STUDY OF RELATIONSHIP BETWEEN SP1 POLYMORPHISM IN THE COLLAGEN TYPE I ALPHA -1 (COLIA1) GENE AND OSTEOPROSIS IN PATIENTS WITH BETA-THALASSEMIA.

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ABSTRACT

Background:-Osteoporosis is an important cause of morbidity in patients with β-thalassemia. The pathogenesis of reduced bone mineral density (BMD) is multifactorial. A range of genetics factors have been implicated in patients with osteoporosis. Polymorphism at the Sp1 binding site of the collagen type I A 1*(COLIA1*) gene is thought to be an important factor in development of osteoporosis.

Objective:-is to study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

Methods:-A total of 40 patients with β-thalassemia(21 females &19 males)aged 6-18 yearsand 40 healthy subjects cross matched with age and sex. Serum osteocalcin, serum alkaline phosphatase, calcium and DEXA were examined in all patients. The *COLIA1* Sp1genotypes (SS, Ss, ss) were measured by digestion using restriction enzyme (*Bal*1) of DNA amplified by the polymerase chain reaction (PCR).

Results:-SS (G/G) genotype in β-thalassemia major was30.7% and in β-thalassemia intermedia was 49.4% and the Ss (G/T) genotype in βT Major was 55.63% and in βT intermedia was 50.6%, the ss genotype (T/T) type in βTM was 13.67% only. The difference was statistically significant (p= 0.040). There was highly significant difference between thalassemia major and thalassemia intermedia compared to control group as regard Ca, OC, ALP and DEXA (P= 0.000).

Conclusions:-Detection base substitutions at the Sp1 binding site on the *COLIA1* gene in early years considering an important role in preventing osteoporosis in children with beta -thalassemia.and early mangment of these patients.

Key words:
Beta-thalassemia, *COLIA1*,osteoprosis.

INTRODUCTION

Beta thalassemia syndromes are a group of genetic diseasesfamous by a genetic defect in the formation of β-globin chains. This genetic deficiency leads to marked transfusion-dependent anemia in the homozygous type of beta thalassemia (thalassemia major) while in the heterozygous type of the β-thalassemia (thalassemia minor) leads to mild to moderate microcytic anemia. Patients with clinical severity of the disease lie between thalassemia major and thalassemia minor are defined as thalassemia intermedia. Many different genotypes are associated with thalassemia intermedia [1].

In spite of the improved treatment of the hematologic disorder and its complications, β-thalassemia patients exhibit an unbalance in bone mineral turnover with increased resorptive rates and suppression of osteoblast activity, resulting in diminished bone mineral density (BMD) more evident in the lumbar spine [2].

Osteoporosis is a debilitating bone disease which detected by a decrease low bone mass

and density and results inhigh risk of fracture. In low bone mass, together environmental and polygenic factors are the cause of osteoporosis [3].

Many researches detected that the collagen one gene genotype diagnoses bone fracture by ways that are partly not dependent of bone mineral density [4]. There is high risk fracture in relation to SP1 polymorphism although of mild relation with bone mineral density [5].

Polygenic factors has an important role in the development of osteoporosis, containing variability in many genes for example collagen type I alpha 1 (COLIAI), vitamin D receptors, estrogen receptors and interleukin-6 (IL-6) which monitor bone mineral density and bone shape and structure[6].

Collagen type I considers the main component of the bone matrix. Sp1 binding site Polymorphism in the transcriptional control region of the COL1A1 gene causes alleles with a G-base at the Sp1 binding are defined as 'S', while alleles with a T-base are defined as 's'. Many studies have shown that patients with 's' allele are mostly have reduced BMD and osteoporotic fractures in several populations [7].

The aim of this work was to study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with Beta thalassemia.

SUBJECTS AND METHODS

Study design and sample selection:

This study was conducted in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on 40 thalassemic patients at their regular follow-up visits and forty age- and sex-matched healthy children as a control group.

This study included 80 subjects, They were classified as the following:

Group 1:

This group included 40 patients (21 females &19 males) on follow up of β-thalassemia and they are undergoing blood transfusion and iron chelation therapy(desferal) which their ages ranged from 6-18 years and classified as:

Group 1A: 22 patients with β- thalassemia major (TM).

Group 1B:18 patients with β- thalassemia intermedia (TI).

Group 2:

This group included 40 apparently healthy subjects cross matched with age and sex.

An informed written consent of participation in the study was signed by the parents or legal guardians of the studied subjects. This study was approved by the Ethical Research Committee , Faculty of medicine , Zagazig University.

Each member of this research was admitted to complete history talking and full clinical observation and laboratory investigation as complete blood count done by sysmex XN, Hb electrophoresis done by full automated capillary electrophoresis, Serum Calcium level, alkalin phosphatase done by Cobas 8000, Bone Density by DEXA, Serum osteocalcine level and COLIAI gene polymorphism by using polymerase chain reaction – restriction fragment length polymorphism technique (PCR-RFLP).

- ***Bone Density by DEXA***:

Bone mineral density (BMD) of the lumber spine (second, third and fourth lumber vertebrae) was measured by dual energy X-ray absorptiometry (DEXA). All children were scanned in the supine position, BMD data were expressed as grams per centimeter. Squared and were compared with BMD values of normal children of the same age.

WHO measurement of osteoporosis using BMD measurement at the spine, hip or forearm:	
Normal	BMD is within 1 SD of a young normal adult (T-score at -1.0 and above)
Low Bone Mass (Osteopenia)	BMD is between 1.0 and 2.5 SD below that of a young normal adult (T-score between -1.0 and -2.5)
Osteoporosis	BMD is 2.5 SD or more below that of a young normal adult (T-score at or below -2.5)
Severe/Established Osteoporosis	BMD is 2.5 SD or more below that of a young normal adult (T-score at or below -2.5), plus there have been ≤1 fractures

DNA analysis

DNA was extracted from leukocytes of peripheral blood samples using the QIAamp® UltraSens virus® extraction kit (Qiagen) USA according to manufacture protocol. The extracted cfDNA was stored at -20°C till analysis.

PCR amplification:

DNA was amplified using Maxime PCR PreMix Kit composed of Ready- to- Go PCR Beads which are designed and manufactured by (iNtRON Biotechnology). PCR reaction with 25µl final volume was prepared by adding12.5ul Master mix, 1.0µl Forward primer, 1.0µl Reverse primer, 10µlextracted DNA and 0.5 µl Sterile high quality water into PCR wells.

PCR was done by the following conditions: initial denaturation at 94 º C for 3 min, 35cycles (94 º C for 50 sec for denaturation, 62 º C for 10sec for annealing, 72 º C for 15 sec for extention) and for Final extention step72 º C for 5 min.

The PCR products were stored at -80°C until used.

collagen type I alpha -1gene Primers:

Forward primer (5′ GTCCAGCCCTCATCCTGGCC -3′).

Reverse primer(5′-TAACTTCTGGACTATTTGCGGACTTTTTG G -3′).

Detection of amplified PCR product by agarose gel electrophoresis:

5 µl of each sample were slowly loaded into sample well and 5 µl PCR marker was also loaded into one of the wells.

The power supply was programmed as 150 volts and 100 milli amperes for 20 minutes **(Consort E 844).** Then The gel was placed on the filter area of the ultraviolet transilluminator **(Biometra).**

The amplified PCR product gave rise to 264 bp fragment.

Restriction digest reaction:

The amplified DNA was digested by using **(Bal 1)(**BioLabs, UAS, part No. R0534S, 250 Units) (5,000 units/ml).

10 µl of amplified product added to 16µl sterile distilled water then add 2µl of restriction enzyme and 2µl of reaction buffer then incubated at 37°C.

Detection of the band of polymorphism:

Then the digested segments were subjected to electrophoresis on eight percent non denaturing polyacrylamide gel; then the gel stained using ethidium bromide (one mg/ml) for thirty minutes at room temperature then followed by visualization by the ultraviolet (UV) trans-illuminator.

The mplified product 264 bp fragment with *Bal 1* restriction enzyme gave rise to:

- Undigested 264bp fragment indicated the presence of S allele and identified as G-T substitution
- Appearance of 246bp fragment indicated the presence of s allele.

Thehomozygous variant SS (G/G) result in one fragment at 264 bp, homozygous variant ss (T/T) result in one fragment at 246bp while the heterozygous variant Ss (G/T) result in two fragments at 264 bp and 246 bp.

described in Fig. 1.

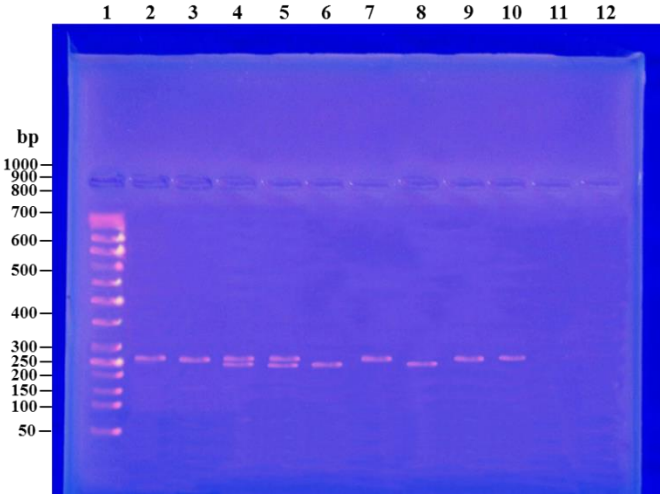

Fig. (1): A representation of collagen gene polymorphism by PCR-RFLP.
Lane 1: Ladder (50bp).
Lane2,3,7,9,10: represent the homozygouse cases (SS) genotype of collagen gene (G/G).
Lane 4,5: represent the heterozygouse cases (Ss) genotype of collagen gene (G/T).
Lane 6,8: represent homozygouse cases (ss) genotype of collagen gene (T/T).

Statistical analysis:-

For statistical analysis using statistical program SPSS version 10 for Windows (SPSS, Chicago, Illinois, USA).

Presentation of data in the form of median, frequency, percentages.

Comparing two independent groups using Mann–Whitney test (U). For comparing between independent proportions using Chi squared test (χ^2) and for non- parametric data using kruskal wallis test (K).

P value < 0.05 was considered statistically significant.

RESULTS

Table (1): Demographic data of the studied groups.

Demographic data	Group 1 (n=40)		Group 2 (n=40)		Test	p-value (Sig.)
Age (years) Mean ± SD	10.50±2		10±1.9		1.146•	0.255 (NS)
Sex	No.	%	No.	%		
Male	20	50.0%	20	50.0%	0.000#	1.000 (NS)
Female	20	50.0%	20	50.0%		
Family history	No.	%	No.	%		
Positive	14	35%	0	0%	16.97#	0.000 (HS)
negative	26	65%	40	100%		

• T-test
Chi-square test (χ^2).
p< 0.05 is significant.
Sig.: Significance.

This table shows no significant difference between cases and controls as regard age (p=0.255) and sex (p=1.000).And highly significant difference in family history (p=0.000).

Table (2): Comparison between patients and control groups as regard onset and frequency of blood transfusion and duration of iron chelation.

	Group 1a	Group 1b	U Test	p
Onset of transfusion in years				
Median	3.4	3.1	0.8	>0.05
Range	(0.5-3)	(0.5-3)		(NS)
Frequency of transfusion (weeks)				
Median	4.1	3.7	2.1	<0.05
Range	(2-4)	(2-4)		(S)
Duration of chelators (years)				
Median	11.2	7.8	4.3	<0.001
Range	(7-14)	(3-11)		(HS)

Mann Whitney test (U) for nonparametric data and comparison between 2 groups.
p< 0.05 is significant

It was found statistical significant variation between 2 groups as regard frequency of blood transfusion and duration of chelation therapy and no significant differenceas regardonset of frequency of blood transfusion.

Table (3): Serum level of Calcium, Osteocalcin and Alkaline phosphatase in all studied groups.

Laboratory finding	Group 1a (n=22)	Group 1b (n=18)	Group 2 (n=40)	K Test	p-value (Sig.)
calcium (mg/dL)					
Median	8.78	8.96	10.96	39.7	0.000
Range	(5.02-9.14)	(5.2-9.32)	(7.24-11.28)		(HS)
OC (ng/mL)					
Median	10.62	10.43	17.11	35.6	0.000
Range	(6.12-11.92)	(6.07-11.59)	(5.45-25.57)		(HS)
ALP (u/L)					
Median	418.44	406.29	239.9	35.4	0.000
Range	(166.74-666.34)	(182.25-626.53)	(87.82-388.18)		(HS)

kruskalwallis test (K) for non-parametric results and comparison between 3 groups.

- p< 0.05 is significant.
- Sig.: Significance.
- OC: Osteocalcin.
- ALP: Alkaline phosphatase.

This table showed highly significant difference between thalassemia major and thalassemia intermedia compared to control group as regard Ca, OC and ALP (P= 0.000).

Table (4): Comparison between two groups of thalassemia (TM, TI) as regard Serum level of Calcium, Osteocalcin and Alkaline phosphatase.

Laboratory finding	Group 1a (n=22)	Group 1b (n=18)	U Test	p-value (Sig.)
calcium (mg/dL)				
Median	8.78	8.96	163.50	0.346(NS)
Range	(5.02-9.14)	(5.2-9.32)		
OC (ng/mL)				
Median	10.62	10.43	185.00	0.722
Range	(6.12-11.92)	(6.07-11.59)		(NS)
ALP (u/L)	418.44	406.29	169.50	0.437
Median	(166.74-666.34)	(182.25-626.53)		(NS)
Range				

- Mann-whintey test (U).
- P < 0.05 is significant.
- Sig.: Significance.

This table revealed no significant difference between two studied groups of thalassemia as regard calcium, osteocalcin and alkaline phosphatase (p>0.05).

Table (5): DEXA finding concerning Bone Mineral Density and Z-score in the studied groups.

Item	Group 1a (N=22)	Group 1b (N=18)	Group 2 (N=40)	K Test	p-value (Sig.)
BMD					
Median	1.88	1.83	2.03	150.7	0.000
Range	(0.467-0.716)	(0.424-.656)	(0.462-0.97)	7	(HS)
Z-score					0.001
Median	-2.9	-1.95	-1.4		(HS)
Range	(-3.06:-0.30)	(-1.5:0.40)	(-0.19:-0.18)	90.88	

Kruskalwallis test (K) for non-parametric results.
P< 0.05 is significant.
Sig.: Significance.
DEXA: Dual-Energy X-ray Absorptiometry.
BMD: Bone Mineral Density.

This table showed highly significant difference between thalassemia major and intermedia compared to control group (p<.001).

Table (6): DEXA finding concerning Bone Mineral Density and Z-score in thalassemic patients.

Item		**Group 1a** (n=22)	**Group 1b** (n=18)	**U Test**	**p-value** (Sig.)
	BMD				
Median		1.88	1.83	2.63	0.012
	Range	(0.467-0.716)	(0.424-.656)		
	Z-score				
Median		-2.9	-1.95	-5.132	0.000
	Range	(-3.06:-0.30)	(-1.5:0.40)		

Mann-Whitney test (U).
P< 0.05 is significant.
Sig.: Significance.

This table showed significant difference between thalassemia major and intermedia as regard DEXA finding concerning BMD and Z-score.

Table (7): Correlation between Onset of transfusion, Frequency of transfusion, Duration of iron chelators and Serum level of Calcium, Osteocalcin, Alkaline phosphatase and DEXA.

Item	**Onset of transfusion in years**	**Frequency of transfusion in years**	**Duration of iron chelators**
Calcium			
r coefficient	0.7	-0.25	0.05
P-value	0.03	0.8	0.6
	(S)	(NS)	(NS)
OC			
r coefficient	0.6	-0.05	0.39
P-value	0.01	0.6	0.7
	(S)	(NS)	(NS)
ALP			
r coefficient	0.8	0.33	-0.4
P-value	0.04	0.4	0.75
	(S)	(NS)	(NS)
DEXA			
r coefficient	0.7	-0.2	0.05
P-value	0.02	0.3	0.6
	(S)	(NS)	(NS)

P< 0.05 is significant.
Sig: Significance.

This is table illustrates:

- Positive correlation between onset of transfusionand laboratory finding (calcium, osteocalcin and ALP) and DEXA ($p<0.05$).It denotes the earlier onset of blood transfusion the less decrease in calcium and osteocalcin levels and DEXA. And the less increase of alkaline phosphatase level.
- No significant correlation betweenfrequency of transfusion, duration of iron chelation and laboratory finding (calcium, osteocalcin and ALP) and DEXA.

Table (8): Distribution of COLIA1 genotype in thalassemic patients and healthy control.

	Total (N=80)						χ^2**Test**	**p-value** (Sig.)
	Group 1a (n=22)		**Group 1b** (n=18)		**Group 2** (n=40)			
COLIA1 genotype	No.	%	No.	%	No	%		
Homozygous 264(SS)	10	30.7%	8	49.4%	25	79.5%		
Heterozygous 264+246(Ss)	9	55.63%	10	50.6%	15	20.5%	10.02	.040
Homozygous 246(ss)	3	13.67%	0	0%	0	0%		(S)

Chi-square test (χ^2).
$p< 0.05$ is significant.

Comparison of COLIA1 genotyping in patients with βTM, βTI, healthy group showed highly significant difference in which βTM had 30.7%Homozygous (SS), 55.63%Heterozygous (Ss) and 13.67%Homozygous (ss) and βTI had 49.4%, 50.6%, 0% compared to control group ($p=.012$).

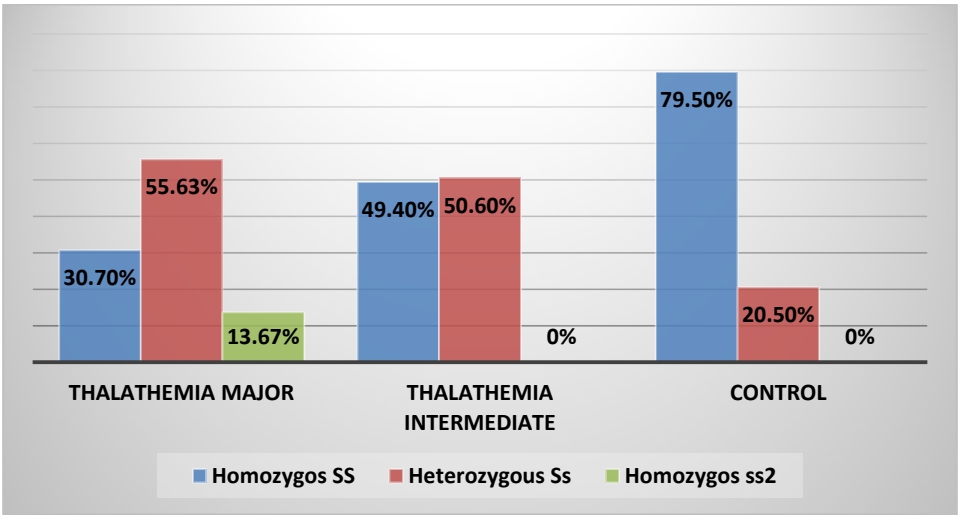

Figure (2): Distribution of COLIA1 genotype in thalassemic patients and healthy control.

Multiple bar chart showed high percentage of Ss, ss genotype in thalassemia major (55.63%, 13.67%) than thalassemia intermedia (50.60%, 0%) compared to control group.

Table (9): Comparison between Thalassemia major and Thalassemia intermedia as regard COLIA1 genotype.

Item	Group 1a (n=22) no	Group 1a (n=22) %	Group 1b (n=18) no	Group 1b (n=18) %	χ^2 Test	p-value (Sig.)
Homozygous SS genotype	19	30.7%	8	49.4%		
Heterozygous Ss genotype	9	55.63%	10	50.6%	2.904	0.234 (NS)
Homozygous ss genotype	3	13.67%	0	0%		

Chi-square test (χ^2).
p< 0.05 is significant.

This table showed no significant difference between two types of beta thalassemia in SP1 polymorphism in the collagen type I alpha -1 (COLIA1). There was three cases with homozygous **ss** genotype of collagen gene that associated with low calcium level, low osteocalcin level, high alkaline phosphatase level and low DEXA finding concerning BMD and Z-score.

Table (10): Relation between COLIA1 genotype distribution and laboratory data, DEXA among studied groups.

Laboratory findings	Total (N=80) Homozygous 264 (SS) (N=43)	Heterozygous 264+246 (Ss) (N=34)	Homozygous 246 (ss) (N=3)	K Test	p-value (Sig.)
Ca (mg/mL) Median	10	10.27	7.3	8.192	0.02 (S)
OC(ng/ml) Median	14.4	13.64	10.2	1.93	0.381 (NS)
ALP (u/L) Median	301.69	347.7	443.56	5.178	0.075 (NS)
DEXA Median	1.949	2.154	1.967	1.455	0.483 (NS)

Kruskal Wallis Test (K)
p< 0.05 is significant

Test showed significant relation between COLIA1 genotype and Calcium level and no significant relation between COLIA1 genotype and alkaline phosphatase, osteocalcin level and DEXA (p>0.05).

DISCUSSION

In β- thalassemia, low bone mass represents a chronic, degenerativedisease, even betweengood transfused and iron chelated prepubertal and adult cases [8]. The inheritance of bone mass is affectedgeneticeffect, although the genes responsible for bone mass are lessreported[9].New researches showed the relation between Sp1polymorphism at first intron of collagen one gene and bone thickness in many people[10],[11].

Our result has been analyzed to detect the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with β-thalassemia

The present study detected significant variation betweenTM,TI groups as regards frequency of blood transfusion and duration of iron chelation (p < 0.05) although it thought that absence significant variation between them as regard onset of blood transfusion.

Our data showed significant hypocalcaemia which was 100% in TM and 55.5% in TI compared to control group 25%.

In agreement with our data that reported biochemical hypocalcaemia in beta thalassemia patients [12],[13],[26],[15],[16].

In contrast, there was no difference in plasma level of calcium in thalassemic patients compared with healthy control subjects [17].

Our result showed the low level of serum osteocalcin and high alkaline phosphatase in beta thalassemic patients than healthy control. Although there is absence significant variations between TM, TI as regard calcium, osteocalcine and alkaline phosphatase (p<0.05).

Low level of serum osteocalcin and high alkaline phosphatase in thalassemic patients were observed in several studies [18],[19],[17],[14],[20].

In contrast with current study, there was no statistically significant variations in alkaline phosphatase levelsofblood transfusion dependent TMcases [21].

In the present study, there was significantly lower BMD and Z-score values in patient groups than controls both at the lumbar and femoral levels (p<0.001) which is highly significant. Alsoit found significant variations between TM, TI (p value less than 0.05).

Several previous studies showed lower BMD in thalassemic patients [22],[23],[24],[25],[26]which due to their low physical improvement which affected by iron overload and iron chelation that can affect the liver and the endocrine system.

In contrary to our result, Christoforidis et al. [27] reported that BMD Z-scores were within normal.

In the present study, the **SS** genotype *(G/G)* in βTM, βTIand control was 30.7%, 49.4%, 79.5% respectively. The **Ss** genotype *(G/T)* in βTM, βTI and control was 55.63%, 50.6%, 20.5% respectively. The **ss** genotype *(T/T)* in βTM was 13.67% only. The difference was statistically significant (p= 0.012).Identifying the frequency of alleles inβ-thalassemia persons and health persons, it was found that the s allele higher in the TM (13.7%) than TI and control. The low percentage of **ss** genotype in thalassemic personsmaydue to the limited size ofthe examined group or caused by ethnic cause.It was found absenceof significant variation between TM, TI inCOLIAI genotype (p>0.05).

Singh et al. [7]reported in his study that SP1 polymorphism in β-thalassemia, 19% of β -TM patients have **SS** *(G/G)* genotype, 40% have **Ss** *(G/T)* and 43% have **ss** *(T/T)*.And Kritanjali et al. [13] 19.8% **SS** *(G/G)*, 35.8% **Ss** *(G/T)* and 43.4% **ss** *(T/T)* with 37.7% **S**, 61.3 % **s** allelic frequencies in TM persons.

Galal et al. [6] reported that80.6% of the β -thalassemia patients were homozygous for **SS** *(G/G)* and 19.4% were heterozygotes for **Ss** *(G/T)* polymorphism. Guzeloglu et al. [28] revealed that there was no **ss** genotype. There was **Ss, SS** and **ss** genotypes in 24.3% , 40.5% and 35.2% of patients, and in 16%, 84% of the control group, respectively. Also Arisal et al.

[9] detected that 64.3% **Ss** *(G/T)* and 35.7% **SS** *(G/G)* in β -TM. 60% **Ss** *(G/T)* and 40% **SS** *(G/G)* in β -TI which was significantly higher compared to control group (p=0.029**).**

In a study done by Perrota et al. [29], the genotypes of **Ss** and **ss** were accompanied by low bone mineral density than **SS** genotype. The absent relation between bone mineral density and COLIAI polymorphism in thalassemic persons due to low frequency of the **s** allele, also the low sample size. And BMD is to be detected by a manypolygenic and acquired factors, and its inheritance is due to be under polygenic effect [30].

Jin-Ai et al. [31] reported that collagen gene one alpha one genotype diagnosebone fracture by techniquesthat are partly nondependent of bone mineral density and revealed high risk of osteopenic fracture in relation to SP1 transcription factor polymorphism although of mild relation with bone mineral density. There was a study revealed absent correlation between SP1/BMD in TM patients had low bone mass [32].

Limitations of the study were that a small number of thalassemic patients were evaluated.

CONCLUSION

The frequency of low Bone Mineral Density (BMD) and osteoporosis are high among the children with β- Thalassemia and SP1 mutation is one of the factors that influences bone density in these patients with no significant difference between thalassemia major and intermedia. The findingsincrease the rolewhich genotyping at SP1 site may consider an important cause for detecting the thalassemic persons with a risk of achieve osteoporosis and bone fractures thataccount a possible role for improvement of osteoporosis management in those thalassemic persons.

Conflict of interest:- The authors have no conflict of interest to declare.

REFERANCES

[1] **Hapgood G, Walsh T, Cukierman R, Paul E, Cheng K, Bowden DK.** Erythropoiesis is not equally suppressed in transfused males and females with β-thalassemia major: are there clinical implications? .*Haematologica*2015; 100 (8): 292-294.

[2] **Carlo Perisano, EmanueleMarzetti, Maria Silvia Spinelli, Cinzia Anna Maria Callà, CalogeroGraci, GiulioMaccauro.** Physiopathology of Bone Modifications in – Thalassemia, *Anemia*2012; 5:68-77.

[3] **Zhang J, Ai Z, Ning X, Liu X, Tang W, Luo Y.** The Association between Vitamin D Receptor FokI Gene Polymorphism and Osteoporosis in Postmenopausal Women: A Meta-Analysis. *J OsteoporPhys Act*2015;3:143.

[4]**MaheshChandMeena,AlokHemal, MukulSatija, ShilpaKhannaArora, and ShahinaBano**. Comparison of Bone Mineral Density in Thalassemia Major Patients with HealthyControls.*Advances in Hematology*2015; 4.

[5] **Kostik MM, Smirnov AM, Demin GS, Mnuskina MM, Scheplyagina LA, Larionova VI.**Genetic polymorphisms of collagen type I a1 chain (COL1A1) gene increase the frequency of low bone mineral density in the subgroup of children with juvenile idiopathic arthritis. *EPMA J* 2013; 4(1):1–8.

[6] **Galal A, Hanan M. Hameda, Mona E.L. Ghamrawyb,Abd El Azeem K, Hussein IR, Mona Fayez Abd-Elgawad MF.** An SP1-binding site polymorphism in the COLIAI gene and osteoporosis in Egyptian patients with thalassemia major.*Blood Coagulation and Fibrinolysis*2011; 22:81–85.

[7] **Singh K, Agarwal S, Gupta S .** An SP1-binding site polymorphism in the COLIAI gene: may be a strong predictor for low bone density in thalassemia major. *Gene TherMolBiol* 2013; 15:112–119.

[8] **Tantawy AA, El Kholy M, Moustafa T, Elsedfy HH.** Bone mineral density and calcium metabolism in adolescents with beta-thalassemia major. PediatrEndocrinol 2008; 6 (1suppl):132–135

[9] **Arisal O, Deviren A, Fenerci EY, Hacihanefioglu S, Ulutin T, Erkmen S, et al.**Polymorphism analysis in the COLIA1 gene of patients with thalassemia major and intermedia. *HaematologiaBudap*2002;32:475–482.

[10] **Grant SF, Reid DM, Blake G.** Reduced bone density osteoporosisassociated with a

polymorphic Sp1 binding site in the collagen type a1 gene. *Nat Genet* 1996; 14:203–205.

[13]**Goyal M, Abrol P,Lal H.** Parathyroid and calcium status in patients with thalassemia.*Indian J ClinBiochem*2010;25: 385-387.

[11] **Ji GR, Yao M, Sun CY, Zhang L, Han Z.** Association of collagen type Ialpha1 (COLIA1) Sp1 polymorphism with osteoporotic fracture inCaucasian postmenopausal women: a meta-analysis. *J Int Med Res* 2009;37:1725–1732.

[12] **Bandebuche S, SagarV ,Sontakk A.** Serum total Calcium level in patients with Thalassemia. *International Journal of Medical Researchand Review*2013; 1(4):190-192.

[13] **Kritanjali Singh, SaritaAgarwal, Sushil Gupta.**An SP1-binding site polymorphism in the COLIAI gene: may be a strong predictor for low bone density in thalassemia major.*Gene Therapy and Molecular Biology*2013; 15,112-119.

[14]**Saboor Muhammad, Qudsia Fatima, QamarKhansa, MoinuddinMoinuddin.**Levels of Calcium, Corrected Calcium, Alkaline Phosphatase and Inorganic Phosphorus in Patients' Serum with β-Thalassemia Major on Subcutaneous Deferoxamine.*J HematolThromb Dis*2014*;* 2(2):2329-8790.

[15] **Shah S.**Assessment of serum calcium and phosphorus levels among transfusion-dependent βeta thalassemia major patients on chelation therapy. *J Postgrad Med Inst* 2015; 29(3): 168-171.

[16] **SultanS, Syed Mohammed Irfan, Syed Ijlal Ahmed.**Biochemical Markers of Bone Turnover in Patients with β-Thalassemia Major. *Advances in Hematology* 2016;
avaliable at http://dx.doi.org/10.1155/2016/5437609**.**

[17] **Morabito N, Gaudio A, Lasco A, Atteritano M, Pizzoleo MA, Cincotta M, La RosaM, Guarino R, Meo A, Frisina N.**Osteoprotegerin and RANKL in the pathogenesis of thalassemia-induced osteoporosis: new pieces of the puzzle. *J Bone Miner Res*2004;19:722–727.

[18] **Asif M, Manzoor Z, Farooq MS,Kanwal A, Shaheen U, Munawar SH, et al.** Correlation between serum ferritin level and liver function tests in thalassemic patients receiving multiple blood transfusions. *Int J Res Med Sci*2014**;** 2(3): 988-994.

[19] **DundarUmit, KupesizAlphan, OzdemSebhat,GilgilEradl, TuncerTiraje, YesilipekAkif, et al.** Bone metabolism and mineral density in patients with beta-thalassemia major. *Saudi Med J*2007; 28(10):1425-1429.

[20] **Salama OS, Al-Tonbary YA, Shahin RA, Eldeen OA.** Unbalanced bone turnover in children with beta-thalassemia. *Hematology* 2006; 11: 197-202.

[21] **Goyal M, Abrol P,Lal H.** Parathyroid and calcium status in patients with thalassemia. *Indian J ClinBiochem*2010;25: 385-387.

[22] **AyferGozuPirinccioglu, VeysiAkpolat, OrhanKoksal,KenanHaspolat, Murat Soker.** Bone mineral density in children with beta-thalassemia major in Diyarbakir.*Bone*2011;49(4):819-823.

[23]**DomrongkitchaipornSomnuek,VorachaiSirikulchayanonta,PantepAngchaisuksiri,WasanaStitchantrakul,ChavasakKanokkantapong, RajataRajatanavin.** Abnormalities in Bone Mineral Density and Bone Histology in Thalassemia. *Journal of Bone and Mineral Research* 2003;18(9): 1682–1688.

[24] **FouziaIshaq ,Asma Anwar ,MazherIqbal.**Frequency of Low Bone Mineral Density and Osteoporosis in Children with Beta Thalassemia Major.*P J M H S.* 2015; 9 (3): 1073.

[25]**MehranKarimi, AlirezaFotouhiGhiam, AlirezaHashemi,Saied Alinejad, MahmoodSoweid, Sara Kashef.**Bone Mineral Density in Beta-Thalassemia Major and Intermedia.*Indian Pediatrics* 2007; 44: 29-31.

[26]**RashidMerchant,AmishUdani,ViplaPuri,ValentinaD'cruz,Deepak Patkar,AartiKarkera.**Evaluation of Osteopathy in Thalassemia by Bone Mineral Densitometry and Biochemical Indices.*Indian J Pediatr 2010*; 77:987–991.

[27] **Christoforidis A, Kazantzidou E, Tsatra I,Metaxa MA.**Normal lumbar bone mineral density in optimally treated children and young adolescents with B-thalassemia major.*Hormones*2007; 6: 334-340.

[28] **Guzeloglu-Kayisli O, Cetin Z, Keser I, Ozturk Z, Tuncer T.** Relationship between Sp1 polymorphism, osteoporosis in b

thalassemia major patients. *PediatrInt*2008; 50:474–476.

[29] **Perrotta S, Cappellini MD, Bertoldo F.** Osteoporosis in b-thalassemia major patients: analysis of the genetic background. Br J Haematol 2000;111:461–466.

[30] **Mahachoklertwattana P, Chuansumrit A, Sirisrio R, Choubtum L,Sriphrapradang A.** Bone mineral density, biochemical and hormonalprofiles in suboptimally treated children and adolescents with betathalassemiadisease. ClinEndocrinol 2003; 58:273–279.

[31] **Jin-Ai Mary Anne Tan, Ping-Chin Lee, Yong-Chui Wee, Kek Heng Chua.** High Prevalence of Alpha- and Beta-Thalassemia in the adazandusuns in East Malaysia: Challenges in Providing Effective Health Care for an Indigenous Group. *Journal of Biomedicine and Biotechnology* 2010; available at: http://dx.doi.org/10.1155/2010/706872.

[32] **Gaudio A, Morabito N, Xourafa A, CurroM, Czccamo D, Ferlazzo N, et al.** Role of genetic pattern on bone mineral density in thalassemic patients. *ClinBiochem* 2010; 43:805–807.