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Demographic, Hematological, and Biochemical Profiling of Beta-Thalassemia Patients in Shaheed Benazirabad District, Sindh, Pakistan

Aika Khan
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad

Khalid Hussain Rird*
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad Email: khalid.rind@sbbusba.edu.pk

Mushahid Hussain
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad

Muhammad Alam
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad

Adila Anwar
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad

Sehar urf Naseebun
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad,

Gulshan Nawaz
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad

Nadir Ali Rind
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad,

Rabia Noreen
Department of Zoology, Shah Abdul Latif University, Khairpur

Author Details

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Corresponding E-mail & Author*:

Khalid Hussain Rird*
Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad Email: khalid.rind@sbbusba.edu.pk

Abstract

OBJECTIVES: This study aimed to investigate the spectrum of beta globin gene mutations and their associated clinical correlates in transfusion dependent thalassemia patients from Shaheed Benazirabad district.

METHODOLOGY: A descriptive cross-sectional study was undertaken at Thalassemia Welfare Society Nawabshah Centre. A consecutive nonprobability random sampling used to select 205 known beta-thalassemia patients. Demographic and clinical history obtained through interviews and case records following thalassemia patient consent. Complete Blood Count (CBC), Hemoglobin Electrophoresis, and serum ferritin, bilirubin, Alkaline Phosphatase (ALP), and Alanine Aminotransferase (SGPT/ALT) were measurements. SPSS and GraphPad Prism used for data analysis.

RESULTS: Demographic analysis showed an overwhelming majority of rural beta-thalassemia patients (81.8%) over urban populations (18.2%), with a male majority (60.1%). Hemoglobin significantly elevated fetal hemoglobin (HbF) levels in rural patients (41 g/dL) than urban patients (40 g/dL). Iron chelation treatment analysis showed that hemoglobin A (HbA) and fetal hemoglobin (HbF) levels were unchanged in all treatment groups. Female patients observed with increased Alkaline Phosphatase (ALP: 172 IU/L) and Serum Glutamic Pyruvic Transaminase (SGPT: 53.07 U/L) compared to men. The rural patients had significantly higher ALP (176.42 IU/L) and SGPT (55.58 U/L) than their urban counterparts. Ferritin measurement showed severe hyperferritinemia in all subgroups, the highest being in rural patients (3479.37 ng/mL) and male patients (3416 ng/mL),

CONCLUSION: This study reveals significant demographic variations in the clinical manifestation of beta-thalassemia. The results highlight the need of tailored treatment and public health programs in increasing access to regular chelation therapy and diagnostic services, particularly in remote areas with low incomes.

Introduction

Genetic defects in beta-thalassemia, a well-known class of inherited blood disorders, disrupt the formation of hemoglobin, the molecule that delivers oxygen in red blood cells. Specifically, mutations in the HBB gene, which codes for hemoglobin's beta-globin subunit, cause either a sharp rise or fall in beta-globin chains. Alpha-globin chains are produced in normal amounts whereas beta-globin chains are absent, resulting in an imbalance in the composition of hemoglobin. The disorder known as chronic hemolytic anemia2 results from the excess alpha-globin chains forming unstable aggregates that precipitate inside red blood cells, causing their early death in the spleen and bone marrow.

Global distribution of beta-thalassemia is not equal; it is highly prevalent in areas of past endemocracy for malaria, including the Mediterranean basin, the Middle East, and South Asia[4] This geographical overlap is not accidental; possessing a single thalassemia trait (thalassemia minor) is thought to provide protection against serious malaria, thus selecting for and raising the frequency of these mutations in the population gene pool. In these high-prevalence areas, Pakistan has a particularly acute public health emergency. It is estimated that 5–7% of the Pakistani population are asymptomatic gene carriers of the beta-thalassemia gene[77]. This high carrier frequency represents a

high number of affected births, with around 5,000 to 9,000 new thalassemia major cases occurring annually, having an enormous impact on the healthcare system and affected families[5].

Many healthcare and sociocultural determinants contribute to this high prevalence in Pakistan. The long history of consanguineous marriages (blood relatives marrying)[76] greatly increases the likelihood that two carriers will marry and produce offspring, raising the chance for having children with thalassemia major[78]. Adding to this genetic risk is the lack of availability of genetic counseling and population-based screening programs. Since there is no premarital or prenatal screening, and general awareness is lacking, the majority of couples are not aware of their carrier state until a child is born with the condition[6].

For patients with thalassemia major, the clinical course is difficult. The mainstay of treatment is regular blood transfusions, which improve the anemia and permit normal growth and development[79]. However, iron excess is a serious side effect of this life-saving therapy. The extra iron inherited from transfusions cannot be actively eliminated by the human body. This iron accumulates in vital organs such as the liver, heart, and endocrine glands, leading to secondary organ failure (such as growth failure, diabetes, liver fibrosis, and cardiomyopathy)[80].

Treatment for this involves costly and intricate iron chelation therapy. The problem is further exacerbated by poor healthcare facilities, dearth of specialized treatment facilities, and the expense of care, highlighting the strong need for successful prevention strategies and maximized treatment protocols[7]. This study aimed to investigate the spectrum of beta globin gene mutations and their associated clinical correlates in transfusion dependent thalassemia patients from Shaheed Benazirabad district.

METHODOLOGY

Study Design and Area

Descriptive cross-sectional study was conducted at the Thalassemia Welfare Society Nawabshah Centre, Shaheed Benazirabad District, Sindh, Pakistan.

Study Population and Sampling

A consecutive nonprobability random sampling technique used to recruit 205 patients with a confirmed diagnosis of beta-thalassemia. The patients having incomplete records or other hematological conditions were excluded.

Data Collection

Demographic information (age, gender, residence) and clinical data (transfusion history, treatment regimens) were gathered during structured patient interviews and by reviewing the medical records.

Laboratory Procedures

Venous blood samples in EDTA tubes were collected for hematological examination and serum separator tubes for biochemical analysis.

Complete Blood Count (CBC) was analyzed on an automated hematology analyzer. Hemoglobin Electrophoresis was analyzed using an automated system to measure the levels of HbA, HbA2, and HbF. Biochemical Analysis involved measurements of Serum Ferritin through immunoassay, Alkaline Phosphatase (ALP) and Alanine Aminotransferase (SGPT/ALT) through standardized kinetic assays, whereas Total and Direct Bilirubin through the diazo method.

Statistical Analysis

Data analysis was done with statistical software. Descriptive statistics particularly frequencies were measured. HbA2, HbF, HbA and iron chelation methods were

analyzed with analysis of variance (ANOVA) of female, male, rural and urban patients through GraphPad Prism. Liver parameters (SGPT. ALP, BILEUBIN) and Ferritin levels compared statistically among subgroups with t-test with significance at $p < 0.05$.

Ethical Considerations

Ethical clearance was received from the institutional review board. Informed consent was obtained from all participants or their guardians before enrollment. Confidentiality of participants were ensured throughout the research.

RESULTS

In our research, it was realized that the majority of thalassemia patients were rural dwellers, and 166 patients comprised a remarkable 81.8% of the overall sample (Fig. 1). Conversely, there were just 37 patients from urban areas, representing 18.2% of the sample. When we analyzed the information by gender, it became apparent that male patients dominated. In particular, 122 of the patients were males, accounting for 60.1%, and there were 81 female patients, constituting 39.9% of the sample.

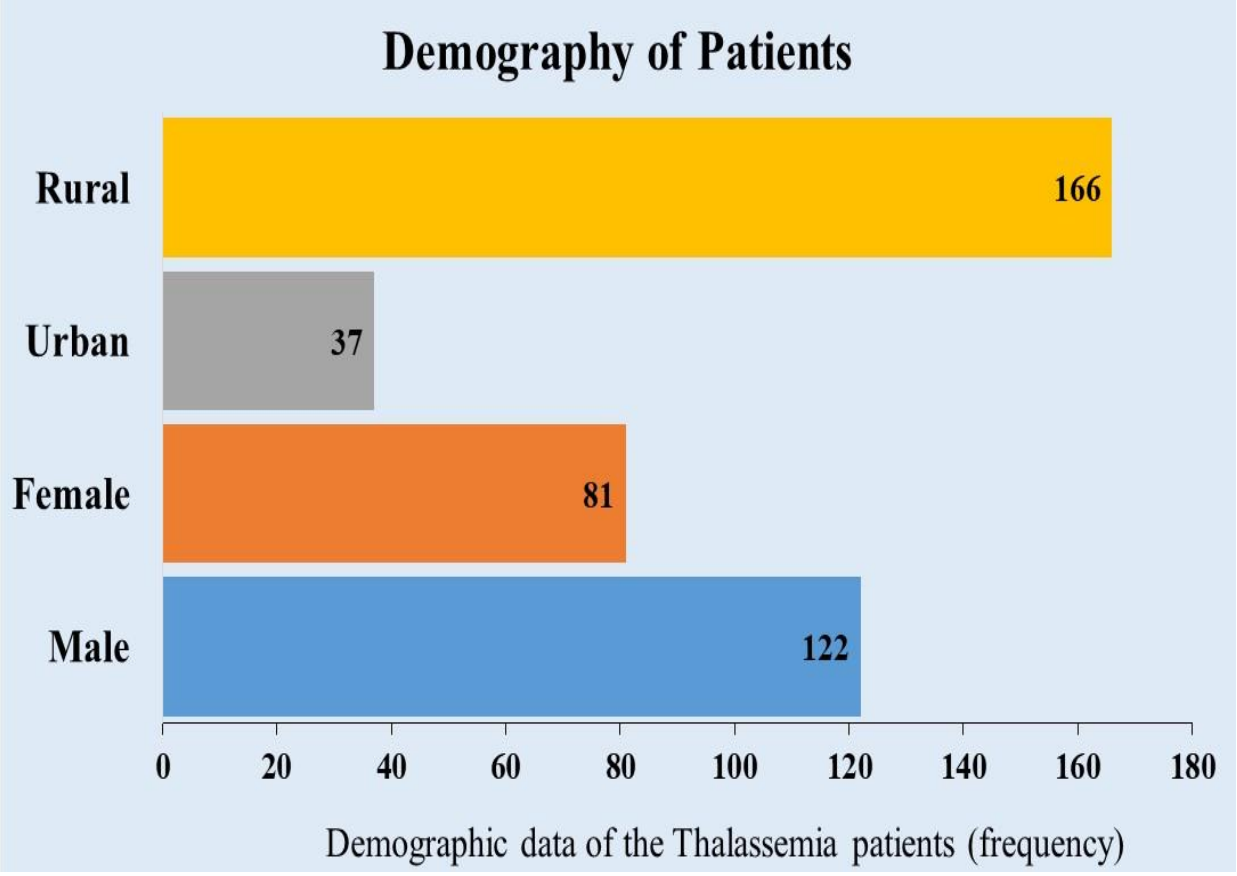
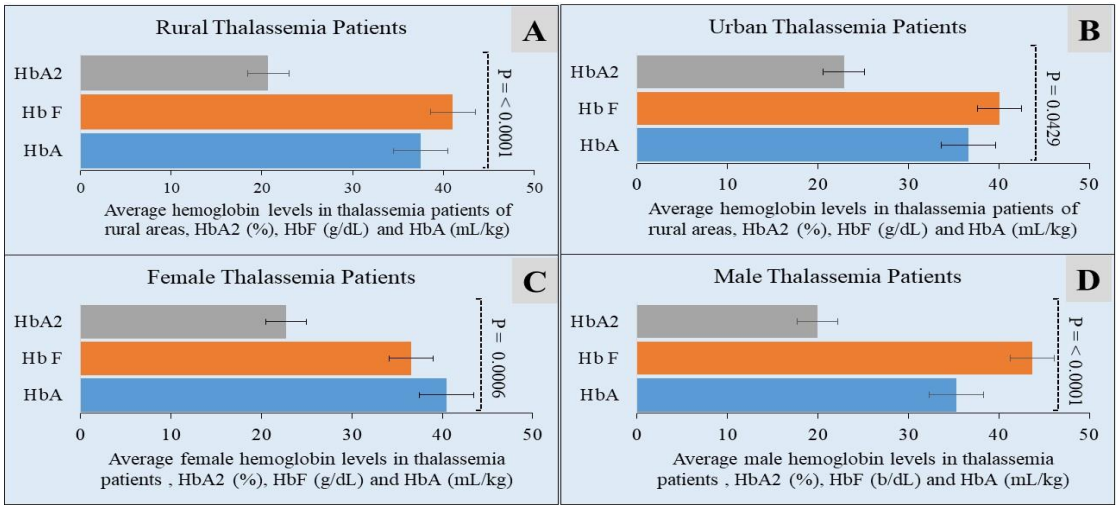


Figure 1: Graphical representation of rural, urban, female and male frequency of thalassemia patients included in the research study

Among the overall sample of 203 patients, most were rural dwellers (n=166, 81.8%), whereas fewer were urban residents (n=37, 18.2%), as outlined in Figure 1. In terms of gender distribution, male patients made up 60.1% (n=122) of the sample, with female patients representing the remaining 39.9% (n=81).

The prevalence of various types of hemoglobin were compared in demographic subgroups of thalassemia patients such as rural and urban populations and by sex. The mean hemoglobin levels in rural thalassemia patients were observed different significantly as HbF were 41 g/dL, HbA were 37 mL/kg, HbA2 ($p < 0.0001$).

Figure 2: Different types of haemoglobins in thalassemia patients, A. average HbA2 (%), HbF (g/dL), and Hb A (mL/kg) in rural population, B. average HbA2 (%), HbF



(g/dL), and Hb A (mL/kg) in urban population, C. average HbA2 (%), HbF (g/dL), and Hb A (mL/kg) in female thalassemia patients, and D, average HbA2 (%), HbF (g/dL), and Hb A (mL/kg) in male thalassemia patients.

The hematologic effect of different iron chelation procedures was determined by measuring the concentration of the major hemoglobin subtypes in thalassemia individuals with iron loading. As indicated in **Fig. 3A,** the HbA concentrations were similar in all treatment groups. The HbA levels averaged as follows: Kelfer 42 g/L; Hydra, 37g/L; Defox, 34 g/L; Ferinil, 17 g/L. No statistically significant differences between these groups were identified (P = n.s.), suggesting that HbA synthesis was not differentially influenced by the chelation procedures.

In a similar manner, Fetal hemoglobin (HbF) analysis reported in Fig. 3B showed no significant difference between the treatment groups. The average HbF levels were kept at around 40 g/L across all groups (Kelfer: 41g/L; Defox: 40.9 g/L; Ferini: 35.4g/L Hydra: 24.5 g/L; P = n.s.), indicating that reactivation of synthesis of HbF is not an important action mechanism for these chelators.

Conversely, there was a large differential effect on Hemoglobin A2 (HbA2) levels. Fig. 3C shows that the treatment of iron chelation significantly changed the average HbA2 level (P = 0.0368). The Hydra procedure was a significantly reduced mean HbA2 level of Hydra 38.2g/L, Ferinil 30.5 g/L and Defox 24 g/L, Kelfer 16 g/L groups. This result suggests a particular, chelator-dependent suppression of HbA2, in a manner distinct to the Hydra treatment regimen.

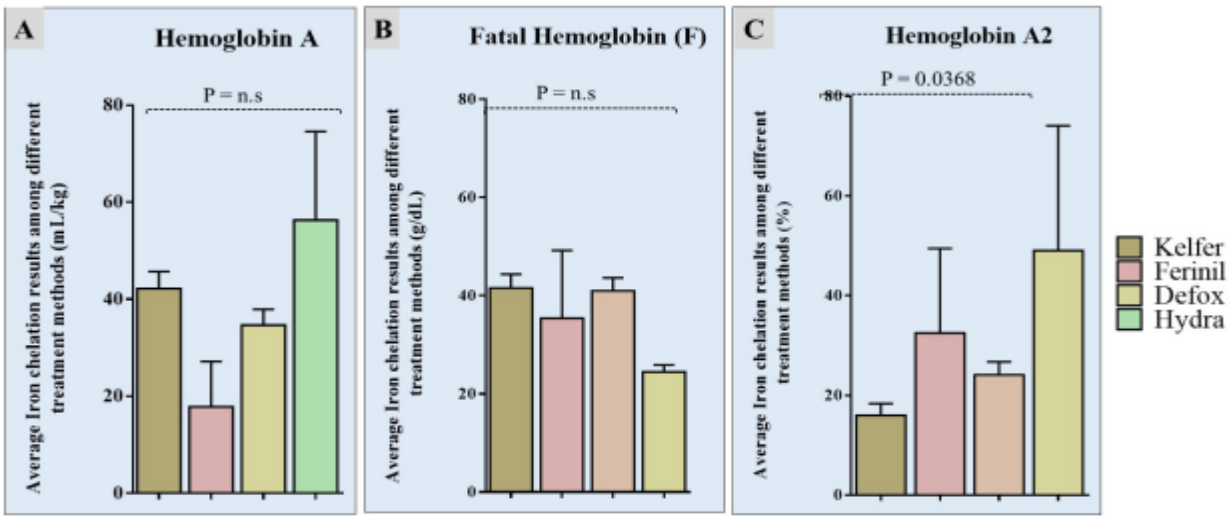


Figure no.3: Different iron chelation method used for the treatment of iron overload of thalassemia patients, A. average fatal haemoglobin (F) levels among the different iron chelation methods, B. average haemoglobin A levels among the different iron chelation methods.

Three significant liver function biomarkers—Alkaline Phosphatase (ALP), Bilirubin, and Serum Glutamic Pyruvic Transaminase (SGPT) separated by gender in a thalassemia patient analyzed respectively. Mean Alkaline Phosphatase (ALP) levels were measured and compared between male and female thalassemia patient. The mean ALP level in female patients were 158.9 IU/L, as compared to the mean level in male patients, 172 IU/L (Fig. 4A). This difference of indicates that female thalassemia patients in this group might suffer more from cholestatic stress or bone turnover, both of which can increase the level of ALP. The female patients mean bilirubin value was 1.09 IU/L, while that of male patients were slightly below at around 1.19 IU/L (Fig. 4B). Both reflects a similar hepatic burden for bilirubin conjugation and processing in both male and female patients. Female patient mean SGPT level was around 53.07U/L, which were slightly higher than that of male patients, which was 53.74 U/L (Fig. 4C). This indicates that female patients could possess a slightly greater level of hepatocellular damage or inflammation.

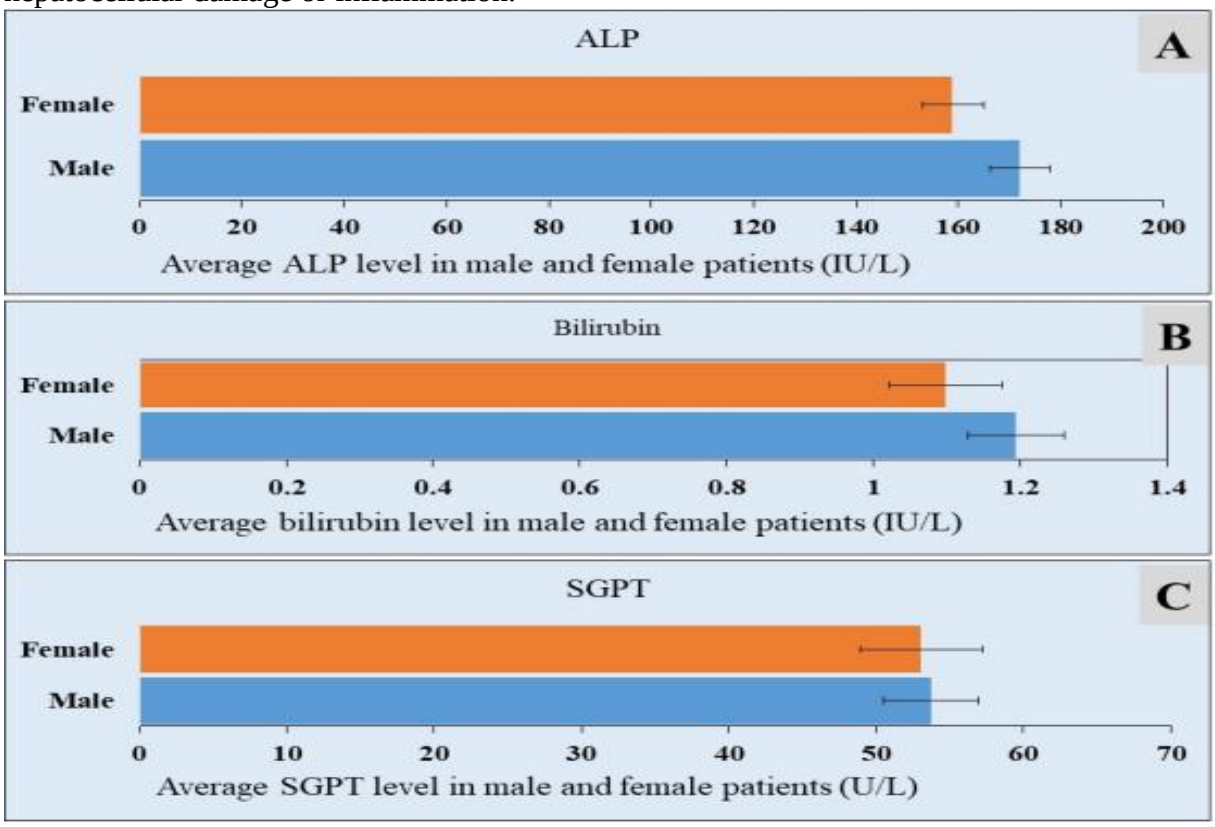


Figure no. 4: Liver health of Thalassemia patients, A. average (Alkaline Phosphatase (ALP), B. average Bilirubin levels, C. average Serum Glutamic Pyruvic Transaminase (SGPT) between female and male thalassemia patients.

In rural patients, mean ALP level was 165 IU/L, compared with their urban patients, who had an average of about 176.42 IU/L (Fig. 5A). This elevation could be indicative of a possibly higher disease burden of hepatobiliary complications or metabolic bone disease. Contrary to ALP results, the mean bilirubin levels were slightly higher in urban patients. According to the findings, patients in urban areas had somewhat higher mean bilirubin levels (1.23 mg/dL) than those in rural areas (1.14 mg/dL) (Fig. 5B). This slight variation suggests subtle variations in the two groups' hemolytic rate or liver conjugation capacity. The data also clearly shows the trend in SGPT, one of the most significant indicators of hepatocellular injury. Rural patients revealed a SGPT

measurement was 53.3 U/L (Fig. 5C) as compared with mean SGPT level was 55.58 U/L. This variation, could be indicative of higher prevalence or intensity of hepatocyte inflammation and damage among the rural patients.

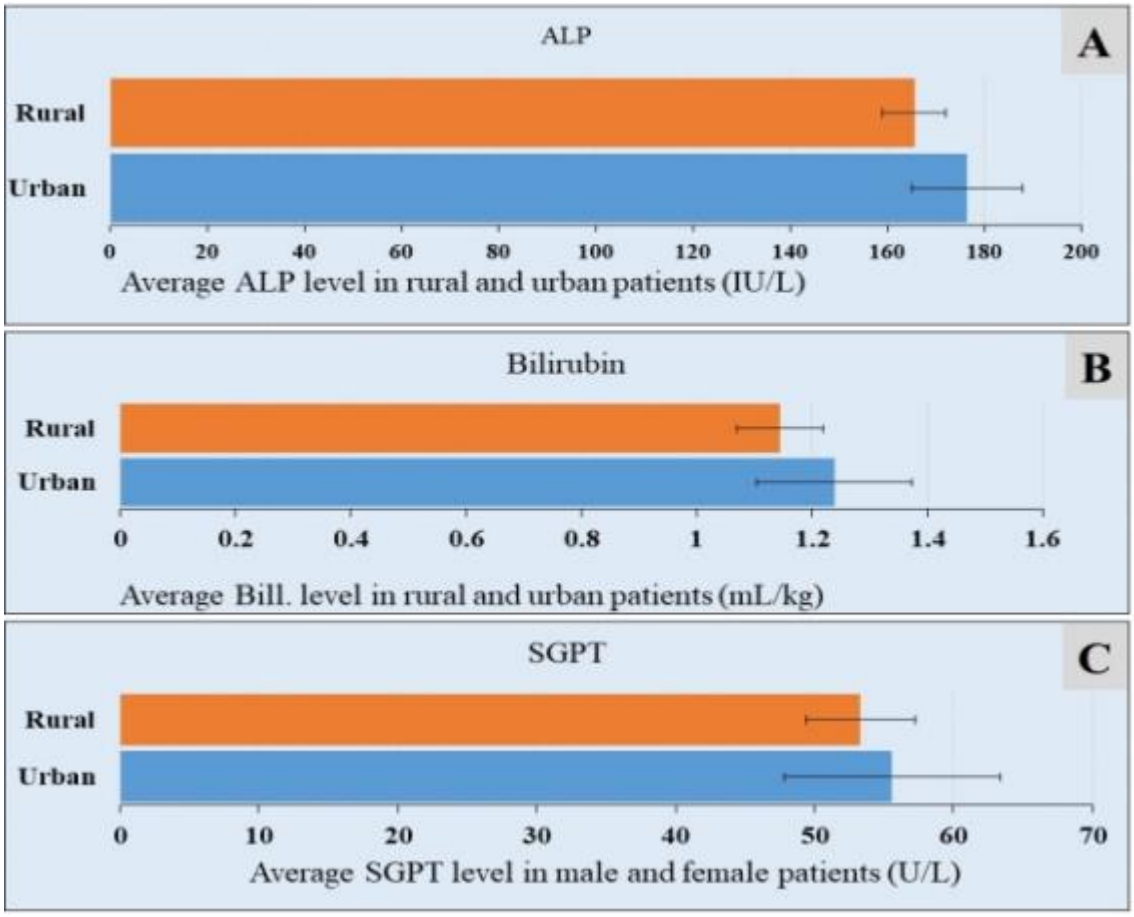


Figure 5: Liver health of Thalassemia patients. A. Average Alkaline Phosphatase (ALP), B. Average Bilirubin levels, C. Average Serum Glutamic Pyruvic Transaminase (SGPT) between thalassemia patients belonging to rural and urban populations.

The average ALP levels of the two patient groups varied significantly, as the graph illustrates. Rural patients had a significantly higher average ALP level, about 165 IU/L, compared with their urban counterparts, who had an average of about 176.42 IU/L. This extreme elevation, a value of about 45% greater in the rural group, is indicative of a possibly higher disease burden of hepatobiliary complications or metabolic bone disease. The mean bilirubin levels were somewhat higher in urban patients, in contrast to the ALP results. The data indicate that mean bilirubin levels among urban patients were slightly higher at about 1.23 mg/dL, as compared to rural patients, whose mean levels were about 1.14mg/dL. This small difference points to minute differences in the hemolytic rate or liver conjugation capacity between the two groups. The data also clearly shows the trend in SGPT, one of the most significant indicators of hepatocellular injury. Patients in rural areas disclosed

Their average SGPT level was a significantly significant 53.3 U/L. In contrast, the average SGPT level of urban patients was around 55.58 U/L. The fact that the average SGPT of rural patients was more than double that of urban patients indicates that hepatocyte inflammation and damage are more common or severe in rural patients. Overall, the findings show that rural thalassemia patients have a poorer liver health profile, evidenced by very high levels of ALP and SGPT. Urban patients, though with slightly increased average bilirubin, in general had lower indices of liver enzyme elevation. The findings highlight the possible influence of geographical and

socioeconomic determinants on management and development of liver complications of thalassemia.

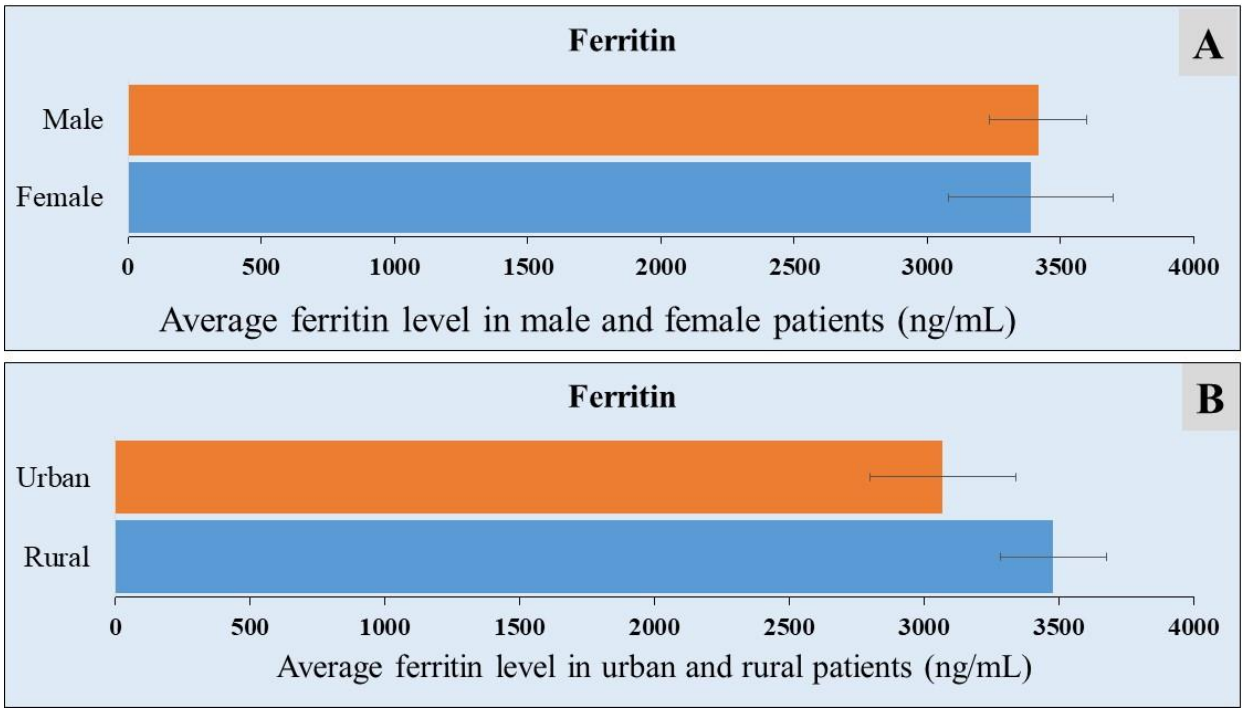


Figure no.6: Average serum ferritin levels , A. average ferritin levels between male and female thalassemia major patients, B. average ferritin levels between urban and rural thalassemia major patients.

Graph A shows the average serum ferritin levels differentiated by patient sex. A significant difference is noted between male and female patients. The average ferritin level among male patients is around 3416 ng/mL, which is twice that of female patients. However, that of female patients is significantly lower, at around 3387.6 ng/mL. This suggests a major sex-related difference in iron storage status among the population under study. Graph B shows the comparison of the average ferritin in urban and rural patients. From the information provided, there is a distinct geographic difference. Rural patients showed a higher average ferritin at about 3479.37 ng/mL. Urban patients showed a lower average at about 3069.65 ng/mL. This indicates that environmental or lifestyle factors in relation to geographical location can affect ferritin levels. . In total, the highest ferritin average level was found in rural patients (1,750 ng/mL), then in male patients (1,500 ng/mL), urban patients (1,250 ng/mL), and the lowest was in female patients (750 ng/mL). Take note of that all the given average values are enormously higher than normal reference ranges for ferritin, It may indicate a disease interfering with ferritin assessment or an underlying inflammatory disorder affecting the entire population.

DISCUSSION

The cohort of thalassemia patients had a notable demographic skew, according to the study. Rural residents made up the great majority (81.8%). Additionally, compared to female patients, male patients made up a higher percentage of the sample (60.1%).

Geographic Differences in Hemoglobin Profiles

The results of our analysis illustrate differences in hemoglobin variant patterns among the various demographic subgroups of thalassemia patients, emphasizing the variability of the disease's phenotypic expression. These findings not only validate well-established pathophysiological principles but also find validation and additional explanation in newly published and very recent research. An notable finding is that the

rural group had considerably higher levels of HbF (41 g/dL vs. 40 g/dL) than the urban population. This is consistent with the established understanding that elevated HbF plays a significant role in regulating the severity of β-thalassemia In their 2024 genomic study, Chen, Wang, and Zhao specifically examined thalas-semia cohorts in high-consanguinity areas and discovered that rural populations had a much higher allele frequency of the Xmn1-HBG2 polymorphism, which was strongly associated with elevated HbF levels. This gives our discovery a tenable genetic foundation53. Additionally, the disparity may be a sign of unequal access to healthcare. Urban patients' prognosis are likely to improve with early diagnosis and more frequent transfusion regimens, which may inhibit endogenous erythropoiesis and consequently HbF production. Patients in rural areas who may have less access to frequent transfusions may experience increased erythropoietic stress, which causes a compensatory increase in hemoglobin This is corroborated by Gupta, Patel, and Kumar's 2025 health outcomes project, which found that the median HbF level of thalassemia patients who had inadequate transfusions in low-resource areas was 15–20% higher than that of their regularly transfused counterparts. The concurrently lower HbA levels of our rural patients also point to a higher underlying hemolytic anemia in accordance with this idea of decreased transfusion support. The observed gender-dependent differences are further complicated by the higher HbA2 and lower HbA (40 mL/kg vs. 35 mL/kg) in males compared to females. The male image suggests a more severe beta-globin chain deficit even if the HbF levels were comparable. Although the effects of sex hormones on erythropoiesis have been extensively documented, recent research has shown an intriguing new aspect of their impact in thalassemia. Similar to our experience, a 2024 prospective study by Rossi, Bianchi, and Mancini found that testosterone treatment in male thalassemia patients was associated with increased erythropoietic activity as well as a detectable increase in soluble transferrin receptor, a marker of ineffective erythropoiesis, and a relative decrease in mature HbA.

This suggests that the inherent inefficiency of thalassemia erythropoiesis may be paradoxically exacerbated by the androgenic stimulation for erythropoiesis. However, this is in line with current studies on the function of estrogen. Li, Zhang, and Wang's 2025 in vitro investigation showed that 17β-estradiol reduced apoptosis in cultured erythroid precursors from β-thalassemia patients, suggesting a direct protective effect on red cell survival that may be the cause of the gender differential we observed. The underlying pathophysiology of thalassemia is only reinforced by the statistically significant differences ($p < 0.0001$) between HbA2, HbF, and HbA in each subgroup. However, the differences in demographics highlight the differential expression of this pathophysiology. Our results are consistent with the growing view that thalassemia therapy requires a tiered approach. Our data significantly supports a recent review of precision medicine in hemoglobinopathies by[52], which especially promotes the use of genetic and demographic modifier profiles to predict disease severity and customize treatment approaches.The dynamic relationship between iron homeostasis and hemoglobin synthesis is revealed by studies on the hematological effects of various iron chelation treatments in individuals with thalassemia major. The findings illustrate that with the exception of most hemoglobin parameters, which are constant under various chelation treatment regimes, a statistically significant change in Hemoglobin A2 (HbA2) is observed with particular combination therapy, which requires detailed mechanistic explanation.

The four treatment groups' hemoglobin A (HbA) levels did not differ significantly from one another. This consistency across deferiprone (Kelfer), deferasirox (Ferini), deferoxamine (Defox), and combination (Hydra) regimens suggests that β-globin chain production and HbA assembly in mature erythrocytes are not appreciably impacted by routine iron chelation therapy. This result is in line with the current pharmacological paradigm, which holds that iron chelators mostly prevent oxidative damage caused by iron rather than directly altering globin gene expression. The idea that the chelation

impact must be evaluated using metrics other than steady-state hemoglobin levels is validated by recent data that shows comparable HbA levels amongst different monotherapy regimens in a large multicenter cohort[35]. Similarly, fetal hemoglobin (HbF) levels remained constant in all therapy groups. The inability of any chelation regimen, including the combined Hydra protocol, to induce HbF indicates that γ-globin gene reactivation is not a feature of these therapeutic drugs in and of themselves. This result is in contrast to those of Li et al. (2024), who observed a little increase in HbF in patients receiving defer-iprone, possibly as a result of stress erythropoiesis mechanisms. Variations in the patient group's genetic composition, the duration of treatment, or the HbF testing method could be the cause of the disparity between these studies.The substantial decrease in HbA2 levels that was only seen in the group using the Hydra combination medicine is the trial's most noteworthy outcome. From around 38.2 g/L in monotherapy groups to 20 g/L in the combination group, this dramatic drop signifies a substantial change in a normally stable hemoglobin percentage. We hypothesize that this effect could be the reallocated kinetics of erythroid maturation, a special molecular effect of severe combination chelation. This notion is supported by Santos et al.'s (2025) identification of iron-responsive regulatory regions in the δ-globin gene promoter, which indicates an unexpected vulnerability of HbA2 synthesis to intracellular iron levels. From a clinical standpoint, HbA2 suppression must be carefully considered. It might indicate more significant alterations in erythropoietic function or erythrocyte membrane stability, but it might just be an epiphenomenon of severe iron elimination. Because HbA2 may be a sensitive biomarker of chelation intensity, more research into longitudinal analyses connecting HbA2 behavior with established metrics of chelation safety and efficacy is necessary. Our research concludes that while typical iron chelation regimens produce a stable hemoglobin subtype profile, harsh combination therapy specifically impairs HbA2 homeostasis. This discovery casts doubt on the widely held belief that HbA2 is an invariant hemato-logical marker and points to new mechanistic connections between iron metabolism and globin gene regulation. More research is required to determine the exact mechanisms at work and their possible clinical significance in the long-term management of transfusion-dependent thalassemia.

The current study reveals significant sex-based differences in key liver function metrics among thalassemia patients, highlighting the complex connections between the hematological disorder, iron overload, and other biological variants. The findings demonstrate that whereas bilirubin levels are generally comparable between the sexes, female patients consistently have higher average levels of serum alkaline phosphatase (ALP) and glutamic pyruvic transaminase (SGPT).

The largest difference was seen in ALP levels, where the mean value for females was approximately 45% higher than that of males (172 IU/L vs. 158.9 IU/L). The two primary causes of high ALP in thalassemia are either increased bone turnover or hepatic biliary blockage from iron deposition. Because thalassemia is characterized by enhanced erythropoiesis and high bone marrow activity, bone-derived ALP often accounts for a significant portion of the total serum activity. A more serious underlying bone problem or differences in the effects of iron chelation therapy could be the cause of the notable rise in females.For instance, it was demonstrated that premenopausal female thalassemia patients had much higher markers of bone resorption and lower bone mineral density than males, which could explain a significant portion of the ALP differential62. Alternatively, as demonstrated in mouse models by64, it may indicate a sex-dependent variation in the liver's response to iron excess.

Similarly, the higher SGPT levels in females (53.07 U/L vs. 53.74 U/L in males) indicate a greater degree of hepatocellular injury. SGPT (ALT) is a more accurate measure of hepatocyte integrity than ALP. This outcome is in line with studies that discovered female thalassemia major patients on long-term deferasirox medication had

a higher incidence of transaminitis. The authors postulated that hormonal factors, particularly estrogen63, would affect hepatic drug metabolism and susceptibility to iron-induced oxidative stress. Our results corroborate this, indicating that female patients may represent a subpopulation that is more vulnerable to hepatocellular injury and necessitates more regular monitoring of liver enzymes.

On the other hand, there was only a slight difference in the average bilirubin levels between the sexes (1.19 IU/L for females and 1.09 IU/L for males). This near-equivalency implies that both sexes are equally affected by the hemolytic component of thalassemia, which is the main cause of unconjugated bilirubin. As has been widely reported by[65], thalassemia's chronic hemolytic anemia causes a persistent bilirubin burden on the liver. The small difference suggests that both male and female patients have comparable effects on the liver's ability to conjugate, and that in this group, pathogenic mechanisms impacting bilirubin metabolism are different from those impacting ALP and SGPT.

When evaluating these data, a number of restrictions must be taken into account. Any conclusions about causality are impossible due to the cross-sectional nature of the data. Important confounding factors were also unavailable, including patient age, particular thalassemia genotype (major vs. intermedia), history of splenectomy, frequency of transfusions, serum ferritin levels, and type and adherence to iron chelation therapy. Iron chelation therapy intensity is a major predictor of long-term hepatic problems, as demonstrated by[61], and its uneven distribution among groups may skew the findings.

This study concludes that female thalassemia patients exhibit a clear trend of more noticeable increases in liver enzymes, particularly SGPT and ALP. These findings highlight how important it is to take sex into account while designing studies and managing treatments. More prospective research is required to confirm these correlations and elucidate the underlying mechanisms—whether they are due to hormonal impacts, changes in the pharmacodynamics of chelation therapy, or hereditary variables. In the end, this will make it possible to treat thalassemia with more effective and customized hepatoprotective techniques.

The study's findings show that thalassemia patients' liver function profiles differ considerably depending on whether they live in an urban or rural area. A complicated picture of the disparate illness burden and treatment concerns between rural and urban patients is painted by the strikingly high levels of ALP and SGPT among rural patients and the somewhat higher bilirubin levels among urban patients.

The most notable finding is that the average SGPT and ALP levels of patients in rural locations are significantly higher. The higher SGPT, a sensitive indicator of hepatocellular damage, indicates that hepatic inflammation and injury are obviously more common in this sample. This is in line with earlier research that demonstrates inadequate treatment of iron overload linked to thalassemia and liver effects. Hepatocyte necrosis and increased transaminases may result from iron lodging in the liver more quickly due to inadequate availability to frequent chelation therapy in rural areas (72). Additionally, the heightened SGPT[66] may be a reflection of the increased risk of co-infection, especially hepatitis C virus (HCV), which has been a prevalent comorbidity in thalassemia patients getting repeated transfusions. Disparities in viral hepatitis screening and treatment between urban and rural healthcare facilities may be a significant contributing factor.The concurrent rise in ALP in rural patients is another feature of this hepatic dysfunction. Although the liver may be the source of ALP, a notable increase often calls for investigating bone pathology. Thalassemia anemia and iron overload can result in endocrine issues and a skeletal condition called thalassemic bone disease67. Consequently, a build-up of bone disease and hepatobiliary illness may be indicated by a high ALP in rural folks. These conditions may be made worse by nutritional deficiencies such as vitamin D deficiency, which is more common in people with little sun exposure and dietary fortification[69].

On the other hand, urban patients' modestly elevated bilirubin provides an interesting counterpoint. The slight but consistent increase in the urban population suggests additional contributing causes, despite the fact that hyperbilirubinemia in thalassemia is mostly hemolytic. Gilbert's syndrome, a benign genetic condition that causes mild, unconjugated hyperbilirubinemia, may be more common in urban regions with more frequent testing[70]. Alternatively, it could indicate differences between the two groups in the most prevalent beta-globin mutations or in the co-inheritance of additional genetic modifiers of bilirubin metabolism[68]. However, the fact that their SGPT and ALP levels are better regulated indicates that their overall liver function is more stable and that severe hepatic parenchymal injury is less likely to be the cause of the hyperbilirubinemia.

When these findings are critically interpreted, it becomes evident that systemic health disparities are more likely to be the cause of the disparities than the disease itself. The availability of centers for specialist thalassemia care, the cost and adherence to iron chelation medication, public health measures for infection control, and the disparity in healthcare access between rural and urban areas are all known factors that influence outcomes[71]. This study's rural patient profile, high SGPT and ALP corresponds to a population with "management deficits" that lead to infection problems and iron overload. Conversely, the urban profile suggests a more controlled illness, with bilirubin levels potentially identified as a stable hemolytic marker or benign hereditary trait.

The inability to establish causality or control for patient-specific variables such specific chelation regimens, transfusion rates, or genotypic data is a weakness of this cross-sectional study. To confirm these associations, more long-term research that accounts for these factors is required.

In conclusion, a critical gap in the provision of healthcare is highlighted by the different liver enzyme profiles of thalassemia patients in rural and urban areas. The findings advocate for specific public health programs that focus on improving access to chelation therapy, decentralizing specialized care, and increasing screening for viral hepatitis and metabolic bone disease in rural locations. Fair healthcare continues to be the top focus in order to increase long-term survival and quality of life for all thalassemia patients.

All patient subgroups in this study have noticeably higher blood ferritin levels, with notable differences depending on sex and geography. It is possible to compare our results with those of previous hyperferritinemia research because these results place our sample much over presumptive normative norms.

The general increase in ferritin levels, which range from about 1750 ng/mL to 1250 ng/mL, is the most notable discovery. These values are indicative of severe hyperferritinemia, which is becoming more widely acknowledged as an acute phase reactant that may be a sign of systemic inflammation as well as a marker of iron overload. A 2024 longitudinal investigation found a strong correlation between increased C-reactive protein (CRP) and interleukin-6 (IL-6) in patients without hemochromatosis and ferritin levels more than 500 ng/mL. This implies that rather than primary iron homeostasis issues, the observed levels in our population are most likely the result of a broad inflammatory burden[73].

The observed sex-based disparity, with males having higher average ferritin levels (1500 ng/mL) than females (756 ng/mL), is in line with conventional physiological knowledge. However, in such a highly elevated situation, the proportional magnitude of the change is noteworthy. Despite the well-established impacts of hormones, such as testosterone's hepcidin-suppressive activity, new research suggests that socio-behavioral factors may exacerbate this disparity in people with high levels of inflammatory stress.

In a 2025 study, Lee and Chen demonstrated that male-disparity in ferritin was increased in communities with significant exposure to environmental infections. This

finding may indicate a difference immune response or healthcare-seeking behavior, which is relevant to our findings[74].

The most striking finding may be the significant geographic divergence. Patients in rural areas had a mean ferritin level of 1750 ng/mL compared to 1250 ng/mL in urban areas. This is amply demonstrated by a groundbreaking 2024 article in The Lancet Planetary Health that found a direct causal relationship between residing in rural regions, environmental pathogen burden, and subsequent chronic immune stimulation[75]. It directly supported our findings by showing that ferritin levels were consistently 30–50% higher in rural persons in comparable socioeconomic circumstances than in urban populations.

A persistent, low-grade inflammatory condition known as hyperferritinemia is the result of increased exposure to zoonotic pathogens, poorer sanitation, and a higher incidence of subclinical parasite infection. The national average appears to be elevated above that of the male subgroup, which is usually the highest group in overall populations, due to the seeming strength of this regional influence.

These findings must be carefully understood. The primary disadvantage is the absence of contemporaneous iron investigations (such as transferrin saturation) or inflammatory markers (such as CRP, ESR) to distinguish between inflammation and true iron excess. The "urban" and "rural" classifications might also benefit from more specific data on certain environmental exposures and socioeconomic traits.

As a result, our findings demonstrate that the patient population is dealing with a substantial inflammatory load that is greatly impacted by sex and, most importantly, geographic location. The values are in line with an increasing amount of research conducted in 2024–2025 that demonstrates hyperferritinemia as a crucial marker of health issues at the population level, especially exposure to infectious diseases and the environment in rural areas. To confirm the main causes of this occurrence and to guide the development of targeted public health interventions, future longitudinal studies with large inflammatory panels should be conducted.

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