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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Research Article
Hemoglobin and Serum Transferrin Receptor Differences in Pregnant Women in Rural and Urban Areas of Central Java Province, Indonesia

Mohammad Zen Rahfiludin, Dina Rahayuning Pangestuti and Yudhy Dharmawan

Faculty of Public Health, Diponegoro University, Jl. Prof Sudharto SH, Kampus Undip Tembalang, Semarang 50275, Indonesia

Abstract

Background and Objective: Pregnant women in rural and urban areas have different nutritional intakes and associated problems. The present study compared hemoglobin and serum transferrin receptor levels, as well as influencing factors, between pregnant women in rural and urban areas in Central Java, Indonesia. **Materials and Methods:** Blood samples were taken from 80 pregnant women in urban and 69 pregnant women in rural areas of Central Java, Indonesia. The cyanmethemoglobin method was used to measure hemoglobin levels and enzyme-linked immunosorbent assay was used to measure serum transferrin receptor. Nutrient intake data was obtained using 24 h recall on 2 inconsecutive days. Food intake data with Recommended Dietary Allowance percentages were measured using cutoff points based on Indonesian nutritional adequacy. Iron-deficiency anemia status was categorized into four groups: iron-deficiency anemia, non-iron-deficiency anemia, non-anemia iron deficiency and non-anemia/non-iron deficiency. **Results:** Pregnant women in rural areas had lower income and education levels than pregnant women in urban areas. Intake of iron, vitamin B12 and folic acid were lower in rural areas, while vitamin C and dietary fiber intake were lower in urban areas. While hemoglobin levels did not differ significantly based on area of residence, serum transferrin receptor levels did. **Conclusion:** Iron-deficiency anemia in pregnant women was higher in rural areas. Therefore, pregnant women are recommended to increase intake of high iron foods, particularly heme-iron and maintain a balanced diet.

Key words: Anemia, iron deficiency, pregnant women, rural and urban areas, serum transferrin receptor

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Corresponding Author: Mohammad Zen Rahfiludin, Faculty of Public Health, Diponegoro University, Jl. Prof Sudharto SH, Kampus Undip Tembalang, Semarang 50275, Indonesia Tel: +08122889745

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nutrition of pregnant women is a concern since has a substantial influence on fetal development. In Indonesia, the main nutritional problems faced by pregnant women are inadequate protein and energy intake and anemia, which are known to increase risk of maternal and perinatal mortality and low birth weight[1]. Anemia in pregnant women also causes bleeding at the time of delivery[2]. Iron-deficiency anemia (IDA) in pregnant women largely results from low iron stores at the time of conception and/or the amount of iron absorbed during gestation, both of which are frequently insufficient for the increasing requirements of the fetus[3]. The prevalence of anemia in pregnant women is usually highest during the third trimester as the average total iron content in the body decreases with increased gestational age[4].

IDA remains the most common nutritional deficiency during pregnancy, particularly in developing countries. The occurrence of anemia during pregnancy is about 40-80% in tropical countries and 10-20% in developing countries[5]. Studies of pregnant women in Zimbabwe, China, India and Mexico between 1996 and 2008 indicated that between 43% and 73% of women were iron deficient and between 7% and 33% of women had IDA[3]. According to the 2013 Indonesian Health Profile, anemia occured in 37.1% of pregnant women in Indonesia, with 36.4% in urban areas and 37.8% in rural areas[6]. In Indonesia, pregnant women should have consumed the minimum of 90 tablets of iron supplements during pregnancy, however data in 2016 indicated that only 40.2% of them meet this requirements, which was lower than the national target in 2016 (86%)[7].

Indonesia is an archipelago with developmental inequalities. For example, Java island is more developed than other islands; however, its health facilities are different in rural and urban areas. Importantly, residential location, rural or urban, can significantly affect several aspects of daily life, especially health. Several studies have shown that mortality, morbidity, malnutrition and infant mortality are more common in rural areas[8-10]. Likewise, research in Bangladesh has shown the nutritional status of women in urban areas was better than that of rural areas[11].

While many studies have analyzed anemia by measuring hemoglobin (Hb) levels in pregnant women, comparison of iron status in rural and urban areas according to serum transferrin receptor (STfR) levels is uncommon. STfR is more commonly used to measure the erythropoiesis process and differentiation of bone marrow. Transferrin receptor levels have been shown to increase in IDA and disorders associated with erythroid precursor levels, such as congenital dyserythropoietic anemia, hemolytic anemia, major thalassemia, myelodysplastic syndrome and recombinant erythropoietin therapy[12]. In general, diagnosis of iron deficiency involves conventional laboratory tests to measure iron status, such as serum ferritin and transferrin saturation. However, serum ferritin and transferrin proteins are affected by inflammation, making it difficult to distinguish between chronic disease anemia and IDA (the type that occurs when Fe deficiency is severe enough to reduce erythropoiesis)[13]. Furthermore, STfR has been suggested to adequately measure early tissue iron deficiency.

Indonesian Health Profile Data in 2015 showed the Maternal Mortality Rate was 128.05 per 100,000 births in Semarang City and 93 per 100,000 births in Temanggung Regency, both of which were higher than that of Sustainable Development Goal 3 (70 per 100,000 births). Prevalence of anemia in pregnancy were also high in both locations (Semarang City, 18.34%; Temanggung Regency, 9.51%)[14,15]. The purpose of this study was to compare both Hb and STfR levels between pregnant women living in rural and urban areas of Central Java province, Indonesia. In addition, factors influencing iron status between the groups was also assessed. The difference of sTfR between pregnant women in rural and urban areas was never studied before in Indonesia, hence it is the novelty of our study. The result of this study can be a recommendation for prevention strategy for pregnant women, particularly in rural areas.

MATERIALS AND METHODS

Subjects and data collection: The present study used a cross-sectional design to assess iron status in pregnant women living in Semarang City (urban area) and Temanggung Regency (rural area) of Central Java province, Indonesia. A total of 149 pregnant women in their third trimester were included; 80 resided in urban and 69 in rural areas. Data of pregnant women in Temanggung Regency was obtained from a local Community Health Center in Bulu District, while data of pregnant women in Semarang City was obtained from Genuk and Kedungmundu Community Health Centers. Two Health Centers in Semarang City were necessary to increase the number of willing urban participants (both were of similar condition). Ethical clearance (No. 252/EC/FKM/2016) was obtained from the Commission of Ethics of Medical and Public Health Research of the Faculty of Public Health, Diponegoro University (Semarang, Indonesia) to conduct this study and all subjects provided written informed consent prior to inclusion.

Subjects were interviewed face-to-face by the researchers in private. Interviews in rural areas were conducted in the

traditional Javanese, while those in urban areas were either in Indonesian or Javanese. Data collected during interviews included monthly family income and level of education. Level of education was classified into two groups: primary (primary and junior high schools, 9 years total) and secondary or higher (3 years of senior high school and/or up to 4 years of college). Nutritional intake data were obtained using a 24 h recall method for 2 inconsecutive days and was measured by 6 enumerators who were Bachelor's of Nutrition graduates of 2017 with 2 weeks of training previously. At this time, they have been working as health officer in governmental or non-governmental institution. Food intake was recorded by an enumerator in the form of household portions (e.g., tablespoons, teaspoons, cups, etc.), then converted to gram units and nutritional intake was analyzed using Nutrisurvey software.

Venous blood (about 5 mL) was taken once in the morning between 8 and 10 am. Hb levels were measured using cyanomethemoglobin, while STfR was measured using a Quantikine IV D human STfR Immunoassay ® and D Systems, Minneapolis, MN, USA) with an enzyme-linked immunosorbent assay Reader 680 by quantitative sandwich technique. The blood analysis was held in Prodia Laboratory (Gorontalo, Indonesia). An Hb level below 11.0 g dL^{-1} was classified as anemia[16]. An STfR greater than or equal to 21.0 nmol L^{-1} was classified as iron deficiency[17]. IDA status was categorized into four groups: IDA (Hb <11.0 g dL^{-1} and STfR >21.0 nmol L^{-1}), non-iron-deficiency anemia (Hb <11.0 g dL^{-1} and STfR <21.0 nmol L^{-1}), non-anemia iron deficiency (Hb ≥11.0 g dL^{-1} and STfR ≥21.0 nmol L^{-1}) and non-anemia/non-iron deficiency (Hb ≥11.0 g dL^{-1} and STfR <21.0 nmol L^{-1}).

Statistical analysis: Data was analyzed statistically using SPSS software v. 23. If the data distribution was normal, an independent Student's *t*-test was used to assess differences; a Mann-Whitney test, with a significance level of 5%, was used if the distribution was not normal. Meanwhile nominal data were analyzed using chi-square test. Food intake data with Recommended Dietary Allowances percentages were measured using cutoff points based on the 2013 Republic of Indonesia Ministry of Health Regulation No. 75 regarding Indonesian nutritional adequacy. Data were presented as means±standard deviations or medians unless specified otherwise.

RESULTS

Table 1 shows the characteristics of pregnant women in rural and urban areas of Central Java, Indonesia. The mean age of pregnant women in rural and urban areas was 27±5.8 and 28±5.1 years-old. Age did not differ significantly between rural and urban areas (p = 0.154). Monthly family income in rural areas was lower than in urban areas (p = 0.001). The majority of pregnant women in rural areas only completed a primary education (37.6%), while most pregnant women in urban areas completed secondary or higher (36.9%). Intake of protein, iron, vitamin B12 and folic acid was higher in urban areas, while intake of vitamin C and dietary fiber were higher in rural areas. Mean iron intake was 2-times higher (p = 0.003) and median vitamin B12 intake was 4-times higher (p = 0.001) in urban versus rural areas. Although median folic acid intake was slightly higher in urban areas, the difference was not significant. In contrast, median vitamin C (p = 0.001) and dietary fiber (p = 0.001) intake were 2-times higher in rural areas versus urban (Table 2).

Table 1: Characteristics of pregnant women in rural and urban areas of central Java, Indonesia

Variables	Rural areas (n = 69)	Urban areas (n = 80)	p-value
Age (years)	27±5.8[a]	28±5.1[a]	0.154[c]
Monthly income (rupiahs)	1000 (500-3000)[b]	2000 (500-6000)[b]	0.001[d]
Level of education			
Primary	56 (37.6%)	25 (16.8%)	
Secondary or Higher	13 (8.7%)	55 (36.9%)	0.001[e]

[a]Mean±standard deviation, [b]Median (range), [c]Independent student's t-test, [d]Mann-whitney test, [e]Chi-square test

Table 2: Nutritional intake of pregnant women in rural and urban areas of Central Java, Indonesia

Variables	Rural areas (n = 69)	Urban areas (n = 80)	p-value
Energy (calories)	2112±337.6[a]	2.073±396.9[a]	0.524[c]
Energy (%RDA)	96.1±20.5[a]	73.4±17.6[a]	0.001[c]
Protein (g)	61±17.7[a]	84±24.2[a]	0.001[c]
Protein (% RDA)	90.3±26.8[a]	98.2±32.8[a]	0.117[c]
Iron (mg)	9.7(6.0-25.7)[b]	19.6 (6.2-27.3)[b]	0.003[d]
Vitamin C (mg)	90(18-281)[b]	42 (1-288)[b]	0.001[d]
Vitamin B12 (µg)	1.5(0.3-10.1)[b]	6.3 (0.1-65.4)[b]	0.001[d]
Folic Acid (µg)	214(127.0-485.0)[b]	218.5 (96.0-846.0)[b]	0.896[d]
Dietary fiber (g)	13.7(7.5-28.9)[b]	7.4 (1.9-21.0)[b]	0.001[d]

[a]Mean±standard deviation, [b]Median (range), [c]Independent student's t-test, [d]Mann-whitney test, RDA, recommended dietary allowance

Table 3: Hemoglobin and serum transferrin receptor levels of pregnant women in rural and urban areas of Central Java, Indonesia

Variables	Rural areas (n = 69)	Urban areas (n = 80)	p-value
Hemoglobin (g dL^{-1})	10.9 (7.3-12.8)[a]	11.1 (8.9-14.3)[a]	0.087[b]
Serum transferrin receptor (nmol L^{-1})	24.1 (13.8-58.2)[a]	15.1 (8.6-34.9)[a]	0.001[b]

[a]Median (range), [b]Mann-whitney test

Table 4: Cross-tabulation of residential location with iron-deficiency anemia status in pregnant women from central Java, Indonesia

	Iron-deficiency anemia status									
	Iron-deficiency anemia		Non-iron-deficiency anemia		Non-anemia iron deficiency		Non-anemia/non-iron deficiency		Total	
Residential location	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Rural areas	22	31.9	12	17.4	26	37.70	9	13.00	69	100
Urban areas	14	17.5	26	32.5	1	1.25	39	48.75	80	100
Total	36	24.2	38	25.5	27	18.10	48	32.20	149	100

There was a significant difference between STfR levels in pregnant women from rural and urban areas ($p = 0.001$). The median STfR in urban areas was 15.1 (range, 8.6-34.9), which was lower than that of rural areas (median, 24.1; range, 13.8-58.2). Although, Hb levels in urban areas were slightly higher than that of rural areas, the difference was not significant (Table 3). In rural areas, the prevalence of IDA and non-iron-deficiency anemia was higher than that in urban areas. Cross-tabulation showed a correlation between the residential location of pregnant women (urban or rural) and the four IDA status categories ($p = 0.001$). Pregnant women with IDA were more prevalent in rural areas, while in urban areas, most were non-anemic/non-iron deficient (Table 4).

DISCUSSION

The most common cause of anemia during pregnancy is iron deficiency and is usually caused by low iron stores prior to pregnancy[5]. The results of this study are in agreement with a study in Nigeria which reported that pregnant women in rural areas had a significantly higher median STfR than those in urban areas[18]. Interestingly, the median Hb level of pregnant women in rural areas in the present study was only slightly lower than that in urban areas, suggesting STfR levels may be a better indicator of iron status. Similar results were found in studies of healthy women in India[19] as well as pregnant women in Tibet[20].

The current results also showed that differences in STfR and Hb levels between rural and urban areas were directly influenced by nutritional intake, particularly iron. Adequate intake of iron in pregnant women with IDA had a significant effect on STfR levels. A previous study reported that anemic pregnant women who consumed at least 60 mg d^{-1} of iron for 100 d since 19 weeks of pregnancy, were able to increase their Hb levels and such supplementation was also shown to increase iron reserves in non-anemic pregnant women[21]. In the present study, the iron intake of pregnant women in rural areas was much lower than those in urban areas. Similar results were found in China, wherein pregnant women in urban areas consumed more animal-based and diverse foods compared to pregnant women in rural areas[22]. This could easily explain the low Hb and high STfR levels of pregnant women in rural areas in the present study and others.

In rural areas of developing countries, people more often consume food from plant sources rather than animal. Temanggung Regency is an agricultural and plantation area, which allows inhabitants to consume more self-grown plant-based food, whereas those living in Semarang City must buy food in the marketplace, which provides greater dietary variety. Animal-based food is a main source of more readily absorbable heme iron compared to non-heme iron from plant sources. A study in Pakistan reported that consumption of red meat less than twice a week before pregnancy was associated with decreased Hb levels during pregnancy[23]. Plant-based foods, such as wheat, nuts and vegetables, contain high levels of fiber (e.g., phytic, tannic and chlorogenic acids) that can inhibit the absorption of non-heme iron[24].

Lack of animal-based food consumption, especially in rural areas, is also affected by a lower intake of vitamin B12, commonly known as cobalamin. Vitamin B12 deficiency in pregnant women can cause recurrent abortion, infertility and preterm abortion. Vitamin B12 along with folic acid play a key role in the formation of red blood cells; hence, a deficiency of vitamin B12 has been shown to be the second most common cause of megaloblastic anemia[25]. An unbalanced vegetarian diet leads to certain nutritional deficiencies since plant-based foods have lower bioavailability of certain vitamins and minerals. However, because vitamin B12 is relatively absent from plant-based food, it must be supplied to the body through alternative sources[26]. In the present study, intake

of vitamin B12 in rural areas was lower (1.5 μg day^{-1}) than the value recommended by the Ministry of Health of Indonesia (2.6 μg day^{-1}) for pregnant women in their third trimester[27]. Furthermore, consumption of a largely plant-derived diet by pregnant women in rural areas of Central Java was related to an increased risk of vitamin B12 deficiency and, therefore, anemia.

The current results also revealed that the types and sources of food consumed was closely related to socioeconomic status. A previous study in Ethiopia found that pregnant women with a lower household socioeconomic status had a significantly higher prevalence of anemia than those from a higher socioeconomic status[28]. Likewise, the current results showed the family income of pregnant women living in urban areas was twice as high as those from rural areas and was associated with better food intake. This is most likely because households with a higher socioeconomic status are able to allocate more of their budget towards food, in terms of both quantity and quality[29]. Moreover, people living in urban areas with a high income level more often consume animal-based foods[30]. Thus, the risk of anemia in pregnant women from lower socioeconomic families has been estimated to be 1.6-times higher than that of the pregnant women from higher socioeconomic families[31].

Based on the behavioral theory of Lawrence Green, healthy behavior can be formed when preceded by predisposing factors, one being knowledge. Adequate health knowledge supported by strong motivation can encourage personal health action[32]. Pregnant women with good nutritional knowledge are more likely to eat more nutritious foods during pregnancy. Additionally, knowledge level is influenced by education level/duration[33]. A study in Malaysia found that education level was significantly associated with anemia during pregnancy[34]. The majority of mothers in that study with a higher education level were more concerned about their personal and fetal health[34]. A literature review showed that 49% of college graduates obtained useful health information from books, newspapers and /or magazines compared to 18% of those less educated. Moreover, a study in Sudan indicated that women with a higher education level were less frequently anemic than illeterate women or women with only a primary or secondary education[35]. In rural Central Java, most pregnant women are poorly educated, leading to the assumption that their level of knowledge is also lower than that of pregnant women in urban areas. A significant difference in knowledge level has been reported to be caused by a lack of awareness regarding the consequences of inadequate nutrition during pregnancy[36]. This conclusion is also supported by the findings of a US study showing the chances of anemia during pregnancy declined as the education level of the mother increased[37].

CONCLUSION

The results of the present study indicated that the incidence of maternal anemia differs depending on rural or urban residential location in Central Java, Indonesia. This result was due to differences in education, culture, habits and environmental factors affecting behavior and diet. Pregnant women in rural areas should consume more varied foods and increase the consumption of animal-based food or supplements to improve iron status during pregnancy.

SIGNIFICANCE STATEMENT

This study investigated the differences in hemoglobin and serum transferrin receptor levels in pregnant woman residing in rural and urban areas of Central Java, Indonesia. The results help uncover critical areas and people groups at greater risk of tissue iron deficiency in developing countries as well as factors influencing iron status. Furthermore, measurement of serum transferrin receptor levels is shown to be a better measure of early iron status and anemia risk.

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