Original Article

Variation of DNA Repair Genes *APE1* and *RAD18* in β-Thalassemia Patients

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Abstract

Background: In the last decade, beta-thalassemia (β-thalassemia) has been recorded in high percentages among the Iraqi population and this disorder has a deficiency in β-globin chains causing striking heterogeneity of molecular disorder. The study subjects' characteristics showed nonsignificant differences between age, body mass index, and the percentage of gender between patients and control. **Objectives:** The objective of the study was to detect the apurinic/apyrimidinic endonuclease enzyme (*APE1*) Asp148Glu (rs3136820) and *RAD18* Arg302Gln (rs373572) in β-thalassemia patients. **Materials and Methods:** A case–control study was conducted, which included β-thalassemia patients. Thirty acute myeloid leukemia patients attended the Hematology Consultation Clinic and were diagnosed by a specialized hematologist belonging to disease criteria. The control group included 28 healthy individuals, blood samples were collected with approval from each contributor, and ethical approval was given according to the Ministry of Environment and Health in Iraq. **Results:** A significant elevation in iron and ferritin level were observed in the patient group ($P = 0.000$) the *RAD18* genotypes showed nonsignificant differences between Gln/Gln and other genotypes, and between Gln/Arg and Arg/Arg, in allele frequency, there was nonsignificant difference also, the Gln/Arg genotype frequent in slightly changes in patients and control group, Arg/Arg did not observe in the control group. The *APE1* genotype showed that GG and TT were more frequent in patients compared with the control group, GT was more frequent in the control compared with the patient group, and all changes were nonsignificant. The allele frequency showed that the T was more frequent in the patient group with nonsignificant differences. **Conclusion:** From this study, we can conclude there was a nonsignificant association between *APE1* and *RAD18* at the single-nucleotide polymorphisms *RAD18* Arg302Gln (rs373572) the *APE1* Asp148Glu (rs3136820).

Keywords: *APE1*, β-thalassemia patients, DNA repair genes, *RAD18*, variation

Introduction

Beta-thalassemia (β-thalassemia), is a process of erythropoietic alteration, which causes missed or reduced β-globin chains synthesis leading to over synthesis of free α-globin chains in the erythroid cells. The changes in these chains like aggregation, degradation, and denaturation result in insoluble molecules causing red blood cell (RBC) membrane damage, the common consequences of this disease are iron overload according to ineffective erythropoiesis and premature hemolysis in the major organs like the heart, liver, and endocrine glands.[1,2] In the last decade, β-thalassemia has been recorded in high percentages among the Iraqi population and this disorder has a deficiency in β-globin chains causing striking heterogeneity of molecular disorder.[3,4] Despite some molecular defects contributing to the structural β gene directly, some gene down-regulation by distal *cis* impacts and rare *trans*-acting mutations have been observed. The inherited β-thalassemia is sometimes based on the Mendelian recessive fashion or as a dominant negative (unraveling the molecular basis of β-thalassemia has explained a paradigm for clarification of some of human genetics.[5]

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The base excision repair is a pathway used for deoxyribonucleic acid (DNA) lesion correction and it is also involved in DNA, which is stimulated by reactive oxygen species and reactive nitrogen species. One of the base excision repair enzymes is apurinic/apyrimidinic endonuclease enzyme (*APE1*),[6] which splits the 5′ phosphodiester bond, production 3′OH, and 5′ deoxyribose phosphatetermini in three ways, three ways used by *APE1* to generate 3′OH terminus for priming and the amino acids that necessary of these functions were clarified by different studies.[7]

RAD18 is a conserved enzyme that has numerous functions, its E3 ubiquitin ligase regulates some pathways of DNA repair and damage tolerance like homologous recombination, postreplication repair pathways, break-induced replication, and Fanconi anemia.[8,9] *RAD18* is involved in the instability of the genome, driven by controlled the ubiquitination of the primary *RAD18* molecules, proliferating cell nuclear antigen, which represented as a ring-shaped homotrimer encircling DNA as a sliding clamp component of the replisome.[10,11]

Materials and Methods

Study subjects

A case–control study was conducted, which included β-thalassemia patients. Thirty acute myeloid leukemia patients attended the Hematology Consultation Clinic and were diagnosed by a specialized hematologist who belonged to the disease criteria. The control group included 28 healthy individuals, blood samples were collected with approval from each contributor, and ethical approval was given according to the Ministry of Environment and Health in Iraq. DNA was isolated by FavorPrep™ Whole Blood RNA Mini Kit, the primers were selected for *RAD18* Arg302Gln (rs373572) detection: "F1-ATACCCATCACCCAT CTTC and R1-GTCTTCTCTATATTTTCG ATT TCT T for the Gln allele (146 pb) and F2-TTAACAGCTGCTGAAATAGTTCG and R2-CTGAAATAGCCCATTAAC ATACA" for the Arg allele (106 bp). Common band A 206 bp was detected by allele-specific polymerase chain reactionand the *APE1* Asp148Glu (rs3136820) was detected by "F1-CCTACGGCATAGGTGAGACC and R1-TCCTGATCATGCTCCTCC and F2-TCTGTTTCATTTCTATAGGCGAT and R2-GTCAATTTCTTCATGTGC CA." The G allele 167 bp, T allele 236 bp, and 360 bp common band at 60°C[12] amplification results were vision by electrophoresis (1% agarose gel, 100 V, 20 mA for 40 min), then gel staining was done with ethidium bromide.

Ethical approval

The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee in Babylon University/College of Medicine, Babylon, Iraq, according to document number 12 on May 8, 2023, to get this approval.

Results

The study subject characteristics showed nonsignificant differences between age, body mass index, and the percentage of gender between patients and control, significant elevations were observed in iron and ferritin ($P = 0.000$) [Table 1].

The present study focused on the DNA repair gene variation in β-thalassemia patients, the *RAD18* genotypes showed nonsignificant differences between Gln/Gln and other genotypes, and between Gln/Arg and Arg/Arg, in allele frequency, there was a nonsignificant difference also [Figure 1], the Gln/Arg genotype frequent in slightly changes in patients and control group, Arg/Arg did not observe in the control group.

The *APE1* genotype showed that GG and TT were more frequent in patients than control group, and GT was more frequent in the control compared with the patient group. All changes were nonsignificant. The allele frequency showed that the T was more frequent in the patient group with nonsignificant differences [Figure 2].

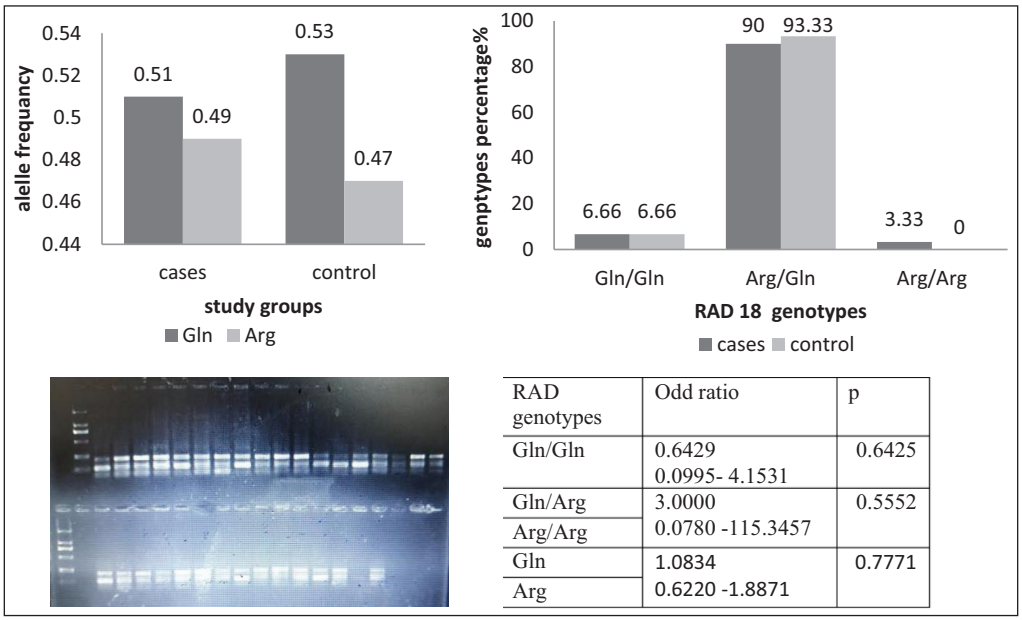
Discussion

The present study deals with (β-thalassemia) patients who are characterized by several clinical features including pallor poor, weight changes, and stunted growth. The lab findings were the absence of iron deficiency increased ferritin level, severe microcytic hypochromic anemia with anisopoikilocytosis, and nucleated RBCs on peripheral blood smear.[13] β-Thalassemia disorder has been reported in a high percentage in Iraq in recent years, some reports deal with this disease.[14] In 2017, the thalassemia prevalence in Iraq was a bit elevated despite low incidence. The carriers screening, intensified premarital screening, and counseling programs coupled with robust legislation are used in other limitation incidence rates,[15] whereas cases with β-thalassemia in Iraq, have been observed in a lower age and low adequately transfused

Table 1: Study subject characteristics (mean ± SE, independent sample *t* test, *P* value 0.05)

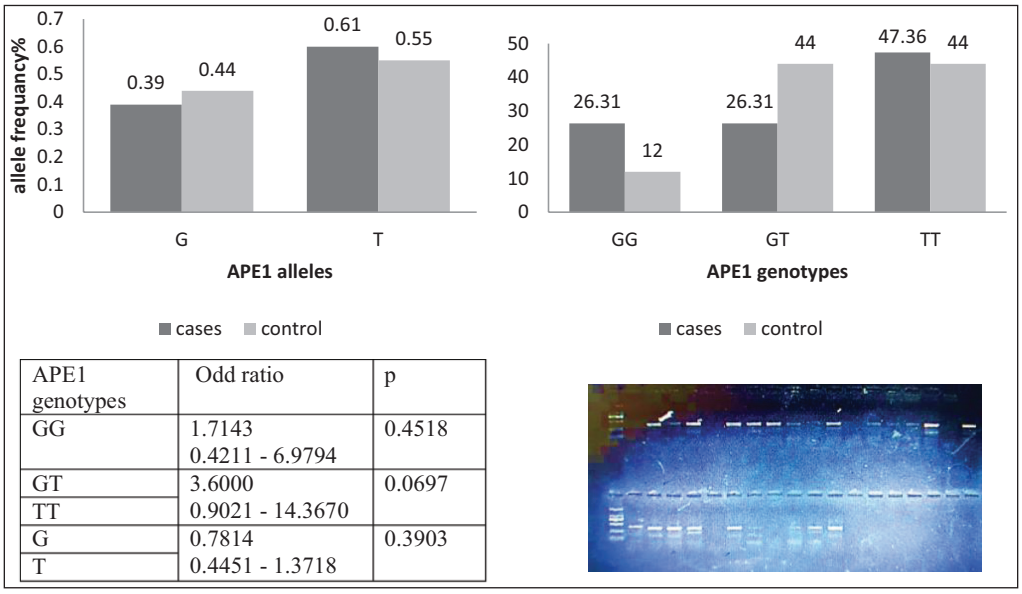
Subjects	β-Thalassemia	Control	*P* value
Age	23.90 ± 1.51640	22.3125 ± 1.606	0.480
BMI	23.235 ± 0.65740	25.32 ± 0.909	0.065
Iron level	191.55 ± 2.77	86.00 ± 5.419	0.000
Ferritin level	3543.30 ± 471.43	57.43 ± 12.07	0.000
Sex			
Male (%)	42.85	56.66	0.3316
Female (%)	57.14	43.33	

BMI: body mass index



RAD genotypes	Odd ratio	p
Gln/Gln	0.6429 0.0995- 4.1531	0.6425
Gln/Arg	3.0000 0.0780 -115.3457	0.5552
Arg/Arg		
Gln	1.0834 0.6220 -1.8871	0.7771
Arg		

Figure 1: The *RAD18* genotypes analysis in the study groups, electrophoresis pattern of Arg302Gln (rs373572) amplification products, histogram of alleles frequency, genotypes percentage in the study groups, and the statistical analysis of allele frequency



APE1 genotypes	Odd ratio	p
GG	1.7143 0.4211 - 6.9794	0.4518
GT	3.6000 0.9021 - 14.3670	0.0697
TT		
G	0.7814 0.4451 - 1.3718	0.3903
T		

Figure 2: The *APE1* genotypes analysis in the study groups, electrophoresis pattern of Asp148Glu (rs3136820) amplification products, histogram of alleles frequency, genotypes percentage in the study groups, and the statistical analysis of allele frequency

with more prominent consequent skeletal alteration and growth retardation.[16] The association of DNA repair gene variations and β-thalassemia were poorly studied, thus the present study was suggested as one of the serious reports that study DNA repair genes that may be affected in β-thalassemia patients. Apurinic/apyrimidinic endonuclease is a protein that has more than one function that contributes to oxidative stress responses, using endonuclease activity to act on both alkylative and oxidative DNA damage[17] and increase various transcription factors activities by redox signaling,[18] like in RNA clearance from damaged bases.[19] The association between β-thalassemia and *APE1* Asp148Glu (rs3136820) gene variation was nonsignificant in the present study but the role of this gene has been important to oxidative stress impacts, despite the primary etiology of thalassemia is not related to oxidative stress but its mediated several disease pathologies. The degradation of unstable hemoglobin and iron overload are the main reasons for oxidative stress that stimulates free radicals overexpression.[20] The oxidative stress aggravated symptoms include increment, ineffective erythropoiesis, and vital organs failure like liver and heart, in addition to external and internal such as genetic makeup, physical activity, health conditions, age,

nutrition, and the environment, such as air pollution and radiation. In addition, oxidative stress is enhanced by the disease clinical manifestations like unpaired globin chains, iron overload, and anemia.[21] The relation of *RAD18* with β-thalassemia was not observed in the present study at least with the Arg302Gln (rs373572) single-nucleotide polymorphism (SNP), in the previous literature, there was poor information about this association but it deals with the role of DNA rapier With oxidative stress and DNA damage.[22] The present study needs further investigations to prove the role of repair in blood disorder diseases like β-thalassemia and other SNPs in *APE1* and *RAD18* genes.

Conclusion

From this study, we can conclude there was a nonsignificant association between *APE1* and *RAD18* at the SNPs *RAD18* Arg302Gln (rs373572) and the *APE1* Asp148Glu (rs3136820).

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Nil.

Conflicts of interest

There are no conflicts of interest.

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