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Identification of Genomic Markers by RAPD-PCR Primer in Leukemia Patients

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Abstract: The aim of this study is to ascertain the possible application of Random Amplification of Polymorphic DNA (RAPD) analysis as a genetic test to investigate DNA polymorphisms and detection of genomic markers in various types of leukemia. The results showed unique profiles of amplified DNA fragments produced in genomic DNA of three types of leukemia by an arbitrary primer of decamer oligonucleotides OPA-09. The primer produced four types of amplified DNA fragments (980, 1659, 2187 and 3162 bp). The smallest amplified DNA fragment (980 bp) appeared in 14.3 and 13.3% of tested acute myeloid leukemia and chronic myeloid leukemia patients, respectively; but was absent in genomic DNA of chronic lymphoid leukemia and normal individuals. Whereas the largest amplified fragment (3162 bp) was present in 12.5, 20 and 75% of chronic lymphoid leukemia, chronic myeloid leukemia and normal individuals, respectively and was absent in acute myeloid leukemia. On the other hand, the two amplified fragments (1659 and 2187 bp) were present in normal and leukemia patients. Cluster analysis of amplified DNA fragments grouped the leukemia patients in two main groups. The detected DNA polymorphisms by the arbitrary primer OPA-09 might find application in developing efficient RAPD primer for diagnosis of leukemia.

Key words: Leukemia, RAPD-PCR analysis, genetic polymorphisms, DNA markers

INTRODUCTION

Random Amplified Polymorphic DNA (RAPD) is one of the molecular techniques that has benefited from the advent of the PCR (Saiki *et al*., 1988; Williams *et al*., 1990). This molecular method has found applications in various types of organisms, e.g., bacteria (Yamamoto *et al*., 2001), plants (Awasthi *et al*., 2004; Awamleh *et al*., 2009) and animals (Gu *et al*., 1999; Mollah *et al*., 2005, 2009).

Various studies have applied RAPD-PCR analysis for investigating genetic instabilities associated with tumor development. It has been used as a mean for identifying the genetic alterations in human tumors and revealed frequent occurrence of genetic polymorphisms in various types of tumors, for example, lung cancer (Ong *et al*., 1998), squamous cell carcinoma of the head and neck (Maeda *et al*., 1999), brain tumor (Misra *et al*., 1998), ovarian cancer (Sood and Buller, 1996), breast cancer (Singh and Roy, 2001; Papadopoulos *et al*., 2002), hepatocellular carcinoma (Zhang *et al*., 2004; Xian *et al*., 2005), lymphoma (Scarra *et al*., 2001) and in leukemia (Odero *et al*., 2001).

Recently, it has been reported that molecular genetic analysis by RAPD primer OPA-09 revealed novel pattern of amplified DNA fragments in genomic DNA of Acute Lymphoblastic Leukemia (ALL) patients (Ibrahim *et al*., 2010). It is worth mentioned that there are other three types of leukemia namely, acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and chronic lymphoid leukemia (CLL) (Bolufer *et al*., 2006). Genetic analysis by RAPD primer OPA-09 of three types of leukemia (AML, CML and CLL) might support the value and usefulness of OPA-09 primer in investigating the genomic polymorphism and detection of novel genomic markers. Therefore, the objective of this study is to ascertain the ability of primer OPA-09 for genetic polymorphism analysis and detection of DNA markers in genomic DNA of AML, CML and CLL.

MATERIALS AND METHODS

Blood samples collection and DNA extraction: Blood samples were collected from leukemia patients attending Al-Bairuni University Hospital and Al-Assad University Hospital from different regions of Syria during the period from 20/3/2007 to 30/12/2007 according to reported methods (Goossens *et al*., 1991). Five-millimeters of blood were obtained from each patient and control (normal

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individual), placed in tubes containing anti-coagulant (K_3EDTA) and kept at -20°C till further use for genomic DNA extraction.

Genomic DNA was extracted from whole blood following the instructions of the DNA purification kit obtained from Promega Company.

DNA amplification: The reaction mixture (25 µL) consisted of 2 µL template DNA (25 ng µLG^1), 2.5 µL primer (10 pmol mLG^1), 8 µL mili Q water and 12.5 µL PCR Master mix {0.05 units µLG^1 Taq polymerase, 4 mM $MgCl_2$ and dNTPs (0.4 mM of each of dATP, dCTP, dGTP, dTTP)}. The mixture was incubated in the Appollo thermocycler (with heating lid) programmed for 40 cycles, each one consisting of a denaturation step (30 sec at 94°C), one annealing step (60 sec at 38°C) and an extension step (2 min at 72°C), an extra extension step was performed for 10 min at 72°C (Xian *et al*., 2005; Ibrahim *et al*., 2010). The amplification was carried out with primer OPA-09, the primer was obtained from Operon Technologies, Alameda, AL, USA.

Gel electrophoresis: The reaction products were separated by electrophoresis on an Agarose gel (1.5%) containing ethidium bromide with final concentration of 0.5 µg mLG^1 were prepared in 1X TBE buffer. The DNA ladder size marker (Fermentas) used in this study contained 10 discrete fragments (in base pair): 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp from nearest distance to the well to the far one from the well respectively. This Ladder was used as a molecular size indicator in the experiments of this study. The DNA bands obtained were visualized under ultraviolet light and the molecular sizes of DNA fragments (bands) were estimated according to standard curve represented the relationship between band molecular sizes of the Ladder measured by base pairs and distant of migration measured by millimeter (Sambrook and Russell, 2001).

Data analysis: The reproducible amplified DNA fragments from three experiments were scored, each RAPD-PCR product was assumed to represent a single locus. For considering a marker as polymorphic, the absence or addition of an amplified product in at least one sample was used as a criterion (Seufi *et al*., 2009). The bioinformatics toolkit in MATLAB was used for the cluster statistical analysis for grouping the RAPD-PCR bands into respective categories for creating phylogenetic tree to investigate the relationships (genetic distance) among genomic DNA of leukemia patients.

RESULTS

The results presented in Table 1 classified leukemia patients into three groups: Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia (CML) and Chronic Lymphoid Leukemia (CLL). The predominant groups were AML (38.9%) and CML (44.4%), whereas the percentage of CLL was 16.7.

RAPD-PCR analysis was carried out on selected DNA samples obtained from blood of leukemia patients which showed no clotting and represented the three classes of leukemia. The profiles of amplified DNA fragments obtained by primer OPA-09 from each class of leukemia showed discrete bands with unique patterns. The primer produced four types of amplified DNA fragments from genomic DNA extracted from normal males and females and three classes of leukemia patients. The molecular sizes of amplified DNA fragments were 980, 1659, 2187 and 3162 bp (Table 2).

The results obtained in this study showed that the incidence of the two amplified DNA fragments of 980 and 3162 bp in the genomic DNA of acute myeloid leukemia (AML) patients were 14.3 and 0%, respectively (Table 2). The results in Fig. 1 demonstrated the pattern of amplified DNA bands produced by primer OPA-09 in AML genomic DNA. On the other hand, the obtained data indicated the absence of 980 bp DNA fragment in tested patients of Chronic Lymphoid Leukemia (CLL) patients (Fig. 2); however, this fragment was present in 13.33% of CML patients (Fig. 3). Whereas the largest amplified DNA fragment (3160 bp) was present in 12.5 and 20% of chronic lymphoid leukemia and chronic myeloid leukemia, respectively.

The RAPD-PCR analysis of genomic DNA obtained separately from eight normal individuals using OPA-09 was performed to investigate the presence and/or absence

Table 1: Percentages of various types of leukemia patients diagnosed during the period of study

Types of leukemia	No. of patients	Percentage
AML	21	38.9
CLL	9	16.7
CML	24	44.4

Table 2: Percentages of four types of amplified DNA fragments generated by OPA-09 in three types of leukemia. Sizes of DNA bands are indicated in base pairs (bp)

		Percentage of amplified DNA bands			
Types of leukemia	No. of tested	3162 bp	2187 bp	1659 bp	980 bp
AML	7	0.0	100.0	100	14.286
CLL	8	12.5	87.5	100	0.000
CML	15	20.0	100.0	100	13.330
Control	8	75.0	100.0	100	0.000

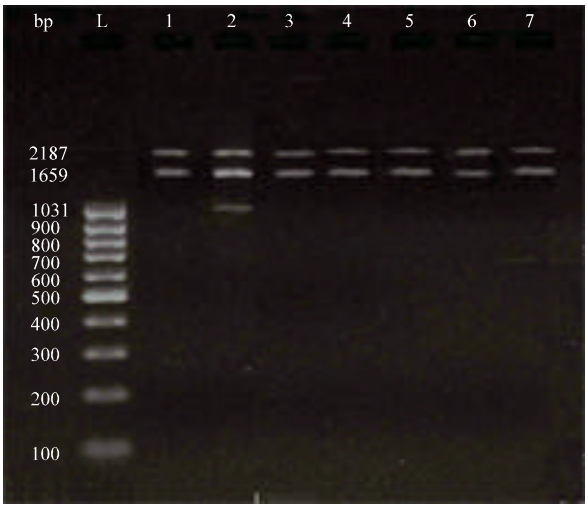

Fig. 1: RAPD-PCR amplified DNA fragments patterns of AML genomic DNA obtained by OPA-09 primer. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt cm^{-1}. Lanes from 1 to 4 represent the AML female patients; lanes from 5 to 7 represent AML males' patients. Lane L indicates the 8 DNA as a ladder, bp base pairs

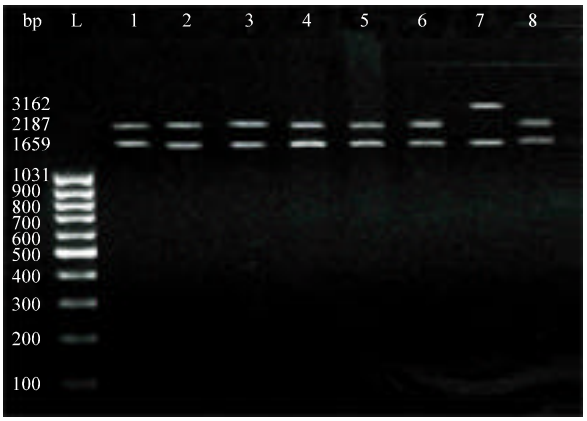

Fig. 2: RAPD-PCR patterns of DNA fragments amplified from genomic DNA of CLL patients obtained by primer OPA-09. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt cm^{-1}. Lane 1 represents the CLL female patient; lanes from 2 to 8 represent CLL male patients. Lane L indicates the 8 DNA as a ladder

of four bands appeared in leukemia patients. The results presented in Fig. 4a and b showed the absence of small band (980 bp) and the presence of large band (3160 bp) at a percentage of 75 in the eight normal individuals (Table 2).

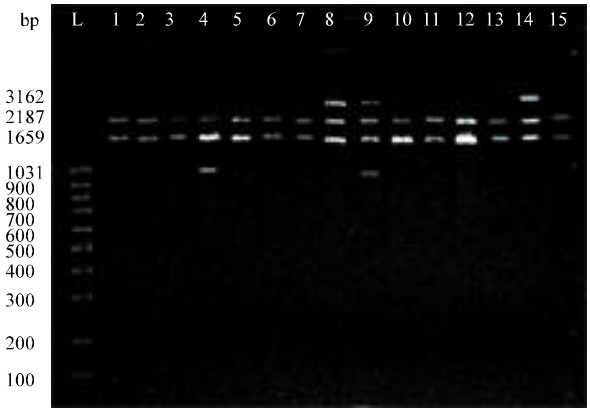

Fig. 3: RAPD-PCR patterns of amplified DNA fragments obtained by primer OPA-09 from genomic DNA of CML patients. Electrophoresis was performed on (1.5%) agarose gel and run with 3 volt cm^{-1}. Lanes from 1 to 8 represent the CML female patients; lanes from 9 to 15 represent CML male patients. Lane L indicates the 8 DNA as a ladder

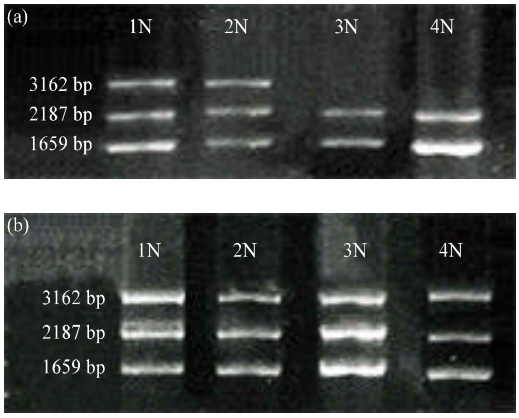

Fig. 4: RAPD-PCR patterns of amplified DNA fragments obtained by primer OPA-09 from genomic DNA of (a) normal males and (b) females

Dendogram was developed using squared euclidean distance and hierarchal clustering of 88 amplified DNA fragments generated from RAPD-PCR of primer OPA-09 (Fig. 1-4), the obtained results provided valuable information on genetic relatedness among genomic DNA of AML, CLL and CML patients and control group (Fig. 5). Six out of eight normal male and female individuals were found clustered in one subgroup, whereas six AML patients and 18 CLL and CML patients were clustered in two distinct subgroups.

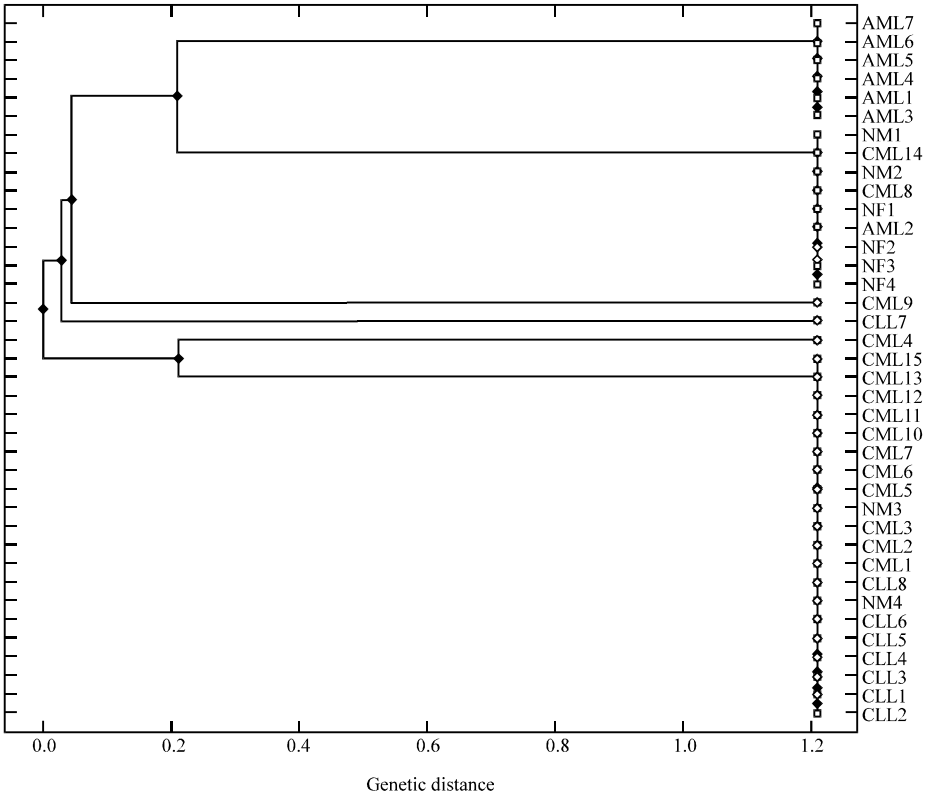

Fig. 5: Dendogram generated by cluster analysis of the DNA fragment (bands) shown in Fig. 1-4. AML1 to AML7 represent AML patients, CLL1 to CLL8 represent CLL patients, CML1 to CML15 represent CML patients, NM1 to NM4 represent normal males and NF1 to NF4 represent normal females

DISCUSSION

The RAPD-PCR analysis has been applied to a variety of tumor types to study genomic instability and to identify novel DNA amplifications patterns (Ong *et al*., 1998; Papadopoulos *et al*., 2002; Xian *et al*., 2005). Although, RAPD-PCR provides an easy way to screen the genome for DNA markers, the application of RAPD amplified DNA fragments to develop molecular markers for detection of cancer has not yet been achieved. It is worth mentioned the results of our study showed frequent occurrence of DNA polymorphisms in genomic DNA of leukemia patients in contrast to normal persons, this might due to binding properties of nucleotides sequence of RAPD primer in question and on the aberrations of genotype of leukemia patients. Earlier studies indicated that the genetic polymorphism of leukemia patients includes addition, deletion or mobility shift of amplified bands (Ong *et al*., 1998; Papadopoulos *et al*., 2002).

Primer OPA-09 was one of twenty three decamer RAPD primers used by our group in this study and previous study (Ibrahim *et al*., 2010). This primer showed novel amplified DNA fragments in genomic DNA of leukemia patients as compared with the obtained results of other 22 tested RAPD primers. The outcome of present study on genomic DNA of AML, CLL and CML support the results of our earlier investigation on genomic DNA of ALL (Ibrahim *et al*., 2010) and showed the possibility of using primer OPA-09 to identify deletion and addition of DNA fragments in genomic DNA of leukemia patients as compared with normal individuals. The observation of newly added DNA fragment of 980 bp in the genomic DNA of CML, AML and ALL might suggest the possibility of using this DNA fragment as a molecular marker for diagnosis and prognosis of leukemia. However, further work is required on larger sample of normal individuals and leukemia patients to validate the results presented in this study. The possible application of DNA markers in diagnosis of leukemias might prove more practical than current methods which started from cytomorphology and cytogenetic techniques to collection of different methods that are necessary not only for the diagnosis and classification but also for individual

treatment decision. Nowadays, new techniques perform multi-parameter flow cytometry (MFC), with metaphase cytogenetic in every case, in which leukemia is suspected, the latter has to be accompanied by FISH analysis (Haferlach *et al*., 2005). However, during this decade investigators have ascertained DNA polymorphism by means of RAPD-PCR analysis to develop new molecular methods for diagnosis of cancer (Papadopoulos *et al*., 2002; Xian *et al*., 2005). The results of current investigation support the possible application of RAPD-PCR analysis for screening specific molecular markers by using arbitrary primers. These markers might be useful in the diagnosis and to assess prognosis of various types of leukemia.

CONCLUSIONS

RAPD decamer primer OPA-09 produced 980 bp DNA fragment in genomic DNA of acute myeloid leukemia and chronic myeloid leukemia patients, this DNA fragment was not detected in tested normal individuals and chronic lymphoid leukemia. The amplified DNA fragment might be proved useful for further development of molecular marker for diagnosis of leukemia. The study gives new evidence of potential promise of RAPD-PCR analysis in cancer research for detecting and selecting novel genomic markers.

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