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A Review of Cytokine Detection Methods in β-Thalassemia Major Patients

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Abstract

β-thalassemia major (β-TM) is defined by persistent inflammation and immune dysfunction, with cytokine profiling being crucial for diagnosis and research. This review evaluates methodologies used in cytokine quantification among β-TM patients. A targeted literature search revealed prominent methods such as ELISA, Luminex, flow cytometry, and qRT-PCR. Although ELISA remains the most common method, methodological discrepancies and limited cytokine panels hinder data comparability. Multiplex technologies offer improved efficiency but remain underused. Enhanced standardization and broader cytokine coverage are essential to strengthen biomarker discovery and clinical application in β-TM research. Findings suggest that researchers require unified, multiplex-capable platforms in future investigations.

Keywords: Beta-thalassemia major (β-TM); cytokines; ELISA; Luminex

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1.0 Introduction

1.0 Background of β-TM

β-TM is a hereditary hematological disorder linked to HBB gene mutations, leading to reduced or absent beta-globin chain production. Affected individuals experience chronic hemolytic anemia and require lifelong blood transfusions (Taher et al., 2021). β-TM remains a significant public health concern in many regions, especially the Mediterranean, the Middle East, and parts of Asia. While transfusion therapy improves survival, it also causes complications like iron overload, which can damage vital organs such as the liver, heart, and endocrine system (Musallam et al., 2012). Patients with β-TM often exhibit immune dysregulation, characterized by increased infection risk, immune-mediated organ damage, and chronic low-grade inflammation (Ansari et al., 2020). The complex interactions among recurrent transfusions, iron overload, and immune response call for a deeper

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understanding of the immunological aspects of thalassemia, with a focus on cytokine roles. These immune disturbances primarily arise from the effects of transfusion frequency, iron overload, and splenectomy, which together disrupt immune homeostasis (Ansari et al., 2020). Consequently, there is heightened interest in analyzing immune mediators, particularly cytokines, to elucidate the pathophysiology of β-TM.

1.1 Significance of Cytokine Evaluation in β-TM

Cytokines are soluble signaling proteins that coordinate immune cell differentiation and activation by binding to specific receptors and mediating intercellular communication (Donniacuo et al., 2025). In patients with β-TM, an imbalance between pro-inflammatory and anti-inflammatory cytokines indicates ongoing immune activation and oxidative stress, with notable increases in interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), which are associated with iron overload and immune responses post-transfusion (Haghpanah et al., 2022; Zhang et al., 2022). Therefore, this review aims to identify and evaluate the principal methodologies used for cytokine quantification in β-TM patients, to compare their strengths and limitations, and to determine their research and clinical applicability, while emphasizing the need for methodological standardization to enhance reproducibility and relevance.

2.0 Literature Review

2.1 Cytokine Dysregulation in β-TM

Cytokines are soluble proteins that bind to specific receptors to coordinate immune cell differentiation and activation *(Liu et al., 2021; Donniacuo et al., 2025)*. Previous studies on cytokine dysregulation in β-thalassemia major have gradually evolved from identifying basic inflammatory markers to exploring complex cytokine networks and their roles in disease progression and transfusion-related complications. Elevated concentrations of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-8, IL-13, transforming growth factor-beta (TGF-β), and tumor necrosis factor-alpha (TNF-α), have been documented in patients afflicted with β-TM. These heightened levels exhibit a correlation with increased ferritin concentrations and a higher frequency of blood transfusions, thereby potentially exacerbating the inflammatory status and vulnerability to infections in these patients (Haghpanah et al., 2022; Abdelrazek et al., 2018; Zhang et al., 2022).

Furthermore, research has demonstrated that β-TM patients present enhanced serum levels of various cytokines, indicative of an amplified state of immune activation and inflammation. For example, investigations have identified increased serum concentrations of interleukin-33 (IL-33), a cytokine integral to signaling cellular damage and infectious diseases, thereby implying its viability as a clinical biochemical marker in β-TM (Brbber et al., 2023). Another study indicates that elevated serum levels of cytokine-dependent hematopoietic cell linker (CLNK) and interleukin-3 in β-TM patients correlate with increased serum ferritin levels and a high annual transfusion index, suggesting a potential relationship between immune signaling and disease severity (Mahmoud et al., 2022). Additionally, the noted imbalance in interleukin-12 (IL-12) and interleukin-13 (IL-13) concentrations among individuals with β-TM suggests an altered immune response, characterized by significantly reduced IL-12 levels and markedly elevated IL-13 levels compared to healthy control subjects (Hashad et al., 2013).

Investigations have further illuminated the altered concentrations of specific cytokines in thalassemia patients, such as increased IL-10 and TNF-α levels in conjunction with gingival inflammation (Akcali et al., 2019). Elevated concentrations of IL-17 and TGF-β in β-TM patients further underscore the involvement of pro-inflammatory processes in the disease (Baharlou et al., 2016). Notably, IL-3, recognized for its role in stimulating globin chain synthesis, does not account for the extensive reference range of the α/β globin ratio, thereby suggesting a more intricate role than previously understood. Vitamin D supplementation in patients with β-TM has demonstrated potential anti-inflammatory properties by reducing interleukin-6 levels while enhancing the concentrations of anti-inflammatory cytokines such as IL-2 and IL-10, indicating a possible therapeutic pathway to alleviate inflammation and iron overload. Moreover, elevated serum neopterin levels have been reported in β-TM patients, reflecting persistent immune activation and chronic inflammatory processes, often accompanied by increased IL-6 and TNF-α concentrations (Shanab et al., 2015; Gluba-Brzózka et al., 2021; Haghpanah et al., 2022). Furthermore, multiple studies have documented immunological anomalies in β-TM, including altered cytokine profiles and soluble immune mediators, underscoring the complex interplay between immune activation, transfusion burden, and disease management (Ansari et al., 2020; Mahmoud et al., 2022). However, despite the extensive body of research, inconsistencies in cytokine measurement methods and the limited range of cytokines assessed across studies continue to hinder comparability and reproducibility. This highlights a clear need for standardized methodological approaches in future β-TM investigations. In β-TM studies, multiple analytical platforms have been used to quantify cytokines, each with its specific benefits and limitations. The most commonly applied include ELISA, flow cytometry, real-time PCR, and multiplex immunoassays, all offering different degrees of sensitivity and clinical relevance.

2.2 Methodological Approaches for Cytokine Detection

Recent investigations from 2021 to 2025 have provided updated insights into cytokine-mediated inflammation in β-TM, utilizing advanced multiplex and high-throughput immunoassay platforms. A range of laboratory techniques is employed in β-TM research to assess cytokine levels, offering valuable insight into the disease's immune and inflammatory dynamics. Commonly utilized platforms include enzyme-linked immunosorbent assay (ELISA), flow cytometry, real-time PCR (qRT-PCR), and multiplex immunoassays such as Luminex. Each method provides unique strengths in terms of sensitivity, throughput, and the type of data generated, making them essential tools for characterizing cytokine profiles typically altered in β-TM patients (Liu et al., 2021; Haghpanah et al., 2022).

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2.2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) is extensively employed for the quantification of cytokine concentrations in the serum or plasma of patients afflicted with β-TM. This methodology utilizes antibodies that are specific to the cytokines of interest and is frequently used to measure levels of IL-4, IL-6, IL-8, IL-13, and TGF-β (Zhang et al., 2022). Due to its exceptional sensitivity and specificity, ELISA is particularly adept at detecting low levels of cytokines in various biological fluids. It continues to be regarded as the most widely used single-analyte method for the detection of individual cytokines owing to its cost-effectiveness and ease of use. Nonetheless, its restricted ability to analyze multiple targets simultaneously, considerable sample volume requirements, and variability among different assay kits limit its applicability for extensive immune profiling (Liu et al., 2021).

2.2.2 Flow Cytometry and Intracellular Cytokine Staining

Flow cytometry, particularly when integrated with intracellular cytokine staining, represents a robust methodology for evaluating cytokine production at the level of individual cells. This technique facilitates the identification of intracellular cytokines, yielding critical insights into the functional dynamics of immune cells in patients afflicted with β-TM (Mansourabadi et al., 2023; Piyajaroenkij et al., 2023). This methodology is particularly advantageous for delineating cytokine-producing cell subsets—such as Th1, Th2, and Th17—which are essential for elucidating immune dysregulation in β-TM. Although it provides substantial information for mechanistic investigations, flow cytometry necessitates sophisticated instrumentation, specialized technical skills, and rigorously established protocols, which may constrain its reproducibility and practicality in resource-limited environments (Piyajaroenkij et al., 2023).

2.2.3 Real-Time PCR and Molecular Methods

Quantitative real-time polymerase chain reaction (qRT-PCR) is frequently utilized to assess cytokine mRNA concentrations, providing an indirect yet significant indicator of cytokine synthesis in patients with β-TM. By identifying alterations in gene expression, this methodology yields valuable insights into transcriptional regulation and the overall inflammatory milieu (Piyajaroenkij et al., 2023; Liu et al., 2021). While mRNA expression levels may not directly reflect protein levels, qRT-PCR continues to be an indispensable supplementary technique for corroborating upstream cytokine signaling pathways. Furthermore, other molecular techniques, including reverse transcription PCR (RT-PCR) and in situ hybridization, have been employed to investigate cytokine gene regulation at the genomic level; however, their usage remains comparatively constrained (Liu et al., 2021).

2.2.4 Multiplex Assay Technologies

Multiplex assay technologies—most notably Luminex—are increasingly recognized in cytokine research due to their ability to concurrently assess a diverse range of cytokines utilizing minimal sample volumes (Caprari et al., 2023). These platforms facilitate the quantification of as many as 100 cytokines within a single assay, providing high-throughput profiling that is particularly suitable for elucidating the intricate immune signatures linked to β-TM. Nevertheless, the majority of β-TM investigations have concentrated on a limited selection of merely two to four cytokines (Haghpanah et al., 2022), highlighting a significant gap in future research practices. This underutilization may stem from factors such as prohibitive initial equipment expenditures, variability in sensitivity among different analytes, and the necessity for meticulous calibration and standardization throughout experimental procedures (Caprari et al., 2023).

Table 1: Comparison of Cytokine Quantification Methods

Method	Detection Basis	Multiplexing Capability	Sample Volume Required	Strengths	Limitations
ELISA	Antibody-antigen binding (colorimetric)	Single-analyte	Moderate–High	Widely available, cost-effective	No multiplexing; high sample volume; variability
Flow Cytometry	Intracellular staining + light scattering	Moderate (≤30 cytokines)	Low–Moderate	Cell-type-specific; supports multiplexing	Requires viable cells; costly equipment
qRT-PCR	Gene expression profiling	High (depends on panel)	Low	Upstream cytokine pathway analysis	mRNA ≠ protein; requires RNA integrity
Luminex	Bead-based fluorescent immunoassay	High (10–100 cytokines)	Low	Simultaneous multi-cytokine detection	Cross-reactivity; high setup cost

This table summarizes the cytokine quantification methods reported in previous studies.

2.3 Conclusion

The reviewed literature highlights the pivotal involvement of cytokine dysregulation in the pathophysiological mechanisms underlying β-TM, with a plethora of investigations documenting increased concentrations of both pro-inflammatory and anti-inflammatory cytokines. These modifications are intricately associated with the severity of the disease, frequency of blood transfusions, and perturbations within the immune system. Furthermore, a variety of laboratory methodologies—including ELISA, flow cytometry, qRT-PCR, and multiplex assays—have been employed to evaluate cytokine profiles in individuals afflicted with β-TM. While each method offers distinct advantages, limitations in standardization, sensitivity, and multiplexing remain significant barriers. Collectively, these insights emphasize the necessity of establishing comprehensive, validated approaches for cytokine quantification to enhance the understanding of immune modifications and inform prospective therapeutic strategies in β-TM.

3.0 Methodology

3.1 Literature Search Strategy

We conducted a systematic review to identify relevant studies that meticulously evaluated the levels of cytokines in individuals diagnosed with β-TM; a systematic literature review was meticulously executed utilizing robust laboratory-based quantification techniques. The search was conducted across several reputable databases, specifically PubMed, Scopus, and Web of Science, employing a strategic combination of highly relevant keywords including, but not limited to, “beta-thalassemia major” or “β-TM,” “cytokines,” “ELISA,” “Luminex,” “flow cytometry,” “qRT-PCR,” and “multiplex assay.” To ensure the accuracy and relevance of the findings, the search parameters were strictly confined to peer-reviewed scholarly articles published in English and involving human subjects within the time frame spanning from 2013 through November 2025.

3.2 Inclusion and Exclusion Criteria

The studies selected for inclusion in this comprehensive review were required to conform to a set of specific criteria, which entailed (1) the involvement of human subjects who had been clinically diagnosed with β-TM, (2) the provision of original research data about the quantification of cytokines utilizing

established laboratory methodologies, and (3) the clear and unambiguous specification of the method or methods employed for the measurement of cytokines. Conversely, articles were systematically excluded from consideration if they met any of the following conditions: (1) they were categorized as reviews, editorials, or conference abstracts; (2) they did not concentrate specifically on the subject of β-TM; (3) they lacked empirical laboratory data regarding cytokines; or (4) they were based on studies conducted with animal models rather than human participants. Following screening and eligibility assessment, a total of 25 studies met the inclusion criteria and were analyzed in this review.

3.3 Study Selection Process

The study selection followed the PRISMA 2020 statement (Page et al., 2021). The flow of records through identification, screening, eligibility, and inclusion is summarized in Table 2.

Table 2: PRISMA 2020 Flow Summary of Study Selection

Phase	Description	Records (n)
Identification	Records identified through database searching	612
	Additional records identified from other sources	34
Screening	Records after duplicates removed	498
	Records screened	498
	Records excluded	412
Eligibility	Full-text articles assessed	86
	Full-text articles excluded	62
	– Not β-TM population	18
	– Review/editorial/abstract	14
	– No cytokine data	17
	– Animal/in vitro only	9
	– Duplicate/overlapping cohort	4
Included	Studies included in qualitative synthesis	25

Note. This table summarizes the study selection process according to PRISMA 2020.

3.4 Data Extraction and Analysis

A thorough extraction of relevant data from included studies was performed, considering variables such as geography, sample size, age demographics, cytokine types, sample nature, and quantification techniques like ELISA, flow cytometry, Luminex, and qRT-PCR. The number of simultaneously measured cytokines in multiplex studies was highlighted for its significance in cytokine analysis. Due to methodological diversity, quantitative data were not aggregated; instead, a narrative synthesis was utilized, concentrating on methodological trends, assay frequency, and cytokine panel diversity. A narrative synthesis approach was chosen due to heterogeneity in assay types and study designs, precluding quantitative pooling.

4.0 Findings

4.1 Frequently Assessed Cytokines and Their Clinical Relevance

Among the 25 studies subjected to review, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) were consistently recognized as the most frequently evaluated pro-inflammatory cytokines. These markers were commonly elevated in individuals with β-TM, showing a notable correlation with increased serum ferritin levels and transfusion requirements, suggesting their role as indicators of disease severity and systemic inflammation.

In contrast, anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) were less frequently studied. These showed inconsistent expression patterns, which may reflect variability in their regulatory roles amid chronic immune activation in β-TM.

4.2 Predominant Cytokine Detection Techniques

The enzyme-linked immunosorbent assay (ELISA) was the most widely utilized method, referenced in over two-thirds of the included studies. ELISA's widespread use can be attributed to its affordability, accessibility, and specificity for single-analyte detection. However, its use often restricted cytokine panels to only two or three markers, limiting the depth of immune profiling.

In contrast, advanced platforms such as Luminex multiplex assays and flow cytometry were used in a smaller subset of more recent, technologically equipped studies. These methods enable simultaneous quantification of over 10 to 20 cytokines using minimal sample volumes. Nonetheless, many of these studies still limited their panels to fewer than five cytokines, indicating underutilization of the full capabilities of these high-throughput systems. A detailed summary of the most frequently assessed cytokines and their corresponding detection methods in β-TM studies is presented in Table 3.

Table 3: Summary of Frequently Assessed Cytokines and Detection Methods in β-TM Studies

Cytokine	Inflammatory Role	Common Detection Method(s)	Associated Findings / Clinical Relevance

IL-6	Pro-inflammatory	ELISA / Luminex	Elevated; correlates with ferritin, overload, transfusion frequency
TNF-α	Pro-inflammatory	ELISA / Luminex	Systemic inflammation; immune activation
IL-10	Anti-inflammatory	ELISA / Luminex / qRT-PCR	Variable patterns; compensatory regulation
TGF-β	Anti-inflammatory / Fibrotic	ELISA / Luminex	Fibrosis; immune modulation
IL-8	Pro-inflammatory / Chemotactic	ELISA	Neutrophil activation; vascular inflammation
IL-33	Pro-inflammatory / Tissue damage	ELISA	Oxidative stress / injury marker
IL-17	Pro-inflammatory	ELISA	Th17 activation; chronic inflammation
IL-2	Regulatory / T-cell response	ELISA / qRT-PCR	Decreased; impaired immune activation

This table summarizes the cytokines most frequently quantified in β-thalassemia major (β-TM) studies, their immunological roles, commonly used detection platforms, and associated clinical or pathological relevance.

4.3 Trends and Limitations in Cytokine Profiling

A recurring limitation across the reviewed studies was the lack of methodological standardization. Variations in assay platforms, cytokine targets, handling procedures, and data presentation formats often hindered direct comparisons between studies. Only a limited number of studies included healthy control groups or stratified their findings based on transfusion frequency or iron overload, further constraining clinical interpretation.

Geographically, the majority of studies were concentrated in Middle Eastern and Southeast Asian populations, consistent with the regional epidemiology of β-TM. However, this concentration also emphasizes the need for broader representation from high-prevalence but underreported regions, such as Africa and South Asia.

4.4 Summary of Methodological Preferences

While ELISA continues to dominate as the primary method for cytokine analysis in β-TM studies, its limited capacity for multiplexing restricts comprehensive immune profiling. The increasing adoption of Luminex and flow cytometry in more recent research indicates a trend towards high-throughput, integrative approaches. Nevertheless, challenges remain in terms of cost, technical expertise, and assay standardization. These findings underscore the importance of expanding cytokine panels and harmonizing detection protocols to improve comparability and clinical relevance in future β-TM studies.

5.0 Discussion

This review underscores the persistent phenomenon of cytokine dysregulation in β-TM, with particular emphasis on the heightened expression of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These cytokines have consistently been identified across the analyzed studies as significant inflammatory biomarkers and were positively associated with clinical parameters indicative of disease severity, such as iron overload and the frequency of transfusions (Hagag et al., 2022; Zhang et al., 2022). In contrast, anti-inflammatory cytokines like interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) were investigated in a lesser number of studies and demonstrated inconsistent expression patterns, indicating variable regulatory responses to chronic immune activation (Ansari et al., 2020; Akcay et al., 2019).

From a methodological standpoint, enzyme-linked immunosorbent assay (ELISA) emerged as the predominant platform for cytokine quantification in β-TM investigations due to its economic viability, user-friendliness, and specificity for single-analyte detection. Nonetheless, its constrained multiplexing capabilities and reliance on relatively substantial sample volumes limit its analytical scope and clinical applicability (Zhang et al., 2022). Multiplex assays, including Luminex and sophisticated techniques such as flow cytometry, provide the capacity to concurrently analyze over 20 cytokines, thus facilitating a more comprehensive immune profile (Caprari et al., 2023). Despite these advantages, such methodologies remain underutilized owing to their technical and financial requirements, the necessity for standardized protocols, and restricted access in resource-constrained environments (Mahmoud et al., 2022).

Although gene expression analysis methodologies, such as quantitative reverse transcription polymerase chain reaction (qRT-PCR), yield complementary insights at the transcriptional level, they should not be regarded as substitutes for protein-level quantification. These techniques function as auxiliary approaches to assess upstream signaling processes and the transcriptional dynamics of cytokines (Zhou et al., 2020). The integration of both gene-level and protein-based methodologies could afford a more nuanced comprehension of cytokine dynamics and immune dysfunction in β-TM.

Numerous methodological challenges were identified throughout the reviewed studies, including a dearth of standardized assay protocols, variations in sample types (serum versus plasma), discrepancies in cytokine panels, and inconsistent reporting practices. These factors compromise the reproducibility and comparability of findings. Furthermore, the limited employment of control groups and insufficient stratification based on clinical parameters further obstructed interpretability. Geographically, the majority of investigations were concentrated in Middle Eastern and Southeast Asian populations, with minimal representation from high-prevalence regions such as Africa and South Asia (Caprari et al., 2023). This highlights the imperative for broader geographic inclusivity in forthcoming research.

To mitigate these challenges, a multifaceted approach is essential. First, the adoption of harmonized assay protocols for sample handling, calibration, and cytokine reporting is critical. Second, subsequent research endeavors should utilize high-throughput, multiplex-capable platforms and broaden the spectrum of cytokines evaluated beyond IL-6 and TNF-α to encompass a more extensive immunological profile. Third, the incorporation of appropriate control groups and stratified analyses grounded in disease severity and treatment history will enhance clinical applicability. Finally, augmenting the geographic scope of studies will fortify global generalizability and present a more holistic perspective on immune dysregulation in β-TM.

6.0 Conclusion and Recommendations

This review highlights the crucial importance of cytokine profiling in understanding immune dysregulation in β-TM. Elevated levels of IL-6 and TNF-α, and to a lesser extent IL-10, indicate inflammatory and regulatory issues related to disease severity and transfusion needs. Nonetheless, the clinical applicability of these findings is limited by methodological inconsistencies in the literature.

ELISA is prevalent in cytokine studies in β-TM for its simplicity and cost-effectiveness, although its shortcomings in multiplexing and analytical depth are becoming more apparent (Zhang et al., 2022). Advanced techniques such as Luminex, flow cytometry, and qRT-PCR provide greater analytical capabilities but are often underutilized due to challenges in infrastructure and standardization (Caprari et al., 2023; Mahmoud et al., 2022).

To progress, it is vital to bridge methodological gaps through standardized protocols and enhanced technological integration. Standardization in assay execution and a broader range of cytokines will improve reproducibility and clinical relevance. Furthermore, increasing geographic diversity and implementing stratified analyses will enhance the applicability of results.

With these advancements, cytokine profiling could transition from a descriptive research method to a practical aspect of personalized medicine in the management of β-TM.

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Paper Contribution to Related Field of Study

This manuscript delivers a comprehensive overview of the most prevalent laboratory techniques employed to identify cytokines in β-TM(β-TM) patients. It aids researchers and healthcare practitioners in discerning which methodologies are most efficacious, as well as elucidating their advantages and limitations. The review further underscores the necessity for enhanced, more standardized techniques in forthcoming investigations to augment patient surveillance and therapeutic strategizing.

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