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A New Method To Detect IDH1 Synonymous SNP 105 C >T rs11554137

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Abstract

Alteration of cellular metabolism is one of the oncogenetic mechanisms. Furthermore, the defect of isocitrate dehydrogenase (*IDH*) enzymes lead to abnormal cellular metabolism. Mutation in the *IDH1* gene is common in glioblastoma multiforme (GMB), glioma, and acute myeloid leukemia (AML). The *IDH1* single nucleotide polymorphism (SNP) in codon 105 C>T rs11554137 is a prognostic factor in patients with glioma and AML. Currently, the most common technique used for *IDH1* SNP 105 C>T detection is the sequencing technique that expensive and time-consuming. We designed a new method through the Restriction Fragment Length Polymorphism (RFLP). Ninty Eight unrelated healthy volunteers comprising 49 males, and 49 females, which were randomly selected. Their ages ranged from 1 to 64 years with a median age of 33 years. Polymerase chain reaction (PCR) and *R*saI fragmented *IDH1* SNP105 C >T were identified in 16.3% of our cases. So we concluded that we may use the PCR-RFLP assay instead.

Keywords: *IDH*, SNP, RFLP, AML, Glioma

1. Introduction

Isocitrate dehydrogenase (*IDH*) enzyme encoded from *IDH* gene is one of the β-decarboxylating dehydrogenase family of enzymes which can convert the isocitrate to α-ketoglutarate (α-KG) in the tricarboxylic acid (TCA) cycle. This conversion is a vital biochemical pathway for the production of amino acids, nucleotides, and lipids [1–3]. *IDH* enzymes have three isoforms with different subcellular localization in mammalian cells: the cytosolic *IDH1* enzyme and mitochondrial *IDH2* and *IDH3* enzymes [1, 3–7]. The location of *IDH1* gene is on chromosome band 2q33.3 [3] and its SNP located in codon 105 in exon 4 [8].

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IDH mutations are usually heterozygous with one wild-type allele and one mutant allele [8]. Furthermore, they are common mutations in patients with low-grade gliomas and secondary glioblastoma. It is also found in de novo AML, secondary AML (sAML) [9], cholangiocarcinomas, in both benign and malignant central cartilaginous tumors [10], angioimmunoblastic T cell lymphomas, thyroid cancer, colorectal cancer, and prostatic cancer [11]. *IDH1* synonymous SNP, representing a GGC to GGT conversion at the glycine residue 105 [12] is associated with bad prognosis in patients with CN-AML [8, 13], AML with $NPM1^{-}$/$CEPB\alpha^{-}$ mutations [14] and in Glioblastoma multiforme GMB [15].

Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) is a widespread technique for genetic investigations. It has been used for the detection of interspecies and intraspecies variations [16]. PCR-RFLP technique has many advantages including inexpensiveness and does not require advanced instruments or an extensive training of laboratory staff. The technique is also applicable for the analysis of single nucleotide polymorphisms as well as the availability for all molecular biology laboratories and it is easy to design [16].

2. Materials and Methods

2.1 Subject recruitment

Ninety-eight unrelated healthy volunteers comprising 49 males and 49 females were selected from blood donors. Their ages were ranged from 1 to 64 years with a median age of 33 years. All volunteers or their guardians for this study have signed a consent. PCR-RFLP was used for the assessment.

2.2 Analysis of *IDH1* SNP105 C>T rs11554137 by PCR-RFLP

DNA was extracted from EDTA anti-coagulated blood samples from normal volunteers by using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Genomic DNA was amplified specifically for exon 4 of *IDH1* gene, using forward primer: 5'- CCAAGGATGCTGCAGAAGC- 3' and reverse primer: 5'-CCCCATAAGCATGACGACC-3'. PCR was performed in 20 µl reaction mixture containing 10 µl ready to use 2X master mixed solution (i-Taq) (Intron Biotechnology, Inc, Korea) containing dNTPs, 25 mM MgCl2, PCR-buffer, i-Taq DNA polymerase and DNA loading dye, 100 ng/dl of genomic DNA, 0.4 µl of 10 pmol/µl forward primer and 0.4 µl of 10 pmol/µl reversed primer (Invitrogen - Thermo Fisher Scientific, Inc.) and the total volume was completed with nuclease-free distilled water. PCR condition was as follows: initial denaturation at 95°C for 150 seconds, followed by 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds followed by final extension step at 72°C for 10 minutes. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. Nebcutter V.2-0 application (http://www.labtools.us/nebcutter-v2-0) was used to define suitable restriction enzyme for cutting variant type at the correct site of the SNP. PCR products were digested with *R*saI enzyme (Fermentas - Thermo Fisher Scientific, Inc.), the reaction was done by addition of 10 µl PCR product, 20 µl reaction master mix containing (17 µl nuclease-free distilled water, 1 µl *R*saI FastDigest enzyme and 2 µl FastDigest 10X buffer) and incubated at 37°C for 60 min instead of 5 min to be sure the digestion is completely done.

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2.3 Statistical analysis

Data were analyzed using the SPSS statistical package for social science version 16 (SPSS, Inc., Chicago, IL, USA). Median and range as appropriate were determined to represent numerical data. Frequency and percentage were used to express qualitative data. T-test was used for comparison of quantitative parametric data and Chi-square test for categorical variables. P value was set at 0.05 level.

3. Results and Discussion

3.1 Detection method of *IDH1* SNP 105 C >T rs11554137

The amplified product of *IDH1* gene produced a band at 240 bp (Figure 1). Restriction Fragment Length Polymorphism (RFLP) is a new method used to identify the *IDH1* SNP 105 C>T, where the digestion of PCR product is by enzyme *R*saI, that recognizes the sequence GTAC and digests the sequence that possesses the variant type *IDH1* SNP into 146 bp and 94 bp fragments (Figure 2). The reactions run in simple thermocyclers, which are available in every laboratory working in molecular biology, and there is no need for a dedicated software to assess the results. This is the first analysis of the *IDH1* SNP 105 C>T by the PCR-RFLP technique.

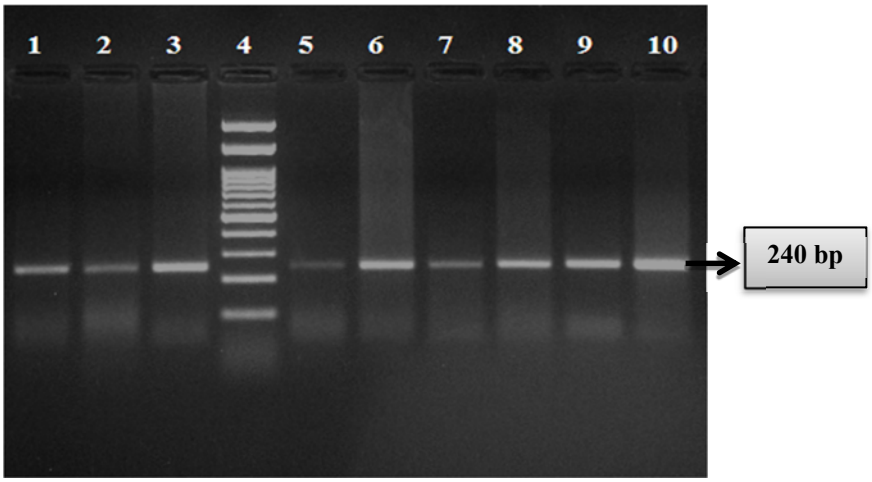

Figure 1. PCR product of *IDH1* SNP 105 C>T separated on 2% agarose gel. Lane 4 represents 100 bp marker and other lanes represent 240 bp amplified products.

Sequencing technique is the most common method which used to detect *IDH1* SNP 105 C>T rs11554137, but it is expensive and needs to specific machines that did not available in most laboratories. Many studies used sequencing technique in *IDH1* SNP 105 (C>T rs11554137 detection) [8, 12–15, 17–20] while some studies used other techniques and followed by sequencing as Single Strand Conformation Polymorphism (SSCP) [21] and PCR-Denaturing High-Performance Liquid Chromatography (PCR-DHPLC) [9, 22]. In addition, some studies use other methods like a light cycler based melting curve assay [12] and real time PCR [23] (as shown in Table 1).

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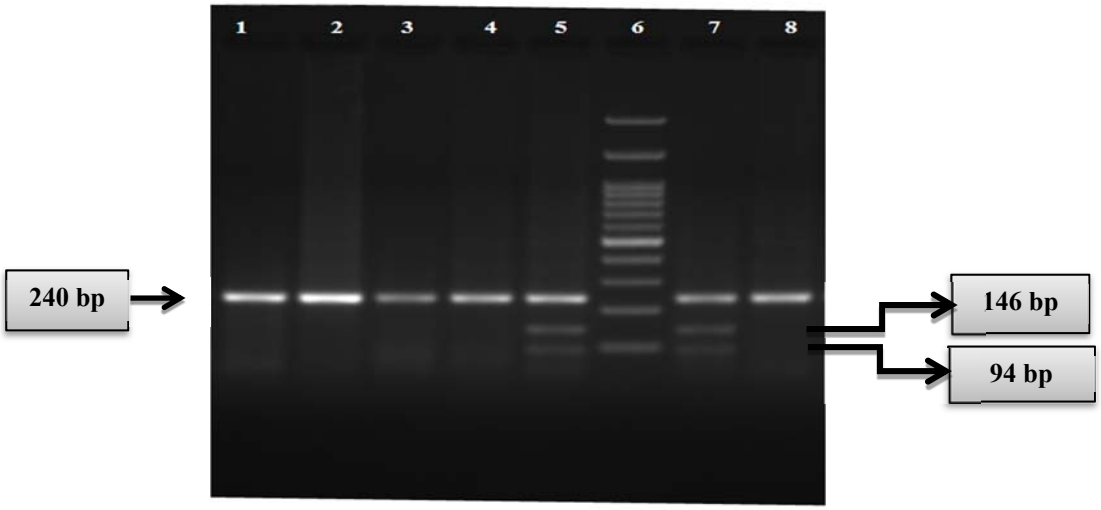

Figure 2. PCR-RFLP detection of *IDH1* SNP 105 C>T separated on 2% agarose gel. Lanes 1, 2, 3, 4 and 8 represent the wild type of *IDH1* SNP, Lanes 5 and 7 represent the variant type of *IDH1* SNP (Heterozygous) and Lane 6 represents 100 bp marker.

3.2 Frequency of *IDH1* SNP 105 C >T rs11554137 in normal volunteers

IDH1 SNP 105 C >T was found in 16 out of 98 healthy volunteers (16.3%), which all of them were heterozygous. Besides, there were no significant correlations between sex and age with *IDH1* SNP 105 C>T (P= 0.274 and P=0.136 respectively) (Table 2).

The Egyptians had the highest frequency of variant type *IDH1* SNP105 C >T rs11554137 (16/98, 16.3%) in normal controls compared with other studies of Germans, Chinese, Thai, and Italians [14]. Germans found *IDH1* SNP rs11554137 in (14 / 120) 11.7% [13] and in (42/475) 9% [12]. Moreover, Italian had found a lower frequency (6/109) of 5.5% in healthy controls [20] while Chinese did not find *IDH1* SNP105 C >T in healthy controls (0/270, 0%) [18, 21]. Thai did not find *IDH1* SNP105 C >T in healthy controls (0/20, 0%) [22] as well.

IDH1 synonymous SNP 105 C>T rs11554137 found in many types of malignancies associated with poor prognosis in patients with glioma [15], CN-AML [13], and AML with negative NPM1 and CEPBα mutations [14]. *IDH1* SNP 105 C>T had a great variability in its incidence in normal volunteers according to the population number and ethnical variations.

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Table 1. Detection methods of *IDH1* SNP in many diseases and ethnic populations

Method	Disease	Country	Reference
Direct sequencing	AML	Egypt	[14]
Direct sequencing	AML	China	[18]
	Control		
Direct sequencing	AML	Asia, Africa and America	[17]
Direct sequencing	AML	America	[3]
Direct sequencing	AML	Sweden	[8]
Direct sequencing	Control	Germany	[12]
Direct sequencing	CN-AML	Germany	[13]
	Control		
Direct sequencing	Control	Brazil	[19]
Direct sequencing	Malignant glioma	France and Germany	[15]
SSCP Followed by sequencing	Hematological disorders	China	[21]
	Control		
PCR-DHPLC followed by Direct sequencing	Control	Thailand	[9]
PCR-DHPLC followed by Direct sequencing	AML	Thailand	[22]
	Control		
NGS	Brain Tumors	Italy	[20]
	Control		
Real time PCR	Chronic Myeloproliferative Neoplasms	Turkey	[23]
Light cycler-based melting curve assay	AML	Germany	[12]

Abbreviations: AML; Acute Myeloid Leukemia, SSCP; Single Strand Conformation Polymorphism, DHPLC; Denaturing High Performance Liquid Chromatography, NGS; Next Generation Sequencing.

Table 2. Association between sex and age with *IDH1* SNP 105 C>T

	All cases n=98	Wild type n=82	Variant type n=16	P
Sex				0.274
Male, n%	49 (50%)	39 (47.6%)	10 (62.5%)	
Female, n%	49 (50%)	43 (52.4%)	6 (37.5%)	
Age, Median & Range	33 (1-64)	36 (1-64)	28 (10-48)	0.136

4. Conclusions

Sequencing technique with RFLP in our study can be used because of an accurate, simple, sensitive, inexpensive, available and speedy method for *IDH1* SNP105 C >T rs11554137 instead of the complicate method.

5. Acknowledgements

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