

Unraveling Renal Complexities in Thalassemia Major: A Comprehensive Nephrological Inquiry in Central Punjab, Pakistan

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ABSTRACT

This research investigates the intricate nexus between thalassemia major and renal dysfunction in Central Punjab, Pakistan. A comprehensive analysis was conducted on a diverse cohort of beta-thalassemic patients to explore the prevalence and factors influencing renal profiles. The study encompassed demographic factors, family history, and caste systems, shedding light on their associations with beta-thalassemia. Furthermore, gender and age-specific assessments were performed, focusing on serum creatinine and urea levels to provide a nuanced understanding of renal function. The findings revealed a higher prevalence of beta-thalassemia in the 11-20 years age group, emphasizing the critical role of adolescence and early adulthood in the manifestation of this genetic disorder. Family history emerged as a significant factor, with patients having positive family histories showing a higher prevalence. Caste-based analysis highlighted variations in prevalence, with Arains and Rajpoot exhibiting the highest rates. Renal profile assessments, including serum creatinine and urea levels, indicated stable renal function across genders and age groups. No significant variations were observed, reaffirming the stability of renal parameters in beta-thalassemic patients. The study contributes valuable insights for targeted healthcare strategies, genetic counseling, and awareness programs in managing thalassemia major in the Central Punjab population.

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Authors' Contribution

MW contributed as a co-researcher, provided technical assistance and formatted the research article. RB participated in the revision process and contributed to manuscript preparation. KM conducted the research work. MA supervised and guided the overall research.

Key words

Thalassemia major, Renal dysfunction, Serum creatinine, Serum urea

INTRODUCTION

Thalassemia, a complex and heterogeneous group of disorders, is recognized as an autosomal recessive blood disorder with a global prevalence across all races (Badeli *et al.*, 2019). This genetic and inheritable disease is characterized by the defective production of hemoglobin, the crucial protein responsible for oxygen transport (Bakr *et al.*, 2014). First identified in the early 1920s by Dr. Denton Cooley, thalassemia major presented a distinctive clinical profile in seven children of Italian or Greek origin (Borgna-Pignatti, 2010). The term thalassemia, originally coined by Sarrai (2012), Mary *et al.* (2017), finds its roots in the Greek words thalassa (sea)

and haima (blood), underlining the condition's impact on blood composition (Galanello and Origa, 2010).

Beta thalassemia major, the focal point of this inquiry, involves three pivotal pathophysiological factors: chronic anemia hemolysis, ineffective erythropoiesis, and secondary iron overload (Bekhit *et al.*, 2017). Associated symptoms encompass a spectrum of clinical manifestations, including failure to thrive, progressively pale color, feeding problems, diarrhea, recurrent fever episodes, delayed growth, jaundice, dark urine, poor muscle development, chronic hypoxia, hepatosplenomegaly, and the formation of masses due to extramedullary hematopoiesis (Raza *et al.*, 2016). Skeletal alterations arising from bone marrow expansion contribute to long bone deformities in the legs, while distinctive craniofacial changes involve skull protuberance, prominent malar eminence, nasal bridge depression, and a tendency for exposed upper teeth (Sultan *et al.*, 2016).

In addition to the hematological challenges, thalassemia major poses a significant risk of renal complications. Renal failure, indicative of the kidneys' inability to perform excretory functions, results in the retention of nitrogenous waste products in the blood. The renal profile, a comprehensive measure of kidney functions, encompasses key markers such as creatinine,

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urea, uric acid, and glomerular filtration rate (GFR). Chronic kidney disease, defined by kidney damage or a GFR less than 60 mL/min per 1.73 m² for three months or more, is a formidable concern for individuals with thalassemia major (Demosthenous *et al.*, 2019). Notably, renal issues rank as the fourth most common cause of morbidity, following endocrine, cardiovascular, and hepatic concerns, underscoring the multifaceted nature of thalassemia major and its impact on overall health (Demosthenous *et al.*, 2019).

Creatinine levels in serum serve as a widely utilized test for assessing renal function. Creatinine, a breakdown product of creatine phosphate in muscle, is typically produced at a constant rate by the body, dependent on muscle mass (Economou *et al.*, 2010). While freely filtered by the glomeruli, under normal conditions, Creatinine is not re-absorbed by the tubules to any appreciable extent. Elevated blood creatinine levels are indicative of marked nephron damage, making it less suitable for detecting early-stage kidney disease. The apparent differences in serum creatinine levels may be attributed to factors like age and weight, with lower values potentially reflecting the influence of younger age and smaller body weight, as serum Creatinine is affected by muscle mass (Economou *et al.*, 2010).

Urinary N-actly-D-beta glycosidase (NAG) excretion emerges as a valuable tool for early detection of proximal tubular epithelial cell malfunction in beta thalassemia major patients, offering a reliable marker for tubular cell status (Bekhit *et al.*, 2017). Identifying high-risk patients prone to renal damage is crucial for implementing timely interventions to mitigate the progression of renal injury and reduce the incidence of renal impairment. Recognizing the significance of urinary NAG excretion in monitoring tubular cell status aids in tailoring specific measures for individual patients (Bekhit *et al.*, 2017).

Chronic anemia, iron overload, and iron chelation therapy are implicated in the etiology of these abnormalities. Anemia induces alterations in renal hemodynamics, disrupting renal plasma flow and glomerular filtration rate (GFR) in beta thalassemia patients (Bekhit *et al.*, 2017). Despite an initial increase in renal plasma flow due to glomerular hyperfiltration and renal hyperperfusion resulting from anemia, prolonged anemia leads to a significant decrease in GFR over time. Additionally, non-progressive increases in serum creatinine levels have been noted following exposure to certain iron chelators. Case reports, such as a 62-year-old man experiencing renal function effects with Deferasirox, underscore the importance of monitoring and managing iron chelation therapy to prevent adverse renal outcomes (Brosnahan *et al.*, 2008; Musallam and Taher, 2012).

Post-marketing surveillance has revealed several cases of acute kidney injury associated with the oral chelator Deferasirox, reinforcing the need for careful consideration and monitoring in thalassemia patients undergoing iron chelation therapy (Musallam and Taher, 2012).

In conclusion, this research endeavors to shed light on the intricate nexus between thalassemia major and renal dysfunction in Central Punjab, Pakistan. By comprehensively characterizing the prevalence and genetic aspects of thalassemia major in this unique demographic, the study aims to unravel the specific nature of renal complications associated with the disorder. Additionally, the research seeks to identify early predictors of renal dysfunction, exploring the potential utility of markers such as urinary N-actly-D-beta glycosidase (NAG) excretion. Through a meticulous examination of factors influencing renal function, including chronic anemia, iron overload, and iron chelation therapy, the study aspires to provide region-specific insights that inform both clinical management and public health strategies. Ultimately, this research contributes to the global understanding of thalassemia major and offers tailored insights for optimizing healthcare practices in the Central Punjab, Pakistan population.

MATERIALS AND METHODS

The experimental study was conducted at the Department of Zoology, University of Education Lower Mall Campus Lahore, Pakistan. Blood samples were collected between August 2019 and June 2020 from individuals diagnosed with thalassemia major at Fatimid Foundation Johar Town Lahore, Pakistan. A total of 185 blood samples were obtained from patients whose thalassemia status was confirmed through genetic and hemoglobin analyses.

Study subjects

The study included 185 patients diagnosed with thalassemia major based on comprehensive genetic and hemoglobin analyses.

Sample collection

Blood samples were collected via vein puncture, with meticulous attention to proper site sterilization using antiseptic measures to prevent contamination. Sterile, disposable, 23-gauge Shifad syringes (5ml) were used for blood sampling, with 3ml of blood collected from each patient. The collected samples were deposited into serum separating tubes (SST or yellow top vials) containing anticoagulant dipotassium ethylenediamine tetra acetic acid (K2-EDTA). Subsequently, samples were centrifuged (Cryofuge 16) for 2 min at 3500 rpm, and the serum

samples were stored at 2-8 °C until further analysis.

Laboratory analysis

Quantitative determination of creatinine in serum utilized the CREJ2 diagnostic kit (lot no. 04810716) with a measuring range of 0.17-24.9 mg/dL. Urea levels in serum were quantitatively determined using the diagnostic kit (Cat. No. 04460715) with measuring ranges of 3.0-240 mg/dL urea. For uric acid quantification in serum, the diagnostic kit Cat. No. 03183807 with a measuring range of 0.2-25.0 mg/dL was employed. These analyses were conducted using the Cobas c 111 Roche automatic analyzer. The selection of these specific diagnostic kits and analyzer ensured accurate and standardized measurements for the assessment of renal function parameters in thalassemia major patients.

Statistical analysis

The data collected for the study underwent meticulous coding and entry using the statistical package for social science (SPSS) software. The dataset was comprehensively summarized utilizing measures such as mean and standard deviation to provide a robust overview of the key parameters. Group comparisons were performed employing the Kruskal-Wallis test, which is particularly suitable for non-parametric data. The threshold for statistical significance was set at a P value of ≤ 0.05 , ensuring a rigorous evaluation of the study outcomes.

RESULTS

Gender wise prevalence of β -thalassemia

The gender-wise distribution of beta-thalassemia patients, as illustrated in Figure 1, revealed intriguing patterns. Categorized into male and female groups, the prevalence of beta-thalassemia was notably higher among males, accounting for 54.54%, compared to females, where the distribution stood at 45.45%. This significant gender-based disparity in the prevalence of beta-thalassemia underscores the need for further exploration into potential biological or societal factors influencing the observed variations. Incorporating the Kruskal-Wallis test into the statistical analysis ensured robust assessments of group differences, providing a more comprehensive understanding of the data. The utilization of SPSS software for data management and analysis adds a layer of reliability to the study's findings, enhancing the credibility of the observed trends.

Age wise prevalence of β -thalassemia

Examining the age-wise prevalence of beta-thalassemia (Fig. 2) across four distinct age groups (1-10 years, 11-20 years, 21-30 years, and 31-40 years)

revealed noteworthy insights. The prevalence of beta-thalassemia in the 11-20 years age group was strikingly higher at 45.45%, highlighting a significant concentration during adolescence and early adulthood. In contrast, the 31-40 years age group exhibited the lowest prevalence at 1.2%, indicating a potential decrease in beta-thalassemia cases with advancing age. While in the age groups of 1-10 and 21-30 the prevalence of beta-thalassemia was noted 30.30% and 23.03%, respectively. The prevalence order across different age groups was observed as 11-20 > 1-10 > 21-30 > 31-40, emphasizing the pivotal role of screening and intervention strategies, particularly during the adolescent years. These findings provide valuable data for targeted healthcare initiatives and underscore the need for age-specific awareness programs in addressing beta-thalassemia prevalence.

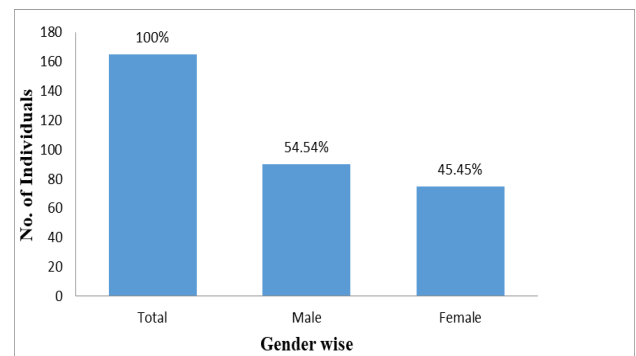


Fig. 1. Gender wise prevalence of β thalassemia.

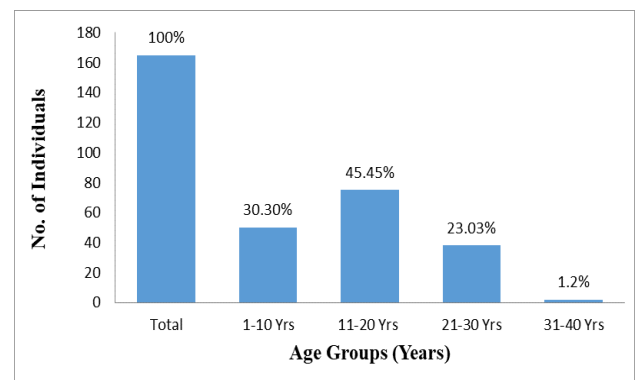


Fig. 2. Age wise prevalence of β thalassemia.

Family history among β -thalassemic patients

The analysis of family history among beta-thalassemic patients revealed two distinct categories: Positive and negative family histories (Fig. 3). The data demonstrated that 57.58% of beta-thalassemia cases had a positive family history, while 42.42% had a negative

family history. This observation underscores a higher prevalence of beta-thalassemia among individuals with a positive family history compared to those without. The significant influence of familial factors on the occurrence of beta-thalassemia highlights the importance of genetic screening and counseling within affected families for effective preventive measures.

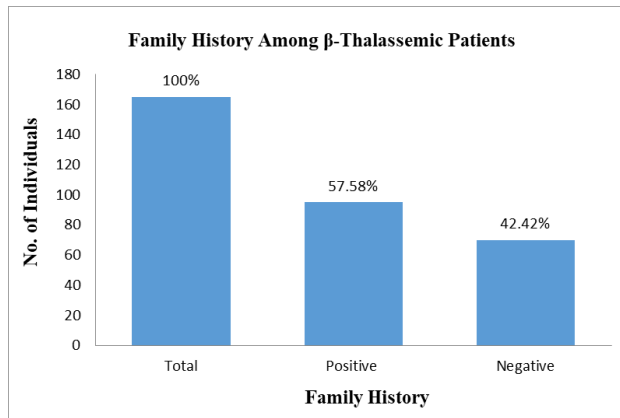


Fig. 3. Family history among β thalassemic patients.

Prevalence of β -thalassemia in different cast systems

Figure 4 shows the assessment of beta-thalassemia prevalence among various caste systems in Pakistan. The Arains and Rajpoot shared the highest prevalence at 19.39%, surpassing other castes. Following closely were Sheikh, Jutt, and Khokhar, with prevalence rates of 12.12%, 9.09%, and 4.84%, respectively. Notably, thalassemia frequency in other castes collectively formed 35.15%, with prevalence less than 5%, providing a distinct subgroup for further analysis. This caste-based distribution underscores the significance of considering diverse sociodemographic factors in understanding beta-thalassemia prevalence, advocating for targeted awareness campaigns and genetic screening initiatives within high-risk caste groups.

Gender wise serum creatinine and urea level (mg/dl) in β thalassemic patients

Table I provides insights into the gender-wise levels of serum creatinine (mg/dl) in beta-thalassemic patients, categorizing them into male (n=88) and female (n=72) groups. The measurement of serum creatinine was integral to understanding renal function as part of the renal profile assessment. The reference value for normal serum creatinine is 0.4 to 1.2 mg/dl. Upon comparing serum levels between genders, no statistically significant difference was observed ($p=0.09$). The mean value of serum creatinine in males was 0.539 ± 0.213 , while in females, it was 0.483 ± 0.201 . The results indicated that

serum creatinine values fell within the reference range, suggesting no variation in serum creatinine levels among beta-thalassemic patients. This observation provides reassurance regarding the stability of renal function within the studied population.

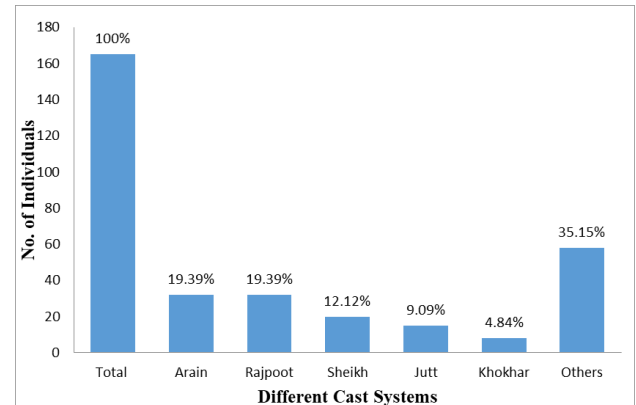


Fig. 4. Prevalence of beta thalassemia in different Cast system.

Table I. Gender wise comparison of serum creatinine level (mg/dl) and urea level (mg/dl) in beta thalassemic patients.

Gender	No.	Mean± SD	p-value
Creatinine level			
Males	88	0.539±0.213	0.09
Females	72	0.483±0.201	
Serum urea level (mg/dl)			
Males	79	20.19±11.33	0.06
Females	63	16.9±9.48	

Table I shows the gender-wise levels of serum urea (mg/dl) in beta-thalassemic patients, encompassing a study population of 142 individuals, of which 79 were males and 63 were females. Serum urea (mg/dl) was measured comprehensively in all beta-thalassemic patients to assess their renal function as part of the renal profile. The reference value for serum urea level (mg/dl) is 10-50 (mg/dl). The mean value of serum urea in males was 20.19 ± 11.33 , and in females, it was 16.9 ± 16.9 . Upon comparing serum urea levels (mg/dl) between genders, no statistically significant difference was observed ($p=0.06$). The results indicated that values of serum urea level (mg/dl) showed no variations, consistently falling within the reference range. This finding suggests stability in renal function across genders among beta-thalassemic patients.

Age wise serum creatinine and urea level (mg/dl) in Beta thalassemic patients

Table II delved into the age-wise levels of serum creatinine (mg/dl) in beta-thalassemic patients, categorizing them into three distinct age groups: 1-10 years (n=46), 11-20 years (n=71), and 21-30 years (n=37). The mean serum creatinine level (mg/dl) in the 1-10 years age group was 76.37, 74.22 in the 11-20 years age group, and 85.20 in the 21-30 years age group. Serum creatinine (mg/dl) measurement served as a crucial aspect of the renal profile assessment. Upon comparing serum creatinine levels in these age groups, no statistically significant difference was observed ($p=0.45$). The results indicated that values of serum creatinine level (mg/dl) exhibited no variations, as they consistently fell within the reference range. This finding suggests stability in renal function across different age groups among beta-thalassemic patients.

Table II. Age wise comparison of serum creatinine level (mg/dl) and serum urea level (mg/dl) in beta thalassemic patients.

Age groups	No.	Mean	P-value
Creatinine level (mg/dl)			
1-10 years	46	76.37	
11-20 years	71	74.22	
21-30 years	37	85.20	
Urea level (mg/dl)			
1-10 years	38	74.36	0.549
11-20 years	68	66.23	
21-30 years	33	72.76	

Table II provided insights into the age-wise levels of serum urea (mg/dl) in beta-thalassemic patients studied in Central Punjab of Pakistan, categorized into three distinct age groups: 1-10 years (n=38), 11-20 years (n=68), and 21-30 years (n=33). The mean level of serum urea (mg/dl) in the 1st age group was 74.36, 66.23 in the 2nd age group, and 72.76 in the 3rd age group. Serum urea (mg/dl) measurement served as a crucial aspect of the renal profile assessment. Upon comparing serum urea levels (mg/dl) in these age groups, no statistically significant difference was observed ($p=0.06$). The results indicated that values of serum urea level (mg/dl) exhibited no variations, consistently falling within the reference range. This finding suggests stability in renal function across different age groups among beta-thalassemic patients in Central Punjab, Pakistan.

CONCLUSION

Thalassemia, an autosomal recessive blood disorder with a global prevalence across all races, is characterized by the defective production of hemoglobin. The heightened prevalence of thalassemia major observed in the 11-20 years age group underscores the critical period of adolescence and early adulthood in the manifestation of thalassemia major. Positive family histories emerged as significant indicators, emphasizing the role of genetic factors in the prevalence of the disorder. Caste-based analysis further delineated variations, with specific castes exhibiting higher rates. The meticulous examination of renal profiles, encompassing serum creatinine and urea levels, demonstrated consistent stability across genders and age groups. These findings have crucial implications for healthcare strategies, genetic counseling, and targeted awareness programs tailored to the unique characteristics of the Central Punjab, Pakistan population.

DECLARATIONS

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Ethical statement and IRB approval

Ethical approval of this study was obtained from the University's Ethical Committee. All procedures adhered to the principles outlined in the Declaration of Helsinki.

Statement of conflict of interest

The authors have declared no conflict of interest.

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