jakarta". Results show that the average levels of Ret-He Endline normal (> 25.0 pg), but when compared between treatment with control, then the control group had an average much higher than the mean average treatment groups, especially in the age group > 14 years. Shows that although the average value of IRF is still below the standard value (<11 %). However, when compared to the value of IRF treatment group and control group, the higher in the treatment group, because it was nearing standard. The average level of RPI endline, where all three age groups in the treatment group and the control group for all three age groups showed an average RPI is lacking (<1.0%). When compared between the treatment groups with the control, then the control has on average a higher than average treatment groups, especially in the age group > 13 years. In conclusion (1) Concentration of Ret-He in the control group had an average higher than the average treatment groups, especially in the age group > 14 years. (2) Concentration IRF higher in the treatment group and already nearing the standard compared to the control group. (3) The concentration of RPI in the control group is higher than the treatment group.

Key Words: Foods Fortification, Reticulocyte Hemoglobin, Immature Reticulocyte Fraction and Reticulocyte Production Index

INTRODUCTION

The World Health Organization (WHO) in 2009 states that in Southeast Asia as much 25-40% of young women suffer from *Iron Deficiency Anemia* (IDA) both mild to severe. The prevalence of anemia in adolescents and young men aged 15-24 was 18.4% and 13.7%, respectively. Riskesdas in 2007 showed that the national prevalence of anemia was 12.8%, of which 70.1% is IDA. Increased become 21.7% in 2013. For school age children was 29.0% are anemic and young men was 18.4% (Riskesdas, 2013). This indicates that the largest proportion of anemia is *Iron Deficiency Anemia* (IDA).

Anemia is a certain condition in which red blood cells (erythrocytes) is not able to provide enough oxygen into the body tissues, mostly due to iron deficiency (Ngui R, et.al, 2012). Iron deficiency can occur because of inadequate iron intake and less absorption, increased iron requirements during growth, and loss excess of iron (Pasricha SR, et.al, 2013). Patients with IDA can result in death (WHO, 2009). Iron deficiency in school age can affect immunity (Ekiz C et.al. 2005), growth rate (G.Samuelson, 1995), intellectual performance, neurological function and intelligence (Iqbal K, et.al 2015 and FY Mesfin et.al, 2015).

Food fortification is certain nutrient-enriched foodstuffs (Gera T, et.al, 2012 and Hurell RF, 2012). Example of food fortification of iron that has been done is soy sauce, fish sauce, flour and rice with iron in the People Republic of China (PRC), flour with iron in India, flour fortification with iron in Philippines, noodles and rice with iron in Thailand, fish sauce with iron in Vietnam, flour with iron in Indonesia (KFI, 2011). Food fortification should focus on foodstuffs that are consumed locally, in order to be implemented later in a sustainable manner on a large scale, allowing people to get the nutritional value is met from the food consumed (Allen LH, et.al 2006 and Arcanjo F, et.al, 2013).

Reticulocyte is a precursor of red blood cells (Silvesthern DUL, 2013). It as immature erythrocytes, released in peripheral blood after a period of maturation in bone marrow, experienced a further differentiation in mature erythrocytes (Yesmin S1, et.al, 2011). Ret-He is a parameter defined in *the National Kidney Foundation*, as a guide for assessing initial iron status of patients with chronic kidney disease with hemodialysis as efforts to recover iron in patients (Scott Kirn, 2009). Hypoxia stimulates the cells that produce erythropoietin will make and release erythropoietin into the blood and will be circulated to the tissue that need it especially *progenitor erythroid* cells in the bone marrow to stimulate erythrocyte formation process. The result is an increased release of reticulocytes into the peripheral blood so that it can deal with complaints of anemia (Chang KH, et.al, 2004). Reflex of Ret-He monitoring is a short-term guide of iron for erythropoiesis and its response to iron therapy (Schoorl M, 2015).

Immature Reticulocyte Fraction (IRF) is a newly routine parameter in hematology analysis, which can give an idea on early morphological changes for bone marrow recovery, before other tests to be positive after chemotherapy (Yesmin S, et.al, 2011). In order to knows the number of reticulocytes in earlier then necessary to examine the number of IRF (Setiawaty et.al, 2007). IRF is defined as the ratio of young reticulocytes to the total number of reticulocytes. IRF is an examination of bright reticulocyte fraction with a high content of RNA (Schoorl M, 2015). Reticulocyte Production Index (RPI) is a number that reflects the absolute index of erythrocyte production by the bone marrow in patients with anemia (Hilman RS, 2002). When reticulocytes are released early from bone marrow, this immature reticulocyte can be in circulation for 2-3 days. Especially, this occurs in severe anemia that causes increased erythropoiesis (Schrier SL, 2011). Examination of RPI calculation is an important diagnostic tool, in addition to

using other specific markers, in which reticulocytes as a reflection of increased production of erythrocytes in bone marrow (Setyawati, et.al, 2007).

MATERIALS AND METHODS

A. Location and Period

1. Location

The research has been conducted in Pondok Pesantren Annihayah, Rawamerta sub-district, Karawang, Province of West Java.

2. Period

The research was conducted from May 2015 until March 2016, within 10 months, consisting of: 2 months for preparation, 6 months for intervention and 2 months for data management and report writing.

MATERIALS AND METHODS

The research design in this efficacy study is a *Double Blind Randomized Control Trial* or DB-RCT. With several provisions as follows:

1. The sample was selected randomly in the Islamic School.
2. The sample was grouped into 2 (two) groups as treatment and control groups.
3. The given of fortified rice or not was conducted in *double blind* in which all research team and researched subject does not know whether consumed is fortified rice or not.

B. Population, Sample and Sample Size

1. Population

The population of this research was all students at the Islamic School Annihayah, Rawamerta sub-district, Karawang, totaling 603 people. This research as a part of "Efficacy Study of Fortified Rice with Premix Local on Hb Concentration, Ferritin, Folic Acid, Zinc and Prevalence of Anemia for students," through a projects of JFPR-9132-INDO by National Planning Board (Bappenas) through Asian Development Bank (ADB).

2. Sample

a. Subject

The sample in this research was students aged 12-15 years.

b. Sample Size

The sample size was calculated based on information from Hussaini SZ, et.al, 2014, it calculated and obtained: n = 36.

To anticipate Drop Out and other reasons, then *n* is simulated, up to *n* = 40 students per group x 2 groups (cases and controls) = 80.

Reference: Lemeshow, et al. (1995). Imelda Angeles-Agdeppa, et al. (2008). Int Science Index Vol.6 9, 2012. Waste. org/Publication/1277.

3. Sampling Procedure

a) Choosing the Islamic Schools that have 603 students (male).
b) Screening anemia by measuring hemoglobin concentration of selected students.
c) Make a list of students who concentrations of Hb $\geq$ 8 mg% to $\leq$ 12 mg%.
d) Perform *random allocation* to the students from a list created in 3rd point into 2 (two) groups, each group consisted of 40 students, for one class.

C. Criteria of Inclusion and Exclusion of Sample

1. Inclusion criteria: a. Male b. Age 12-15 years
   a. Having Hb concentration : $\geq$ 8 mg% to $\leq$ 12 mg%
   b. Willingness to be sampled, signed *Informed Consent*.
2. Exclusion criteria:
   a. Having chronic infections e.g tuberculosis, malaria, thalassemia
   b. Non-infectious disease i.e. heart disease, blood vessels, cancer, chronic kidney and diabetes.
   c. Acute and chronic bleeding.
3. Dropout:
   a. Recurrent acute bleeding
   b. The frequency and duration of diarrhea is high and recurrent
   c. Not willing to drawn their blood at midline and endline.
   d. Withdrew from the trial.

D. Instrument of Research

The instrument used in this research includes:

1. Student identity form for screening and sample selection
2. Form the willingness to be respondents (*informed consent*)
3. Questionnaires of characteristic data, infectious disease history
4. Laboratory tool for measuring the concentration of hemoglobin, ferritin, RET-He and IRF.

E. Procedure of Research

1. Field preparation:
   a. Selection of Islamic School site
   b. Approaches the headmaster and teachers
   c. Socialization to: teachers and students as well as the kitchen worker
2. Preparation of materials and tools used, as follows:
   a. Respondent identity includes the name, age, education and address
   b. Gauges the concentration of Hb, Ferritin, Ret-He, and IRF
   c. Fortified rice
   d. Completeness preparation, field workers, school teachers and kitchen worker
   e. Training of research team
3. Implementation and data collection
   a. Approaches the subject of research by explaining the intent and purpose of the research
   b. Each student performs early diagnose, and selected based on criteria
   c. Asking the willingness of candidates to be respondents
   d. Prepare tools and materials for research. Gauges the concentration of Hb, Ferritin, Ret. He, IRF. Materials interventions used.
   e. Measuring the levels of Hb, Ferritin, Ret. He, IRF by Prodia Laboratory
   f. Respondents were given worm medicine albendazole, before intervention.
4. Implementation of Intervention:
   a. Taking rice from the warehouse according to type of sack (Forti A and Forti B)
   b. Weighting the fortified rice in each 4 Kg
   c. Washing the rice according to Rasforti A and Rasforti B
   d. Cooking the rice in Rice Cooker A and Rice Cooker B
   e. Scoop out and weighting the fortified rice A by group I, fortified rice B by group II, respectively 200 g/eat. Rice is weighed use "TANITA Digital" scale, an accuracy of 0.1 gram, then inserted into a Lunch Box.
   f. Distributing meal to target students in the classroom or in Showroom, with compositions as in table 1.
   g. To monitor the students who eat and weigh leftover rice if any
   h. This intervention was given 2 (two) times a day, that is lunch and afternoon.

Table 2-1: Composition of fortified rice and nutrition needs by age

Variable	Energy	Vitamin				Mineral	
		B1 (mg)	B3 (mg)	(Folat) (µg)	B12 (mg)	Fe (mg)	Zn (mg)
Fortified rice per (100 grm)	183	0,447	18	400	2,6	60	15
AKG Male 12-15 yr	2100	1,1	12	400	1,8	13	14

F. Packaging and Data Processing

Data from laboratory tests: hemoglobin and ferritin were taken, packed and processed by the Prodia laboratory personnel in Jakarta.

G. Analysis and Data Presentation

Descriptive analysis was conducted to determine the profile of hemoglobin and ferritin as indicators of hematology for the students. The data were analyzed by using SPSS ver. 18 and Microsoft Excel.

RESULTS AND DISCUSSIONS

A. Monitoring and Follow-up

After observation run first 3 months (Baseline/BL to Midline/ML), then occurs drop-out and transfer of students to another school, so that total remaining students is 73, divided into 37 as treatment group and 36 as controls. Furthermore, at the end of intervention or second 3 months (ML to Endline/EL), also still occur drop- out and transfer of students to another school, so that total remaining students is 64, divided into 32 as treatment group and 32 as controls at the time of EL.

B. Characteristics of Socioeconomic

Table 3.1: Distribution of socio-economic of samples

Characteristics	Anemia Treatment Group	Anemia Control Group
Class, n (%) : 1 (n = 43) 2 (n = 28) 3 (n = 9)	n = 40 19 (47,5) 16 (40,0) 5 (12,5)	n = 40 24 (60,0) 12 (30,0) 4 (10,0)
Race of Parent, n (%) Father, Sunda (n = 49) Mother, Sunda (n = 58)	 22 (27,5) 29 (36,2)	 27 (33,7) 29 (36,2)
Occupation of Parent, n (%) Father, Entrepreneur (n = 40) Mother, Household (n = 58)	 24 (30,0) 29 (36,2)	 16 (20,0) 29 (36,2)
Education of Parent, n (%) Father, Senior High School (n = 20) Mother, Senior High School (n = 24)	 14 (17,5) 16 (20,0)	 6 (7,5) 8 (10,0)

Based on table 3.1 indicates that the distribution of socio-economic of sample, in which case group (intervention) by 47.5 is Class 1, 40.0% Class 2 and 12.5% Class grade 3. In the control group by 60.0% is Class 1, 30.0% Class 2 and 10.0% Class 3. Race of parents, the greatest is Sunda father as treatment group is 27.5%, while 36.2 is Sunda mother. The control group was 33.7% Sunda father, while 36.2% is Sunda mother.

C. Laboratory Result

1. Mean distribution of Ret-He Endline Level by Age

Table 3.2: Mean distribution of Ret-He endline level by age

Age (Year & Month)	Reticulocyte - He (pg)			
	Treatment	n	Control	n
12 yr – 12 yr 11 mo.	27.7	16	27.9	17
13 yr – 13 yr 11 mo.	25.7	11	26.3	13
14 yr – 14 yr 11 mo.	26.2	5	30.4	2
Total	Mean : 26.8 SD : 3.2	32	Mean : 27.4 SD : 3.3	32

Table 3.2 indicate the mean of RET-He Endline level, where all three age groups in both treatment and control groups, the three age groups showed a mean of Ret-He is normal (> 25.0 pg). However, when compared between the treatment and control groups, then in the control group had a mean is higher than treatment, especially in the age group > 14 years.

The results of this research on table 3.2 indicates that the mean of RET-He Endline level is normal (> 25.0 pg), but when compared between treatment and control, then the control group had a mean is higher than treatment groups, especially in the age group > 14 years. It further indicates that the intervention of fortified rice had little effect. Because Ret-He is more sensitive to IDA, compared with other markers, it should be showing a good effect in the treatment group, not vice versa. The argument of Chang KH, et.al (2004) above that low concentration of reticulocyte in patients with infections in particular, it may be caused by inadequate production of erythrocytes or erythroid response that is sub-optimal.

When acquired low concentration of reticulocyte in patients with infection, then this is caused by inadequate production of erythrocytes or erythroid response that is sub-optimal. Then the results of another study reported that changes in levels of count result of reticulocytes count only illustrate the release of immature reticulocytes from bone marrow, and is not a sign of the expansion of erythropoiesis process. For this reason, it is more important to know the response of erythropoiesis to the administration of iron than only see reticulocyte index only (CL Chuang, et.al 2003).

RET-He (reticulocyte hemoglobin) is the number of count results in the content of hemoglobin in reticulocytes, (Brugnara et.al, 2006). Ret-He is often abbreviated as CHr (*Reticulocyte Hemoglobin Count*). CHr is more reflects the quantitative measure of erythropoiesis, while reticulocyte parameter is more provides information about the

condition of reticulocytes quality (Suega K, 2010).

Recently, various studies have reported that CHr as a reticulocyte index that is widely used in the clinic. This is due to the content of hemoglobin was constant throughout the life of the erythrocytes, unless there are structural changes that lead to impaired function and intracellular fragmentation. CHr is the latest reflection of hemoglobin production in the bone marrow, and also as a reflection of their reserves of adequate iron. It is more beneficial than the examination of iron grains in the bone marrow which is a rough estimate of reserves of iron in the reticuloendothelial system (Brugnara et.al 1999).

2. Mean distribution of IRF Endline level By Age

Table 3.3: Mean distribution of IRF Endline level by age

Age (Year & Month)	IRF (%)			
	Treatment	n	Control	n
12 yr – 12 yr 11 mo.	8.3	16	6.8	17
13 yr – 13 yr 11 mo.	9.8	11	8.4	13
14 yr – 14 yr 11 mo.	9.0	5	6.2	2
Total	Mean : 8.0 SD : 3.2	32	Mean : 7.5 SD : 3.2	32

Table 3.3 indicate the mean of IRF endline level, where all three age groups in the treatment group and control, all three age groups showed a mean of IRF is less than normal (< 11.0%). However, when compared between the treatment and control, then the treatment group had higher mean (closer to normal) of a mean of control, especially in the age group > 14 years.

The results of this research on table 3 indicate that although the mean value of IRF is still below the standard value (< 11%). However, when compared to IRF value of treatment and control groups, the treatment group is higher, because it closer to standard. It showed that the treatment group had an increase in the ratio of immature reticulocyte on the amount of total reticulocyte although it not yet to standard value. As noted above that although only occur 1 % in the increasing of immature reticulocyte, it can be an indicator that there are regeneration of erythroid.

Count result of IRF is expressed in the percentage of its normal value ranged between 1 – 2%. Beside, other ways is using flow cytometer. By this way in addition to counting the number of reticulocytes it can also determine the level of maturation of reticulocytes is based on the amount of RNA content, which more and more of RNA, the cell of reticulocytes is immature (Buttarello M, et.al in 2004 and Riley RS et.al, 2001).

The increased accuracy of reticulocyte measurements are automated and the possibility to can measure the number of IRF, it provided an opportunity to assessing a change of status effect of iron in the cells of population today.

The increasing of IRF level is showing an increase in erythropoiesis, during pregnancy trimester. The increasing of IRF as the increasing of *zinc protoporphyrin* (ZPP) and the decreasing of RET-He as an indication of iron deficiency functions, although ZPP cannot distinguish between reality with iron deficiency functions, (Choi JW and Pai SH, 2001). IRF or this immature reticulocyte fraction can be used as an indicator of erythropoietic activity. IRF reflects the degree of erythropoiesis, but not as an indicator of the iron-restricted erythropoiesis (Goodnoughm LT, 2000).

Finally, the phenomenon occurs when the body's iron store is still adequate but iron cannot be used by the bone marrow. It is often encountered as in the cases of infection and inflammation (Bartels PCM, et.al 2006). Measurement of mature erythrocytes have an instructions on automated hematologic analysis is an indicator that is not sensitive to early iron deficiency, due to the slow of erythrocyte turnover (120 days) and various variable between individuals (Mast AE, et.al 2007). The fact that the levels of reticulocytes can be stable after storage for 48 to 72 hours, but not so with IRF that can only be stable for up to 8 hours.

Immature reticulocytes should be distinguished from mature reticulocytes by means of increased levels of RNA and fragments of cytoplasmic organelles. Immature reticulocytes are released over a period of erythropoietic stimulation, such as in response to iron or therapeutic stimulation causes erythroid (Davies BH et.al 1996 and Thomas DW et.al, 2013).

3. Mean distribution of RPI Endline level by age

Table 3.4: Mean distribution of RPI Endline level by age

Age (Year & Month)	RPI (%)			
	Treatment	n	Control	N
12 yr – 12 yr 11 mo.	0.69	16	0.73	17
13 yr – 13 yr 11 mo.	0.62	11	0.97	13
14 yr – 14 yr 11 mo.	0.62	5	0.74	2
Total	Mean : 0.65 SD : 0.24	32	Mean : 0.27 SD : 0.24	32

Table 3.4 indicate the mean of RPI Endline level, where all three age groups in the treatment and control group for all three age groups showed a mean of RPI is less (<1.0%). When compared between the treatment and control, then the control has a higher mean than the treatment group, especially in the age group > 13 years.

The results of this study on table 4 indicate that the mean of RPI level in the control group is higher than the treatment group. It is likely in line with Ret-He, where RPI is calculated based on the number of reticulocyte and hematocrit percentage, and compares it with the production count of reticulocyte on individual without anemia. Thus, the count of RPI will be normal when reticulocyte and hematocrit production are also been normal. A number of factors affecting the production of reticulocyte and hematocrit, and also a factor that affecting RPI calculation.

We know that the occurrence of anemia, with the stimulation of erythropoietin, there will be a release of immature reticulocyte that should not time yet removed from the bone marrow, so that these cells will require a longer time for maturation, compared with other cells. Under normal circumstances, the time required for the maturation process of reticulocyte is 2 days, while the time required by reticulocyte cells that stimulated for release for erythropoietin (*stress reticulocyte*) is approximately 2-4 days, depending on the severity of anemia, (Dessypris EN 2003).

Factors affecting the calculation of corrected reticulocytes are the release of premature reticulocytes in circulation in patients with anemia. Where usually reticulocytes in the blood for 24 hours before release its RNA and then into erythrocytes (Schrier SL, 2011-(B). Furthermore, if reticulocytes are released early from the bone marrow, the immature reticulocyte can be circulated for 2-3 days. It is common, especially in patients with severe anemia that causes increased erythropoiesis (Schrier SL, 2011-A).

High IRF with *Absolute Retikulocyte Count* (ARC) is normal or low found in diseritropoiesis conditions as the case of acute leukemia, myelodysplastic syndromes, aplastic or megaloblastic anemia. The maturity of normal reticulocyte in patients with conditions such as decreased erythropoiesis in patients with chronic renal failure and iron deficiency anemia (Riley Hospital, (A) et al 2001). Reticulocytosis or increased number of reticulocytes in blood, normally found in cases of anemia with bone marrow function is still good (Suega K, 2010). High levels of IRF and the increase in RPI count, often due to acute hemorrhage (Riley Hospital, (A) et.al, 2001).

Limitation of Research

The examination of Ret-He, IRF and RPI concentrations are only done at the Endline (EL), until unable to compare the effect of treatment between baseline and endline.

CONCLUSIONS AND RECOMMENDATIONS

A. Conclusion

1. Concentration of Ret-He in the control group is higher mean than treatment

groups, especially in the age group > 14 years.
2. Concentration of IRF of the treatment group is higher and already closer to the standard compared to the control group.
3. Concentration of RPI in the control group is higher than the treatment group

B. Recommendation

To perfection on future studies it is necessary to examine the complete markers, such as Ret-He, IRF since Baseline, Midline and endline, or minimal Baseline with endline.

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