IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Dysregulated Expression of miR-222 and miR-15a in Transfusion-Dependent Thalassemia: Associations with Torque Teno Virus and Cytomegalovirus Infections

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Received: 21, May, 2024
Accepted: 09, Aug, 2025

ABSTRACT

Background: Beta-thalassemia is a hereditary blood disorder characterized by reduced synthesis of the beta-globin chain. MicroRNAs (miRs) are small RNA molecules that regulate gene expression and have been implicated in beta-thalassemia. To explore dysregulated miR-222 and miR-15a expression in transfusion-dependent beta-thalassemia and assess their potential associations with Torque Teno Virus and cytomegalovirus infections.

Materials and Methods: This study included 57 TDT patients registered at the Thalassemia Clinic affiliated with the Hematology Research Center, Shiraz, Iran. The expression levels of miR-222 and miR-15a were analyzed using the real-time SYBR Green PCR method. TTV and CMV infections were detected by analyzing the presence of their genomic DNA using an in-house semi-nested PCR protocol.

Results: The expression level of miR-222 was significantly up-regulated (47.5-fold, P≤0.001) in TDT patients compared to healthy controls. However, the expression of miR-15a in TDT patients was slightly decreased compared to healthy controls, but the difference was not statistically significant (P=0.193). TTV infection was observed in 21.1% of TDT patients, while CMV infection was detected in 5.2% of the patients. Although miR-222 and miR-15a gene expression levels were higher in TTV-positive patients compared to TTV-negative patients, the differences were not statistically significant (P=0.926 and P=0.243, respectively).

Conclusion: MiR-222 was up-regulated in TDT patients, but miR-15a did not show a significant difference. TTV and CMV infections were detected, but their association with miR expression was not significant, possibly due to the small sample size. Larger studies are needed for a more comprehensive evaluation.

Keywords: Transfusion-dependent beta-thalassemia; MicroRNA; Expression levels; Viral infection prevalence; miRNA

INTRODUCTION

Beta-thalassemia is a diverse group of inherited blood diseases with more than 300 different mutations affecting the beta-globin gene. This results in short survival of red blood cells, ineffective erythropoiesis, and chronic anemia[1]. Beta-thalassemia is more prevalent in the Mediterranean region, Southeast Asia, the Indian subcontinent, and the Middle East[1]. In comparison with normal subjects, patients with beta-thalassemia demonstrate marked hyperplasia of the erythroid, and in these patients, the number of

DOI:

orthochromatic erythroblasts is less than the number of polychromatophilic erythroblasts [2].

MicroRNAs (MiRs) are small, non-coding RNAs containing 19-25 nucleotides utilized in the prognosis and diagnosis of many diseases. These specific RNAs are the primary gene expression regulators that normalize cellular proliferation, differentiation, and apoptosis[3-5]. MicroRNAs (miRNAs) were first discovered to play a significant role in erythropoiesis and hematopoiesis in 2004 and 2005 [6, 7]. These small RNA molecules have been found to regulate the expression of globin genes and participate in the differentiation of erythroid cells. In the context of beta-thalassemia, a genetic blood disorder, alterations in miRNA expression have been observed, including the upregulation of miR-181 and downregulation of miR-222 [8, 9].

MiR-222 has been identified as having functional relevance in erythropoiesis, specifically through its interaction with the c-kit protein. It has been shown to decrease erythroblast proliferation and hemoglobin F (HbF) levels while also playing a role in the regulation of human perinatal hemoglobin switching[10-13]. Interestingly, the expression levels of miR-222 were significantly lower in beta-thalassemia patients compared to individuals without the disorder [9]. By increasing the levels of the KIT protein, miR-222 promotes the differentiation of erythropoietic cells while reducing the proliferation of CD34+ precursor cells[14]. It is important to note that miR-222-3p has been identified as functioning as an oncogene in certain types of tumors and as a tumor suppressor in others, indicating its context-dependent role in cancer development, progression, metastasis, and drug resistance[15]. Furthermore, miR-222 likely plays a regulatory role in the terminal stages of hematopoietic differentiation[16]. These findings highlight the complex and multifaceted functions of miRNAs in the intricate processes of erythropoiesis and hematopoiesis.

MiR15a, via transcription factors, does posttranscriptional regulation and modulates gene expression. For instance, through transcription factor MYB, causes an increase in γ globin gene expression [17] and consequently fetal hemoglobin (HbF) production[18]. It was reported that hsa-microRNA-15a-5p was downregulated in pediatric β-thalassemia patients[17].

MiR-15a is a microRNA that exerts posttranscriptional regulation and plays a role in modulating gene expression. Specifically, it has been found to interact with transcription factors, such as MYB, to regulate gene expression. Through its interaction with MYB, miR-15a promotes an increase in the expression of the γ globin gene, leading to an increase in fetal hemoglobin (HbF) production [17, 18]. In pediatric β-thalassemia patients, the expression of hsa-microRNA-15a-5p is downregulated, indicating a potential disruption in the regulatory role of miR-15a in these patients' γ globin gene expression and HbF production. Further research is needed to fully understand the implications and therapeutic possibilities of miR-15a dysregulation in β-thalassemia.

Torque Teno Virus (TTV) is a non-enveloped, circular, single-stranded DNA virus with negative polarity. Initially, it was believed to be associated with viral hepatitis infections. However, extensive research has shown that TTV is present in approximately 50% of healthy individuals[19, 20]. In thalassemia patients, the prevalence of TTV varies, with higher rates observed in transfusion-dependent individuals[22]. The significance of TTV in TDT patients lies in its high prevalence, potential role as a marker of immune status, and possible contribution to transfusion-related complications or disease pathogenesis. Understanding the role of TTV in TDT could lead to improved monitoring, risk stratification, and therapeutic strategies for this vulnerable patient population. Studies have reported different prevalence rates of TTV among thalassemia patients, ranging from 64.4% to 39.4% [21, 22]. Cytomegalovirus (CMV) belongs to the Herpesviridae family and can be transmitted among humans through various means, including hematopoietic or stem cell transplantation [23]. In healthy individuals, primary CMV infection typically presents as an asymptomatic or self-limiting mononucleosis-like fever. However, immunocompromised patients may experience severe CMV infections[26]. The prevalence of CMV in developing countries ranges from 85% to 100% [27]. In the beta-thalassemia population, studies have

shown that there is an adequate immune response to CMV, as indicated by negative viral DNA detection and high IgG avidity [24].

In this study, our aim was to investigate the expression levels of MiR-222 and MiR-15a in transfusion-dependent beta-thalassemia (TDT) patients compared to healthy individuals. Additionally, we sought to determine the frequency of Torque Teno Virus (TTV) and Human Cytomegalovirus (CMV) infections in TDT patients and explore any potential association with the expression of MiR-222 and MiR-15a. Dysregulation of MiR-222 and MiR-15a has been linked to cancers, hematological disorders, and thalassemia. Investigating their expression in transfusion-dependent beta-thalassemia (TDT) patients provides insights into the molecular mechanisms of thalassemia pathology. Additionally, exploring TTV and CMV infections in TDT patients is crucial due to their impact on health and management. Understanding the association of these infections with MiR-222 and MiR-15a expression could guide therapeutic strategies, contribute to diagnostic markers, and improve clinical outcomes in TDT.

MATERIALS AND METHODS

Patients

In this cross-sectional study, we recruited 57 registered patients with transfusion-dependent beta-thalassemia (TDT) at a single thalassemia clinic affiliated with the Hematology Research Center of Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. The recruitment was done using a convenience sampling method from January to June 2017. All patients who tested positive for thalassemia were included, and no exclusions were made. Additionally, a control group of 50 healthy individuals was included in the study. It is important to note that recruiting a larger number of TDT patients was not feasible due to the rarity of the condition in adults[25].

The study was conducted following the approval of SUMS' ethics committee (IR.SUMS.REC.1397.652 - grant number=1396-01-32-15455), and informed consent was obtained from the patients or their legal parents (Ethical considerations, such as obtaining informed consent, and practical issues, such as patient willingness to participate, may have limited the number of participants).

Inclusion criteria include patients with a confirmed diagnosis of TDT requiring regular blood transfusions (every 2–4 weeks), age between 5 and 50 years, regular transfusion history for at least 2 years, clinically stable at the time of enrollment (no acute infections or complications) and willingness to provide informed consent.

Exclusion Criteria include patients with non-TDT (thalassemia intermedia or minor), significant comorbidities (HIV, hepatitis B or C, active malignancies), recent acute infections or hospitalizations (within the past 3 months), pregnant or lactating women

Incomplete medical records or insufficient clinical data.

Sample collection and ribonucleic acid isolation

Five ml peripheral blood was collected in Ethylenediaminetetraacetic acid (EDTA)-containing tubes from each patient. The peripheral blood mononuclear cells that play a part in response[326]. viral infections, were obtained from each patient using Ficoll-hypaque density gradient centrifugation. Total RNA was extracted by RNX-Plus solution (CinnaGen, Tehran, Iran). The quantity of the extracted RNA was evaluated by Nanodrop (Thermo Fisher Scientific, USA). Total RNA was converted into cDNA using Prime Script RT Reagent Kit (Takara, Japan) according to the manufacturer's instruction in the T100 thermocycler (Bio-Rad Laboratories, USA) by specific stem-looped designed primers for each MiR[27-29].

SYBR green real-time polymerase chain reaction

For the quantitative analysis of MiR-222 and MiR-15a mRNAs expression level, the SYBR Green Real-Time polymerase chain reaction (PCR) method was performed using SYBR® Premix Ex Taq ™ II (TliRNaseH Plus) (Takara, Japan) and designed primers specific for each miRNA in an iQ5thermocycler (BioRad Laboratories, USA). U6 gene was used as an internal control. The Real-Time PCR reaction program and primer sequences and controls are summarized in table 1. Melt curve analysis was performed to confirm the specificity of

the reaction at the end of the program. The expression level of the MiR-222 and MiR-15a band and MiR-15a were calculated by [2-ΔΔCt] method, where ΔΔCt = [ΔCt (patient) - ΔCt (control)] and ΔCt dicer mRNAs was normalized to the U6 gene. The changes in the relative expression levels of MiR-222 = [Ct (sample) –Ct (housekeeping gene)]. All real-time PCR was performed in duplicate wells.

Table 1: Real-Time PCR reaction program and primer sequence for RT-PCR

Gene	Primer sequences (5'->3')	Thermocycling condition
MiR-15a	**Forward:** ATCCTGTAGCAGCACAUAATGG	94°C/2 min, 40
miR-15a Stem loop (SL)	**Reverse:** GTGCAGGGTCCGAGGT GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAG CCAACCACAAA	cycles of 94°C/30 sec, 56.5°C/20 sec and 70°C/30 sec
MiR-222	**Forward:** GCATGTCATCACTCAGTAGCCAGTGTA	94°C/2 min, 40
miR-222 Stem loop (SL)	**Reverse:** CCAGTGCAGGGTCCGAGGTA GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGAT ACGAC	cycles of 95°C/30 sec, 57.5°C/20 sec and 70°C/30 sec
U6	**Forward:** CTCGCTTCGGCAGCACA	95°C/2 min, 40
	Reverse: AACGCTTCACGAATTTGCGT	cycles of 95°C/30 sec, 57.5°C/20 sec

Molecular detection of TTV infection and cytomegalovirus

The TTV infection and CMV were analyzed based PCR method. Briefly, the TTV and CMV genomic DNA was extracted from blood using a dinitrophenol (DNP) kit (Cinna Gen Inc., Tehran, Iran) according to manufacturer instruction. TTV and CMV genomic DNA presentation was analyzed in beta-thalassemia patients using an in-house semi-nested-PCR protocol, as previously described [30-32].

Statistical analysis

Data were analyzed by SPSS software version 18. The Kolmogorov-Smirnov test checked normality of data. Descriptive data were expressed as mean, standard deviation, and appropriate charts. Quantitative variables were compared by Student's t-test between two groups of patients. P-value less than 0.05 was considered statistically significant.

RESULTS

The mean age of the patients was 28±1.4 years (5-55 year) and 47.4% were female. The laboratory characteristics of patients with Thalassemia displayed that the mean WBC count at diagnosis was 50341±80241/cm. The average platelet count was 61228±4351/mL and the mean Hb and LDH levels in these patients were 7.3±.13 g/dL and 1798±154 U/L, respectively. Markers were compared against normal ranges.

Changes in MiR-222 and MiR-15a expression amongst beta-thalassemia patients

The mRNA expression of MiR-222 and MiR-15a was measured in TDT patients (Figure 1). The expression of MiR-222 was significantly higher (47.5-fold) in thalassemia patients compared to healthy controls (-0.27 ± 5.9 *vs.* 5.3 ± 4.1, $P<0.001$). Expression of MiR-15a was not significantly different in these patients than in healthy controls (3.2 ± 4.1 *vs.* 1.8 ± 6.08, $P=0.193$).

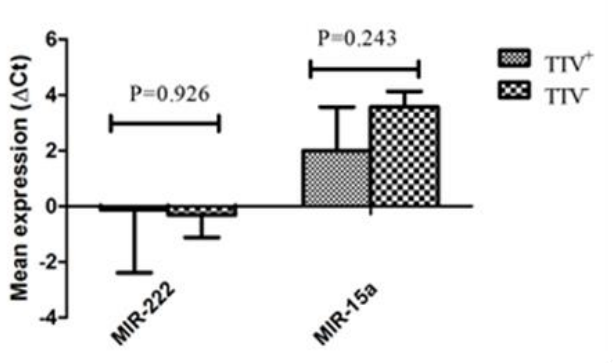

Figure 1. Change in MiR-222 and MiR-15A expression in transfusion-dependent thalassemia patients and Control

Association of MiR-222 and MiR-15a expression with TTV infection in patients

TTV was detected in 12 of 57 (21.1%) TDT patients. According To the TTV infection status, the mean expression of MiR-222 and MiR-15a was compared in patients (Figure 2). MiR-222 and MiR-15a gene expression levels were higher in TTV^+ patients compared to TTV^- patients, albeit the difference was not statistically significant (-0.13±7.8 *vs*. -0.31±5.4; *P*=0.926, for MiR-222 and 1.9±5.4 *vs.* 3.5±3.7; *P*=0.243, for MiR-15a).

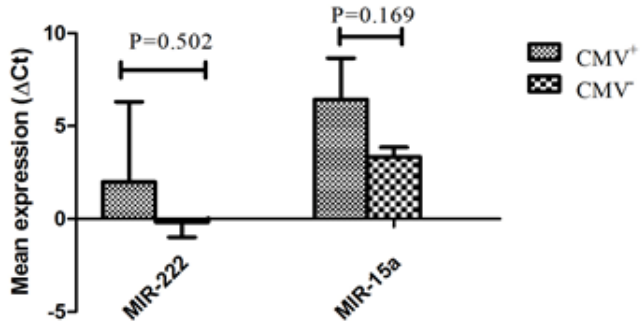

Figure 2. The relation Change in MiR-222 and MiR-15a expression with TTV infection in transfusion-dependent thalassemia patients

Association of MiR-222 and MiR-15a expression with CMV infection in patients

CMV infection was detected in 3 of 57 (5.2%) TDT patients. Figure 3 shows the baseline expression levels of MiR-222 and MiR-15a in TDT patients according to the CMV infection status. Our results demonstrated that MiR-222 and MiR-15a expression levels were less in CMV^+ patients compared to CMV^- patients, albeit the difference was not statistically significant (1.9±7.4 *vs.* -0.4±5.9; *P*=0.502, for MiR -222), (6.4±3.8 *vs*. 3.06±4.09; *P*=0.169, for MiR-15a).

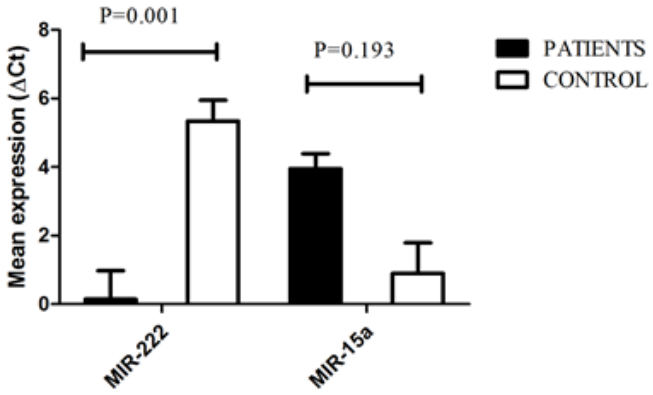

Figure 3. The relation Change in MiR-222 and MiR-15A expression with CMV infection in transfusion-dependent thalassemia patients

DISCUSSION

In this study, expression levels of MiR-222 and MiR-15a in 57 TDT patients were evaluated. In comparison with the healthy controls, the expression of MiR-222 was significantly higher (47.5-fold). MiR-15a expression level showed no significant difference between TDT patients and controls.

In TDT patients, miR-15a levels are often altered compared to healthy individuals, reflecting the impact of disease on miRNA profiles[33]. Studies have shown that TDT patients have higher levels of particular miRNAs, including miR-144-3p, which may be connected to the expression of miR-15a and its functions in regulating erythropoiesis[34]. Dysregulation of miR-15a can impact hematopoiesis-related pathways, which may result in TDT-related complications like organ dysfunction and elevated apoptosis in different cell types [34, 35]. The presence of altered miRNA profiles, including miR-15a, may also influence the response to treatments like hydroxyurea, which aims to increase gamma-globin expression[36]. Conversely, although miR-15a has been related to the pathophysiology of β-thal, more research is needed to fully understand

the complexity of miRNA interactions in this disorder, as well as its precise function and potential for therapeutic targeting. TDT patients experience chronic anemia, iron overload, and oxidative stress, which may trigger compensatory mechanisms to maintain cellular homeostasis. Other microRNAs or regulatory pathways that offset variations in miR-15a expression may be involved in these mechanisms[33, 36, 37]. Epigenetic changes, such as DNA methylation or histone modifications, could influence miR-15a expression in TDT patients. For example, hypermethylation of the miR-15a promoter region might suppress its expression, while other regulatory mechanisms could counteract this effect[38, 39].

Moreover, the expression of both MiR-222 and MiR-15a was higher in TTV+ patients than TTV- patients; however, the difference was not statistically significant miR-222 has been implicated in various viral infections, often playing a role in modulating viral replication or the host immune response. A study by Murakami *et al*. found that miR-222 is upregulated in HCV-infected liver tissues and contributes to viral replication by targeting the cyclin-dependent kinase inhibitor p27. This suggests that miR-222 can play a direct role in viral pathogenesis[40].Peta *et al*., reported that miR-222 is overexpressed in HPV-associated cervical cancer and promotes cell proliferation by targeting the tumor suppressor p53. This highlights the context-dependent roles of miR-222 in viral infections and cancer[41].

miR-15a is known to regulate cell cycle, apoptosis, and immune responses, but its role in viral infections is less well-studied.

The absence of a correlation between miR-15a and TTV/CMV infection status in TDT patients might indicate that miR-15a is not a key regulator of these infections or that its expression is influenced by other factors, such as the underlying thalassemia pathology.

The observation that miR-222 and miR-15a expression levels were lower in CMV+ TDT patients compared to CMV- patients, albeit not statistically significant, is an intriguing finding. In HBV-associated hepatocellular carcinoma, miR-15a was downregulated and played a tumor-suppressive role by targeting BCL2. While this study did not focus on CMV, it highlights the context-dependent regulation of miR-15a[41]. In HIV-1 infection, miR-15a was involved in regulating viral replication. However, its role in CMV infection remains unclear, and the lack of significant changes in your study suggests that miR-15a might not be a key regulator of CMV pathogenesis[42]. CMV infections in TDT patients might be latent or present at low viral loads, leading to minimal changes in host miRNA expression. Active CMV infections are more likely to induce significant changes in miRNA profiles, as seen in studies involving HUVECs[43]. The authors can thoroughly examine the function of miR-15a in TDT by integrating in vitro and in vivo functional studies, target gene expression analysis, extensive transcriptomics, and longitudinal patient monitoring. This multi-faceted approach will help clarify whether miR-15a contributes to disease pathology despite unchanged expression levels and may identify novel therapeutic avenues. Transcription factors or co-activators that bind to the miR-15a promoter region might counteract epigenetic silencing by recruiting chromatin remodelers or histone acetyltransferases (HATs) to activate transcription[44]. miR-15a can regulate factors that influence its own expression through feedback loops involving cell cycle regulators (CDK4, Cyclin D1) and apoptotic pathways (BCL2), thereby stabilizing its levels and functional effects in cells[45, 46]. RBPs could stabilize miR-15a precursors or mature forms, ensuring consistent expression levels even if transcription is partially suppressed[47]. RNA editing or alternative splicing of miR-15a precursors might produce functional miRNAs despite transcriptional repression[48]. miR-15a expression might be regulated differently in various tissues or under different physiological conditions. In TDT, the bone marrow microenvironment or stress responses associated with chronic anemia might create unique regulatory conditions that maintain miR-15a levels[49]. Other miRNAs with overlapping functions might compensate for changes in miR-15a expression. For instance, to preserve regulatory balance, miR-16, which shares targets with miR-15a, may be upregulated[50]. Other miRNAs or regulatory mechanisms might compensate for changes in miR-

222 or miR-15a expression, masking any significant differences. For instance, miR-155, a well-known immune-regulatory miRNA, might play a more dominant role in the response to CMV infection[51]. Ferreira *et al.,* showed that MiR-24, 221, 222, and 223 downregulated in beta-thalassemia patients [9]. It was in contrast with our result that the expression of MiR-222 was significantly higher (47.5-fold, *P*<0.001) in patients compared to healthy controls. The patient populations in the two studies may have differed in terms of their age, sex, severity of disease, and other factors. These differences could have affected the expression levels of MiR-222. Also differences in sample sizes (9 TDT in their study and 57 in ours) have provided more power to detect a significant difference in miR-222 expression between patients and controls. This discrepancy may also be due to different methods of measuring miR-222 expression, quality of the RNA samples, the efficiency of the reverse transcription reaction, and the specificity of the primers and probes used for qRT-PCR.

Felli *et al.* described, in β-thalassemia, miR-221 and miR-222 were found to be upregulated in erythroid precursors, where they modulate erythropoiesis by targeting the stem cell factor receptor (c-KIT). This supports the idea that miR-222 plays a role in erythroid differentiation and anemia-related pathologies[10]. The inhibitory effect of specific MiRs (miR-16-1/15a/-26b/-23a/-451/-27a) during expression of the β globin genes, can have a potential effect for upregulation of fetal hemoglobin production and γ globin gene expression[13, 52.]

Other studies demonstrated that miR-222 is upregulated in response to iron overload and oxidative stress in sickle cell disease. Given that TDT patients often experience iron overload due to frequent transfusions, this could explain the significant upregulation of miR-222 in your study[53]. In acute myeloid leukemia (AML), miR-222 was found to be upregulated and associated with poor prognosis. This further supports the idea that miR-222 plays a role in dysregulated hematopoiesis[55]. However, our results revealed that no significant difference in the expression levels of MiR-15a between TDT patients and controls.

Cimmino *et al.* demonstrated that miR-15a and miR-16-1 are downregulated in chronic lymphocytic leukemia (CLL) and play a tumor-suppressive role by targeting the anti-apoptotic protein BCL2. However, in TDT, miR-15a expression was not significantly altered, suggesting that its regulation might be context-dependent[54]. Felli et al. found that miR-15a regulates erythroid differentiation and apoptosis. However, the study did not report significant changes in miR-15a expression in thalassemia patients compared to controls, which aligns with your findings[10.] One possible explanation for this finding is that miR-15a may be dysregulated in TDT patients in a different way than in other diseases where it has been shown to be involved in fetal hemoglobin production. For example, it is possible that miR-15a is expressed at normal levels in TDT patients, but that it is not able to function properly due to other changes in the gene expression profile of these cells[13]. Another possibility is that miR-15a is not a major player in regulating fetal hemoglobin production in TDT patients[55].Sangokoya *et al.* reported that modulating oxidative stress responses. However, the study did not report significant changes in miR-15a expression in response to iron overload, which might explain the lack of significant differences in your study[53].

Other microRNAs, such as miR-16-1/-26b/-23a/-451/-27a, may be more important in regulating this process in TDT. It is also possible that the sample size of our study was too small to detect a significant difference in miR-15a expression between TDT patients and controls. A larger study would be needed to confirm or refute this finding.

Beta-thalassemia patients are prone to various transfusion-transmitted infections (TTIs) such as CMV and HBV[56]. Frequency of CMV infection in thalassemia patients in our study (5.2%) was similar to many other reports from Iran [57-59]. Choobineh *et al.,* reported a higher prevalence of 12.9 % of CMV IgM antibody, in a group of Iranian thalassemia patients less than 15 years old [60]. Moreover, Aghaeipour *et al.* determined a prevalence of 9.1 % IgM antibodies seropositivity for CMV in thalassemia patients which was higher than our reported frequency in thalassemia patients[61]. We did not observe any significant difference in the

expression levels of MiR-222 and MiR-15a between CMV positive patients and CMV negative patients. However, it should be noted that the number of CMV-positive patients in our study was too small to interpret the results accurately.

TDT patients frequently have comorbidities such as iron overload, prolonged transfusions, and other conditions that affect their immune systems. These factors might affect the expression of microRNA and its association to viral infections[62]. The relationship between the measurement of microRNA expression and the timing of TTV or CMV infection may also be significant. Depending on how recent or latent the infection was, microRNA expression may be impacted differently[63]. The regulation of miR-222 and miR-15a might be influenced by factors other than viral infections, such as oxidative stress, inflammation, or iron overload, which are common in TDT patients[64]. The study population might be heterogeneous in terms of age, transfusion history, chelation therapy, or other clinical parameters. This heterogeneity could contribute to variability in microRNA expression, making it harder to detect a significant correlation[62].

In the present study, the frequency of TTV positive in TDT patients was 21.1% which was less than the report of Zandieh *et al.,* with a prevalence of 57.2% in beta-thalassemia patients and 20% in healthy individuals in Khuzestan province, located in south-eastern Iran (P<0.0001). TTV infection prevalence among Mazandaran Province was more similar to our result (26.8%)[65]. Several investigations showed that TTV was highly present in beta-thalassemia patients in Italy population (73% present in children with beta-thalassemia and 69.1% present in the adult with beta-thalassemia)[66, 67] and 61% in the Turkey population[68]. Hassuna *et al* detected a prevalence of 60% in TTV positive children with thalassemia but 57% in healthy children[69]. We did not determine the frequency of TTV and CMV infection in healthy controls, but for comparison, we cited the reported prevalence in healthy individuals in the literature. The slight increase in miR-15a expression in TTV+ TDT patients might reflect a mild host response to TTV, but the lack of statistical significance suggests that this response is not robust. TTV is a ubiquitous virus with unclear pathogenicity, and its interaction with host miRNAs is poorly understood. Few studies have investigated miRNA regulation in TTV infection, making it difficult to draw direct comparisons[70] . Both TTV and CMV have the ability to cause latent infections, which are characterized by low levels of immune responses and viral activity. If the majority of infected patients in the study had latent rather than active infections, this could explain the lack of significant changes in microRNA expression[71, 72]. Immune-related miRNA responses are frequently restricted to infected or inflammatory tissues rather than circulating blood, and TTV and CMV miRNAs show tissue-specific expression patterns. MiRNA levels in blood may not be correlated with viral activity or immune responses that primarily occur in other tissues, like the liver or spleen, due to tissue specificity[72-74]. the threshold effect of viral load or immune activation is critical in determining whether miRNA expression is significantly altered during TTV or CMV infection[75, 76].

The results of this study have important implications for the understanding and management of TDT. The significant upregulation of miR-222, despite contrasting findings with previous studies, suggests its potential role in the pathophysiology of TDT. The lack of significant difference in miR-15a expression in TDT patients indicates a different regulatory mechanism or a minor role in fetal hemoglobin production compared to other diseases. The identification of CMV and TTV infections highlight the importance of monitoring and managing these infections in TDT patients. These findings provide insights into potential diagnostic biomarkers, therapeutic targets, and interventions to improve treatment outcomes in TDT. However, further research, including larger studies, is warranted to validate these findings, explore the functional significance of dysregulated miRNAs, and understand the clinical implications of TTIs in TDT patients.

LIMITATIONS

The limitations of our study include the volume and freshness of the collected samples, as well as constraints related to the research budget.

Additionally, we explicitly acknowledge the lack of data on splenectomy status and ferritin levels, which were not evaluated in this study. Future studies should focus on larger, multi-center cohorts and incorporate more comprehensive analyses to validate the findings. Functional assays, cross-disease comparisons, integration with Multi-Omics Data, more thorough immune profiling, and viral load quantification may all help determine the roles of miR-222 and miR-15a in TDT pathology. Additionally, recommending specific experimental approaches would guide further study and support the development of TDT biomarkers or targeted therapies.

CONCLUSION

In this study, we determined the up-regulation of only miR-222 in TDT patients compared to controls. The frequency of TTV infection was approximately less than the reported prevalence in other parts of the world. The absence of a significant relationship between the expression levels of the evaluated MiRs and infections might be due to the small sample size in subgroups. Further, more extensive studies are suggested for more accurate evaluation.

FUNDING

The study was supported by a grant from Shiraz University of Medical Sciences, Shiraz, Iran.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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