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Assessment of Thyroid Hormones and Vitamin D Levels in Patients with Hereditary *Hemoglobinopathies*

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***Abstract*- Background: Hemoglobinopathies are ultimately developed many complications with a high of morbidity, secondary to iron overload. Thyroid dysfunction and bone disorders are the most common clinical manifestations.**
Methods: A case control study that included (140) patients with hemoglobinopathies and (50) healthy participants as a control. Anthropometric and biochemical measurements were estimated for all participants by standard methods.
Results: This study were shown no significant differences (p > 0.05) for vitamin D, calcium, parathyroid hormone (PTH), thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroid hormone (FT4). On other hand, showed significant (p <0.05) increase levels of phosphorus and ferritin in patient's group comparison with control.
Conclusions: There were high level of ferritin; while, normal levels of thyroid hormones, vitamin D and parathyroid hormone in patients with hemoglobinopathies in comparison with control. There was risk of progression to overt state as a frequent complication of iron overloaded. Ferritin represents a prognostic marker for hemoglobinopathies and a predictive factor for organs dysfunction progression.

Keywords: *Thyroid hormones, Parathyroid hormone, Vitamin D, Hemoglobinopathies.*

List of Abbreviates

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, FT3: Free triiodothyronine, FT4: Free thyroxine, PTH: Parathyroid hormone, SCD: Sickle cell disease, β-TM: β-thalassemia major, TSH: Thyroid stimulating hormone, BMI: body mass index

I. INTRODUCTION

Inherited hemoglobin disorders represent a significant health problem. Thalassemia major is a hereditary blood disease characterized by reduced or absent production of β-globin chains. Sickle cell anemia (SCA) is a hereditary blood disease, which results from point mutation replace of thymine with adenine in one of the amino acids forming the globin chain of hemoglobin (Lahhob, Mohammed, and Abbas, 2021). However, multiple red blood cell transfusions over a long period of time with poor compliance to chelation therapy result in iron overload, which causes increased morbidity and mortality in those patients, including damage to the heart, endocrine glands, pancreas and liver. Despite the improved survival of β-thalassemia major (β-TM) patients, especially with optimum transfusion therapy and iron chelation, complications are still common (Omar and Ismail, 2017; Wu *et al.*, 2017). Vitamin D affects bone mineralization, through its stimulation of intestinal calcium and phosphorus absorption. Vitamin D deficiency or hypoparathyroidism play a role in causing hypocalcemia in patients with hemoglobinopathies (Abbassy *et al.*, 2019). Hypoparathyroidism can lead to hypocalcemia and osteoporosis. Osteopenia in hemoglobinopathy patients is multifactorial and is mainly predisposed by defective function of the parathyroid gland, vitamin D deficiency and excessive iron deposition (De Sanctis *et al.*, 2018). The etiology of thyroid disorders in hemoglobinopathy patients is substantially different from that of the general population. Therefore, the knowledge of risk factors influencing the development of hypothyroidism is a critical component for long-term monitoring and treatment of those patients (De Sanctis *et al.*, 2019).

The aims of this study were to assess the levels of thyroid hormones and bone related biomarkers (parathyroid hormone, vitamin D, calcium and phosphorus) in hereditary hemoglobinopathy patients. In addition to assessment organs disfunctions and risk stratifications in these patients.

II. MATERIALS AND METHODS

A case control study that included 140 patients with hemoglobinopathies (50 with β- thalassemia major, 40 with β-thalassemia intermediate, and 50 with sickle cell anemia), who attended hemoglobinopathies Center in Basrah Governorate, Iraq; throughout the period from October 2021

to March 2022, in addition to age match 50 healthy volunteers as a control group.

All patients were diagnosed by specialist physicians, which depended on clinical and laboratory investigations. The patient's ages ranged from (5-18) years. Old ages or other acute and chronic diseases, such as diabetes, renal and liver diseases were excluded.

The blood samples were collected in a gel tube by venipuncture, from all participants, and left for 15 minutes to clot at room temperature. Then, the blood samples were centrifuged at 4000 rpm for 10 minutes. Serums were kept in the freezer at (-20ºC) until use.

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Spectrophotometry kits (Biolabo/France) were used for measuring serum of urea (Urease/Salycilate Enzymatic method), creatinine (enzymatic method), calcium (orthocresolphthalein complexone) and phosphorus (ammonium molybdate); while, chemiluminescence technique were used for measuring serum ferritin, vitamin D, PTH, TSH, FT4 and FT3 determinations by Cobas E411 (Roche/Germany).

Statistical analysis was performed by using a statistical package for social sciences (SPSS) version 26. One way ANOVA test and chi-square test were used. Data were expressed as mean and standard deviations (SD). $P < 0.05$ was considered statistically significant.

III. RESULTS

The demographic characteristics of patients and control were shown in (Table 1). There were no significant differences in means of age and gender among study groups ($p = 0.239$). While there was a significant difference in BMI. It is a measure of body fat based on height and weight that applies to adult men and women.

TABLE 1: Demographic characteristics of control subjects and patients with hemoglobinopathies.

Variables	Control $n = 50$ Mean ±SD	SCA $n = 50$ Mean ±SD	B-TM $n = 50$ Mean ±SD	B-TIM $n = 40$ Mean ±SD	*P. value*
Age (years)	10.3 ± 3.5	10.9 ± 3.4	11.1 ± 3.4	9.7 ± 3.7	0.239
Gender					
Males, no. (%)	24 (47.5 %)	23 (46 %)	27 (54 %)	21 (52.5 %)	0.839
Females, no. (%)	26 (52.5 %)	27 (54 %)	23 (46 %)	19 (47.5 %)	
Weight					
BMI (kg/m^2)	20.8 ± 2.5	17.3 ± 6.1	18.1 ± 7.6	15.8 ± 2.5	< 0.001

P-value less than 0.05 was consider significant
SD: standard deviations, BMI: body mass index

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There were no significant differences (p. value >0.05) in the means values of each of vitamin D, urea, creatinine, TSH, FT4, FT3, and calcium in SCA, β-TM and β- TIM, when compared with control. While, there were significant differences (p. value < 0.01) in the means of each of PTH, ferritin, phosphorus, ALT, AST and LDH in SCA, β-TM and β- TIM when compared with control, as shown in Table 2.

TABLE 2: Comparison of mean values of parameters in control and patients with hemoglobinopathies

Variables	Control and SCA			Control and B-TM			Control and B-TIM		
	Control n = 50 Mean ±SD	SCA n = 50 Mean ±SD	P. Value	Control n = 50 Mean ±SD	B-TM n = 50 Mean ±SD	P. value	Control n = 50 Mean ±SD	B-TIM n = 40 Mean ±SD	P. Value
Vit D (ng/mL)	23.4±12.3	21.7±9.2	0.82	23.4±12.3	23.7±7.0	0.944	23.4± 12.3	24.9±9.6	0.869
Urea (mg/dL)	20 ± 8.33	17 ± 5.44	0.275	20 ± 8.33	17 ± 6.74	0.613	20 ± 8.33	16 ± 5.36	0.232
Creatinine (mg/dL)	0.49 ± 0.3	0.46 ± 0.9	0.994	0.49 ± 0.3	0.45 ± 0.11	0.983	0.49 ± 0.3	0.45 ± 0.9	0.991
TSH (mIU/L)	0.8 ±0.42	0.95 ± 0.78	0.547	0.8 ± 0.42	0.87 ± 0.7	0.883	0.8 ± 0.42	0.73 ± 0.46	0.979
FT4 (pmol/l)	16.3 ±3.55	16.8 ± 4.5	0.795	16.3 ± 3.55	16.6 ± 3.8	0.987	16.3 ± 3.55	14.6± 4.0	0.821
FT3 (nmol/l)	4.6 ± 1.04	4.6 ± 1.05	0.995	4.6 ± 1.04	4.4 ± 1.01	0.664	4.6 ± 1.04	4.3 ± 0.94	0.503
Ca (mg/dL)	9.6 ±0.64	9.24 ± 0.86	0.25	9.6 ± 0.64	9.28 ± 0.9	0.363	9.6 ± 0.64	9.13 ± 1.12	0.095

PTH (ng/mL)	36.8 ±17.5	29.6 ± 13.6	0.076	36.8 ± 17.5	32.6 ± 12.8	0.54	36.8± 17.5	27.8 ± 11.2	0.023
Ferritin (ng/mL)	66.05 ±80.16	3637 ± 1927	<0.01	66.05±80.16	3365±1760	<0.01	66.05±80.16	3794± 2097	<0.01
Phosphorus (mg/dL)	3.65 ± 0.5	4.4 ± 0.87	<0.01	3.65 ± 0.50	4.57 ± 0.88	<0.01	3.65 ± 0.50	4.65 ± 0.78	<0.01
Hb (g/dL)	11.5 ±1.49	9.2 ± 1.34	<0.01	11.5 ±1.49	9.2 ± 1.15	<0.01	11.5 ±1.49	9.0 ± 1.68	<0.01
ALT (U/L)	14.4 ± 4.12	20.9 ± 7.5	<0.01	14.4 ± 4.12	19.06 ± 6.96	<0.01	14.4 ± 4.12	20.4 ± 7.45	<0.01
AST (U/L)	15.24 ± 5.13	32.8 ± 11.77	<0.01	15.24± 5.13	32.5 ± 8.78	<0.01	15.24 ± 5.13	27.5 ± 6.29	<0.01
LDH (U/L)	95 ± 24	115 ± 17	<0.01	95 ± 24	120 ± 23	<0.01	95 ± 24	119 ± 19	<0.01

B-TM: *beta thalassemia major*; ***B-TIM:*** *beta thalassemia intermediate*; **SCA:** sickle cell anemia; ***n*:** number of cases; **SD:** standard deviation.

There were no significant differences (p. value > 0.05) in the means values of all parameters among patients' groups; accept, AST between B-TIM groups and B-TM and SCA groups, Table 3.

TABLE 3: Comparison of mean values of biomarkers among hemoglobinopathies groups

Variables	B-TM and SCA			B-TM and B-TIM			SCA and B-TIM		
	B-TM n = 50 Mean ±SD	SCA n = 50 Mean ±SD	P. Value	B-TM n = 50 Mean ±SD	B-TIM n = 40 Mean ±SD	P. Value	SCA n = 50 Mean ±SD	B-TIM n = 40 Mean ±SD	P. Value
Vitamin D	**23.7±7.0**	**21.7±9.2**	**0.989**	**23.7±7.0**	**24.9±9.6**	**0.58**	**21.7±9.2**	**24.9±9.6**	**0.389**
Urea	**17 ± 6.74**	**17 ± 5.44**	**0.937**	**17 ± 6.74**	**16 ± 5.36**	**0.878**	**17 ± 5.44**	**16 ± 5.36**	**0.997**
Creatinine	**0.45 ± 0.11**	**0.46 ± 0.9**	**>0.99**	**0.45 ± 0.11**	**0.45 ± 0.9**	**>0.99**	**0.46 ± 0.9**	**0.45 ± 0.9**	**>0.99**
TSH	**0.87 ± 0.7**	**0.95 ± 0.78**	**0.933**	**0.87 ± 0.7**	**0.73 ± 0.46**	**0.698**	**0.95 ± 0.78**	**0.73 ± 0.46**	**0.356**
FT4	**16.6 ± 3.8**	**16.8 ± 4.5**	**0.935**	**16.6 ± 3.8**	**14.6± 4.0**	**0.641**	**16.8 ± 4.5**	**14.6± 4.0**	**0.309**
FT3	**4.4 ± 1.01**	**4.6 ± 1.05**	**0.798**	**4.4 ± 1.01**	**4.3 ± 0.94**	**0.988**	**4.6 ± 1.05**	**4.3 ± 0.94**	**0.64**
Calcium	**9.28 ± 0.9**	**9.24 ± 0.86**	**0.996**	**9.28 ± 0.9**	**9.13 ± 1.12**	**0.859**	**9.24 ± 0.86**	**9.13 ± 1.12**	**0.939**
PTH	**32.6 ± 12.8**	**29.6 ± 13.6**	**0.698**	**32.6 ± 12.8**	**27.8 ± 11.2**	**0.375**	**29.6 ± 13.6**	**27.8 ± 11.2**	**0.936**
Ferritin	**3365±1760**	**3637 ± 1927**	**0.835**	**3365±1760**	**3794 ±2097**	**0.595**	**3637 ± 1927**	**3794 ± 2097**	**0.968**
Hb	**9.2 ± 1.15**	**9.2 ± 1.34**	**0.99**	**9.2 ± 1.15**	**9.0 ± 1.68**	**0.892**	**9.2 ± 1.34**	**9.0 ± 1.68**	**0.974**
Phosphorus	**4.57 ± 0.88**	**4.4 ± 0.87**	**0.664**	**4.57 ± 0.88**	**4.65 ± 0.78**	**0.962**	**4.4 ± 0.87**	**4.65 ± 0.78**	**0.402**
ALT	**19.06 ±6.96**	**20.9 ± 7.5**	**0.502**	**19.06 ±6.96**	**20.4 ± 7.45**	**0.782**	**20.9 ± 7.5**	**20.4 ± 7.45**	**0.981**
AST	**32.5 ± 8.78**	**32.8 ±11.77**	**>0.99**	**32.5 ± 8.78**	**27.5 ± 6.29**	**0.036**	**32.8 ± 11.77**	**27.5 ± 6.29**	**0.021**
LDH	**120 ± 23**	**115 ± 17**	**0.598**	**120 ± 23**	**119 ± 19**	**0.976**	**115 ± 17**	**119 ± 19**	**0.87**

B-TM: *beta thalassemia major*; ***B-TIM:*** *beta thalassemia intermediate*; **SCA:** sickle cell anemia; ***n*:** number of cases; **SD:** standard deviation.

There were highly significant prediction values of phosphorus (odd Ratio= 4.685), AST (odd Ratio=1.36) and ALT (odd Ratio= 1.21) to predict risk in SCA, Table 4.

TABLE 4: Multivariable logistic regression analysis of clinical and biochemical markers to predict risk in SCA patients

Variable	Regression Coefficients	Standard Error	P-value	Odd Ratio	Lower 95% confidence limits	Upper 95% confidence limits
Gender	0.08	0.401	0.841	1.084	0.494	2.377
Age	0.141	0.08	0.078	1.151	0.985	1.346
BMI	-0.193	0.06	<0.01	0.825	0.734	0.927
TSH	0.459	0.353	0.194	1.583	0.792	3.164
FT3	-0.045	0.197	0.821	0.956	0.65	1.407
FT4	0.048	0.052	0.355	1.049	0.947	1.162
PTH	-0.029	0.014	0.035	0.972	0.946	0.998
Vit. D	-0.015	0.019	0.449	0.985	0.949	1.023
Ferritin	0.082	27.791	<0.01	1.085	1.045	1.125
Phosphorus	1.544	0.365	<0.01	4.685	2.293	9.574
Ca	-0.61	0.297	0.04	0.544	0.304	0.973
Hb	-1.477	0.328	<0.01	0.228	0.12	0.434
AST	0.307	0.07	<0.01	1.36	1.186	1.559
ALT	0.19	0.044	<0.01	1.21	1.109	1.32
Urea	-0.055	0.033	0.094	0.947	0.888	1.009
Creatinine	-0.18	0.895	0.841	0.835	0.145	4.824
LDH	0.059	0.014	<0.01	1.061	1.032	1.091

The regression analysis of clinical and biochemical markers to predict risk in β- TM was showed in Table 5. There were significant (p- value < 0.05) prediction values to predict risk in patient with β- TM for each of age (odd Ratio= 1.182), ferritin (odd Ratio= 1.196), phosphorus (odd Ratio= 5.84), AST (odd Ratio= 1.366) and ALT (odd Ratio= 1.172), LDH (odd Ratio= 1.062).

TABLE 5: Identification of risk with multivariable logistic regression analysis in β- TM

Variables	Regression Coefficients	Standard Error	P-value	Odd Ratio	Lower 95% confidence Limits	Upper 95% confidence limits
Gender	-0.24	0.401	0.549	0.786	0.358	1.725
Age	0.167	0.081	0.039	1.182	1.009	1.386
BMI	-0.141	0.06	0.018	0.869	0.773	0.976
TSH	0.283	0.358	0.43	1.327	0.657	2.677
FT3	-0.231	0.203	0.254	0.794	0.533	1.181
FT4	0.02	0.056	0.719	1.02	0.914	1.139
PTH	-0.016	0.013	0.22	0.984	0.958	1.01
Vit. D	-0.011	0.02	0.606	0.99	0.951	1.03
Ferritin	0.179	72.017	<0.01	1.196	0	

Phosphorus	1.765	0.369	<0.01	5.84	2.832	12.043
Ca	-0.494	0.277	0.074	0.61	0.355	1.049
Hb	-1.777	0.398	<0.01	0.169	0.078	0.369
AST	0.312	0.065	<0.01	1.366	1.204	1.55
ALT	0.159	0.045	<0.01	1.172	1.072	1.281
Urea	-0.031	0.028	0.279	0.97	0.918	1.025
Creatinine	-0.249	0.876	0.776	0.779	0.14	4.34
LDH	0.06	0.014	<0.01	1.062	1.033	1.091

The regression analysis of clinical and biochemical markers to predict risk in β-TIM was showed in Table 6. There were highly significant (p value < 0.01) prediction values to predict β-TIM for ferritin (odd Ratio= 1.196), phosphorus (odd Ratio= 9.014) and AST (odd Ratio= 1.423), Table 6.

TABLE 6: Identification of risk with multivariable logistic regression analysis in beta- thalassemia intermediate patients

Variables	Regression Coefficients	Standard Error	P-value	Odd Ratio	Lower 95% confidence Limits	Upper 95% confidence limits
Gender	-0.314	0.302	0.299	0.731	0.404	1.32
Age	-0.024	0.021	0.253	0.976	0.937	1.017
BMI	-0.785	0.16	<0.01	0.456	0.333	0.624
TSH	-0.288	0.504	0.568	0.75	0.279	2.015
FT3	-0.319	0.224	0.154	0.727	0.469	1.127
FT4	-0.054	0.059	0.355	0.947	0.844	1.063
PTH	-0.04	0.016	0.012	0.961	0.931	0.991
Vit. D	0.016	0.022	0.472	1.016	0.973	1.061
Ferritin	0.194	74.2	<0.01	1.214	1	1.428
Phosphorus	2.199	0.452	<0.01	9.014	3.719	21.848
Ca	-0.567	0.26	0.029	0.567	0.341	0.945
Hb	-1.593	0.39	<0.01	0.203	0.095	0.436
AST	0.353	0.076	<0.01	1.423	1.226	1.651
ALT	0.181	0.047	<0.01	1.198	1.093	1.314
Urea	-0.059	0.035	0.087	0.942	0.88	1.009
Creatinine	-0.2	0.907	0.826	0.819	0.139	4.841
LDH	0.061	0.015	<0.01	1.063	1.033	1.094

In order to evaluate the diagnostic performance of biochemical markers in patients with SCA, receiver operator characteristic (ROC) curve analysis was performed. The results of serum LDH, AST, ALT, phosphorus and ferritin were expressed high validity (sensitivity and specificity). Ferritin was considered as gold standard test or test of choice as were showed in Table 7.

TABLE 7: Characteristics of biochemical markers as predictors of sickle cell anemia diagnosis

Variables	AUC	95% CI	Cut-off	Sensitivity %	Specificity %	PPV	NPV	P. value
TSH	0.515	0.413 -0.617	<0.97	78	36	54.9	62.1	0.794
Ft3	0.516	0.413 - 0.617	≤3.6	80	26	51.9	56.5	0.791
Ft4	0.552	0.449 - 0.652	≤20.1	86	38	58.1	73.1	0.378
PTH	0.615	0.513 - 0.711	<32	68	56	60.7	63.6	0.042
Vit D	0.531	0.428 - 0.631	<19.7	46	68	59	55.7	0.601
Ferritin	1.00	0.964 - 1.000	>75	100	100	100	100	<0.01
Phosphorus	0.803	0.711 - 0.876	>4	78	84	83.0	79.2	<0.01
Calcium	0.606	0.504 - 0.703	≤9.6	66	60	62.3	63.8	0.065
Hb	0.865	0.782 - 0.925	≤10.8	98	60	71	96.8	<0.01
AST	0.946	0.882 -0.981	>19.3	92	96	95.8	92.3	<0.01
ALT	0.773	0.679 -0.851	>17	68	80	77.3	71.4	<0.01
Urea	0.585	0.482 -0.683	≤14	36	84	69.2	56.8	0.139
Creatinine	0.519	0.417 -0.620	>0.58	4	58	8.7	37.7	0.778
LDH	0.887	0.808 -0.941	>99	100	84	86.2	100.0	<0.01

AUC: area under the curve; **CI**: confidence interval; **PPV**: positive predictive value; **NPV**: negative predictive value.

The ROC curve analysis of β-TM was showed in Table 8. The results of serum LDH, AST, ALT, phosphorus and ferritin were expressed high validity. Also, ferritin was considered as gold standard test.

TABLE 8: Characteristics of biochemical markers as predictors of β-TM

Variables	AUC	95% CI	Cut-off	Sensitivity %	Specificity %	PPV	NPV	P. value
TSH	0.534	0.432 - 0.635	≤0.4	34	82	65.4	55.4	0.564
Ft3	0.562	0.460 - 0.661	≤3.6	32	80	61.5	54.1	0.279
Ft4	0.501	0.399 - 0.602	≤15.7	42	46	43.7	44.2	0.991
PTH	0.546	0.443 - 0.645	≤57.1	94	22	54.7	78.6	0.437
Vit D	0.502	0.401 to 0.604	>11.8	94	24	55.3	80	0.967
Ferritin	1.00	0.964 - 1.000	>75	100	100	100	100	<0.01
Phosphorus	0.818	0.728 - 0.888	>4	76	84	82.6	77.8	<0.01
Calcium	0.593	0.490 - 0.690	≤8.4	22	100	100	56.2	0.105
Hb	0.875	0.794 to 0.933	<10.8	100	60	71.4	100	<0.01
AST	0.947	0.883 - 0.982	>19.3	90	96	95.7	90.6	<0.01
ALT	0.700	0.600 - 0.787	>19	44	88	78.6	61.1	<0.01
Urea	0.550	0.447 -0.649	≤14	38	84	70.4	57.5	0.394
Creatinine	0.510	0.408 - 0.611	>0.22	100	36	61	100	0.877
LDH	0.890	0.812 - 0.944	>99	100	84	86.2	100	<0.01

AUC: area under the curve; **CI**: confidence interval; **PPV**: positive predictive value; **NPV**: negative predictive value.

The ROC analysis of β- TIM was performed and the results were showed in Table 9. Also, serum LDH, AST, ALT, phosphorus and ferritin were expressed high validity, and ferritin was considered as gold standard test.

TABLE 9: Characteristics of biochemical markers as predictors of beta thalassemia intermediate diagnosis

Variable	AUC	95 % CI	Cut-off	Sensitivity %	Specificity %	PPV	NPV	P. value
TSH	0.580	0.471 - 0.683	<0.5	60	70	61.5	68.6	0.213
Ft3	0.584	0.476 -0.687	≤4.2	47	68	54.3	61.8	0.164
Ft4	0.567	0.458 - 0.671	≤14	45	78	62.1	63.9	0.299
PTH	0.639	0.530 - 0.737	<27.1	67.5	60	57.4	69.8	0.019
Vit D	0.583	0.475 - 0.687	>20.4	82.5	46	55.0	76.7	0.172
Ferritin	1.00	0.960- 1.000	>75	100	100	100	100	<0.01
Phosphorus	0.854	0.764 - 0.919	>4	85	84	81.0	87.5	<0.01
Calcium	0.580	0.471 - 0.683	≤8.5	27.5	96	84.6	62.3	0.201
Hb	0.867	0.779 - 0.929	≤10.7	100	60	66.7	100	<0.01
AST	0.936	0.864 - 0.977	>19.3	87.5	96	94.6	90.6	<0.01
ALT	0.739	0.635 - 0.826	>18	60	84	75.0	72.4	<0.01
Urea	0.585	0.477 - 0.688	≤15	50	68	55.6	63	0.164
Creatinine	0.514	0.406 - 0.620	>0.3	100	38	56.3	100	0. 837
LDH	0.886	0.802 - 0.943	>100	100	88	87.0	100	<0.01

AUC: area under the curve; **CI**: confidence interval; **PPV**: positive predictive value; **NPV**: negative predictive value.

IV. DISCUSSION

The findings of this study were showed no significant differences of gender between patients and control groups, (Table 1); which agree with (Jorgensen et al., 2019).

Vitamin D showed no significant difference level in B-TM compared to the control groups, (Table 2). The result of this study had similar findings to previous studies (Darvishi-Khezri *et al*. 2020; Wong *et al*. 2013). While, this study wasnot matched (Bordbar *et al*., 2019). Vitamin D is critical for calcium homeostasis and mineralization of the skeleton, especially during periods of rapid growth (infantile and pubertal growth periods). Vitamin D deficiency leads to rickets and osteomalacia *(Soliman et al*., 2013). Thalassemia is a cause of secondary osteoporosis; therefore, age limitations are less relevant in this context but still, the lack of measurements in middle-aged patients makes the estimation of the actual burden of bone disease in patients with thalassemia even more challenging. The frequency of vitamin D insufficiency in patients with thalassemia has been shown to be high in every single trial shortlisted in the review (Bordbar *et al*., 2019).

In this study, serum PTH level was not significantly different between B-TM and control, Table 2. This agreed with previous study (Sharma *et al.*, 2016). The incidence of hypo parathyroid varies from 0% to 22.5% of (Sharma *et al*., 2016). Majority of cases of hypoparathyroidism in Beta- thalassemic children were seen in second decade of life. Parathyroid hormone levels are chiefly regulated by alteration in calcium levels in the body. Hence, it is suggested that supplementation with vitamin D and calcium would greatly help in normalization of function of parathyroid gland and serum parathyroid hormone levels (Kumar *et al* 2018).

Also, vitamin D was shown no significant difference when compared B-TIM group with the control (Table 2). The result of this study had similarity with previous studies (Darvishi-Khezri *et al.*, 2020, Soliman *et al.*, 2013). Beta- thalassemia intermediate is a form of non-transfusion dependent thalassemia, although they may be needed occasionally in certain clinical conditions. Vitamin D deficiency, frequently reported in B-TIM populations (Baldini *et al.*, 2014). Nonetheless, bone demineralization of varying severity and low 25OH-vitamin D levels were present in most cases.

Although the mean of vitamin D was lower in patients with SCA than control group; but there was no significant difference, (Table 2). The result of this study had similarity with previous study (Melchionda *et al*., 2013); while, disagree with previous study (Nolan *et al*., 2015).

Thyroid estimation hormone, free triiodothyronine and free thyroid hormone were showed no significant differences in B-TM when compared to the control, (Table 2). The result of this study had similarity with

previous studies (Al-Naama, *et al*., 2020); on other hand, disagreed with (Singhal and Goyal, 2020). Transfusional iron overload and increased iron uptake from the gastrointestinal tract causes deposition of iron in the thyroid gland, with consequent fibrosis of the glandular parenchyma, and progressive thyroid dysfunction going through different degrees of severity up to overt hypothyroidism. Thyroid dysfunction is known to occur frequently in thalassemia major, but its prevalence and severity varies in different cohorts (Kurtoglu, and Temizkan 2012). Iron overload are implicated in over 90% of morbidity and mortality in patients with B-TM. Therefore, the knowledge of risk factors influencing the development of hypothyroidism represents a critical component of long-term monitoring and treatment of patients affected by B-TM (De Sanctis *et al.*, 2019). The prevalence of hypothyroidism in thalassemia major patients ranges from 3.3% to 24.4% in various countries. Some studies had reported a high prevalence of primary hypothyroidism reaching up to 17%–18% (Singhal and Goyal 2020).

Thyroid estimation hormone, free triiodothyronine, free thyroid hormone levels and parathyroid hormone were showed no significant differences between B-TIM and control, (Table 2). The result of this study had similarity to (Yassin *et al*. 2019). The most frequent endocrine complications reported in B-TIM are growth retardation, delayed puberty, hypogonadism, diabetes, impaired thyroid, parathyroid and adrenal functions, and dyslipidemias (Inati *et al.*, 2015). Iron overload in B-TIM patients and deposition of iron in the thyroid gland with consequent fibrosis of the glandular parenchyma may play a role in the development of thyroid dysfunction of variable degrees of severity, reaching up to overt hypothyroidism (Abdel-Razek *et al.*, 2013). The mean of thyroid and parathyroid hormones within the normal level, the reason may be the young age (<18 years) which taken in current study; therefore, the iron deposition rate is less. As in the previous study, which suggested the reason that the rate of hormones is within the normal range due to the young age of the patients; also, the B-TIM patients with milder anemia than patients with β-thalassemia major, so the complication ineffective erythropoiesis, chronic hemolytic anemia, and iron overload are less than B-TM (Zekavat1 *et al.*, 2014).

Thyroid estimation hormone, free triiodothyronine, free thyroid hormone levels and parathyroid hormone were showed no significant difference level in SCA compared to the control, (Table 2). The prevalence of hypothyroidism ranged between 2-6% of patients in different studies. Increase TSH response to TSH-releasing hormone in SCD compared with controls and thus were suggestive of primary thyroid failure. In a previous study, children and adolescents with SCD showed increased incidence of hypothyroidism (6%) of both central and primary hypothyroidism. However, other study reported normal thyroid function in children with SCD (Özen *et al*., 2013). The etiology of thyroid dysfunction in SCD is not clear; however, most affected patients have received multiple transfusions consistent with severe iron overload. Autopsy reports in some patients have shown significant iron deposition in the thyroid gland, suggesting that the etiology of the primary thyroid failure might well be transfusional hemosiderosis and subsequent cellular damage to the thyroid gland. The result of this study had similarity with previous studies (Soliman *et al.* 2017); while, disagreed with (Garadah *et al.*, 2016).

In B-TM group, calcium level showed no significant difference level compared to the control group, (Table 2). This study was agreed with previous studies (Arman Bilir *et al.*, 2020); but, disagreed with (Nafady *et al.*, 2018; Şahin *et al.*, 2020), who reported that vitamin D deficiency and low level of parathyroid hormone are main factors that cause the low level of Ca^{2+} in thalassemia major patients. In this study the opposite results for calcium, parathyroid hormone and vitamin D, and the reason might due to diet, life stile or sample size.

In B-TIM group the calcium level showed no significant difference compared to the control group, (Table 2). This study was matched with (Goyal, Abrol and Lal, 2010), and disagreed with (Baldini *et al.*, 2014). In present study the opposite of the expected results, the reason might be due no significant decrease of PTH and vitamin D, therefore the calcium of the patients of the present study was normo-calcemic.

The calcium level in this SCA group also showed no significant difference level compared to control (Table 2), this study was disagree with previous studies (Digban and Matthew, 2016; Antwi-Boasiako *et al.*, 2019). These observations were suggested that calcium homeostasis was affected in sickle cell disease due to the hemoglobin S that found in the erythrocyte of sickle cell patients auto-polymerize faster than the normal hemoglobin also deoxygenation of sickle cell is known to increase cation permeability of Ca^{2+} (Pandey *et al.*, 2012). While, in this study the opposite of the expected results, the reason, that there was no deficiency of vitamin D, as well as the parathyroid hormone.

Phosphorus in B-TM showed a significant difference increase level compared to the control group (Table 2), this study was agreed with previous studies (Nafady *et al*., 2018), and disagree with (Arman Bilir *et al.*, 2020). Many studies have suggested that parathyroid gland damage occurs in beta thalassemia major may be due to oxidative stress caused by iron overload or chronic hemolysis or

metabolic irregulation, oxidative stress capable of causing oxidative damage to macromolecules leading to damage parathyroid gland and cause hypoparathyroidism was most frequent causes for phosphorus increase, which was thought to be due to the decrease tubular excretion of phosphates in these patients (Bazydlo, *et al.*, 2014; Sultana and Akhter, 2018).

In B-TIM group, phosphorus level was shown significant difference increase level compared to the control (Table 2); also, due to same causes as in B-TM. Hypoparathyroidism is one of the most common causes of phosphorus increase (Bazydlo, et al., 2014; Sultana and Akhter, 2018).

In SCA group, phosphorus showed significant increase level compared to the control group (Table 2), this study was agreed with previous studies (Yousif, Hassan and Al-Naama, 2018); this was thought to be due to decreased excretion of renal phosphate was most frequent causes for phosphorus increased which may be due to hypoparathyroidism, renal dysfunction, or and resistance to PTH (Bazydlo, et al., 2014).

There were significant prediction values to predict risk in sickle cell anemia for each of ferritin (Odd ratio =1.085), aspartate aminotransferase (Odd ratio =1.36), alanine aminotransferase (Odd ratio=1.21), phosphorus (Odd ratio=4.685), and lactate dehydrogenase (Odd ratio =1.061), Table 4.

There was a highly positive significant prediction value to predict risk in beta thalassemia major for each of ferritin, aspartate aminotransferase, alanine aminotransferase, phosphorus, and lactate dehydrogenase at p value < 0.01, Table 5. The area under the ROC curve for ferritin was (1) in B- TM, B-TIM and SCA, sensitivity and specificity for ferritin was (100 % & 100 %), receptively. Ferritin was excellent acceptable for disease status and next extant was AST, Table 7, 8, 9.

aminotransferase, phosphorus, and lactate dehydrogenase at p value < 0.01, Table 5.

There was a highly significant prediction value to predict beta thalassemia intermediate for each of ferritin, aspartate aminotransferase, alanine aminotransferase, phosphorus (OR=9.014), and lactate dehydrogenase. The changes that occur in thalassemia intermediate are the same as those that occur in thalassemia major, but they are less severe compared to thalassemia major, Table 6.

Ferritin was excellent acceptable for disease status and next extant was AST, Table 7, 8, 9.

V. CONCLUSIONS.

There were high levels of ferritin and phosphorus level; whereas vitamin D, parathyroid hormone, thyroid hormones and calcium showed no differences in patients with hemoglobinopathies compared with control.

Organs disfunctions are frequent complication in iron overloaded patients with risk of progression.

The efforts to restore the ferritin levels to the normal range may be effective in reducing the morbidity or complications associated with iron overload.

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REFERENCES

Abbassy, H. A., Elwafa, R. A. A. and Omar, O. M. (2019) 'Bone mineral density and vitamin D receptor genetic variants in egyptian children with beta thalassemia major on vitamin D supplementation', *Mediterranean journal of hematology and infectious diseases*, 11(1).

Abdel-Razek, A. R. A. *et al.* (2013) 'Study of thyroid function in Egyptian children with β-thalassemia major and β-thalassemia intermedia', *Journal of the Egyptian Public Health Association*, 88(3), pp. 148–152.

Al-Naama, L. M., Mea'ad Kadhum Hassan, M. M. and Karim, A. (2020) 'Growth Hormone and Insulin-Like Growth Factor-1 Status in Pediatric Patients with β-Thalassemia Major', *Growth*, 25(9), pp. 3183–3193.

Antwi-Boasiako, C. *et al.* (2019) 'Total serum magnesium levels and calcium-to-magnesium ratio in sickle cell disease', *Medicina (Lithuania)*, 55(9), pp. 1–8.

Arman Bilir, Ö. *et al.* (2020) 'Renal function and the oxidative status among children with thalassemia major and healthy controls: A cross-sectional study', *Transfusion and Apheresis Science*, 59(4), pp. 10–13.

Baldini, M. *et al.* (2014) 'Beta-thalassaemia intermedia: Evaluation of endocrine and bone complications', *BioMed Research International*, 2014.

Bazydlo, L. A. L., Needham, M. and Harris, N. S. (2014) 'Calcium, Magnesium, and Phosphate', *Laboratory Medicine*, 45(1), pp. 44–50.

Bordbar, M. *et al.* (2019) 'Prevalence of endocrine disorders and their associated factors in transfusion-dependent thalassemia patients: a historical cohort study in Southern Iran', *Journal of Endocrinological Investigation*, 42(12), pp. 1467–1476.

Darvishi-Khezri, H. *et al.* (2020) 'Moderate to severe liver siderosis and raised AST are independent risk factors for vitamin D insufficiency in β-thalassemia

patients', *Scientific Reports*, 10(1), pp. 1–8.

Digban, A. K. and Matthew, A. (2016) 'Evaluation of Some Micronutrients in Sickle Cell Disease', *International Journal of Innovative Research and Development*, 5 (August), pp. 309–313.

Doulgeraki, A. *et al.* (2012) 'Bone health evaluation of children and adolescents with homozygous β-thalassemia: Implications for practice', *Journal of Pediatric Hematology/Oncology*, 34(5), pp. 344–348.

Fung, E. B. *et al.* (2006) 'Multi-Centre Study of Iron Overload Research Group: Increased prevalence of iron-overload associated endocrinopathy in thalassaemia versus sickle-cell disease', *Br J Haematol*, 135(4), pp. 574–582.

Garadah, T. S. *et al.* (2016) 'Hormonal and echocardiographic abnormalities in adult patients with sickle-cell anemia in Bahrain', *Journal of Blood Medicine*, 7, pp. 283–289.

Inati, A. *et al.* (2015) 'Endocrine and bone complications in β -thalassemia intermedia: Current understanding and treatment', *BioMed Research International*, 2015.

Jorgensen, J. M., Crespo-Bellido, M., & Dewey, K. G. (2019). Variation in hemoglobin across the life cycle and between males and females. *Annals of the New York Academy of Sciences*, 1450(1), 105–125.

Kumar, D. V., Aggarwal1 and Dr Vineet Popli2, D. V. U. (2018) 'Assessment of Vitamin D levels and Parathyroid Hormone levels in children with Beta Thalassemia major , and to co.

Kurtoglu, A. U., Kurtoglu, E. and Temizkan, A. K. (2012) 'Effect of iron overload on endocrinopathies in patients with beta-thalassaemia major and intermedia Wpływ przeładowania żelazem na występowanie endokrynopatii u chorych', 63(4), pp. 260–263.

Lahhob, Q. R., Mohammed, N. Y. and Abbas, H. J. (2021) 'Study of Some Minerals and Trace Elements Levels in patients with Sickle Cell Anemia and Sickle Cell Anemiathalassemia in South of Iraq', *Biochemical and Cellular Archives*, 21(1), pp. 1091–1095.

Magro, S. *et al.* (1990) 'Hypothyroidism in patients with thalassemia syndromes', *Acta Haematologica*, 84(2), pp. 72–76.

Mahachoklertwattana, P. *et al.* (2003) 'Bone mineral density, biochemical and hormonal profiles in suboptimally treated children and adolescents with β-thalassaemia disease', *Clinical Endocrinology*, 58(3), pp. 273–279.

Melchionda, F. *et al.* (2013) 'Letter to the editor a novel WT1 Mutation in Familial Wilms Tumor', *Pediatric Blood & Cancer*, (February), pp. 1388–1389.

Merchant, R. *et al.* (2010) 'Evaluation of osteopathy in thalassemia by bone mineral densitometry and biochemical indices', *Indian Journal of Pediatrics*, 77(9), pp. 987–991.

Miller, R. G. *et al.* (2006) 'High prevalence and correlates of low bone mineral density in young adults with sickle cell disease', *American Journal of Hematology*, 81(4), pp. 236–241.

Nafady, A. *et al.* (2018) 'Alteration of trace elements and T-cell subsets in patients with β-thalassemia major: influence of high ferritin level', *The Egyptian Journal of Haematology*, 43(2), p. 55.

Napoli, N. *et al.* (2006) 'Low serum levels of 25-hydroxy vitamin D in adults affected by thalassemia major or intermedia', *Bone*, 38(6), pp. 888–892.

Nolan, V. G. *et al.* (2015) 'Prevalence of Vitamin D deficiency in sickle cell disease: A systematic review', *PLoS ONE*, 10(3), pp. 1–12.

Özen, S. *et al.* (2013) 'Frequency and risk factors of endocrine complications in Turkish children and adolescents with sickle cell anemia', *Turkish Journal of Hematology*, 30(1), p. 25.

Pandey, S *et al.* (2012) 'Biochemical indicator of sickle cell disease: Preliminary report from India', *Indian Journal of Clinical Biochemistry*, 27(2), pp. 191–195.

Parshad, O. *et al.* (1989) 'Abnormal thyroid hormone and thyrotropin levels in homozygous sickle cell disease', *Clinical & Laboratory Haematology*, 11(4), pp. 309–315.

Phillips Jr, G. *et al.* (1992) 'Hypothyroidism in adults with sickle cell anemia', *The American journal of medicine*, 92(5), pp. 567–570.

Pirinççioğlu, A. G. *et al.* (2011) 'Assessment of thyroid function in children aged 1-13 years with beta-thalassemia major', *Iranian Journal of Pediatrics*, 21(1), pp. 77–82.

Şahin, A. *et al.* (2020) 'Sodium, Magnesium, Calcium, Manganese, Iron, Copper, and Zinc in Serums of Beta Thalassemia Major Patients', *Biological Trace Element Research*.

De Sanctis, V. *et al.* (2018) 'Bone disease in β thalassemia patients: past, present and future perspectives', *Metabolism*, 80, pp. 66–79.

De Sanctis, V. *et al.* (2019) 'Thyroid disorders in homozygous β-thalassemia: Current knowledge, emerging issues and open problems', *Mediterranean Journal of Hematology and Infectious Diseases*.

Sharma, R. *et al.* (2016) 'Endocrinopathies in adolescents with thalassaemia major receiving oral iron chelation therapy', *Paediatrics and International Child Health*, 36(1), pp. 22–27.

Singhal, A. and Goyal, H. (2020) 'Thyroid dysfunction in beta thalassemia major patients', *Thyroid Research and Practice*, 17(2), p. 70.

Sleem, G. A. A. M., Al-Zakwani, I. S. and Almuslahi, M. (2007) 'Hypoparathyroidism in adult patients with beta-thalassemia major', *Sultan Qaboos University Medical Journal*, 7(3), pp. 215–218.

Soliman, A., De Sanctis, V. and Yassin, M. (2013) 'Vitamin D status in thalassemia major: An update', *Mediterranean Journal of Hematology and Infectious Diseases*, 5(1).

Soliman, A. T. *et al.* (2017) 'Chronic anemia and thyroid function', *Acta Biomedica*, 88(1), pp. 119–127.

Sultana, M. A. and Akhter, Q. S. (2018) 'Serum calcium and serum phosphate levels in transfusion dependent beta thalassemia', *Journal of Bangladesh Society of Physiologist*, 13(2), pp. 54–58.

Tantawy, A. A. *et al.* (2008) 'Bone mineral density and calcium metabolism in adolescents with beta-thalassemia major.', *Pediatric endocrinology reviews: PER*, 6, pp. 132–135.

Vogiatzi, M. G. *et al.* (2009) 'Differences in the prevalence of growth, endocrine and vitamin D abnormalities among the various thalassaemia syndromes in North America', *British journal of haematology*, 146(5), pp. 546–556.

Wong, P. *et al.* (2013) 'Thalassemia bone disease: The association between nephrolithiasis, bone mineral density and fractures', *Osteoporosis International*, 24(7), pp. 1965–1971.

Wong, S. H., Omar, J. and Ismail, T. S. T. (2017) 'Multiple Endocrinologic Complications in Thalassemia Major', *The Korean Journal of Clinical Laboratory Science*, 49 (4), pp. 495–497.

Wu, H. P. *et al.* (2017) 'Survival and complication rates in patients with thalassemia major in Taiwan', *Pediatric Blood and Cancer*, 64(1), pp. 135–138.

Yassin, M. A. *et al.* (2019) 'Final height and endocrine complications in patients with β-thalassemia intermedia: Our experience in non-transfused versus infrequently transfused patients and correlations with liver iron content', *Mediterranean Journal of Hematology and Infectious Diseases*, 11(1), pp. 1–9.

Yousif, O. O., Hassan, M. K. and Al-Naama, L. M. (2018) 'Red Blood Cell and Serum Magnesium Levels Among Children and Adolescents With Sickle Cell Anemia', *Biological Trace Element Research*, 186(2), pp. 295–304.

Zekavat1 *et al.* (2014) 'Hypothyroidism in β-Thalassemia Intermedia.pdf'.