DNA profiling in blood, buccal swabs, and hair follicles of transplantation patients

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1. Introduction
Allogeneic peripheral blood stem cell transplantation (allo-PBSCT) has been widely used in the treatment of malignant or nonmalignant hematological diseases over the past three decades. Today, approximately 15,000 allo-PBSCTs are performed annually worldwide, and the number of patients undergoing allo-PBSCT is increasing each year (1). Stem cell transplantation is a procedure in which hematopoietic stem cells obtained from a donor are implanted into a recipient to replicate and form a part or whole of the patient's hematopoietic system (2).

In allo-PBSCT, complete hematopoiesis from donor cells is necessary to sustain the engraftment and prevent relapse of the underlying disease (1). The qualitative and quantitative assessments of donor-specific cells in the recipient's body are important in the follow-up of patients after transplantation; moreover, chimerism analysis has become necessary for determining the success or failure of allo-PBSCT (3).

It is accepted that the transplanted bone marrow stem cells have the potential to transdifferentiate or dedifferentiate into neural, bone, cartilage, muscle, liver, intestine, alveolar, epidermal, or endothelial cells; this phenomenon is referred to as adult stem cell plasticity. Thus, using peripheral blood samples is not an appropriate method for identifying and determining paternity/kinship in individuals after successful allo-PBSCT (1,4,5).

Similarly, some studies have reported donor chimerism in patients who underwent liver transplantation. This method is used in the early diagnosis of some complications such as graft-versus-host disease (GVHD) after liver transplantation (6).

Peripheral blood is frequently used in forensic identification and paternity/kinship determination. However, because of the potential of chimerism in individuals after transplantation, it may be more appropriate to use buccal swab or hair follicle samples to assess DNA profiles instead of peripheral blood (1,6).

The aim of this study is to demonstrate the utility and reliability of DNA analysis of hair follicle and buccal swab samples in forensic identification after allo-PBSCT and liver transplantation.

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2. Materials and methods

2.1. Collection of the samples

After obtaining approval from the Noninvasive Research Ethics Committee of Dokuz Eylül University (30.06.2011 - 2011/22-07) and then obtaining informed consent, 35 patients who underwent liver transplantation and follow-up in the General Surgery Department of the Dokuz Eylül University Faculty of Medicine and five patients who underwent allogeneic stem cell transplantation and follow-up in the Hematology Science Department were voluntarily enrolled in the study. The following samples were obtained from each patient and maintained at –20 °C: blood samples (2 ml) were collected in EDTA-coated tubes, buccal swab samples were obtained from the oral cavity mucosa using sterile cotton swabs (the patients rinsed their mouths with clean water three times before the samples were obtained), and 5–7 strands of hair were plucked from the scalp.

2.2. DNA extraction and profiling

Genomic DNA from the samples was extracted using a QIAGEN MagAttract DNA Mini M48 Kit. Fifteen autosomal unlinked loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) and an additional sex-determining marker, amelogenin, were amplified using the AmpFlSTR® Identifiler® Plus Polymerase Chain Reaction (PCR) Amplification Kit (Applied Biosystems, Foster City, CA, USA). Detection and separation of PCR products were carried out using the ABI 3130 XL Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions, and the samples were genotyped using GeneMapper ID v3.2 software (Applied Biosystems).

The following clinical data of the patients were analyzed using computer software: sex, disease, age, complication development, relationship with the recipients, the duration after transplantation, and the possible difference between the DNA profiles.

2.3. Statistical analysis

The data are presented as mean and standard deviation. The paired t-test was used to compare the three sample groups. P < 0.05 was considered statistically significant. SPSS 15.0 was used for statistical analysis.

3. Results

The five patients who underwent allo-PBSCT included three female and two male patients with a mean age of 31.6 ± 13.2 years (range: 18–53 years). The time of obtaining samples ranged between 2 months and 30 months after transplantation. All five patients underwent allo-PBSCT for malignant hematological diseases. The clinical information of the volunteers who underwent allo-PBSCT is provided in Table 1.

The 35 patients who underwent liver transplantation included eight female and 27 male patients with a mean age of 46.3 ± 15.1 years (range: 7–67 years).

The DNA profiles obtained from the blood samples of four patients who underwent allo-PBSCT were 100% donor type. The hair follicle and buccal swab samples from these patients were 100% recipient type. The DNA profiles of all the three samples from the fifth patient were 100% recipient type. This patient, who underwent allo-PBSCT 4 months before sample collection, later developed GVHD, because of which a retransplantation is being scheduled. This finding was statistically significant (P = 0.025). Statistical analysis revealed no statistically significant relationships between the DNA profiles of the samples and the age and sex of the donor/recipient, disease diagnosis, and duration after transplantation. None of the patients had mixed chimerism (Table 2).

The DNA profiles of the blood, hair follicle, and buccal swab samples of all 35 patients who underwent liver transplantation were determined to be 100% recipient type. Statistical analysis revealed no statistically significant relationships between the DNA profiles of the samples and the age and sex of the donor/recipient, disease diagnosis, and duration after transplantation.

Table 1. Clinical information of allo-PBSCT patients (AML: acute myeloid leukemia; GVHD: graft-versus-host disease).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Recipient sex</th>
<th>Donor sex</th>
<th>Donor–recipient relationship</th>
<th>Disease</th>
<th>Elapsed duration after transplantation</th>
<th>GVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>Female</td>
<td>Male</td>
<td>Sibling</td>
<td>AML</td>
<td>10 months</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>Female</td>
<td>Male</td>
<td>Sibling</td>
<td>B-cell lymphoblastic lymphoma</td>
<td>2 months</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Female</td>
<td>Female</td>
<td>Sibling</td>
<td>AML</td>
<td>5 months</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>Male</td>
<td>Male</td>
<td>Sibling</td>
<td>AML</td>
<td>30 months</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>Male</td>
<td>Male</td>
<td>Son</td>
<td>AML</td>
<td>4 months</td>
<td>Yes</td>
</tr>
</tbody>
</table>
4. Discussion

Currently, allo-PBSCT is widely used in the treatment of many hematological diseases (3). It is considered inappropriate to use peripheral blood samples in forensic identification or paternity/kinship determination in individuals who have undergone allo-PBSCT because of the potential for chimerism; therefore, the aim of our study was to evaluate whether buccal swab or hair follicle samples are better for DNA sampling than peripheral blood samples.

In studies conducted so far, it has been shown that the DNA profiles of blood samples obtained after successful allo-PBSCT were of the donor genotype, those of the buccal swabs showed variability in genotype, and those of hair follicles were unaffected by the donor genotype (1,2,4). In our study, the DNA profiles of the peripheral blood samples were completely different from those of hair follicle and buccal swab samples in four of the five patients who underwent allo-PBSCT. The fifth patient had an identical (recipient) genotype in all three samples. This patient is being scheduled to undergo retransplantation because of engraftment failure. These findings demonstrate the usefulness of DNA profiling of hair follicle and buccal swab samples for determining the genotype in recipients.

After aplasia develops during the preparatory regimen, donor cells find their way to the bone marrow, where they reestablish the normal production of blood cells; this process is called engraftment (7). According to previous studies, granulopoiesis and erythropoiesis begin 2–4 weeks after transplantation, and thrombopoiesis becomes adequate after a few months (8). The shortest elapsed time of sample collection after the allo-PBSCT in our patients was 8 weeks. Thus, all patients had sufficient time for complete donor chimerism.

The DNA profiles of buccal swab and hair follicle samples of all five patients who underwent allo-PBSCT were identical and showed 100% recipient genotype. In some studies, it has been reported that some buccal swab samples showed mixed chimerism (1,2,4), suggesting that donor leukocytes may migrate to the oral mucosa, get secreted in the saliva, and be the source of the donor DNA in the buccal swab samples. In normal saliva, the number of the leukocytes varies between 2 and 13,600 cells/mL, and this number may increase to $1.1 \times 10^6$ cells/mL in patients with oral inflammation (9,10). None of our patients showed findings of oral inflammation (e.g., mucositis). Chimerism in buccal swab samples is related to the number of migrated leukocytes. Thus, it has been suggested that the duration after transplantation is an important factor for the number of donor cells observed in buccal swab samples. Zhou et al. reported that the chimerism levels increased 99–147 months after transplantation compared with 3–16 months after transplantation. Tran et al. showed in vivo that the bone marrow cells may migrate to the buccal mucosa and transform into buccal epithelial cells. Therefore, donor cells may increase in the mouth of allo-PBSCT transplant patients over time. In our allo-PBSCT patients, the longest elapsed duration after transplantation was 30 months. The lack of chimerism in the buccal swab samples is probably because of the limited number of patients and the limited duration after transplantation.

Currently, the detection of chimerism levels is an important method for monitoring outcomes after allo-PBSCT (5,11). In our study, one of the patients had the same genotype in all three samples; this patient is being scheduled to undergo retransplantation because of transplantation failure.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Elapsed duration after transplantation</th>
<th>GVHD</th>
<th>Blood sample genotype</th>
<th>Buccal swap sample genotype</th>
<th>Hair follicle genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>10 months</td>
<td>No</td>
<td>100% donor</td>
<td>100% recipient</td>
<td>100% recipient</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>2 months</td>
<td>No</td>
<td>100% donor</td>
<td>100% recipient</td>
<td>100% recipient</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>5 months</td>
<td>No</td>
<td>100% donor</td>
<td>100% recipient</td>
<td>100% recipient</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>30 months</td>
<td>No</td>
<td>100% donor</td>
<td>100% recipient</td>
<td>100% recipient</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>4 months</td>
<td>Yes</td>
<td>100% recipient</td>
<td>100% recipient</td>
<td>100% recipient</td>
</tr>
</tbody>
</table>

The existence of GVHD and 100% recipient DNA profile in three samples was statistically significant (P = 0.025).
in the recipient's blood is important for monitoring disease progression and treatment response (12). Some studies have reported that donor chimerism after liver transplantation disappears within 1–3 weeks; however, in the presence of GVHD, this period is further extended (12,16). Dauber et al. reported that a patient who died in the 18th week after liver transplantation because of GVHD showed only donor genotype in blood samples obtained 2 days before death. In this patient, the buccal swab sample had a mixed genotype, and the genotype of the hair follicle sample showed 100% recipient genotype. In the field of forensic sciences, changes in recipient DNA profiles in liver transplant patients are as important as they are in allo-PBSCT patients (2,6). To date, no study has conducted a long-term follow-up of patients after liver transplantation using short tandem repeat analysis. In our study, 35 patients were examined 1–125 months after liver transplantation. The DNA profiles of blood, hair follicle, and buccal swab samples of all patients were the same and no chimerism was detected. The fact that there were no differences in the DNA profiles was interpreted as the absence of GVHD in all of the patients.

In the present study, two liver transplant patients had acute rejection episodes. Domiati-Saad et al. previously showed that there were no significant relations between the level of chimerism and the incidence of acute rejection episodes in patients who had undergone liver transplantation (12). No differences were detected in the DNA profiles of all three samples in the two patients with acute rejection episodes in our study.

Blood and buccal swabs are not appropriate for the assessment of a patient's pretransplant or true genotype profile because these samples are not devoid of donor-derived cells. For hair follicle samples, no donor chimerism has been observed; thus, they can be used as a reliable biological source for personal identification by DNA profiling. Great care must be taken to avoid possible contamination while collecting the samples (17). It has been shown that peripheral blood DNA profiles of recipients show donor chimerism after successful allo-PBSCT. In addition, the DNA profiles of buccal swab samples can change over time after transplantation and have a high risk of salivary contamination during sample collection. However, the DNA profiles of the hair follicle samples shown both in this study and previous studies showed 100% recipient genotype.

In forensic science, DNA samples recovered from a crime scene have become standard forensic evidence for the investigation of a wide spectrum of crimes, including murder and rape. Forensic scientists use DNA from various biological samples found at crime scenes to identify criminal suspects by DNA profiling. Different types of DNA samples could be used for DNA analysis according to the hypothesis that all the cells in the human body have an identical DNA profile (18). As in every area of forensic science, it is also important to gain accurate and complete genetic information as well. Therefore, the medical history and records of individuals must be known so that forensic identification or paternity/kinship determination can be accurately performed.

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References
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