IN THE NAME OF GOD

دانشگاه علوم پزشکی و خدمات بهداشتی درمانی زنجان

Title: Frequency of HFE gene C282Y and H63D mutations and their correlation with iron status in Iranian beta-thalassemia major patients

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- Thalassemias are the most common monogenic diseases in man.

Inherited, autosomal recesive

- Beta Thalassemia major:
  - Primary abnormality is the absence or severely reduced rate of Beta globin chain synthesis.
  - Defined by imbalance αβ ratio.
  - Associated with severe microcytic hypochromic anemia.
  - Need lifelong transfusion therapy.
  - Definitive treatment : BMT

Epidemiology of thalassemia in Iran

- a -thalassemia is rare in Iran.
- ß-thalassemia is fairly common.
- The gene frequency of ß-thalassemia is about or more than 10% around the Caspian sea, and Persian Gulf.
- In Isfahan, the frequency rises again to about 8%.
- In the Fars Province, in southern Iran, the gene frequency is also high and reaches 8-10%.

Epidemiology of thalassemia in Iran

- It was found that the b-thalassemia frequency is 1.2-2% for Zanjan region.
- The prevalence of ß-thalassemia in other areas is between 4% to 10%.

Epidemiology of thalassemia in Iran

- More than two million carriers of ß-thalassemia live in Iran.
- 20,000 patients affected with ß-thalassemia are living in Iran.

Supportive therapy for beta thalassemia patients

Regular Red Blood cell Transfusion is the most common Supportive therapy for Beta thalassemia patients

Sunnybrook
HEALTH SCIENCES CENTRE
when it matters MOST

Blood transfusion complications

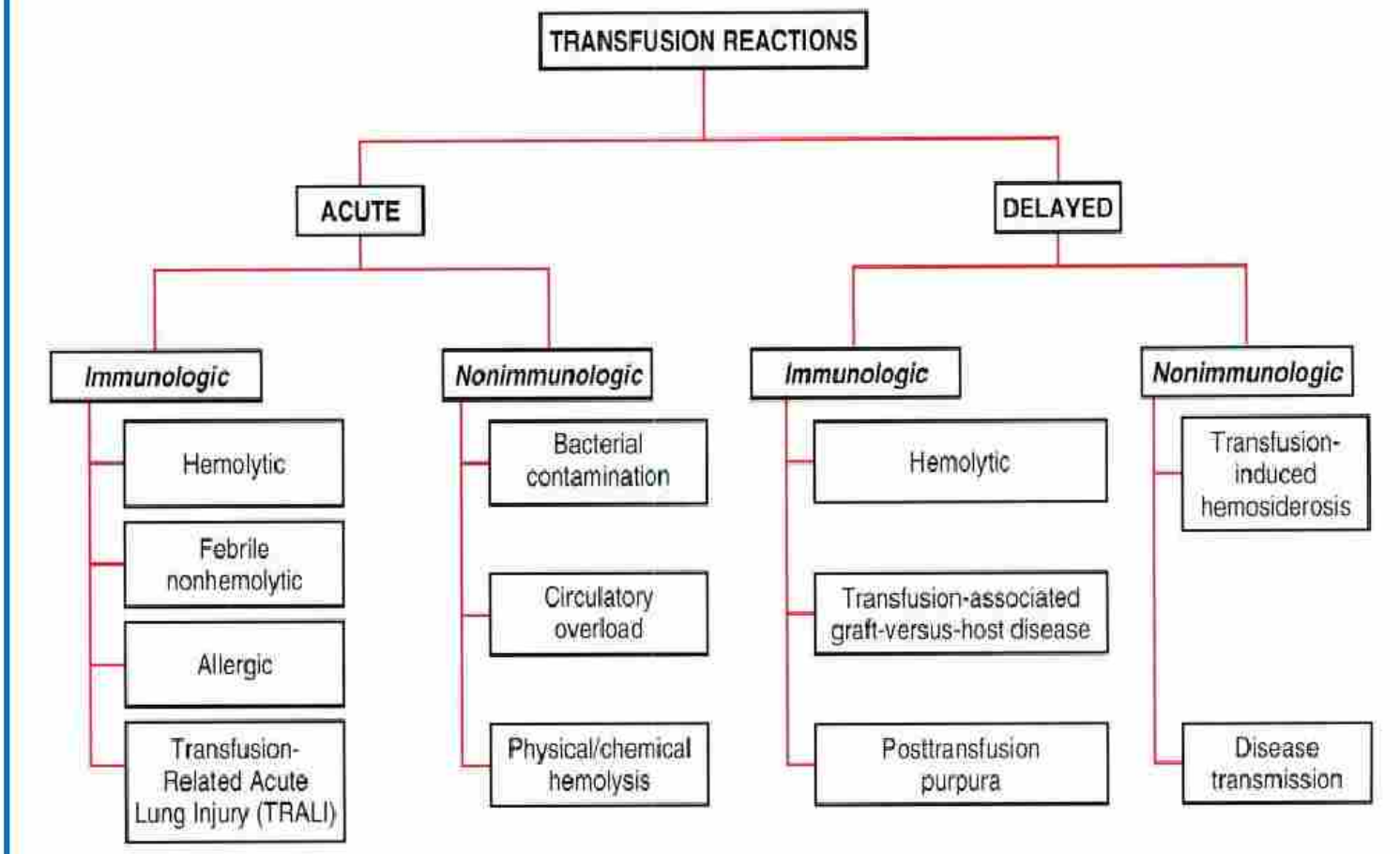


Fig 15-1 Transfusion reactions.

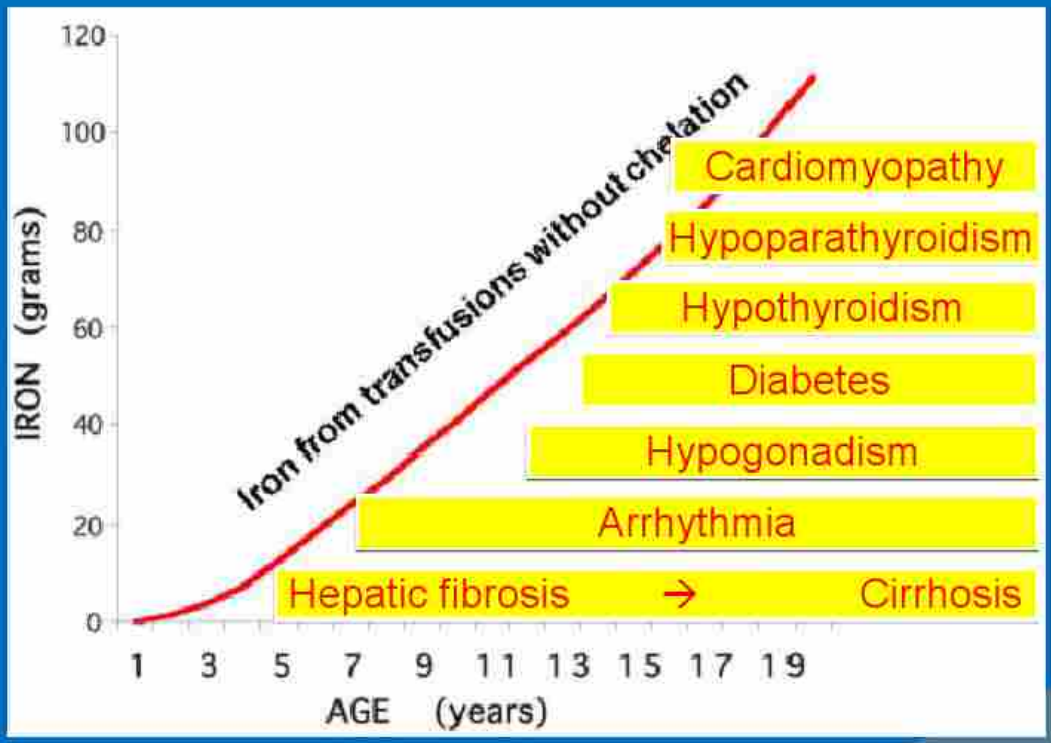
Blood transfusion overwhelms the iron balance

- Normal daily iron flux:

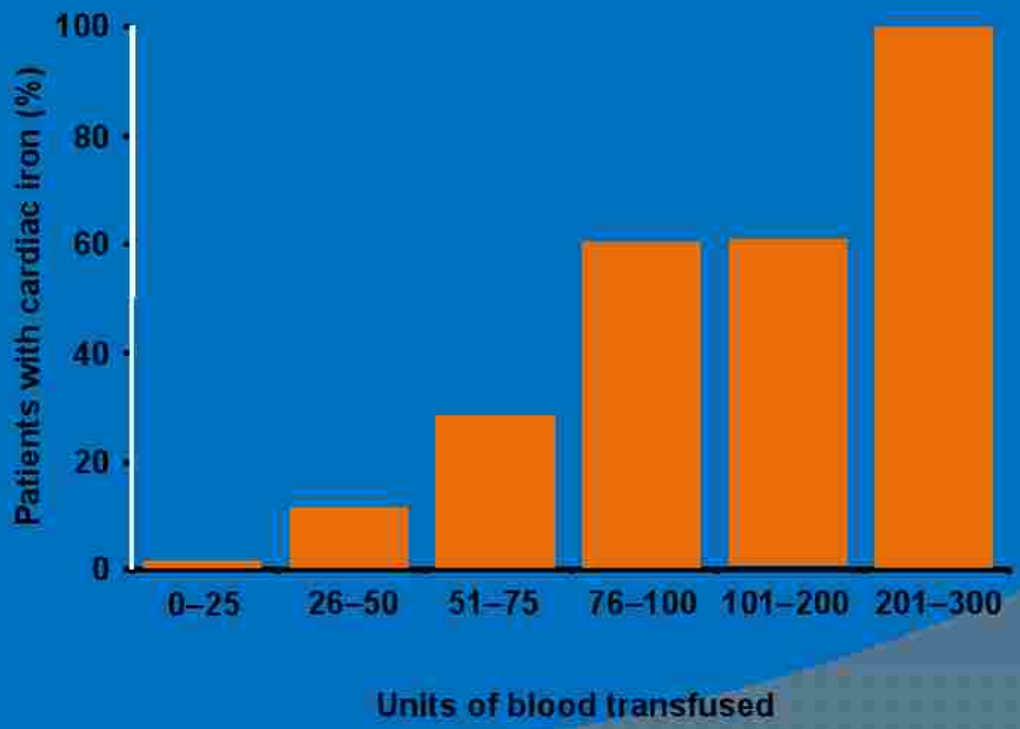
1-2 mg

- Each unit of PRBC:

Iron overload complication



Cardiac iron deposits correlate with blood transfusions



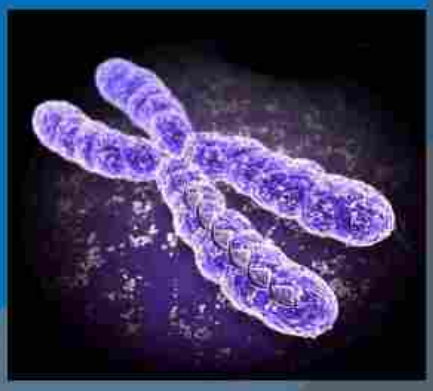
Study Design:
Heterogeity in Iron overload complication

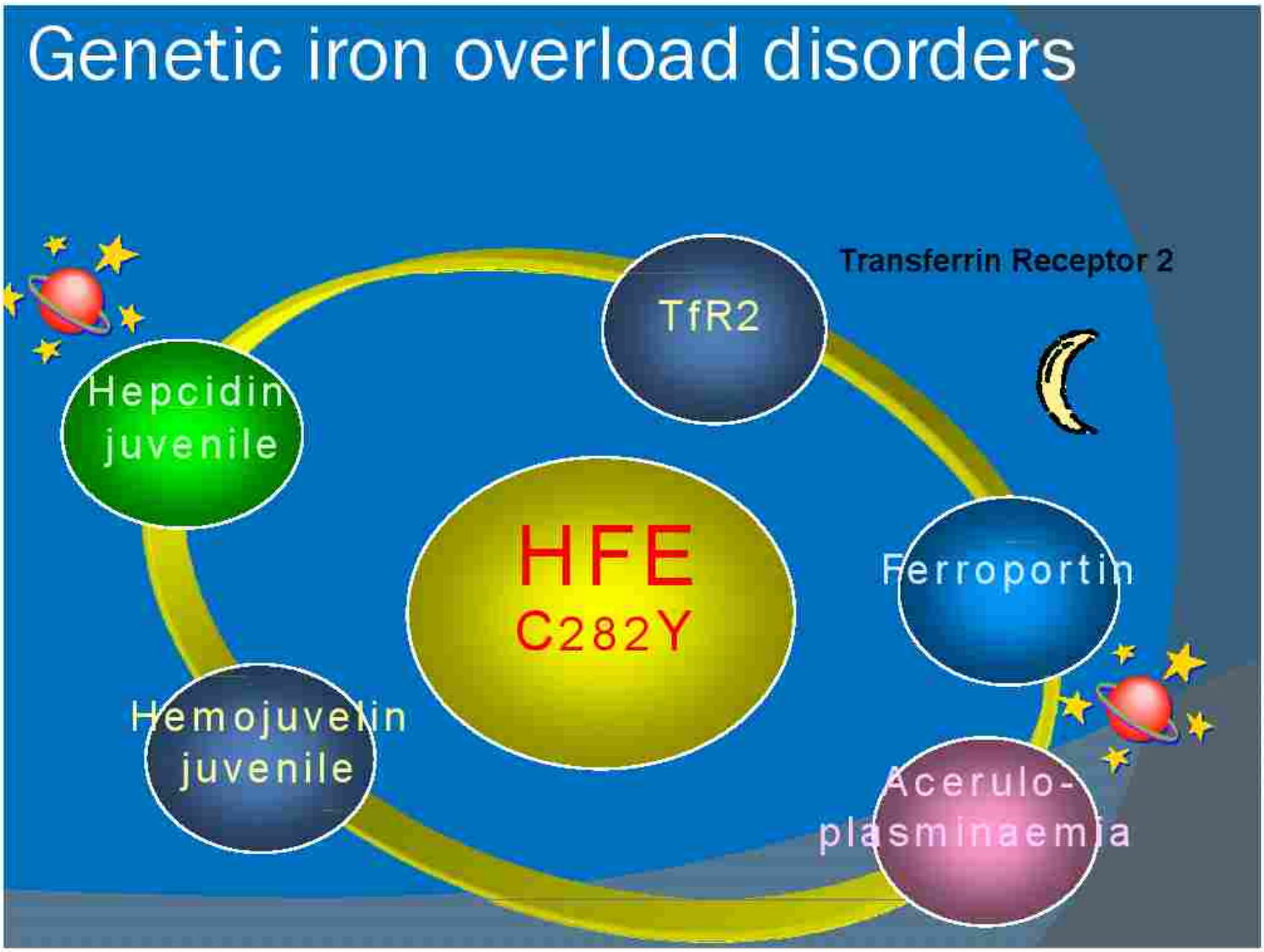
- The Iron overload complication is more severe and more common in some patients than others.
- Organ specific hemosiderosis is more common in some patients than others.
- In spite of regular and intense iron chelation therapy, some patients develop Iron overloading while others do not.

Causes of iron overload in BTM patients

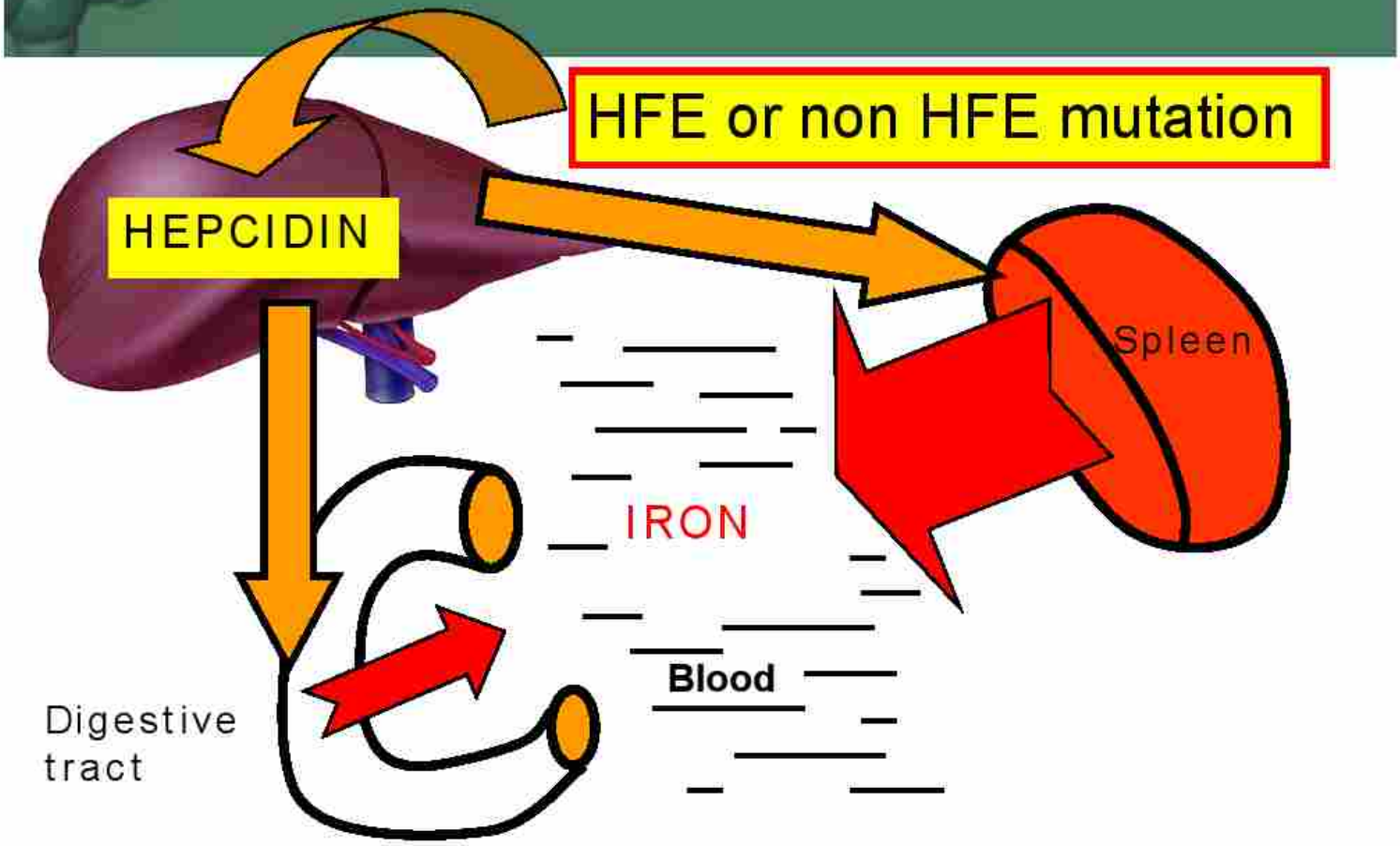
- Transfusional iron overload

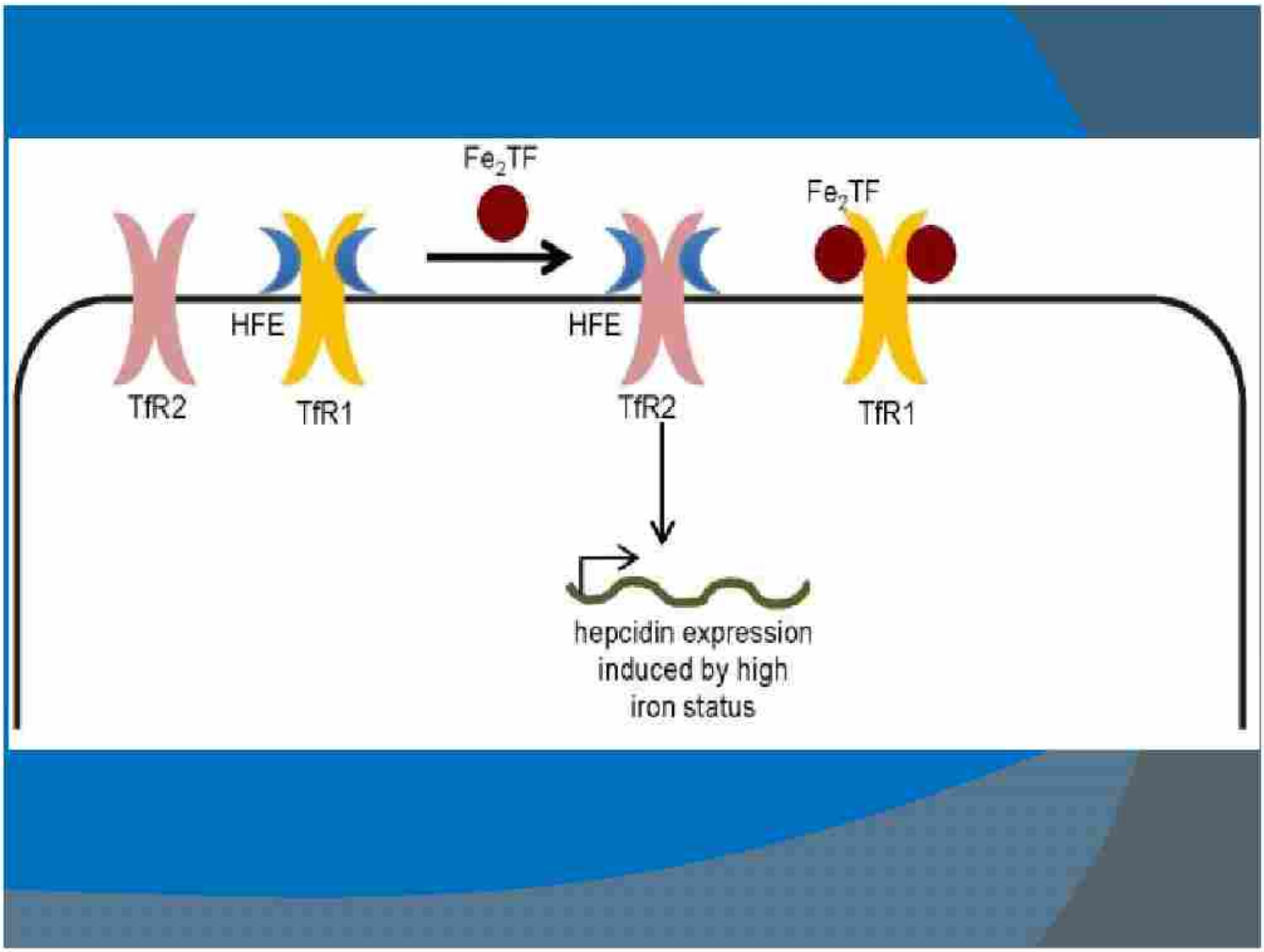
- Genetic iron overload




Genetic iron overload disorders
Transferrin Receptor 2
TfR2
Hepcidin juvenile
HFE
C282Y
Ferroportin
Hemojuvelin juvenile
Acerulo-plasminaemia

iron overload




Fe2TF
Fe2TF
HFE
HFE
TfR2
TfR1
TfR2
TfR1
hepcidin expression
induced by high
iron status

- **The C282Y mutation:**
  preventing the HFE molecule from interacting with b2-microglobulin

- **HFE H63D mutation:**
  Reduces the HFE- transferrin receptor affinity

The study objectives:

- Prevalence of HFE mutations in BTM patients and control subjects.
- Association between HFE H63D mutation with biochemical iron markers and ferritin.
- Correlation between HFE H63D mutation with cardiac and hepatic hemosiderosis.
- Correlation between plasma ferritin levels with cardiac and hepatic hemosiderosis.

How do we know if there's too much iron?

- Serum ferritin concentration
  - Used in clinical practice globally
- Liver biopsy
  - Reference methodology ('gold standard')
- Magnetic resonance imaging (MRI)
  - Investigational, potential for broad access

Materials and Methods:

- Study population: consisted of 65 BTM patients including 31 women and 34 men (mean age 17.73 ± 9.26) and 200 healthy subjects as controls.

- All patients were on regular Red blood cell transfusion at 2-4 weak intervals to maintain Hemoglobin levels between 7-9 g/dl.

Materials and Methods:

- **Iron markers:** Routine colorimetric assays
- **Ferritin levels**: Enzyme immunoassays or Chemiluminescence.
- **HFE H63D, HFE C282Y mutations**: PCR-RFLP

Table 1: PCR products and enzyme digesting fragments				
Mutation	P.S	Wt allele (bp)	Mu allele(bp)	R.E
C282Y	390bp	250,140	250,111,29	RsaI
H63D	207bp	138,69	207	BclI
P.S: product size, Mu: mutant, Wt: wild type, R.E: Restriction enzyme				

Materials and Methods:

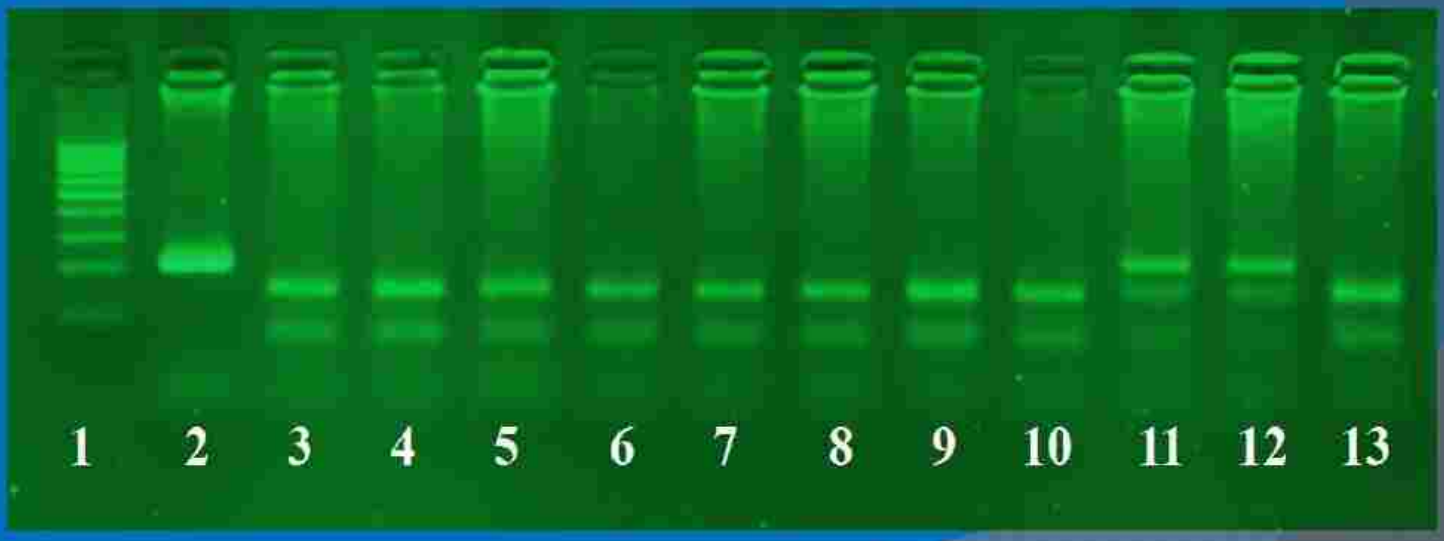
Table 1 Guideline used for assessment of cardiac and hepatic iron overloading in thalassemia patients

Group	Cardiac T2*MRI (ms)	Hepatic T2*MRI (ms)	LIC
1, Normal	>20	>6.3	<2
2, Mild	14-20	2.8-6.3	2-5
3, Moderate	10-14	1.4-2.7	5-10
4, Severe	<10	<1.4	>10

LIC: Liver iron concentration

- Results:

Mutation analysis of HFE H63D: The PCR products were digested by restriction enzyme BclI I: 1 (100 bp ladder); Lanes 3-10,13 wild type genotype (138bp, 69bp); Lanes 11, 12 heterozygote genotype (207 bp ,138 bp,69 bp); Lane 2 homozygote genotype (207bp).



Mutation analysis of HFE C282Y:

The PCR products were digested by restriction enzyme RsaI I: 1 (100 bp ladder); Lane 2: Undigested product (390bp); Lanes 2-12 wild type genotype (250 bp ,140 bp).

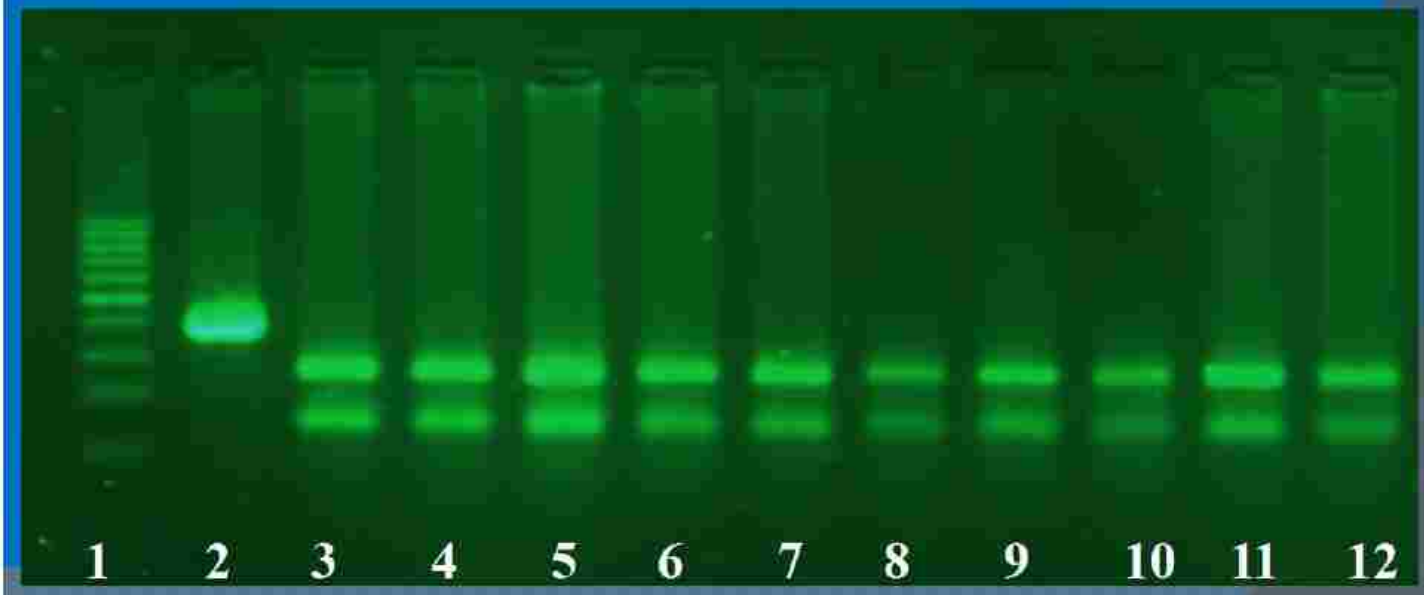


Table 2: Genotype frequency of HFE H63D and C282Y gene mutations among thalassemia major patients and controls

Gene mutation	Patients (n=65)	Controls (n=200)	P value	OR (95%CI)
HFE H63D				
Wild	52 (80.00%)	158 (79.00%)	1	Reff
Hetero	10 (15 .38%)	37 (18.50%)	0.614	0.82 (0.38-1.77)
Homo	03 (04.62%)	05 (2.50%)	0.421	1.82(0.42-7.89)
HFE C282Y				
Wild	65 (100%)	197 (98.5%)	1	Reff
Hetero	0 (0.0%)	03 (1.50%)	0.857*	undefined
Homo	0 (0.0%)	0 (0.00%)	undefined	undefined

Table 3: Analysis of HFE H63D gene mutation among thalassemia major patients and controls using dominant, recessive and allelic genetic models

Genetic Model	Genotype/Allele	Patients (n=65)	Controls (n=200)	P value	OR (95%CI)
----------	Wild	52 (80.00%)	158 (79.0%)	1	Ref
Dominant	Hetero + Homo	13 (20.0%)	42 (21.0%)	0.862	0.94 (0.46-1.88)
----------	Wild + Hetero	62 (95.38%)	195 (97.5%)	1	Ref
Recessive	Homo	03 (4.62%)	5 (2.5%)	0.394	1.88 (0.44-8.12)
----------	Wild allele	114(87.69%)	353(88.25%)	1	Ref
Allelic	Mutant allele	16 (12.31%)	47 (11.75%)	0.864	1.05 (0.57-1.93)

Table 4: Profile of β-thalassemia major patients with and without HFE H63D mutation

Parameters	HFE H63D carrier n= 13	HFE H63D non-carrier n=52	P value
Ferritin (µg/L)	1930 ± 912	1221 ±751	0.005
Iron (µg/dL)	213.68±48.46	165.87±57.80	0.008
TSI (%)	63.34±15.37	46.54± 14.23	0.042
MTB (ml/kg/year)	225.82± 53.47	203.52± 63.34	0.247
Splenectomy	5/13	12/52	0.260

Table 5: Genotype frequency of HFE H63D and C282Y gene mutations among thalassemia patients or carriers in different ethnic populations

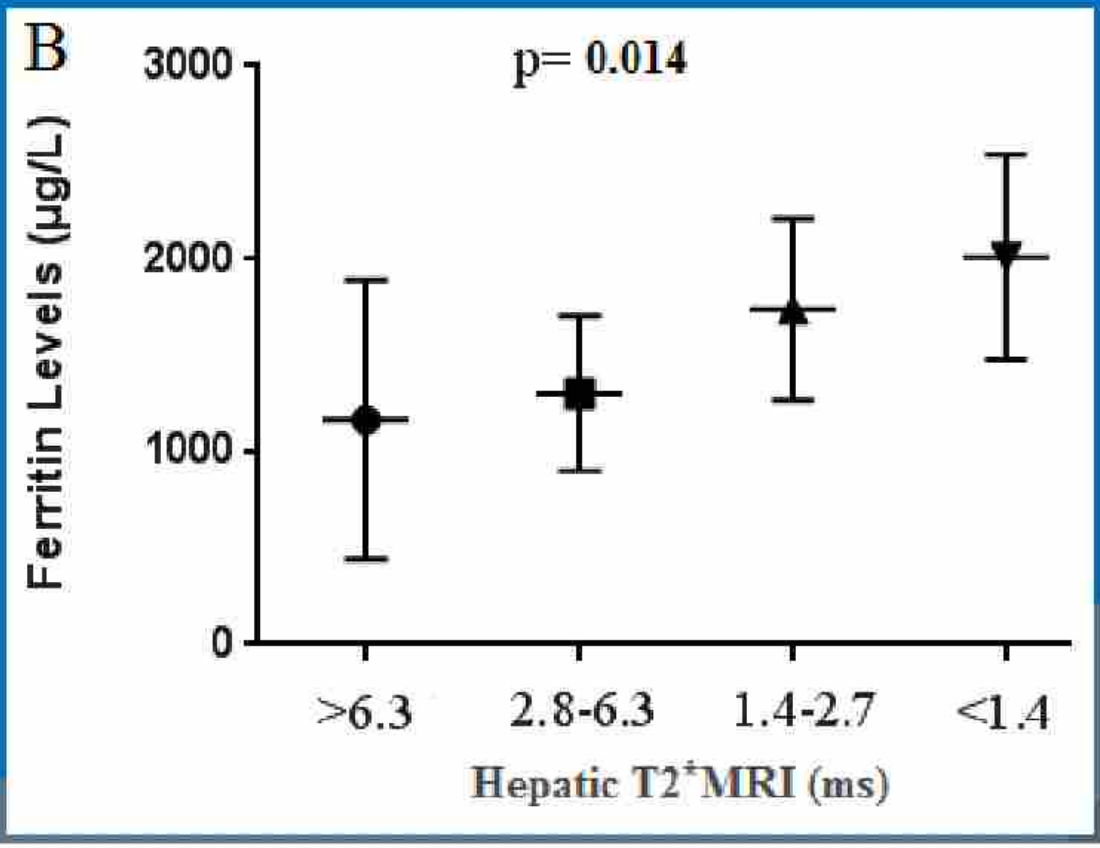
Population (Reference)	Number of studied patients	H63D (%)		C282Y (%)	
		Heterozygote	Homozygote	Heterozygote	Homozygote
Brazil [9]	168	22.61	2.38	4.76	0
Turkey [10]	33	15.15	3.03	0	0
North Indian [11]	308	13.96	0.97	0	0
Italy [12]	71	21	1.4	1.4	0
Tunisian [13]	50	26	04	0	0
Portugal [14]	101	30.7	0	2.97	0
Egypt [4]	75	37.3	10.7	0	0
Iran (current study)	65	15.38	4.62	0	0

Frequency of hepatic and cardiac hemosiderosis based on MRI*T2 results

- Cardiac hemosiderosis: 23 of the BTM patients (38.33 %).
- Hepatic hemosiderosis: 25 of the BTM patients (41.66%).

Table 5 Association between hepatic and cardiac hemosiderosis and some biochemical iron markers in beta thalassemia major patients			
Patients parameters	Ferritin (µg/L)	Iron (µg/dL)	TSI
Hepatic hemosiderosis	1633.83±414.56	219.49 ± 51.53	59.63±17.67
Non- hepatic hemosiderosis	1162.14±723.44	158.13±56.78	45.89±12.53
P value	0.021	0.001	0.010
Cardiac hemosiderosis	1710.72±463.28	231.67±48.59	61.18±16.58
Non-cardiac hemosiderosis	1130±663.75	153.88±56.14	47.66± 13.14
P value	0.008	0.001	0.012
TSI: Transferrin saturation index			

Comparison of plasma ferritin levels among the four groups of patients with thalassemia regarding T2* MRI of liver (B).



Comparison of plasma ferritin levels among the four groups of patients with thalassemia regarding T2* MRI of heart (B).

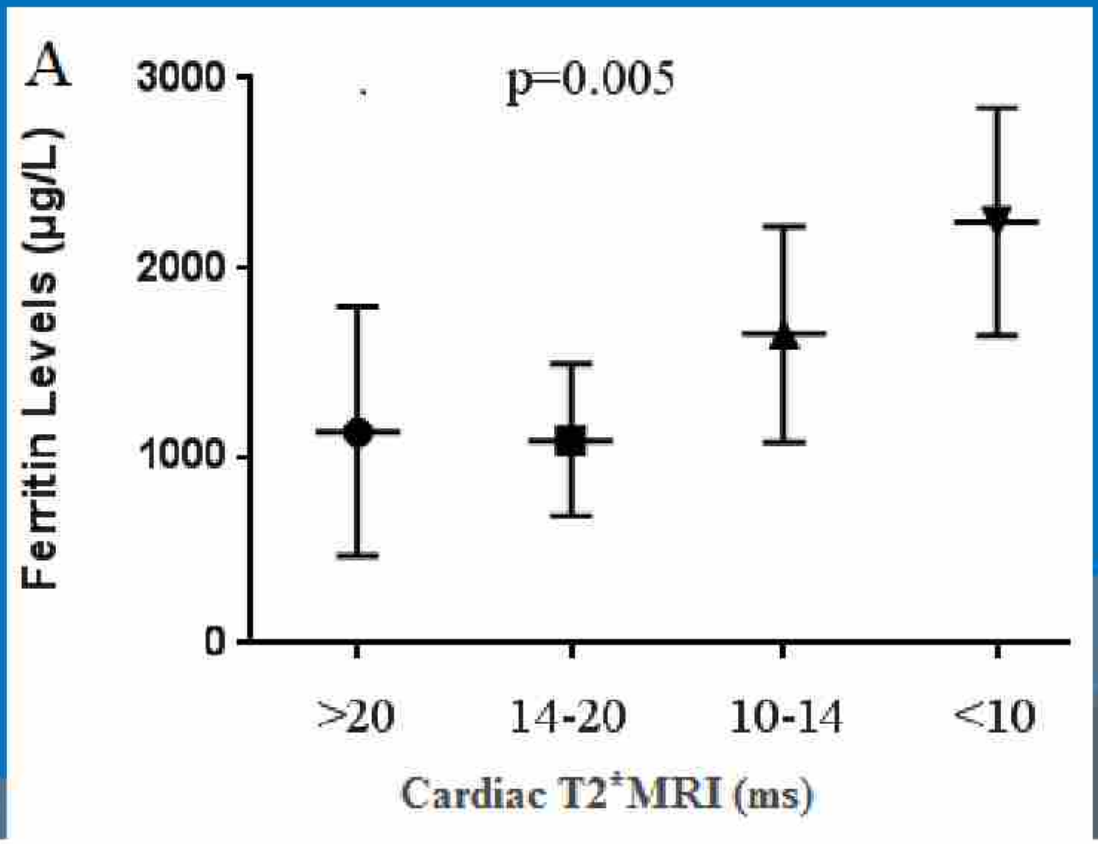


Table 3 Correlation of plasma ferritin level with T2* MRI of heart and liver and liver iron concentration in patients with beta thalassemia major

Variable	Value	P value
T2*MRI Heart	-	<0.001
Correlation coefficient	-0.781	
%95 Confidence Intervals	-0.901-0.653	
T2*MRI Liver	-	0.002
Correlation coefficient	-0.637	-
%95 Confidence Intervals	-0.850-0.553	-
Liver Iron Content	-	<0.001
Correlation coefficient	+ 0.815	-
%95 Confidence Intervals	0.627-0.845	-

Table 4 The comparison of some genetic and biochemical markers between hepatic and cardiac hemosiderosis patients

Genetic or biochemical markers	Hepatic hemosiderosis patients (n= 25)	Cardiac hemosiderosis patients (n=23)	P value
HFE H63D carrier	7 (28%)	6 (26.1%)	0.910
Ferritin (µg/L)	1633.83±414.56	1710.72±463.28	0.546
Iron (µg/dL)	219.49 ± 51.53	231.67±48.59	0.404
TSI (%)	59.63±17.67	61.18±16.58	0.813

TSI: Transferrin saturation index

Table 5 The association between hepatic hemosiderosis and HFE H63D mutation in beta thalassemia major patients

HFE H63D mutation	Hepatic hemosiderosis patients (n= 25)	Non-hepatic hemosiderosis patients (n=35)	P value	OR (%95CI)
Wild	18 (72%)	30 (85.7%)	-	1
Hetero + Homo	6+1(28%)	4+1(14.3%)	0.19	2.33 (0.64-8.45)
Allele H	42 (84%)	64 (91.43%)	-	1
Allele D	08 (16%)	06 (08.57%)	0.21	2.03 (0.65-6.27)

Table 6 The association between cardiac hemosiderosis and HFE H63D mutation in beta thalassemia major patients

HFE H63D mutation	Cardiac hemosiderosis patients (n=23)	Non-cardiac hemosiderosis patients (n=37)	P value	OR (%95CI)
Wild	17 (73.9%)	31 (83.8%)	-	1
Hetero + Homo	5+1 (26.1%)	5+1 (16.2%)	0.35	1.82 (0.50-6.52)
Allele H	39 (84.78%)	67 (90.54%)	-	1
Allele D	07 (15.22%)	07 (9.46%)	0.34	1.71 (0.56-5.26)

Discussion and conclusion

The main finding of our study

- No significant differences were observed between BTM patients and control subjects regarding the frequency of HFE H63D and C282Y mutations.

The main finding of our study

- HFE H63D mutation is not a risk factor for organ specific hemosiderosis but is a contributing factor for elevated plasma ferritin levels.

The main finding of our study

- Plasma ferritin levels, iron levels and transferrin saturation index correlated significantly with the **hepatic** and **cardiac** hemosiderosis.

The main finding of our study

- Plasma ferritin levels, iron levels and transferrin saturation index were similar between patients with hepatic and cardiac hemosiderosis indicating the ineffectiveness of these values in discriminating of organ specific hemosiderosis.

The main finding of our study

Because of good correlation between elevated plasma ferritin levels and T2* MRI results of heart and liver,

In unequipped centers, ferritin levels can be used as a primary criterion for iron overload assessment.


Thanks for
your attention