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ORIGINAL RESEARCH

The Effect of Serum Ferritin Level on Gonadal, Prolactin, Thyroid Hormones, and Thyroid Stimulating Hormone in Adult Males with Sickle Cell Anemia

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Background: Sickle cell anaemia (SCA) is an inherited hemoglobinopathy resulting in sickling of erythrocytes that cause micro-vascular obstruction leading to acute complications and chronic organ damage. Adults with SCA have endocrine complications and metabolic alterations. The aim of this study was to assess the association between gonadal and thyroid hormones with iron indices and to explore the potential association between serum ferritin levels and sex hormones in adult males with sickle cell disease in New Prince Saud Bin Jalawy Hospital (NPSBJH) in Hofuf city (Eastern region of Saudi Arabia) where there are many patients with SCA.

Methods: A cross-sectional analytical study was carried out in the Haematology Clinic at NPSBJH in 2018. A total of eighty (80) male patients with sickle cell anaemia were included in this study and were divided into two groups according to serum ferritin level. Group I (G-I): Included 40 male patients with high serum ferritin level and group II (G-II): included 40 male patients with normal serum ferritin level.

Results: There was a significant difference in height/cm between GI and GiI, P value= 0.006. Serum ferritin was significantly higher in GI (P value= 0.000), and serum TIBC was significantly higher in G-II. (P value= 0.022). Testosterone level was significantly higher in G-II (P value= 0.018). Luteinizing hormone (LH) was significantly higher in group I (P-value 0.019). There was a significant relation between serum ferritin level in G-I and the following: serum iron, TIBC, serum testosterone, LH, prolactin, free T3 and free T4.

Conclusion: Adult males with SCD with high serum ferritin level were shorter than adult males with SCD who had normal serum ferritin level and had a significant lower level of serum testosterone and significant high level of LH and this was most likely due to endocrine dysfunction secondary to high ferritin level and iron overload.

Keywords: sickle cell disease, ferritin, testosterone, prolactin

Introduction

Sickle cell anaemia (SCA) results from an inherited abnormality of human adult haemoglobin structure. The resultant abnormal haemoglobin is called Sickle haemoglobin (Hb SS) which has a glutamic acid to valine substitution at position six of the beta-globulin chain. Hb S is normally carrying oxygen however begins to produce intracellular polymers at low oxygen tension. This polymerization is the key event of SCA pathophysiology.[1,2] The SCA is characterized by haemolytic anaemia, intermittent

episodes of vaso-occlusion in microcirculation and organ dysfunction.[3] Although blood transfusion can prevent a number of SCA complications, it leads to iron overload in the body, besides the excess iron absorption due to anaemia.[4] Another factor for increased iron in SCA patients is haemolysis with subsequent recycling and accumulation of iron. In steady-state sickle cell anaemia, there are several studies reported an increase in serum ferritin level.[4,5] Ferritin is a protein found inside the cells of the body and only a small amount is secreted into the serum. It is well known in the literature that some factors, like liver disease or chronic inflammation, may increase serum ferritin level. The primary role of ferritin is the storage of iron and there is a positive correlation between the ferritin level and the total amount of body iron. Hence, serum ferritin is used as a reliable indicator of the total amount of iron in the body.[6] So, it is one of the most important tools in the measurement of the state of iron balance in steady-state sickle cell disease.[5] The damaging effect of SCA on endocrine organs is indirect, it occurs through chronic anaemia, tissue hypoxia, and iron overload.[7] The endocrine system dysfunction is reported as the most common and earliest complication of SCA.[8] Infertility appears to be a big problem among males than females with SCA. Delayed puberty, gonadal insufficiency and thyroidal disorders were frequently reported in patients with SCA.[8] It is well known that iron overload leads to gonadal atrophy. Therefore, this study aimed to assess the association between Thyroid hormones, luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Testosterone hormone, Prolactin hormone, and Thyroid-Stimulating Hormone (TSH) with serum ferritin level and other iron indices in males with SCA.

Patients and Method

A cross-sectional analytical study was carried out in the Hematology Clinic Department at the New Prince Saud Bin Jalawy Hospital (NPSBJH) between January and August 2018. A total of eighty (80) male patients with Sickle Cell Anemia (Hb SS) were included in our study and distributed into two groups according to the serum ferritin level. The normal range of serum ferritin level is 20 to 270 ng/mL in adult male, generally, the serum ferritin level of more than 300 ng/mL in male is considered as elevated.

Group I: Including 40 male patients had a serum ferritin level >300 ng/mL with their age ranged between 18 and 45 years.

Group II: Including 40 male patients had a serum ferritin level >20 < 270 ng/mL with their age ranged between 19 and 43 years.

Inclusion criteria:

- An adult male of age from 18 to 45 years.
- A patient diagnosed with SCA (Hb SS).
- Patients are on regular follow-up

Exclusion criteria:

- Heart failure
- Severe liver disease
- Renal failure.
- Blood transfusion within 4weeks prior to study entry
- Vaso-occlusive crisis within the last 4 weeks.
- Patient admitted with fever and infection or sepsis
- Patient on hydroxyurea
- Patient on chronic oral narcotics

Ethical Considerations

Approval for the study was obtained from the research and ethics committee at the hospital. The study was conducted in accordance with the Declaration of Helsinki, and informed written consent was obtained from all participants in the study.

All patients were subjected to the following:

Complete medical history was taken and complete clinical examination was done and blood tests were taken for complete blood count (CBC), iron profile (serum iron, serum ferritin, and total iron-binding capacity) and hormonal assay (serum testosterone, prolactin, LH, FSH, TSH-free T3 and T4 levels) as well as liver function test (ALT-AST- INR-PT) and renal function test (serum creatinine-blood urea).

Electrocardiogram.

Specimen Collection and Handling

Five mL of venous blood was withdrawn in a plastic syringe under complete aseptic condition using routine precautions observed in venipunctures and divided into two parts.

1. Two mL were added to ethylenediaminetetraacetic acid (EDTA) vacutainers for complete blood count (CBC) including differential white blood cells count which was done on a Leishman stained

peripheral blood smear with evaluation using a fully automated cell counter (Sysmex XN, Germany).

2. Three mL were taken in plain vacutainers and left to clot for 30 mins, then centrifugations were done at 1500 xg for 10 mins. Serum was separated for assessment of ferritin, FSH, LH, prolactin, testosterone, T3, T4 and TSH using chemical auto analyzer (Cobas 401), Germany and kits supplied by Roche Diagnostic Kits according to manufacturer instructions.

Complete blood count:

- White blood cell count (WBC) (N0. Range in adult 3.000–10.500 million cells/microliter) (mcL)
- Red blood cell count (RBCs) (N0. Range in adult 4.32–5.72 million cells/microliter) (mcL).
- Haematocrit (Hct)
- Haemoglobin (Hbg)
- Mean corpuscular volume (MCV) (N0. Range in adult male 80–100 femtoliter/red cell)
- Mean Corpuscular Hemoglobin (MCH) (N0. Range in adult male 27 – 31pg/red cell)
- Mean corpuscular Hemoglobin concentration (MCHC) (N0. Range in adult male 32–36 g/dl).

Iron profile:

- Serum Iron (No. range in adult male 35–15 µg/dL)
- TIBC (No. range in adult male 252–479 µg/dL)
- Serum Ferritin (No. in adult male 20 to 270 ng/mL)
- Liver function test (ALT-AST- INR-PT)
- Renal function test (serum creatinine-blood urea)

Hormonal assay:

- Thyroid-Stimulating Hormone (TSH) (N0. Range 0.4 mL/L- 4.0 µlU/mL in adult male).
- Free Thyroxin (free T4) (No. 9.00–26.00 pmol/L in the adult male).
- Free triiodothyronine (free T3) (No. 2.80–7.10 pmol/L in the adult male).
- Serum Prolactin hormone (No. <15 ng/mL in adult male).
- Luteinizing hormone (LH) (No. 1–10 mIU/mL in the adult male).
- Follicle-stimulating hormone (FSH) (No. 1.5–12.4 IU/L in the adult male).

- Testosterone hormone (No. range 2.70–10.0 ng/mL in male with 20 to 39 years) (No. range 3.50–8.90 ng/mL in male with 40 to 59 years).

Statistical Analysis
Data were analysed using SPSS version 20. Descriptive statistics was provided. Continuous data were reported in the form of mean, median, range, standard deviation. The t-test was used to assess the significant difference between continuous variables. A significant difference between categorical variables was assessed by using the Chi-squared test. The significance level was set at 95% and the P value was considered significant if <0.05%.

Results
The Characteristics of Data Between Groups
Table 1 shows the comparative study of the clinical and laboratory characteristics data between the study groups. The current study enrolled 80 male SCA (Hb SS) patients who were divided into; 40 patients with serum ferritin level above 300 ng/mL as Group I and 40 patients with normal serum ferritin level as Group II. There was no statistically significant difference between G-I and G-II with respect to the age/year (G-I: 31.09 ± 8.64 vs G-II: 31.89 ± 11.07, P-value = 0.682) and weight/Kg (G-I: 59.58 ± 13.84 vs G-II: 73.26 ± 10.33, P-value = 0.371). But there was a significant difference of height/cm between G-I (152.15 ± 4.94) vs G-II (154.79 ± 4.74), P value= 0.006.

Comparison of Groups Based on the Complete Blood Count
It was found that, there was a statistically significant difference between G-I and G-II as regarding the RBCs (G-I 3.73 ± 0.89 vs G-II 4.27 ± 0.84, P value = 0.002) and Hb g/dl (G-I: 9.25 ± 1.50 vs G-II: 10.12 ± 1.30, P-value = 0.002) as shown in Table 1.

While there was no statistically significant difference between G-I and G-II as regarding MCV/femtoliter (G-I: 73.26 ± 10.33 vs GII: 74.48 ± 9.71, P value= 531), MCH pg/red cell (G-I: 25.48 ± 4.01 vs G-II: 25.49 ± 3.96, P value=0.994), and MCHC g/dL (G-I: 34.00 ± 0.99 vs G-II: 33.80 ± 2.16, P value=0.553). Also, there was no significant difference between the median of platelet count 10^9/L in G-I 211 (175–400) and G-II (287 (181–410), P value=0.604, as shown in Table 1.

Table 1 Comparison Between the Two Groups of SCA Male Patients

Parameter	Group 1 (Ferritin>300 ng/mL)	Group II (Ferritin 20–270 ng/mL)	P value
Age in year (Mean ± SD)	31.09 ± 8.64	31.9 ± 11.07	0.682
Body weight/Kg (Mean ± SD)	59.58 ± 13.84	61.80± 11.50	0.371
Height/cm(Mean ± SD	152.15 ± 4.94	154.79 ± 4.74	0.006
RBCs/mcL (Mean ± SD)	3.73 ± 0.89	4.27 ± 0.84	0.002
Hb g/dl(Mean ± SD)	9.25 ± 1.50	10.12 ± 1.30	0.002
MCV/femtoliter(Mean ± SD)	73.26 ± 10.33	74.48 ± 9.71	0.531
MCH pg/red cell(Mean ± SD)	25.48 ± 4.01	25.49± 3.96	0.994
MCHC g/dL(Mean ± SD)	34.00 ± 0.99	33.80 ± 2.16	0.553
Platelet/10^9/L(Median)	211	287	0.604
Serum Iron µg/L(Mean ± SD)	12.81 ± 5.08	11.44 ± 4.89	0.159
TIBC/µg/dL(Mean ± SD)	54.25 ± 14.54	61.24 ± 16.39	0.022
Serum Ferritin/ng/mL (Median)	346.5	113.6	0
Testosterone/ng/mL(Mean ± SD)	4.11 ± 1.61	5.18 ± 2.85	0.018
FSH/IU/L(Mean ± SD)	5.15± 2.49	6.09 ± 3.37	0.105
LH mIU/mL (Mean ± SD)	8.3	4.14	0.019
prolactin ng/mL(Mean ± SD)	8.02± 2.96	8.65 ± 5.63	0.475
Free T3 pmol/L(Mean ± SD)	5.5 3± 1.72	5.38 ± 1.07	0.606
Free T4 pmol/L(Mean ± SD)	13.47 ± 2.65	12.39 ± 3.10	0.057
TSH µIU/mL(Mean ± SD)	2.0 3± 1.59	1.95 ± 0.98	0.734

Comparison of Groups Based on Iron Profile

In Table 1 the serum iron/µg/L was a non-significant difference between G-I (12.81 ± 5.08) and G-II (11.44 ± 4.89) (P value= 0.159). The median of serum Ferritin/ng/mL was significantly higher in G-I: 346.5 (312.1–590) than the median in G-II 113.6 (48.9–225.8) (P value= 0.000) and serum TIBC/µg/dL was significantly higher in G-II (61.24 ± 16.39) than G-I (54.25 ± 14.54) (P value= 0.022).

Comparison of Groups Based on Hormonal Assay

In Table 1 the testosterone ng/mL level was significantly higher in G-II (5.18 ± 2.85) than G-I (4.11 ± 1.61) (P value= 0.018). While the median (IQR) of LH mIU/mL was significantly higher in G-I: 8.3 (2.57–10.50) than G-II: 4.14 (3.50–4.99) (P value= 0. 019). The serum level of FSH/IU/L was the statistically non-significant difference when compared between G-I (5.15 ± 2.49) and G-II (6.09 ± 3.37) (P value= 0.105). Also, there was a non-significant difference of serum prolactin ng/mL level when compared between G-I (8.02 ± 2.96) and G-II (8.65 ± 5.63) (P value= 0.475).

Moreover, there were non-significant differences in free T3 pmol/L, free T4 pmol/L and TSH µIU/mL levels in G-I (5.53 ± 1.72, 13.47 ± 2.65, and 2.03 ± 1.59), respectively, when compared with G-II (5.38 ± 1.07, 12.39 ± 3.10, and 1.95 ± 0.98), respectively (P value=0.606, P value= 0.057, and P value= 0.734), respectively, as shown in Table 1.

The Correlation Study

Pearson's correlation showed a non-significantly association between serum ferritin level and serum Testosterone/ng/mL level (r= 0.181, P= 0.194), serum FSH/IU/L level (r= 0.124, P= 0.375), prolactin ng/mL level (r= 0.020, P= 0.889) and Free T4 pmol/L level (r= −0.206, P= 0.140) in G-II, as shown in Table 2. Also, there was a non-significantly association between serum ferritin level and serum FSH/IU/L level (r= 0.175, P= 0.211) in G-I, but there was significant relation between serum ferritin level in G-I and the followings: serum iron (r= 0.27, P=0.04), TIBC (r=0.3, P=0.01), serum testosterone (r= 0.32, P= 0.01), LH (r=−0.30, P=0.02), prolactin (r= 0.56, P=0.00), free T3 (r= 0.30, P=0.02), and free T4 (r=−0.37, P=0.006) as shown in Table 2.

Discussion

This cross-sectional analytical study which was carried out in the Hematology Clinic Department at the New Prince Saud Bin Jalawy Hospital (NPSBJH) consisted of eighty (80) male patients with Sickle Cell Anaemia (Hb SS) and were divided

Table 2 Correlation Between Serum Ferritin Level and Other Parameters Within Group I and Group II

Parameter	Group I (Ferritin>300 ng/mL)		Group II(Ferritin 20–270 ng/mL)	
	r	P-value	r	P-value
Serum Iron µg/L	0.274	0.047	0.534	0
TIBC/µg/dL	−0.34	0.013	−0.315	0.021
Testosterone/ng/mL	0.324	0.018	0.181	0.194
FSH/IU/L	0.175	0.211	−0.124	0.375
LH mIU/mL	−0.306	0.26	−0.516	0
prolactin ng/mL	0.582	0	−0.02	0.889
Free T3 pmol/L	0.301	0.029	−0.377	0.005
Free T4 pmol/L	−0.376	0.006	−0.206	0.14

into two groups according to serum ferritin level, group I (G I) with high serum ferritin level and group II (GII) with normal serum ferritin level. Each group consisted of 40 patients. It showed that the height of patients who had serum level of ferritin above 300ng/mL were significantly shorter than the control who had serum ferritin below 270 ng/mL, this shorter height could be due to abnormal endocrine function in these patients either secondary to iron overload which was reflected by high serum ferritin level or it could be repeated vasoocclusive crises in microcirculation leading to endocrine glands dysfunction. Also, it was reported that both red cell count and Hb level were significantly lower in the same group with shorter height. This was in keeping with several studies that showed decreased height in men with SCA, Friedman et al in 1974, Olambiwonnu et al in 1975, Platt et al study in 1984 and Zemel et al in 2007where they found that 38% of patients with SCA were below the 5th percentile for height.[9] Ferritin was significantly higher in G-I compared to G-II (P value= 0.000), and serum TIBC was significantly higher in G-II. (P value= 0.022). This high serum ferritin level could be explained by increased iron absorption due to anaemia or it could be due to increased haemolysis in these SCD patients with subsequent recycling and accumulation of iron or it could be due to repeated blood transfusion in these patients, anyway this could be assumptive rather than conclusive. This was similar to the study carried out by Hagag et al in Egypt in females with SCA.[10] Testosterone level was significantly higher in G-II than G-I (P value= 0.018), while Luteinizing hormone (LH) was significantly higher in group I (P-value 0.019), the lower level of testosterone in GI (SCA with high ferritin level) could be attributed to gonadal hypo-function in patients

with SCA secondary to iron overload or it could be due to intra-vascular sickling vasoocclusive occlusion infraction or hypoxia associated with chronic anaemia causing testicular failure. This was in keeping with the result of the study carried out by Garadah et al in Bahrin in adult patients with SCA where they found low serum testosterone level,[11] and also similar to the result of the study done by Ezeiruaku F.C in Nigeria where he found significant low level of testosterone in patients with SCA compared to control.[12] It was also similar to the results of the study done by Martins et al in 2015, were they found low testosterone level in males with SCA.[9] The levels of follicle-stimulating hormone (FSH), prolactin hormone, free T3, free T4 and thyroid-stimulating hormone (TSH) were statistically non-significant between the two groups. Pearson's correlation showed a significant relation between serum ferritin level in GI and the followings: serum iron (r= 0.27, P=0.04), TIBC (r=0.3, P=0.01), serum testosterone (r= 0.32, P= 0.01), LH (r=−0.30, P=0.02), prolactin (r= 0.56, P=0.00), free T3 (r= 0.30, P=0.02), and free T4 (r=−0.37, P=0.006), this was similar to the result of the study done by Garadah et al in Bahrin in adult patients with SCA where they found, low serum T4 level.[11] This was also similar to the study done by Ozen et al in 2013 among males with SCA, where they found a low free T4 level with normal TSH.[9] There was a non-significant association between serum level of ferritin and serum levels of Testosterone/ng/mL l FSH/IU/L, prolactin and Free T4 in G-II. Also, there was a non-significantly association between serum ferritin level and serum FSH/IU/L level in GI.

Conclusion

From this study, we can conclude that adult males with SCD Who had high serum ferritin level were shorter than adult males with SCD who had normal serum ferritin level and they had also significant lower level of serum testosterone and significant high level of LH and this was most likely due to endocrine dysfunction secondary to high ferritin level and iron overload, anyway, these results were not conclusive and ferritin was an only indicator of iron overload and other factors like vaso-occlusive occlusion, infraction or hypoxia associated with chronic anaemia could be responsible for endocrine dysfunction in patients with SCD. There was a significant relation between serum ferritin level in patients with SCA and high ferritin level and the following: serum iron, TIBC, serum testosterone, LH, prolactin, free T3 and free T4. These results could have serious impact on the fertility of male with SCD.

Recommendations

Male patients with SCA with high ferritin level may have gonadal hormone deficiency, so they should be subjected to regular medical follow-up with good monitoring to iron profile studies and receiving chelating agents for iron to prevent gonadal damage. More researches are needed to address if disease severity affects hormonal levels in patients with SCA.

Disclosure

The authors report no conflicts of interest in this work.

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