

## The Effect of Xmn1 Gene Polymorphism on Blood Transfusion Dependency and Hemoglobin Concentration among Iranian Thalassemia Patients with IVSII-1 Mutation

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### Abstract

**Background:** One of the most important phenotypic modifying factors for thalassemia is the presence of Xmn1 polymorphism. This retrospective study was performed to investigate the overall prevalence of Xmn1 polymorphism among Iranian  $\beta$ -thalassemia patients with homozygote IVSII-1 mutation and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy.

**Materials and Methods:** The present cross sectional study included 112  $\beta$ -thalassemia patients with homozygote IVSII-1 mutation. Laboratory investigations included complete blood count and routine hematological indices were measured by Sysmex K1000 (Japan) blood auto analyzer. To find the state of Xmn1 polymorphism, blood samples were collected from patients using EDTA containers for genomic DNA analysis. DNA extraction and amplification-refractory mutation to determine the Xmn-1 polymorphism were performed.

**Results:** In total, 206 thalassemia patients including 112 patients diagnosed as thalassemia major and 94 patients diagnosed as thalassemia intermediate entered the study. The mean age at the start of transfusion was  $5 \pm 6.4$  years old, and all of the patients received hydroxyurea. Twenty eight patients (14%) did not show any Xmn 1 polymorphisms (- / -), and 178 patients (86%) showed polymorphism either in one loci (- / +, 44 patients, 21.3%) or both loci (+ / +, 134 patients, 65%). Patients with Xmn1 polymorphism showed significantly higher age at diagnosis ( $p=0.002$ ), higher age at start of transfusion ( $p=0.001$ ), higher hemoglobin levels after treatment with hydroxyurea ( $p=0.005$ ), and lower transfusion dependency ( $P=0.044$ ).

**Conclusion:** The presence of Xmn1 polymorphism led to a delay in onset of blood transfusions, higher hemoglobin levels, better response to hydroxyurea treatment and milder phenotypic presentation among thalassemia patients with IVSII-1 mutation.

**Keywords:** Blood Transfusion, Hemoglobin, Polymorphism, Thalassemia.

### Introduction

Beta thalassemia is the most common form of chronic hemolytic anemia due to impaired globin chain synthesis which has carrier frequencies of 1 to 20% in Mediterranean region, Africa, and Southeast Asia, representing a major public health problem in these regions (1). Each year, at least 40,000 people

worldwide are born with  $\beta$ -thalassemia including 23,000 people with  $\beta$ -thalassemia major (2). Iran is located on thalassemia belt and  $\beta$ -thalassemia is the most common genetic disorder in our country (3). According to available statistics, 25,000  $\beta$  thalassemia major patients and about two million carriers of this disease live in Iran (4). The spread of

this disease varies among different regions of Iran, but it is most prevalent around the Caspian Sea and the Persian Gulf (5).

The severity of  $\beta$ -thalassemia symptoms ranges from transfusion-dependent anemia to milder conditions observed among thalassemia intermediate and minor patients (2). One of the clinical challenges for the management of  $\beta$ -thalassemia is to identify the phenotype of patients as early as possible especially in those patients who are between the transfusion-dependant thalassemia major and the non transfusion dependant thalassemia intermedia (6).

There has been progress in predicting phenotype from genotype among thalassemia patients indicating that variable phenotypes may occur based on different  $\beta$ -globin gene mutations (6).

One of the most important phenotypic modifying factors for thalassemia is the presence of Xmn1 polymorphism (7). The association of Xmn1 has been shown to correlate with increased hemoglobin (Hb) production in studies conducted in different countries as well as in Iran (6-9). Xmn-1 polymorphism resulted from a C > T base substitution at the-158 position of  $G\gamma$  globin (HBG2) gene (8). This polymorphism resides in close proximity to locus control region of  $\beta$ -globin gene ( $\beta$ -LCR), which controls differential expression of  $\beta$ -like globin genes throughout the life (10). In the present study, the aim was to investigate the overall prevalence of Xmn1 polymorphism among Iranian  $\beta$ -thalassemia patients with homozygote IVSII-1 mutation, and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy. Hydroxyurea increases the production of fetal hemoglobin (HbF) via reactivation of gamma genes (11). It also suppresses the  $\beta$  globin expression through its cytotoxic effects, which may ameliorate the effects of the alpha/non alpha chain imbalance (12). In this study, only homozygote IVSII-1 patients were

included to exclude the effect of alpha-globin gene mutations and other beta globin gene mutations.

## Materials and Methods

This was a retrospective study which included 206  $\beta$ -thalassemia patients with homozygote IVSII-1 mutation. All patients were regularly followed up at Zafar Thalassemia Clinic, Tehran, Iran. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (1397/5/110741), according to the tenants of declaration of Helsinki, regarding the human studies. All patients' medical history and clinical examination results were extracted from their files. Laboratory investigations included complete blood count and routine hematological indices measured by Sysmex K1000 (Japan) blood auto analyzer.

### Detection of Xmn1 polymorphism

Blood samples were collected from patients into EDTA containers for genomic DNA analysis. DNA extraction and amplification-refractory mutation to determine the Xmn-1 polymorphism were performed as previously described by Miri et al.(8). In summary, amplification-refractory mutation system PCR (CinnaGen Company, Karaj-Iran) and TaqDNA polymerase (CinnaGen Company, Karaj-Iran)) were conducted to determine the Xmn-1 polymorphism as previously described (8).

### Statistical analysis

To present data, mean, standard deviation, median, and range were used. To compare the groups, t-test, Mann-Whitney test, one way analysis of Variance, Kruskal Wallis test, Chi-square, and Fisher exact test were utilized. Some statistics were reported with their 95% confidence interval. All statistical analysis was performed by SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.). P-values less

than 0.05 were considered statistically significant.

## **Results**

In total, two hundred and six thalassemia patients including 112 females and 96 males were included in the present study. These patients were classified as thalassemia major and intermedia, based on their hematological and clinical phenotype. Patients were from various provinces of Iran and all of them had homozygous form of IVSII-1 mutation. Participants in the present study consisted of 112 patients diagnosed as thalassemia major and 94 patients diagnosed as thalassemia intermediate patients. The mean age at the start of transfusion was  $5 \pm 6.4$  years old, and all of patients received hydroxyurea. The baseline demographic and clinical characteristics are illustrated in Table I.

The patients were divided into three major groups based on Xmn1 polymorphism including Xmn-1 +/+, -/+, and -/- groups. Out of 206 patients, 28 patients (14%) did not show any polymorphism (- / -), and 178 patients (86%) showed polymorphism either in one loci (-/+ , 44 patients, 21.3%) or both loci (+/+ , 134 patients, 65%). There was a statistically significant difference between two sexes regarding the Xmn1 polymorphism, while female patients showing higher percent of polymorphism ( $p=0.012$ ) (Table II). No

statistically significant difference was observed between patients with thalassemia major or those with thalassemia intermedia regarding the Xmn1 polymorphism ( $p=0.133$ ) (Table II). Table III shows the age at diagnosis, age at start of transfusion, splenectomy, hemoglobin levels, and transfusion frequency related to the presence or absence of Xmn1 polymorphism. There was statistically significant relation between age at diagnosis and Xmn1 polymorphism status indicating lower age at diagnosis for patients without polymorphism compared to patients with polymorphism ( $p=0.002$ ). The age at start of transfusion was also significantly lower among patients without Xmn1 polymorphism ( $p=0.001$ ). There was a statistically significant difference between patients with Xmn1 polymorphism and those patients without polymorphism in terms of hemoglobin levels after treatment with hydroxyurea indicating higher levels of hemoglobin among patients with polymorphism ( $p=0.005$ ). There was also statistically significant difference between different Xmn1 polymorphism groups regarding the transfusion dependency indicating a higher percentage of transfusion dependency (0.044) among patients without Xmn1 polymorphism compared to those patients with Xmn1 polymorphism.

Table I: Demographic data of patients

Variable		Total
Type of Thalassemia	TM	94 (45.6)
	I	112 (54.4)
Sex	Male	94 (45.6)
	Female	112 (54.4)
Height	Mean $\pm$ SD	159 $\pm$ 13
	Median (range)	160 (99 to 183)
Weight	Mean $\pm$ SD	51 $\pm$ 10
	Median (range)	52 (15 to 74)
Age at Diagnosis	Mean $\pm$ SD	4.2 $\pm$ 5.1
	Median (range)	3 (0.3 to 30)
Age at start of Transfusion (Years)	Mean $\pm$ SD	5 $\pm$ 6.4
	Median (range)	3 (0.3 to 40)
Dose of Hydroxyurea	Mean $\pm$ SD	7.8 $\pm$ 2.2
	Median (range)	7 (2 to 14)
Ferritin Before Hydroxyurea	Mean $\pm$ SD	1565 $\pm$ 2036
	Median (range)	1100 (100 to 23834)
Hb Before Hydroxyurea	Mean $\pm$ SD	9.2 $\pm$ 1.2
	Median (range)	9.1 (6.6 to 14)
Splenectomy	Yes	119 (58.0)
	No	86 (42.0)
Age of Splenectomy (Years)	Mean $\pm$ SD	12 $\pm$ 7
	Median (range)	10 (1 to 36)

Table II: Drug usage, age, sex, and type of thalassemia distribution related to Xmn1 polymorphism

Variable		Total	Xmn 1 polymorphism			P
			- / -	- / +	+ / +	
Sex	Male	94 (45.6)	19 (67.9)	23 (52.3)	52 (38.8)	0.012*
	Female	112 (54.4)	9 (32.1)	21 (47.7)	82 (61.2)	
Type of Thalassemia	TM	94 (45.6)	17 (60.7)	22 (50.0)	55 (41.0)	0.133*
	TI	112 (54.4)	11 (39.3)	22 (50.0)	79 (59.0)	
Height		159 $\pm$ 13	164 $\pm$ 9	160 $\pm$ 12	158 $\pm$ 13	0.148†
		160 (99 to 183)	165 (143 to 180)	163 (130 to 178)	160 (99 to 183)	
Weight		51 $\pm$ 10	56 $\pm$ 8	51 $\pm$ 10	50 $\pm$ 10	0.032†
		52 (15 to 74)	56 (38 to 68)	53 (28 to 74)	51 (15 to 69)	
Dose of Hydroxyurea		7.8 $\pm$ 2.2	7.9 $\pm$ 1.4	7.7 $\pm$ 1.9	7.8 $\pm$ 2.5	0.943†
		7 (2 to 14)	7 (7 to 10)	7 (5 to 14)	7 (2 to 14)	

† Based on One-way Analysis of Variance. , \* Based on Chi-Square test., TM: Thalassemia Major ,TI: Thalassemia intermedia

*Table III: Age at diagnosis, age at start of transfusion, splenectomy, hemoglobin levels, and transfusion frequency related to Xmn1 polymorphism*

		Total	Polymorphism Xmn1			P
			- / -	- / +	+ / +	
<b>Age at Diagnosis</b>		4.2 ± 5.1	2 ± 2.1	3.8 ± 4.5	4.8 ± 5.6	0.002‡
		3 (0.3 to 30)	1 (0.5 to 8)	1.8 (0.5 to 20)	3 (0.3 to 30)	
<b>Age at start of Transfusion</b>		5 ± 6.4	2.4 ± 2.4	3.8 ± 4.5	5.9 ± 7.2	0.001‡
		3 (0.3 to 40)	1 (0.5 to 10)	1.8 (0.5 to 20)	4 (0.3 to 40)	
<b>Splenectomy</b>	Yes	119 (58.0)	16 (57.1)	29 (65.9)	74 (55.6)	0.486*
	No	86 (42.0)	12 (42.9)	15 (34.1)	59 (44.4)	
<b>Age of splenectomy</b>		12 ± 7	9 ± 5	13 ± 7	13 ± 8	0.135†
		10 (1 to 36)	8 (1 to 22)	10 (5 to 36)	11 (2 to 35)	
<b>Hemoglobin</b>	Before	9.2 ± 1.2	8.9 ± 0.9	9.3 ± 1.2	9.2 ± 1.2	0.326†
	After	9.4 ± 1.4	8.8 ± 0.8	9.9 ± 1.7	9.3 ± 1.3	0.005†
<b>Transfusion</b>	Dependency	98 (100%)	19 (19.4%)	21 (21.4%)	58 (59.2%)	0.044‡
	Independency	107 (100%)	9 (8.4%)	22 (20.6%)	76 (71.0%)	
<b>Annual Transfusion (N/Year)</b>	Before	13 ± 5	14 ± 5	13 ± 6	13 ± 6	0.773†
	After	5 ± 5	4 ± 4	4 ± 3	5 ± 5	0.158†
<b>Ferritin</b>	Before	1565 ± 2036	1286 ± 877	1668 ± 1433	1596 ± 2367	0.18†
	After	1421 ± 1460	1144 ± 628	1592 ± 1355	1423 ± 1608	0.456†

## Discussion

This study was conducted to evaluate the frequency of receiving PRBC transfusion in preterm infants admitted to NICU of Shahid Sadoughi Hospital. Multiple complications of transfusion and immune system disorders in infants may be related to the blood transfusion. In the current study, the mean birth weight of Apgar was significantly lower in neonates with transfusion. It seems that this may be due to the effect of hypoxia on functional dysfunction of various organs. Other factors, including increased risk of coagulation disorder and consequently

increased need for transfusion in these infants may also play a significant role. Complications such as sepsis, respiratory failure, and pneumothorax were significantly higher in patients undergoing transfusion. Perciaccante et al., (22) reported that RBC transfusion increased the incidence of necrotizing enterocolitis. RBC transfusion induces a pro-inflammatory answer which may confirms the pathogenesis of transfusion related necrotizing enterocolitis (13). Dani et al. evaluated 20 premature infants and measured the level of several cytokines before and after transfusion. The findings

showed the increase of interleukins, namely IL-1 $\beta$ , IL-17 and IL-8 (23). A study showed that transfusion was related to developing of retinopathy of prematurity (24). Dani et al., also reported that RBC transfusion volume was associated with ROP in infants with a birth weight of < 1,250 g (23). Wang et al., reported that red blood cell transfusion was associated with intraventricular hemorrhage (24). Early RBC red blood cell transfusion suppresses production of erythropoietin (25). Hirano et al., reported that after blood transfusion, plasma non-transferrin bound iron (ferrous form) was significantly higher in preterm infants due to the reduction of ferric iron (Fe<sup>3+</sup>) by ascorbic acid and the low ferroxidase activity. Moreover, Hirano et al., reported that most preterm infants developed retinopathy of prematurity and/or chronic lung disease (26). According to results of these studies, it seems that transfusion is associated with serious complications. Borna et al. assessed the frequency and risk factors of blood transfusions in newborns. In their study, 19.98% of patients needed transfusion. Moreover, significant relation was seen between frequency of blood transfusion with using of ventilator and during hospital stay. They reported that decreasing length of hospital stay and duration of mechanical ventilation led to reduction of the frequency and complications of blood transfusions. Moreover, given the wide range of differences between different communities for choosing the type of product, the time, and correct dose of consumable products, physicians should consider transfusion guideline to prevent unnecessary consumption of blood products (19).

In the current study, there was significant relation between blood transfusion and birth weight of infants. Mosayebi et al., reported that there was a significant relationship between birth weight and blood volume (27). Strauss et al., reported significant relation between birth weight and the need for blood, which is consistent

with findings of our study. However, Rafati et al., demonstrated inverse relation between blood transfusion and birth weight (19). This difference can be due to different blood transfusion protocols in hospitals and different guidelines used in different parts of the world.

In total, the mean blood transfusion for each infant was 16.51 cc /kg. Bednarek et al., suggested that the mean blood transfusion was 24 cc/kg per baby (28). In another study by Patterson et al., transfusion rates in infants were reported 29% (33). Moreover, 55.9% of patients received at least one RBC transfusion in Brazil (29). The mean volume of wasted blood in current study was 488.39 ml. It seems that this difference may be due to different blood transfusion protocols in different hospitals. Rafiei et al., found that the pattern of blood transfusion in the Besat hospital was not ideal. They also observed that excessive demand for blood did not only reduce the quality of blood, but also imposed additional financial burdens on health centers (30). Contrary to the current study, most studies did not investigate the amount of wasted blood, which was a hallmark of this study. Presence of Xmn1 polymorphism has been reported to be one of the main phenotypic modifying factors of  $\beta$ -thalassemia (6-9). In the present study, the aim was to investigate the overall prevalence of Xmn1 polymorphism among Iranian  $\beta$ -thalassemia patients with homozygote IVSII-1 mutation and to assess the relationship between Xmn1 polymorphism with patients' hemoglobin levels and the response to hydroxyurea (Hu) therapy. In the present study, the overall frequency of Xmn1 gene polymorphism was 86%, which happened either in one loci (- / +, 21%) or both loci (+ / +, 65%). Said et al., studies Egyptian patients with thalassemia major or intermedia patients, and yielded that only 8% of patients showed Xmn1 polymorphism, which was significantly lower than the present study (6). In another study by Miri-Moghaddam et al.,

in South East of Iran Xmn-1 polymorphism was observed in 62% of patients which is more similar to the present study findings. It seems that the Xmn1 polymorphism has a variable geographical distribution among patients from different countries (8).

The frequency of positive heterozygote Xmn1 gene polymorphism in thalassemia major and intermedia was previously compared among Egyptian patients with  $\beta$ -thalassemia (13, 14). These studies showed a frequency of 9% polymorphism in  $\beta$ -thalassemia intermedia patients and 4% in  $\beta$ -thalassemia major patients (13, 14). The findings of the present study were in line with previous findings indicating no statistically significant difference regarding the frequency of positive Xmn1 gene polymorphism between patients with thalassemia intermediate or thalassemia major.

In the present study, significant differences in transfusion dependency was observed, indicating that Xmn1 positive patients were statistically less transfusion dependent and the hemoglobin level after treatment with hydroxyurea was higher among patients with Xmn1 polymorphism compared to those patients without polymorphism. This was in line with earlier studies which revealed that the presence of the Xmn1 polymorphism might contribute to higher hemoglobin levels among thalassemia patients (6-9). In addition, patients with Xmn1 polymorphism in the present study were more frequently transfusion independent and had an older age at first transfusions.

We also observed that Xmn1 polymorphism had statistically significant effect on the response to hydroxyurea treatment. This was in concordance with previous studies that reported correlation between the presences of Xmn1 polymorphism and response to hydroxyurea therapy in  $\beta$ -thalassemia patients (15-18). For example Bradai et al., in a study including nine patients with thalassemia intermedia and 45 patients with thalassemia major (TM), found that

Xmn1-/- was associated with a worse response to hydroxyurea treatment. However, other researchers reported that the response to hydroxyurea was not significantly correlated with the presence of Xmn1 polymorphism (19,20). For example, Koren et al., in a study on 18 beta-thalassemia patients treated with HU, found no correlation between response to therapy and the presence of Xmn1 polymorphism, which might be attributed to their relatively small sample size (19). Moreover, in another study by Dixit et al., on thirty-seven patients with beta-thalassemia intermedia, no association was detected between response to hydroxyurea and Xmn1 polymorphism (20).

Some shortcomings of the present study are the retrospective nature of the study as well as the relatively low number of patients in thalassemia major and intermediate groups, which might make the results regarding the absence of Xmn1 polymorphism difference between these two groups of patients less reliable.

## **Conclusion**

In conclusion, we suggested that the presence of Xmn1 polymorphism might lead to a delay in onset of blood transfusions, higher hemoglobin levels, better response to hydroxyurea treatment, and milder phenotypic presentation among thalassemia patients with IVSII-1 mutation.

## **Conflict of interest**

There is no conflict of interest.

## **References**

- 1- De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, et al.  $\beta$ -Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. *Mediterr J Hematol Infect Dis* 2017; 9(1):e2017018-e2017021.
- 2- Cappellini MD, Viprakasit V, Taher AT. An overview of current treatment

- strategies for  $\beta$ -thalassemia. *Expert Opin Orphan Drugs* 2014; 2(7): 665-679.
- 3- Hashemieh M, Timori Naghadeh H, Tabrizi Namini M, Neamatzadeh H, Hadipour Dehshal M. The Iran Thalassemia Prevention Program: Success or Failure? *Iran J Ped Hematol Oncol* 2015;5(3):161-166.
- 4- Saleh-Gohari N, Bazrafshani M. Distribution of  $\beta$ -Globin Gene Mutations in Thalassemia Minor Population of Kerman Province, Iran. *Iran J Public Health* 2010; 39(2):69-76.
- 5- Zeinalian M, Nobari RF, Moafi A, Salehi M, Hashemzadeh-Chaleshtori M. Two decades of pre-marital screening for beta-thalassemia in central Iran. *J Community Genet* 2013; 4(4):517-522.
- 6- Said F, Abdel-Salam A: XmnI polymorphism: Relation to  $\beta$ -thalassemia phenotype and genotype in Egyptian Children. *Egypt J Med Hum Genet* 2015: 16(2): 123-127.
- 7- Dadheech S, Jain S, Madhulatha D, Sharma V, Joseph J, Jyothy A, et al. Association of XmnI -158  $\gamma$ G variant with severity and HbF levels in  $\beta$ -thalassemia major and sickle cell anaemia. *Mol Biol Rep* 2014; 41(5):3331-3337.
- 8- Miri-Moghaddam E, Bahrami S, Naderi M, Bazi A, Karimipoor M. XmnI-158 gammaG Variant in  $\beta$ -Thalassemia Intermediate Patients in South-East of Iran. *Int J Hematol Oncol Stem Cell Res* 2017; 11(2):165-171.
- 9- Agouti I, Badens C, Abouyoub A, Khattab M, Sayah F, Barakat A, et al. Genotypic correlation between six common beta-thalassemia mutations and the XmnI polymorphism in the Moroccan population. *Hemoglobin* 2007; 31(2):141-149.
- 10- Motovali-Bashi M, Ghasemi T. Role of XmnIG Polymorphism in Hydroxyurea Treatment and Fetal Hemoglobin Level at Isfahanian Intermediate  $\beta$ -Thalassemia Patients. *Iran Biomed J* 2015; 19(3):177-182.
- 11- Cokic VP, Smith RD, Beleslin-Cokic BB, Njoroge JM, Miller JL, Gladwin MT, et al. Hydroxyurea induces fetal hemoglobin by the nitric oxide-dependent activation of soluble guanylyl cyclase. *J Clin Invest* 2003; 111(2): 231-239.
- 12- Mabaera R, West RJ, Conine SJ, Macari ER, Boyd CD, Engman CA, et al. A cell stress signaling model of fetal hemoglobin induction: what doesn't kill red blood cells may make them stronger. *Exp Hematol* 2008; 36(9):1057-1072.
- 13- Kaddah N, Rizk S, Kaddah AM, Salama K, Lotfy H. Study of possible genetic factors determining the clinical picture of thalassemia intermedia. *J Med Sci* 2009; 9:151-155.
- 14- Tantawy AA, Andrawes NG, Ismaeil A, Kamel SA, Emam W. Prevalence of XmnI G $\gamma$  polymorphism in Egyptian patients with  $\beta$ -thalassemia major. *Ann Saudi Med* 2012; 32(5):487-491.
- 15- Bradai M, Pissard S, Abad MT, Dechartres A, Ribeil JA, Montalembert M. Decreased transfusion needs associated with hydroxyurea therapy in Algerian patients with thalassemia major or intermedia. *Transfusion* 2007; 47(10):1830-1806.
- 16- Sharma N, Das R, Kaur J, Ahluwalia J, Trehan A, Bansal D, et al. Evaluation of the genetic basis of phenotypic heterogeneity in north Indian patients with thalassemia major. *Eur J Haematol* 2010; 84(6):531-517.
- 17- Alebouyeh M, Moussavi F, Haddad-Deylami H, Vossough P. Hydroxyurea in the treatment of major beta-thalassemia and importance of genetic screening. *Ann Hematol* 2004; 83(7):430-433.
- 18- Yavarian M, Karimi M, Bakker E, Hartevelde CL, Giordano PC. Response to hydroxyurea treatment in Iranian transfusion-dependent beta-thalassemia patients. *Haematologica* 2004; 89(10):1172-1178.
- 19- Koren A, Levin C, Dgany O, Kransnov T, Elhasid R, Zalman L, et al. Response to hydroxyurea therapy in beta-



thalassemia. *Am J Hematol* 2008; 83(5):366-370.  
20- Dixit A, Chatterjee TC, Mishra P, Choudhry DR, Mahapatra M, Tyagi S, et al. Hydroxyurea in thalassemia intermedia-a promising therapy. *Ann Hematol* 2005; 84(7):441-446.