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Characterization of General Urine Examination in Diagnosed Thalassemia Patients

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Abstract

The present study aimed to investigate urine composition in patients with thalassemia. A total of 60 thalassemia patients and 40 healthy control subjects were enrolled in this case-control study. Fresh urine specimens are collected from patients and controls and tested using standard test strips to investigate physical, chemical, and microscopic characteristics. The urine colour changed to an amber colour, and specific gravity was significantly higher (p=0.0001) in thalassemia patients (2.71±1.3) compared to the control group (1.03±0.037). The urine was significantly (p=0.031) more alkaline in thalassemia patients (pH=6.25±0.722) than in controls (6±0). Nitrite, protein sugars, bilirubin, ketones, and urobilinogen revealed differences between control and thalassemia patients. Leukocyte testing revealed a significantly (p=0.021) higher level in control subjects (2±0) compared to thalassemia patients (1.88±0.324). The pus cell counts in the urine of thalassemia patients (6.22±1.904) were markedly elevated (p=0.001) versus control subjects (1.43±0.747). The RBCs in urine in thalassemia patients (9.3±1.018) showed a significant increase (p=0.001) versus control subjects (0.75±1.463). The average count of epithelial cells in urine of thalassemia patients (0.78±0.480) were significantly higher (p=0.0001) versus control subjects (0.403±0.2). The average urine casts in thalassemia patients (0.44±0.1) were significantly higher (p=0.001) versus control subjects (0.221±0.05). These results provided direct clue for subclinical renal changes in thalassemia patients, demanding frequent followup of renal function in these patients.

Keywords: Thalassemia, Urine sample, Urinary crystals, Urine specific gravity, Bilirubin

Introduction

Thalassemia is an inhirited hematological disease that upsets normal erythropoiesis, precisely intervening with the globin structure of haemoglobin, resulting in chronic haemolysis and ineffective erythropoiesis (Sanchez-Villalobos *et al*., 2022; Bate *et al*., 2023). The body compensate by increasing erythropoiesis, ensuing a higher red blood cells (RBC) breakdown rate (Nagdalian *et al*., 2024; Theocharaki *et al*., 2025). The elevated breakdown rate leads to increased cellular waste byproducts, including uric acid, which trigger hyperuricemia and lead to complications of thalassemia (Mehrzad *et al*., 2022; Sakhnenkova *et al*., 2023; Kunlayawutipong *et al*., 2024). Patients presented with clinical symptoms, typically splenomegaly, skeletal abnormalities, and severe anaemia (Hajimoradi *et al*., 2021, Shoghi & Kian, 2022; Huyen *et al*., 2023).

Structurally, haemoglobin made up of four parts, two alpha-globin chains and two beta-globin chains (Yu, 2022; Huyen *et al*., 2023). A modification in any single gene encoding for this protein can result in anaemia (Petronis *et al*., 2023; Pinto *et al*., 2025). Genetic and environmental factors can upset the normal chain, initiating blood disorders (Sadeghi *et al*., 2021; Cantile, 2024). Genetic factors contribute more to the disease than environmental factors (Thanoon *et al*., 2025). Haemoglobin structural disassembly can disrupt haemoglobin qualitatively and quantitatively (Kontoghiorghes & Kontoghiorghe, 2020). Quantitative deficits alter haemoglobin synthesis rates, whereas qualitative deficits affect the synthesis of protein chains within the haemoglobin tetramer, ultimately causing thalassemia (Sadiq *et al*., 2024).

Thalassemia patient prognosis and survival rate have largely improved through blood transfusion, iron chelation therapy, and advanced knowledge about the disease's pathophysiology (Caprari *et al*., 2023). In Western countries, the incidence of beta-thalassemia births was significantly reduced through prenatal screening programs (Basu *et al*., 2023). However, thalassemia patients still face multi-organ complications that impact heart and lung function, endocrine glands, and liver function (Sanchez-Villalobos *et al*., 2022). The renal functions are affected through the clearance of thalassemia metabolic byproducts and chelation drugs or their metabolites (Tanous *et al*., 2021). Urine composition may change; therefore, more studies testing urine composition are required to build knowledge and fill gaps in the scientific literature, especially in countries with a high prevalence of thalassemia. The present study aimed to investigate the urine composition in thalassemia patients from the same centre and locality.

Materials and Methods

Study Design

In this case-control clinical study, participants have been informed about the study and the investigation of the urine analyses for both the patient and control groups. The study was approved by the College of Pharmacy at the University of Mosul (Iraq). The patients included in this study were recruited from Ibn-Alatheer

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pediatric hospital during the period from February 18, 2024, to April 8, 2024.

The present study consisted of two groups of subjects:

Control Group

which was the control group consisting of 40 subjects (including 15 females and 25 males), their age range was from 3 to 16 years.

Patient Group

which consists of 60 patients with β-thalassemia (including 22 females and 38 males). All populations of this group were transfusion-dependent β-thalassemia patients receiving only regular blood transfusions and iron chelation therapy, and their age ranged from 3 to 20 years.

Sampling

Urine samples were obtained from all subjects included in the study in special, clean, disposable containers. The fresh urine specimen is collected from patients and controls.

Urine Test Strips

The test strips used in the present study were purchased from the Alltest company (UK). The test begins by dipping the test strip directly into the urine sample collected, and each reagent pad starts to interact with specific parameters present in the collected specimen. This single test commences multiple colourimetric reactions, each specified to identify distinct urine markers through chemical reactions. The outcomes were interpreted through comparison with colour charts printed on the container of the strips. Each marker needs individual colour assessment after the chromogenic reactions commenced independently and produced distinct hues relative to analyte concentrations **(Figure 1)**.

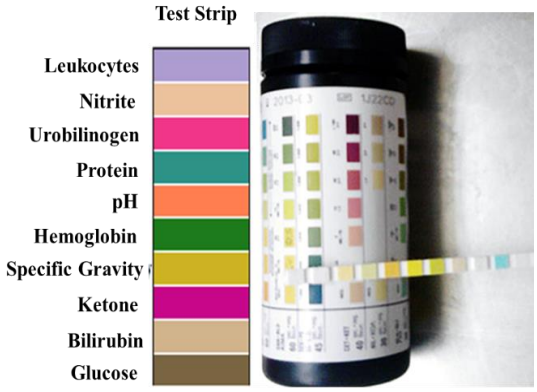

Figure 1. Test strips are used for the detection of urine constituents.

Microscopic Examination

A 10ml of urine specimen of thoroughly mixed and centrifuged (1000rpm for 10 min) until a slightly cohesive pellet is collected at the bottom of the tube. The supernatant is discarded, and 0.5ml is left inside the tube. The sediment is suspended in the remaining supernatant by flicking the bottom of the tube several times. A drop of re-suspended sediment is poured onto a glass slide and covered with a coverslip.

The collected resuspended pellets is checked under a low-power field (LPF) to detect most crystals, casts, squamous cells, and other large objects. Example: 5-10 cast/LPF. Due to variation in presence of these particles between fields, more than one field were checked and average determined. Then, same samples were detected under high power field (HPF) to investigate crystals, cells, and bacteria. The different types were described by the number of each type found per HPF.

Renal Crystal Detection

The analysis starts with centrifugation of 10-15 millilitres of urine at 1000rpm for 5 min. This will cause cellular elements and crystals to settle to the bottom. Most of the clear supernatant was decanted, and the leftover sediment was gently re-suspended, creating a concentrated sample. The samples were first scanned under bright light, and the whole slide was examined. Crystals are presented in geometric shapes in the fields. Under polarised light, calcium oxalate crystals appeared as brilliant colours against a dark background. These crystals appeared as envelope-shaped dihydrates or oval monohydrates. However, uric acid crystals remain dark, appearing as yellowish-brown pleomorphic structures, under polarised light, because they absorb rather than bend the light. Struvite crystals appeared under polarised light as the characteristic coffin lid shape, with beveled edges.

Results and Discussion

The urine colour in the control group was light yellow transparent versus amber-colored transparent urine in the thalassemia subjects, with no odor differences between them. The specific gravity of urine in thalassemia patients (2.71±1.3) was significantly (p=0.0001) higher compared to the control group (1.03±0.037) **(Table 1)**.

Table 1. Urine physical examination parameters in the control versus thalassemia patients.

Parameters	Control (n=40)	Patients (n=60)	P value	Normal range
Colour	Light yellow	Amber		Light yellow
Appearance	Transparent	Transparent		Transparent
Specific gravity	1.03±0.037	2.71±1.3	0.0001	1.010-1.025
Odor	Mild smell	Mild smell		Mild smell

The urine was significantly (0.031) more alkaline in thalassemia patients (pH=6.25±0.722) versus controls (6±0). Nitrite levels demonstrated no significant difference (p=0.32) between groups, control group 2±0 and thalassemia patients showing 1.98±0.129. Protein levels revealed no significant (p=0.156) difference between control (2±0) and thalassemia patient (1.95±0.22). Sugars, bilirubin, ketones, and urobilinogen demonstrated equal results in both groups (p>0.05) **(Table 2)**.

Table 2. Urine chemical examination parameters in the control versus thalassemia patients.

Parameters	Control (n=40)	Patients (n=60)	P value	Normal Value
Reaction	6±0	6.25±0.722	0.031	5.5-8.0
Nitrite	2±0	1.98±0.129	0.32	-ve
Protein	2±0	1.95±0.22	0.156	Negative to trace
sugars	2±0	2±0	1	-ve
Bilirubin	2±0	1.97±0.181	0.303	-ve
Ketones	2±0	2±0	1	-ve
Urobilinogen	2±0	2±0	1	0.1-1.0 EU/dl

Leukocyte testing revealed a significantly (p=0.021) higher level in control subjects (2±0) compared to thalassemia patients (1.88±0.324). The pus cell counts in urine in thalassemia patients (6.22±1.904) showed marked elevation (p=0.001) compared to control subjects (1.43±0.747). The RBCs in urine in thalassemia patients (9.3±1.018) showed a significant increase (p=0.001) compared to control subjects (0.75±1.463). Moreover, Epithelial cell counts in urine in thalassemia patients (0.78±0.480) showed significant elevation (p=0.0001) compared to control subjects (0.403±0.2). Urinary casts in thalassemia patients (0.44±0.1) were significantly (p=0.001) higher compared to control subjects (0.221±0.05). Bacterial presence was negative in thalassemia compared to the control **(Table 3)**.

Table 3. Microscopic examination parameters in the control versus thalassemia patients.

Parameters	Control (n=40)	Patients (n=60)	P value	Normal Value
Leukocyte	2±0	1.88±0.324	0.021	-ve
Pus cells	1.43±0.747	6.22±1.904	0.001	-ve
RBCs	0.75±1.463	9.3±1.018	0.001	-ve
Epithelial	0.403±0.2	0.78±0.480	0.0001	0-1
Casts	0.221±0.05	0.44±0.1	0.001	-ve
Bacteria	-ve	-ve		-ve

Amorphous urate crystals in thalassemia patients (1.32±0.504) demonstrated significant (p=0.001) elevation compared to the control group (0.15±0.427). Calcium oxalate, amorphous phosphate, and struvite crystals were absent in the control group, while thalassemia patients reported detectable limits at 0.853±0.47, 2.893±0.73, and 0.555±0.12 **(Table 4)**.

Table 4. Urine crystals in the control versus the thalassemia patients.

Parameters	Control (n=40)	Patients (n=60)	P value	Normal Value
Amorphous urate	0.15±0.427	1.32±0.504	0.001	
Calcium oxalate	-ve	0.853±0.47		-ve
Amorphous phosphate	-ve	2.893±0.73		-ve
Struvite or triple phosphate	**-ve**	**0.555±0.12**		**-ve**

Thalassemia has slightly changed the physical characteristics of urine, since thalassemia patients present with amber-colored urine. Perhaps, this colour change reflects the pathophysiology of thalassemia (Montgomery *et al*., 2023). These changes might be due to increased bilirubin elimination or modulated metabolic byproducts from hemolysis and iron overload (El-Hawy *et al*., 2023). However, the colour of urine is transparent and free from cloudiness, reflecting that the pathology in thalassemia does not affect the filtration process of the kidneys (Sadeghi *et al*., 2021; Khandker *et al*., 2023). Nonetheless, thalassemia patients presented with elevated specific gravity compared to healthy subjects, reflecting increased urinary solute composition in thalassemia patients (Kavouras *et al*., 2021), due to exaggerated excretion of metabolic byproducts, iron-related compounds, or other thalassemia-specific byproducts that increase the urine density (Kontoghiorghes & Kontoghiorghe, 2020). Characteristics of urine odour are unchanged in thalassemia, suggesting that thalassemia introduces no volatile byproducts that would alter the urine composition (Iqbal *et al*., 2018).

Thalassemia has slightly changed the chemical characteristics of urine; urine pH in thalassemia was slightly more alkaline compared to normal. Nitrite levels demonstrated no changes, suggesting the absence of bacteriuria in thalassemia (Pinto *et al*., 2025). No changes in protein levels were reported, indicating that thalassemia is associated with no proteinuria or glomerular filtration dysfunction (Ziyadeh *et al*., 2013; Deveci *et al*., 2016). Sugar and ketone levels remained constant (Musharraf *et al*., 2017; Schnedl *et al*., 2017; Gomber *et al*., 2018). Bilirubin concentration in urine revealed no increase despite the hemolytic nature of thalassemia (Fasano *et al*., 2022; Mufarrij *et al*., 2025). Urobilinogen concentration in urine preserved constant, reflecting normal hepatic function and bile metabolism in both populations (Sanchez-Villalobos *et al*., 2022).

The leukocyte concentration in urine were low in thalassemia patients, reflecting that the leukocyte concentration in urine s may be altered due to haematological defects linked to the condition (Buttari *et al*., 2020). Pus cells concentration in urine of thalassemia patients demonstrated that extensive increase in thalassemia, reflecting an extensive inflammatory reaction happen inside the urinary system of thalassemia patients, perhaps reflecting the chronic inflammation associated with continuous hemolysis and iron accumulation conjoined with the disease (Gluba-Brzózka *et al*., 2021; Hatairaktham *et al*., 2021; Caprari *et al*., 2023). Thalassemia patients revealed extensive increase of RBC, due to a chronic hemolytic process (Fasano *et al*., 2022). Thalassemia patients shown higher epithelial cell concentration in urine and cast numbers, perhaps due to tubular damage (Sadeghi *et al*., 2021; Sripathy *et al*., 2024). Bacterial presentation in urine were negative in thalassemia (Nonejuie *et al*., 2024; Obed *et al*., 2024).

The crystalluria revealed high metabolic derangements in thalassemia patients. Amorphous urate crystals demonstrated elevation in thalassemia patients; this reflects diminished purine catabolism and a deficit in renal handling of uric acid, or dehydration propensities occur in thalassemia patients due to increased metabolic demands (Tzounakas *et al*., 2022;

Theocharaki *et al*., 2025). The accumulation of calcium oxalate crystals could be explained in the context of thalassemia perturbed calcium homeostasis and oxalate metabolism (Shee & Stoller, 2022; Rodriguez *et al*., 2025), alongside changes in calcium metabolism due to bone deficits associated with thalassemia (Wong *et al*., 2016), altered absorption patterns (Pathrapol Lithanatudom *et al*., 2010), or interference with renal tubular function affecting oxalate handling mechanisms (Quinn *et al*., 2011). The struvite and triple phosphate crystals increased in thalassemia patients (Wong *et al*., 2017; Sayani *et al*., 2022), which optimally formed in alkaline urine, usually linked to UTI (Kunlayawutipong *et al*., 2024). However, the sterile urine reflects that metabolic dysregulation rather than infectious causes their formation (Pinto *et al*., 2025).

Conclusion

The findings of the present study confirmed significant alterations in urinary parameters among thalassemia patients, including changes in physical and chemical urine characteristics, alongside increased pus cells, RBCs, epithelial cells, and urinary casts. Crystals were also detected.

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Conflict of interest: None

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Ethics statement: The study registered by College of Pharmacy, Univesity of Mosul and recorded at approval number CP/UoM10 on 13 April 2025.

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