http:// ijp.mums.ac.ir
Original Article (Pages: 18306-18313)

Premarital Screening for Beta-Thalassemia in Birjand City

* Tayebeh Chahkandi [1], Bita Bijari [2], Sajjad Akbari [3]

[1] MD, Birjand atherosclerosis and coronary artery research center, Associate professor of pediatrics, Birjand University of Medical Sciences (BUMS), Birjand, Iran.

[2] MD, Birjand atherosclerosis and coronary artery research center, professor of community Medicine, Birjand University of Medical Sciences (BUMS), Birjand, Iran.

[3] MD, School of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, Iran.

Abstract

Background: Thalassemia is a common inherited anemia; and population-level screening followed by molecular evaluation is the most effective way to prevent it. Given the significance of beta-thalassemia minor and its prevalence in communities, this study aimed to identify the prevalence of beta-thalassemia minor among participants in the pre-marital screening program in Birjand City, located in South Khorasan province, Iran.

Methods: The study included individuals who underwent pre-marital testing between 2014 -2019 and were categorized as high-risk for beta-thalassemia based on their blood indices and medical diagnosis. Data on their blood indices and follow-up results were collected from Birjand City's health center using a pre-designed form. The data were analyzed using statistical tests to assess significance.

Results: Out of 35,801 couples who underwent premarital testing, 195 (390 individuals) were identified as the high-risk group for beta-thalassemia. Among these high-risk couples, 67.2% responded to iron therapy. Thalassemia prevalence was 9.7% among high-risk couples, 31.6% among individuals at actual risk, and 76% among those who underwent Prenatal Diagnosis (PND). The mean values of MCV, MCH, and Mentzer indices were significantly higher in the group that responded to iron therapy compared to the group eligible for PND.

Conclusion: It was demonstrated that the prevalence of beta-thalassemia carriers among marriage applicants attending the health center in Birjand City is currently low. However, the implementation and continuity of beta-thalassemia screening programs for couples in this region can effectively prevent the birth of infants with thalassemia major and prevent unexpected medical treatment expenses.

Key Words: Beta-thalassemia, Birjand, Iran, screening.

* Please cite this article as: Chahkandi T, Bijari B, Akbari S. Premarital Screening for Beta-Thalassemia in Birjand City. Int J Pediatr 2023; 11 (10):18306-18313. DOI: **10.22038/ijp.2023.74029.5339**

Corresponding Author:

Tayebeh Chahkandi, MD, Birjand atherosclerosis and coronary artery research center, Associate professor of pediatrics, Birjand University of Medical Sciences (BUMS), Birjand, Iran. Email: na_chahkandi@yahoo.com

Received date: jul.20,2023; Accepted date: Oct.21,2023

1- INTRODUCTION

Thalassemia disorders are the most common single-gene inherited diseases worldwide (1), and approximately 3% of the global populations are carriers of the beta-thalassemia gene (2). This group of disorders is caused by the lack of synthesis, reduced synthesis, or instability of one or more globin chain(s), and depending on the type of involved chain, they are classified into different types such as α, β, εβ, and so on (3,4). The inheritance of beta thalassemia is autosomal recessive (3, 5, 6).

Although thalassemia has been reported in most countries worldwide, its highest prevalence is observed in the malaria belt, including countries in the Mediterranean region, parts of West and North Africa, the Middle East, the Indian subcontinent, and Southeast Asia (2, 4). Iran is also located within this thalassemia belt (4, 7) and faces a relatively high prevalence of thalassemia (4). Around 23,000 individuals in our country are affected by this disease, and approximately 1,500 new cases are added annually (2). The prevalence of beta-thalassemia carriers in Iran is approximately 4%, varying across regions. It is less than 2% in provinces such as Tehran, East Azerbaijan, West Azerbaijan, Khorasan, Hamadan, and Yazd, while it is higher than 8% in provinces like Kerman, Hormozgan, Sistan and Baluchestan, and Mazandaran (7).

Beta-thalassemia, clinically and histologically, manifests in three forms: beta-thalassemia minor, thalassemia intermedia, and thalassemia major (8). In each pregnancy resulting from the marriage of two carriers of beta-thalassemia minor, there is a 25% chance of having a child with thalassemia major (6, 9, 10, 11, 12). Compared to thalassemia major, which presents with severe anemia and requires lifelong blood transfusions (9), cases of thalassemia minor are usually asymptomatic and can be detected through routine blood tests (3, 13).

In order to prevent the occurrence of genetic diseases, specialists have always been seeking appropriate screening methods to identify carriers of genetic disorders. Comprehensive screening programs that encompass the entire community should be designed in a way that all individuals present in a population are examined in a uniform manner.The primary objective of screening programs is to raise awareness and increase knowledge among all individuals about genetic diseases and their risk of occurrence. As a result, from an ethical perspective, it enhances individual autonomy, enabling them to make better decisions regarding marriage and having children. The second objective of screening programs is to reduce the number of individuals suffering from genetic diseases (14).

Since 1997, the national thalassemia control program has been integrated into Iran’s healthcare network as the first non-communicable disease control program. The project's overall objective is to prevent the occurrence of new cases of thalassemia by enhancing the awareness and attitudes of individuals and discouraging marriages between couples who are both carriers of the thalassemia gene (15, 16, 6).

According to the latest revision of the national guidelines in the year 2014, suspicious couples, after undergoing the first stage of testing, including CBC, HbA2, and iron therapy if recommended by the thalassemia consultant physician, will be categorized into high-risk and low-risk groups in the second stage if no response is observed. The couple is classified as high-risk if either both partners have similar beta-thalassemia indices (MCV and MCH less than 75 and 26, respectively, and HbA2 greater than 3.2) or if one partner has indices similar to the beta pattern and the other partner exhibits HbF greater than 3 in

electrophoresis.They are considered candidates for pre-implantation genetic diagnosis known as Prenatal Diagnosis (PND) investigations. PND is conducted using samples such as chorionic villus sampling and amniotic fluid, through several molecular methods (17).

At present, there are no available studies on the effectiveness of the low-risk and high-risk classifications for screening couples concerning beta-thalassemia mutations and the prevalence of beta-thalassemia minor in our region, particularly in the recent years following the implementation of the new thalassemia screening guidelines.This research aims to investigate the effectiveness of the national protocol in identifying thalassemia carriers and assess the prevalence of beta-thalassemia carriers (based on PND testing) among high-risk couples attending healthcare centers in Birjand City. The study will cover five years from the beginning of 2014 to the end of (2019), starting from implementing the new national guidelines.

2- MATERIALS AND METHODS

In this cross-sectional study, after coordination with a healthcare center in Birjand city and obtaining the necessary approvals, all high-risk couples who visited the healthcare center in Birjand from 2014 to 2019 were included. The data collection tool consisted of a checklist containing demographic information and details related to initial tests, including MCV, MCH, Hb, HbA2, and the results of the PND test. The data were completed based on the participants' medical records for high-risk couples or, when needed, through direct communication with them.

The collected data were subjected to analysis using statistical software, including Mann-Whitney U test, independent t-test, and Chi-square test, with a significance level of α=0.05, using SPSS version 15.

3- RESULTS

Out of the total of 35,801 couples who visited for premarital testing, 195 couples (390 individuals) were categorized as the initial high-risk group for beta-thalassemia, which accounts for 0.54 % or 54 per thousand. The demographic and laboratory characteristics of the participants are presented in **Table 1**.

Table-1: Demographic and Laboratory Characteristics of High-Risk Couples

Characteristics	All(High-Risk Couples)	Female	Male	Non-response to treatment group
	Mean± SD	Mean± SD	Mean± SD	Mean± SD
Age	29.1±7.3	27.5±7.1	30.6±7.2	30.63±8.89
Hb	13.3±1.7	12.4±1.2	14.3±1.5	13.32±1.5
RBC	5.6±0.89	5.5±0.72	6.09± 0.66	5.8±0.54
MCV	76.1±6.8	77.6±5.6	74.6±7.6	72.3±5.7
MCH	31.07±1.6	30.7±1.4	31.3±1.7	22.72±2.08
HbA2	2.8±0.92	2.6±0.7	2.9±1.05	2.9±0.91
Mentz	13.8±2.8	13.4±2.4	12.1±2.3	12.5±1.9

From among the 195 initial high-risk couples, 67.7% responded positively to iron therapy and were no longer considered high-risk. Additionally, 19 couples migrated out of Birjand City, 16 couples did not attend follow-ups despite repeated contact attempts by the healthcare center personnel, and three couples decided not to proceed with the marriage.

Only 25 couples (12.8%) underwent PND testing

The prevalence of thalassemia couples who underwent pre-marital testing was 0.53 per thousand, and it was 9.7% among the initial high-risk couples. The prevalence was 31.6% among individuals who were identified as actual high-risk carriers, and 76% among those who underwent PND testing (**Table 2**).

Table-2: Relative Frequencies of Thalassemia Types in the participants

Variable	To high-risk couples (n=195)	To real high-risk couples (n=60)	To the couples who did PND (n=25)
Thalassemia carrier couples (19 couples)	9.7%	31.6%	76%
Alpha thalassemia carrier couples (9 couples)	4.6%	15%	36%
Beta thalassemia carrier couples (7 couples)	3.6%	11.6%	28%
Couples whom one of them is Alpha and the other one Beta (3 couples)	1.5%	5%	12%

The mean hemoglobin and RBC levels in the group responding to iron therapy and the group eligible for PND testing did not show a statistically significant difference. However, the mean values of MCV, MCH, and Mentzer indices were significantly higher in the group that responded to iron therapy compared to the group eligible for PND testing (**Table 3**).

In the group eligible for PND testing, MCV was less than 75, HbA2 between 3.5-7, and Mentzer index less than 13, which were significantly higher than those in the group responding to iron therapy (**Table 4**). The mean indices between individuals with alpha-thalassemia and beta-thalassemia did not show a statistically significant difference ($p>0.05$).

4- DISCUSSION

In this study, which aimed to investigate the prevalence of beta-thalassemia minor among participants in the premarital screening program in Birjand, the capital of South Khorasan province in eastern Iran, 0.54% of couples were classified in the initial high-risk group. The prevalence of high-risk couples in the present study is lower than that reported in most of the previous studies. In the province of Kurdistan, the prevalence of high-risk couples among prospective marriage candidates was 2.1%., among whom 49.8% responded to iron therapy, and 20% of the initial high-risk couples were referred for PND testing. Among those referred, 9.8% were diagnosed with beta-thalassemia (18).

Table-3: Comparison of Hematological Indices among High-Risk Couples

Index	Mean± SD		P Value
	Respond to iron therapy	PND performed	
Hb	13.4±1.7	13.32±1.5	0.24
RBC	5.6±0.94	5.8±0.54	0.18
MCV	77.7±6.3	72.3±5.7	<0.001
MCH	24.21±1.99	22.72±2.08	<0.001
HbA2	2.7±0.84	2.9±0.91	0.005
Mentz	14.3±2.9	12.5±1.9	<0.001

Table-4: Comparison of Abnormal Hematological Indices among High-Risk Couples

Index		Respond to iron therapy N (%)	PND performed	P Value
MCV	<75	45(17)	31(62)	<0.001
	>75	219(83)	19(38)	
MCH	<26	256(97)	49(98)	0.92
	>26	8(3)	1(2)	
HbA2	<3.5	238(90.2)	38(9.8)	0.001
	3.5-7	26(9.8)	8(17.4)	
Mentz	<13	69(27.5)	30(65.2)	<0.001
	>13	182(72.5)	16(34.8)	

In Sharifi et al.'s study on couples volunteering for marriage in Ilam province, 0.5% of the couples were identified as having final suspicions (19). In the present study, the prevalence of beta-thalassemia among all participating couples was 0.24 per thousand individuals. In the study by Ghatee et al, the prevalence of thalassemia in candidate couples for marriage in Kohgiluyeh and Boyer-Ahmad province was 28.9 per thousand individuals studied (20). The study conducted in Javanroud City in 2013-2014 showed that 0.2% of the couples were carriers of thalassemia (21). Fathi et al. conducted a study in Ardabil province and found that the prevalence of beta-thalassemia minor among all participants was 2.4%. Notably, the estimated prevalence was higher in couples with MCV < 80, reaching 4.94% (22).

As shown in different studies, the prevalence of the beta-thalassemia gene varies in different regions of Iran. Abolghasemi et al. conducted an epidemiological study on thalassemia in Iran, revealing that beta-thalassemia minor prevalence among individuals eligible for marriage was 9.7% in Mazandaran, 19.4% in Ahvaz, and 26.3% in Isfahan, showing a significant increase compared to Birjand (23). Hayat bakhsh reported a prevalence of 5.7% for beta-thalassemia among individuals volunteering for marriage in Kerman (24). In Fars province, a prevalence of 4.9% was also reported (24).

Chahkandi et al. conducted a study in 2001 on students in Birjand, reporting a prevalence of 1% for beta-thalassemia minor based on the criteria of MCV < 80 and A2 > 3.7% (25). This prevalence is higher than that found in the current study, which was conducted based on a new screening protocol. The difference could be attributed to changes in the screening guidelines in previous years. In the study by Chahkandi, out of 480 students, 34 individuals had microcytosis. After receiving iron supplementation, microcytosis persisted in 14 individuals, and among them, 5 had HbA2 levels higher than 3.7%, which led to their classification as carriers of beta-thalassemia (25).

In our study, 9.7% of the initial high-risk couples, one-third of the actual high-risk couples (31.6%), and 76% of PND candidates were beta-thalassemia carriers. This indicates that the national protocol with this format plays a significant and influential role in identifying thalassemia carriers and preventing the birth of major thalassemia cases. Although in studies conducted based on the previous guidelines (MCV less than 80 or MCH less than 27), a higher percentage of high-risk couples has been reported (26). Determining which protocol is more efficient in identifying beta-thalassemia

carriers requires larger-scale studies with larger sample sizes.

In the current study, among the initial high-risk couples, only three couples (5.1% of high-risk couples) decided not to proceed with the marriage. In the study by Hayat Bakhshin Kerman, out of 110 carrier couples, only eight couples (7.2%) decided to cancel their marriage plans (24). In the study of Rahmani in Kurdistan province, none of the high-risk couples decided to cancel their marriage plans (21). In the study conducted by Hedayati Nia in Kurdistan, out of 7 couples who were candidates for PND, three couples (42.8%) decided to withdraw from marriage plans (18). The expansion of genetic services in the country, including the possibility of prenatal and therapeutic diagnosis, can be a reason for reducing the rate of marriage cancellations. Additionally, the ethnic and cultural roots of each region can also have a significant impact on the decision to cancel marriage.

The current study showed no significant difference in the mean hemoglobin and RBC levels between the group that responded to iron therapy and those eligible for PND. However, the mean values of MCV, MCH, and Mentzer indices were significantly higher in the group that responded to iron therapy compared to the group eligible for PND.

In the study by Shaianmehr et al., in Urmia, HBA2 levels were significantly higher in the beta-thalassemia group than in the iron-deficiency anemia group. Furthermore, the beta-thalassemia group exhibited significantly lower MCV and MCH values than the alpha-thalassemia and iron-deficiency anemia groups (27).

In the present study, there was no statistically significant difference in mean indices between individuals with alpha-thalassemia and those with beta-thalassemia. Based on the fact that individuals with alpha-thalassemia usually have higher RBC parameters, which are close to the upper limit (28); and in this study, individuals with MCV and MCH values, respectively, less than 75 and 26 were included. So, the present study is not practically helpful in investigating the prevalence of alpha-thalassemia.

5- CONCLUSION

This 5-year study revealed a low prevalence of thalassemia carriers among individuals visiting the studied health center in Birjand City. These findings confirm a significantly lower prevalence of beta-thalassemia carriers and a reduced risk of giving birth to infants with thalassemia major in this region compared with many other areas in Iran. However, considering the importance of beta-thalassemia major and the high rate of consanguineous marriages, along with the low rate of marriage cancellations among carriers, implementing and sustaining the beta-thalassemia screening program for couples in this city can effectively prevent the birth of infants with thalassemia major, alleviate the emotional burden, and reduce the unplanned healthcare costs associated with the disease.

6- ETHICAL CONSIDERATIONS

The Ethics Committee of Birjand University of Medical Sciences approved the study with the ethics code IR.BUMS.REC.2020.535.

7- CONFLICTS OF INTEREST

None.

8- ACKNOWLEDGMENTS

The authors would like to thank Dr. SaidehRohani for her collaboration in gathering information related to the participating couples in the study.

9- REFERENCES

1. Yadav SS, Panchal P, Menon KC. Prevalence and Management of β-Thalassemia in India. Hemoglobin. 2022 Jan 2; 46(1):27-32.

2. Lichtman, M.A., Kaushansky K, Kipps TJ, Prchal JT, Levi MM. Williams Manual of Hematology. 2011: McGraw-Hill.

3. Hashemizadeh HA, Noori R. Premarital screening of beta thalassemia minor in north-east of Iran. Iranian journal of pediatric hematology and oncology. 2013; 3(1):210.

4. Mobasheri L, Chahkandi T, Talebpour A, Sarab GA. Red blood cell alloimmunization among transfusion-dependent thalassemia major patients in Northeastern Iran. Asian Journal of Transfusion Science. 2023 May 11.

5. Chahkandi, T., Norouziasl S, Farzad M, Ghanad F. Endocrine disorders in beta thalassemia major patients. International Journal of Pediatrics, 2017. 5(8): p. 5531-5538.

6. RezabeigiDavarani E, MohseniTakaloo F, Vahidnia A, Daneshi S, RezabeigiDavarani M, Khanjani N, Hushmandi K, Raei M. Epidemiological investigation of a twenty-year major β-thalassemia surveillance in Kerman, Iran. Archives of Hygiene Sciences. 2020 Oct 10; 9(4):265-74.

7. HadipourDehshal M, TabriziNamini M, Hantoushzadeh R, YousefiDarestani S. β-Thalassemia in Iran: things everyone needs to know about this disease. Hemoglobin. 2019 May 4; 43(3):166-73.

8. Origa R. β-Thalassemia. Genet Med. 2017 Jun; 19(6):609-619. doi: 10.1038/gim.2016.173. Epub 2016 Nov 3. PMID: 27811859.

9. Wendt AS, Brintrup J, Waid JL, Kader A, Lambrecht NJ, Gabrysch S. Thalassemia and hemoglobinopathy prevalence in a community-based sample in Sylhet, Bangladesh. Orphanet Journal of Rare Diseases. 2023 Jul 19; 18(1):192.

10. Aslamkhan M, Qadeer MI, Akhtar MS, Chudhary SA, Maryam M, Ali Z, Khalid A, Irfan M, Khan Y. CULTURAL CONSANGUINITY AS CAUSE OF β-THALASSEMIA PREVALENCE IN POPULATION. medRxiv. 2023:2023-06.

11. Tuli, A. and E.D. Yenilmez, Invasive and Noninvasive Approaches in Prenatal Diagnosis of Thalassemias. Thalass Other Hemolytic Anemias, 2018

12. Hasanshahi F, Khanjani N. Investigating the reasons for marriage among couples with thalassemia minor, in Iran. Journal of Community Genetics. 2021 Oct; 12:507-13.

13. Valaei, A., Karimipoor M, Kordafshari A, Zeinali S. Molecular basis of α-thalassemia in Iran. Iranian Biomedical Journal, 2018. 22(1): p. 6.

14. Nussbaum, R.M., R.R. McInnes, and H.F. Willard, Thompson & Thompson geneticsin medicine e-book. 2015: Elsevier Health Sciences.

15. Khorasani, G., Kosaryan M, Vahidshahi K, Shakeri S, Nasehi MM. Results of the national program for prevention of β-thalassemia major in the Iranian Province of Mazandaran. Hemoglobin, 2008. 32(3): p. 263-271.

16. Samavat, A. and B. Modell, Iranian national thalassaemia screening programme. Bmj, 2004. 329(7475): p. 1134-1137.

17. Najmabadi, H., Ghamari A, Sahebjam F, Kariminejad R, Hadavi V, Khatibi T, Samavat A, Mehdipour E, Modell B, Kariminejad MH. Fourteen-year experience of prenatal diagnosis of thalassemia in Iran. Public Health Genomics, 2006. 9(2): p. 93-97.

18. The prevalence of thalassemia trait in premarital consulting, couples center in the city of Sanandaj in year 89-91. Zanko J Med Sci 2014; 14 (43):11-18.

19. Sharifi, A., Aminzadeh bookani M, Pourmoghaddam Z, Jozian F, Mahdieh N. A survey of Beta-Thalassemia Trait in Marriage Volunteers in Ilam: The Impact

of National Guidelines for Prevention and Control of Thalassemia. Journal of Ilam University of Medical Sciences, 2015. 23(4): p. 148-157.

20. Ghatee, M., et al., Study of prevalence of Thalassemia and comparison of hematological indices in types of Thalassemia in marriage candidate patients in Kohgiluye&Boyerahmad province in 1392.Armaghanedanesh, 2016. 21(1): p. 84-94.

21. Rahmani S, Rostami S, Moradi G,Balbasi F, safaee A. The survey of thalassemia minor epidemiological in volunteers of marriage referred to health centers of javanroud city in 2013-14. 2015.

22. Fathi A, Amani F, Mazhari N. The Incidence of Minor β-thalassemia Among Individuals Participated in Premarital Screening Program in Ardabil Province: North-west of Iran. Materia Socio-Medica, 2019. 31(4): p. 294.

23. Abolghasemi, H., Amid A, Zeinali S, Radfar MH, Eshghi P, Rahiminejad MS, Ehsani MA, Najmabadi H, Akbari MT, Afrasiabi A, Akhavan-Niaki H, Hoorfar H. Thalassemia in Iran: epidemiology, prevention, and management.Journal of Pediatric Hematology/Oncology, 2007. 29(4): p. 233-238.

24. Hayatbakhsh AM, Bahrampour A. determination of the rate of incidence of thalassemia minor among marriage condidates in kerman. 2002.

25. Chahkandi T, Sadat JS. Prevalence of the minor-β thalassemia in birjand city. 2001.

26. Gholamreza BM. The efficacy of the national screening guidelines for identification of betathalassemia carrier couples by using parental tests of patients with thalassemia major and intermedia. Razi J Med Sci (Journal Iran Univ Med Sci. 2020;27(1):9-16

27. Shaianmehr M, Sadaghiyanifar A, Zareipour MA, Abassi M, Eftekhar E. Premarital screening of beta thalassemia in Urmia County of northwest Iran during 2014: a short report. Journal of Rafsanjan University of Medical Sciences, 2016. 14(10): p. 903-910.

28. Zarbakhsh B, Eghbalpour F, Farshadi E, Fallah MS, Karimipoor M, KaeiniMoghadam Z, Zeinali S. Frequency of alpha thalassemia carriers detected in Tehran premarriage screening using molecular techniques. Scientific Journal of Iran Blood Transfus Organ. 2013 Jan 10; 9(4):414-21.