

## **The demographic status and seroprevalence of SARS-CoV-2 antibodies in B- thalassemia patients**

**Maira Riaz<sup>1</sup>, Mahnoor Basit<sup>1,\*</sup>, Rida Dawood<sup>1</sup>, Mariym Asghar<sup>1</sup>, Sania Javaid<sup>2</sup>, Zoya Naveed<sup>1</sup>, Yasir Nawaz<sup>3</sup>, Ayesha Abbas<sup>3</sup>, Ahmed Yar Khoso<sup>4</sup>, Sobia Mushtaq<sup>5</sup>, Basit Nawaz<sup>6</sup>**

1 Institute of Microbiology and Molecular genetics, University of the Punjab, Lahore, Pakistan

2 Department of Biochemistry, School of Biological Sciences, University of the Punjab, Lahore, Pakistan

3 Jiangsu Key Laboratory for Microbes and Functional Genomics, School of Life Sciences, Nanjing Normal University, Nanjing 210023, China

4 Department of Zoology, University of Karachi, Karachi, Pakistan

5 Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

6 Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

### **Corresponding author:**

mahnoor98basit@gmail.com

### **Abstract**

Beta thalassemia is a blood disorder in which there is mutation in gene encoding for betaglobin chain. Reduced or absent beta globin chain lead to ineffective erythropoiesis and immature red blood cell production. Covid 19 also known as SARSCoV-2 causes acute respiratory infectious disease that transmits through respiratory droplets. People with underlying secondary conditions are more prone towards developing severe form of the virus. This study investigates the prevalence of SARS-CoV-2 antibodies in patients with  $\beta$ -thalassemia major ( $\beta$ -TM) in Pakistan, focusing on the impact of iron chelation therapy and associated comorbidities on COVID-19 outcomes. This work involved 200  $\beta$ -TM patients aged 10 to 30 years. Blood samples were collected and analyzed for hematological, biochemical, and immunological parameters. The study highlights the complex interaction between iron overload, common in  $\beta$ -TM patients due to frequent transfusions, and susceptibility to severe COVID-19. The role of iron chelation therapy, which has antiviral properties, was also examined. Despite the lack of a direct link between hemoglobinopathies and respiratory diseases, underlying conditions such as cardiac, liver, and renal diseases, exacerbated by iron accumulation, were found to increase the risk of severe COVID-19 outcomes. In conclusion, addressing the unique challenges faced by thalassemia patients in the context of COVID-19 requires a multifaceted approach. A robust data system and targeted research will be vital in developing strategies for prevention, treatment, and long-term care for this vulnerable group.

**Keywords:** Beta thalassemia, Covid 19, SARSCoV-2, Iron, Pakistan

## **Introduction**

Thalassemia can be defined as heterogenous group of genetic disorders that are passed down from parents to their off springs as per autosomal recessive pattern of inheritance. There are two types of defects in hemoglobinopathies i.e., qualitative and quantitative. Sickel cell anemia is classified as a qualitative or structural defect of hemoglobin while thalassemia is categorized as a quantitative defect of hemoglobin synthesis. In Thalassemia, rate of hemoglobin production is either partially or fully suppressed due to a reduction of  $\alpha$ - or  $\beta$ -chain synthesis (1). This gives rise to hypochromic microcytic anemia. There are more than 1 million transfusion dependent patients in Pakistan as per the report of 2020 (2).

Coronavirus is a family of viruses that can be classified majorly into alpha, beta, gamma and delta due to serology and genetic basis. Covid-19 belongs to the beta-lineage (3). This virus first emerged in Wuhan city, Hubei province of China. Patients' sufferings from SARS-Cov-2 experience flu-like symptoms. The mean incubation time for the virus is 2-14 days. The onset of symptoms includes sore throat, coughing, sneezing, runny nose (in some cases), mild to moderate fever, diarrhea, vomiting, abdominal pain but later on all these symptoms add up to the severity of the disease resulting in a very high fever, inflammation, hypoxia, thrombosis and cytokine storm which ultimately leads to the death of the infected patient (4). Its route of transmission is respiratory droplets or aerosols (5).

Haemoglobin disorders including thalassemia are not directly linked to respiratory diseases but different underlying disorders make patients more susceptible against viral infection like Covid-19. The multiple transfusions result in iron accumulation in different organs and cause many complications. This process initiates with iron storing in the RES (Reticuloendothelial System) mainly the bone marrow and the spleen and it causes bone expansion and splenomegaly. Later it headways towards hepatocytes, the heart and the endocrine glands. So, cardiac diseases, liver diseases, diabetes and renal diseases etc are the risk factors for the severity of Covid-19 and they are associated with increased chance of death and illness in thalassemia patients (6). Iron chelation therapy provides antiviral and immunomodulatory effect which could help in providing protection from deadly Covid-19. RNA viruses require iron for their replication and propagation. Restricting iron availability by the use of chelators contains the spread of virus in the body tissues (7). Moreover, the immune status of thalassemia major patients is quite active due to the continuous DFO treatment that upregulates their B cells and enhances the production of neutralizing antibodies (8). This study was aimed to collect the demographic history, medical profile and the biochemical analysis of patients with SARS-COVID with  $\beta$ -thalassemia.

## **Material and methods**

### **Study Design and population**

This experimental research was conducted between December 2020- July 2021 at Sundas Foundation Shadman, Lahore. The proposed study was multicentre as blood samples were collected from different centers of Sundas foundation located in Lahore, Gujranwala, Sialkot and Faisalabad. Research performa having important parameters were prepared and they were filled by patients after signing an ethical consent. Blood samples were taken and

analysed for hematological, biochemical and immunoassays. Data was collected from 200 patients that met the inclusion criteria i.e. Patients suffering from Thalassemia Major (age  $\geq 10 \leq 30$ ) participated in study willingly.

### **Ethical approval and consent to participate**

The study was done by following the declarations of Helsinki. Ethical committee of Sundas Foundation approved the presented study and there was no violation of rules and bioethics during this study. The patients consent form was obtained to gather data and samples.

### **Questionnaire and data collection**

The questionnaire was designed to collect data about patients. The trained phlebotomist drew blood from median cubital vein. The site of venipuncture was cleaned using isoamyl-alcohol swabs and air dried. The blood sample was drawn by using 5ml syringe. 2ml blood was collected in clotted vials whereas remaining sample was collected in Ethylenediaminetetraacetic acid vials. Appropriate labelling of sample vials was ensured.

### **Biochemical Assays**

The clotted vials were used for serum collection. These sample vials were centrifuged at 4500rpm for about 10-15 minutes. Serum from each sample was separated in a new Eppendorf.

Ferritin levels were checked on Abbot ARCHITECT i1000SR immunoassay analyzer. It is chemiluminescent microparticle immunoassay (CMIA) used to check ferritin levels in blood serum or plasma (9).

### **Semi Quantitative ELISA Test and Qualitative Enzyme Immunoassay (EIA)**

The ELISA kit (Bio-shield 2019-nCoV Total Immunoglobulins) was used to determine IgA, IgM and IgG against SARS-CoV-2 in serum or plasma of humans. This kit was manufactured by ProGnosis Biotech S.A. Its working principle was sandwich Elisa. The comparison of optical density of specimen to optical density of Cut-off control determines the presence of total antibodies present in human sample (10).

The Foresight EIA kit of SARS-CoV-2 IgM and IgG is an enzyme immunoassay which is used for qualitative detection of IgM and IgG antibodies against Covid-19 in human plasma or serum. This EIA kit is manufactured by Acon labs. The working principle is qualitative indirect enzyme immunoassay (10)

### **Statistical study**

The study was analyzed by Microsoft Excel 2010 to calculate the frequencies and chi square test to obtain the probability value i.e.,  $p > 0.05$  was considered as non-significant difference (11, 12).

## **Results**

### **Demographic status of patients**

In this study, 200 patients with  $\beta$ -Thalassemia Major were enrolled, and demographic data was collected through a structured questionnaire. Of the participants, 109 were male (54.5%) and 91 were female (45.5%). Blood group B was the most prevalent, found in 67 patients (33.5%), followed by blood groups A (23%), O (31%), and AB (7%). The Rh+ blood type was observed in 177 patients (88.5%), while 12 patients (6.0%) had the Rh- blood type.

Socioeconomic status varied, with 39 patients (19.5%) classified as having poor socioeconomic status, 87 (43.5%) belonging to the middle class, and 3 (1.5%) from the upper class. Consanguinity was reported in 136 patients (68.0%), while 56 (28.0%) reported no consanguineous relationships. Educationally, 140 patients (70%) had attended high school, while 50 (25%) were either illiterate or had only completed primary school. In terms of social interaction, 139 patients (69.5%) reported frequent interactions, 31 (15.5%) interacted rarely, and 21 (10.5%) had no social interaction at all. This is shown in table 1.

Table 1: The demographic status of patients

Variables		Frequency	Percentage
<b>Gender</b>	Male	109	54.5%
	Female	91	55.4%
<b>Blood Group ABO</b>	A	46	23%
	B	67	33.5%
	AB	14	7.0%
	O	62	31.0%
<b>Blood Group Rh</b>	Rh+	177	88.5%
	Rh-	12	6.0%
<b>Socioeconomic Status</b>	Poor	39	19.5%
	Middle Class	87	43.5%
	Affluent	3	1.5%
<b>Consanguinity</b>	Yes	136	68.0%
	No	56	28.0%
<b>Education</b>	Illiterate or primary school	50	25.0%
	High school or above	140	70.0%
<b>Social Interaction</b>	None	21	10.5%
	Rare	31	15.5%
	Frequent	139	69.5%

### Clinical symptoms among thalassemia major patients

The most common symptom observed is a runny nose, affecting 16% of the patients. This is followed by diarrhea, which is reported by 9% of the patients, and a loss of appetite, experienced by 8%. Both joint pain and loss of taste and smell are present in 8% and 7.5% of the patients, respectively. Muscle ache and chills each affect 6.5% and 6%

of the patients, while malaise is reported by 5%. Nausea and shortness of breath are less common, affecting 4% and 3.5% of the patients, respectively. The least common symptom is pneumonia, observed in only 0.5% of the patients. This distribution indicates that while some symptoms are relatively prevalent, others are rare, suggesting variability in how Thalassemia Major affects different patients. This is shown in figure 1.

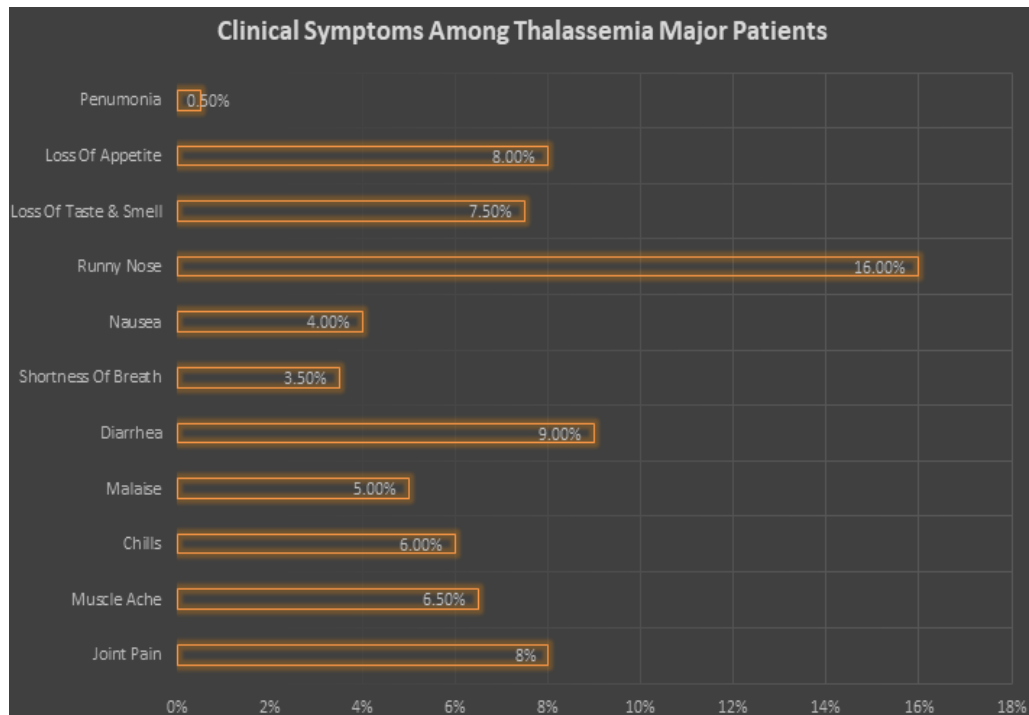


Figure 1: The clinical symptoms among thalassemia patients

### Medical history of patients

The results indicate that self-reported COVID-19, family history of COVID-19, cardiac diseases, lung diseases, liver diseases, and renal diseases do not show a statistically significant association, as their p-values are greater than 0.05. However, splenectomy shows a statistically significant association with the condition being studied, with a p-value of 0.019. Diabetes is noted, but no statistical test was applied to this parameter.

The analysis examines the frequency of various symptoms and their association with a particular condition using the Chi-square test. The results indicate that joint pain, muscle ache, chills, malaise, diarrhea, shortness of breath, nausea, running nose, loss of appetite, and pneumonia do not show a statistically significant association, as their p-values are greater than 0.05. Notably, the only symptom that demonstrates a statistically significant association is the loss of taste and smell, with a p-value of 0.047, suggesting a potential link with the condition being studied. This is shown in Table 1.

Table 1: The disease related history of patients

<b>Variables</b>		<b>Frequency</b>	<b>Percentage%</b>
<b>Self-Covid 19</b>	Yes	2	1.00%
	No	198	99.00%
<b>Family history –Covid 19</b>	Yes	12	6.00%
	No	188	94.00%
<b>Cardiac Diseases</b>	Yes	10	5.00%
	No	190	95.00%
<b>Chronic Lung diseases</b>	Yes	1	0.50%
	No	199	99.50%
<b>Diabetes</b>	No	200	100.00%
<b>Liver Diseases</b>	Yes	38	19.00%
	No	162	81.00%
<b>Renal Diseases</b>	Yes	1	0.50%
	No	199	99.50%
<b>Splenectomy</b>	Yes	16	8.00%
	No	184	92.00%
<b>Joint Pain</b>	Yes	16	8%
	No	184	92.00%
<b>Muscle Ache</b>	Yes	13	6.50%
	No	187	93.50%
<b>Chills</b>	Yes	12	6.00%
	No	188	94.00%
<b>Malaise</b>	Yes	10	5.00%
	No	190	95.00%
<b>Diarrhea</b>	Yes	18	9.00%
	No	182	91.00%
<b>Shortness of Breath</b>	Yes	7	3.50%
	No	193	96.50%
<b>Nausea</b>	Yes	8	4.00%
	No	192	96.00%
<b>Runny Nose</b>	Yes	32	16.00%
	No	168	84.00%
<b>Loss of Taste and Smell</b>	Yes	15	7.50%
	No	185	92.50%
<b>Loss of Appetite</b>	Yes	16	8.00%
	No	184	92.00%
<b>Pneumonia</b>	Yes	1	0.50%
	No	199	99.50%

### Seroprevalence of SARS-CoV-2 Antibodies In $\beta$ TM Patients

The most common symptom observed is a runny nose, affecting 16% of the patients. This is followed by diarrhea, which is reported by 9% of the patients, and a loss of appetite, experienced by 8%. Both joint pain and loss of taste and smell are present in 8% and 7.5% of the patients, respectively. Muscle ache and chills each affect 6.5% and 6% of the patients, while malaise is reported by 5%. Nausea and shortness of breath are less common, affecting 4% and 3.5% of the patients, respectively. The least common symptom is pneumonia, observed in only 0.5% of the patients. This distribution indicates that while some symptoms are relatively prevalent, others are rare, suggesting variability in how Thalassemia Major affects different patients. This is shown in figure 2.

According to biochemical analysis, the S. Ferritin was observed with patient's shows positive and negative cases with COVID. Less number of patients was observed with COVID positive while more number of people shows negative results with COVID.

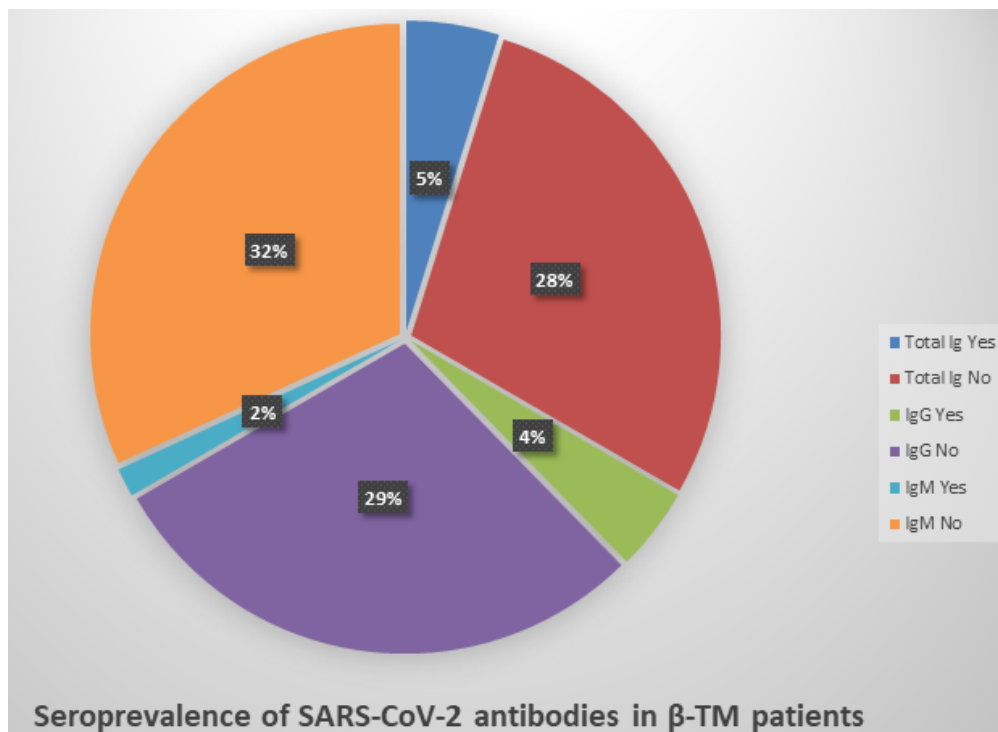


Figure 2: Seroprevalence of SARS-COV-2 antibodies in B-TM patients

### Discussion

Thalassemia is a quantitative genetic blood disorder which is characterized by genetic mutations in the  $\beta$ -chain of hemoglobin. December 2019 marked the emergence of novel corona virus in Wuhan, China after which it spread to all parts of the world. It was in February that the first case was reported in Pakistan and to this date Pakistan has experienced three waves of SARS-CoV-2 (13). Beta Thalassemia Major Patients are considered to be

immunocompromised due to iron overloading, impaired immunity and underlying comorbidities. That is why they are considered to be at a higher risk as compared to the normal population (14).

In our study, we determined the occurrence of novel SARS-CoV-2 in  $\beta$ -Thalassemia patients. About 14.5% of 200 people tested positive for antibodies against corona when their prevalence was checked by using ELISA kits. As compared to the normal population in which 8.35% people tested positive in 200 individuals. The ratio of virus is higher in patients of beta thalassemia major. Worldwide, the greatest number of cases have been reported in men due to their dysfunctional antiviral response and age factor (15). However, the gender ratio remains constant in BTM patients. For conclusive findings, more data needs to be gathered and prevalence needs to be checked in each gender.

Our study showed that the occurrence of corona virus was the most in B-blood group. This is due to the likeliness of B blood group to develop cardiovascular diseases and blot clots Thus, individuals with B blood group are more susceptible to develop severe form of the virus (16). Seeing the socio-economic status of people and their educational level it was also observed that a lack of basic education and awareness led to the virus being more prevalent in those particular regions. Counseling and understanding of SOPs could lead to the implementation of safety measures and contain the spread of virus among their population. It was also observed in our study that the social interaction of such patients was much more than the normal population as these children had to visit hospitals or medical centers for their fortnightly blood transfusion. This enhanced their exposure amidst the pandemic. Through our research we found out that the RT-PCR test ratio was much less in such individuals. This is owing to the price hike of the test as well as negligence of the people towards getting them tested timely. If any patient is suffering from symptoms, he or she should be advised immediately by the doctor to get tested for timely detection and medical intervention.

IgM is the first antibody to be produced at the onset of infection. There is a great amount of it in the beginning. As the infection is fought by our immunity, amount of IgG in blood stream is raised indicating that the body has developed resistance against the virus. IgM diminishes quickly but IgG prevails for a long time and can be used in the detection of antibodies even after the infection has ended. This is the reason why our results showed higher positives for IgG (13%) and not for IgM (4.5%). Analyses of quantitative variables was done to see an association between the serum ferritin levels of Beta Thalassemia patients and covid'19. There was no significant co-relation as the p value was found to be 0.135. Normal people who had corona experienced high serum ferritin levels as compared to the normal threshold. However, BTM patients already have hyperferritinemia in their blood so no significant change was observed in covid positive BTM patients.

The most intriguing aspect of our research was the asymptomatic state of Beta Thalassemia patients even after direct exposure to covid positive individuals within the family. Even if they contracted the virus, BTM patients experienced mild to moderate symptoms that reversed during a short period of time. No patient experienced severe symptoms or developed cytokine storm despite their underlying secondary conditions of heart problems, diabetes, renal and liver issues. We think this may be due to the viral clearance of the blood stream owing to their fortnightly



blood transfusion therapy. This way viral titre reduces considerably and helps in the prevention of RBC hemolysis. Moreover, it reduces the risk of coagulation in covid positive BTM patients.

Secondly, iron chelation therapy plays a great role in binding free iron in the blood stream. RNA viruses require iron for their replication and propagation. Restricting iron availability by the use of chelators contains the spread of virus in the body tissues (17). Thirdly, the immune status of such patients is quite active due to the continuous DFO treatment that upregulates their B cells and enhances the production of neutralizing antibodies. Multiple transfusion is linked with the constant stimulation of allo-antigens as well as production of alloantibodies (18). This modifies their immune system and defense mechanism to deal with foreign pathogens. Last but not the least, BTM patients take dietary supplements daily like vitamin C and E. These are very good antioxidants and protect cells from damage due to oxygen radicals. Hesperidin is a component of citrus fruits that blocks the attachment of virus to lungs and has proved to be beneficial for thalassemia patients consuming this on regular basis (19).

### **Conclusion**

To conclude, the study founds 14.5% were positive for SARS-CoV-2 antibodies. The ongoing pandemic has posed a significant challenge to the scientific community, particularly in protecting vulnerable populations. Patients with hemoglobinopathies, such as thalassemia, require special attention, yet our understanding of COVID-19 progression in this group remains limited. It is essential to closely monitor infected individuals and track their clinical symptoms to gather data that can enhance our understanding of the virus's prognosis in this population. Advancing computational, epidemiological, and molecular research is crucial to establish connections between thalassemia and COVID-19, potentially leading to therapeutic interventions and effective management strategies for hemoglobinopathy patients at risk. Moreover, addressing the unique challenges faced by thalassemia patients in the context of COVID-19 requires a multifaceted approach. A robust data system and targeted research will be vital in developing strategies for prevention, treatment, and long-term care for this vulnerable group.

### **Acknowledgments**

Authors are thankful to the university department to complete the work.

### **Conflict of interest**

None

### **Funding**

None

### **Author's contribution**

All authors contributed equally in the manuscript

## References

- 1) Al-Zwaini IJ. *Thalassemia and Other Hemolytic Anemias: BoD–Books on Demand*; 2018.
- 2) Zaheer HA, Waheed U, Abdella YE, Konings F. *Thalassemia in Pakistan: A forward-looking solution to a serious health issue. Global Journal of Transfusion Medicine.* 2020;5(1):108.
- 3) Fani M, Teimoori A, Ghafari S. *Comparison of the COVID-2019 (SARS-CoV-2) pathogenesis with SARS-CoV and MERS-CoV infections. Future Virology.* 2020;15(5):317-23.
- 4) Kumar S, Nyodu R, Maurya VK, Saxena SK. *Morphology, genome organization, replication, and pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronavirus Disease 2019 (COVID-19): Springer; 2020. p. 23-31.*
- 5) Almanar MA. *The shifting of face to face learning to distance learning during the pandemic Covid-19. Globish: An English-Indonesian Journal for English, Education, and Culture.* 2020;9(2):111-8.
- 6) Farmakis D, Giakoumis A, Cannon L, Angastiniotis M, Eleftheriou A. *COVID-19 and thalassaemia: A position statement of the Thalassaemia International Federation. European Journal of Haematology.* 2020;105(4):378-86.
- 7) Aleem A, Shakoor Z, Alsaleh K, Algahtani F, Iqbal Z, Al-Momen A. *Immunological evaluation of beta-thalassemia major patients receiving oral iron chelator deferasirox. J Coll Physicians Surg Pak.* 2014;24(7):467-71.
- 8) Perricone C, Bartoloni E, Bursi R, Cafaro G, Guidelli GM, Shoenfeld Y, et al. *COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy. Immunologic research.* 2020:1-12.
- 9) Sumbal MA, Mehmood SS, Ali H, Fayyaz T, Faisal M. *CROSS ANTIGENICITY OF SALMONELLA TYPHI AND NOVEL CORONAVIRUS ANTIBODY IN SECOND WAVE OF COVID-19 PANDEMIC.* 2022.
- 10) 10Tsatsakis A, Vakonaki E, Tzatzarakis M, Flamourakis M, Nikolouzakis TK, Poulas K, et al. *Immune response (IgG) following full inoculation with BNT162b2 COVID-19 mRNA among healthcare professionals. International Journal of Molecular Medicine.* 2021;48(5):1-10.
- 11) Dong T, Wang M, Liu J, Ma P, Pang S, Liu W, et al. *Diagnostics and analysis of SARS-CoV-2: current status, recent advances, challenges and perspectives. Chemical Science.* 2023;14(23):6149-206.
- 12) Drefahl S, Wallace M, Mussino E, Aradhya S, Kolk M, Brandén M, et al. *A population-based cohort study of socio-demographic risk factors for COVID-19 deaths in Sweden. Nature communications.* 2020;11(1):5097.
- 13) Javed W, Abidi SHB, Baqar JB. *Seroprevalence and characteristics of Coronavirus Disease (COVID-19) in workers with non-specific disease symptoms. BMC Infectious Diseases.* 2022;22(1):481.
- 14) Taher AT, Bou-Fakhredin R, Kreidieh F, Motta I, De Franceschi L, Cappellini MD. *Care of patients with hemoglobin disorders during the COVID-19 pandemic: An overview of recommendations. American Journal of Hematology.* 2020;95(8):E208.
- 15) Galbadage T, Peterson BM, Awada J, Buck AS, Ramirez DA, Wilson J, et al. *Systematic review and meta-analysis of sex-specific COVID-19 clinical outcomes. Frontiers in medicine.* 2020;7:348.
- 16) Zhang F, Yang D, Li J, Gao P, Chen T, Cheng Z, et al. *Myocardial injury is associated with in-hospital mortality of confirmed or suspected COVID-19 in Wuhan, China: A single center retrospective cohort study. MedRxiv.* 2020:2020.03. 21.20040121.
- 17) Aleem MT, Munir F, Shakoor A, Gao F. *mRNA vaccines against infectious diseases and future direction. International Immunopharmacology.* 2024;135:112320.
- 18) Oymak Y, Karapinar TH. *COVID-19 pandemic and thalassemia major patients: Transfusion practice and treatment assessment. Journal of Pediatric Hematology/Oncology.* 2021;43(8):e1073-e6.
- 19) Fung TS, Liao Y, Liu DX. *Regulation of stress responses and translational control by coronavirus. Viruses.* 2016;8(7):184.