Renal Function in β-Thalassemia Major Patients: A Decade of Follow-Up

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β-Thalassemia (β-T)

Hereditary disorder characterized by a genetic deficiency in the synthesis of β-globin chains

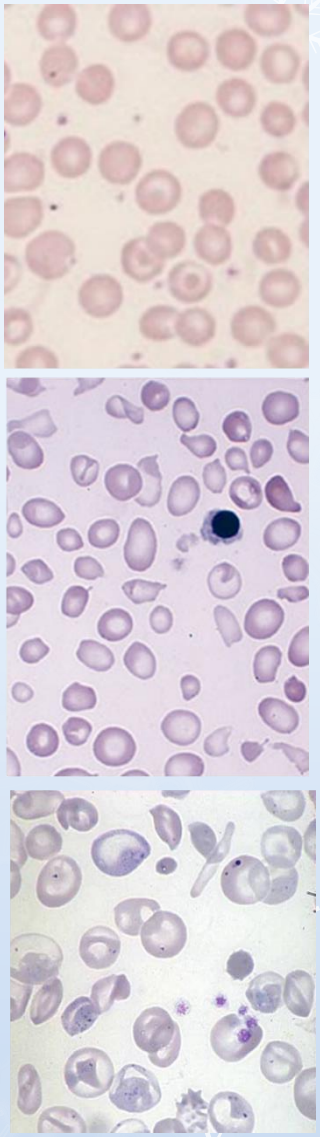
- Three main "Classical" forms
  - **β-T minor** (the heterozygous "asymptomatic" state)
  - **β-T intermedia** (β-TI) homozygous
  - **β-T major** (β-TM) homozygous

- Two main "Treatment based" forms

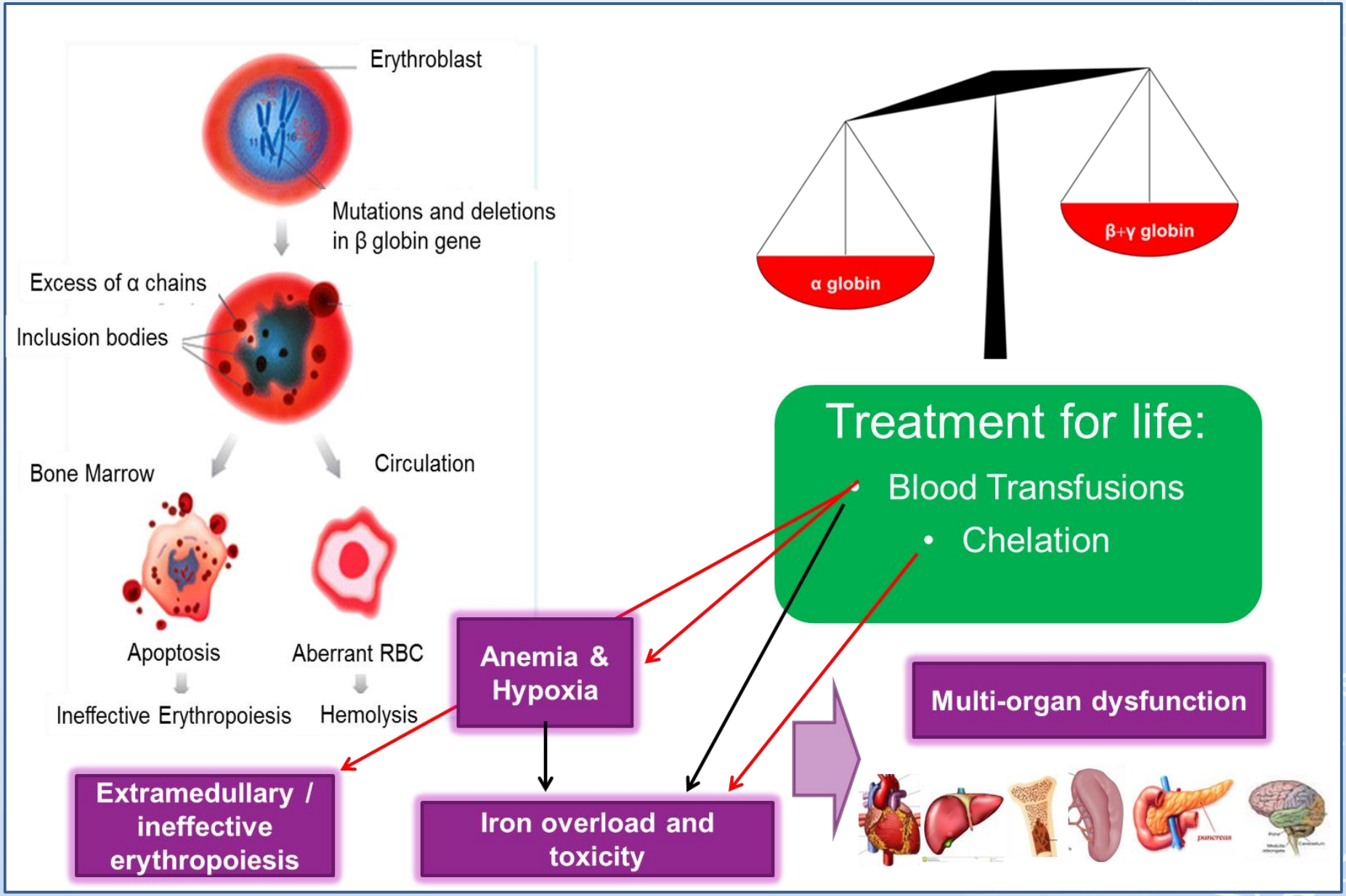
- Nontransfusion dependent β-T (NTDT) requires occasional or short-course of regular transfusions

- Transfusion dependent β-T (TDT) present in early childhood with severe anemia that requires lifelong regular transfusion therapy for survival

- Adequate treatment improved survival. As patients get older previously unrecognized complications emerge, such us renal abnormalities



β-Thalassemia pathophysiology



β-Thalassemia Therapy

- **Regular blood transfusions**: to ensure growth and prevent symptoms of anemia ineffective erythropoiesis and bone deformities. Every 2 to 5 weeks, pre transfusion Hb levels ~9.5g/dl

- **Iron chelation**: > 2 years of age, (>10–20 transfusions)

Three agents are available for iron chelation

- Parenteral deferoxamine (DFO) SC or IV
- Oral deferiprone (DFP)
- Oral deferasirox (DFX)

- **Others**
- Allogenic Stem Cell Transplant
- Gene therapy /β globin replacement
- Fetal globin reactivation
- Targeting Hepcidin to Reduce Iron Overload
- Ineffective Erythropoiesis Signaling Modulators
- Reducing Cardiac Iron

General monitoring recommendations across age groups in TDT

Iron intake	Record at every transfusion | Yearly assessment of iron intake based on transfusion burden		
Serum ferritin	Q 1-3 months		
LIC[a]	>6 years	Q 2 years if <3 mg/g | Q 1 year if 3-15 mg/g | Q 6 months if >15 mg/g or rapidly increasing trend in serum ferritin/LIC	
Cardiac T2*[a]		Q 2 years if ≥30 ms | Q 1 year if ≥10 to <30 ms | Q 6 months if <10 ms	
LVEF[b]		Q 1 year if ≥56% | Q 3-6 months if <56% | Q 1-3 months if <56% and symptomatic | TRV assessment can also be done as needed and simultaneous ECG measurement should be conducted	
Growth	Weight every visit | Standing/Sitting Height Q 6 months | Bone Age Q 1 year if delayed puberty/growth		Weight every visit
Sexual development		Tanner staging Q 1 year	Routine assessment for infertility, secondary hypogonadism, impotence
Liver status	Enzymes[c]: Q 3 months | Q 1 month if >5 ULN | Virology: Q 1 year		
Liver US			Q 1 year | Q 6 months if abnormal. TE assessment may also be done if available.
Endocrine labs[d]		Q 6 months to 1 year | Q 3 to 6 months as needed in patients with abnormality	
BMD		Q 2 years | Q 1 year as needed in patients with abnormality	
Other	Psychosocial assessment for patient and family		
Age	<10 years	10-18 years	>18 years

Endo & Fertility tests as indicated: calcium, phosphate, vitamin D, thyroid, parathyroid , luteinizing, follicle-stimulating, gonadotropin-releasing hormones, testosterone, estradiol; fasting blood sugar, oral glucose tolerance test.

BMD, bone mineral density; LIC, liver iron concentration; LVEF, left-ventricular ejection fraction; Q, every; TE, transient elastography; TRV, tricuspid-regurgitant jet velocity.

Taher & Cappellini, How I manage medical complications of β-thalassemia in adults, Blood, 2018

General monitoring recommendations related to chalators in TDT

Monitoring for adverse effects of iron chelation

Test	Frequency of Monitoring
All chelators	
Visual acuity and dilated ophthalmology examination	Annually
Audiology examination	Annually
Vitamin C level	Annually
Zinc level	Annually
Deferasirox	
Urinalysis for proteinuria	Every 3 mo
Liver function testing	Every 2 wk × 2 after initiation, then monthly
Renal and tubular function—creatinine, potassium, phosphorus, bicarbonate	Monthly
Deferiprone	
Complete blood count with differential	Weekly
Liver function testing	Every 3 mo

E. Khandros & J. Kwiatkowski. Beta Thalassemia Monitoring and New Treatment Approaches *Hematology/Oncology Clinics of North America*,2019

Renal complications in β-T

- **Glomerular dysfunction**

Etiology	Mechanism	Evaluation
Chronic anaemia/hypoxia	Reduced vascular resistance, elevated RPF	Urine dipstick Serum creatinine Urine protein/creatinine Serum cystatin CrCl eGFR
	Damage and loss of peritubular capillaries, epithelial-mesenchymal trans differentiation of tubular cells to myofibroblasts, tubulointerstitial injury, glomerulosclerosis	
Iron overload	Damage and loss of peritubular capillaries, epithelial mesenchymal trans differentiation of tubular cells to myofibroblasts, tubulointerstitial injury, glomerulosclerosis	
Infections (e.g. HIV, HCV, HBV)	Glomerulonephritis	
Iron chelators	Relative iron depletion, mitochondrial dysfunction in tubular cells, tubuloglomerular feedback, vasoconstriction of the afferent arteriole	
NSAIDs, COX-2 inhibitors	Vasoconstriction of the afferent arteriole	
ACE inhibitors, ARBs	Vasodilation of the afferent and efferent arterioles	

Demosthenous et al, β-Thalassemia and renal complications. A narrative review of pathophysiologic mechanisms Integr *Mol Med,* 2018

Renal complications in β-T

- **Tubular Dysfunction**

Etiology	Mechanism	Evaluation
Chronic anaemia/hypoxia	Oxidative stress, lipid peroxidation, endothelial damage and loss of peritubular capillaries	Serum β2-M Urine calcium/creatinine Urine β2-M/creatinine Urinary NAG Urinary NAGL Urinary a1-microglobulin Urinary RBP
Iron overload	Oxidative stress, lipid peroxidation	
Iron chelators	Nephrotoxicity	
Aminoglycoside, intravenous radiocontrast agents, NSAIDs	Cytotoxicity, renal vasoconstriction, acute tubular necrosis.	
β-lactames	Mitochondrial dysfunction, lipid peroxidation, acute tubular necrosis	
Ampicillin, ciprofloxacin, sulphonamides	Crystal precipitation within the distal tubular lumen	

- **Deferasirox** (DFX) **induced Fanconi syndrome** (Yacobovich et al, 2010)
  - Heightened form of proximal tubular wasting manifested by hypophosphatemia, normal anion gap metabolicacidosis, glucosuria, and proteinuria
  - Deferasirox's lipophilic properties allow it to penetrate cell membranes and to accumulate in the proximal tubular cells, cause direct nephrotoxic effects or deplete mitochondrial iron proximal and tubular dysfunction

Renal complications in β-T

- **Hematuria**

Etiology	Mechanism	Evaluation
Nephrolithiasis	Hypercalciuria, hyperuricosuria, cystinuria, struvite stones	Dipstick urinalysis

- **Nephrolithiasis**

Etiology	Mechanism	Evaluation
Vitamin D, calcium supplementation, deferasirox, tubular dysfunction	Hypercalciuria, calcium stones	Urine dipstick Serum electrolytes Serum creatinine 24-hour urine collection Radiographic studies
Tubular dysfunction	Cystinuria, cystine stones	
Splenectomy increased red cell turnover, tubular dysfunction	Hyperuricosuria, uric acid stones	
Urinary tract infections by urease-producing bacteria (e.g. Proteus spp, Klebsiella spp, S.epidermidis, Mycoplasma spp, yeast species)	Struvite stones	

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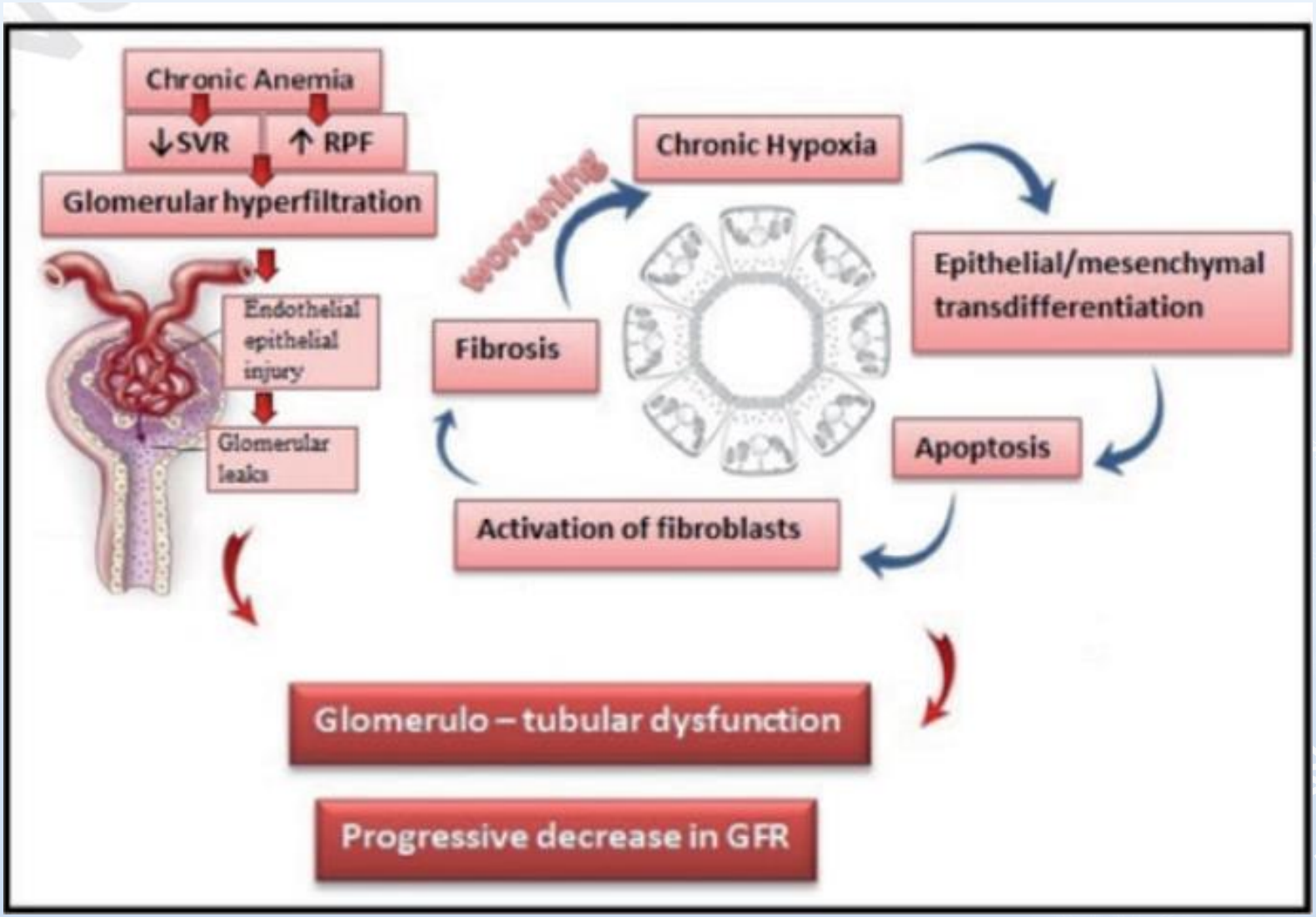
Mechanisms of renal complications in β-T

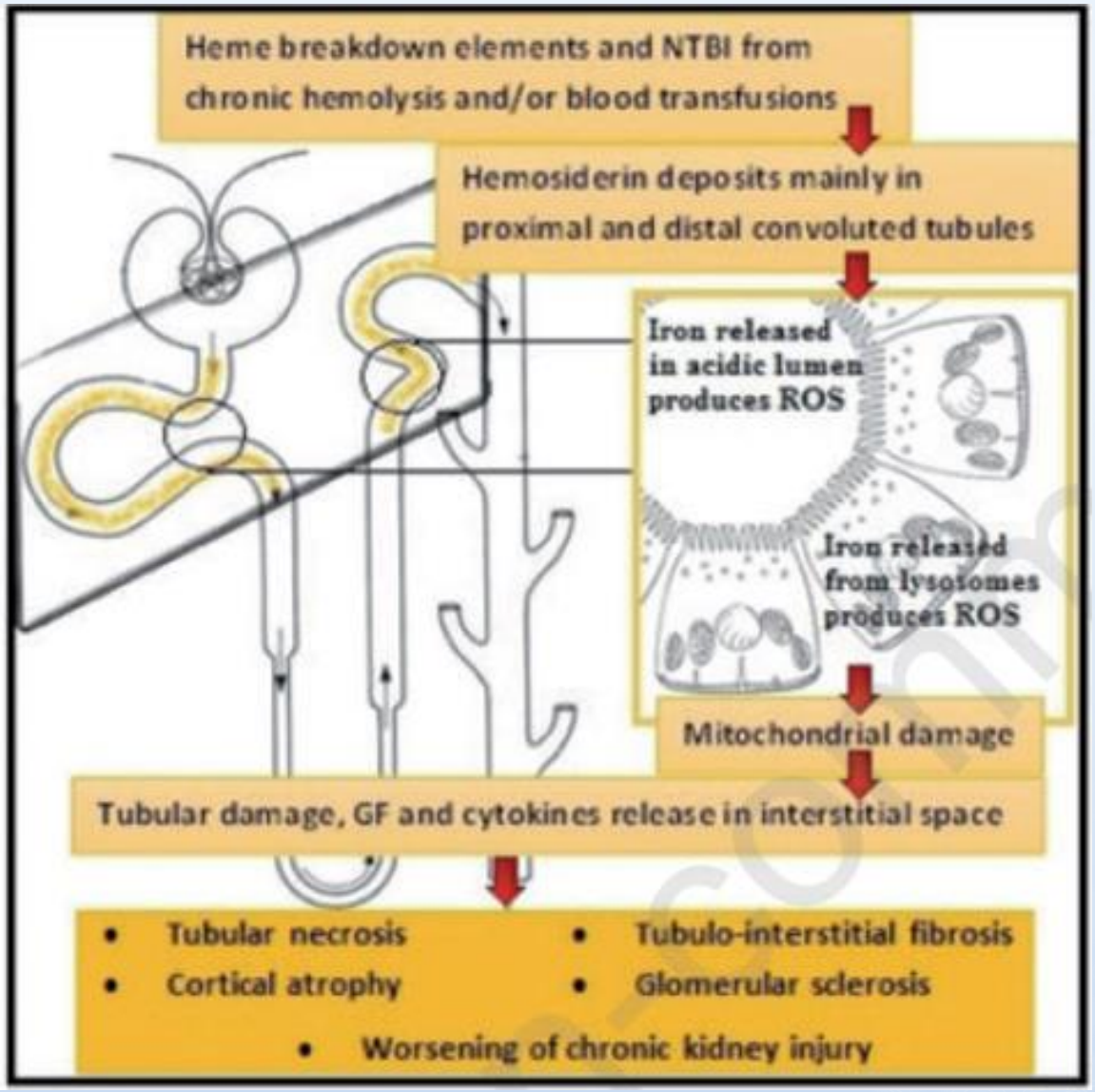
- Chronic anemia



SVR, systemic vascular resistance; RPF, renal plasma flow

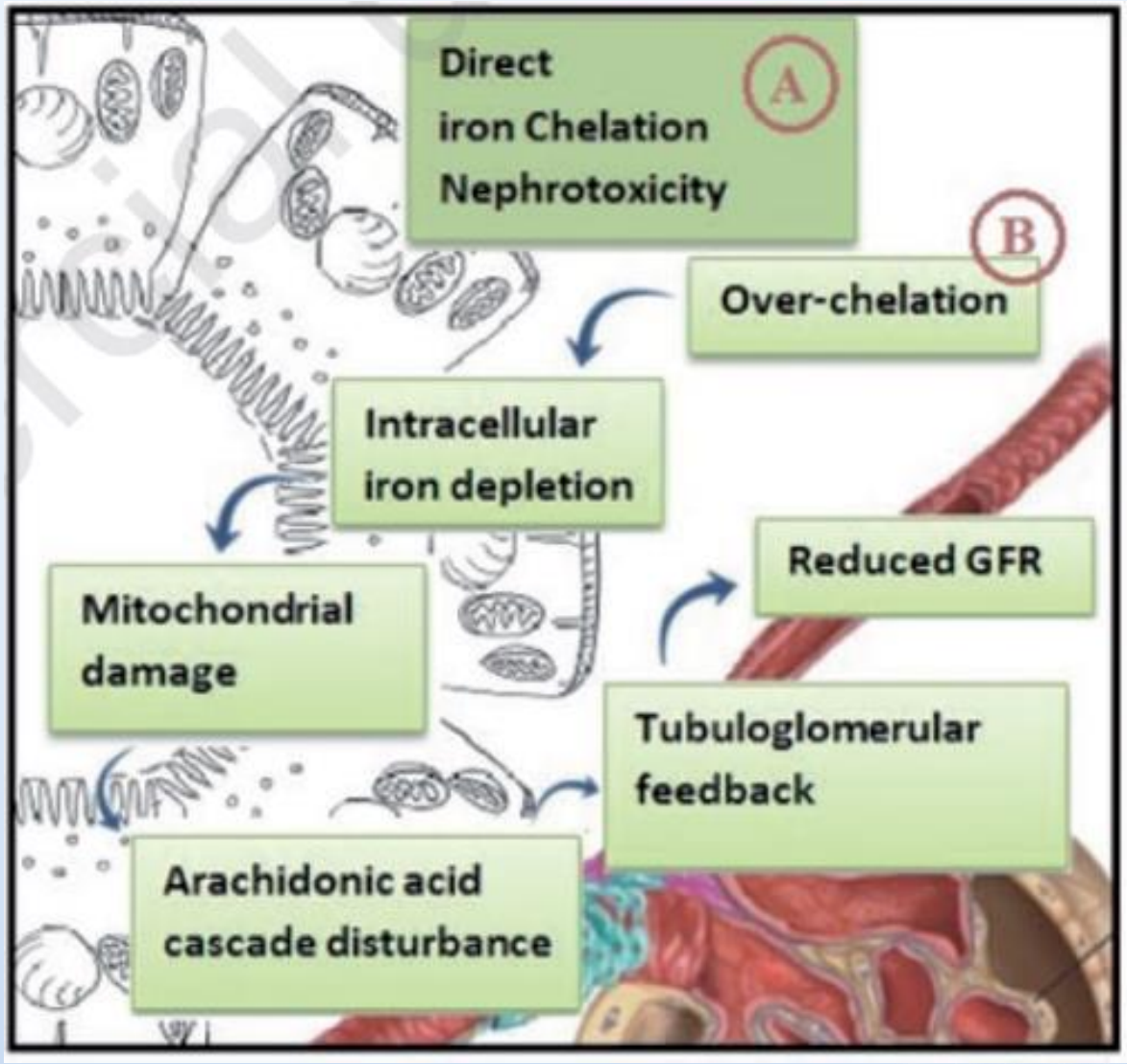
Mechanisms of renal complications in β-T

- iron overload



Mechanisms of renal complications in β-T

- Chelation therapy



Studies indicated or no tubular dysfunction in patients with β-T

Authors	Study type	Number of patients	Age (years)	Chelation therapy	Biomarkers	Results-Conclusions
[18]**Koliakos** et al, 2003	observational	91 TM with no evidence of renal disease	17.2 ± 7.2	DFO	Urine NAG Urine IgG Urine albumin Urine β2M Serum ferritin	- high incidence of renal proximal tubular dysfunction. -iron overload as the main cause of this dysfunction
[87]**Papassotiriou** et al, 2010	observational	150 TM with no evidence of renal disease	29.2 (6.4–44.2)	DFX	Plasma NGAL Cys C NT-proBNP ferritin	Cys C concentration may be influenced by hemodynamic parameters as a result of therapy with DFX. Any changes in cys C do not reflect renal impairment
[16]**Jalali** et al, 2011	case-control study	140 TM with no evidence of renal disease	7-16	DFO	urine NAG blood sample for biochemical and ferritin tests	- Kidney dysfunction in thalassemia increases with increasing age, duration, and levels of blood transfusion and hypercalciuria. - The presence of severe renal dysfunction in thalassaemic patients should be investigated using sensitive and specific tests, mainly NAG, to prevent progress
[15]**Mohkam** et al, 2008	cross-sectional study	103 TM with no evidence of renal disease	12.5+/-5.53	DFO	Urine sodium (Na), potassium (K), calcium (Ca), creatinine (Cr), phosphate, uric acid (UA), NAG and amino acids	Urinary NAG excretion can be a reliable index of the tubular toxicity and a possible predictor of proteinuria, aminoaciduria and eventual renal impairment in these patients.
[38]**Michelakakis** et al, 1997	case-control study	36 TM with no evidence of renal disease	5-22	DFO	urine specimens Urine NAG a-Mannosidase ferritin	Iron overload resulted in increased urinary levels of the lysosomal enzyme NAG. Reduction of iron load, achieved by regular DFO infusion, resulted in normalization of the urinary enzyme levels.
[30]**Smolkin** et al, 2008	case-control study	37 TM and 11 TI	2.4 - 27	DFO	Urine and blood samples Urine NAG	Renal tubular function is impaired in children with TM and TI. It is not known whether these functional abnormalities would have any long-term effects on the patients.
[22]**Kalman** et al, 2005	case-control study	32 Tmin	5.8 +/- 3.1	-	Urinary calcium excretion zinc glucosuria (mg/dL), β2M (mg/dL), NAG, sodium, magnesium uric acid and tubular phosphorus reabsorption	Renal tubular dysfunction has not been determined in children with TMin.
[44]**Tantawy** et al, 2014	cross-sectional, case-control study	66 TM and 26 TI	2.5-16	DFP	Serum ferritin, bicarbonate, plasma osmolality and urinary total proteins, microalbuminuria, NAG, RBP, α-1 micro globulin, bicarbonate, osmolality, creatinine clearance (CrCl)	Asymptomatic renal dysfunctions are prevalent in young β-TM and β-TI patients that necessitate regular screening
[121]**Aydinok et al**, 1999	case-control study	40 TM	6-24	DFO	Urinary NAG creatinine, zinc	Urinary NAG indices (U/g Cr) were significantly higher in the patients compared to controls. Urinary zinc excretion was correlated with the urinary NAG indices.
[14]**Ong-ajyooth** et al, 1998	case-control study	95 beta-thal/ Hb E	na	na	Urine NAG, β2M Plasma and urine MDA	This is the first report of renal tubular defects found associated with beta-thal/Hb E disease. The mechanism leading to the damage is not known but it might be related to increased oxidative stress secondary to tissue deposition of iron, as indicated by the raised levels of serum and urine MDA.
[26]**Patsaoura** et al, 2014	case-control study	35 TI	8-63	-	Plasma NGAL, STfR, NTBI, Cys C, β2M, hs-CRP	The increased NGAL levels reported for the first time in TI patients in agreement with the elevated expression of NGAL observed in TI mouse models. The induction of NGAL may represent either a survival response, facilitating the survival of the less damaged thalassaemic erythroid precursors, or a consequence of the abnormal iron regulation in TI.
[27]**Roudkenar et al,** 2008	case-control study	25 adult's TM and 9 paediatric TM	24.33 ± 7.09 and 8.28 ± 1.49	na	Plasma NGAL with PCR and ELISA	- In all adult cases, except one sample, NGAL protein was expressed more compared to the controls - Positive correlation with ferritin - Negative correlation with sex, age - NGAL upregulation was not found in paediatric β- thalassemia patients. Iron overload and oxidative status in β-thalassemia patients induce NGAL/Lcn2 expression. Upregulation of NGAL in this disorder may play a beneficial role in decreasing ROS or chelating iron. Obviously, chelating of iron is one of the major therapeutic goals in b-thalassemia.

Study aims and Methods

- **Aims:**

To evaluate tubular and glomerular function of TDT

To correlate the renal tubular function with the iron overload status and the type of chelation (2 different iron ICT regimes).

To compare renal function with the previous study (Smolking V. et al, 2008 Ped Neph)

- **Population:**

TDT patients at the Pediatric Hematological Unit, Emek Medical Center

Treated by standard protocols: PC every 2-3 weeks and ICT:

1-Deferasirox (DFX) (20-40mg/kg/day)

2-Deferoxamine (DFO) 20-40mg/kg/day S.C +/- Deferiprone (DFP) ~75mg/kg per day

- **Evaluations:**

Estimated glomerular filtration rate (eGFR) [Schwartz formula in pediatric pts and CKD EPI for adults].

Fractional excretion of: sodium (FeNa), potassium (FeK); calcium to creatinine ratio (Ca/Cr), uric acid excretion (UAE), tubular phosphorus reabsorption (TPR) and urinary N-acetyl-b-D-glucosaminidase (uNAG) as a marker of tubular injury.

Demographic, clinical, laboratory and iron-overload markers

Results:

- 36 TDT pts (18 M/18 F), mean age 19.42 ±9.3 (range 5- 45)
- 26 received DFX and 10 DFO+/- DFP
- The sCr, K, Na, FeNa and eGFR was in normal levels in all pts
- Hypercalciuria (Ca/Cr>0,25) in 28% of pts, increased FeK (>15%) in 33%, high UAE (>0.56 mg/dl GFR) in 64%, and high TmP/GFR in 25%
- **The DFX group compared to DFO+/- DFP :**

- Younger age (mean 19.5 vs 24.8ys) and lower ferritin levels (2500 vs 4800 ng/dl; p=0.006)
- Hypercalciuria in 30% vs.10%
- eGFR was slightly lower (100.9±17.09 vs 114.0±22.31 mL/min/1.73m2; P=0.0676)
- The urinary NAG was significant higher (10.4 vs. 5.3 IU/l, p=0.012). Abnormal values found in 30% vs 10% respectively
- No correlation was found between NAG and transfused iron while DFO+/- DFP group showed + correlation. (Similar to the first study).

Results:

In 20 pts treated with DFX vs. 9 treated with DFO+/- DFP the renal function was previously evaluated (Smoking et al, 2008; all under with DFO treatment) and compared with the current values.

- The sCr significantly increased from the previous study in pts treated with DFX but not in pts treated with DFO+/- DFP (mean 0.51 ±0.9 vs 0.67±0.1, p=0.0008).

- The same observation was found regarding urine Ca/Cr (mean 0.08±0.11 vs 0.176±0.12, P=0.001).

- The eGFR was significantly lower than the previous study in pts treated with DFX but not in pts treated with DFO +/- DFP (mean GFR first study 113.5 ±26 vs 100.1±17, p=0.0093

Conclusions

- A high prevalence of renal tubular abnormalities was observed in our TDT patients; most evident in patients treated with DFX.
- The uNAG - marker of tubular injury - was associated with the transfusional iron burden in the DFO+/- DFP group, but not in the DFX treated pts; proposing an alternative mechanism than iron overload for the pathogenesis of tubular injury in DFX treated patients.
- The glomerular function remained within the normal range in all patients; however a significant decline in glomerular function in respect a decade earlier was observed only in the patients currently treated with DFX.
- The clinical consequences, reversibility and log term implications of renal dysfunction are still unknown
- Strict follow up of renal function in β-T pts, especially children is warranted.

EMEK MEDICAL CENTER

Pediatric Hematology Unit

Thank you

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