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CORRELATION BETWEEN SERUM FERRITIN, TRANSFERRIN SATURATION AND PITUITARY MRI T2 RELAXATION TIMES AND FSH, LH AND TESTOSTERONE LEVELS IN MALE TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS

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ABSTRACT

Objective: The purpose of this study is to see the correlation between iron overload with the hypogonadal state by analyzing the correlation between ferritin serum, transferrin saturation and pituitary MRI T2 relaxation time with FSH, LH and testosterone levels.

Methods: This is a cross-sectional study of 32 male subjects with transfusion-dependent thalassemia. The subjects were collected with a consecutive sampling technique in the thalassemia outpatient clinic in National Hospital in Indonesia. Measurements of serum ferritin, transferrin saturation, FSH, LH and testosterone were taken using ELISA technique. Pituitary MRI T2 relaxation time was done using MRI Avanto 1.5 Tesla.

Results: In this study, secondary sexual characteristics were not fully achieved in 62.5% of patients. Low testosterone levels were found in 25% of patients. There was a negative correlation between transferrin saturation and pituitary MRI T2 relaxation time in the normal testosterone level group.

Conclusion: This study showed a high rate of patients who had not achieve puberty, but a low rate of patients with low testosterone, which means there is a weak negative correlation between transferrin saturation and pituitary MRI T2 relaxation times.

Keywords: Hypogonadism, Pituitary MRI T2 relaxation time, Transfusion-dependent thalassemia

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INTRODUCTION

Hypogonadism, a disorder that leads to decreasing testosterone levels in men, may increase the risk of sexual dysfunction, mood disturbances and changes in bone mineral density and body composition [1]. Primary hypogonadism (hypogonadotropic hypogonadism) is indicated with low serum testosterone levels, but high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH); while secondary hypogonadism is characterized by low testosterone, FSH and LH levels [2, 3].

Hypogonadism is an endocrinal complication that occurs in 70–80% of major thalassemia patients. In these cases, hypogonadism is caused by iron build-ups in the pituitary gland, testes or both. This disorder is a hereditary condition caused by a defect in globin synthesis, which leads to impaired hemoglobin and requires patients to undergo blood transfusions. Beta-thalassemia is most common in the Mediterranean, Middle East, Asia, India, Southern China, Northern Africa and South America. The highest carrier frequency has been reported in Cyprus (14%), Sardinia (10.3%) and Southeast Asia [4-6].

Thalassemia major patients require regular red blood cell transfusions; this severe type of thalassemia is called transfusion-dependent thalassemia (TDT). Transfusions results in excessive iron accumulation, which can lead to iron toxicity. The examination of blood iron levels is conducted by measuring the transferrin saturation (TSAT) and serum ferritin [4, 7–9].

A radiology technique, Magnetic Resonance Imaging (MRI), has been used and validated in various clinical trials as a useful modality for measuring iron deposits in an organ and has become a new standard for attaining these measurements. The assessment uses MRI T2* in the liver, heart and pancreas. However, due to the small size of the pituitary gland, spin-echo imaging (T2) is likely to perform better than T2* methods [8, 10-12].

Therefore, the aim of this study is to determine the correlation between serum ferritin, transferrin saturation, and excess iron levels in the pituitary gland (assessed by pituitary MRI T2). Along with, the correlation between excess iron levels in the pituitary gland and FSH, LH and testosterone levels in transfusion-dependent thalassemia in the Indonesian population.

MATERIALS AND METHODS

An analytic cross-sectional method was used in this study. The study was conducted in the adult thalassemia clinic and in the Department of Radiology of National Hospital in Indonesia, in the period of March–July 2019. The samples used were from male transfusion-dependent thalassemia patients aged>18 y of age who were receiving transfusions in the thalassemia clinic during the period of the study. The exclusion criteria included the following:

1. Subjects who experienced drug-induced hyperprolactinemia
2. Subjects with a pituitary tumor
3. Subjects with history of testicular trauma, irradiation or surgery
4. Subjects with history of pituitary surgery

Following approval from the Ethics Committee of the Faculty of Medicine in our university, informed consent was obtained from each subject. The convenient sampling technique was used. Based on the correlation test conducted, the minimum number of subjects needed were 32 patients. Data analysis was processed using the SPSS 21.0 program. Results were correlated and statistical analysis was conducted using Pearson's correlation *(r)* for normally distributed data, and Spearman's correlation (rho) test was used for data that was not normally distributed.

Patients included in the study were recurring thalassemia patients who were receiving blood transfusions. The diagnosis of thalassemia was previously determined using high-performance liquid chromatography (HPLC) or a microcapillary examination. Plasma concentrations of serum ferritin and transferrin saturation were calculated using mean over a period of 12 mo. Serum ferritin measured using electrochemiluminescence immunoassay (ECLIA)

and transferrin saturation were obtained by dividing the serum iron total by the total iron-binding capacity.

MRI examinations were performed using the Siemens MAGNETOM® Avanto 1.5T MRI to produce T2 SE sequences. The calculation of pituitary T2 relaxometry value was conducted using CMRtools™ software to determine iron deposits in various organs.

The pituitary MRI T2* was assessed by multi-echo spin-echo (SE) sequence images (TR = 2000 ms; TE = 15, 30, 45, 60, 75, 90, 105 and 120 ms; slice thickness = 3 mm; matrix size = 256 x 256; field of view [FOV] = 20 cm) taken from the coronal view with a focus on the anterior pituitary gland. The scan duration was 8 min and 36 seconds. Calculations of the pituitary T2 SE relaxometry were done on the coronal plane.

We determined the region of interest (ROI) on the T2 map of the pituitary gland without crossing organ boundaries and while avoiding bone structures. The segment option was then taken to provide the boundaries of the pituitary that were previously determined as the ROI. By selecting the analyze option, the T2 value for all images was automatically calculated, resulting in the average T2 value with a standard deviation in *ms*-units. The pituitary length measurement was done on the sagittal plane in *mm*-units.

RESULTS

Table 1 shows the subjects' characteristics. Most of the patients were thalassemia ß-major (93.7%), while the rest were thalassemia ß-HbE (6.3%). A statistical test on age was conducted, and an abnormal distribution was obtained, with the median age of 22 y (minimum-maximum range is 18–39 y).

Of the patients' complaints relating to hypogonadism, erectile dysfunctions and decreased libido were reported in 9.4% and 21.8% of the cases, respectively. Not all patients have a family history related to delayed sexual growth. The body mass index (BMI) examination reported that 50% of the subjects were underweight, 12% of the subjects were normal weight, 9.4% of the subjects were overweight and 3.1% of the subjects were obese. Of the 32 transfusion-dependent thalassemia patients, 62% did not reach puberty according to their age (Tanner stage V), and 6 of them (18.75%) have hypogonadotropic hypogonadism.

Table 1: Subjects' characteristics

Characteristics	Total n = 32
Age (years, median, minimum–maximum	22 (18–39)
Thalassemia type, n (%)	
Thalasemia ß major	30 (93.7)
Thalasemia ß HbE	2 (6.3)
Age, first received transfusion (months, median, minimum-maximum)	9 (3–72)
Age, first received chelating agents (years), mean (SD)	6.28 (3.51)
Chelating agents n (%)	
Deferiprone (Feriprox®)	17 (53.1)
Deferasirox (Exjade®)	5 (15.6)
Deferiprone and Deferasirox	8 (25)
Deferiprone and Deferoxamine (Desferal®)	2 (6.3)

Table 1: Subjects' characteristics (continued)

Clinical, n (%)	
Family history related to delayed sexual growth	
No	32 (100)
Yes	0 (0)
Erectile dysfunction	
No	29 (90.6)
Yes	3 (9.4)
Decreased libido	
No	25 (78.1)
Yes	7 (21.8)
Physical examination, n (%)	
Body mass index, kg/m^2, mean (SD)	
Underweight	18.85 (3.14)
Normal weight	17 (53.1)
Overweight	11 (34.4)
Obese	3 (9.4)
High-pitched voice	
No	21 (65.6)
Yes	11 (34.4)
Acne	
No	25 (78.1)
Yes	11 (34.4)
Facial hair	
No	25 (78.1)
Yes	7 (21.9)
Axillary hair	
No	17 (53.1)
Yes	15 (46.9)
Spleen size	
Not palpable	10 (31.3)
S 1–4	15 (46.8)
S 5–8	3 (9.4)
Post-splenectomy	4 (12.5)
Penile length (cm), median (minimum–maximum)	5.0 (2.0–7.0)
Testicle size (ml), median (minimum-maximum)	20 (3–20)

Table 2 shows the patients' sexual development clinical status. Sexual development is divided into clinical hypogonadism (Tanner stages I–IV) and fully developed of puberty (Tanner stage V). The clinical hypogonadism group showed higher serum ferritin and transferrin saturation than the normal testosterone group. Lower levels of FSH, LH and testosterone were observed, compared to the normal clinical group. Lower pituitary MRI T2 relaxation times and shorter pituitary lengths were also observed in the clinical hypogonadism group when compared to the normal clinical group.

Table 2: The study's basic characteristics, based on clinical hypogonadism

Characteristics	Tanner stages I–IV	Tanner stage V
	n=8	n=24
Age (years), median (minimum–maximum)	22 (18–35)	21.5 (18–39)
Serum ferritin (ng/ml), median (minimum–maximum)	7,320.87 (3,039.6–20,657)	6,364.03 (1,824.48–11,299.47)
Transferrin saturation (%), mean (SD)	87.45 (10.14)	83.9 (11.5)
FSH (mIU/ml), mean (SD)	5.98 (3.97)	7.2 (2.57)
LH (mIU/ml), mean (SD)	6.09 (4.38)	8.4 (3.57)
Testosterone (nmol/l), mean (SD)	17.13 (12.68)	33 (14.84)
Pituitary MRI T2 time (ms), median (minimum–maximum)	69.44 (46.55–148.85)	74.21 (60.83–126.7)
Pituitary length (mm), mean (SD)	5.7 (1.89)	6.06 (1.84)

Descriptive data based on the patients' testosterone levels can be seen in table 3. The low-testosterone group had higher serum ferritin and transferrin saturation, compared to the normal-testosterone group. Lower pituitary length was also observed in the low-testosterone group, compared to the normal-testosterone group.

Table 3: The study's basic characteristics, based on testosterone levels

Characteristics	Low testosterone	Normal testosterone
	n=8	n=24
Age (years), median (minimum–maximum)	23 (18–35)	21.5 (18–39)
Serum ferritin (ng/ml), mean (SD)	9,870.05 (6,421.65)	6,846.90 (3,115.13)
Transferrin saturation (%), mean (SD)	86.78 (8.95)	85.95 (11.30)
Pituitary MRI T2 time (ms), median (minimum-maximum)	79.67 (46.55–96.55)	69.90 (47.71–148.85)
Pituitary length (mm), mean (SD)	5.1 (1.7)	6.14 (1.84)

There was no significant correlation found between serum ferritin and FSH levels, but there was a positive correlation between transferrin saturation and FSH level (table 4).

Table 4: Correlation between FSH and serum ferritin levels and transferrin saturation

Variables	FSH	
	r value	p
Serum ferritin	0.013	0.942
Transferrin saturation	0.432	0.013

There was no significant correlation found between serum ferritin and LH levels and transferrin saturation (table 5).

Table 5: Correlation between LH level and serum ferritin levels and transferrin saturation

Variables	LH	
	r value	p
Serum ferritin	0.029	0.875
Transferrin saturation	0.157	0.390

There was no significant correlation found between serum ferritin and testosterone levels and transferrin saturation; but there was a positive correlation between FSH and testosterone levels, and between LH and testosterone levels (table 6).

Table 6: Correlation between testosterone and serum ferritin levels and transferrin saturation

Variables	Testosterone	
	r value	p
Serum ferritin	-0.021	0.911
Transferrin saturation	0.087	0.635
FSH	0.526	0.002
LH	0.755	0.000

There was no correlation found between pituitary MRI T2 relaxation time and serum ferritin, FSH, LH and testosterone levels and transferrin saturation (table 7).

Table 7: Correlation between pituitary MRI T2 relaxation time with the serum ferritin, FSH, LH and testosterone levels and transferrin saturation

Variables	Pituitary MRI T2 Relaxation Time	
	r value	p
Serum ferritin	- 0.178	0.329
Transferrin saturation	- 0.211	0.247
FSH	- 0.125	0.494
LH	0.029	0.873
Testosterone	- 0.089	0.630

This study found a negative correlation between serum ferritin levels and pituitary length. There was no correlation between pituitary length, pituitary MRI T2 relaxation time, transferrin saturation, and FSH and LH levels (table 8).

Table 8: Correlation of pituitary length with pituitary MRI T2 relaxation time, serum ferritin, FSH and LH levels and transferrin saturation

Variables	Pituitary Length	
	r value	p
Pituitary MRI T2 relaxation time	-0.170	0.353
Serum ferritin	-0.442	0.011
Transferrin saturation	0.067	0.715
FSH	0.116	0.527
LH	0.063	0.730

In both the low-and normal-testosterone groups, there was a negative correlation between serum ferritin and pituitary MRI T2, even though this correlation was not statistically significant. In the normal-testosterone group, there was a positive correlation between pituitary length and pituitary MRI T2 relaxation time, and a negative correlation between transferrin saturation and pituitary MRI T2 relaxation time. There were significant correlations between FSH and LH levels in both groups (table 9).

Table 9: Correlation between pituitary MRI T2 relaxation time with the serum ferritin, transferrin saturation, FSH and LH levels in low- and normal-testosterone groups

Variables	Pituitary MRI T2 relaxation time			
	Low testosterone		Normal testosterone	
	r value	p	r value	p
Pituitary length	0.251	0.548	0.463	0.023
Serum ferritin	-0.492	0.215	-0.053	0.807
Transferrin saturation	0.592	0.122	-0.424	0.039
FSH	0.558	0.151	-0.322	0.125
LH	0.444	0.270	0.054	0.802

DISCUSSION

Correlation between serum ferritin and transferrin saturation and FSH, LH and testosterone levels

In this study, excessive serum ferritin and transferrin saturation was found in 100% of the cases. However, serum ferritin levels did not correlate with FSH and LH levels. This is consistent with the study conducted by Abo-Elwafa *et al.*, which stated that there was no correlation found between serum ferritin level and FSH, LH, oestradiol and testosterone levels after an analogue GnRH test was carried out [13]. Habeb *et al.* also found no significant association between serum ferritin levels and hypogonadism [14].

This current study also found a positive correlation between transferrin saturation and FSH level, but not between transferrin saturation and LH levels. FSH has a longer half-life than LH15. It was found that FSH has a complex secretion regulation due to various molecular factors that influence it, such as adiponectin, synaptotagmin 9 (syt-9) and ACTH [16]. We also found that both serum ferritin and transferrin saturation have negative correlations with testosterone levels, although this correlation was not statistically significant (r =-0.021, p = 0.991 and r =-0.016, and p = 0.933, respectively).

Several factors are thought to be related to hypogonadism and thalassemia patients. Chronic anemia can lead to hypoxic tissue, which impairs the function of endocrine organs. Belhoul *et al.* found that patients who experienced splenectomy are related to hypogonadism and not dependent on a patient's ferritin levels, although the mechanism has not yet been explained. Liver damage, diabetes and hypothyroidism were reported to contribute to the prevalence of hypogonadism in the thalassemia population [17].

Correlation between pituitary MRI T2 time and serum ferritin, FSH, LH and testosterone levels and transferrin saturation

In this study, there was no correlation found between MRI T2 values and blood-iron status (serum ferritin and transferrin saturation). Similarly, no significant correlation was found between pituitary MRI T2 values and FSH, LH and testosterone levels. This shows that iron build-ups in tissue causes an irreversible impact, even though blood iron levels decreased. In their study on this subject, Chatterjee *et al.* asserted that minimal organ damage was seen in patients with slightly increased iron levels and that this would lead to reversible hypogonadotropic hypogonadism. To the contrary, irreversible hypogonadism was seen in patients with excessive iron levels [18].

Correlation between pituitary length and MRI T2 relaxation time, serum ferritin, FSH and LH levels and transferrin saturation

This study found a negative correlation between pituitary length and serum ferritin levels. The correlation between pituitary length and pituitary MRI T2 relaxation time was not found to be statistically significant. This is consistent with studies conducted by Cetincakmak *et al.* and Noetzli *et al.*; both studies reported that anterior pituitary height and pituitary volume did not correlate with T2* values. While iron build-ups in the pituitary gland can be reversible, the decrease in pituitary size is irreversible [12, 19].

There was no correlation also found on pituitary length with FSH and LH level. To date, there have been no studies conducted on the relation of pituitary length to FSH and LH levels.

Correlation between pituitary MRI T2 times and serum ferritin, FSH and LH levels and transferrin saturation in low-and normal-testosterone groups

To improve the analysis, patients in this study were divided into subgroups based on their testosterone levels. The first group consisted of subjects whose testosterone level was<11.54 ng/ml (300 nmol/l), and the testosterone level of the subjects in the second group was>11.54 ng/ml. There was a negative correlation between pituitary MRI T2 relaxation time and transferrin saturation values ($r = -0.424$, $p = 0.039$) in the normal-testosterone group; no such correlation was found in the low-testosterone group. There was a positive correlation between pituitary length and pituitary MRI T2 relaxation time in the normal-testosterone group; in the low-testosterone group, the correlation between these two factors was not significant, which is likely due to smaller pituitary size. The pituitary MRI T2 relaxation time examination is more challenging to conduct on patients with shorter pituitary length because it is more difficult to determine the ROI.

CONCLUSION

Based on the conducted study, high serum ferritin levels and transferrin saturation levels were found, but these levels did not correlate with FSH, LH and testosterone levels in male transfusion-dependent thalassemia subjects. Moreover, there was no correlations found between serum ferritin levels and MRI T2 relaxation times; transferrin saturation levels and pituitary MRI T2 relaxation times; and pituitary MRI T2 relaxation times and FSH, LH and testosterone levels in male transfusion-dependent thalassemia subjects.

We recommend that further research using a dynamic test (GnRH stimulation test) be conducted, followed with an evaluation of serial FSH and LH levels. In addition, a study with a control design should be conducted to observe changes in the pituitary structure and the impact of these changes on hormone levels and sexual development in transfusion-dependent thalassemia patients. Finally, further diagnostic-based research should be undertaken to determine the limits of hemosiderosis values in the pituitary gland.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES

1. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 y: the HIM study. Int J Clin Pract 2006;60:762–9.
2. Dandona P, Rosenberg MT. A practical guide to male hypogonadism in the primary care setting. Int J Clin Pract 2010;64:682–96.
3. Seftel A. Male hypogonadism. Part II: Etiology, pathophysiology, and diagnosis. Int J Impot Res 2006;18:223–8.
4. Taher AT, Musallam KM, Inati A. Iron overload: consequences, assessment, and monitoring. Hemoglobin 2009;33:S46–57.
5. Srisukh S, Ongphiphadhanakul B, Bunnag P. Hypogonadism in major thalassemia patients. J Clin Transl Endocrinol 2016;5:42–5.
6. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
7. Shalitin S, Carmi D, Weintrob N, Phillip M, Miskin H, Kornreich L, *et al.* Serum ferritin level as a predictor of impaired growth and puberty in major thalassemia patients. Eur J Haematol 2005;74:93–100.
8. Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. Int J Hematol 2008;88:7–15.
9. Hantrakool S, Tantiworawit A, Rattarittamrong E, Chai-Adisaksopha C, Nawarawong W, Srichairattanakool S, *et al.* Elevated serum ferritin levels are highly associated with diabetes mellitus and hypothyroidism in thalassemia patients. Blood 2012;120:5174.
10. Azarkeivan A, Hashemieh M, Shirkavand A, Sheibani K. Correlation between heart, liver and pancreas hemosiderosis measured by mri t2* among thalassemia major patients from Iran. Arch Iran Med 2016;19:96–100.
11. Wood JC. Magnetic resonance imaging measurement of iron overload. Curr Opin Hematol 2007;14:183–90.
12. Bozdag M, Bayraktaroglu S, Aydınok Y, Çallı MC. MRI assessment of pituitary iron accumulation by using pituitary-R2 in β-thalassemia patients. Acta Radiol 2017;59:732–9.
13. Abo-Elwafa HA, Hamid SA, Heshmat MM, Ahmed ZS. Impact of ferritin load on gonadal reserve among regular transfused β-thalassemia. OJBD 2017;7:65–78.
14. Habeb AM, Al-Hawsawi ZM, Morsy MM, Al-Harbi AM, Osilan AS, Al-Magamsi MS, *et al.* Endocrinopathies in beta-thalassemia major. Prevalence, risk factors, and age at diagnosis in Northwest Saudi Arabia. Saudi Med J 2013;34:67–73.
15. Wei C, Davis N, Honour J, Crowne E. The investigation of children and adolescents with abnormalities of pubertal timing. Ann Clin Biochem 2017;54:20–32.
16. Das N, Kumar TR. Molecular regulation of follicle-stimulating hormone synthesis, secretion and action. J Mol Endocrinol 2018;60:R131–R155.
17. De Sanctis V, Soliman AT, Yassin MA, Di Maio S, Daar S, Elsedfy H, *et al.* Hypogonadism in male thalassemia major patients: pathophysiology, diagnosis and treatment. Acta Biomed 2018;89:6–15.
18. Chatterjee R, Katz M. Reversible hypogonadotrophic hypogonadism in sexually infantile male thalassaemic patients with transfusional iron overload. Clin Endocrinol (Oxf) 2000;53:33–42.
19. Noetzli LJ, Panigraphy A, Mittelman SD, Hyderi A, Dongelyan A, Coates TD, *et al.* Pituitary iron and volume predict hypogonadism in transfusional iron overload. Am J Hematol 2012;87:167–71.