Mal J Nutr 21(2): 155 - 166, 2015

Helminth Infection and Iron Status of Pregnant Women in Two Geo-Climatic Areas in Indonesia

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ABSTRACT

Introduction: Helminthiasis is known to contribute to iron deficiency (ID). The aim of this study was to identify the associations between helminth infection and iron status among pregnant women in two different geo-climatic areas in Indonesia. **Methods:** A cross-sectional study was conducted among pregnant women in two districts, stratified by wet-lowland (*n*=135) and wet-highland (*n*=120) areas. Data on general characteristics, helminth infection, and iron status were obtained. Helminth infection was determined using the Kato-Katz and Harada Mori technique. Iron status was measured using simple-sandwich ELISA techniques for plasma ferritin (PF) and transferrin receptor (TfR), while haemoglobin (Hb) values were determined by the haematology analyser, and the TfR/PF ratio for body iron stores (BIS) was calculated. **Results:** Prevalence of hookworm infestation was significantly higher (30% vs. 17.8%)while *T. trichiura* was significantly lower (0.8% vs. 11.9%) in wet-highland compared to wet-lowland areas. The overall iron status was relatively good and not significantly different between the two areas: 17.3% anaemia (Hb<110 g/L), 6.9% depleted iron store (PF<15 μg/L), 2.4% tissue ID (TfR>8.5 mg/L), 3.3% had BIS<0 mg/kg body weight, and 2.3% anaemia with ID. Prevalence of tissue ID and negative body iron store (BIS) was significantly higher among helminth infected women than in the non-infected women ($p<0.05$). **Conclusions:** Differences in helminthiasis profiles, but not in the iron status of the pregnant women, were found in the two geo-climatic areas studied. Prevalence of helminth infection especially hookworm was high, and significantly higher in wet-highland area. Hookworm infection was weakly correlated with TfR concentration and BIS. There is a need for parasitic control in the form of health education to be in place.

Key words: Geo-climatic areas, helminthiasis, Indonesia, iron status, pregnant women

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INTRODUCTION

Iron deficiency (ID) with or without anaemia has important consequences for health (WHO/CDC, 2005), especially during pregnancy (Scholl, 2011). ID among pregnant women may contribute to maternal morbidity and mortality, negative effects on the foetus and infant, and negative pregnancy outcomes (Pavord *et al.*, 2011). Pregnant women are highly vulnerable to ID since iron requirements are substantially high (Larocque *et al.*, 2005) due to rapid cell and tissue development involved in foetal growth (Burke, Leon & Suchdev, 2014). The causes of ID are multifactorial, including diet, infection and genetics (Brooker, Hotez & Bundy, 2008). Indonesian pregnant women are more vulnerable to ID, since dietary iron intake among the population is low i.e. below 2/3 of Recommended Dietary Allowance (RDA) due to low intake of animal source foods and rice as their main staple contains little iron and is rich in phytate which inhibits iron absorption (Kurniawan *et al.*, 2006). Among individuals with inadequate iron intake, blood loss from helminth especially hookworm can result in ID and iron deficiency anaemia (IDA) (Smith & Brooker, 2010).

The tropical climate of Indonesia is highly favourable for the persistence of helminth infection associated with ID and IDA such as hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* (Margono, 2003). Helminth infection especially hookworm infection may contribute to ID and IDA by causing blood loss through ingestion and mechanical damage of the intestinal mucosa, and by affecting nutrient absorption for erythropoiesis (Larocque *et al.*, 2005; Nguyen *et al.*, 2015). Hookworm infection in pregnancy has been negatively correlated with haemoglobin (Hb) concentration (Larocque *et al.*, 2005) and serum ferritin (Nurdiati *et al.*, 2001). Pregnant women who are infected by helminth, either hookworm, *T. trichiura* or *A. lumbricoides* have higher risk (relative risk: 1.56 (95% CI 1.43-1.69)) of suffering from IDA compared to pregnant women who are not infected (Rodriguez-Morales *et al.*, 2006).

An earlier study among the rural population in Indonesia showed that prevalence of hookworm is significantly higher in wet-highland compared to wet-lowland, dry-highland and dry-lowland areas (44.5%, 28.5%, 14.6% and 12.8%, respectively) (Widjana & Sutisna, 2000). One area in Indonesia which has wet-highland as well as wet-lowland topography and high prevalence of helminth infection is Central Java Province (BAPPEDA JATENG, 2008).

ID is defined as a spectrum ranging from iron depletion to IDA (Pavord *et al.*, 2011). However, prevalence of ID, commonly calculated from surveys of anaemia prevalence, is measured using Hb indicator (Lynch, 2012). Hb concentration alone cannot be used to diagnose ID (Lynch, 2012; Salvi,Braga & Filho, 2014). A limitation of Hb measurement is that Hb level will decrease only when ID is already severe (UNICEF/UNU/WHO, 2001). Until now, there have been few publications on helminth infection in wet-lowland and wet-highland areas and on the iron status among pregnant women in Indonesia using iron status indicator and Hb concentration. This study aimed to assess the iron status and prevalence of helminth infection of Indonesian pregnant women 12-20 weeks gestational age, living in wet-highland and wet-lowland areas of Central Java Province, and to identify the association between helminth infection and their iron status.

METHODS

This study was part of a food intervention study on the effect of fermented soyabean and vitamin C rich fruit on iron status of pregnant women (registered as an International Standard Randomised

Controlled Trial No. ISRCTN13994081). Detailed methodology and results related to this study have been published (Wijaya-Erhardt, Muslimatun & Erhardt, 2011; Wijaya-Erhardt, Muslimatun & Erhardt, 2014). For the purpose of the present analysis, only baseline data of the subjects i.e., general characteristics, anthropometry measurement, blood and faecal assessments are shown.

Study design and subjects recruitment

A cross-sectional study was conducted from November 2007 to March 2008 among pregnant women in 12-20 weeks of gestation at Karanganyar and Demak Districts, Central Java Province, Indonesia.

The sample size calculation was based on estimating population proportion (Lwanga & Lemeshow, 1991). At a confidence level of 95%, the prevalence of helminth infection in Karanganyar was 43% (Sakti *et al.*, 1999), absolute precision 10%, design effect of 2 and allocation for any incomplete data 10%, a total of 209 pregnant women were needed for this study. However, we analysed all eligible subjects (n = 255) participating in the intervention study. There were two groups of subjects; stratified by wet-lowland (n=135) and wet-highland (n=120) areas. Requirements for eligibility included 15-49 years old, 12-20 weeks of gestation, apparently healthy, and resident of study area. The gestational age was calculated from the first day of the last menstrual period and confirmed by fundal height palpation by midwives; physical examination was performed by a medical doctor from a primary health centre.

Data collection

Interviews and observation were carried out by trained enumerators using structured questionnaire to describe the general characteristics of subjects, housing condition, household belongings, as well as knowledge and practice of helminth infection, anaemia and nutrition.

Anthropometric measurements of weight and height were assessed to obtain nutritional status of the subjects. Body weight was measured to the nearest 0.1 kg by using weighing scale SECA 890 (Hamburg, Germany) and body height was measured by using the roll-up measuring tape "*microtoise*" to the nearest 0.1 cm. The anthropometric indices were expressed as body mass index (BMI).

Biochemical analysis

A sample of 3 ml of venous blood was taken from each subject; 2.5 mL was drawn into Li-Heparin tubes (Greiner, Kremsmuenster, Austria) for the analysis of plasma ferritin (PF), transferrin receptor (TfR), C-reactive protein (CRP) and α-1-acid glycoprotein (AGP). The remaining 0.5 mL blood was drawn into Na-EDTA tubes for Hb analysis. Blood samples were kept in a cool box during transportation to a local laboratory. Within 6 hours of blood sampling, plasma was separated by centrifugation (MLW T51.1, Germany) at room temperature for 10 min at 2750 rpm and drawn into Eppendorf Safe Lock vials that were frozen at -7°C for ± 1 month and subsequently at -70°C until further analysis.

Hb concentration was measured using haematology analyser (Nihon Kohden MEK-6318 K, Tokyo, Japan or Coulter HmX) at the local laboratories. Hb concentration <110 g/L was defined as anaemia, and was adjusted + 2 g/L and + 5 g/L to account for altitude ≥1000-1499 m and ≥1500 m , respectively (UNICEF/UNU/WHO, 2001). Hb concentration 100-109 g/L, 70-99.9 g/L, and below 70 g/L was defined as mild, moderate, and severe anaemia (Singh, Khan & Mittal, 2013). Seven blood samples could not be analysed for Hb concentration due to coagulation during transportation.

For analyses of PF, TfR, CRP, and AGP; frozen plasma samples were transported in dry ice to DBS-Tech, Legelshurst, Germany.

The plasma was analysed with a simple-sandwich enzyme-linked immunosorbent assay (ELISA) technique (Erhardt *et al.*, 2004). PF <15 μg/L was considered as iron store depletion (WHO/CDC, 2005) and PF <30 μg/L was considered as early iron depletion (Pavord *et al.*, 2011). TfR >8.5 mg/L was considered as tissue ID. Body iron store (BIS) was calculated from the TfR/PF ratio as follows: body iron (mg/kg) = – [log(TfR/PF ratio) – 2.8229] / 0.1207. Positive value indicates the amount of iron in stores, negative value indicates the deficit in tissue iron (Cook, Flowers & Skikne, 2003). Subjects were categorised as IDA when the Hb <110 g/L in the presence of ID. The presence of ID was indicated by depleted iron stores (PF <15 μg/L) (WHO/CDC, 2005; UNICEF/UNU/WHO, 2001) or PF<30 μg/L (Pavord *et al.*, 2011), or tissue ID (TfR >8.5 mg/L), or negative body iron stores (BIS <0 mg/kg body weight) (Cook *et al.*, 2003). CRP and AGP concentrations were used to identify the presence of acute and chronic inflammation and to control high PF concentration in the presence of infection (UNICEF/UNU/WHO, 2001). Cut-off used to determine inflammation for CRP concentration was >10 mg/L, and for AGP >1 g/L. For PF and body iron store analyses, 9 subjects who had high CRP and/or AGP concentrations were excluded.

For analyses of helminth infection, fresh stool samples were collected in provided containers and analysed within 24 h by using Kato-Katz technique to identify the presence of *A. lumbricoides,* hookworm and *T. trichiura* eggs. The presence of hookworm infection was also confirmed through Harada-Mori culture technique (Shahid *et al.*, 2010). Stool samples were analysed in the parasitological laboratory of the Faculty of Medicine of Sebelas Maret University, Solo (for subjects from Karanganyar District) and the parasitological laboratory of Faculty of Medicine of Diponegoro University, Semarang (for those from Demak District).

Statistical analysis

Descriptive data analysis was carried out to characterise iron status and related indicators. N-Par Kolmogorov-Smirnov normality test was used to check the normality of data distribution. Normally distributed data were presented as mean ± SD, while non-normally distributed data were transformed and presented as geometric mean ± SD, or median and interquartile range.

Independent *t*-test was used to examine differences in means for normally distributed data, while Mann-Whitney *U* test was used to examine differences in means for non-normally distributed data. Analysis of covariate was used to examine the differences between Hb concentration in two areas adjusted for altitude. Chi square or Fisher Exact test was used to examine differences in proportion. Spearmann rank's correlation test was used to examine correlation between helminth infection and iron status indicators. Differences were considered as significant at P <0.05 (two-tailed). All statistical analyses were performed by using SPSS software for Windows version 16.

Ethical consideration

The study was approved by the Ethical Committee of the Faculty of Medicine, University of Indonesia. Permission was solicited from local government and local health offices, and each participating woman gave written consent before data collection was started. Participation was voluntary.

RESULTS

Subject characteristics

All pregnant women (n = 255) were from one ethnic group (Javanese) and 96.5% were Moslem. Most of them (82.4%) were in the age range of 20-35 years, 11.7% were below 20 years, and 5.9% were more than 35 years. Gestational age ranged from 11.1 to 20.6 weeks, and 47.1% were in the first

Table 1. General characteristics, helminth infection and inflammation status of study participants

	Wet-lowland *n* = 135	*Wet-highland* *n* = 120	*All subjects* *n* = 255
	Mean ± SD§	*Mean ± SD*§	*Mean ± SD*§
Age (years)	26.3 ± 5.4	26.5 ± 5.1	26.4 ± 5.3
BMI (kg/m²)	21.5 ± 3.2	21.6 ± 3.1	21.5 ± 3.2
Age of gestation (weeks)	15 (13, 16)¶	16 (13, 19)** ¶	14 (13, 15) ¶
Education (years)	6 (6, 9)¶	9 (6, 9)* ¶	9 (6, 9) ¶
Number of pregnancy	2 (1, 3)¶	2 (1, 3) ¶	2 (1, 2) ¶
Number of iron tablet consumed	93.3ʃ	81.7ʃ	87.8ʃ
Helminth infection	28.9ʃ	48.7ʃ	29.8ʃ
Hookworm†	17.8*ʃ	30.0ʃ	23.5ʃ
Hookworm‡	8.9ʃ	10.0ʃ	9.4ʃ
T. trichiura	11.9**ʃ	0.8ʃ	6.7ʃ
A. lumbricoides	2.2ʃ	0.0ʃ	1.2ʃ
CRP (mg/L)	1.61 (0.66, 3.20) ¶	1.80 (0.73, 3.79) ¶	1.67 (0.73, 3.54) ¶
CRP >10 mg/L	2.2ʃ	5.0ʃ	3.5ʃ
AGP (g/L)	0.53 ± 0.13	0.61 ± 0.13	0.57 ± 0.14
AGP>1g/L	0.0ʃ	1.7ʃ	0.8ʃ

§ or otherwise indicated
¶ median (P25th, P75th)
ʃ %
† Analysed using Harada-Mori technique.
‡ Analysed using Kato-Katz technique.
*P<0.05, **P<0.01 for value difference between geo-climatic areas, calculated using Mann Whitney U-test for non-normally distributed variables and t-test for normally distributed variables, or calculated using Fisher Exact test for proportion difference between two areas.

trimester while 33.7% were primiparous. In terms of miscarriage, stillbirth, and child died before 5 years of age, the figures were 10.6%, 0.8%, and 5.1%, respectively. Among those who had a child previously (*n* = 159), 2.5% had less than 2 years of birth spacing. About 13% of subjects were classified as underweight (BMI <18.5 kg/m²), and 11.1% were still underweight in the second trimester. More than 80% of the women consumed iron tablet. Only 3.5% of subjects had high CRP (>10 mg/L) and 0.8% had high AGP (>1 g/L) (Table 1).

Helminth infection

About 30% of pregnant women had helminth infections, with 28.2% having a single infection while 1.6% were infected by two species. Among those who had a single infection, 22.8% were infected by hookworm, 5.1% by *T. trichiura* and 0.4% by *A. lumbricoides*. Among those who were infected by two species, 0.8% were infected by hookworm and *T. trichiura*, and 0.8% were infected by hookworm and *A. lumbricoides*. The median egg per gram of hookworm, *T. trichiura* and *A. lumbricoides* were 51 (4-2888) epg, 15 (5-835) epg and 55 (10-245) epg, respectively. Infection was mainly at a light intensity. Only 0.4% of women were infected at medium intensity of hookworm (2888 epg, data not shown). There was no significant difference in intensity of helminth infection between wet-highland and wet-lowland areas. Prevalence of hookworm infection was significantly higher in wet-highland than that in wet-lowland areas (30% vs. 17.8%,

Table 2. Iron status according to types of geo-climatic areas

	Wet-lowland		Wet-highland		Total	
	n	*Mean ± SD*	*n*	*Mean ± SD*	*n*	*Mean ± SD*
Hb (g/L)	129	115.9 ± 15.3	119	128.7 ± 11.9	248	121.9 ± 15.1
12-14.9 wks	50	119.7 ± 19.0	67	129.1 ± 10.7	117	125.2 ± 15.6
15-20 wks	79	113.5 ± 11.9	52	127.6 ± 13.3	131	119.2 ± 14.3*
Hb< 110 g/L, n (%)	129	42 (32.6)	119	1 (0.8)	248	43 (17.3)
100-109.9 g/L, n (%)		25 (19.4)		1 (0.8)		26 (10.5)
70-99.9 g/L, n (%)		17 (13.2)		0 (0.0)		17 (6.8)
PF (µg/L)‡	132	40.94 ± 1.96	114	37.40 ± 1.93	246	39.26 ± 1.94
PF < 15 µg/L, n (%)		7 (5.3)		10 (8.8)		17 (6.9)
PF < 30 µg/L, n (%)		45 (34.1)		40 (35.1)		85 (34.6)
TfR (mg/L)‡	135	5.19 ± 1.25	120	5.23± 1.25	255	5.21± 1.25
TfR> 8.5 mg/L, n (%)		5 (3.7)		1 (0.8)		6 (2.4)
BIS (mg/kg body wt)	132	5.97 ± 2.81	114	5.73 ± 2.87	246	5.80 ± 2.85
< 0 mg/kg body wt, n (%)		4 (3.0)		4 (3.5)		8 (3.3)
Anaemia with ID						
Hb< 110 g/L and PF < 15 µg/L, n (%)	111	5 (4.2)	100	0 (0.0)	211	5 (2.3)
Hb< 110 g/L and PF < 30 µg/L, n (%)	111	14 (12.6)	100	0 (0.0)	211	14 (6.6)
Hb< 110 g/L and TfR >8.5 mg/L, n (%)	129	4 (3.1)	119	0 (0.0)	248	4 (1.6)
Hb< 110 g/L and BIS < 0 mg/kg body wt, n (%)	111	1 (0.9)	100	0 (0.0)	211	1(0.4)

Mean ± SD, unless otherwise indicated
‡ Geometric mean ± SD
*P < 0.05, adjusted difference for altitude (analysis of covariance)

respectively). Prevalence of *T. trichiura* infection was significantly higher in wet-lowland area (11.9% vs. 0.8%, respectively) (Table 1).

Iron status

Hb concentration was 12.8 g/L higher (*P* <0.001) in wet-highland area than in wet-lowland area (128.7±11.9 g/L vs. 115.9±15.3 g/L). Only 17.3% of pregnant women were anaemic, i.e.10.5% had mild anaemia (Hb 100-109.9 g/L) and 6.9% moderate anaemia (Hb 70-99.9 g/L). Only 6.9% of pregnant women had depleted iron store (PF <15 µg/L), 34.6% had early depleted iron stores (PF <30 µg/L), 2.4% had tissue ID (TfR >8.5 mg/L) and 3.3% had negative body iron store (BIS <0 mg/kg body wt). There were no significant differences in PF, TfR and body iron stores between the two areas (Table 2). Among those who were anaemic (Hb <110 g/L, *n* = 43), 32.6% had depleted iron store (PF <30 µg/L), 9.3% had tissue ID (TfR >8.5 mg/L) and 2.3% had negative BIS (<0 mg/kg body wt). About 67.4% of anaemic women had good iron status. Finally, only 2.3% of all subjects were anaemic with ID, while 6.6% were anaemic with early depleted iron stores.

Association between helminth infection and iron status

Mean of Hb, PF, TfR, and body iron concentrations were within the normal range in both helminth-infected andun-infected women. Hb concentration was significantly lower among those infected by *T. trichiura*. Helminth-infected women had higher geometric mean of TfR concentration than the un-infected women (5.5±2.1 mg/L vs. 5.3±1.1 mg/L, P =0.004). Prevalence of tissue ID (TfR >8.5 mg/L) was significantly higher among helminth infected women (5.3% vs. 1.1%, respectively, P =0.004). Negative body iron (<0 mg/kg body wt) was also significantly higher among helminth infected women (5.5% vs. 2.3%, respectively, P =0.030). There was no significant difference in Hb concentration, PF, anaemia prevalence, and depleted iron store prevalence between helminth-infected and un-infected women as well as between hookworm-infected and hookworm un-infected women (Table 3). The data was not comparable for *A. lumbricoides* infection.

Concentrations of CRP (2.76±3.72 mg/L vs. 2.81±3.29 mg/L) and AGP (0.56±0.13 g/L vs. 0.57±0.14 g/L) were similar among helminth-infected and un-infected women. The prevalence of inflammation based on CRP and AGP indicators was low and similar in both wet-lowland and wet-highland areas (data not shown).

DISCUSSION

The present study showed that iron status among Indonesian pregnant women at 12-20 weeks of gestation was relatively good. Prevalence of ID and IDA was low and similar between those who lived in wet-highland and wet-lowland areas. Prevalence of helminth infection especially hookworm was high, and significantly higher in wet-highland area. Hookworm infection was weakly correlated with TfR concentration and BIS .

Only 17.3% of pregnant women in our study were categorised as anaemic, indicating a mild public health problem (5.0-19.9%) (UNICEF/UNU/WHO, 2001). Low prevalence of anaemia in our study population may have been associated with gestational age of the subjects. A previous study among pregnant women in Nepal showed that prevalence of anaemia in early stage of pregnancy was low but increased in the 2nd and 3rd trimesters (Singh *et al.*, 2013). Haemodilution during pregnancy caused Hb concentration to decline throughout the 1st and 2nd trimesters, and reach the lowest point in the 2nd or early 3rd trimester (Scholl, 2011).

ID was not prevalent in our study area. Only 6.9% of pregnant women in our study suffered from depleted iron stores (PF <15μg/L) and only 2.4% had tissue ID (TfR >8.5 mg/L). However, 34.6% were in the early stage of depleted iron stores (PF <30μg/L). When PF <12 μg/L was used as cut-off point, the prevalence of depleted iron store in our study was lower than those in a previous study at Purworejo District (6.1% vs. 16.7%, respectively) (Nurdiati *et al.*, 2001). There are several explanations for this difference. Firstly, higher prevalence and more severe helminthiasis were found in Purworejo District, i.e. hookworm 23.8%, *T. trichiura* 49.3%, *A. lumbricoides* 18.8% with the intensity of 344.0±629.8 epg, 297.0±626.2 epg, 5385.8±11339.7 epg, respectively (Nurdiati *et al.*, 2001). Secondly, fewer pregnant women consumed iron tablet in Purworejo (Nurdiati *et al.*, 2001) than women in this study (55.7% vs. 87.8%, respectively).

About two-thirds of anaemic women in this study were not ID. The results found in this study were in line with that found in previous studies, in which anaemia in developing countries is multifactorial (Lynch, 2012; Salvi *et al.*, 2014). It is possible that deficiencies in other key micronutrients such as vitamin A, vitamin B12 or folate in the aetiology of anaemia occurred among the study population (Nguyen *et al.*, 2015). To assess iron status or ID prevalence among Indonesian population, we should consider the use of PF and TfR indicators instead of Hb measurement only (WHO/CDC, 2005; UNICEF/UNU/WHO, 2001). The use of Hb measurement alone might not

Table 3. Iron status according to the helminth infection

Iron status	*Helminth Infection*		*Hookworm Infection*		*T. trichiura Infection*	
	Infected	*Not Infected*	*Infected*	*Not Infected*	*Infected*	*Not Infected*
Hb (g/L)	120.8 ± 14.4	122.5 ± 15.5	122.7 ± 14.3	121.8 ± 15.4	113.2 ± 11.4*	122.6 ± 15.2
Hb< 110 g/L†	12 (16.2)	31 (17.8)	8 (13.6)	35 (18.5)	4 (25.0)	39 (16.8)
PF (μg/L) ‡	47.2 ± 32.1	48.6 ± 30.9	44.2 ± 31.2	49.4 ± 31.2	60.3 ± 37.0	47.3 ± 30.6
PF < 15 μg/L, n (%)	5 (6.8)	12 (6.9)	4 (7.0)	13 (6.9)	1 (5.9)	16 (7.0)
PF < 30 μg/L, n (%)	28 (38.4)	57 (32.9)	25 (43.9)	60 (31.7)	4 (23.5)	81 (35.4)
TfR (mg/L) ‡	5.5 ± 2.1	5.3 ± 1.1*	5.6 ± 2.3	5.3 ± 1.1*	5.2 ± 1.2	5.4 ± 1.5
TfR> 8.5 mg/L, n (%)	4 (5.3)	2 (1.1)*	3 (5.0)	3 (1.5)	1 (5.9)	5 (2.1)
BIS (mg/kg body wt)	5.6 ± 3.0	5.9 ± 2.8	5.3 ± 2.9	6.0 ± 2.8*	6.7 ± 3.1	5.7 ± 2.8
BIS< 0 mg/kg body wt, n (%)	4 (5.5)	4 (2.3)*	3 (5.3)	5 (2.6)	1 (5.9)	7 (3.1)

Mean ± SD, unless otherwise indicated
‡ Geometric mean ± SD
*P < 0.05, calculated using independent *t*-test or chi square test for differences between two groups.

directly identify ID as it may overestimate the prevalence of ID of a population.

Low prevalence of ID in this study might also be caused by good iron absorption mediated by co-factors obtained from rice-based meals combined with meat and/or fish, and fruit and/or vegetables containing ascorbic acid (Hallberg, 1985), and fermented food (FAO/WHO, 2001). The other possibility of the low ID prevalence in this study might be due to the use of too low PF threshold, i.e. 15 μg/L (WHO/CDC, 2005). A previous study showed that a threshold of PF at 30 μg/L was more appropriate for the determination of the ID status by bone marrow staining than the other thresholds (van den Broek *et al.*, 1999). Further, low ID might be caused by unusual sources of iron that were not assessed in this study, like iron cooking vessels, soil or dust contamination (FAO/WHO, 2001), or high iron content in ground water (Merril *et al.*, 2012). Iron contamination is more frequent in developing countries where the amount of such contamination in the meal may be several times greater than the amount of food iron (DeMaeyer *et al.*, 1989). The absorption of iron contaminant is influenced by the same factors as the native to the food substance (Hallberg, 1985). Iron contaminants in food are sometimes found in large amounts, thus it is considered nutritionally important, especially if consumed together with the enhancing absorption factors (Hallberg, 1985; FAO/WHO, 2001).

Our study areas were categorised as hookworm endemic areas as assessed by Harada Mori technique (prevalence >20%) with light intensity (<2,000 epg) (Brooker *et al.*, 2008;WHO, 2011). A higher prevalence of hookworm was found in wet-highland, similar to an earlier study in Bali, Indonesia (Widjana & Sutisna, 2000). Prevalence of *T. trichiura* and *A. Lumbricoides* infection was also low (<10%). High prevalence of hookworm and low prevalence of other soil transmitted helminthes in this study fitted the common profile that hookworm infection is more common among adults (Hotez *et al.*, 2005).

This study also showed that pregnant women infected by one or more species of helminthes had higher prevalence of tissue ID (TfR >8.5mg/L) and negative body iron store (BIS <0 mg/kg body weight). Although the prevalence of hookworm infection was high, the intensity was light, which might explain why anaemia was not prevalent in this population.

This result is in line with previous research which showed that the association between hookworm infection and Hb concentration is significant only when the intensity is moderate or heavy (Larocque *et al.*, 2005; Brooker *et al.*, 2008). Correlation between helminth infestation and anaemia often exists among populations with low iron stores (Hotez *et al.*, 2005). If hookworm infection is endemic and anaemia is very prevalent, hookworm is likely to be an important cause of anaemia (UNICEF/UNU/WHO, 2001).

The strength of this study was the use of more than one indicator to detemine iron status: Hb, PF, TfR, BIS, CRP and AGP. The analysis of TfR, PF, BIS, and CRP or AGP offers the best indicator for iron status assessment (WHO/CDC, 2005; UNICEF/UNU/WHO, 2001). PF is the best indicator to identify depleted iron store. However, the concentration of PF would increase during inflammation although there is ID (WHO/CDC, 2005). TfR is a sensitive indicator of ID in pregnancy because it is able to detect onset of mild ID, and its concentration is not influenced by inflammation or hormonal alteration during pregnancy (Pavord *et al.*, 2011; Burke, 2014; Lynch, 2012). Therefore measurements of CRP and AGP are needed to control for inflammation in the data analysis (Thurnham *et al.*, 2010).

In summary, the health status of pregnant women in the study areas was relatively good as indicated by their iron status. The prevalence of helminth infection was high, but the intensity was light; and therefore, it was difficult to find an association between helminth infection and iron status. Since helminth infection, especially hookworm infestation of light intensity was present in this study population, there is no need for antihelmintic adminstration (Kumar, 2009). However, parasitic control in the form of health education needs to be in place. Furthermore, iron intake (both from food sources and supplements) is needed to maintain iron status throughout the pregnancy period.

ACKNOWLEDGEMENTS

The study was supported by grants from Nestlé Foundation, Switzerland with additional support from SEAMEO-RECFON, University of Indonesia. There was no conflict of interest.

WR developed the project, carried out the fieldwork, undertook analysis and wrote the manuscript. SM and MW-E managed the project, supervised the work and reviewed the manuscript. JGE developed laboratory methods, undertook blood assessment and contributed to the manuscript. AK developed laboratory methods in parasitology and reviewed the manuscript.

We acknowledge the valuable advice and contribution in stool analysis provided by dr. Bambang Sukilarso Sakiman, MS and his staff at Department of Parasitology, University of Sebelas Maret, Solo. We thank all pregnant women who participated in this study. We also thank the field team members (Yuridayati, Keszia Tuni Sulistyo Utami, Wawin Mysterianingtyas) the local field staff (Devi, Purwanti, Darwanto, Setyo, Dana, Susi, Eko, Wahyu); the staff of the Karanganyar District General Hospital; Clinical Pathology of Diponegoro University, Semarang; Puskesmas: Ngargoyoso, Jenawi, Tawangmangu, Bonang 1, Karangawen 2, and Demak 2, for their contribution to data collection.

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