The Influence of Iron Depletion and Chronic Energy Deficiency on the Risk of Hypothyroidism in Pregnant Women Living in Iodine Deficiency Disorders Endemic Areas in Badegan Ponorogo District East Java, Indonesia

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Summary Several minerals are needed for thyroid hormone to work properly, such as iodine and iron. Iron is one of important essential trace elements that serves as co-factor for thyroid peroxidase (TPO). Those with iron deficiency have lower ferritin serum level than those who are normal. In particular, pregnant women with chronic energy deficiency (CED) have higher anemia risk than the otherwise. The study aimed to measure ferritin level, CED and hypothyroidism among pregnant women living in iodine deficiency disorders (IDD) endemic areas. A total of 37 pregnant women aged 18–45 y-old with pregnancy of 2 to 8 mo living in ID Dendemic of Dayakan and Watu Bonang Villages of Ponorogo District were include in the study. Two different measurements were taken namely, anthropometric measurement of mid-upper arm circumference (MUAC) or upper circumference (UAC) which is more correct term to use, and blood markers of iodine and iron status were assessed using thyroid stimulating hormone (TSH), free thyroxine (fT4), and blood ferritin. Among pregnant women, 27% had hypothyroidism and 54.05% had iron depletion, with 30% had both conditions. The hypothyroidism was higher in pregnant women had CED than those non-CED, 50% vs. 18.5%. No significant correlation ($p \geq 0.05$) between hypothyroidism and both ferritin and CED, but adjusted odds ratio (AOR) was found at 7.7 (95% CI: 1.2–52.9), indicated more than four times higher risk of hypothyroidism in pregnant women with CED than the otherwise. Hypothyroidism in pregnant women living in Ponorogo was not caused by iron deficiency, but allegedly by lack of other nutritional intake. Conclusion: Integrated and sustainable efforts to improve nutritional status is needed since pre-conception and throughout pregnancy.

Key Words chronic energy deficiency, ferritin, hypothyroidism, iodine deficiency, pregnant women

Normal thyroid hormones metabolism involves such minerals as iodine, iron, selenium, and zinc. Aside from iodine, iron is considered an essential element in thyroid hormones metabolism. It serves as a co-factor in the catalysis of a variety of bioenzymes, including thyroid peroxidase (TPO). This enzyme is involved in the catalysis of the first two reactions in thyroid hormones biosynthesis, namely iodide oxidation and iodination of tyrosyl residues of thyroglobulin. Iron deficiency leads to disturbances to the body's ability to synthesize thyroid hormones resulted from lowered TPO efficiency. Ferritin is an indicator of iron reserves in the body, and the level of serum ferritin is reported to decline in hypothyroidism. The level of serum ferritin is indicative of iron deficiency (1, 2). Pregnancy is constantly associated with physiological changes which give rise to macro- as well as micronutrients deficiency.

Some nutrients seeing an increase in demand during pregnancy include iron, iodine, vitamin C, vitamin A, and protein. Iodine deficiency during pregnancy will

cause an adverse effect on the fetus. Daily iodine intake is represented by urinary iodine excretion (UIE). The 2013 Riskesdas data (Basic Health Research) reveal that the median UIE for pregnant women was 163 $\mu\text{g/L}$, but 24.3% of the pregnant women had UIE of $<100 \mu\text{g/L}$ (3). Iron deficiency in pregnancy with hypothyroidism will worsen pregnant women's condition. Anemia in pregnancy can cause childbirth-related death, low birthweight, susceptibility of fetuses and pregnant women to infection, miscarriage, and risk of preterm labor. The World Health Organization (WHO) reported iron deficiency prevalence in pregnant women to hit 41.8%, and the percentage increases with the gestation period. In Indonesia, the anemia prevalence in pregnant women were at 37.1% (3, 4).

Anemia in pregnancy related to complication during pregnancy and could increase maternal mortality. In developing countries, most maternal mortality cases occur in pregnant women with anemia, which is caused by either acute bleeding or poor nutritional status. Aminin reported that chronic energy deficiency (CED) occurred more often in pregnant women who suffered

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from anemia (88.9%) than those who did not (11.1%) (5). Meanwhile, another study reported that pregnant women with CED were at risk four times higher than those who did not have CED (6). Whilst, CED could be attributed to multiple micronutrient deficiencies.

Further, hypothyroidism and anemia make matters worse for pregnant women who have already suffered from chronic energy deficiency as well as the fetuses, necessitating the implementation of various policies and intervention programs. Earlier detection of iron deficiency, chronic energy deficiency, and other nutritional deficiencies in pregnant women with or without hypothyroidism in IDD areas can be conducted to prevent worse effects such as LBW, abortion, pregnancy complications, and death risks for the pregnant women and their fetuses.

This research aimed at figuring out the ferritin contents, CED and hypothyroidism-suffering pregnant women residing in IDD endemic areas.

MATERIALS AND METHODS

This research is a cross-sectional study, conducted in four (sub-villages) in Dayakan Village and three in WatuBonang Village, Badegan District, in which iodine deficiency disorders (IDD) cases occurred. These locations were selected purposively. The respondents of this research were all pregnant women living in the locations, aged 18–45 y and 2–8 mo pregnant.

Data for pregnant women characteristic were collected by interview using standardized structured questionnaire. Nutrition status was categorized by anthropometric measurement for middle upper arm circumference (MUAC) to address chronic energy deficiency (CED). CED measurement was performed by a 1 mm accuracy level MUAC tape, with readings below 23.5 cm indicating that the pregnant women were suffering from CED and were at risk of giving birth to infants with low birth weight (LBW) (7).

Iodine status was collected by spot urine in continuously to examine urine iodine concentration using spectrophotometry method in accredited laboratory. The iodine level would be categorized as deficient if the UIE was $<150 \mu\text{g/L}$, normal if the UIE was $150\text{--}249 \mu\text{g/L}$, and overloaded if the $\mu\text{g/L}$ was $>250 \mu\text{g/L}$ (10).

Thyroid function was measured a thyroid stimulating hormone (TSH), and free thyroxine (fT4) and usually conducted routinely at a laboratory for a thyroid dysfunction diagnosis (13). TSH testing was run by the ELISA method with procedure including venous blood drawing and reagent preparation as follows: pipette standard and sample; add CON (mixed and incubated in a shaker); remove the content of the wells into hypochloric sol, wash five times with wash solution (program washer: TSH serum); add SUB; incubate in the shaker; add stop reagent; mix with care; and read on the microplate reader at 450 nm wavelength.

fT4 testing was run by the ELISA method with procedure including reagent preparation as follows: prepare the microplate wells to use for reference serum, control, and sample; pipette the standard, control, and sample

into the designated microplate wells; add thyroxine enzyme conjugate solution, stir; add working substrate solution; incubate in the dark at room temperature; put the reaction to a halt by adding 3NHCl (stop solution); mix slowly; and read on the microplate reader at 450 nm wavelength.

Based on the Human Kit, one would be categorized as suffering from hypothyroidism if their TSH contents was $>6.2 \mu\text{IU/mL}$ (11) and the fT4 contents was $<0.76 \mu\text{IU/mL}$ (12). The UIE, TSH, and fT4 testings were run at the Chemistry Laboratory, Health Research and Development Center, Magelang. Iron depletion was addressed by blood sampling for ferritin. For the serum ferritin testing, enzyme-linked immunosorbent assay (ELISA) was used for its accurate, reliably quantitative measurement capability (8). The WHO (1993) set a recommended cut-off of 11.0 g/dL for pregnant women in the second trimester (9). Ferritin serum testing was conducted as per the enzyme-linked immunosorbent assay (ELISA) procedure for its accurate, reliable quantitative measurement capability (8).

The data collected were analyzed statistically by a descriptive test to figure out the mean and standard deviation and by a chi-square test to figure out the relationship between variables, followed by a logistic regression to measure CED risk and iron depletion on two groups of hypothyroidism and no hypothyroidism. The analysis was conducted with the software Statistical Package for the Social Sciences (SPSS).

RESULTS

Anthropometric measurement and urinary and blood biochemical markers

The mean ferritin, chronic energy deficiency, and UIE, TSH, and fT4 contents are presented in Table 1.

The results show that some hypothyroidism and anemia cases were still encountered in pregnant women living in the IDD areas of interest, but some pregnant women in normal condition (no hypothyroidism) were also found. Some others had UIE of $\geq 300 \mu\text{g/L}$. In the past few years, the Regency of Ponorogo has been staying as a mild IDD area with a TGR of 11.9%, which virtually equaled the National TGR. However, a recent urinary test's results show that a portion of the population had higher median than normal and the national value. People with UIE of $\geq 300 \mu\text{g/L}$, twice as the national value, made up 66% of the population, meaning that some of the population had iodine excess (14).

Iron stored status in pregnant women

Thyroid dysfunction is a global phenomenon. Mostly it takes the form of hypothyroidism in women, which exhibits a constantly elevated prevalence in pregnancy. In the majority of developing countries, lacking such nutrients like iron, vitamin B12, and folic acid is predominant and common in pregnant women and can be the cause of maternal and foetal complications (15). The ferritin contents of the pregnant women in this study can be seen in Table 1.

Hypothyroidism occurred in 45.9% of the pregnant women, 54.05% of whom were ferritin-deficient.

Table 1. Anthropometric measurement and urinary and blood biochemical markers of the participants ($n=37$).

Indicator	Mean $\pm$ SD	Min–Max
Biochemical markers		
Blood ferritin (please use g/dL as indicated in text, instead of $\mu\text{g/L}$)	21.4 $\pm$ 18.7	4.1–74.2
Iron depletion (<11 g/dL)	45.9 (17) ^a	
TSH ($\mu\text{IU/dL}$)	3.1 $\pm$ 2.2	0.3–12.1
fT4 (ng/dL)	1.18 $\pm$ 0.65	0.60–3.37
Hypothyroidism (>6.2 $\mu\text{IU/mL}$ for TSH and <0.8 ng/dL) fT4	45.9 (17) ^a	
UIE	1.18 $\pm$ 0.65	0.60–3.37
Anthropometric marker	105.68 $\pm$ 78.24	2–429
MUAC (cm)	24.9 $\pm$ 2.7	21.0–32.0
At risk of CED (<23.5 cm)	21.6 (8) ^a	

^a Value is presented in% and number of participants in parentheses was 37.

Fig. 1. Proportion of ferritin deficiency among pregnant with hypothyroidism.

Hypothyroidism was identified in 30% of pregnant women with iron depletion and in 23% of pregnant women with normal ferritin contents. Between these two groups, no significant difference was identified ($p>0.05$).

Relationship between ferritin contents and hypothyroidism in pregnant women

A study found that the decline in serum ferritin contents was more significant in patients with hypothyroidism than those without (16). Another study similarly discovered that patients with hypothyroidism showed a more significant drop in iron and ferritin contents than the control group (17). The relationship between ferritin contents and hypothyroidism in pregnant women is presented in Fig. 1.

The research results show that prevalence of hypothyroidism in pregnant women with iron depletion did not differ from that in pregnant women with normal ferritin contents. Ferritin reflects the iron reserves in the body, which become the most predominant clinical indicator in anemia. Iron depletion is caused by the increase

in pregnant women's demand for iron for maintaining the tissues of their bodies, placentas, and fetus, especially in the second and third trimesters of pregnancy. Inadequate nutritional intake is also responsible for the low levels of ferritin in pregnant women. Inadequate nutritional intake can lead to iron deficiency during pregnancy. Some studies provide an explanation that the nutrient absorption capacity of the small intestine influences ferritin concentrations during pregnancy (18, 19). This research's results are in line with those of Turkey- and Thailand-based studies reporting that thyroid profile did not have any significant effect on iron depletion (20, 21).

Contrary to the abovementioned studies, a study reported a more significant decrease in the serum ferritin levels of patients with hypothyroidism than that in the serum ferritin levels of normal subjects. Ferritin is an iron-storing protein found in nearly all body tissues. Akhter et al. (1) explains in their research that thyroid function disorders in patients with iron depletion are reflective of problems in the activity of thyroid peroxidase which cause disturbances to the thyroid hormones metabolism. Meanwhile, Hess et al. (22) state that iron plays a role in the thyroid hormones transport into cells, thus iron deficiency causes an accumulation of thyroid hormones and leads to hypothyroidism. This disparity may be a result from geographical or characteristic differences between the research samples.

Research by Mogahed et al. (15) also reported that TSH contents were lower in pregnant than in non-pregnant women. Thirty-five point five percent of the respondents were suffering from subclinical hypothyroidism, 10% were suffering from hypothyroidism, and 2.2% were suffering from hypothyroidism and anemia at the same time. 15 Iron deficiency occurred in pregnant women during the second trimester, with 11.4% of pregnant women having iron concentrations lower than 12 $\mu\text{g/L}$ and 7.6% suffering from anemia (15). Maternal iron status is the determinant of TSH concentration among pregnant women in the second trimester (19).

Relationship between UAC and hypothyroidism in pregnant women

The nutrient status before and during pregnancy is of a considerable importance to producing optimal pregnancy output. The most apparent nutritional deficiency cases in pregnant women are those of chronic energy deficiency and micronutrient deficiency. Nutritional deficiency is known to have a good, acute, or chronic effect on thyroid hormone physiology, particularly in the peripheral hormone metabolism. In protein-energy malnutrition, there is a disturbance to the deiodinase process, affecting the conversion of T4 into T3. Both T4 and T3 hormones are related to the protein levels in human plasma. Meanwhile, in individuals with protein-energy malnutrition, the concentrations of binding proteins, thyroxine binding globuline (TBG), thyroxine binding prealbumin (TBPA), and albumin are of low extreme values, and the serum T4 and T3 decrease significantly (23). The relationship between

Table 2. Inter-relationships between all biochemical marker (TSH, fT4, Ferritin, UIE) and anthropometric measurement of MUAC.

	TSHs		ft4		Ferritin		UIE		MUAC		<i>n</i>
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	
Biochemical markers											
TSHs	1.00	—	−0.18	0.28	−0.17	0.33	0.32	0.05	0.00	0.98	37
ft4	−0.18	0.28	1.00	—	−0.28	0.10	−0.26	0.11	0.15	0.37	37
Ferritin	−0.17	0.33	−0.28	0.10	1.00	—	0.25	0.14	−0.03	0.87	37
UIE	0.32	0.05	−0.26	0.11	0.25	0.14	1.00	—	0.25	0.14	37
Anthropometric Measurement of MUAC											
MUAC	0.32	0.98	0.15	0.37	−0.03	0.87	0.25	0.14	1.00	—	37

Table 3. Simple logistics regression model of odd ratios (OR) of the risk of hypothyroidism compared with normal thyroid status of pregnant women.

	Risk of hypothyroidism (TSH>6.2 μ IU/mL and fT4<0.8 ng/dL)	
	Crude Odds ratio (95%, CI)	<i>p</i> -value
Prevalence		
All indicator		
Age group		
- Younger age (<35 y)	1.0 (Referent)	0.26
- Older age (>35 y)	4.1 (0.3;51.4)	
Education level		
- Low	1.0 (Referent)	0.78
- Middle to High	1.2 (0.2;8.5)	
MUAC (cm)		
- CED (<23.5 cm)	7.7 (1.1;55.5)	0.04
- Normal ($\geq$ 23.5 cm)	1.0 (Referent)	
Blood ferritin		
- Iron depletion (<11 g/dL)	0.8 (0.1;5.4)	0.87
- Normal	1.0 (Referent)	
UIE		
- Iodine deficiency (<150 ug/dL)	0.1 (0.1–1.1)	0.07
- Normal	1.0 (Referent)	
	Adjusted Odds Ratio (95% CI)	<i>p</i> -value
Models in Equation		
MUAC		
- CED (<23.5 cm)	8.2 (1.2;52.9)	0.03
-Normal (>23.5 cm)	1.0 (Referent)	
UIE		
- Iodine deficiency (<150 ug/dL)	0.2 (0.02;1.14)	0.07
- Normal	1.0 (Referent)	

UAC and hypothyroidism is presented in Table 2.

In this research, it was found that hypothyroidism prevalence was higher in CED pregnant women than in their non-CED counterparts with an adjusted odds ratio (AOR) 4.4 times the AOR of non-CED pregnant women. However, statistically speaking, no significant difference was found between the two groups, which was assumed to be caused by inadequate research sample. Chronic energy deficiency in pregnant women is marked with

UAC of less than 23.5 cm. Energy deficiency is known to have an impact on thyroid hormones metabolism as it triggers a disturbance to stimulation of insulin release, inhibiting the conversion of T4 to T3 (24). It was also found that the TSH and T4 contents did not show any correlation with body mass index (BMI) (25).

The data from previous research point out the effect of malnutrition on thyroid function, especially on T3 and T4 levels, and T3 was reported to be subnormal

(23). A study in Iran reported that goiter, which mostly is triggered by thyroid hormones deficiency, exhibited a higher prevalence in populations with malnutrition (26).

The 2013 Riskesdas data indicate that the anemia prevalence in pregnant women stood at 37.1%, while the proportion of pregnant women with CED was 24.2%. The median UIE (urinary iodine excretion) of pregnant women was below 150 $\mu\text{g/L}$ (categorized as deficient in Indonesia if the percentage surpasses 40%) and the median urinary iodine concentration (UIC) fell into normal borderline (27).

According to the 2018 Riskesdas data, CED occurred in 17.3% of pregnant women of childbearing age and 14.5% of non-pregnant women of childbearing age. Over the five-year period, the CED prevalence in pregnant women of childbearing age was reported to go down. The CED prevalence in pregnant women of childbearing age was above that in non-pregnant ones, with more cases involving pregnant women occurring in rural areas (19.3%) than in urban areas (15.7%) (28).

Pregnant women with UAC of <23.5 cm are at risk of giving birth to infants with low birth weight (LBW) twice higher than those with UAC of >23.5 cm. Thus, to minimize CED risk, it is necessary to address LBW and anemia prior to gestation period. It is also important to address the combined risk of CED and anemia to LBW (29).

DISCUSSION

Ferritin is an iron-bearing intracellular protein. Lack of iron may affect thyroid functions. The iron contents of pregnant women are determinant to TSH concentrations during pregnancy (19). Ferritin is an iron-storing protein pervasive in nearly all body tissues. The levels of serum ferritin have been reported to change in patients with thyroid diseases, suggesting a relationship between serum ferritin concentration and thyroid functions (16).

Iron deficiency anemia refers to the anemia disease caused by the loss of iron reserves within body tissues. As the consequence of iron deficiency (mild anemia) in pregnant women, the oxygen delivery to placenta will be disturbed as the weight, volume, and surface area of the placenta are reduced. The decrease in the levels of hemoglobin and serum ferritin is more significant in the second trimesters than in the first and third trimesters of pregnancy (9). Meanwhile, lower levels of hemoglobin, ferritin, and vitamin B12 are more common in the first and second trimesters of pregnancy. This condition urges the need for early detection of thyroid status. Thus, screening of pregnant women for thyroid dysfunction should be considered immediately (15).

Thyroid hormones metabolism and iron are interlinked; iron deficiency can cause hypothyroidism, and, on the other side, thyroid hormones inadequacy affects iron deficiency. It is deemed necessary, then, to have iron reserves for hypothyroidism treatment (17). Ferritin contents changes have been reported to have taken place in patients with thyroid function disorders (16).

In patients with hypothyroidism, iron and ferritin contents were found to have decreased more significantly than in the control group (17). Hence, hypothyroidism is associated with low ferritin contents. Serum ferritin estimation will be helpful in understanding the etio-pathogenesis, diagnosis, and monitoring of patients with hypothyroidism (16).

The significant difference in the ferritin levels between patients with hypothyroidism and normal, healthy control group is a reflection of the iron-dependent enzyme activity. It is, necessary to carry out ferritin testing before and after thyroid hormones therapy to provide useful information for thyroid disease diagnosis (16).

Pregnancy is a physiological condition that brings about complex changes to hormones and the immune system. Pregnancy influences the body in a number of ways, one of which causing iron deficiency, leading to an adverse effect on the thyroid metabolism (30).

Thyroid dysfunction may occur during pregnancy, which is influenced by a wide variety of factors, including iron deficiency.¹⁹ This reflects the importance of iron to thyroid functions (25). Hypothyroidism prevalence is high in pregnant women with iron and vitamin B12 deficiencies, making it important to conduct evaluation of nutritional adequacy in pregnant women for earlier treatment to prevent health disorders in them and their fetuses for normal pregnancy outcomes (15).

Iodine and iron deficiencies can be prevented by continuous intervention programs. These programs are important because iodine and iron deficiencies can increase death risk during childbirth and have been reported to be implicated in 20% of maternal deaths (31). Among the intervention programs are iron supplementation for pregnant women to reduce intellectual damages to their offsprings.¹⁹ and malnutrition prevention during pregnancy (15).

Evaluation of thyroid functions during pregnancy in relation to iron, vitamin B12, and folic acid intake that meets the changing need throughout all trimesters of pregnancy is necessary because iron deficiency in pregnant women can cause increased TSH and decreased T4 contents (30).

In Indonesia, the median UIC belonged to the national normal range, but in some sub-populations, especially in pregnant women, the UIC was categorized as subnormal. To prevent iodine deficiency and to maintain the median UIC in the normal range, regular monitoring of iodine status, especially in pregnant women, should be conducted (27).

This research comes with limitation. The sample used in this research included all pregnant women in the research locations, but it is deemed necessary to include more locations. Further research should be conducted to look into the impacts of iron depletion in pregnant women with hypothyroidism on infant growth/nutritional status.

CONCLUSION AND RECOMMENDATION

Iron depletion and CED was found in pregnant women and also in pregnant with hypothyroidism and

those without hypothyroidism in the IDD areas in the Regency of Ponorogo. However, the hypothyroidism cases there were not caused by iron and iodine deficiencies only. They were likely to be caused by other nutritional deficiencies as well. Normal and healthy pregnancy output can be achieved by evaluation of thyroid functions and adequacy of iron and other nutrients throughout different trimesters of pregnancy. Therefore, integrated and sustainable nutritional improvement efforts since pre-conception and during gestation period should be performed. And it is recommended to screen pregnant women to prevent thyroid dysfunction and to run continuous supplementation in relation to urgent needs during pregnancy due to physiological and hormonal changes.

Disclosure of state of COI

We declare that this research does not contain any conflict of interest.

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