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Kidney Functions and Physiological Complications in Pediatric Thalassemia Patients of Indian Origin

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Abstract--

Background: Thalassemia patients developed progressive anemia and require regular blood transfusion to sustain life as adverse effects starts with hemolysis of red blood cells (RBCs).

Objectives: The present study elucidates physiological complications and the effect of hypoxic stress on physiology of kidney function in pediatric thalassemia.

Methods: Current study includes 45 thalassemia patients of age between 3-16 years. A sufficient urine and blood samples were obtained from all the participants. Samples were analyzed for the biochemical parameters such as pH, Creatinine, Protein, Urea, Sodium (Na+), Potassium (K+); Uric acid, ferritin, albumin, hemoglobin, fetal hemoglobin and Malonaldehyde (MDA). Physiological complications were also investigated with the help of patient data sheet, blood and urine biochemical investigations and microscopic examinations of urine.

Results: Most of the patients show severe physiological complications including recurrent fever, joint and chest pain, shortness of breath, swelling of abdomen and unusual headache. Serum creatinine level, serum uric acid and hemoglobin levels in thalassemia is less while fetal hemoglobin level is significantly elevated in thalassemia patients. Urine protein value, creatinine level, urea and MDA were significantly high in thalassemia patients. High molecular weight proteins ranging from the molecular weight 10-260 kDa appeared in urine of thalassemia patients.

Conclusion: Most of the urine and serum biochemical parameters are significantly altered indicating glomerular dysfunction may be due to glomerular damage, tubular damage and nephritis.

Keywords--- *renal function, microscopic investigations, malonaldehyde, hemolysis, fetal hemoglobin*

Acronyms---

ICMR: Indian council of medical research

RBC's: Red blood cells

NAG: (N-acetyl-beta-D-glucosaminidase

eGFR: estimated glomerular filtration rate

HPLC: High performance liquid chromatography

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I. INTRODUCTION

Thalassemia is the most common haemoglobinopathies which is prevalent in some parts of the world such as in Mediterranean region, north and west of Africa and Indian subcontinent (Weatherall and Clegg, 2001). Mukherji was the first to report thalassemia in India (Mukherji, 1938). In thalassemia patient red blood cells (RBCs) are found to be defective which leads to premature hemolysis. These patients depend on repeated blood transfusion therapy. Most of the patients succumb before the attainment of age of twenty. The main cause of death is accumulation of iron in cardiac tissue resulting heart failure. Repeated blood transfusion and thus accumulation of iron accounts for tubular damage in thalassemia (Smolkin *et al*., 2008). About 10% of total thalassemia homozygotes and heterozygotes babies are born that may cause burden on general health of the nation(Sukumaran, 1975). According to an ICMR report, thalassemia affects different ethnic and geographical groups with prevalence of 2-16% in different regions (Joint, 1993). Thalassemia is widespread in India among several tribes and several ethnic groups like Sindhis, Jat Sikhs and Bramhins. Some cases of thalassemia trait are also reported in Muslim community (Balgir, 2000). Kidney is an important organ affected in thalassemia disease due to generation of oxidative lipid peroxides, iron deposition, renal hypoxia, anemia and toxic effect of iron chelating drugs(Sumboonnanonda *et al*., 2003; Prasannan *et al*., 2003; Cianciulli *et al*., 1992). Significant alterations in renal biochemical parameters prove proximal tubular damage secondary to oxidative peroxidation of lipids due to overload of iron (Aldudak *et al*., 2000). Patients with β-thalassemia major usually suffer with severe anemia. Thus there is a requirement for these patients for regular blood transfusion for survival. Repeated blood transfusions results into multiple organ dysfunctions (Brittenham *et al*., 1994).

Hypoxic condition in thalassemia may affect the kidney function in the pediatrics in selected population which needs to be addressed. The aim of the present study is to investigate the effect of hypoxic stress on physiology of kidney. Biochemical investigations of blood, urine and microscopic examination reveal that the renal tubular functions ofthalassemia patients are highly affected in pediatric thalassemia.

II. MATERIAL AND METHODS

The present study was approved by Institutional Human Ethics Committee (205/12-13). Study was conducted in district Nashik of India. Forty five patients of Thalassemia undergoing regular blood transfusion and chelation therapy at Thalassemia Center, Jankalyan Blood Bank Nasik were randomly selected. The mean age of the participants was 6.75±0.66 years. All patients selected were earlier diagnosed for thalassemia major based on clinical manifestations, electrophoresis and High Performance Liquid Chromatography (HPLC). This study was conducted during January 2010 to August 2012. The study protocol and laboratory examinations were discussed with patients and their parents and written consent was taken.

All patients selected were in a stable phase of their diseased condition with regular blood transfusion once or twice in month. Participants were requested to fast overnight before attending the clinic and were advised not to consume vitamin or mineral supplement for 24 hrs. Controls were selected from the same locality. Clinical parameters like recurrent fever, joint and chest pain, recurrent tiredness, shortness of breath, swelling of abdomen,

unusual headache, jaundice, delayed growth, recurrent occurrence of pain episodes, yellow eyes etc. were investigated by a physician and were recorded in patient history form.

A) Blood collection and study of blood parameters:

5ml of blood samples werecollected in EDTA coated tubes at Thalassemia Centre Nashik, India and transported in ice bags and stored at 4^{o}C. Hemoglobin F (Hb F) was estimated byAlkali Denaturation Method(Sood, 1992) while Hemoglobin (Hb) bycyanmethemoglobinMethod (Drabkin, 1932). Serum ferritin was detected by commercial Immunometric Enzyme Immunoassay for the quantitative determination of Ferritin in serum or plasma (ORGENTEC Diagnostika) and absorbance was read at 450 nm on ELISA reader. Estimation of serum Albumin, serum creatinine and serum uric acid were carried out by using commercial kit on B2B chemical analyzer.

B) Urine collection and study of urine parameters:

Fresh urine samples were collected fromall the participants. Urine samples were preserved in 4% formaldehyde, transported and stored at 4^{o}C. Quantitative detection of proteinuria was performed by colorimetric method of Lowry (Lowry *et al*., 1951). Urine Sodium (Na^{+}), Potassium (K^{+}) ion, creatinine, uric acid and urea estimation were performed by using commercial kit on B2B chemical analyzer. Creatinine and Sodium (Na^{+}) Potassium (K^{+}) ratio was calculated for each voided random urine samples. Glomerular Filtration Rate (GFR) was calculated according to the Schwartz formula. Physical parameters like color of the urine sample were observed and compared with those of normal urine samples**.**pH of urine sample was measured by pH Tutor (Make: Oakton). Specific gravity of urine sample was measured by Urinometer (Make: Davinder Glass works, New Delhi) and also by dipsticks. SDS-PAGE of urine samples was performed.

C) Microscopic Analysis of Urine:

Cleansing solutions chlorhexidine were used to avoid the erroneous results. 1 ml of urine sample was centrifuged at 3,000 rpm for 5 minutes. Following aspiration of the supernatant to a marked level, the pellet was agitated into a homogeneous mixture followed by a sampling pipette (Tarson, India). A drop of prepared urine sample was pipetted onto a slide and a cover slip was placed. The slide is then examined without staining. The urine sediment is routinely examined by bright field microscopy under both low and high power without staining. For the present study, prepared slide was observed under the Magnus inclined trinocular microscope (Olympus MLX series). Slides were observed for the normal and abnormal cellular components like RBCs, WBCs, Epithelial cells, renal tubules cells, Bacteria, Yeast and Protozoan under high-power field. Non cellular components like Tyrosine, Cholesterol, A/T Phosphate, Fibers, Hyline, Uric Acid, Oxalate and Cystine were observed.

Statistical analysis:

Data was presented as arithmetic mean; standard deviation and standarderror were calculated by SPSS and Mega stat software. Comparative student t-test and Poisson's correlation between each parameter were calculated. p value of 0.05 was considered as statistically significant value for current study.

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III. RESULTS

A) Study of physiological complications:

Around 79.17% patients suffer from recurrent fever,87.5 % patients suffer from joint and chest pain, 75% experience tiredness, 54.17% experience shortness of breath, swelling of abdomen was found in 35.71 % patients, 41.67% experience unusual headache. Yellow eyes were observed in 35.71 % thalassemia patients indicating probable liver malfunction. In all the patients, growth was found to be delayed. Recurrent occurrence of pain episodes was observed in 25% patients (Fig. 1).

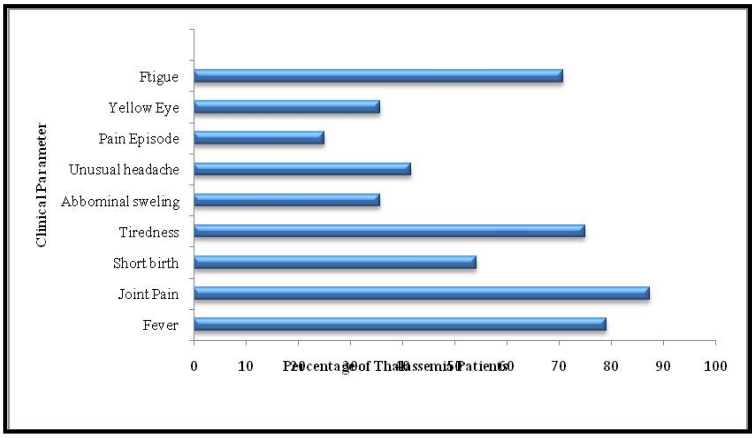

Figure 1: Severity of physiological complications in thalassemia patients. X-axis represents % of thalassemia patients while Y-axis represents clinical parameters.

B) Study of blood parameters:

Values of serum biochemical markers concerning renalfunction were significantly altered in thalassemia patients (Table 1). Serum creatinine, serum uric acid and hemoglobin level was low while as fetal hemoglobin level was significantly higher in thalassemia. It was observed that ferritin level in serum of thalassemia was significantly high in males and also in females than normal patients. Level of ferritin in thalassemia male patients were 1413.64 ng/ml and in thalassemia females were 780 ng/ml of serum. These values are highly significant as compared to values of ferritin in normal male and female. The ferritin level in serum of normal male was found to be 310 + 4.006 and in normal female it was only 112 + 2.08. Serum albumin mean value in normal was 5.5mg in 100ml while in thalassemia patients were 1.548+0.15 mg/ml significantly significant.

Table 1: Comparative analysis of biochemical parameters in blood of thalassemia patients and control samples.

Biochemical parameter	Thalassemia	Control
Hemoglobin (Hb)	7.44±0.11*	16± 0.2
Fetal Hemoglobin (Hb F)	32.14±9.77*	2.0±0.76
Serum creatinine (mg/dl)	0.24± 0.02*	2.0± 0.23
Serum uric acid (mg/dl)	3.71±1.01*	6.0 ± 1.56

All values are expressed in mean± SE: *p<0.05 with respect to control

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C) Study of urine parameters:

Urine pH, specific gravity and uric acid, urine sodium, urine potassium, urine uric acid levels were not significantly altered in thalassemia patients. Urine protein, urine creatinine, urine urea were significantly high in thalassemia patients; however the value of urine MDA level was significantly high indicating oxidative stress (Table2).

Table 2: Comparative analysis of biochemical parameters of urine samples in thalassemia patients and control samples.

Parameters	Control	Thalassemia
pH	6.53±0.17	7.39±0.19
Specific gravity	1.014±0.001	1.006±0.001
Uric acid (mg/dl)	2.22±0.02	2.4±0.39
Creatinine (mg/dl)	0.83±0.45	4.83±0.16*
Urea (mg/dl)	47.7±2.7	177.04±7.27*
Proteins (mg/dl)	0.53±0.29	3.36±0.51*
Na^+mmol/L	201.56±2.52	145.73±12.48*
K^+mmol/L	53.31±6.12	51.59±2.53*
Malonaldehyde	1.11±0.14	2.05±0.31*

Data represented as Mean ±SE. *p<0.05 as compared to control

D) SDS-PAGE of urine proteins:

High molecular weight proteins ranging from the molecular weight 10-260 kDa appeared in urine samples of thalassemia patients (Fig. 2). The proteins in the range of molecular weight such as 143kDa, 123kDa, 97kDa, 31kDa, 121kDa and 74kDa appeared in the urine of thalassemia patients. These proteins could be heme oxygenase/α1 microglobulin, oxidized low density lipoprotein/ hepatoglobulin, CD38 ligand, N-acetyl-beta-D-glucosaminidase/IgG and inositol polyphosphate 5-phosphatase/ transferrin respectively.

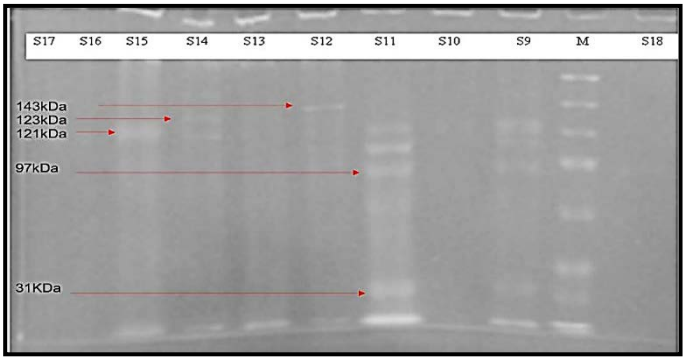

Figure 2: SDS-PAGE analysis of urine of thalassemia patients. S=sample, M= marker lane

E) Correlation of different parameters in thalassemia major patients:

In thalassemia patients, various biochemical parameters show positive correlations with each other, Sodium to Potassium ratio showed positive correlation with protein to creatinine, sodium, uric acid, urine creatinine, urine urea, Body Mass Index, specific gravity (Table 3).

Table 3. Comparative view of correlation of and among different biochemical parameters in thalassemia major patients

	AGE	**Ucr**	**Upr**	**Sg**	**pH**	**HA**	**Uua**	**NA^+**	**K^+**
Ucr	-0.123								
p value	0.595								
Upr	-0.514	0.735							
p value	0.017	0.000							
Sg	0.117	0.178	-0.029						
p value	0.615	0.440	0.900						
pH	-0.322	-0.148	0.162	-0.486					
p value	0.154	0.522	0.484	0.026					
HA	-0.601	0.633	0.806	-0.114	0.262				
p value	0.004	0.002	0.000	0.624	0.252				
Uua	0.123	-0.222	-0.239	0.271	0.098	-0.182			
p value	0.596	0.333	0.296	0.234	0.673	0.429			
Na^+	0.669	-0.442	-0.781	0.215	-0.504	-0.642	0.233		
p value	0.001	0.045	0.000	0.349	0.020	0.002	0.309		
K^+	-0.157	0.040	-0.080	0.161	-0.090	0.129	0.058	0.229	
p value	0.496	0.864	0.729	0.485	0.699	0.577	0.802	0.319	
UUN	0.415	-0.195	-0.347	0.039	-0.085	-0.338	-0.027	0.264	-0.258

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p value	0.062	0.398	0.124	0.866	0.714	0.134	0.908	0.247	0.259
Pr/Ucr	-0.441	-0.143	0.525	-0.227	0.331	0.187	-0.070	-0.565	-0.252
p value	0.046	0.536	0.015	0.323	0.143	0.416	0.762	0.008	0.271
Na^+/ K^+	0.751	-0.391	-0.748	0.214	-0.523	-0.693	0.232	0.934	-0.110
p value	0.000	0.079	0.000	0.352	0.015	0.000	0.312	0.000	0.636
Pr/Ucr	-0.182								
p value	0.429								
Na^+/ K^+	0.320	-0.539							
p value	0.157	0.012							

F) Microscopic Examination of Urine:

Cellular components of urine:

RBCs were observed in the urine of 65% patients, WBCs in 57% samples, epithelial cells in 100%, renal tubules cells in 24%, bacteria in 8% patients and yeast in 7% thalassemia patients (Table 5).

Table 5: Physiological complications associated in urine samples of pediatric Thalassemia patients.

Cell Type	**% of sample**	**Clinical manifestation**
RBCs	64.04	Glomerulonephritis, Inflammation
WBCs	56.14	Kidney Infections, (pyelonephritis), inflammation somewhere in the urinary tract
Squamous Epithelial cells	99.12	Infections in urinary tract/Normal
Renal tubular Cells	23.68	Infections / Normal
Bacteria	7.89	Urinary tract infection
Yeast	7.89	Vaginal yeast infection in women
Protozoan	6.14	Vaginal infection

G) Non-Cellular components in urine of thalassemia patients:

Various abnormal crystals were observed in the urine samples of thalassemia patients. Tyrosine was observed in 10%, cholesterol in 72 %, phosphate in 48%, while hyline was found in 76% patients, Uric acid 50%, Oxalate 57% and Cystine 9% in thalassemia patients (Fig. 3).

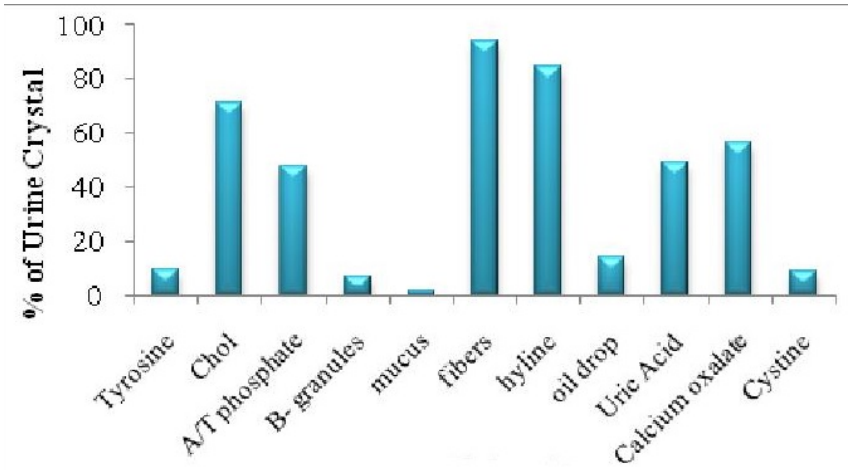

Figure 3: Percentage of non-cellular components in urine samples of thalassemia patients.

H) Quantitative analysis of microscopic components of urine samples in thalassemia patients:

RBCs and WBCs were very high in number 17020 and 412 in cu/mm respectively as compared to normal samples indicating severe hematuria. Squamous Epithelial cells were found to be 13.25 mm/cu and renal cells were 60 mm/cu. Some crystals were also very high in number like Calcium Oxalate (31.39 mm/cu) while Calcium Carbonate were 977.27. Tyrosine crystals were 5.56 cu/mm suggesting impaired amino acid metabolism. Triple Phosphate was 30.0 cu/mm. (Table 4).

Table 4: Quantitative analysis of Microscopic components of Urine samples of Thalassemia Patients.

Cells /crystals in urine	**Mean±SEM (cu/mm)**
RBCs	17020±7426.3
WBCs	412±165.4
Squamous Epithelial cells	13.25±7.07
Renal Cells	60±39
Calcium Oxalate	31.39±18.4
Calcium Carbonate	977.27±503.2
Uric acid	30.56±14.2
Triple Phosphate	13.89±3.8

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Fibre	1.67±0.5
Hyline	55.28±41.8
Cholesterol	2.22±1.07
Tyrosine	5.56±5.2
Cast	86.39±56.2

IV. DISCUSSION

In the present study of the selected pediatrics thalassemia population, renal tubular function ofthalassemia patients were found to be affected maximum. The urine and serum parameters were considerably altered an indication of glomerular dysfunction, glomerulonephritis, tubular damage and pyelonephritis, impaired renal function, inflammation urinary tract etc. Similar work has been carried out (Bekhit *et al*., 2017) which shows the presence of glomerular and/or tubular dysfunction in children with β-thalassemia major using routine and early marker tests such as urinary NAG (N-acetyl-beta-D-glucosaminidase) and correlated the urinary NAG level with other clinical and laboratory findings. Glomerular filtration rate levels were lower in patients, and serum levels of creatinine were higher in 40% of patients.

Present study shows that around 80% patients were suffering from recurrent fever,87.5% from joint and chest pain, 75% experience tiredness, 54.17% experience shortness of breath, swelling of abdomen was observed in 35.71% patients and 41.67% experience unusual headache. Osteopenia is a common complication in β-thalassemia major patients is multifactorial and was mainly predisposed due to defective function of parathyroid gland and unusual headache and, excessive iron deposition in various body organs (El-Nashar *et al*., 2017).

Serum creatinine, serum uric acid and hemoglobin levels were low and fetal hemoglobin level was more in thalassemia. Serum creatinine, urinary Ca^{+2}/creatinine ratio and urinary uric acid is high in thalassemia patients as compared to the control (Bekhit *et al*., 2017). Blood urea nitrogen, serum creatinine, creatinine clearance, serum sodium, urine osmolality and fractional excretion of sodium, potassium and uric acid, were not different between healthy controls and thalassemia major patients (Aldudak *et al*., 2000). High molecular weight proteins appear in the urine of thalassemia patients which is an indication of glomerulonephritis and kidney damage. Thalassemia patients had normal values of serum sodium, potassium, calcium, and phosphate. There was no significant differences in serum urea, creatinine and eGFR values but thalassemia group had a significantly higher urine protein-to-creatinine ratio than control. Serum levels of potassium, phosphorus, uric acid, and UProtein/Cr levels were higher in β-thalassemia major patients than in normal subjects (Aldudak *et al*., 2000). This proteinuria may be due to impairment of proximal tubular reabsorption, result of severe iron overload in the tissues which generate reactive oxygen radicals and ultimately resulting in cellular injury (Kassab*et al*., 2003). Albuminuria was present in the majority of patients, but it was not consistently associated with the intensity of transfusion therapy (Quinn *et al*., 2011). Renal hyper-filtration, hypercalciuria, and albuminuria are common in thalassemia patients (Quinn *et al*., 2011). The mean urinary protein to creatinine (UProtein/Cr) ratio was significantly higher in patients compared to the healthy subjects with significantly increased U(NAG/Cr) and U(NGAL/Cr) in β-thalassemia patients compared

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with healthy controls (Şen*et al*., 2015). Thalassemia patients had significantly lower hemoglobin, hematocrit and red blood cell counts and higher serum ferritin levels (Şen*et al*., 2015)which was similar to our findings. High concentration of urine malondialdehyde, a lipid peroxidation metabolite, in thalassemia patients, suggesting a role of lipid peroxidation in renal injury (Sumboonnanonda *et al*., 2003). Kidney is affected in thalassemia major at the glomerular level by lobular and segmental sclerosis and in the tubules by atrophy and interstitial fibrosis (Ali *et al*., 2008). There is a positive correlation between estimated glomerular filtration rate (eGFR) and age, height, weight and 1st blood transfusion age in thalassemia patients (Stevens and Levey, 2005). The increase in urinary excretion of uric acid and alpha-1M in selected groups was observed. The observation indicated that Fe^{++} Na^{+} levels increased as an indicator of poor renal function in β-thalassemia patients(Mula *et al*., 2011). Tubular dysfunction and glomerular filtration rate abnormalities were observed in the pediatric population and the pathogenesis of these abnormalities could be attributed to iron overload, too aggressive iron removal, and/or the underlying anemia (Ponticelli *et al*., 2010). This study was confined to one research Centre and thalassemia patients need to be recruited from other centers of the country to check the prevalence and other pathophysiology of thalassemia at country level

V. CONCLUSIONS

In the selected pediatric thalassemia population renal tubular functions ofthalassemia patients is highly affected. The most of the urine and serum biochemical parameters are significantly altered indicating glomerular dysfunction, may be glomerulonephritis, tubular damage and pyelonephritis, impaired renal function, inflammation in urinary tract etc. It is therefore recommended to detect the presence of severe renal dysfunction in thalassemia patients using sensitive and specific tests.

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Disclosure statement

The authors declare that they have no conflict of interest.

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