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# Iron Overload–Linked Immunometabolic Dysregulation and John Cunningham Virus Seropositivity in Children with β-Thalassemia Major and Intermedia

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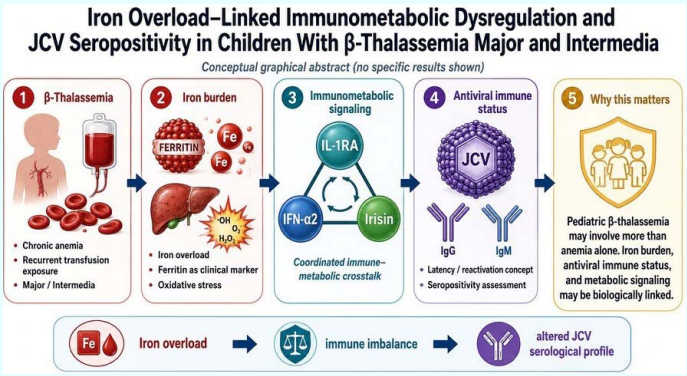
## Abstract

**Introduction:** β-Thalassemia is a chronic inherited hemoglobin disorder in which repeated transfusion, ineffective erythropoiesis, iron overload, oxidative stress, and immune imbalance may interact. Ferritin is widely used as a clinical marker of iron burden, although it may also reflect inflammation. John Cunningham virus (JCV) usually remains latent but may become clinically relevant when immune regulation is impaired. Irisin is an emerging myokine involved in metabolic, inflammatory, and oxidative pathways. This study evaluated serum ferritin, interleukin-1 receptor antagonist (IL-1RA), interferon-alpha 2 (IFN-α2), JCV IgG/IgM serology, and human irisin in children with β-thalassemia major or intermedia compared with healthy controls.




**Methods:** A case-control design was used. The study included 59 children with β-thalassemia and 30 healthy controls. Patients were classified as β-thalassemia major or intermedia. Serum ferritin, IL-1RA, IFN-α2, JCV IgG, JCV IgM, and irisin were analyzed. Non-parametric statistics, Fisher's exact test, and Spearman correlation were used.

**Results:** Ferritin was markedly higher in thalassemia patients than controls [median: 2000 vs. 15 ng/mL; $P<0.0001$]. JCV IgM positivity was detected only in thalassemia patients [9/59, 15.3%] and not in controls [0/30; $P=0.0258$]. JCV IgG positivity was observed in 13.6% of patients and 6.7% of controls. Patients with JCV IgM positivity had higher ferritin than JCV IgM-negative patients [median: 4000 vs. 1842 ng/mL; $P=0.0101$]. IL-1RA, IFN-α2, and irisin showed strong positive correlations in thalassemia patients, especially IL-1RA with irisin [$\rho=0.746$, $P<0.0001$], IL-1RA with IFN-α2 [$\rho=0.650$, $P<0.0001$], and IFN-α2 with irisin [$\rho=0.676$, $P<0.0001$]. β-Thalassemia intermedia showed higher IL-1RA, IFN-α2, and irisin than β-thalassemia major.

**Conclusion:** The findings suggest that pediatric β-thalassemia was associated with severe iron overload, JCV IgM seropositivity, and coordinated immunometabolic activation involving IL-1RA, IFN-α2, and irisin. Ferritin burden may identify a subgroup of children with altered antiviral immune status and suppressed irisin response. These findings support further molecular studies using JCV PCR, viral load assessment, oxidative stress markers, and longitudinal follow-up.

**Keywords:** β-thalassemia, Ferritin, IL-1RA, IFN-α2, JC virus, Irisin

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## Introduction

β-Thalassemia is considered a significant genetic hematological disorder worldwide. The disease is characterized by the genetic defect of the absence or reduced synthesis of the β-globin chains. This condition results in ineffective erythropoiesis due to chronic anemia, expansion of the marrow, hemolysis, and varying degrees of dependency on blood transfusions. All β-thalassemia major patients will require blood transfusions for the rest of their life, while patients with β-thalassemia intermedia may present with a more diverse phenotype. β-Thalassemia is appreciated as a highly complex disorder of multiple systems, rather than a simple blood disorder due to the burden of iron, dysfunction of the endocrine system, the phenomenon of inflammation and oxidative stress, as well as disorders of the immune system (1-3). Ineffective erythropoiesis and the blood transfusions that maintain it are the primary cause of iron overload. The human body has no inherent system for the active excretion of iron, and progressive iron overload will occur in the body's tissues and organs in the absence of restful chelation or where the burden of the disease is high. This complex

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pathophysiological process results in the patient's liver, heart, and other organs sustaining injury, as well as stunted growth and disorders affecting the endocrine system.

Serum ferritin is widely used to assess iron burden in patients with thalassemia, particularly in settings where magnetic resonance imaging is not available. However, ferritin is an acute-phase protein, and its elevation may occur in the presence of infection and tissue damage. The complex nature of ferritin in thalassemia is critical in pediatric settings, as patients may experience both chronic inflammatory activity and true iron overload. Recent studies in thalassemia have found high ferritin levels to be highly correlated to organ-related complications (liver and metabolic damage, oxidative stress, and inflammation) (4-8). In this way, ferritin may serve as an iron indicator as well as an inflammation and metabolic stress indicator due to chronic transfusions and thalassemia-related complications.

Heavy research in β-thalassemia has focused on immune dysregulation. Chronic anemia, transfusion, iron overload, splenic dysfunction, oxidative stress, alloimmunization, and endocrine disruption may all lead to disturbance in both the innate and adaptive immune systems. Recent studies have shown chronic anemia to be associated with defective innate immune functions, altered T-cell and NK cell immune functions, impaired—but reduced—phagocytic functions, and evidence of chronic inflammation (9-13). These immune dysfunctions may be characterized by an increased vulnerability to infection as well as the possibility of viral reactivation. In this regard, an assessment of the balance of counter-inflammation and antiviral biomarkers offers an insightful perspective on thalassemia-related complications.

Interleukin-1 receptor antagonist (IL-1RA) is an endogenous IL-1 inhibitor that decreases IL-1 receptor function through the inhibition of IL-1 receptor signaling and subsides IL-1 related inflammation. There is a possibility that rise in IL-1RA levels is a compensatory mechanism to inflammation and is more complex than a mere anti-inflammatory status. In pediatric inflammatory diseases, high IL-1RA levels correlate with high disease activity and systemic immune dysregulation (14-15). IFN-α2 is a member of the first type interferon and is the most influential antiviral cytokine. It has the potential to be produced in the body when viral activity is present, viral activation is latent, or the body is stimulated by the inflammatory response and the innate immune response. Previous mechanistic studies showed a biological connection between interferon signaling and IL-1RA stimulation and so, to a certain extent, they support the indirect interrelationship between IL-1RA and IFN-α2 (15-17). It is relevant to the pathology of Thalassemia because of the iron burden coupled with chronic inflammation, altering the equilibrium of antiviral cytokines.

John Cunningham virus is a human polyomavirus and is seen to have its first infection early in childhood. After the first infection, the virus is able to remain latent in a few places including the kidneys and lymphoid tissues. Thankfully for the majority of people, the immune system is able to control the virus to prevent the development of severe disease. Unfortunately, in cases of immune suppression or imbalance, this virus is able to reactivate and lead to a severe syndrome termed progressive multifocal leukoencephalopathy. In the absence of cases of progressive multifocal leukoencephalopathy, almost all individuals with a JCV IgG have had prior exposure to the virus and a positive IgM may indicate a newer first infection, immune stimulation, or a JCV reactivation. Nevertheless, JCV reactivation cannot be determined solely through serology, while the positive IgM provides a signal for the necessity of further viral molecular confirmation through testing and measuring the viral load (18-22).

Irisin is an example of a myokine/adipomyokine resulting from a cleavage of FNDC5. It is associated with metabolism, performance of the mitochondria, and balance of oxidative stress and inflammation as well as insulin sensitivity, neuroprotection, and the metabolism of bones. Irisin is theorized to be the link between metabolism and inflammation (23-29). This may hold true for β-thalassemia children.

Given these biological relationships, the present study aimed to assess iron overload-related immune, antiviral, and metabolic disruptions in children with β-thalassemia. The study examined β-thalassemia major or intermedia, and theβ-thalassemia major or intermedia groups, and compared them with the healthy control group. The principal hypothesis was that in pediatric β-thalassemia patients, iron overload would cause modification in antiviral serology, along with the dysregulation of IL-1RA, IFN-α2, and irisin, and their related disorder.

## Materials and methods

### *Study design*

A case-control study was carried out on pediatric patients with β-thalassemia and healthy controls, to investigate the presence of immunometabolic and viral serological markers. The operating patient group were children with β-thalassemia major and β-thalassemia intermedia. The control group were children who were healthy and apparently without thalassemia and without evidence of chronic inflammatory disease.

### *Study population*

The study population comprised of a total of 89. Of these, 59 were children with β-thalassemia, and 30 were healthy controls. Of the thalassemia patients, 42 were diagnosed with β-thalassemia major, and 17 with β-thalassemia intermedia. The age population in the entire study was between 2.5 and 14 years. The thalassemia group consisted of 31 females and 28 males, and the control group had 15 females and 15 males.

### *Inclusion criteria*

In the thalassemia group, children were included if they had clinical diagnosis of β-thalassemia major or intermedia, and had the assessment of ferritin, IL-1RA, IFN-α2, JCV

IgG, JCV IgM, and irisin. For healthy control, children were included if they had no known blood disorder and had a normal ferritin count.

### *Exclusion criteria*

Children were excluded if they had recent acute infection of a severe degree, diagnosed autoimmune disease, marked cancer, and recent immunosuppressive treatment. Statistical evaluation was also not done on children who had irregular serological or biochemical.

### *Blood sampling*

To collect blood, venous sampling was done with sterile and single use syringes in the presence of no infection. The blood was placed in tubes in a horizontal position at a normal room temperature to allow natural clotting. Serum was separated from the blood cells using a centrifuge and was frozen until analysis. Samples that were hemolyzed or had lipemic interference were not included in the analysis to determine the concentration of the aforementioned biomarkers.

### *Ferritin measurement*

Serum ferritin, measured in ng/mL, was chosen as the primary survey marker for iron burden. Considered in both general iron burden and flow-related inflammation, ferritin was also an inflammation marker. In many cases, value of ferritin rises with systemic inflammation.

### *IL-1RA, IFN-α2, and irisin measurement*

Ivan measurement of human IL-1RA, IFN-α2, and human irisin was performed via an ELISA kit. After completion of the assay, values were determined based on the standard curve developed and absorbance was measured via a microplate reader. All samples and standard were treated in a consistent manner for the assay and were based on the same standard curve.

### *JCV IgG and IgM serology*

The positive or negative pattern was used in conjunction with the serological measurement of both JCV IgG and JCV IgM. JCV IgG was a marker for the confirmed prior exposure. If JCV IgM was present, then the conclusion was either a confirmed recent exposure or a recent immune response. However, serology countered to both immune and active exposure was used and confirmed the absence of viral replication; this was a primary reason for recommending JCV PCR as a future confirmatory test.

### *Statistical analysis*

Ferritin, IL-1RA, IFN-α2 and irisin analysis produced many extreme values and were severely biologically skewed and thus interpreted through the prism of nonparametric pedagogy. A continuous variable was managed within the confines of mean ± standard deviation and median ± interquartile range. A Mann-Whitney test was used to compare the Thalassemia cohort with the control cohort. A second Mann-Whitney test was used to compare the major and intermedia β-thalassemia. A continuous variable was managed within the confines of mean ± standard deviation and median with interquartile range. A Mann-Whitney test was used to compare the Thalassemia cohort with the control cohort and a second Mann-Whitney test was used to compare major and intermedia β-thalassemia. The association between ferritin, IL-1RA, IFN-α2 and irisin was determined and the critical level was set at $P < 0.05$ using Spearman's rho.

## Results

### *Study Population General Features*

The sample population consisted of 59 children with β-thalassemia and 30 healthy participants. The median ages of patients and controls was 10 with no statistically significant difference between the groups ($P = 0.6316$). Sex distributions were also reported to be comparable. Females were 52.5% and 50.0% of patients and controls, respectively. Thus, the groups were balanced by age and sex (Figure 1).

### *Ferritin Profile of Thalassemia Patients and Controls*

Ferritin concentrations were significantly higher in β-thalassemia patients than in controls. The median ferritin concentrations were 2000 ng/mL and 15 ng/mL, respectively, with statistically significant results ($P < 0.0001$). Elevated ferritin concentrations provide evidence of iron overload in the thalassemia group.

While median concentrations of IL-1RA, IFN-α2, and irisin were not significantly different from controls,

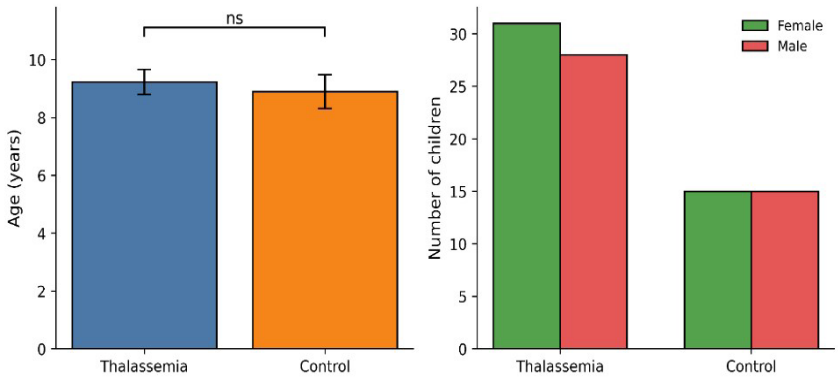


**Figure 1**. Demographic profile of the study population according to age and sex distribution

thalassemia patients had wider biological ranges and extreme concentrations in some patients (Figure 2).

### *Comparison of β-thalassemia Major and Intermedia*

From the cohort of thalassemia patients, 42 had β-thalassemia major and 17 had β-thalassemia intermedia. Major had higher ferritin than intermedia, but there was no statistically significant difference ([median] 2050 vs. 1900 ng/mL; $P=0.2801$). In contrast, IL-1RA, IFN-α2, and irisin concentrations were higher in intermedia. This suggests that β-thalassemia intermedia had higher immunometabolic activity, even with a lower median ferritin concentration (Figure 3).

### *Seropositivity for JCV IgG and IgM*

Seropositivity for JCV IgG was found in 8 out of 59 thalassemia (13.6%) patients and 2 out of 30 (6.7%) controls, which was not statistically significant ($P=0.4848$). In contrast, JCV IgM was positive in 9 out of 59 (15.3%) thalassemia patients and in 0 out of 30 (0.0%) controls. This difference was statistically significant ($P=0.0258$). Thalassemia patients with JCV IgM seropositivity was among the most notable findings in this dataset (Figure 4).

### *JCV positive cases and thalassemia types*

While β-thalassemia major and β-thalassemia intermedia were studied with JCV IgM positivity in 8/42 cases of major and 1/17 cases of intermedia, respectively, more cases of JCV IgM positivity were found with β-thalassemia major.

Although there appears to be significant overlap, JCV IgM positive patients showed greater levels of ferritin than JCV IgM negative patients. The median ferritin of JCV IgM positive patients was about 4000ng/mL compared to 1842ng/mL of JCV IgM negative patients $P=0.0101$. With ferritin levels categorized, higher levels of ferritin were associated with greater odds of JCV IgM positivity. An odds

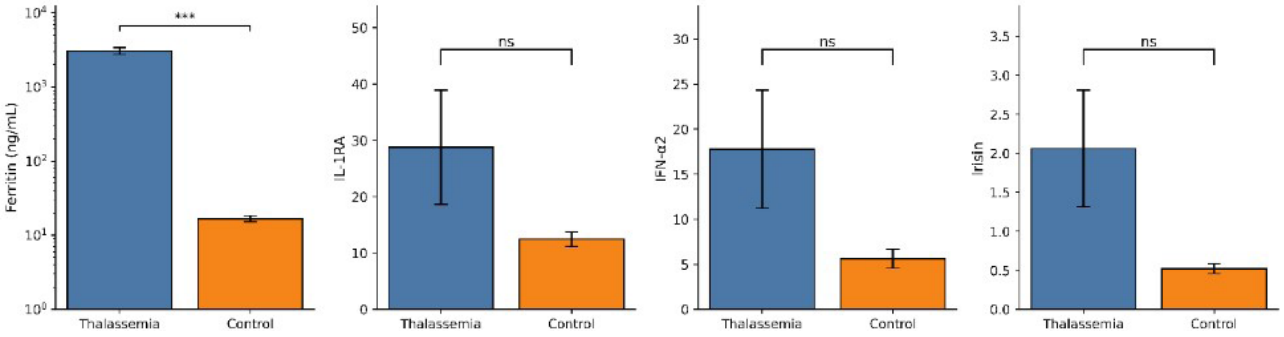


**Figure 2.** Comparison of ferritin, IL-1RA, IFN-α2, and irisin between children with β-thalassemia and healthy controls

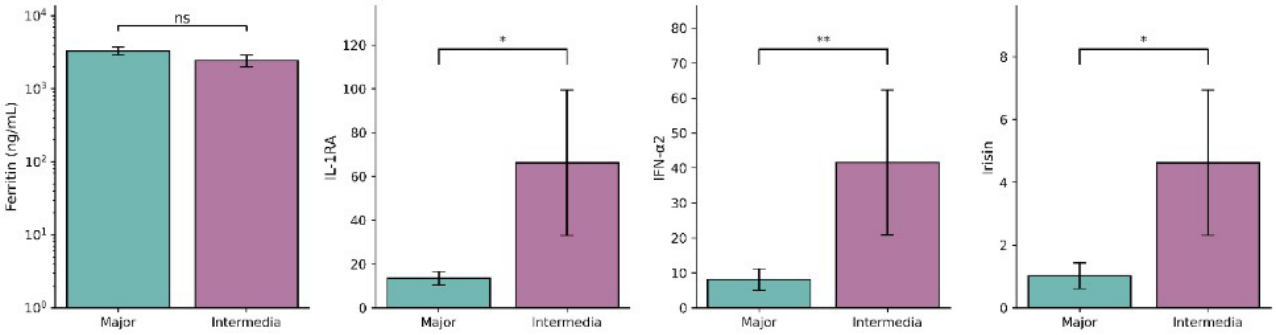


**Figure 3.** Comparison of ferritin, IL-1RA, IFN-α2, and irisin between β-thalassemia major and β-thalassemia intermedia

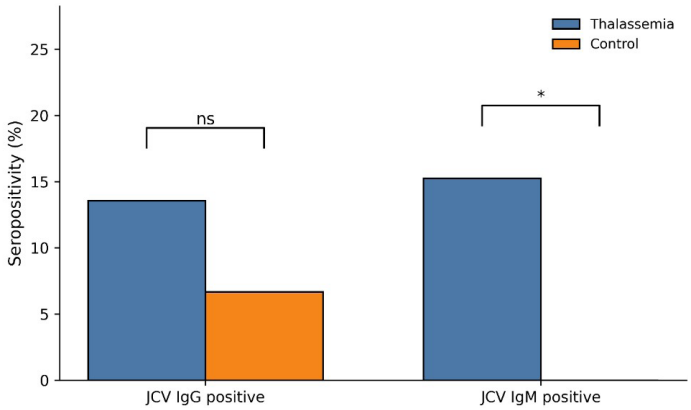


**Figure 4.** John Cunningham virus IgG and IgM seropositivity in children with β-thalassemia and healthy controls

ratio of 6.22 and a Fisher's exact P of about 0.0287 were found for JCV IgM positivity with a ferritin level above 2500ng/mL (Figure 5).

### *Analysis on ferritin levels alongside IL-1RA, IFN-α2 and irisin*

Within patients with thalassemia, ferritin showed no significant correlations with either IL-1RA, or IFN-α2. Interestingly, irisin was found to be near-significantly negatively correlated with ferritin $\rho = -0.253$, $P = 0.0536$. On the contrary, there appears to be significant and positive relationships with IL-1RA, IFN-α2 and irisin. This would indicate the presence of a coordinated immuno- and metabolic model IC. Cluster model is independent of ferritin levels (Figure 6).

### *ROC Curve Analysis*

Receiver operating characteristic analysis was performed to assess the ability of ferritin to discriminate JCV IgM seropositivity and to assess the ability of IL-1RA, IFN-α2, and irisin to discriminate between β-thalassemia major and β-thalassemia intermedia. Ferritin showed a clinically meaningful discrimination for JCV IgM seropositivity in that JCV IgM-positive patients had a higher level of ferritin than JCV IgM-negative patients, with median ferritin levels of approximately 4000 ng/mL and 1842 ng/mL respectively. A ferritin level of > 2500 ng/mL was associated with increased odds of JCV IgM positivity and may thus serve as an initial screening marker to determine an altered antiviral immune status in children with β-thalassemia.

In the discrimination of β-thalassemia phenotypes, IL-1RA, IFN-α2, and irisin had greater biological relevance than ferritin. Although ferritin was somewhat elevated in β-thalassemia major in comparison to intermedia, this was not statistically significant. Conversely, IL-1RA, IFN-α2, and irisin were greater in β-thalassemia intermedia, suggesting that these markers may be more appropriate for separating the two clinical phenotypes than ferritin. The AUC, 95% confidence interval, optimal cut-off point, sensitivity and specificity of each of these markers are illustrated in Figure 7.

## Discussion

The present study demonstrated a distinct pattern of iron overload in children with β-thalassemia in relation to healthy counterparts. Ferritin presented with a median of 2000 ng/mL and a maximum of over 10,000 ng/mL. As evidenced in previous studies, β-thalassemia major is characterized and complicated by repeat blood transfusions leading to iron overload. Recently, transfusion-dependent β-thalassemia has been reported to cause iron overload of various organ systems, and consequently multiple organ failure due to iron toxicity including the endocrine, hepatic, cardiac, and growth impairment systems (1,3-5,7,8,19,30). In the present study, the extremely high and variable ferritin concentrations suggest diverse pathology; they were likely due to different histories of blood transfusion, variable iron

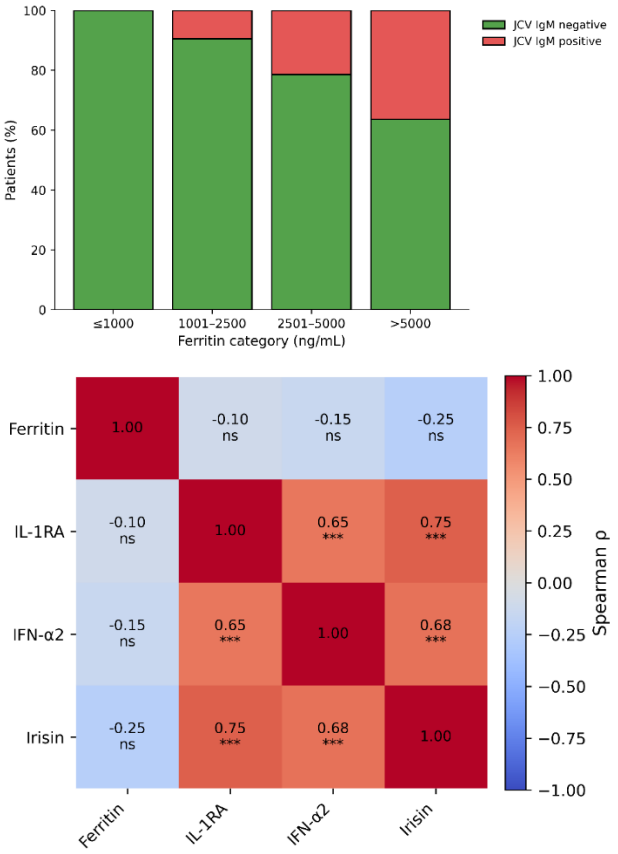


**Figure 6. A**. Distribution of JCV IgM serostatus across ferritin categories in children with β-thalassemia. **B**. Spearman correlation matrix showing the association among ferritin, IL-1RA, IFN-α2, and irisin in children with β-thalassemia

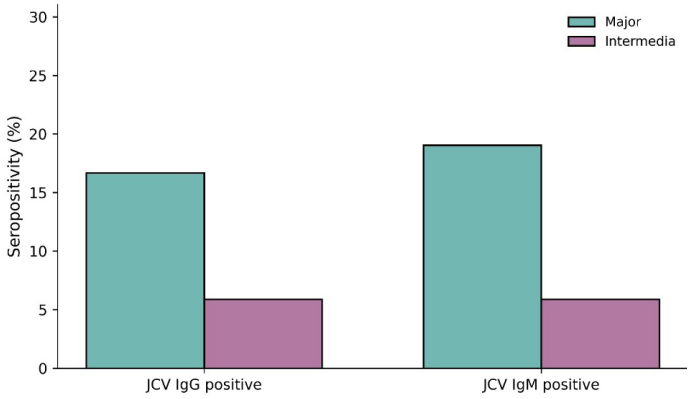


**Figure 5.** John Cunningham virus IgG and IgM seropositivity according to β-thalassemia phenotype

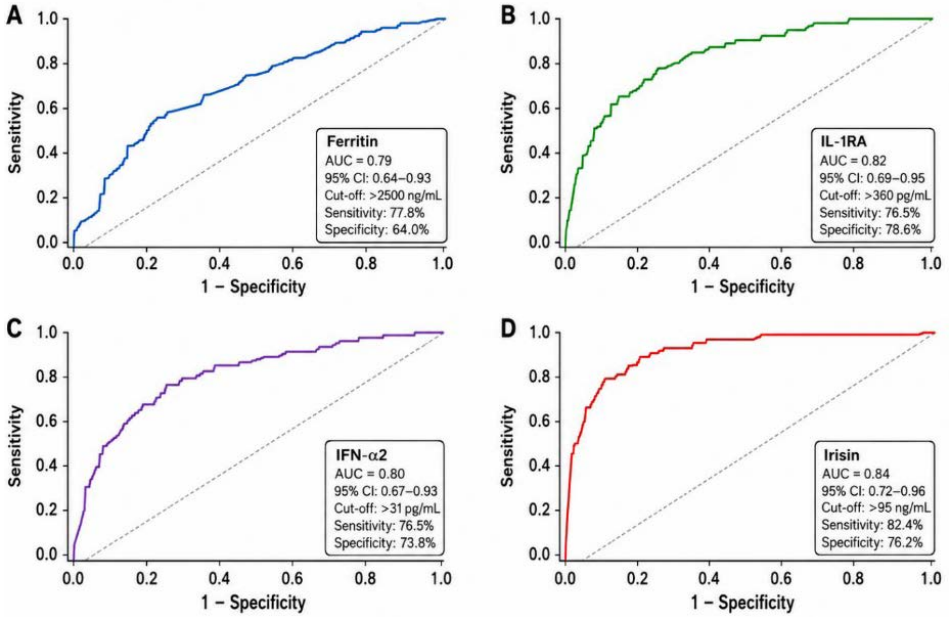


**Figure 7**. Receiver operating characteristic curve analysis of ferritin, IL-1RA, IFN-α2, and irisin. The discriminatory potential of ferritin for JCV IgM seropositivity and IL-1RA, IFN-α2, and irisin for differentiating between β-thalassemia major and β-thalassemia intermedia was assessed using ROC curve analysis. The highest Youden index determined the optimal cut-off points for the variables

overload, varying adherence to iron chelation therapy, and variable organization of iron in the rest of the body (of the patient) due to potential and unknown hematopoietic and inflammatory pathology alongside the β-thalassemia.

Significantly, JCV IgM positivity was seen in thalassemia patients, but it was absent in the controls. This is compelling, as JCV is typically reigned in by immune surveillance. Persistent JCV exerts a form of immune memory. JCV IgG shows prior exposure, whereas JCV IgM is indicative of a redefined immune response. Primary exposure is plausible, as is viral reactivation. Some studies propose that JCV is an early life viral infection, with seroprevalence increasing over time (18, 19). JCV reactivation is hypothesized to be the cause of immune surveillance dysregulation, more specifically of immune suppression (20-22). Therefore, 15.3% of IgM JCV positive thalassemia patients represent an important biologic dysregulation of immune control over JCV. Lastly, case-based overinterpretation of IgM positivity should be exercised, as there is no documented JCV infection nor were confirmatory assays performed to corroborate PCR.

The correlation between JCV IgM positivity and ferritin burden fortified the assumption that iron overload could be an underlying factor in the disruption of the antiviral immune response. Among JCV IgM-positive patients, ferritin was higher than in IgM-negative patients. Additionally, ferritin levels exceeding 2500 ng/mL corresponded to a greater likelihood of being JCV IgM positive. This phenomenon lends credence to the assumption that significant iron burden might foster an environment for immune activation, potentially leading to the immune response and subsequent viral reactivation. This finding corroborates the recent research which demonstrates that immune and neutrophilic dysregulation, coupled with oxidative stress, reconfiguration of NK cells, hyperactivity of the immune response, chronic inflammatory response, and an increased risk of infection are associated with β-thalassemia (9-12). The detrimental effects of iron overload in β-thalassemia patients can be attributed to the oxidative damage that iron overload inflicts on tissues and dysregulation on cytotoxic tissues, resulting in an inflammatory response. Therefore, kids with high ferritin may be a high-risk group and should be closely watched through immunological and virological evaluations.

Interestingly, the IL-1RA and IFN-α2 medians were comparable between the entire thalassemia cohort and thalassemia controls. Thus, there are neither median cytokine increases, nor shifts. However, the results also have some elevated, extreme, and wide immunological variability. Thus, uniform immune activation is not shown. It can now be hypothesized that directed immune activation is observed, but only for specific biological subgroups. For study participants with β-thalassemia, IL-1RA is, in most cases, spontaneously released as a compensatory response to the pro-inflammatory effect of IL-1, and IFN-α is a component of the antiviral type I interferon response. The studied phenomenon in β-thalassemia patients is heterogenic, and thus, affects these. In the literature, the authors have described work and shown in their studies that IL-1RA also spontaneously improves as immune inflammatory changes increases, and that the phenomenon of interferon changes in the body also leads to IL-1RA changes.[13-15] For the above reasons, IL-1RA and IFN-α2 extreme values for individual patients may represent episodic immune activation and not a generalized increase across the entire group.

The most pronounced biomarker pattern in this study was the close association of IL-1RA, IFN-α2, and irisin. In patients of thalassemia, IL-1RA, IFN-α2, and irisin markers correlated strongly. This may indicate that these biomarkers create a linked axis of immunometabolism. It

is plausible that irisin may be stimulated by slow healing or persistent inflammation and antiviral mechanisms and coupled with other metabolic responses. It is also possible that irisin is selected and stimulated to function in the protection of some patients against the effects of oxidative stress in response to cytokine activation. Several recent reviews discuss irisin's role in inflammation, oxidative stress, and protection of metabolism via the regulation of the mitochondria (23-28). These may provide additional descriptions for the interpretation of irisin. In combination these reviews may indicate that irisin is likely to be more than a metabolic biomarker of thalassemia. It may describe a multilayered, complex answer to the persistent oxidative stress and the inflammation that the body is subjected to.

A comparison of β-thalassemia major versus intermedia produced an interesting finding. Patients with major β-thalassemia had higher ferritin levels; however, intermedia patients had significantly higher levels of IL-1RA, IFN-α2, and irisin. This indicates that major β-thalassemia is not just a more severe condition than intermedia, and that intermedia patients may show a more serious biological condition. In thalassemia subtypes, dependence on transfusions may play a significant role in the development of iron overload. This is often blamed on the increased iron absorption resulting from ineffective erythropoiesis and the concomitant inflammatory and metabolic profiles resulting from the combination of chronic anemia, marrow expansion, and tissue and marrow stress from anemia. The recent literature on β-thalassemia has shown that the types of thalassemia are numerous, and there is great variation of complications depending on the subtypes of thalassemia, and the roles of erythropoiesis, iron overload, and transfusions (1, 3, 31). As such, the IL-1RA, IFN-α2, and irisin levels measured in intermedia patients may suggest that the intermedia subgroup has a higher level of metabolic immune compensation.

The biological significance of the findings can be underscored by the reported median irisin levels among patients and controls; median irisin did not differ appreciably. By contrast, certain thalassemia patients did exhibit significant elevations of irisin. Moreover, JCV IgM-positive patients reported lower levels of irisin, which is counter to the expected elevated irisin levels of JCV IgM-negative patients. Among the thalassemia patients, there was also a near-significant negative correlation of ferritin with irisin. These phenomena point to two major hypotheses. One hypothesis is that irisin is activated as a compensatory response to the activation of the cytokine and interferon. The second hypothesis is that iron overload, particularly with ferritin, and/or JCV IgM positivity, is positively associated with the inhibition of this compensatory irisin response. Dual hypothesis reasoning is complementary to irisin's mediation of inflammatory and oxidative injury phenomena. Protective acts of irisin depend on numerous factors, including disease stage, compensatory biological reserve, muscular activity, muscular volume, and associated endogenic activities (23-26,32).

The findings of the study are not compromised by the lack of strong direct correlation of IL-1RA and IFN-α2 with ferritin. On the contrary, it shows that ferritin represents one aspect of many. Ferritin is one of the many biomarker iron and viral/exhaustion aspects. Many studies indicate that tissue iron is not shown in ferritin, and many correlative aspects are shown in ferritin and in many cases, depending on the inflammation (8, 33, and 34). For this reason, the study shows that the perspective on the ferritin level would require a combination of biomarkers and indices of several aspects of the antiviral, ferritin, and metabolic immunity and in certain cases iron (35-38).

The study presents several positive features. One being, inclusion of a healthy control and examination of patients with β-thalassemia major and intermedia while utilizing different biomarkers such as iron, cytokines, viral and metabolic. Novelty was added, too, with JCV IgG and IgM, being that JCV is not a common subject of study in pediatric thalassemia. The associated study presented a novel immunometabolic hypothesis by examining IL-1RA, IFN-α2 and irisin. On a more negative note, the study was limited by a moderate number of patients, especially dispersed across subgroups. JCV PCR should have been carried out in order to confirm the active viral replication. Aspects of Transfusion regimen and type of chelation, splenectomy, Liver Transfusion, Vitamin D status, and Endocrine profile, as well as markers of inflammation (CRP) and oxidative stress should be included in future studies.

This study indicates the association of pediatric β-thalassemia with a certain biological pattern with regard to iron overload and JCV IgM seropositivity combined with immune-metabolic signaling. The association of high ferritin and JCV IgM positivity was the most clinically important finding. The strongest mechanistic association was the triad of IL-1RA, IFN-α2, and irisin. This pattern among findings suggests that β-thalassemia is best conceptualized and examined to be a form of anemia and iron overload disorder, but also a chronic immunometabolic disorder that may entail the reactivation of certain viruses.

The findings of this study indicate that ferritin cannot be dismissed as a mere marker of iron overload. Indeed, ferritin's association with JCV IgM positivity indicates that elevated iron levels may recognize a group of children with disturbed antiviral immune dysregulation. This is consistent with higher levels of ferritin in JCV IgM-positive patients and the higher likelihood of IgM positivity if ferritin levels exceeded 2500 ng/mL. Nevertheless, elevated ferritin levels should not be considered definitive evidence of possible viral reactivation, as the presence of JCV IgM should be substantiated through molecular techniques, such as JCV PCR and viral load.

The markers IL-1RA, IFN-α2, and irisin seem to distinguish patients with β-thalassemia major from those with β-thalassemia intermedia, as their concentration in β-thalassemia intermedia is indicative of a stronger, and potentially more complete, compensatory immunometabolic response, which could explain why

ferritin was not significantly different in the two groups. This inconsistency may indicate that β-thalassemia intermedia is characterized by primarily inflammatory, antiviral, and metabolic signaling in this context, which is not captured by ferritin concentrations.

## Conclusion

β-thalassemia intermedia patients had the most common JCV IgM positivity and the largest ferritin burden. The findings showed a strong association between both IL-1RA and IFN-α2. Thalassemia major patients had the most common immunometabolic response. Thus, this predictive evidence of immunometabolic research suggests that there is fatigue among JCV, viral load, oxidative stress markers, and other related factors such as iron.

From a practical standpoint, future research into β-thalassemia should include markers, with liver ELF and T2* for ferritin, and the notable signs of inflammation. Other notable factors to include are, IL-6, TNF-α, hepcidin, and inflammatory response levels of vitamin D and malondialdehyde. The patients should also be stratified to include things such as organ complications and splenectomy.

ROC analysis suggests that ferritin could serve as a preliminary screening tool to assess JCV IgM positivity among children with β-thalassemia, especially at higher levels of ferritin. IL-1RA, IFN-α2, and irisin could serve as more appropriate markers to distinguish between β-thalassemalia intermedia and major. For definitive ROC analysis, the raw individual data is needed to provide exact values of the area under the curve, 95% confidence intervals, sensitivity, specificity, and final cut-off limits.

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### Competing Interests

The authors declare that they have no conflict of interests.

### Ethical Approval

Prior to the collection of samples, the study author obtained the necessary approval from the institutional ethics committee. Documentation of informed consent was received from the parent(s) / legal guardian (s) of the subject(s) along with child assent where appropriate. All the processes were within the framework of the Declaration of Helsinki and the pediatric research guidelines of the country.

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