Phylogenetic Characterization Of Human Genome Through DNA Fingerprinting Among Indians

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***Abstract*—DNA fingerprinting technique is considered as a powerful technique and is widely used all over the globe today. The basic requirement for it is the availability of biological samples of the individuals. Buccal cells are increasingly used as a source of quality DNA to improve participation rates in molecular studies. These cells are routinely shed and replaced by new cells. We used Random Amplified Polymorphic DNA (RAPD) markers to assess genetic diversity among 6 individuals. The amount of genetic variation was evaluated by polymerase chain reaction amplification with 10-mer oligonucleotide primers. The similarity co-efficient between each pair of accession were used to construct a Dendrogram showing relationships between them using Unweighed Pair Group Method Average (UPGMA).**

Keywords: Buccal cells, DNA Fingerprinting, Random Amplified Polymorphism (RAPD), and genetic variation.

I. INTRODUCTION

The characterization of a DNA sample for individual identity according to its chemistry or sequence information often referred to as DNA "fingerprinting" [1]. The preferred way to obtain genomic DNA is from peripheral blood [2]. Blood sampling, however, may be problematic in cases such as extreme illness or elderly people, babies and people who are unwilling to this invasive procedure [3]. In contrast to blood biological samples such as buccal cells can supply DNA for genetic testing and provide a noninvasive approach [4]. Several cell collection methods are there but mouthwash procedure gives high yield of DNA from buccal cell [5]. DNA Fingerprinting can be defined as a technique that is used for revealing the identity of an organism at the molecular level. Usually fingerprinting is based on the morphological features and is restricted to humans but DNA fingerprinting is a technique of finding the genetic diversity. This is primarily based on the polymorphisms occurring at the molecular level that is on the base sequences of the genome [6]. RAPD based molecular characterization is an important tool to explore genetic biodiversity between morphologically identical species and genetic relatedness between distant species [7]. The random amplified polymorphic DNA (RAPD) method is based on the polymerase chain reaction (PCR) using short (usually 10 nucleotide) primers of arbitrary sequences [8]. Since the technique is relatively easy to apply to a wide array of plant and animal taxa, and the number of loci that can be examined is essentially unlimited, RAPDs are viewed as having several advantages over RFLPs and DNA fingerprints [9]. The RAPD technique has several advantages such as the ease and rapidity of analysis, a relatively low cost, availability of a large number of primers and the requirement of a very small amount of DNA for analysis [10]. DNA purity plays an important role in amplification patterns obtained by RAPD-PCR [11]. Since the primers consist of random sequences, and do not discriminate between coding and noncoding regions, it is reasonable to expect the technique to sample the genome more randomly than conventional methods [9].

In this study DNA is isolated from buccal cells of individuals from different ethnic background. Six subjects from different ethnic background were chosen and the genetic variation among them is analysed by using dendrogram analysis.

II. MATERIALS AND METHODS

A. Collection of buccal cells:

Buccal cells were collected fresh before DNA isolation. One hour after brushing the subjects were asked to scrape the inner cheeks & rinse the mouth with a 10 ml of sterile distilled water for 60seconds and asked to collect the mouthwash in a beaker and transfer to 15 ml centrifuge tubes. During this period the subjects were instructed not to eat anything.

B. DNA Isolation

To the mouthwash collected, 3ml of TNE solution [17 mM Tris/HCl (pH 8.0), 50 mM NaCl and 7 mM EDTA] diluted in 66% ethanol was added and centrifuged for 15 min at 7000 rpm at room temperature & the supernatant was discarded immediately. For second washing 1 ml of TNE was added to resuspend the cells and centrifuged at 7000 rpm for 10 min and the supernatant was discarded. The cell pellet was vortexed vigorously for 5 seconds and 1.3 ml of lysis solution [10mM Tris (pH8.0), 0.5% SDS, 5mM EDTA] and 15 µL of proteinase K were

added. The mixture was vortexed for 5 seconds at medium speed, followed by incubation for 4hrs at 55℃ then 1.4 ml of the mixture was transferred to a 2 ml micro-centrifuge tube. 600µl of a solution containing 8 M ammonium acetate and1 mM EDTA was added, to remove Proteins and other contaminants and vortexing was done at high speed for 5 seconds and centrifuged at 10,000 rpm for 10 min. 900µl of supernatant was carefully poured into two clean 2ml micro-centrifugetubes containing 540µl of isopropanol and is kept for overnight refrigeration. The tubes were gently inverted 20 times & centrifuged at 10000rpm for 10 min, the supernatant

was discarded and the tubes were drained. 2ml of 70% ice cold ethanol was added and mixed by inverting and centrifuged at 10000rpm for 10 min. & the supernatant was discarded. The tube was inverted and drained on clean absorbent paper, then allowed to air dry for 45 to 60 min. The DNA was re-suspended in 10-40µl of TE buffer [10mMTris (pH7.8) and 1mM EDTA]. The DNA sample was run in 0.8% agarose gel and the bands was visualized under UV light using UV Transilluminator and it is then photographed in Gel documentation.

C. RAPD Analysis

The Polymerised Chain Reaction starts with denaturation of the double stranded DNA to form single strands (initial denaturation- 94℃ for 5 min and denaturation-94℃ for 40 sec) followed by annealing (36℃ for 30 sec) and extension steps (extension-72 ℃ for 90 sec & final extension-72℃ for 10 min). Amplification was carried out with a 50µL reaction mixture containing Primer (2µM/µL) -8.0µL, 10X Buffer-5.0 µL, 2mM dNTP Mix- 5.0µL, Taq DNA polymerase (5U/µL)- 0.5µL, Template DNA (50ng)- 2.0µL, Sterile distilled water- 29.5µL. The primers used were PG05 – 5’ GCAGGCTAAC 3’ PG06 – 5’ CCTGGTGGTC 3’ PG07 – 5’ GCTGCAGTAG 3’. About 34 cycles of reactions were carried out and was run in a 2% agarose gel electrophoresis and visualized using UV transilluminator and is photographed.

D. Creating Dendrogram

The data obtained from amplification products by primers were used to estimate genetic similarity among different isolates on the basis of shared amplification products. The RAPD patterns were scored on the basis of presence or absence of band. The similarity coefficients were utilized to generate Dendrogram by using UPGMA (Unweighed Pair Group Method of Arithmetic means) through the programme, Phylip Version 3.69.

III. RESULTS AND DISCUSSION

The isolates of *Homo sapiens* were isolated from different states of India. The samples were collected from people belonging to Jharkhand, Orissa, Kerala, Andhra Pradesh, and Tamil Nadu. DNA Finger printing was done and the RAPD banding pattern was observed stable for each population of the primer. Out of PG 05, PG 06, PG 07, the primer PG 06 alone gave the band variation. The primer PG 05 and PG 07 gave only few bands. Thus the phylogenetic characterization was done through DNA Fingerprinting.

Several protocols have been developed to obtain DNA from buccal cells, but cell collection by mouthwash seems to give higher yields than many other methods. In a similar study conducted, it is revealed that the buccal cells provided usable amounts of DNA, particularly from mouthwash and cytobrush. Previous studies reported by Garcia-Closas *et al*,

[12] shows that the greatest efficiency for DNA extraction was from mouthwash.

The gels scored for computer analysis on the basis of the presence or absence of the amplified products. If the product was present in a genotype, it was designed as ‘0’. A total of 46 bands were observed from six individuals screened from various ethnic backgrounds. The similarity co-efficient between each pair of accession were used to construct a Dendrogram using Unweighed Pair Group Method Average (UPGMA). Cluster analysis was performed on the similarity index calculated by RAPD markers. Through UPGMA programme, the resultant Dendrogram was presented in Figure 3. Figure 3 depicts the analysis of banding patterns & number of polymorphic fragments ranged from 603 to 6557 bp. The polymorphism observed was 39.13 %.

The upper cluster consists of sample of *Homo sapiens* which was isolated from people belonging to Jharkhand (BC1). Lower cluster consists of samples which were isolated from people belonging Tamil Nadu (BC5), Andhra Pradesh (BC4, BC6), Kerala (BC3), Orissa (BC2). Sample collected from Andhra is closely related to Tamil Nadu and Orissa and only distantly related to sample collected from Jharkhand (BC1). Thus the geographical analysis was done from North to the southern regions of India.

In the present study the RAPD markers were more striking because a large number of markers were generated per primer at DNA level for 6 representative isolates. This technique was relatively simple and appeared more effective and positive since genetic diversity can be identified in terms of number of bands based on their presence or absence. The number of

band interpretation is given in Fig. 3., by comparing each samples, it is found that more number of bands were viewed in sample 3. This was in accordance with the findings of Smith *et al*. They reported that RAPD provide useful tools for diagnostic studies in the population, strains and species level. Different thermal cyclers [14], temperature profiles, band of DNA polymerases [15] and the concentration of MgCl2, primer and template DNA can affect the reproducibility of RAPD assay [13]. In our work, we standardised all the above parameters prior to performing our analysis. Stephen J *et al.*, [16] described that animals from near ecological zone show more similarities which is similar to the results of our study represented in Dendrogram (Fig. 3.).

Studies carried out by GU et al. [17] revealed that single canine DNA bands derived from the RAPD assay may contain more than one DNA fragment of similar size, but a different nucleotide sequence. Consistent with this idea, it is possible that each of the amplified fragments could represent more than one DNA sequence. As described by Chansiripornchai *et al.* [18]. The genetic similarity matrix of RAPD data for 5 isolates was constructed based on coefficient of similarity utilized for the construction of Dendrogram using the UPGMA.

The study revealed that the GC rich primer PG 06 generated maximum number of products. Sample BC1 (Jharkhand) and BC3 (Kerala) showed vast genetic diversity, this may be due to climatic factors, food habits etc. Samples BC2 (Orissa), BC6 (Andhra Pradesh) and BC5 (Tamil Nadu), BC4 (Andhra Pradesh) are closely related to each other. This study revealed the phylogenetic reconstruction is an important approach in understanding the genetic diversity.

A. Figures

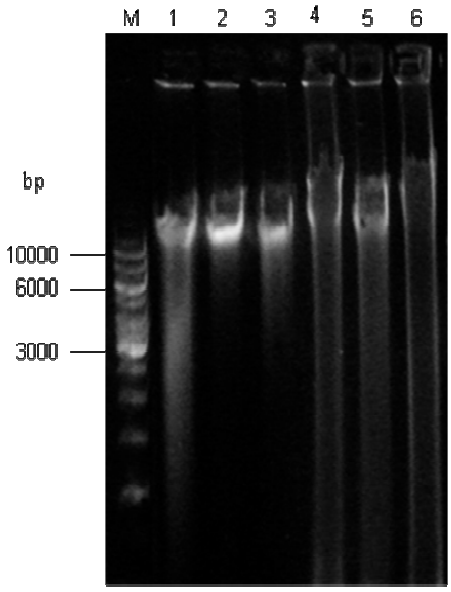

Fig. 1. Agarose gel (0.8%) electrophoresis showing total genomic DNA of Human obtained from mouthwashes
Marker (M) – DNA Marker (1 kb Ladder)
Lane 1 – Jharkhand,
Lane 2 – Orissa,
Lane 3 – Kerala
Lane 4 – Andhra Pradesh
Lane 5 – Tamil Nadu
Lane 6 – Andhra Pradesh

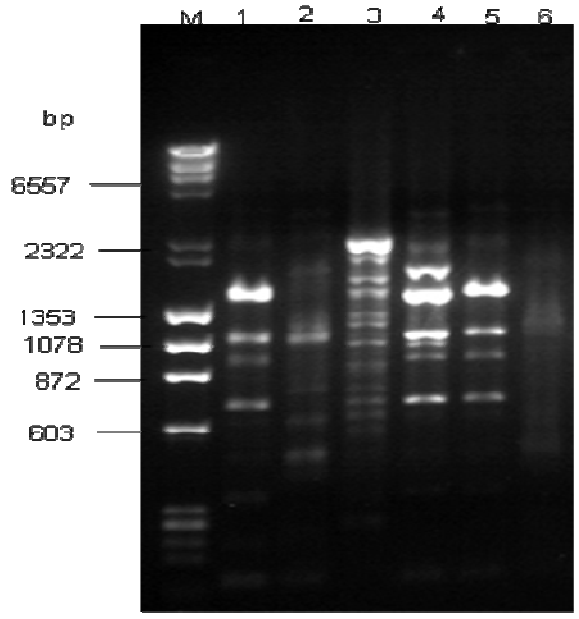

Fig. 2. Agarose gel (2%) electrophoresis showing RAPD pattern of human genomic DNA
Marker (M) – DNA Marker (λ DNA-HindIII and Φ X 174 DNA Hae III digest Mix)
Lane 1 – Jharkhand
Lane 2 – Orissa

Lane 3 – Kerala
Lane 4 – Andhra Pradesh
Lane 5 – Tamil Nadu
Lane 6 – Andhra Pradesh

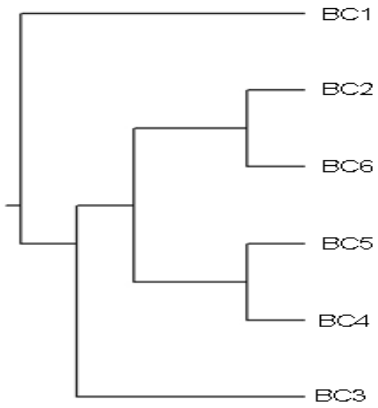

Fig. 3. Dendrogram depicting the genetic diversity among six DNA samples

IV. CONCLUSION

This study shows that genetic diversity among people occurs due to climatic factors and food habits too. The variation among different individuals can be studied through RAPD analysis and the phylogenetic characterization can be arrived.

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