Detection of genomic instability in oral squamous cell carcinoma using random amplified polymorphic DNA based on polymerase chain reaction method (RAPD-PCR)

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ABSTRACT

Background: Oral squamous cell carcinoma is an invasive epithelial neoplasm, occurred most commonly in alcoholic and tobacco using adults. The present study is aimed to identify the genomic instability in OSCC patients using random amplified polymorphic DNA (RAPD), a polymerase chain reaction (PCR) based technique.

Materials and methods: Twenty five blocks of formalin fixed paraffin embedded tissue was used as malignant DNA source and five sample of healthy DNA obtained from the oral tissue and blood. Using DNA extraction kit (Geneaid Minikit) and eleven random sequencing primers to visualize the amplifications pattern under UV.

Results: The primer detectability of genomic instability ranged from 21% in well differentiated OSCC to 68% in poorly differentiated OSCC. Cases T8 and T13 showed highest genomic instability (75%). The results determined numbers of genomic instabilities among OSCC patients by comparing the pattern of amplifications of the primers in both malignant and healthy DNA.

Conclusions: High significance correlation between primers detection rate and histopathological grade of OSCC. Further larger studies are needed to: 1) Obtain RAPD markers useful for OSCC for early diagnosis; 2) investigate different genes directly involved in the etiology of OSCC; 3) analyze chromosomal instability among OSCC patients.

Keyword: Oral squamous cell carcinoma (OSCC), random amplified polymorphic DNA (RAPD), genomic instability.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is associated with major mortality. It is the most common cancer worldwide and its incidence is either stable or slowly increasing in most populations in the world (1). The etiology of oral cancers is complex due to the multigenic nature of the disease and the number of potential environmental agents to which individuals may be exposed (2). Tobacco and alcohol are considered as the major etiological agents involved in development of oral tumors.

Measurement of genomic instability has been performed by techniques like flow cytometry, fluorescent insituhybridization, comparative genomic hybridization (CGH) and allelotyping, which, although informative, are cumbersome to perform and hence, impractical in the assessment of clinical cases (3). Random amplified polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) based fingerprinting technique that amplifies random DNA fragments with short primers of arbitrary nucleotide sequence under low annealing stringency (4,5).

The applications of RAPD technique have been found among several kinds of organism including bacteria, fungi, plants, animals, insects and humans (6-11).

MATERIALS AND METHODS

DNA extraction: Twenty five formalin fixed paraffin embedded tissues blocks of oral squamous cell carcinoma OSCC were included in this study collected from the archives of histopathological department in the college of dentistry/Baghdad University and from histopathological department in Gazi Al-Hariri hospital/Baghdad. While the control group was three samples of fresh blood as a source of healthy DNA and two samples of gingival mucosa. DNA extraction was done using (Geneaid Minikit) which contain Lysis buffers and DNA binding silica filters.

DNA amplification: Total reaction volume of 20 µl of 5µl PCR Master Mix(Bioneer) was used and contains 100 Pmol of each 11 different arbitrary 10-mer primers and 25 to 50 ng of genomic DNA. The names and sequences of these oligoprimer are listed in Table1. The RAPD-PCR amplification reactions were performed in Eppendorf therimal cycler using the following PCR program: one cycle of 95°C for 4 min then amplification was carried out for 40
cycles of 92°C for 1 minute, annealing for 1 minute at 36°C, and 72°C extension for 1 minute, followed by a 5 minute 72°C for final extension. After the amplification, the PCR reaction products were electrophoresed on 10 x 14 cm 1.5%-agarose gel for 2 hours using Tris-borate- EDTA Buffer. The gel was stained with 0.5g/ml of ethidium bromide.

Table 1: RAPD Primers sequences and their GC ratio.

<table>
<thead>
<tr>
<th>No.</th>
<th>Primers codes</th>
<th>Sequencing</th>
<th>GC Ratio</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>OPI-01</td>
<td>5′-GGTCTGAAC-3′</td>
<td>55.6%</td>
</tr>
<tr>
<td>2</td>
<td>OPA-04</td>
<td>5′-TACGGACAC-3′</td>
<td>55.6%</td>
</tr>
<tr>
<td>3</td>
<td>OPA-07</td>
<td>5′-ACGGTACACT-3′</td>
<td>60%</td>
</tr>
<tr>
<td>4</td>
<td>OPC-08</td>
<td>5′-ACGGGCGA-3′</td>
<td>75%</td>
</tr>
<tr>
<td>5</td>
<td>OPA-09</td>
<td>5′-GTCTCCAAGC-3′</td>
<td>70%</td>
</tr>
<tr>
<td>6</td>
<td>OPA-11</td>
<td>5′-CAGGCCCTTC-3′</td>
<td>60%</td>
</tr>
<tr>
<td>7</td>
<td>OPC-12</td>
<td>5′-TAGGCTACG-3′</td>
<td>60%</td>
</tr>
<tr>
<td>8</td>
<td>OPA-13</td>
<td>5′-CCGGCTACGG-3′</td>
<td>70%</td>
</tr>
<tr>
<td>9</td>
<td>OPA-16</td>
<td>5′-TACGGTACTC-3′</td>
<td>60%</td>
</tr>
<tr>
<td>10</td>
<td>OPA-20</td>
<td>5′-AGCTTACGG-3′</td>
<td>60%</td>
</tr>
<tr>
<td>11</td>
<td>GB8</td>
<td>5′-AGGCATTCCC-3′</td>
<td>70%</td>
</tr>
</tbody>
</table>

Data analysis: The analysis is based on the DNA polymorphisms of the tumor comparing with healthy DNA using the same primer to detect deletion, addition, increasing or decreasing in intensity of bands.

RESULTS AND DISCUSSION

Results of genomic instability detected by RAPD-PCR analysis are shown in Table (2). Among all studied cases, genomic instability was demonstrated with at least one primer. Among all studied cases with all primers, the detectability of genomic instability ranged from 21% in well differentiated OSCC to 68% (± SD) in poorly differentiated OSCC. With all used primers, case T8 and T13 showed the highest genomic instability (75%), whereas, the lowest genomic instability was (12.5%) with cases T7, T10.

There are three grades of OSCC according to the histopathological differentiation (Well, Moderate, and Poor). The powerful technique that detects genomic alteration correlated with human tumor is microsatellites analysis(13).

However, this methodology is time consuming and can only detect base-pair expansion or contraction in specific microsatellite loci(4). On the contrary, for genomic instability analysis, it is important to investigate genetic alterations in the entire genome besides microsatellite loci. In contrast, the RAPD method can simply and rapidly detect genetic alterations in the entire genome without knowledge of specific DNA sequence information(13,14). In the RAPD method, genetic alterations appeared as either loss or gain of a band, shift of a band, or decrease or increase of intensities of a band of cancer tissue DNA relative to the corresponding normal tissue DNA(15). Obtained results indicated that RAPD-PCR is an effective tool for identifying genetic alteration and genomic
instability which is in agreement with several various studies\(^{(14)}\). Figure 1 shows the banding profiles of OSCC and corresponding normal DNAs and demonstrate the detected genetic alteration by RAPD technique among OSCC patients in comparison with normal control group. Banding shifts, missing bands and/or banding intensity changes, which indicate genomic instability, were demonstrated in this figure.

![Figure 1: RAPD analysis with primer (OPA-11). Bold arrow refer addition of bands, dashed arrow refer to deletion of bands.](image)

These results might be due to mutations that occurred at the primer-template interaction sites\(^{(15)}\). The summarized results which are illustrated in Table 2 indicated that, there are differences in genetic instability among the studied cases which ranged from 21% with case number T7 to 75% for case T8. These differences might be due to differences in studied OSCC grades (well, moderate and poor).

**REFERENCES**