

Plasma level of matrix metalloproteinase-9 in patients with sickle cell disease and its correlation to myocardial iron overload

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Abstract

Cardiac iron overload is secondary to chronic blood transfusion in patients with sickle cell disease (SCD). Iron overload cardiomyopathy is a restrictive cardiomyopathy associated with systolic and diastolic dysfunction. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases responsible for tissue remodeling. Many studies offer strong evidence for the role of MMP-9 in LV remodeling. We aimed to detect plasma levels of MMP-9 in patients with SCD and its correlation to myocardial iron overload. A case control study was carried out on 50 patients with SCD and 50 age and sex matched healthy controls. Assessment of cardiac iron overload in patients by MRI T2* was performed. Plasma MMP-9 levels were measured for patients and controls using ELISA. SCD patients had significantly higher levels of MMP-9 than controls. There was highly significant correlation between plasma levels of MMP-9 and serum ferritin. Patients with vaso-occlusive crises (VOC) > 5/year had significantly higher levels of MMP-9 than those with VOC \leq 5 /year. No significant correlation was found between MMP-9 and cardiac T2*. MMP-9 seems to be a useful marker in SCD patients. Patients with serum ferritin > 1000 ng/ml, recurrent VOC > 5 /year had significantly higher MMP-9 serum levels than others.

Introduction

Sickle cell disease (SCD) is an inherited chronic hemolytic anemia characterized by recurrent episodes of vaso-occlusion and progressive organ dysfunction. SCD results from mutation in the beta globin chain, leading to production of sickle hemoglobin which polymerizes and results in fragile and deformed red cells that obstruct vascular flow resulting in tissue injury. Though SCD syndromes share a common pathophysiology yet they exhibited great variation in the severity based on the genotype. All are autosomal recessive and include two beta gene mutations, with at least one being a sickle mutation. They include homozygous sickle cell anemia (SS), Hemoglobin SC disease refers to a double heterozygote for S and C mutations and Sickle β thalassemia (either β^0 or a β^+ mutation).¹

Chronic blood transfusions in SCD may lead to myocardial iron deposition in 2%-5% of these patients. This can lead to systolic and diastolic left ventricular dysfunction, heart failure, arrhythmias, and sudden cardiac death.² Cardiac magnetic resonance imaging (MRI) using T2* is the gold standard for diagnosis of cardiac iron overload. There is a clear relationship between degree of complications such as heart failure, and arrhythmias with T2* values <20 milliseconds.³

Matrix metalloproteinases are a family of zinc-dependent endopeptidases. This family is responsible for both physiological and pathophysiological tissue remodeling MMPs cleave all structural elements of the extracellular matrix (ECM), as well as process a variety of non-ECM substrates. MMPs were classified into five groups: collagenases, gelatinases, stromelysins, matrilysins, and membrane type. A numbering system corresponding to the order of discovery was adapted after it was realized that more MMPs existed than originally expected.⁴

MMP-9 plays a major role in the degradation of ECM in many physiological and pathophysiological processes that involve tissue remodeling. MMP-9 is present in developing cardiac tissue in humans and rodents, and is expressed between 16 and 18 days of embryogenesis. MMP-9 plays a significant role in neovascularization through proteolytic degradation of the proteins in basal lamina of the blood vessels and release of the biologically active form of vascular endothelial growth factor.⁵

MMP-9 also plays important roles in immune cell function. MMP-9 deletion promotes the recruitment of eosinophils and Th2 cells into the lungs after exposure to an allergen. In pathophysiological conditions, MMP-9 is up regulated during development and wound healing, as well as during pathologies that involve inflammatory processes, including arthritis, diabetes, and cancer. In these pathophysiological conditions, MMP-9 proteolytic properties contribute to stimulate the immune response to initiate pathogenesis and exacerbate disease progression.⁴

Many studies offer strong evidence for the role of MMP-9 in LV remodeling. In heart failure patients, serum MMP-9 levels are elevated reflecting increased degradation of cardiac collagen . It is well established that an increased expression of MMP-9 is associated with the pathological status in a many inflammatory diseases, including Myocardial infarction, Liver fibrosis, rheumatoid arthritis and periodontal disease.^{4,5}

To the best of our knowledge, No studies have investigated the relationship between MMP-9 levels and cardiac iron overload in patients with sickle cell disease. We aimed to detect plasma levels of matrix metalloproteinase in patients with sickle cell anemia and its correlation to myocardial iron overload in these patients.

Results

Demographic characteristics

The mean age of our patients was 13.8 years. They were 26 males (52%) and 24 females (48%). The mean age of control group was 14.3 years. They were 26 males (52%) and 24 females (48%). Patients and controls were matched as regards age and sex.

Clinical and laboratory characteristics

As regards the initial clinical presentation, 44 (88%) patients were presented by pallor and 6 (12%) patients were presented by VOC. The mean frequency of VOC was 5.6/ year (Range: 0-12/year). The mean serum ferritin was 1719.5 ng/ml (Range: 195-6811 ng/ml). The mean cardiac T2* was 34.2 ms (Range: 23.3-50.9 ms). Clinical and laboratory data were presented in table 1.

Transfusion and chelation characteristics

The mean age of start of transfusion was 2.5 years and the mean transfusion frequency was 12.4/year. 64% patients were on regular transfusion and 36% were irregularly transfused. The mean age of start

chelation was 4.5 years. 44% of our patients were receiving Deferasirox and 28% were receiving Deferiprone while 28% of patients were not receiving any chelating therapy. Transfusion and clinical data were listed in table 2.

Hydroxyurea therapy

56% of our patients received Hydroxyurea as a prophylaxis therapy and 44% didn't receive Hydroxyurea therapy.

Types of Sickle cell disease in patients

40% of our patients were sickle cell thalassemia β^+ , 28% were sickle cell thalassemia β^0 , 24% were sickle cell anemia and 8% were sickle cell trait. Different types were displayed in figure 1.

MMP-9 plasma level in studied groups

Patients with sickle cell disease had significantly higher levels of MMP-9 than controls (150.9 versus 32.0 ng/ml respectively) [Table 3].

Relationship between MMP-9 plasma level and other parameters

Patients with serum ferritin >1000 ng/ml had significantly higher plasma levels of MMP-9 than those with serum ferritin <1000 ng/ml. Also patients receiving chelating therapy had significantly higher plasma levels of MMP-9 than those who didn't receive chelating therapy. Patients with VOC > 5/year had significantly higher plasma levels of MMP-9 than those with VOC < 5/ year. There was no relationship between plasma levels of MMP-9 and any of age, gender, transfusion, hydroxyurea therapy, cardiac T2* or type of sickle cell disease. These relations were presented in table 4.

Correlation between MMP-9 plasma level and each of cardiac T2*, frequency of VOC and serum ferritin

There was significant correlation between MMP-9 plasma and frequency of VOC ($r= 0.09$, $p >0.05$) and serum ferritin($r= 0.66$, $p <0.001$) while there was no significant correlation between MMP-9 and cardiac T2* ($r= 0.23$, $p <0.05$).

Discussion

Chronic hemolysis, anemia and vaso-occlusive crises represent a characteristic triad in patients with SCA. While transfusion may improve disease complications of SCA patients, iron overload is a dreaded and inevitable consequence of ongoing transfusion therapy. The role of iron in the pathogenesis of cardiac damage is undisputed, but the underlying molecular mechanisms remain incompletely understood.³

MMP-9 is one among a large family of zinc-dependent endopeptidases. It plays a crucial role in ECM degradation in many physiological and pathophysiological processes that involve tissue remodeling. A bunch of studies offer strong evidence for the role of MMP-9 in LV remodeling.^{4,5}

To the best of our knowledge, this is the first study to evaluate the relationship between MMP-9 and cardiac T2* in sickle cell disease patients. However there is a large body of literature confirming the role of MMP-9 in cardiovascular diseases including in atherosclerosis, hypertension, heart failure and acute myocardial infarction.^{6,7}

Our results showed that patients with sickle cell disease had significantly higher plasma levels of MMP-9 than controls (150.9 versus 32.0 ng/ml respectively, $p < 0.001$).

These results were consistent with the results of Carvalho et al.⁸ who evaluated hematological, biochemical and immunological parameters in 101 stable SCA patients, 23 SCA patients in vaso-occlusive crisis (crisis state) and 146 healthy control individuals. MMP9 levels were significantly increased in patients with stable SCA (p value < 0.0001) and patients with SCA in VOC (p value 0.0091) group than healthy controls.

In their previous study, Carvalho et al.⁹ found that steady-state SCD patients had the highest values of LTB4, PGE2, TIMP1, MMP9, IL-8, and IL-12 concentration compared with SCA patients in crisis and healthy control groups ($p < 0.0001$).

Our results were also in agreement with a previous study conducted by Franco-Penteado et al.¹⁰ where MMP-9 levels were significantly increased in the plasma of SCD patients (20.99 ± 1.52 , $n=32$) compared to healthy controls (13.96 ± 1.64 , $n=16$, $p=0.02$).

Gümüs et al.¹¹ found significantly higher MMP-9 levels in patients with thalassemia major compared to healthy controls ($p = 0.042$, respectively).

In this study, patients with serum ferritin >1000 ng/ml had significantly higher levels of MMP-9 than those with serum ferritin <1000 ng/ml (197.5 versus 93.9 respectively). Also there was highly significant correlation between serum ferritin and MMP-9 ($r = 0.23$, $p < 0.05$).

The effects of iron on MMPs expression have previously been demonstrated. In normal fibroblasts, iron has been shown to increase MMP-1, 2, 3 and 9 expressions in culture via JNK2 activity. Iron could also stimulate MMP-2 activity in rat hepatic stellate cells leading to an increase in matrix degradation. Furthermore, increased amount of iron released in serum of patients with chronic venous disease could lead to an over-expression of MMP-9 and increased tissue destruction. In agreement with these previous studies, Kaomongkolgit et al.¹² provided new evidence that iron could also upregulate MMP-9 expression in head and neck squamous carcinoma cell lines.

Higher MMP-9 levels in patients with high serum ferritin level has been explained by Zamboni et al.¹³ who reported that the iron-driven pathway is one of the recognized mechanisms of MMP hyperactivation and suggested that, the iron-driven pathway as a further mechanism for MMP hyperexpression leading to tissue lesion.

Our results showed that patients receiving iron chelation had significantly higher levels of MMP-9 than those who did not receive iron chelation therapy <1000 ng/ml (181.5 versus 76 respectively). This can be attributed to iron overload that necessitated chelation.

Vaso-occlusion is the most common outcome and the hallmark of SCD. It is responsible for many clinical complications of SCD, including osteomyelitis, osteonecrosis, stroke, splenic infarct, renal insufficiency, and acute chest syndrome. Endothelial damage plays a crucial role in VOC occurrence in SCD. ¹

Our study showed that, patients with vaso-occlusive crises (VOC) > 5/year had significantly higher levels of MMP-9 than those with VOC \leq 5 /year.

Carvalho et al. ⁹ assessed 129 SCD steady-state patients (SP), 23 SCD in crisis patients (CP), and 67 healthy individuals (HC) age- and sex-matched with patients groups. They found that Steady-state SCD patients had the highest values of MMP9 concentration compared with CP and HC groups ($p < 0.0001$). However, crisis-state SCD patients had the lowest levels of MMP9 ($p < 0.0001$).

In a more recent study, Carvalho et al. ⁸ found both Steady-state SCD patients and SCD patients in crises had higher levels of MMP-9 than healthy controls. However there was no statistical significant difference between Steady-state SCD patients and SCD patients in crises.

One of the limitations of our study was that we did not measure MMP-9 in SCA patients during crises. We only evaluated the relationship between MMP-9 levels and the frequency of VOC/ year.

The finding of difference in inflammatory markers level in steady state and crisis SCD patients suggest that these markers can be used to monitoring patients and to predict crisis events.

While ECG, echocardiography, and serum ferritin levels are very easy and cheap methods for detection of cardiac iron overload, they are either not specific for cardiac overload or not enough sensitive for the detection of iron in the heart. Since iron is able to reduce the magnetic resonance signal, the parameter called T2 measures the amount of iron in heart as the loss of signal in iron-loaded tissue. cMRI-T2, which is based on the evaluation of T2, is the most efficient diagnostic technique for the evaluation of cardiac iron overload. T2* values lower than 20 ms indicate presence of iron overload and, as T2* changes according to the concentration of iron, values lower than 10 ms indicate the presence of severe iron overload with high risk of developing cardiac dysfunction within 1 year. ¹⁴

Indeed, prolonged increase in myocardial fibrotic activity results in stiffening of the heart and is an indicator of adverse outcomes related to systolic and diastolic dysfunction, as well as arrhythmogenesis. Regarding fibrosis, matrix metalloproteinases (MMPs), that are important for pathophysiological tissue repair, appear to be involved in cardiac remodeling. The finding that an iron-binding protein like Lipocalin-2 is induced in a porcine model of heart failure and associates with MMP-9 suggest that iron may also directly influence heart fibrotic response. ¹⁵

Though we have found a significant relationship and correlation between MMP-9 and serum ferritin. Yet, we did not find a significant correlation between MMP-9 and cardiac T2* ($r = 0.09$, $p > 0.05$).

This can be attributed to normal cardiac T2* in all patients with a mean of 34.2 ms and a range of 23.3-50.9 ms and also can be owed to small sample size in our study.

Conclusion

MMP-9 seems to be a useful marker in patients with Sickle cell anemia. Patients with serum ferritin > 1000 ng/ml, recurrent VOC > 5 /year and under chelation therapy had significantly higher MMP-9 serum levels than others. No significant correlation between MMP-9 and Cardiac T2* was observed in our study cohort and further multicenter studies with large scale are still needed to support our findings.

Study Limitations

One of the limitations of this study was small sample size. Also we did not measure MMP-9 levels during vaso-occlusive crises. Larger multicenter studies still warranted to support our findings.

Materials And Methods

A case control study was carried out on 50 patients with sickle cell disease and 50 age and sex matched healthy children as a control group. Patients were recruited from pediatric hematology outpatient clinic of Zagazig university hospitals during the period from June 2019 till February 2020.

Inclusion criteria:

- Approval to sign an informed written consent.
- Children with sickle cell disease.
- Age ≥ 10 years.
- Both sex.

Exclusion criteria:

- Refusal to sign an informed written consent.
- Patients with chronic hemolytic anemia other than sickle cell disease.
- Patients with other systemic diseases.
- Patients with known cardiac diseases other than sickle cell disease.
- Patients < 10 years.

Methods:

- Patients were subjected to full history taking, thorough clinical examination , and routine investigations for sickle cell disease according to our local standards e.g. complete blood count, Hemoglobin electrophoresis, serum ferritin and genetic study.
- Assessment of cardiac iron overload in our patients was assessed by cardiac MRI T2* technique using 1.5 Tesla MRI system (Philips Intera Achieva; Philips Medical Systems, Best, The Netherlands).
- Plasma matrix metalloproteinase 9 levels were measured for patients and controls by ELISA method using MBS722532 ELISA kit (MyBioSource , San Diego, USA).

Statement of ethics

The present study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1964 as revised in 2000, and was approved by the Institutional Review Board, faculty of medicine, Zagazig University. Informed consent and ascent forms were obtained from the study participants and/or their guardians.

Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 24 software (IBM Corp., Armonk, N.Y., USA). Categorical data were presented as number and percentages. Chi square test (χ^2), were used to analyze categorical variables. Quantitative data were tested for normality using Kolmogorov Smirnov test assuming normality at $P > 0.05$. Quantitative data were expressed as mean \pm standard deviation, median and range. Student "t" test was used to analyze normally distributed variables among 2 independent groups. Spearman's correlation coefficient (ρ) was used to assess correlation between non parametric variables. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant).

Declarations

Availability of Materials and Data

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author information

Affiliations

Author Contributions

T.H., M.B. and D.H. designed the study research. M.A., A.E., M.F. D.A. and D.H. performed the research. N.R. performed the laboratory part of the study participants. D.H. collect the clinical and laboratory data. T.H., M.B. and D.H. analyzed the data, perform the statistics and write the manuscript. T.H. submitted the manuscript.

Additional Information

Competing Interests:

The authors declare no competing interests.

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Tables

Table (1): Clinical and laboratory data of patients

Initial clinical presentation	N=50
· Pallor	44 (88%)
· VOC	6 (12%)
VOC/Year	
· Mean ± SD	5.6±3.9
· Range	0-12
· Median	4
Serum ferritin (ng/ml)	
· Mean± SD	1719.5±1714
· Range	195-6811
· Median	1129
Cardiac T2* (ms)	
· Mean ± SD	34.2 ± 7.1
· Range	23.3-50.9

VOC: Vaso-Occlusive Crises; SD: Standard Deviation; ms: Milliseconds

Table (2): Transfusion and chelation characteristics of our patients

N=50	
Transfusion Data	
Age of start (Years)	2.5 ± 2.3 (0.45 – 7.0)
· Mean± SD (Range)	
Regularity	
· Regular	32 (64.0%)
· Irregular	18 (36.0%)
Frequency/Year	
· Mean± SD (Range)	12.4 ± 3.4 (6-24)
Chelation therapy	
· Age of start (Years)	4.5 ± 3.3 (0-17)
· Deferasirox	22 (44.0%)
· Deferiprone	14(28.0%)
· No chelator	14 (28.0%)

SD: Standard Deviation.

Table (3): Plasma level of matrix metalloproteinase-9 in patients and controls.

	Patients N=50	Controls N=50	T	p
MMP-9 (ng/ml)				
· Mean ± SD	150.9±103.8	32.0±14.0	19.1	<0.001
· Range	18.8-294.8	18.3-68.8		
· Median	169	28		

MMP-9: matrix metalloproteinase-9; SD: Standard Deviation.

Table (4): Relationship between matrix metalloproteinase-9 and other parameters

	N.	Plasma MMP-9	t	P
Age (Years)				
· ≤ 15	30	147.4±101	0.26	0.79
· > 15	20	158±112.7		
Gender				
· Males	26	172.7±111.2	1.04	0.3
· Females	24	129±94.7		
S. Ferritin (ng/ml)				
· ≤ 1000	22	93.9±86	2.8	0.009
· >1000	28	197.5±95		
VOC/Year				
· ≤5	30	115.6±90.2	2.23	0.035
· >5	20	200.9±98.3		
Chelating therapy				
· No	14	76±73.3	2.52	0.019
· Yes	36	181.5±100		
Type of chelator				
· Deferiprone	14	215.7±83.7	1.17	0.25
· Deferasirox	22	159.7±107.2		
Transfusion				
Age (Years)				
· ≤ 3	38	149.3±95.1	0.216	0.83
· > 3	12	160.1±138		
Regularity				
· Regular	32	167.6±102.9	1.0	0.32
· Irregular	18	124.1±108.9		
Hydroxyurea				
· No	22	153.2±107	0.05	0.95
· Yes	28	150.9±105		

Cardiac T2*(ms)					
·	≤ 35	28	136.9±107.9	0.8	0.4
·	> 35	22	171±100.0		
Diagnosis					
·	HbSβ ⁺	20	151.6±110.2		
·	HbSβ ⁰	14	115.4±99.5	1.8	0.1
·	HbSS	12	222.9±79		
·	HbSA (Trait)	4	68.8±70.5		

Figures

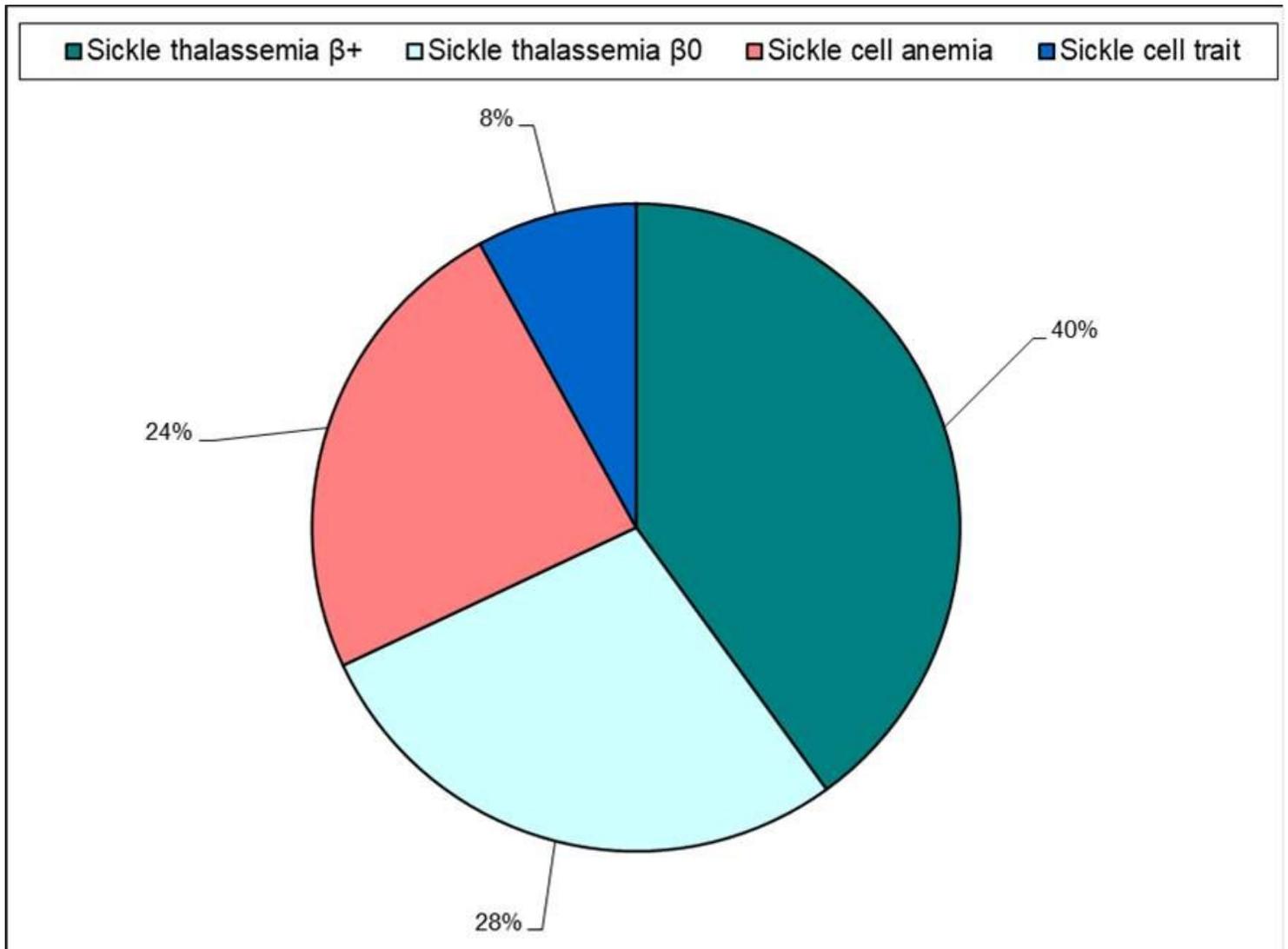


Figure 1

This figure shows that 40% of our patients were sickle cell thalassemia β^+ , 28% were sickle cell thalassemia β^0 , 24% were sickle cell anemia and 8% were sickle cell trait

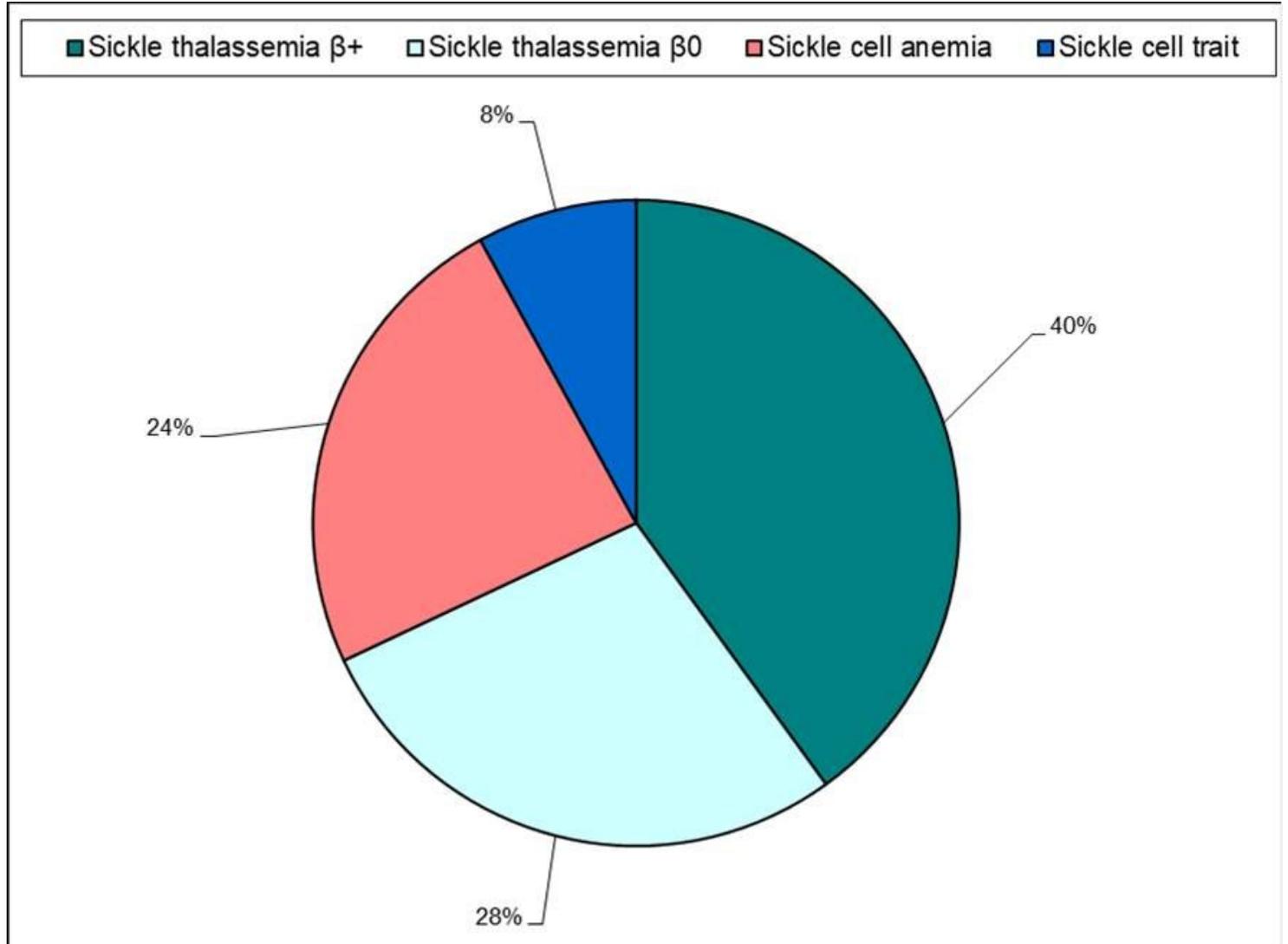


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