MOLECULAR DETECTION OF IVSINT.6 MUTATION ASSOCIATED WITH β-THALASSAEMIA IN IRAQI POPULATION

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ABSTRACT
This study was conducted in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad from August 2007 to March 2008, it is an attempt to determine ethnic distribution of IVSInt.6 mutation associated with β-thalassaemia in Iraqi population. Ninety EDTA blood samples of clinically thalassaemic patients including Arab, Turkman and Kurd were collected from two thalassaemic centers in Iraq. Also blood sample from 30 apparently healthy individuals were collected as a control group. The DNA samples were subjected for molecular detection of IVSInt.6 β-thalassaemia mutation by Amplification Refractory Mutation System (ARMS) based on Polymerase Chain Reaction (PCR). It was found that the ethnic specificity of the studied mutation were reported for the first time within Iraqi population. The ethological distribution of the positive diagnosed mutation showed that the studied mutation was detected only in 10(33.33%) Turkman patients, while it was not detected in Arab and Kurds, which indicated that their was differences between them.

Key words: β-thalassaemia, Molecular detection, ARMS-PCR, Iraq.

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الكشف الجزيئي عن الطفِرة VI Sin.6 المرتبطة بمرض بيتا-ثالاسيميا في المجتمع العراقي

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التغطية

أجريت هذه الدراسة في معهد الهندسة الوراثية والتقنيات الإجبارية للدراسات العليا لمدة من آب 2007 ولغاية آذار 2008 وهي محاولة لتحديد التوزيع العراقي لأحد الطفّرات المصاحبَة لفقد دم البحر الأبيض المتوسط (ثالاسيميا- بيتا) في المجتمع العراقي. جمعت عينات الدم في أنابيب تحتوي على مائع للتخثر من 90 مصاب سريرياً بالثالاسيميا- بيتا (عرب وتركمان وكرد) من مركز ثالاسيميا في العراق في بغداد وكركوك. جمعت عينات الدم أيضاً من 30 شخص من الأصحاب ظاهريَّاً كمجموعة سيطرة. تم استخلاص الحمض النووي وأجري التشخيص للطفّرة المصاحبة للثالاسيميا- بيتا على الحمض النووي باستخدام تقنية ARMS-PCR. بُنيت الدراسة على الخصوصية العراقية التكمانية للطفّرة التي تم دراستها قد سجلت لأول مرة في العراق. وأظهر التوزيع العراقي للطفّرة المشخصة أن 10 مرضى (33.33%) من التركمان مصابين بهذه الطفّرة بينما لم تسجل الطفّرة في كلاً من العرب والكرد مما يشير إلى الاختلاف العرقي بينهم.
INTRODUCTION

Beta-thalassemia is the most common autosomal recessive single gene disorder of haemoglobin synthesis(1). Over 200 mutations have so far been reported in beta-globin gene(2). The spectrum of the disease varies in different populations but fortunately each population at risk has its own set of common mutations. Different ethnic groups have been found with different types of molecular defects. Many social factors such as a preference to marry within ethnic groups and consanguineous marriages have contributed to increase incidence of disease(3,4). Programs based on awareness, carrier screening, genetic counseling and prenatal diagnosis can be very effective in preventing the disease(5). The aim of this study was to determine the frequency of (IVSInt.6) beta-thalassemia mutation in different Iraqi ethnic groups.

MATERIALS AND METHODS

This study was carried out in period between 1 August 2007 to 30 March 2008 in the University of Baghdad, Genetic Engineering and Biotechnology Institute for Postgraduate Studies. A total of 120 EDTA blood samples were collected randomly (except ethnicity and number) from three ethnic group of Iraqi population include Arab, Turkman and Kurd, and 2 major thalassaemia centers (Ibn Al-Balady in Baghdad and Azadi in Kirkuk). The total number of patients group was 90(75%) include Arab 30(25%), Turkman 30(25%) and Kurd 30(25%), while it was 30(25%) for control group distributed as 10(8.33%) for each ethnic group. Genomic DNA was extracted from blood leukocytes using wizard genomic DNA purification kits. The isolation of DNA was based on salting out method(6). The selection of IVSInt.6 mutation based on its distribution as a common mutation of β-thalassaemia in Turks. Mutation detection was carried out by Amplification Refractory Mutation System (ARMS) which based on Polymerase Chain Reaction (PCR) using a specific set of primers (common, mutation, normal and internal control primers). PCR mixture contained 1μg of genomic DNA, 2.5μl(10X) PCR buffer, 50mM MgCl2; 40mM dNTP mixture, 13.5μl dionized water, 0.2μl (5unit/μl)Taq DNA polymerase and 1μl(10 picomols/μl) from common, internal control and either normal or mutant primers. One PCR cycle of 93°C for 3min. (initial denaturation) followed by 25PCR cycles of 93°C for 15sec. (denaturation), 63°C for 30sec. (annealing) and 72°C for 1.5min. (extension), then followed by 1 PCR cycle of 93°C for 1min. (denaturation), 63°C for 1min. (annealing) and 72°C for 3min. (final extension). Spectrum of IVSInt.6 mutation was analyzed in the three different ethnic groups.

RESULTS AND DISCUSSION

The internal control band 861 bp was observed in all samples. An amplified PCR product of M.W. 286bp was observed in 91 PCR reactions (80 normal, 9 homozygous and 1 heterozygous patients). The presence or absence and the position of the band reflected the genotype of the patients (Fig.1).
In this study IVSInt.6 β-thalassaemia mutation was found in 10 (11.11%) of the patients, while no mutations were detected in control group. The all 10 positive diagnosed patients with this mutation were Turkman (33.33%), while no mutation was detected in Arab or Kurd patients. Statistical analysis showed a significant differences at (p<0.01) in frequencies of β-thalassaemia in both patients and control groups.

IVS1nt.6 mutation reported as a mutation of Eastern Mediterranean (taking in consideration the position of Turkey and Cyprus in this area) (3,7,8). Huisman et al. (9); Altay and Gurge (10) were describe the IVS1nt.6 as the second most frequent mutation in Turks (14.75%).

The results of present study indicates that IVS1nt.6 was Turkman specific in Iraqi population because it was not detected in Arab and Kurd. The study indicates that there is an inheritance relationship between Iraqi Turkman and Turks. It was concluded that each ethnic group has its own spectrum of mutations.

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REFERENCES


