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# Prevalence and Severity of Coronavirus Disease 2019 (COVID-19) in Transfusion Dependent and Non-Transfusion Dependent β -Thalassemia Patients and effects of Associated Comorbidities: An Iranian Nationwide study

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**Summary.** *Background:* Coronavirus disease 2019 (COVID-19) outbreak is a global and challenging disease that is accompany with mortality and morbidity. *Aim of study:* We evaluated the prevalence and the impact of comorbidities in thalassemia Iranian patients affected by COVID-19. *Methods:* A multicenter, retrospective, cross-sectional study was conducted across all comprehensive thalassemia centers in Iran, from January to June 15th, 2020. *Results:* Forty-three confirmed COVID-19 thalassemia patients (32 TDT, and 11 NTDT) were detected. The mean age of patients was 35.3 ± 11.5 years (range 9 - 67); 21 females and 22 males. Overall, 78.1% of TDT and 90.9% of NTDT patients were complicated with at least one comorbidity (P: 0.656). The overall mortality rate of thalassemia patients with COVID-19 was 18.6% while 27.3% was in NTDT patients compared to 15.6% in TDT patients (P:0.401). The dead group had a non-significant higher frequency of endocrinopathies compared to the recovered group (62.5% versus 45.7% P:0.457). Ten female thalassemia patients with positive COVID-19 had hypogonadism, six patients were receiving hormone replacement therapy and all of them recovered (zero death) compared to two deaths from 4 patients who were not receiving hormone replacement therapy (P:0.133). Furthermore, the prevalence of COVID-19 in NTDT patients was significantly higher than the general population (45 per 10,000 versus 22.29 per 10,000 respectively, P:0.018) while the prevalence of TDT was almost similar to the normal population (P:0.539). The mortality rate of COVID-19 was 4.71% in the normal Iranian population compared to 18.6% in β-thalassemias (P: <0.001) at the same date. *Conclusions:* It is important to acknowledge that β-thalassemia patients, especially young adults/adults, have a chronic condition which may contribute to increase susceptibility to SARS-CoV-2 infection. A higher susceptibility to the infection was observed in patients with NTDT and in untreated hypogonadal female thalassemic patients. However, to confirm these data, more accurate designed studies are needed.

**Key words:** COVID-19, thalassemias, endocrine disorders, comorbidities, sex steroids

## Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic and challenging disease that is associated with mortality and morbidity. It can involve all people but immunocompromised patients, heart disease, diabetes and obesity are more prone to COVID-19 disease severity than general population. COVID-19 is a potentially severe acute respiratory tract infection with person-to-person transmission (1, 2).

The host predisposing factors significantly determine the illness course, the progression, and the outcome of COVID-19. A meta-analysis of eight studies including 46,248 patients with laboratory confirmed COVID- 19 indicated that those with the most severe disease were more likely to have hypertension (odds ratio 2.36; 95% confidence interval:1.46 to 3.83), respiratory disease (odds ratio: 2.46; 95% confidence interval: 1.76 to 3.44), and cardiovascular disease (odds ratio: 3.42, 95% confidence interval: 1.88 to 6.22) (2).

Patients with β-thalassemias are associated with pre-existing multiple organ damage due to iron overload that predispose them to develop severe form of COVID-19 disease (3) and to be at higher risk of mortality compared to normal general population affected by COVID-19, although its prevalence was recently reported non-significant lower than general population (4).

Several complications are associated with transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT), including chronic liver disease, endocrine complications, heart failure, osteoporosis, extramedullary hematopoiesis (EMH), leg ulcers, gallstones, and thrombophilia. One of the most serious complications in NTDT is pulmonary hypertension (PH) which can be found in 11–50% of patients and leads to heart failure; the most common cause of death in NTDT patients (5,6). The exact cause of this condition is unknown. However, anemia and iron overload (even without blood transfusion) have been proposed as the two main risk factors.

Furthermore, since SARS-CoV-2 virus gains entry to pulmonary cells through binding to membrane ACE2 receptors which are distributed widely in lung, intestine, kidney, and blood vessels, it is possible that increased ACE2 receptor expression in both type 1 and type 2 diabetes (e.g. by angiotensin receptor blockers (ARBs), angiotensin converting enzyme (ACE) inhibitors and non-steroidal anti- inflammatory drugs) may increase SARS-CoV-2 infectivity and illness severity.

Therefore, it is recommended that patients with diabetes maintain a good glycemic control, because it might help reduce the risk of infection itself and may also modulate the severity of the clinical expression of the disease. Moreover, it is advised to ensure adequate stock of medications and supplies for monitoring blood glucose during the period of home confinement (7).

Interestingly, sex differences in inflammation have also been well documented and attributed to various factors. Although most of the immune regulatory genes are encoded by X chromosomes - resulting in women's generally stronger immune response - this sex difference in inflammatory response is postulated to be largely driven by sex hormones (8).

The aim of our study was to evaluate the prevalence of COVID-19 in TDT and NTDT and the impact of associated comorbidities in Iranian thalassemia patients with COVID-19.

## Methods

A multicenter, retrospective, cross-sectional study was performed across all comprehensive thalassemia centers in Iran, from January to June 15th, 2020. All confirmed COVID-19 cases from a total of 15,950 TDT (regular transfusion every 2-4 weeks) and 2,400 NTDT patients registered by the Iranian Ministry of Health (MOH) were investigated.

The term NTDT was used to define a type of non transfusion-dependent thalassaemia, with mild genotype and clinical phenotype not requiring regular transfusions for survival.

Demographics, clinical and paraclinical characteristics including serum ferritin, fasting blood sugar, liver enzymes, hepatitis markers, calcium, phosphorus, vitamin D, thyroid function (FT4 and TSH), PTH, LH, FSH, testosterone, 17 -βestradiol, cortisol, bone mineral densitometry, as well as imaging, associated comorbidities and outcome data were collected analyzing patients' electronic medical records.

The diagnosis of endocrine complications and osteopenia/osteoporosis was based on the guidelines of the International Network on Endocrine Complications in Thalassemia (I-CET) (5).

PH was defined by a mean pulmonary artery pressure of 25 mmHg or higher at rest and 30 mmHg or higher during exercise.

The coronavirus test was performed based on the Berlin protocol by real-time RT-PCR (9).

The study was approved by medical ethics committee and consent form was taken from the patients or their legal guardians.

*Statistical analysis*

Data were analyzed by Statistical Package for the Social Science (SPSS) software version 23 (SPSS Inc., Chicago, Illinois, USA). Descriptive data were presented as mean, standard deviation, frequency, and percentage. Chi-square test and Fissure exact test were used to compare qualitative variables between the two groups. Quantitative variables were compared by Student t-test between the two groups. P value less than 0.05 was considered statistically significant.

## Results

Forty-three confirmed COVID-19 thalassemia patients (32 TDT, and 11 NTDT) were detected up to 15 June 2020. The mean age of patients was 35.3 ± 11.5 years (range from 9 to 67); 21 females and 22 males. Demographic and clinical characteristics of the patients are summarized in table 1. All demographic data, clinical characteristics, and hematologic parameters were comparable between TDT and NTDT patients affected by COVID-19 disease (P: >0.05).

Generally, the most common endocrine disorders in the studied population were osteoporosis (76.7%), hypogonadism (32.6%), and diabetes mellitus (DM: 25.6%). Overall, 78.1% of TDT and 90.9% of NTDT patients were complicated with at least one comorbidity (P: 0.656). The frequency of endocrine disorders consisting of DM, hypogonadism, hypothyroidism, hypoparathyroidism, and growth failure was comparable in both groups of TDT and NTDT patients, as well (46.9% in TDT versus 54.5% in NTDT; P: 0.736). The frequency of reported comorbidities was statistically similar in both groups of the patients except for heart failure (27.3% in NTDT versus 3.1% in TDT patients; P: 0.045). The overall mortality rate of thalassemia patients with COVID-19 was 18.6%. No statistically significant difference was found in NTDT patients (27.3%) compared to TDT patients (15.6%; P: 0.401).

Fourteen β-thalassemia patients with positive COVID-19 had hypogonadism (10 females and four males). In the female group, six patients were receiving hormone replacement therapy and all of them were recovered (zero death) compared to two deaths from 4 patients who were not receiving hormone replacement therapy (50%) (P: 0.133).

Comparison of clinical characteristics and laboratory data as well as the frequency of overall comorbidities and endocrinopathies between dead and recovered thalassemia cases with COVID-19 are illustrated in table 2. TDT /NTDT ratio did not significantly differ between dead and recovered patients (P: 0.401). All dead patients (100%) had comorbidities compared to 77.1% of the recovered patients; however, the difference was not statistically significant (P: 0.316). Similarly, the dead group had a non-significantly higher frequency of endocrinopathies compared to the recovered group (62.5% versus 45.7%; P: 0.457).

Moreover, in a separate analysis, none of the comorbidities showed statistically significant differences between dead and recovered patients (P: >0.05).

Distribution of the frequency of endocrine disorders and other comorbidities in recovered and dead COVID-19 cases with β-thalassemias are illustrated in figure 1. All endocrine disorders except hypogonadism had non-significantly higher frequency in the dead group in comparison with recovered patients (P:>0.05).

The prevalence of COVID-19 was 23.43 per 10,000 in patients with β-thalassemia and 22.29 per 10.000 in the general Iranian population up to June 15$^{th}$, 2020 (P:0.743). The prevalence of COVID-19 was 20 per 10,000 of TDT compared to 45 per 10,000 of NTDT patients, (P: 0.017). Furthermore, the prevalence of COVID-19 in NTDT patients was significantly higher than the general population (45 per 10,000 versus 22.29 per 10,000 respectively: P:0.018)

**Table 1.** Summary of clinical characteristics and laboratory data of confirmed COVID-19 cases in patients with β-thalassemias

| Disease type<br>Parameters | TDT (n=32) | NTDT (n=11) | P value | Total (n=43) |
|---|---|---|---|---|
| **Age** (year) - Mean ± SD | 33.4 ± 11.2 | 41.0 ± 11.0 | 0.061 | 35.3 ± 11.5 |
| **BMI** (kg/m$^2$) | 20.9 ± 2.2 | 22.5 ± 1.3 | 0.050 | 21.3 ± 2.1 |
| **Sex** (M/F) | 14/18 | 8/3 | 0.162 | 22/21 |
| **Splenectomy** (N and %) | 17 (53.1) | 8 (72.7) | 0.309 | 25 (58.1) |
| **Serum ferritin** (ng/ml) Mean ± SD | 2410 ± 3293 | 2064 ± 2805 | 0.758 | 2320 ± 3143 |
| **> 2000 (N %)** | 11 (35.5) | 2 (18.2) | 0.453 | 13 (31) |
| **Hemoglobin** (g/dl) Mean ± SD | 9.10 ± 1.04 | 9.10 ± 1.87 | 0.988 | 9.10 ± 1.30 |
| **WBC** (per μL) Mean ± SD | 12986 ± 8053 | 22790 ± 16828 | 0.090 | 15901 ± 12014 |
| **Platelet count** (per μL), Mean ± SD | 489961 ± 257141 | 650500 ± 194855 | 0.084 | 534555 ± 249622 |
| **Severe vitamin D deficiency** (<10 ng/ml) (N and %) | 5 (15.6) | 0 | 0.306 | 5 (11.6) |
| **Iron overload** (moderate or severe based on LIC) (N and %) | 12 (37.5) | 4 (36.4) | >0.999 | 16 (37.2) |
| **Presence of comorbidities** (N and %) | 25 (78.1) | 10 (90.9) | 0.656 | 35 (81.4) |
| Osteoporosis | 23 (71.9) | 10 (90.9) | 0.409 | 33 (76.7) |
| Diabetes mellitus | 8 (25) | 3 (27.3) | > 0.999 | 11 (25.6) |
| Hypogonadism | 10 (31.3) | 4 (36.4) | > 0.999 | 14 (32.6) |
| Growth failure | 3 (9.4) | 0 | 0.558 | 3 (7) |
| Hypertension | 1 (3.1) | 0 | > 0.999 | 1 (2.3) |
| Heart failure | 1 (3.1) | 3(27.3) | 0.045 | 4 (9.3) |
| Kidney failure | 2 (6.3) | 0 | > 0.999 | 2 (4.7) |
| Pulmonary hypertension | 3 (9.4) | 3 (27.3) | 0.164 | 6 (14) |
| Hypothyroidism | 3 (9.4) | 1 (9.1) | > 0.999 | 4 (9.3) |
| Hypoparathyroidism | 4 (12.5) | 0 | 0.558 | 4 (9.3) |
| Chronic liver disease | 3 (9.4) | 3 (27.3) | 0.164 | 6 (14) |
| HCV positivity | 6 (18.8) | 2 (18.2) | >0.999 | 8 (18.6) |
| **Outcome** | = | = | = | = |
| Recovered | 27 (84.40 | 8 (72.7) | 0.401 | 35 (81.4) |
| Dead | 5 (15.6) | 3 (27.3) | | 8 (18.6) |

***Legend :*** **TDT**: transfusion-dependent β-thalassemia, **NTDT**: non-transfusion-dependent β-thalassemia, **BMI**: body mass index, **LIC:** liver iron concentration, LIC (mg Fe/g dw) was classified as mild (LIC >3 and <7), moderate (LIC >7 and <14), and severe overload (LIC >14).

**Table 2.** Comparison of demographic, clinical characteristics, and comorbidities between the two groups of death and recovery in confirmed COVID-19 patients with β-thalassemia

| Outcome Parameters | Dead (n=8) | Recovered (n=35) | P value |
|---|---|---|---|
| **Disease type** (TDT/NTDT) | 5/3 | 27/8 | 0.401 |
| **Age** (year), Mean ± SD | 36 ± 11.4 | 35.2 ± 11.6 | 0.872 |
| **BMI** (kg/m²) | 20.3 ± 1.5 | 21.5 ± 2.2 | 0.218 |
| **Sex** (M/F) | 5/3 | 17/18 | 0.698 |
| **Splenectomy** (N and %) | 6 (75) | 19 (54.3) | 0.434 |
| **Serum ferritin** (ng/ml) Mean ± SD | 3345 ± 5787 | 2078 ± 2202 | 0.561 |
| > **2000** (N %) | 2 (25) | 11 (32.4) | > 0.999 |
| **Hemoglobin** (g/dl) Mean ± SD | 9.2 ± 1.1 | 9.0 ± 1.3 | 0.775 |
| **Severe vitamin D deficiency** (<10 ng/ml) | 2 (25) | 3 (8.6) | 0.228 |
| **Iron overload** (moderate or severe based on LIC) (N and %) | 3 (37.5) | 13 (37.1) | > 0.999 |
| **Presence of comorbidities** (N and %) | 8 (100) | 27 (77.1) | 0.316 |

***Legend:*** **LIC:** Liver iron concentration, LIC (mg Fe/g dw) was classified as mild (LIC >3 and <7), moderate (LIC >7 and <14), and severe overload (LIC >14).

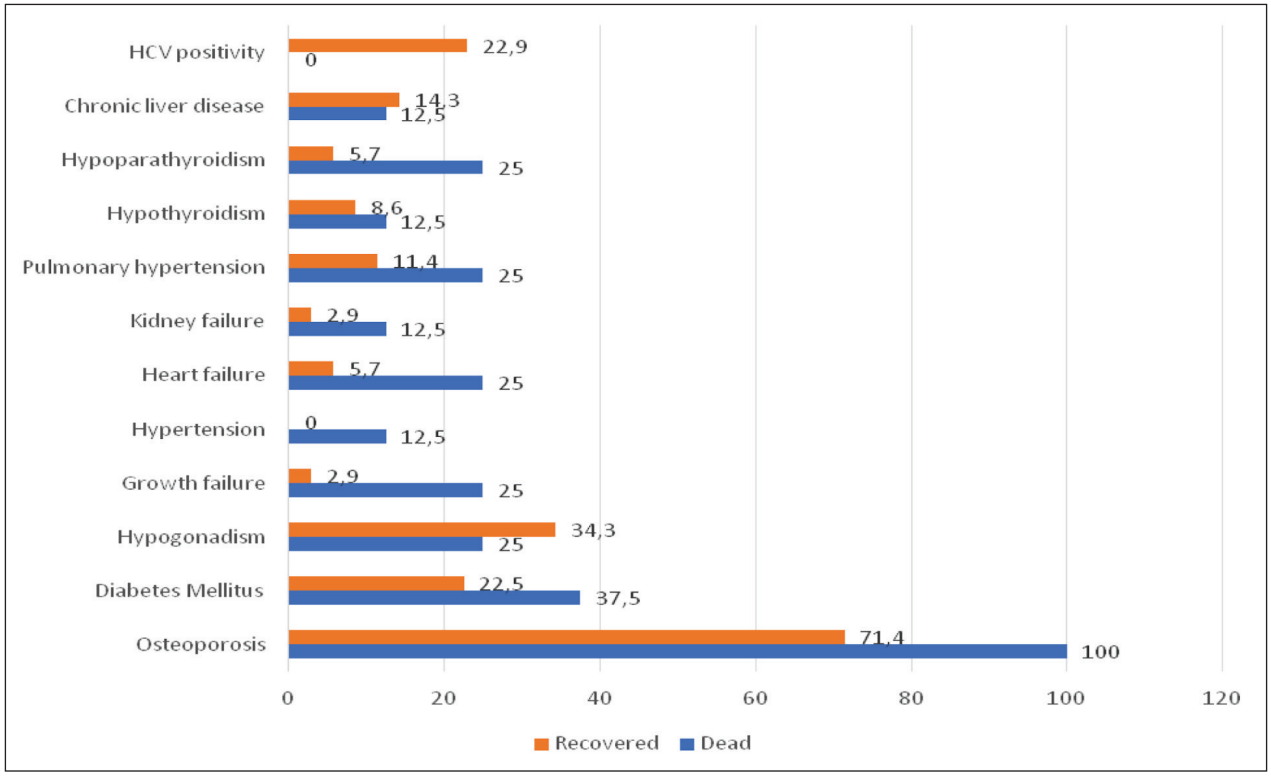


**Figure 1.** Distribution of the frequency of endocrine disorders and other comorbidities in recovered and dead COVID-19 cases with β-thalassemias

while the prevalence of TDT is almost similar to the normal population (P: 0.539). The mortality rate of COVID-19 was 4.71% in the normal Iranian population compared to 18.6% in β-thalassemias (P: < 0.001) at the same date (10).

## Discussion

β-thalassemias are a group of hereditary autosomal recessive anemias caused by either reduced or complete absence of production of β-globin chains of the hemoglobin tetramer. TDT and NTDT patients develop iron-overload that involve the heart, lungs, liver, and endocrine glands. These coexistent comorbidities expose them to a potentially higher risk of complications due to COVID-19, compared to the normal population, especially when they get older (3,4).

There are very limited data on the potential effects of associated comorbidities in thalassemic patients with COVID-19.

One previous comprehensive study (4) and the current updated data did not demonstrate a significant gender prevalence. However, our study documented that all treated hypogonadal female thalassemic patients recovered from COVID-19 disease but 50% of untreated affected female hypogonadism died. Although, the difference was not statistically significant, the clinical observation was particularly notable. In this context, the analysis of a possible hormonal-dependency of the expression and/or activity of ACE2 in the various tissues is of relevance to understand the pathologic mechanisms behind the epidemiologic findings.

Post-menopausal women are reported to have higher levels of proinflammatory cytokines, such as IL-1, IL-6, IL-and TNF-α; however, these levels are reduced with the use of hormone replacement therapy (HRT) (8). Therefore, future research is needed to examine whether HRT may have a "protective" role in female hypogonadal thalassemic patients.

Moreover, our current data showed that NTDT patients were more susceptible to COVID-19 disease severity and higher significant risk of death when were compared to general population. NTDT patients are potentially associated with persistent chronic anemia, hypoxemia and increased circulatory number of nucleated red blood cells which prone these patients to hypercoagulable state and microthrombosis (3,4,11). Therefore, these patients are susceptible to pulmonary artery hypertension that may cause right sided heart failure and death (12). These findings are in accordance with our present data showing a higher rate of mortality in NTDT patients than general population affected by COVID-19 disease.

In conclusion, it is important to acknowledge that thalassemia patients, especially young adults/adults, have a chronic condition which may be associated with several comorbidities linked to the underlying disease as well as complications of chronic transfusions, including heart failure, pulmonary hypertension, hypogonadism and diabetes (3,5,11) that may contribute to increase susceptibility to SARS-CoV-2 infection (3,4,11).

A higher susceptibility to COVID-19 was observed mainly in our patients with NTDT. Therefore, these patients must be monitored regularly especially for cardiac function and evidence of thrombosis. We recommend echocardiography and measure pulmonary artery pressure every 6 months to one year to detect pulmonary artery hypertension leading us to timely diagnosis and management including starting regular blood transfusion, if indicated (12).

Sex differences in the response to inflammation have been documented and can be attributed, at least in part, to sex steroid hormones. Although this relationship is much more complex than it might seem at first glance, in our hypogonadal female thalassemic patients, hormone replacement therapy probably increased the ability to fight against SARS-CoV-2 leading to the less disease severity (13,14).

Our study has several limitations due to the small sample size of patients limiting the representativity of the observations although the cohort of COVID-19 patients with TDT and NTDT was the largest at National level which classified the COVID-19 illness in two main groups: recovered and dead.

At present, the clinical classifications of COVID-19 consist of four main types: mild cases, moderate cases, severe cases, and critically ill cases. Furthermore, it was not possible to record and collect some data that were potentially informative for our analysis in a timely manner.

Nevertheless, we believe that our finding can serve as an informative starting point for further investigations and can help countries to better protect patients with thalassemias by the ongoing COVID-19 pandemic.

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**Contributors**

MK made substantial contributions to the study concept, design the protocol, and in drafting the manuscript. SH performed the statistical analysis and participated in drafting the manuscript. AA, ZZ, MA, AS, SM and AB were responsible for data collection and confirmation. TZ participated in data entry. VDS participated to the preparation of protocol survey and in drafting the manuscript and revising it.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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