

# ***Iron Deficiency is The Main Cause of Anemia in Female Students of Senior High Schools in Sukoharjo Regency with No Polymorphism of Transferrin Receptor 1***

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**Abstract**—Iron deficiency anemia (IDA) in reproductive females remains a public health problem in Indonesia. Transferrin receptor 1 (TfR1) is one of important proteins, which are involved in regulation of the iron metabolism in the human body. Mutation of A210G TfR1 gene increases soluble TFR levels in some patients with type 2 diabetes. This study aimed to investigate the relationship between polymorphism of TfR1 gene and IDA in female students of senior high schools in Sukoharjo regency. This was an analytic observational study with the cross sectional approach. Research subjects were 470 female students who studied in year 10 and 11 of Public Senior High Schools in Sukoharjo Regency, Central Java. IDA diagnosis was determined using haemoglobin (Hb) levels, erythrocyte indexes (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) and plasma ferritin levels. Food recall 1x24 hours was used to determine daily nutrient intake. Polymorphism of TfR1 gene A210G was determined using DNA sequencing. All collected data were analyzed using chi-square and Pearson correlation tests with p value <0.05. Anemia was detected in 18.9% female students and 60% of them had iron deficiency. Intake of carbohydrate ( $r = -0.07$ ;  $p = 0.45$ ), protein ( $r = 0.01$ ;  $p = 0.93$ ) and iron ( $r = 0.04$ ;  $p = 0.32$ ) did not correlate with Hb levels whilst lipid intake had weak correlation with Hb levels ( $r = -0.19$ ;  $p = 0.03$ ). Inadequate food intake was commonly found in female students. A genetic variation of TfR1 gene was not found in female students with IDA. In conclusion, IDA is found in two third of anemic female students of senior high schools in Sukoharjo regency. There is no polymorphism of TfR1 gene in female students with IDA.

**Keywords:** *iron deficiency anemia; polymorphism; transferrin receptor 1; food intake*

## I. INTRODUCTION

Anemia is a global nutritional problem, which is predominantly caused by iron deficiency. More than 2 billion people ( $\pm 40\%$ ) in developed and developing countries have been reported to have anemia [1]. According to data of Indonesian Basic Health Research (Riset Kesehatan Dasar)

(2013), the prevalence of anemia in pregnant women is 37.1% and 22.7% in young females (13-18 years old). Although coverage of iron supplementation in the pregnant women is more than 90%, the prevalence of anemia remains high [2]. In contrast with pregnant women, this iron supplementation program in young females which provides 30 iron tablets for three months has not equally allocated in all regions in Indonesia, including Sukoharjo regency [3].

There are many factors that contribute in etiology of anemia and the prominent factor is lack of iron intake. In addition, genetic factor has clearly been involved in etiology of anemia in some susceptible people. In recent years, several studies have reported that polymorphism of some genes in iron metabolism are associated with IDA [4-6]. Polymorphism of TMPRSS-6 gene, for instance, which encodes matriptase-2 enzyme is responsible for unresponsiveness to oral iron supplementation in some anemic patients [7,8]. In iron metabolism, TfR1 will physiologically interact with iron-transferrin binding complexes in order to modulate hepcidin expression [4]. TfR1 polymorphism in anemic people has not been studied yet although this polymorphism is found in some diabetic patients. Fernández-Real and his colleagues have documented that polymorphism of TfR1 A210G increases soluble TfR1 levels in some patients with type 2 diabetes [9]. Therefore, the aim of this study was to investigate the relationship between polymorphism of TfR1 gene and IDA in young females of senior high schools in Sukoharjo regency.

## II. MATERIALS AND METHODS

The national prevalence of anemia in Indonesian young females was used to select research subjects in this cross-sectional study. They were eligible to follow this study with the inclusion criteria: aged 15-19 years old, were healthy and had BMI  $< 25 \text{ kg/m}^2$  [10,11]. A total of 470 female students in year 10 and 11 of public senior high schools in

Sukoharjo Regency, Central Java participated in this study. IDA diagnosis was determined using Hb level <12 g/dL, mean corpuscular volume (MCV) <80 fl, mean corpuscular haemoglobin (MCH) <30 pg/cell, mean corpuscular haemoglobin concentration (MCHC) <32 g/dL [12], and ferritin level <30 ug/L [13]. Nutrient intake was obtained from 1 x 24 hours food recall method and analyzed using nutrisurvey software. Calculated values of nutrient intake were compared with nutrient intake values of recommended dietary allowance (RDA) [14].

Blood samples were taken from median cubiti vein in lower arms of all subjects and collected into vacutainer tubes with EDTA anticoagulant (BD Biosciences, USA). Hb levels were then measured using the cyanmethemoglobin method whilst plasma ferritin levels were measured using human ferritin ELISA kit (DRG®, USA). Tfr1 gene exon 3 was isolated from peripheral blood nuclear cells using DNA isolation kit (Favorgen®, Taiwan) and amplified with PCR master mix (Promega®, USA). Primers were designed using NCBI primer design tools based on Tfr 1 gene template from GenAtlas. The forward primer was CGCAACACAGTTTGGTGGAG and TGGTCATTCTACCAATCAAGA was the reversed primer. The gene amplification was commenced by denaturation at 94°C for 5 min and followed by 35 PCR cycles: denaturation at 94°C for 20 sec, annealing at 56°C for 30 sec, and elongation at 72°C for 60 sec. Final extension was then carried out at 72°C for 60 sec. Electrophoresis of amplified PCR products used 2% agarose gel and it was visualized using ethidium bromide. PCR products were purified and sequenced with the same primers. All collected data were analyzed using Chi square and Pearson tests with p values <0.05.

**III. RESULTS**

TABLE 1. SUBJECT CHARACTERISTICS OF FEMALE STUDENTS OF SENIOR HIGH SCHOOLS IN SUKOHARJO REGENCY WHO PARTICIPATED IN THIS STUDY.

Characteristics	Non Anemia (n=381)			Anemia (n=89)		
	Min	Max	Mean± SD	Min	Max	Mean± SD
Age (y.o.)	15	18	16.89±0.70	16	19	16.87±0.76
BMI (kg/m <sup>2</sup> )	13.91	24.98	19.73±2.29	15.35	25	19.76±2.63
Menarche (y.o)	7	18	12.97±1.18	7	15	12.64±1.23
Menstruation (d)	3	14	6.30±1.50	4	14	6.41±1.38

Table 1 showed that subject characteristics of non anemia students were similar to that of anemia students in terms of age, BMI, menarche and menstruation. Of 89 (18.9%) female students had anemia. Female students in non-anemia group had average of menarche age (12.97±1.18 years old) slightly older than anemia group (12.64±1.23 years old). In

contrast to menarche age, a marginally shorter of menstrual duration was observed in non-anemia group compared with anemia group.

From 89 female students with anemia, IDA was found in 54 (60.6%) female students. According to Table 2, hematological parameters in IDA group were lower than hematological parameters in non IDA group. The mean of MCH and MCHC in IDA group was two points lower than that of in non IDA group. Whilst IDA group had around 5% lower average of MCV compared with non IDA group. Average of ferritin levels in IDA group was approximately one third of average of ferritin levels in non IDA group.

TABLE 2. RESULTS OF HEMATOLOGICAL TESTS IN 89 ANEMIC FEMALE STUDENTS WITH IDA OR NON IDA OF SENIOR HIGH SCHOOLS IN SUKOHARJO REGENCY. HB LEVELS WERE MEASURED USING THE CYANMETHEMOGLOBIN METHOD AND ERYTHROCYTE INDEXES USED A ROUTINE HEMATOLOGICAL METHOD WHICH WAS APPLIED IN CLINICAL PATHOLOGY LABORATORIES.

Characteristics	IDA (n=54)			Non IDA (n=35)		
	Min	Max	Mean± SD	Min	Max	Mean± SD
Hb (g/dL)	7.70	11.80	10.22±1.16	7.50	11.90	11.05±1.14
MCV (fL)	58.66	79.81	69.91±5.68	58.87	89.40	74.37±7.42
MCH (pg)	15.85	27.00	22.19±2.71	16.33	30.04	24.54±3.48
MCHC (g/dL)	27.03	31.58	30.67±1.11	27.75	33.6	32.90±1.91
Ferritin (ug/L)	13.66	29.33	20.52±3.83	34.16	181.83	66.87±33.50

TABLE 3. NUTRIENT INTAKE OF CARBOHYDRATE, PROTEIN, LIPID AND IRON IN FEMALE STUDENTS WITH OR WITHOUT ANEMIA OF SENIOR HIGH SCHOOLS IN SUKOHARJO REGENCY

Food intake	Anemia (n=45)		Non Anemia (n=69)		p** (CI 95%)	
	n	%	n	%		
Carbohydrate *	Inadequate	38	33.3	57	50.0	1.00
	Adequate	7	6.2	12	10.5	
Protein *	Inadequate	38	33.3	55	48.2	0.69
	Adequate	7	6.2	14	12.3	
Lipid*	Inadequate	39	34.2	60	52.6	1.00
	Adequate	6	5.3	9	7.9	
Iron*	Inadequate	45	39.4	69	60.6	n.d
	Adequate	0	0	0	0	

\* Inadequate <80% RDA and adequate ≥80% RDA; \*\*Chi square test

In this study, we evaluated intake of carbohydrate, protein, lipid and iron in all female students but there were only 114 female students who sent back the food recall questionnaire. As can be seen in Table 3, almost female students had inadequate intake of carbohydrate, protein, lipid and iron. Higher percentage of inadequate nutrient intake was observed in non anemia group compared with anemia group but this was not significant difference (p= 0.69 or higher). The same pattern was also found in adequate nutrient intake in non

anemia group. Surprisingly, all female students with or without anemia were lack of iron intake.

Table 4 indicated that all nutrient intakes had weak correlation with Hb levels in female students with anemia. Negative correlation was found in carbohydrate and lipid intake and Hb levels whereas protein and iron intake positively correlated with Hb levels. The highest correlation was observed in lipid intake ( $r=-0.19$ ) and it reached statistically significance ( $p= 0.03$ ).

**IV. DISCUSSION**

In this study we have documented that prevalence of anemia in female students in Sukoharjo regency is 18.94% and iron deficiency is the main cause of anemia. Iron intake in all female students is low and not sufficient enough to provide iron for Hb production. They also lack of macronutrient intake but non anemia female students have much lower this macronutrient intake. Polymorphism of A210G in exon 3 Tfr1 gene is not found in female students with IDA.

The prevalence of anemia in female students in Sukoharjo regency is lower than the prevalence of anemia in Indonesia and Central Java (approximately 22%) [2,12]. The lower prevalence of anemia in Sukoharjo is probably due to implementation of anemia program in female students (Jum'at Pintar program) in this regency. This program has been implemented since 2014 and provides iron tablets for all female students with and without anemia, as same as the national program of anemia. Iron tablets are distributed every Friday and the students should take it at their schools [15]. Although female students with anemia have obtained iron supplementation, some students still have low Hb levels and erythrocyte indexes. It indicates that their iron intake is inadequate and they should pay attention to consume iron rich foods in their daily life.

There are two possible reasons why the majority of female students have inadequate food intake especially iron intake. Firstly data of food intake is just analyzed from around 36% female students and it does not represent food intake of all research subjects. Collected data of food intake are also conducted 1x24 hours and it differs from the suggested method at least 2x24 hours in alternating days. Secondly, values of macro and micronutrients intake in RDA are too high for Indonesian people because another study has the same results with our study although this study involved people with different age and research location [16]. So we should consider to use another method for data analysis of food intake or to modify values of macro and micronutrients in RDA. Furthermore, these data cannot be used to generalize lack of food intake in female students of secondary schools in Sukoharjo regency.

Previous studies have reported that percentage of Tfr1 polymorphism (A210G) is equal or more than 50% [9,17,18]. Unfortunately, we have not found any mutation of Tfr1 gene in 54 female students with IDA. We used our own protocols and differed from previous studies in terms of primers, optimal temperature for PCR reaction and DNA template. Our oligonucleotide primers are shorter and we used lower temperature for annealing than previous studies, which probably lead to loss of target polymorphism.

**V. CONCLUSION**

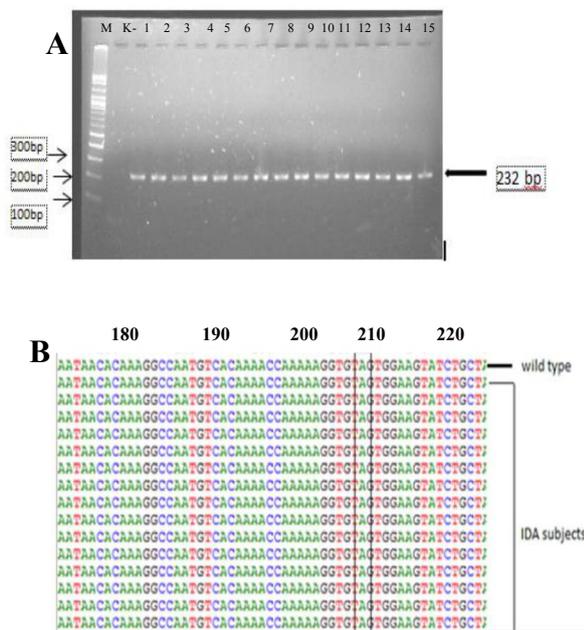
Iron deficiency anemia is a nutritional problem that is frequently found in female students of senior high schools in Sukoharjo regency. There is no polymorphism of exon 3 Tfr1 gene in female students with IDA. Nutrition education

**TABLE 4. CORRELATION BETWEEN NUTRIENT INTAKE AND HB LEVELS IN FEMALE STUDENTS OF SENIOR HIGH SCHOOLS IN SUKOHARJO REGENCY**

Nutrient intake	r	p <sup>*</sup>
Carbohydrate (g/day)	-0.07	0.45
Protein (g/day)	0.01	0.93
Lipid (g/day)	-0.19	0.03
Iron (mg/day)	0.04	0.32

*Pearson test*

PCR products of exon 3 Tfr1 gene were sequenced in order to identify whether or not polymorphism was present in this gene. Figure 1A and B represented the PCR-amplified exon 3 (232 bp) of Tfr1 gene and polymorphism localization at 210 bp downstream from open reading frame respectively. There was no polymorphism, which was detected in this study and all IDA gene sequences had the A nucleotide at 210 bp in length as same as wild type gene sequence.



**Figure 1. Visualization of PCR products and gene sequences of exon 3 Tfr1.** A. Electrophoresis of the amplified PCR products used 2% agarose gel with ethidium bromide staining. B. Sequence alignment of exon 3 Tfr1 gene between wild type and IDA used the Bioedit software version 7.0.5. M was marker, K was positive control and 1-7 lanes were PCR products of IDA students.

should be a part of “Jum’at pintar” program in order to reduce prevalence of anemia in female students.

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