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RESEARCH ARTICLE

Widely distribution of hematological parameters in thalassemia patients with similar α-globin genotype

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BACKGROUND: Thalassemia is known as the commonest monogenic disorder with an imbalanced rate of globin chains production of adult hemoglobin. Despite the available information about the thalassemia etiology, its phenotype varies from each patient to another. This study aimed to evaluate the hematological parameters of patients with the same -α3.7 homozygote and heterozygote genotypes to amend screening programs.
METHODS: In this observational study, we evaluated 1301 thalassemia suspected patients who referred to the Thalassemia and Hemoglobinopathy Research Center of Ahvaz University of Medical Sciences, Khuzestan, Iran during 2014-2016. According to the genotyping studies, patients divided into 2 groups with -α3.7/αα (n = 646) and -α3.7/-α3.7 (n = 181) genotypes. Thereafter, distribution of hematological parameters evaluated in both groups.
RESULTS: The mean age in heterozygous and homozygous groups was 25.7±4.5 and 26±4.4 years old, respectively. The degree of anemia was considerably varied in patients with the same genotype. MCV, RBC and MCH showed a wide distribution in patients.
CONCLUSION: The findings presented here suggest that other molecular mechanisms along with α-globin gene mutations could be involved in determining the phenotypes of alpha thalassemia patients.

Keywords hematological parameters, α-globin genotype, alpha thalassemia

Introduction

Thalassemia is a group of inherited blood disorders caused by the decrease or absence of globin chains synthesis that will be determined with decrease in erythrocyte hemoglobin, increased erythrocytosis and anemia. Alpha(α) and Beta(β) thalassemias are the main classes of thalassemia in which the α and β globin genes are involved (Harteveld and Higgs, 2010). Indeed, the principal pathophysiology of thalassemia is attributed to the deleterious effects of surplus chain. Excess α chains in β-thalassemia damage red cell precursors and cause ineffective erythropoiesis, whiles excess β chains known as β4 molecules or HbH in α-thalassemia are more soluble and often lead to a hemolytic anemia (Singer, 2009). Despite the strong prevention strategies in local health systems, α-thalassemia is the most widely distributed type and the combination of its different mutations besides other hemoglobinopathies results in various clinical pictures. Moreover, because of two α-globin genes per haploid genome, the genetics of α-thalassemia is more complicated than β-thalassemia (Surapolchai et al., 2017). The Mediterranean, middle east and south-east Asia populations carry α-thalassemia variants in a great deal and due to accelerated migration patterns from these regions, it has affected the health systems globally (Galanello and Cao, 2011).

Unlike the β-thalassemia, mutilate synthesis of α-globin is mainly due to deletions within the α-globin gene cluster on

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chromosome 16 (Musallam et al., 2013) and well known α-thalassemia phenotypes are primarily attributed to the deletions on one or both α-globin genes. Phenotypes severity vary from silent α-thalassemia and α-thalassemia trait with mild anemia to HbH disease with moderate hemolytic anemia and its lethal form known as Hb Bart's hydrops fetalis. Despite the many consummated attempts in the underlying role of involved α-globin gene mutations and deletions in α-thalassemia pathophysiology, it seems that the fundamental processes of α-globin gene expression, from mRNA transcription, splicing and protein translation is affected by complicated procedures which result in various phenotypes in patients with similar α-globin genotype (Farashi and Harteveld, 2017). It is too important to continue using the knowledge gained from the well-characterized globin gene disorders to further develop our understanding of the mechanisms by which both common and costly genetic blood diseases may arise.

Material and methods

Subject of study

In this study, we have evaluated 1301 thalassemia suspected people in accordance with their low hematological indexes had referred to Thalassemia and Hemoglobinopathy Research Center of Ahvaz University of Medical Sciences, Khuzestan, Iran during 2014-2016. After obtaining informed consent from all participants, 10 mL of peripheral blood collected in EDTA-containing tubes for further hematological and molecular studies. Patients with confirmed -α3.7 genetic aberration based on molecular analysis were included in the study and other molecular defects in globin gene were excluded. The ethical approval from an ethical committee of the Ahvaz Jundishahpur University of Medical Sciences has improved.

Hematologic analysis

The first step to accede the thalassemia diagnosis, achieved by performing hematological studies. In this regard, a whole blood cell count was performed using an automatic cell counter (Mindray 6800) and both Hb-A2 and Hb-F were measured by column chromatography and hemoglobin electrophoresis on cellulose acetate at acid and alkaline PH, respectively.

Molecular analysis

Genomic DNA extracted from peripheral blood leukocyte using DNA extraction KIT (QiaGen) due to the standard protocol of manufacture. Subsequently, the purity of extracted genomic DNA measured by a nanodrop spectrophotometer (Thermo). To detect four common deletional α-thalassemia including -α3.7, -α4.2, –20.5 and -MED, we utilized multiplex Gap polymerase chain reaction (PCR) (Liu et al., 2000). Poly A1; Poly A (A- > G); AATAAA- > AATAAG (HBA2:c.*94A > G), PolyA2; PolyA (A- > G); AATAAA- > AATGAA (HBA2:c.*92A > G), CD142 (HbCS), Hb Constant Spring (HbCS) (TAA > CAA) (HBA2:c.427 *t* > C)), -a5nt (GAGGTGAGG > GAGG–;HBA2: c.95þ2_95þ6del TGAGG) and codons 93–99 (+ 21 nt) (HBA2: c.280_300dup GTGGACCCGGTCAACTTCAAG) characterized using multiplex amplification refractory mutation system (ARMS) PCR. All of the patients were negative for obtaining β globin genes involvements. Strip Assay hybridization test modified and used to detect non-deletional homozygote and heterozygote mutations. Samples not characterized by mentioned tests, their alpha globin genes were sequenced using DNA sequence, AB-3130 (Applied Biosystems, Foster City, CA, USA). The purification of PCR products carried out by PCR purification KIT (Qiagen GmbH). Afterward, sequenced samples were precipitated in ethanol-sodium acetate for Sanger sequencing (Sanger et al., 1977).

Statistical analysis

The obtained data evaluated with SPSS 19. Normality test carried out to determine the distribution of data. Moreover, descriptive analysis, including mean and standard deviation of data were calculated.

Results

A total of 11 different common mutations in α-thalassemia including 4 deletional: -α3.7, -α4.2, - –20.5 and- -MED and 7 non-deletional: αPA-1α, α-5nt delα, αααanti-3.7, αcd142α, αPA-2α, αcd59α, αICα were investigated (The genotyping data are available in our previous study) (Keikhaei et al., 2018). After that, we focused on-α3.7 mutation as the most common mutation and perused the phenotype of patients with -α3.7 mutation more closely. Based on the genotype, we were able to classify patients into two groups. A total of 646 patients were -α3.7 heterozygous and 181 patients were -α3.7 homozygous confirmed by molecular methods.

Given that HbA2 and HbF percentages besides hematological parameters are the main diagnostic features in thalassemia, we figured on these factors in a cohort of subjects that were heterozygous or homozygous for -α3.7 mutation, with the aim of correct characterizing and related treatment of these patients in Khuzestan population.

According to our survey, the mean age in heterozygous and homozygous groups was 25.7±4.5 and 26±4.4 years respectively and had no correlation with diagnosis and patient phenotypes. The rate of mutation occurrence in both male and female was identical and almost the same in both groups (334 men (51.7%) and 312 women (48.3%) in

heterogeneous group and 83 men (45%) and 98 women (54.1) in homozygous group). However, it was higher in non-Arab patients in comparison with Arab patients (390 non-Arab patients in compared to 256 Arab patients in heterogeneous group and 99 non-Arab patients in compared to 82 Arab patients in homozygous group).

Ninety percent of patients with -α3.7/αα genotype showed silent α-thalassemia with heterogeneous hematological parameters. However, there was an Arab woman with-α3.7/αα genotype showed a normal hematological profile. In addition, two Arab men with -α3.7/αα genotype showed minor α-thalassemia phenotype, unexpectedly.

The mean of HbA2 was 2.44±0.37 and 2.33±0.38 in heterogeneous and homogeneous groups, respectively. The majority had normal HbA2 levels ranging from 1.2 to 3.3% in both groups. The average of HbF level showed a little fall with the mean of 0.56±0.41 and 0.51±0.35 in heterogeneous and homogeneous groups, respectively, and most of patients had HbF levels⩽0.5 (393 patients in hetero group and 110 patients in homo group). Moreover HbA2 and HbF levels showed a significant difference between two groups (p = 0/00).

A complete blood count revealed the mean values for hematological parameters (Table 1). Two groups differed in hematological parameters significantly. The homozygous group had lower levels of Hb, MCV, MCH, HbA2 and HbF and higher RBC in comparison with the heterozygous group ($P < 0.0001$ for all mentioned parameters). Figure 1 and Fig. 2 show the distribution of hematological parameters in -α3.7/αα and -α3.7/-α3.7 groups, respectively. As the results show the distribution of the measured parameters is highly variable in patients with the same genotype.

Discussion and conclusion

Despite the all developments have already achieved in perception of normal Hb production and errors could result in thalassemia formation, uncoordinated cases with existing guidelines and lack of timely identification of some patients, made this context to one of the most central priorities of health systems. Unfortunately, most of these patients are born in developing and low-income countries, that create an enormous health burden (Cao and Kan, 2013). This study concentrates on different phenotypes of patients with the same α-thalassemia genotype owing to its challenges for diagnosis and treatment of thalassemia to help with improving prevention strategies and provide opportunities for professional developments.

Like other genotyping studies, -α3.7 single gene deletion was found as the most common mutation (53%) (Keikhaei et al., 2018). Followed by, patients divided into 2 groups with the same -α3.7/αα (n = 646) and -α3.7/-α3.7 (n = 181) genotypes. This mutation has been reported as the most common mutation in other provinces of Iran, too. However, it has a higher frequency in the south of Iran than northern provinces, that may be attributed to poorly primary prevention strategies or prevalence of consanguineous marriages in tribal culture of these regions (Tamaddoni et al., 2009; Alibakhshi et al., 2015; Derakhshan et al., 2016; Eftekhari et al., 2017). Besides, both genotypes were more frequent in non-Arab population (60% in hetero group and 54.7% in homo group). As we know, the α-thalassemia mutations have different ethnic dispersion and detection the common mutations of any population will help with effective diagnosis and therapeutic policies (Tamaddoni et al., 2009; Onay et al., 2015). α-thalassemia mutations and its various genotypes have been widely studied in Iranian population, but the number of studies investigating the causes of different manifestations of patients with the same genotype is limited (Valaei et al., 2018).

At a glance, hematological findings and related diagnosis in the patients with -α3.7/αα and -α3.7/-α3.7 deletions were comparable with previous reports. Hetero group exhibited a milder degree of MCV, MCH, HbA2 and HbF parameters and RBC increase compared to the homo group that may be related to compensatory effects of normal α-globin gene (Kanavakis et al., 2000; Dehbozorgian et al., 2015). But as the results show, (Figs. 1 and 2) the severity of α-thalassemia phenotype and degree of anemia were variable in patients with the same genotype. These findings beside new different genetic variations from well-known mutations, suggest the role of the other factors in α-thalassemia pathogenesis that could challenge the recognizing of some patients (Higgs and Wood, 2008). The α-globin cluster is located close to the telomere of chromosome 16 and at the 30-70 kb upstream of the α-globin genes, promoters of ζ and α genes and 4 highly conserved noncoding sequences named MCS R1-R4 (multi-species conserved sequence) are involved in regulation of globin gene expression (Ribeiro and Sonati, 2008; Vernimmen et al., 2009). MCS regions carry the binding sites for erythroid transcription factors (GATA1, GATA2, SCL, NFE2, KLF) and MCS-R2 as the main element, plays a key role in chromatin looping and recruitment of RNA polymerase II to

Table 1 Hematological parameters

Alpha Mutation	Age	Hemoglobin	MCV	MCH	RBC	HbA2	HbF
-α3.7 Heterozygote	25.73±4.58	13.16±1.49	77.68±4.45	25.01±1.72	5.29±0.58	2.44±0.37	0.56±0.41
N = 646		9.8-18.5	59-88.5	16.2-30	3.95-7.15	1.4-3.3	0.0-2
-α3.7 Homozygote	26.9±4.44	12.31±1.51	71.68±3.82	22.22±1.43	5.54±0.64	2.33±0.38	0.51±0.35
N = 181		8-16.4	61.7-82.8	16.5-26.8	3.68-7.76	0.6-3.2	0.0-2.9

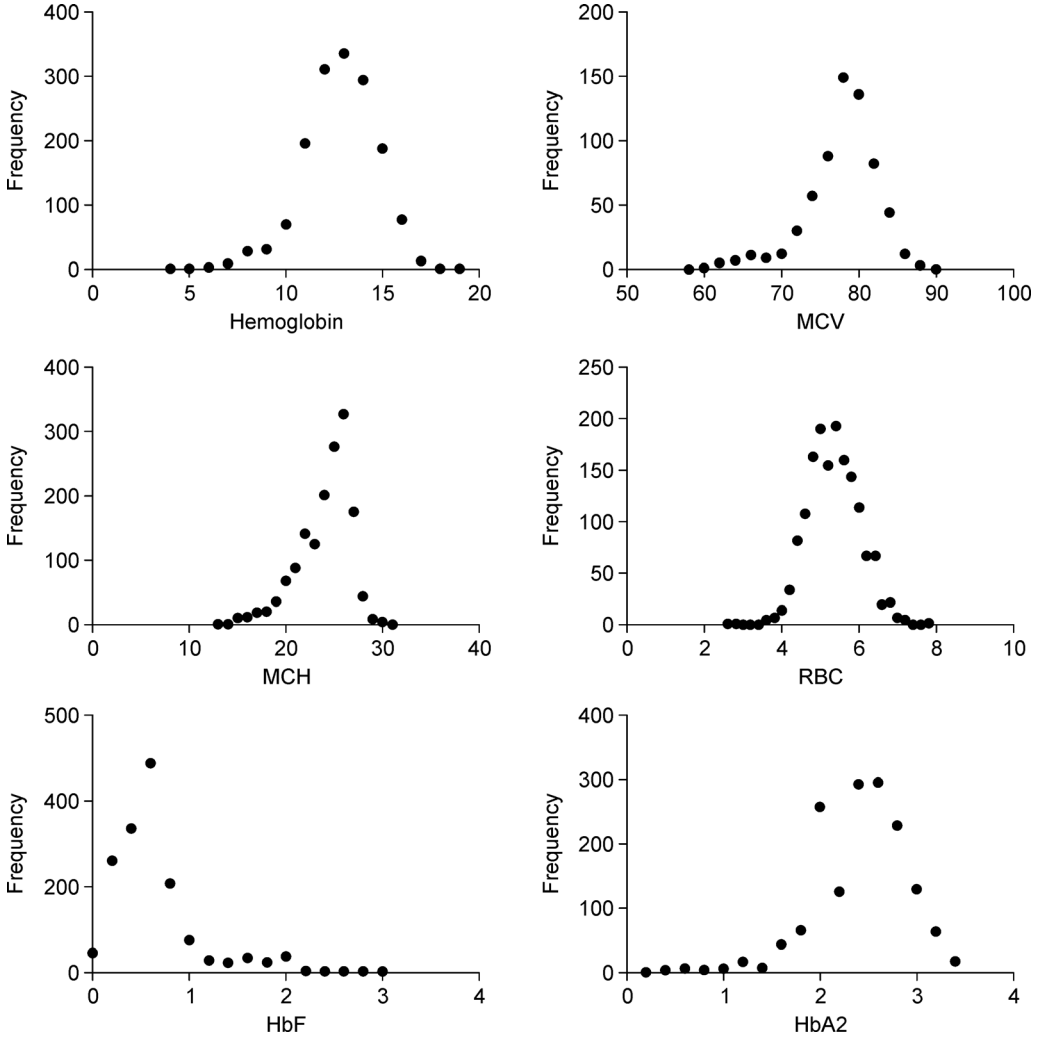

Figure 1 Hematological parameters distribution in patients harboring-α3.7/αα genotype.

the α-globin promoters (Higgs and Gibbons, 2010). Point mutations or deletions of the regulatory elements could affect the α-globin gene expression (Viprakasit et al., 2003). In this regard, a case of HbH disease due to the deletion of MCS-R2 (HS-40) in both chromosomes and MCS-R3 in one chromosome with all α-globin genes intact described in Maria Carla Sollaino study (Sollaino et al., 2010). The absence of MCS-R2 (HS-40) downregulates the expression of α-globin genes strongly, but non-completely abolishes (Coelho et al., 2010). It seems, in α-thalassemia caused by deletions of the MCS-R regions, MCS-R2 is the central regulatory element (Wu et al., 2017). In our study, 3 patients with the same heterozygous-α3.7/αα genotype showed minor α-thalassemia diagnosis, unexpectedly. Regulatory MCS regions specially MCS-R2 could involve in their α-thalassemia pathogenesis.

In addition to the above mentioned, sometimes such variations result from single nucleotide polymorphisms (SNPs). As reported in some α-thalassemia Melanesian patients, the α-globin genes and all of the upstream MCS elements were intact. Further analysis of the entire cluster revealed a SNP that created a functional GATA1 binding site. This new site binds to transcription factors and competes with natural α-globin chain production and could result in α-thalassemia (De Gobbi et al., 2006).

Erythroid transcription factors controlling α and β-globins gene expression interact with regulatory elements and could impress the α-thalassemia phenotype. In a study conducted by Yu et al., α-thalassemia (-α3.7/αα, -α4.2/αα, αws/αα) carriers with mutations on KLF-1 alleles, have a lower level of MCV and MCH, borderline HbA2 and increased HbF levels. Some of these patients may be evaluated as β^{+}-thalassemia wrongly (Yu et al., 2015). Also the results of Stefania Satta study were consistent with the previous study and highlighted that co-inheritance of KLF-1 mutations could modify the hematological figures of α-thalassemia (Satta et al., 2017).

Sometimes, mRNA splicing defects increase pathological conditions of α-thalassemia. RNA dependent protein kinas (PKR) regulates globin mRNA splicing through eIF2α phosphorylation. Mutations in eIF2A or PKR that prevent phosphorylation of the eIF2α or PKR activity, lead to reduced

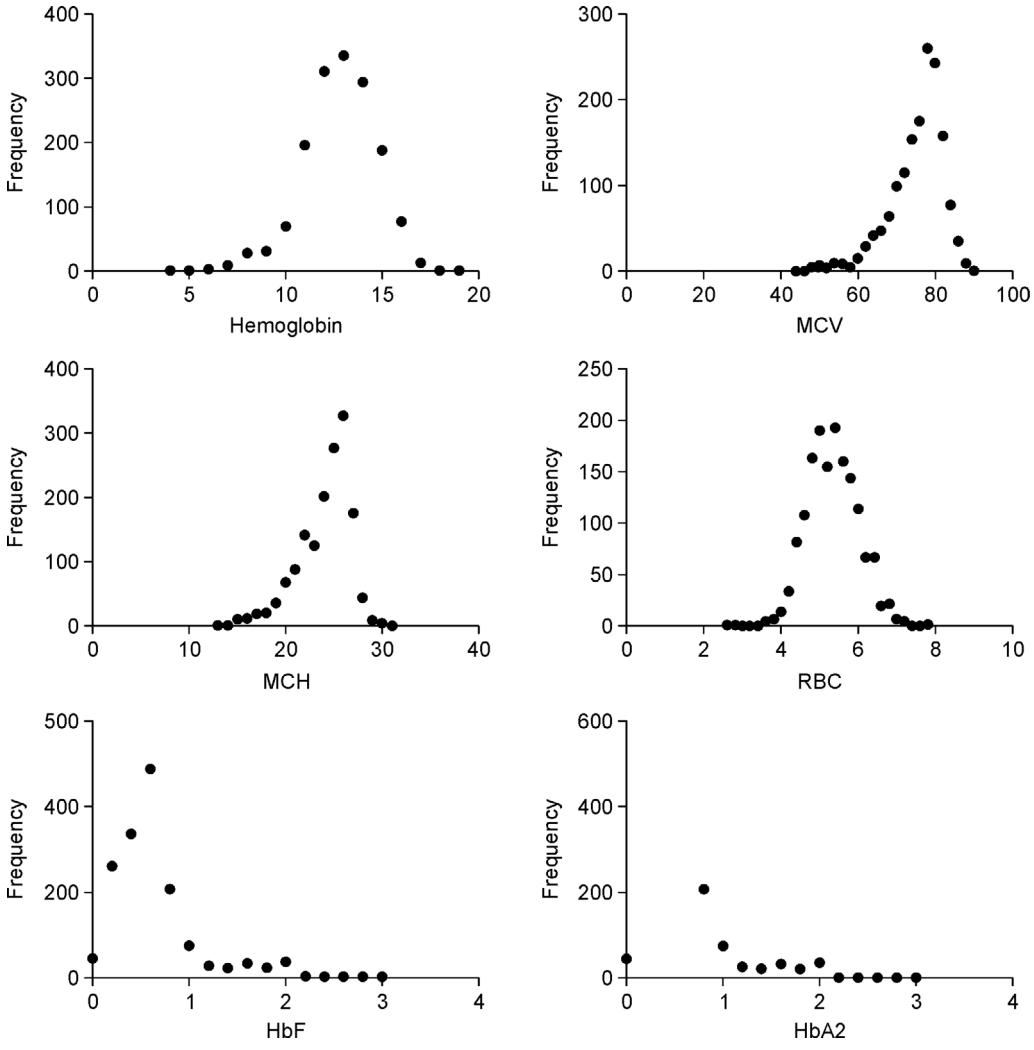

Figure 2 Hematological parameters distribution in patients harboring -α3.7/-α3.7 genotype.

mRNA splicing and translation (Ilan et al., 2017). Even unstable structural variants caused by mutations on α-globin gene that impair α-globin chains and AHSP (Alpha-Hemoglobin Stabilizing Protein) chaperones interaction or reducing the AHSP gene expression, decrease the folding and assembly of the α-globin subunits in hemoglobin molecule and provide pathological conditions for thalassemia formation (Wajcman et al., 2008).

Collectively, because of the most wide distribution of α-thalassemia and effects of α-globin deficiencies in clinical types and severity of hemoglobinopathies, the exact molecular definition and finding specific hematological features in α-thalassemia carriers, hopefully contribute to correct the genotype-phenotype correlation for an accurate screening, genetic counseling and offering new therapeutic targets.

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Ethics approval and consent to participate

The protocol is reviewed and approved by the Medical Ethics Committee of Ahvaz Jundishahpur University of Medical Sciences. All subjects gave informed consent to participate in the study. The authors declare that they have no conflict of interest.

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