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Original Article

A correlation analysis of the relationship between iron metabolism and HEF gene polymorphisms in thalassemia patients with different genotypes

Peng Huang, Jie Wang, Xianjun Gu, Meiyin Huang, Junhua Tan

Department of Nephrology, Affiliated Hospital of Youjiang Medical College for Nationalities, Baise 533000, Guangxi, China

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Abstract: The HEF gene mutation affects iron absorption, and its genetic polymorphism is correlated with multiple diseases. This study analyzed the correlation between HFE polymorphisms and the iron metabolism of thalassemia patients with different genotypes, to help thalassemia treatment. Renal anemia (group A), αron metabolism of thalassemia patients with different genotypes, to help thalassemia treatment. Renal anemia (group A) (n= 30). The serum levels of transferrin receptor (STfR), iron (SI), transferrin saturation (TSAT), ferroprotein (SF), and total iron binding capacity (TIBC) were measured. The PCR-RFLP approach was used to analyze the HEF gene polymorphism at the C282Y and H63D loci. Significant differences existed in the SF and TSAT levels among the four groups, with the highest level in the group B ($P < 0.05$), leaving no significant change in TIBC. STfR presented the lowest level in the Group B ($P < 0.05$). No mutation was found in the HEF C282Y genotype. Genotype frequency (CC, CG, and GG) at HFE H63D loci was 97%, 3%, and 0% in group C, 87%, 13%, and 0% in group A, and B% in group, and 0% in group B ($P < 0.05$). The C and G allele in the H63D loci showed a frequency of 97% and 3% in group C, 93.5% and 6.5% in group A and B wes 91% and 9% in group B3%. The H63D gene polymorphism was not correlated with SF or TIBC but was positively related to SI, TSAT and STfR ($r = 0.225$, 0.478 and 0.380, $P < 0.05$). Thalassemia patients with renal anemia have iron metabolic disorder. The H63D CG genotype frequency is positively correlated with SI, TSAT, and STfR.

Keywords: Thalassemia, Iron metabolism, HFE gene polymorphism

Introduction

Thalassemia is a commonly occurring, single gene mutation, autosomal recessive inheritance disease, with an equal risk among males and females. It severely affects human health and is caused by a mutation of the globin gene [1, 2], which leads to a decreased synthesis of the hemoglobin tetramer peptide chain, leading to an imbalance of the hemoglobin component. In clinics, thalassemia is mainly manifested as chronic and progressive hemolytic anemia, with different severities [3, 4]. Based on specific defects of the globin peptide chin, thalassemia can be further sub-divided into different types including α, β, δ b-d, and γδβδβδβδβid but it is most commonly divided into the t and β and ed monly to difthe gene mutations in type ent types inclare loss-of-function mutations, as point mutations in point mutationua small deletion or insertion. In molecular pathology, type d into different ta high heterogeneity [5, 6]. In severe thalassemia patients, due to chronic hemolytic anemia, the iron absorption by the intestines is remarkably enhanced. Multiple blood transfusions decrease the bio-availability of iron, plus the unregulated hemopoietic function of the body all leads to an excessive iron load, leading to complications, including pulmonary iron-hemoglobin deposition, hemochromatosis, or cardiac dysfunction, which severely affect patienthro quality of life. Some studies showed that the condition of iron load in thalassemia patients was closely correlated with their genotypes [7, 8].

The HFE gene is located in human chromosome 6p21.3, and it belongs to human leukocyte an-

tigen type I like genes. The HFE protein is mainly expressed in human digestive tract epithelial cells. Previous studies showed that HFE gene mutation affected iron absorption. The mutation of the HFE gene regulates the downstream expression of hepcidin, which is an important iron trafficking modulator. With the down-regulation of Hepcidin, iron protein absorption and plasma iron protein levels are elevated, aggravating body iron loading [9, 10]. The polymorphism of the HFE gene is mainly presented at the C282Y and H63D loci. Various studies showed that that HFE gene polymorphism is correlated with various diseases including tumors, liver disease, glucose metabolic disorder, coronary heart disease, hypertension and neurological disorders [11, 12]. Various clinical studies have shown the involvement of iron in multiple biological processes of the body, and the disruption of body iron homeostasis can lead to oxidative stress for further cell apoptosis and damage [13, 14]. Currently few studies have been performed on iron metabolism of thalassemia complicated with renal anemia and the regulatory mechanism of HFE gene, and no study has been conducted that reveals the correlation between iron metabolism of thalassemia plus renal anemia patients with different genotypes and polymorphisms of the HFE gene. This study thus investigated the relationship between the iron metabolism of thalassemia plus renal anemia patients of different genotypes and the HFE gene polymorphism, and it may help to illustrate the pathogenesis mechanism of anemia and iron metabolism disorder in order to provide evidence for the stipulation of iron metabolic disorder and anemia treatment, and it has critical implications for improving patients' survival rate and quality of life.

Materials and methods

Research objects

Single renal anemia (group A), type der and anemia trelicated with renal anemia (group A), type der and anemia trelicated with renal anemia (group B n= 30 each). The diagnosis of renal anemia followed the consensus of diagnosis and treatment criteria of year 2014. There were 15 males and 15 females (aged 21 to 65 years, average age = 44.9 ± 3.45 years) with renal anemia alone, 17 males and 13 females (aged 18 to 53 years, average age = 46.6 ± 4.68 years) having type agthalassemia complicated with renal anemia, 15 males plus 15 females (aged 23 to 62 years, average age = 45.2 ± 3.17 years) with type = 45.2 alassemia complicated with Inclusive criteria: (1) Older than 18 years old; (2) Has not received a blood transfusion and does not take iron supplement drugs; (3) Signed informed consents.

Exclusive criteria: (1) Evidence of recently active or insidious hemorrhage, including surgery, bleeding, or hepatic disease; (2) History of blood transfusion within the past four weeks; (3) History of a malignant tumor, severe liver disease, or chronic hypoxia; (4) Taking an erythrocyte stimulating agent (ESA) or other erythrocyte stimulating factor (iron supplement, folic acid or vitamin B12), or hormonal, immune-suppressant or antibiotics treatment. (5) Patients receiving hemodialysis, peritoneal dialysis, or a kidney transplantation. Another cohort of 30 healthy volunteers were recruited as the control group (group C) with comparable demographic parameters as the disease groups. This study obtained informed consents from all participants.

Equipment and reagent

High-speed centrifuge (Eppendorf, Germany); Automatic blood analyzer (SYSMEX, US); Microplate reader (BIOTEK, US); Automatic gel imaging system (SYNGENE, UK); Radio-immune reagent kit (Northern Biotech, China); Blood genomic DNA extraction kit (Saibaisheng Gene Tech, China); Restriction endonuclease (MBIFermtentas, US).

Assay for indexes

Hemoglobin assay: High performance liquid chromatography (HPLC) was used for the hemoglobin analysis to screen out those with abnormal globin peptide synthesis. The single-tube quadrant PCR-agarose gel electrophoresis approach was used for the gene assay of thalassemia in combination with reverse membrane hybridization to differentiate type c and β and reverse m.

Blood assay: About 2 mL of blood samples were collected from peripheral veins using vac-

uum tubes. The blood samples were sent to the hospital's central lab within 2 h of collection for the blood assay using a fully automatic blood analyzer.

Biochemical index assay: Serum iron (SI), serum ferritin (SF), total iron binding capacity (TIBC) and serum transferrin receptor (STfR) indexes were measured from the 5 mL peripheral venous blood samples collected in the vacuum tubes and were sent to our hospital's central lab within 2 h using a fully automatic biochemical analyzer and a chemiluminescence system. Transferrin saturation (TSAT, in %) = SI/TIBC.

HFE gene polymorphism assay

A total of 120 samples were genotyped for HFE gene polymorphisms at the C282Y and H63D loci using a PCR-restriction fragment length polymorphism (RFLP). PCR amplification was performed on those two target regions of the HFE gene using forward and reverse primers, and the products were analyzed using agarose gel electrophoresis, digested by restriction endonuclease, and then they were tested using agarose gel electrophoresis.

C282Y primer: forward, 5 sis. digested by restriction endonuclease, and was performed on those two target regions of utomatic gel imaging system (SYreverse, 5 using Radio-immune reagent kit) The PCR-RFLP results were analyzed by a UV illuminance apparatus.

Statistical analysis

The data analysis was performed using SPSS 20.0 software. The analysis for mutation frequency adopted the Hardy-Weinberg equilibrium (HWE). The genotype frequency in the disease and control group was tested for HWE using Ailequin software. An HWE was identified when $P > 0.05$. The enumeration data were presented as the meanration da deviation (SD), and one or multi-factorial analyses of variance (ANOVA) were used for comparisons between groups. A Spearman analysis was used for correlation elucidation. The unconditioned logistic regression approach was used to represent the odds ratio (OR) representing the relative risk. Statistical significance was defined when $P < 0.05$.

Results

Higher SI, SF, and TSAT and lower STfR levels in type ype SAT and lower STfR levels i

For SI, group B ype SAT and lower STfR levels in type represent, and group A ($P > 0.05$). In the comparisons involving SF and TSAT, group Balassemia with renal anemiased t the other groups ($P < 0.05$), followed by group BBoup Bmia with renal anemiased to regroup C ware. An HWE was ide the four groups. In the comparison involving STfR, group Bup BIn up BBoup Bmia with r the groups ($P < 0.05$), with higher levels sequentially in group Bd to regro, and group C (**Figure 1**).

HEF H63D gene polymorphism is positively correlated with SI, TSAT and STfR

No mutation was found in the HFE C282Y genotype, as all the samples only showed two bright bands after restriction digestion and electrophoresis, suggesting a normal homozygote at the C282Y loci with the G/G phenotype without a mutation (**Figure 2A**). The frequency of the three genotypes (CC, GG, and GG) at the HFE H63D loci was 97%, 3% and 0% in group C, 87%, 13% and 0% in group A and Bstion and e, and 0% in group B ($P < 0.05$). The C and G alleles at the H63D loci showed frequencies of 97% and 3% in group C, 93.5% and 6.5% in group A and B Bsand 91% and 9% in group Bp C (**Figure 2B**, $P < 0.05$). The polymorphism of the HEF H63D gene was not significantly related with SF or TIBC, but it was positively correlated with SI, TSAT, and STfR ($r = 0.225$, 0.478 and 0.380, $P < 0.05$, **Figure 2C**).

Discussion

Thalassemia is a single gene inheritance mutation and is presented as hemolytic anemia caused by the mutation or deletion of the globin gene within hemoglobin. In China, more than 8% of population are carriers of the type αype iers of e carriers of n are carriers of nted athe type e than 8% of popula [15, 16]. Renal anemia is a condition caused by impaired erythrocyte generation and metabolism secondary to kidney damage, and it's a commonly occurring complication at terminal stage kidney disease derived from chronic kidney dysfunction [17]. At the early stage of kidney dysfunction, renal anemia can occur and aggravates with

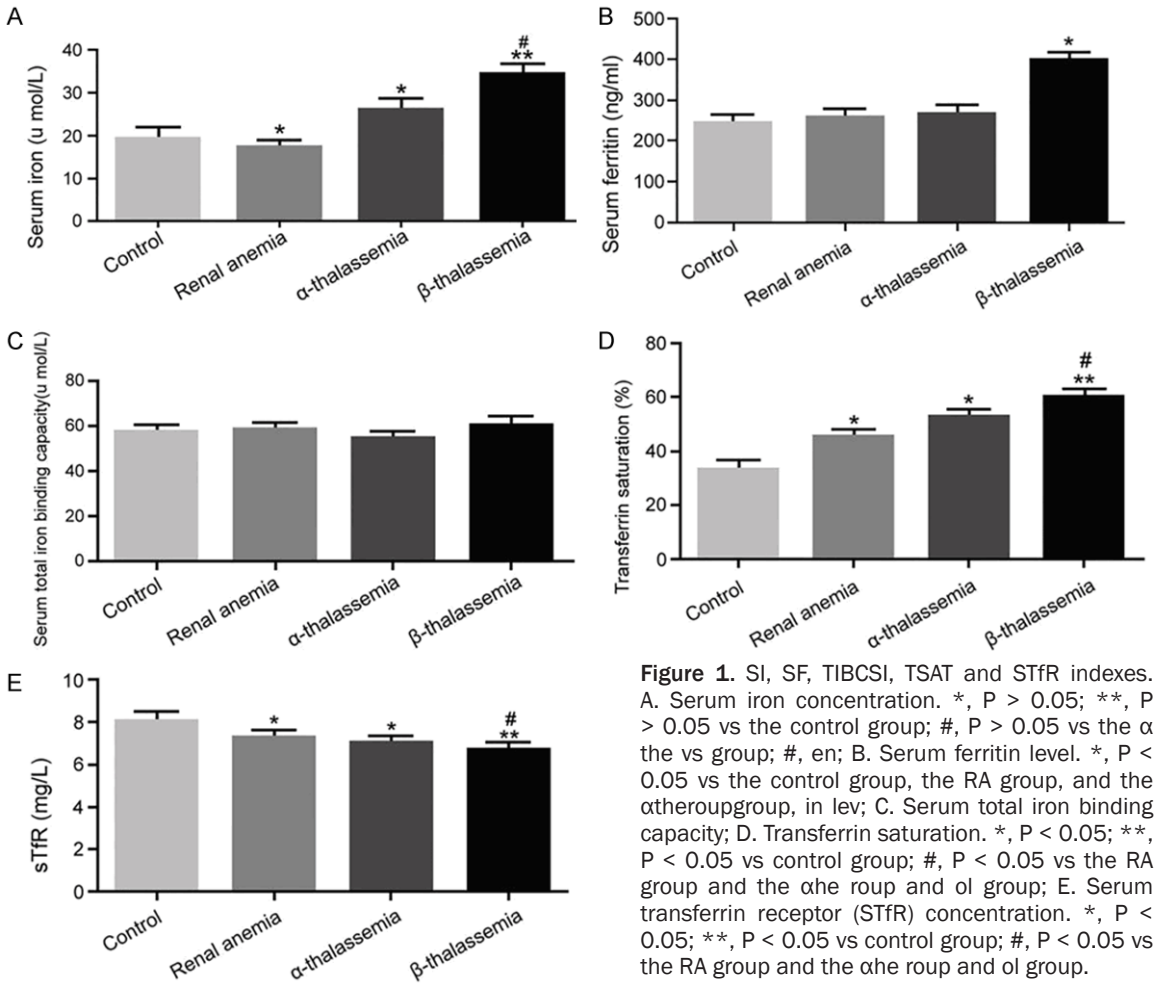

Figure 1. SI, SF, TIBCSI, TSAT and STfR indexes. A. Serum iron concentration. *, P > 0.05; **, P > 0.05 vs the control group; #, P > 0.05 vs the α the vs group; #, en; B. Serum ferritin level. *, P < 0.05 vs the control group, the RA group, and the αtheroupgroup, in lev; C. Serum total iron binding capacity; D. Transferrin saturation. *, P < 0.05; **, P < 0.05 vs control group; #, P < 0.05 vs the RA group and the αhe roup and ol group; E. Serum transferrin receptor (STfR) concentration. *, P < 0.05; **, P < 0.05 vs control group; #, P < 0.05 vs the RA group and the αhe roup and ol group.

progressive kidney failure. The severity of anemia is closely related to kidney function [18]. Iron is an important ingredient for synthesizing hemoglobin, and it's a component of myosin and cytochrome. During erythrocyte generation, metabolism, and DNA synthesis, iron plays important roles. When the body undergoes iron overload, serum transferrin is saturated to produce an unbounded from of free iron. The plasma has strong a reduction potency of iron, leading to lipid peroxidation, DNA strand breakage, genome instability, and tissue oxidative stress, thus causing clinical symptoms including skin pigmentation, liver fibrosis, heart failure, and endocrine dysfunction [19, 20]. In thalassemia patients, due to their chronic hemolysis and consequently excess iron load, iron removal is usually required in clinics. Iron metabolic disorder in renal anemia is mainly manifested as lower serum iron, plus normal or elevated ferritin levels, which is correlated with body iron deficiency, iron storage, and inflammatory disease. In thalassemia with renal anemia, complicated situations exist for the pathogenesis of anemia and iron metabolism. The HFE gene mutation may affect iron absorption. As an important regulatory factor for body iron transporting, hepcidin can bind with transferrin to inhibit the dissociation of iron from macrophage, intestinal mucosa and other cells to the plasma. HFE gene mutation can regulate downstream hepcidin expression. When Hepcidin expression is suppressed, ferritin absorption and plasma ferritin levels are elevated, leading to excess body iron load. The HFE gene polymorphism is mainly manifested at C282Y and H63D. Various studies have shown that the HFE gene polymorphism is correlated with multiple diseases [21, 22]. This study analyzed the gene polymorphism profiles plus iron metabolism among thalassemia patients complicated with renal anemia having different genotypes to provide evidence for correcting iron metabolic disorder and anemia treatment.

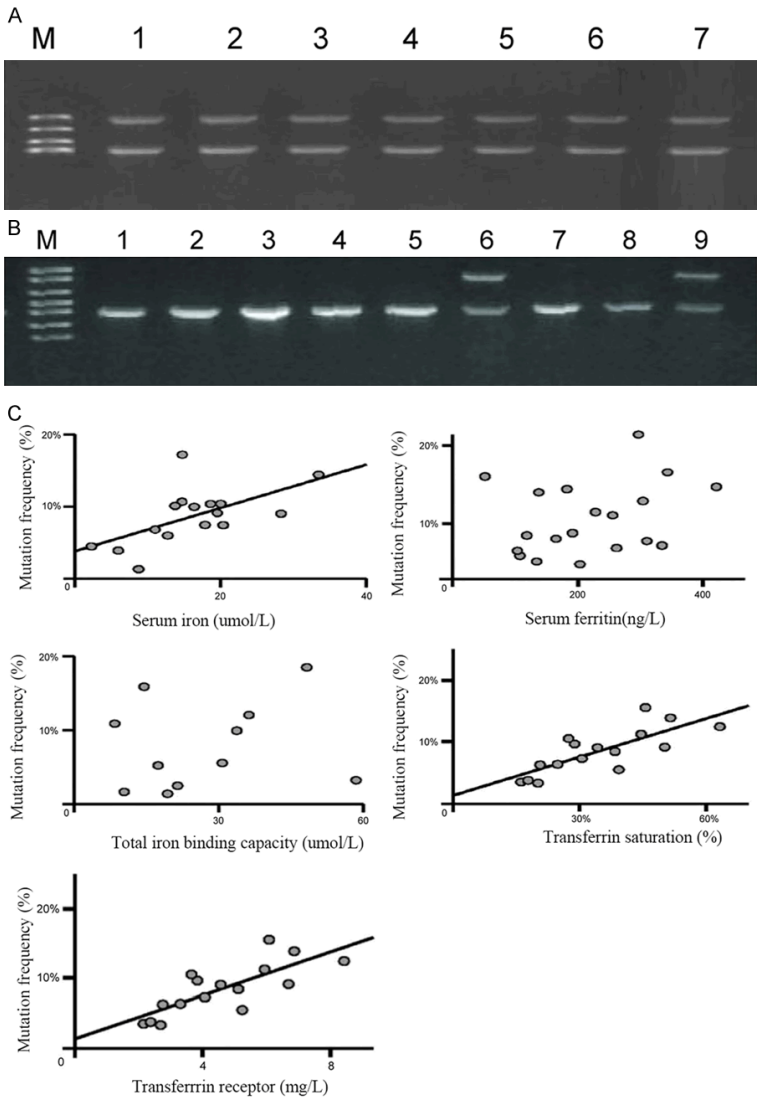

Figure 2. HFE gene polymorphism and iron metabolism of thalassemia patients with different genotypes. A. Electrophoresis band of the HFE C282Y gene; B. Electrophoresis band of the HEF H63D gene. Remarks: Amplification fragment of samples from normal people showed a single Mbo I enzyme digestion site. When the H63D mutation occurs, this site vanishes and cannot be digested. When amplification products were enzymatically digested, two fragments indicated a mutant heterozygous CG genotype, and one fragment indicated a wild type homozygous CC genotype or mutant homozygous GG genotype. Tested samples were enzymatically digested and underwent electrophoresis to visualize two bright bands, and the remaining samples showed one single band, suggesting that most samples were of the H63D wild type, and a few samples were of the heterozygous mutant type; C. Correlation between the HFE H63D gene polymorphism and the SF, TIBC, SI, TSAT, and STfR indexes.

The results of this study showed the existence of different conditions of iron metabolic disorder in thalassemia with renal anemia patients. Among those, type β type of this stua more severe condition of iron metabolic disorder compared to patients with renal anemia alone or to patients with type nts with iron metabolic disorde, as it mainly presented as iron overload. In clinics, long-term follow-up is required for evaluating the iron loads of such patients. However, a routine assay for serum iron or ferritin cannot give the full picture of iron deposition of a specific tissue or organ, and other indexes including transferrin saturation and serum transferrin receptors should be monitored. During the follow-up of these patients, we should keep a close eye on the heart, liver, kidneys, and endocrine (pituitary, gonad hormone and thyroid hormone) functions and try to make an early diagnosis before organ injury caused by ironoverloadanddepositionoccurs, and to initiate iron removal treatment. Some studies found that the HFE gene mutation can enhance serum ferritin and transferrin saturation, and H63D mutant patients presented significantly higher TSAT values than non-carriers, and 90% of C282Y homozygous mutant patients presented TSAT values over 55% [23]. In this study, no C282Y mutation was found among the 120 adult patients. Among the H63D mutant individuals, all of them belonged to the C/G heterozygous mutant group, without the G/G homozygous mutant. We further investigated the iron metabolic indexes using the H63D genotype with the highest frequency in type he iron metabolic i the renal anemia groups. The results showed no correlation between the H63D gene polymorphism and ferritin, or the total iron binding capacity H63D gene polymorphism was positively correlated with serum iron, transferrin saturation, and the serum transferrin binding capacity, consistent with previous reports [22, 23]. These results sug-

gest that such populations of the H63D mutant may participate in the dynamic regulation of the SI, TSAT, and STfR indexes.

This study for the first time investigated body iron metabolism inside the bodies of thalassemia patients with renal anemia, and the relationship between HFE gene polymorphisms and iron metabolism. However, this study did not have a sufficient sample size, and it had no patients who were receiving kidney replacement therapy. A larger sample size is thus required for a detailed description of all patient groups. In contrast to multiple factors, including age, ethnic group, relatives, and drug history, the correlation between iron metabolic features at different stages of chronic renal disease should be investigated in order to better illustrate the disease pathogenesis mechanism for the optimization of an effective treatment plan.

Conclusion

Thalassemia patients complicated with renal anemia all have iron metabolic disorder to different degrees. Type β ifferent degrees. Type plicated with renal anemia almore severe iron metabolic disorder than renal anemia alone or type emia c disorder than ed with renal anemia all have iron metabolic disorder to different degrees. The HFE gene. At the H63D loci, a polymorphism of the HFE gene was found among normal people, renal anemia patients, and thalassemia with renal anemia patients. The elevated CG genotype ratio at H63D of the HFE gene was positively correlated with serum iron, transferrin saturation, and serum transferrin receptor.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jie Wang, Department of Nephrology, Affiliated Hospital of Youjiang Medical College for Nationalities, No. 18 Zhongshan Second Road, Baise 533000, Guangxi, China. Tel: +86-0776-2855284; Fax: +86-0776-2855284; E-mail: yyfywj1@163.com

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