Isolation of DNA from Oral Rinse in HPV Positive Patients

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ABSTRACT

Objective: To extract human genomic DNA from oral rinse in HPV positive patients.

Study Design: Experimental study.

Place and Duration of Study: Research Laboratory, Ziauddin Medical University, from February to July 2011.

Methodology: Two hundred and fifty oral rinse samples from human papilloma virus (HPV) positive subjects were collected in 50 ml corning tubes. DNA was extracted from 10 ml of oral rinse by using Lysis buffer (sodium dodecyl sulfate, sodium chloride, sodium citrate and EDTA), isopropanol and 3 M sodium acetate and final washing with ethanol. The DNA was quantified by using Qubit® dsDNA BR Assay (Qubit® 2.0 invitrogen life technologies USA) and the quality was checked by running an aliquot on 0.6% agarose gel stained with ethidium bromide. PCR were done to amplify β-globin gene on chromosome 11 by using primers GH20/PCO4 of 260 bp fragment.

Results: The mean concentration of all 250 samples DNA was 15.648 ± 10.50 µg/ml determined by using Qubit® 2.0. A single intense band without smearing was seen in almost all cases which confirmed the integrity of DNA. The PCR amplification of human β-globins primers was successfully done.

Conclusion: The oral rinse method was found a simple and highly appropriate means for non-invasive sample collection with easy storage, DNA recovery and subsequent PCR amplification in HPV positive patients.

Key Words: DNA extraction. PCR amplification. β-globin. HPV positive. Non-invasive sample collection. Oral rinse.

INTRODUCTION

With growing advances and remarkable achievements in this field, analysis of DNA has become a routine for the diagnosis of viral and bacterial disease as well as genetic disorders. However, these studies are expensive and there is dearth of such studies in low income countries. Therefore, there was a growing need to develop and standardize, simple, rapid, non-invasive method for collection and extraction of DNA, which can be applied to population based large-scale genomic and epidemiological studies.

Oral rinse or saliva is now being recognized worldwide as a source of cost effective and non-invasive method for DNA extraction, suitable for epidemiological studies in low income countries. It is also now being used for routine diagnostic purposes such as cardiovascular disorder, diabetes, cancer1-5 and in large-scale genomic research studies.

Using exfoliated oral cells as a source of DNA, makes sample collection a very easy, non-invasive and self-administered protocol. Many different kits and protocols are available with variation in collection of oral cells and processing method,6,7 but they too are very expensive and not feasible for large-scale epidemiological studies in low-income studies.

The objective of the study was to develop a simple, non-invasive collection method for oral rinse as well as 5 step (1 hour 30 minutes) method for DNA extraction in patients with human papilloma virus (HPV).

METHODOLOGY

This experimental study was conducted from February to July 2011 and was conducted in Ziauddin Medical University, Karachi. Two hundred and fifty oral rinse samples were collected from subjects gathered in HPV screening camp after filling a consent form. Prior to samples collection, an informed consent and approval was taken from the subjects. The volume of samples were 20 ml collected in 50 ml corning tubes and stored at 4°C till DNA extraction. The subjects were asked to rinse their mouth for one minute with distilled water and then spit in 50 ml corning tubes.

DNA was extracted from 10 ml of the sample centrifuged at 4000 rpm for 10 minutes at room for cell sedimentation. Supernatant was discarded leaving approximately 200 μl at the bottom. This was transferred to 1.5 ml RNase/DNase pyrogen-free tube and 500 μl Lysis buffer (50 g sodium dodecyl sulfate, 8.8 g sodium chloride, 4.4 g sodium citrate and 0.3 g EDTA with the final volume of 1 liter) was added, vortexed for 30 seconds and then incubated at 60°C for 30 minutes in heat block. The tube were then sonicated for one minute and then short spinned. Isopropanol 500 μl and 200 μl 3 M sodium acetate were added and tubes were incubated for 2 minutes at room temperature, vortex for 30 seconds and then centrifuged for 15 minutes at 12500 rpm. Supernatant was discarded without
disturbing the pellet and one ml of freshly prepared 70% ethanol was added. The tubes were vortexed for 30 seconds and then centrifuged for 5 minutes at 12500 rpm. The supernatant was discarded without disturbing the pellet, the tubes were placed vertically upside down on filter paper. The pellet was eluted in 50 µl TE buffer (Tris-EDTA). The DNA was quantified by using Qubit® dsDNA BR Assay kit (Qubit® 2.0 invitrogen life technologies USA) and the quality was checked by running an aliquot on 0.6% agarose gel stained with ethidium bromide.

To check the integrity and source of the DNA PCR and Gel-Electrophoresis were performed. The extracted DNA was amplified by 268 bp sequence of β-globin gene present on chromosome 11; the primers were design by(GeneLink, USA). Sequence of primers is; β-globin-Forward-GH20 5’-CAAACCTCATCCAGGTACC-3’ and β-globin-Reverse-PC04 5’-GAAGAGCGCAAGACGGTAC-3’. The PCR reaction was carried out in 25 µl volume, containing 12.5 µl of GoTaq® Green master mix (GoTaQ® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer pH 8.5, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl2; Promega, USA) 2.5 µl of 1 µM forward primer (GH20), 2.5 µl of 1 µM reverse primers (PC04), 5 µl of DNA template and 2.5 µl of PCR graded water. The PCR was performed in thermal cycler (BioFlux, Korea). The PCR program were first stage followed by pre-denaturation step at 95ºC for 5 minutes, second stage followed by 30 cycles of denaturation at 95ºC for 30 seconds annealing at 51ºC for 30 seconds and extension at 74ºC for 30 seconds, third stage followed by the final extension at 74ºC for 3 minutes. The results were analyzed by running on 2% agarose gel stained with ethidium bromide and visualized under UV transilluminator. A fragment of 268 bp of beta-globin gene was analyzed with negative and positive PCR controls.

Two types of extraction and PCR controls were used for each batch. Negative control was used as blank and human whole blood DNA was used as positive control. Each PCR experiment was repeated at least two times.

RESULTS

Overall 250 samples were checked for DNA extraction from oral rinse and 10 samples were from whole blood from the same persons as controls. A single intense band without smearing was seen in almost all the cases (Figure 1). The average DNA concentration was 15.648 ± 10.50 ug/ml and the average DNA concentration obtained from 10 blood samples was 18.37 ± 6.610 ug/ml using Gene Jet whole blood DNA extraction kit (Fermentas, USA). The maximum DNA concentration obtained from oral rinse was 145 ug/ml and minimum DNA concentration obtained was 4.72 ug/ml.

The band patterns was observed in the agarose gel. Few DNA degradation traces were observed in the DNA samples processed delay after oral cell sample collection (Figure 1).

The suitability of DNA from oral cell, evaluated by PCR amplification of 268 bp fragment of beta-globin gene was found excellent. Also, the two controls, extraction negative and amplification negative were found extremely helpful in optimizing the protocol.

![Figure 1: PCR amplification of beta-globin gene of DNA extracted from oral rinse.](https://example.com/figure1.png)

The study showed that the oral rinse is a good source of DNA and provide DNA in adequate amounts (mean concentration = 14.5 ug/ml) for PCR amplifications. The epidemiological studies by Mulot et al. for establishing DNA bank from a large number of young subjects as well as for adults, found buccal swabs and mouthwash a good source for supply of DNA for genetic testing. They also found the yield and purity of collected DNA more appropriate method for DNA collection and for PCR amplification.

Oral rinse can be a perfect medium for health surveillance and disease follow-up studies. In the last decade, many studies have shown that oral rinse can be explored for state-of-the-art saliva-based biosensors and these salivary biomarkers can be used to discriminate between diseases. Today, around the world, laboratory investigations are mainly based on blood analysis which is an invasive, expensive and paramedical assisted technique. Similarly, urine is also being used for various analytic investigations. Recently, studies conducted showed the possibilities of urine to be used as metabolomics diagnostic tool in various diseases and also as a source for hepatotoxicity markers etc. Yet again, urine collection requires special storage facility with a private and personal collection area, during population based large-scale genomic and epidemiological studies conducted in camps especially, when collection is from women in the country. Secondly, the DNA provided by urine samples has variable yields and contain PCR inhibitors and thus cannot be trusted for use for examining health status, or monitoring disease onset and progression, or scrutinizing treatment outcomes. While, saliva, on the other hand, can offer analysis of biomarkers of same potential through non-invasive means which gives it an
edge over other techniques especially when it comes to children and elderly.

During sample collection in camps, children are a fragile fraction of population group and cannot be forced by the parents after consent. Similarly, elderly show many reservations. With oral rinse, none of these problems were encountered. All participants regardless of age and gender complied willingly. In a study on children with cancer, salivary evaluation of biochemical markers and immunological status of children proved very effective since all children were undergoing antineoplastic treatment. The authors’ attempt to identify saliva as an alternative for a less invasive and less painful monitoring of these patients was very successful.\(^{12}\) Thus, at present, highly desirable goal in health care promotion and delivery,\(^{9}\) should be convergence of major diagnostic testing into salivary diagnostic testing.

Since the earliest report for extraction of DNA from oral cells was published in 1988 by Lench et al.,\(^{13}\) many protocols are published for extraction of genomic DNA from oral cells.\(^ {14,15}\) The protocol followed in this study was simple, cost-effective and did not require elaborate instrumentation. A large number of samples were processed at a very low cost which is an extremely positive point for large scale epidemiological and molecular biological studies in low income countries like Pakistan.

Several methods of buccal cell collection have been described, including swabs,\(^1\) cytobrushes and mouthwash\(^6\) and more recently, treated cards, such as IsoCode cards.\(^ {16}\) In this study, after collection of oral rinse, a gentle brushing all around inside the oral cavity was done with the help of a small brush at the other end of dental floss which is very economical and widely available. The dental floss was left in the oral rinse till DNA extraction. This gave a good quantity of mucosal cells and ultimately good DNA yield. When samples were collected without brushing the DNA yield was compromised. Thus, the isolation of DNA from oral rinse is an attractive, non-invasive method for obtaining relatively large amounts of DNA. This protocol is inexpensive, painless, less arduous, patient friendly, does not require technical assistance, can be collected by ordinary volunteers, anywhere, anytime and should be ideal for large-scale epidemiology and molecular biological studies. Lee et al. considered saliva as an oral fluid which being the ‘mirror of body’ is a perfect medium to be explored for health and disease surveillance.\(^ {17}\)

What was a vision 10 years ago is now a reality and saliva has turned into an ideal translational research tool and diagnostic medium for diseases,\(^ {18}\) proteomics\(^ {19}\) and transcriptome analysis.\(^ {20}\) The need now is to develop sophisticated techniques to detect additional biomarkers for the detections of proteins, metabolomics, genetic material and markers of nutritional status from saliva.

**CONCLUSION**

Oral rinse sample were sufficient to extract good quality and quantity of DNA. From 20 to 40 ml oral rinse, enough DNA can be extracted for genetic analyses.

**REFERENCES**


