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# Ideal Specimen for Identification in Forensic cases after Hematopoietic Stem Cell Transplantation (HSCT): a review.

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ABSTRACT

Nuclear DNA markers like STRs are commonly used for identification, crime investigation and to determine parentage of an individual. In forensic DNA testing, there is always debate about the ideal specimen for identification purpose. Peripheral blood, buccal swab and hair follicles are commonly used specimen for parentage purpose and personal identification. The important issue is addressed here, i.e. , the most suitable biological specimen to be used in a case where subject has undergone hematopoietic stem cell transplantation.

**Keywords:** Chimerism, Short tandem Repeats (STR), DNA profiling

## Introduction

Short tandem repeats marker's (STRs) analysis in forensics has become the most relevant method for human identification testing. No two individuals have same STR,s markers examined at all loci except identical twins. Individuals can be identified by analysis of an appropriate numbers of STR s markers with high probability. STR analysis also helps to differentiate STR profile from two or more individuals and it is independent of source of DNA, date of sampling and age of the source. Before the inception of DNA fingerprinting technique, ABO group antigen, serum proteins and RBC enzymes were used for identification purpose of an individual, however these methods have low polymorphism and poor stability.

Many a times, forensic scientists observe mixed or completely mismatched DNA profile of an individual [1,2]. Such results may lead to erroneous conclusion when the sample analyzed gives mixed or mismatched profile which could be either due to contamination from outside source or the sample analyzed is a genetic mosaic or the sample analyzed is from a person who underwent hematopoietic stem cell transplantation which may also lead to false exclusion or false inclusion of the suspect or person for identification [2,3].

From past 30 years, allogenic peripheral blood stem cell transplantation is used to treat patients with malignant hematological disease or non malignant hematological disease [4]. The main purpose of transplantation is to replace patients stem cell with the stem cell donated by donor after hematopoietic stem cell transplantation recipient cell [4,5]. HSCT can be differentiated into various cells types including buccal epithelial cells. In forensics collection of blood , buccal swab and saliva has been established as a forensic standard to obtain forensic genotype for DNA analysis thus unexpected observation of the donor genotype in these samples of the recipient can cause misinterpretation of DNA evidence.

## Ideal specimen after HSCT

After hematopoietic stem cell transplantation, recipient cells become donor genotype while other cells remain of recipient origin [6]. Thus analysis of donor cells in the recipient blood has great importance. Therefore, analysis of patient/recipient blood can provide a measure of engraftment of bone marrow in recipient. PCR analysis of STRs has improved specificity and sensitivity of these type of analysis . To conduct these analysis it is essential to know genotype of both donor and recipient . Many a times pretransplant material of recipient is not available and pretransplant STR genotype cannot be determined.

Endler et al [7] tested DNA source which could be useful to identify pretransplantation genotype in mouth wash of the recipient after bone marrow transplantation that contained large amount of easily accessible epithelial cells in mouth wash cells pellet . DNA was obtained by boiling mouthwash and followed by phenol-chloroform extraction.PCR amplification of STR loci vWF and TH01 were performed and run on 6% PAGE . The result showed chimerism was observed in eight samples out of 10 DNA , while only two established that of donor type even though mouth wash cell pellets contain 75% of epithelial cells which is presumed of recipient origin and only 25% leukocytes which is presumed of donor origin.

Thiede et al [8] supported the above results, they performed analysis on more than 20 cases and analyzed 13 patients of known profiles of donor and recipient and took samples after 1-24 months after transplantation . The number of leukocytes in normal saliva ranged from 2 to 136000 cells/ml. Patients were asked to rinse mouth with 5-10 ml of water twice and buccal swab were taken by strile cytological brush. DNA was extracted using Qiagen DNA blood kit and analysis was done using multiplex PCR with nine STR labeled with fluorescent primers. They found that mouth wash sample contained high amount of DNA completely derived from donor. DNA samples derived from buccal swab which still contain donor DNA but amount was much lower than in the mouthwash samples.

Further Zhou et al [9] carried out study on seven patients who had undergone allo-perepheral blood stem cell transplantation. Times for the recipients ranged from 3 months to 147 months with a mean time elapsed 47 months after transplantation. 1.5 ml blood were collected from the donor and recipient, 5-7 hairs were plucked from the recipient and epithelial cells were collected from the

both side of the mouth. DNA was extracted from blood, hair, and buccal swab and subjected to DNA profiling using AmpFLSTR SGM plus PCR kit. DNA profiles obtained from blood samples of recipient were 100% donor type. There was no evidence of donor cells in recipient's hair sample that mean the profile was 100% from recipient where as donor cells in buccal swab shows variation between 2-90%. One case showed mixture of two source of DNA i.e. donor and recipient and that is about 54% donor. In another case genotype was almost completely derived from donor that is about 90% of donor. So DNA profile obtained from hair was completely unaffected by donor and hence can be used as a pretransplant sample.

A total no. of 77 adult recipient were analyzed for quantitatively determine state to determine chimeric donor/recipient ratio by Berger et al [2]. Age of the recipient ranged from 19 to 74 years. Total no. of 162 buccal swabs were collected from 77 patients between known time intervals. Patient transplantation intervals(PTI) ranged from 17 to 3,361 days(median 394 days). 162 buccal swabs from majority of the recipient (56%) who sampled several times. Out of 162 samples 120 samples (74%) showed mixture of donor and recipient, 35 samples (22%) showed no chimerism and & samples (4%) showed completely donor type. The median value of 23% shows that recipient STR profile was the major contributor in the mixture in majority of case (mean%30±27). Only 33 samples(20%) gave donor genotype as majority component(mean % >50).In 77 patients, mean chimerism level was found between 10-30% in case of buccal swab and males showed higher chimerism level than female.136 blood samples from 76 patients showed completely donor genotype without any contribution of the recipient . On other hand no donor genotype were found in all 88 hair samples.

Hong et al [10] carried study on 29 patients who underwent engraftment from 15 months to 21.5 years (median 8.2 years) after allogenic HSCT without rejection. Out of 29 patients, 15 were sex matched while 14 were sex mismatched. Peripheral blood, buccal swab and hair follicle were collected. The study was performed by using AmpFLSTR profiler plus kit(Applied Biosystem) to amplify nine highly polymorphic autosomal STR loci with one sex linked loci i.e. Amleogenin. The STR analysis of blood were all donor type except one(which showed mixed chimerism) after 14.5 years of HSCT for buccal swab, mixed chimerism were found ranging from 10 to 96% of donor origin except one who have mixed chimerism for blood and in case of hair follicle shows 100% of reciepient type in all 29 patients.

In a study by Rovo et al [11] who did experiment on 100 patients who are long term survivors (>24 months of follow up) and in addition 15 short term patients(1-12 months of follow up). They took peripheral blood and cellular part of hair that is hair bulb. Hairs were cut to 3 cm including root and washed with phosphate buffer to wash out blood cells attached to roots during plucking of hair. DNA was extracted by QI amp DNA micro kit and PCR was done using nine loci including Amleogenin. PCR product was separated by ABI prism 310 genetic analyzer and fragment analysis done using Gene scan software. All short term and 98% long term follow up patients shows 100% donor type chimerism in blood in contrast all hair follicle shows no chimerism that is 100% patient's alleles.It shows that stem cell transplantation did not triggers chimerism in hair follicles due to its multipotent mesenchymal stem cells capable of differentiating into no blood lineages. This study did not find any evidence to develop plasticity in hair after allo-hematopoietic stem cell transplantation.

Chaudhary et al [12] study showed 19 blood sample out of 25 patients have completely donor chimerism where as 3 patients showed chimerism range from 46.9% to 100% and 3 patients

showed range from 46.9% to 97% at various time intervals/ points. Buccal swabs showed donor chimerism in all samples at all points and that may be due to collection of buccal swab as epithelial cells may be contaminated with granulocyte present in saliva and these cells are of donor type after successful transplantation. Hair follicle showed chimerism but not above 10%, 7 patients showed no chimerism ,3 patients showed chimerism below 1%and 15 patients showed chimerism below 10% in case of hair follicles. Percentage of chimerism was calculated according to peak area of donor and recipient allele for every possible combination like by considering only allele which is not shared between recipient and donor(Type-I),by considering at least one shared and one which is not shared allele between recipient and donor(Type-II) and by considering common allele between recipient and donor (Type-III) . Type-III was non informative as all allele are of donor type and not included in chimerism percent calculation. Sample with more than 90% allele of donor type were classified as no chimerism.

In contrast of the above studies Jacewicz et al [13] revealed that male donor derived genetic material which could be found in recipient's hair follicles after successful allogenic hematopoietic stem cell transplantation .They took 10 female patients and collected blood , buccal swab and hair follicle of recipient and donor as pretransplant samples and posttransplant sample of recipient . Male DNA in female reciepient was checked by quantifiler Y human male DNA quantification kit which detected sex determining region on Y (SRY). Samples were run in 96 well plate to quantify in 9700 RT-PCR system which gave result in post-transplant samples of blood as and buccal swab from all investigated women revealed donor derived male DNA in the range of 0.96 to 19.16 ng/µl and blood revealed donor derived male DNA in the range of 2.57 to 51.18 ng/µl. Hair follicle showed 10 to 70 pg of male DNA in 8 investigated women out of 10 women. To rule out the situation that male fraction in female hair was from their sons or brother whose cell may pass into female circulation due to feto-maternal transfer, they used Y-STR multiplex PCR which revealed complete donor Y – profile in post-transplant samples of blood and buccal swab , similarly male haplotypes was observed in female hair follicles sample after HSCT. 8 females showed all 16 loci donor haplotype where as 2 females give partial profile with 6-8 loci. This undermines the validity of data indicating that hair follicle cell maintain 100% of recipient origin.

### Conclusion and future outlook

According to the present review, it is clear that peripheral blood and buccal swab are not a ideal specimen for identification purpose as these samples are unable to give DNA profile of recipient origin DNA after hematopoietic stem cell transplantation. The present review make it clear, hair follicles can be the reliable source to demonstrate an individual`s origin DNA even after hematopoietic stem cell transplantation, it also serves to highlight that continued research in forensic identification testing is vital.

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## What's New in This paper

### What is already known on this topic?

This is a review article which is a compilation of work already done on chimerism.

## What this study adds?

This article gives a brief idea about the best sample for identification of an individual in medico legal situations after stem cell transplantation.

## Suggestions for further development

s. Hematopoietic stem cell transplantation is very common now a days for the treatment of various GvHD. More study needed to check the status of the patient after transplantation and associated disease. Study needed to check the acceptance and rejection status of the patients at molecular level.