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15 STRS LOCI AND SEX SPECIFIC AMELOGENIN ANALYSIS OF BLOOD SAMPLES FROM FEMALE PATIENTS RECEIVES MASSIVE BLOOD TRANSFUSION USING ABI 310 GENETIC ANALYZER

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ABSTRACT

In forensic context effect of blood transfusion on DNA profiles of an individual is an important issue. In the present study we have investigated the effect of massive blood transfusion, up to 75% blood loss and 65% blood volume replacement of less than 26h old unfiltered whole male blood having large number of leucocytes, in seven female subjects up to 20 KG body weight by PCR based assay on serial post transfusion blood samples. The Amp Fl STR® Identifiler ® PCR Amplification Kit, Applied Biosystem, having primer pair to D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin loci were used for the PCR amplification of 15 STRs and sex specific Amelogenin. Separation and Electropherograms pattern of alleles of amplified multiplex products from pre and post transfusion of seven female recipients and their corresponding donors were generated by ABI PRISM® 310 Genetic analyzer and studied. The DNA profile of Pre-and Post Transfusion of all seven female recipients were found to be consistent with no evidence of the male donor genetic material. It is demonstrated that the currently available PCR based STR and Sex specific Amelogenin loci DNA profiling technique is reliable and informative which can be used in forensic science for paternity and identity purposes in situation of massive transfusion of unfiltered whole male blood (<26h old) to female recipient up to 60 h post transfusion.

KEYWORDS: 15 STRs loci, ABI 310 Genetic Analyzer, Unfiltered Whole Blood, Massive Transfusion, DNA Profiling, Microchimerism

INTRODUCTION

Introduction of donor material (cells and proteins) by transfusion is known to dilute the recipient identity (Wenk and Chiafari, 1997) .At present the use of blood transfusion has become more often than ever before, hence occasional cases with a history of massive transfusion may be encountered in a forensic laboratory for the establishment of identity or paternity. From the standpoint of forensic science it is notable that serologic typing and interpretation of autologous blood group phenotypes and allozymes, conventional red blood cell antigen typing by agglutination or isozyme typing after multiple transfusions could be misleading for identification in such cases (Brauner, 1996; Davidson and Lee, 1999; Huckenbeck and Rand, 1994).

Presently DNA analysis has become routine in laboratories conducting identity and paternity testing, while serological methods are falling into disuse. Therefore the situation arising due to massive blood transfusion has led to a growing interest in typing the DNA from post transfusion blood samples. Reports are available regarding reliable typing of

blood recipients by DNA analysis after transfusion using more than 72 hours old packed red blood cells (Brauner et al., 1997; Reid et al., 2000) and whole blood (Mukherjee and Biswas, 2005; Giroti et al., 2002). A few reports are also available of successful detection of blood microchimerism after transfusion (Carter et al., 1998; Sahota et al.1998). Already blood transfusion has featured in a few cases concerned with forensic and paternity identification (Brauner, 1996; Davidson and Lee, 1999; Huckenbeck and Rand, 1994). Information is not available regarding typing of DNA of recipient after massive transfusion (more than 50 % of blood volume replacement) of fresh (approximately 20-26 hours) unfiltered whole blood. The possibilities of inconsistent DNA profile among blood recipients are highest in massive blood transfusion using less than 26 hours old, unfiltered whole blood.

The use of well-accepted DNA evidence in courts of law has increased since its origin. As the admissibility of this evidence are under strict legal scrutiny (Pitluck, 2001), it is of urgent importance to systematically address the issue of detection of blood microchimerism after blood transfusion in general and massive blood transfusion using unfiltered whole blood in particular. This study was undertaken to determine whether present STRs based DNA profiling methods of forensic and paternity identity testing in ABI PRISM® 310 Genetic analyzer platform are suitable and informative for providing unambiguous identification in cases of massive blood transfusion using unfiltered whole blood between same and opposite sex.

MATERIAL AND METHODS

Subjects Studied: Seven female patients 15 to 20-kilogram body weight, scheduled for neurosurgical procedure requiring blood transfusion were selected for the study. All the seven patients lost more than 50% to 75% of their blood volume (estimated loss 800ml to 1200ml of 1200 to 1600ml of total blood volume of the recipients), for which 2 units of unfiltered approximately 700 ml (average 65% of recipient's blood volume) whole male blood with Total Leukocyte Count (TLC) 7200-8600 (starting 20 hours after donation till 26 hours) was transfused and serial blood samples were obtained as summarized in Table 1. The blood samples obtained from the recipients and the donors were frozen at –70 ^{0}C for later analysis.

Table 1: Transfusion Summary in Female Recipient [a]

Recipient Number	Number of Units	Amount of Blood Transfused (Ml)	Sex of Blood Transfused	Age of Unit	Sampling Period After Transfusion[b]
1	2	750	Male	<26h	Immediately; 1h; 12h;24 & 60h after transfusion.
2	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion
3	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion
4	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion
5	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion
6	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion
7	2	750	Male	<26h	Immediately; 1h; 12h; 24 & 60h after transfusion

[a]All units of blood were less than 26 h old and unfiltered whole blood.

[b]Samples were obtained from all recipients before transfusion in addition to the times indicated.

DNA Isolation: The serial post transfusion sample DNA was extracted from whole blood by using "DNA IQ Extraction kit **,** Promega Corporation, Madison, WI, USA)**"** according to the manufacturer's recommended protocol. High molecular weight DNA was recovered.

Quantification of Extracted DNA: The extracted DNA was quantify by "Quantifiler Duo DNA Quantification kit"(Applied Biosystem,USA) according to the manufacturer's recommended protocol on 7500 Real Time PCR Instrument (Applied Biosystem, USA).

PCR Analysis: About 1 ng of DNA were used for Polymerase Chain Reaction D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin loci were simultaneously amplified by "Amp Fl STR® Identifiler ® PCR Amplification Kit" (Applied Biosystem, USA) according to the manufacturer's recommended protocol. Amplification was carried out in a "Gene Amp® PCR system 2400" thermal cycler (Perkin Elmer Corporation, California, USA).

Electrophoresis and Electropherograms pattern: Separation and Electropherograms pattern of alleles of amplified multiplex products were generated by ABI PRISM® 310 Genetic analyzer (Applied Biosystem, USA**)** according to the manufacturer's recommended protocol and analyzed.

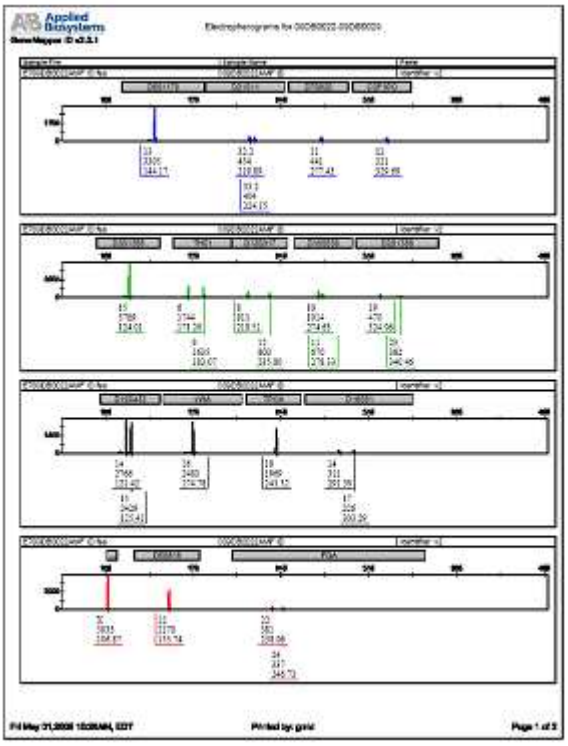
RESULTS

High molecular weight DNA was extracted and quantify from pre-transfusion and the serial post-transfusion samples from the seven recipients and 12 transfusion donors. Pre-transfusion samples from all the recipients, their corresponding donors and the serial blood samples drawn following transfusion were amplified with specific primer pairs to Reaction D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin loci to identify donor alleles. TLC of all the donors were estimated. All the samples were successfully amplified and typed. Examination of the resulting banding pattern of alleles generated by PCR typing from the serial blood samples collected from all the female recipients were indistinguishable from their respective pre-transfusion blood samples. The DNA profile of all the 15 STRs and Amelogenin of pre-transfusion blood samples from 1 representative recipients (recipient 4), the corresponding transfusion donors and the serial post-transfusion blood samples are shown in Table 2, and in Electropherograms 1 – 8A & 8B.

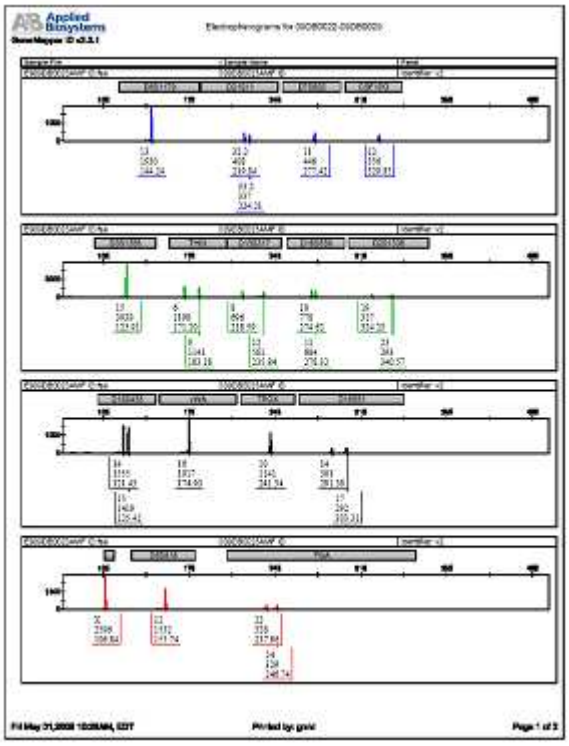
Table 2: Pre and Post-Transfusion CTT Multiplex, FFv Multiplex and Amelogenin Results for Transfusion (tx) Recipients 4 and their Respective Donors

	Donor 1	Donor 2	Subject 4	Immediatel y After tx of Donor 1	Immediatel y After tx of Donor 2	1 hour After tx of Donor 2	12 hour After tx of Donor 2	24 hour After tx of Donor 2	60 hour After tx of Donor 2
D8S1179	13,14	12,12	13,13	13,13	13,13	13,13	13,13	13,13	13,13
D21S11	31,31	30,31.2	32.2,33.2	32.2,33.2	32.2,33.2	32.2,33.2	32.2,33.2	32.2,33.2	32.2,33.2
D7S820	10,13	8,11	11,11	11,11	11,11	11,11	11,11	11,11	11,11
CSF1PO	10,10	13,13	12,12	12,12	12,12	12,12	12,12	12,12	12,12
D3S1358	15,16	15,16	15,15	15,15	15,15	15,15	15,15	15,15	15,15
THO1	9,9.3	7,10	6,9	6,9	6,9	6,9	6,9	6,9	6,9
D13S317	8,11	9,12	8,12	8,12	8,12	8,12	8,12	8,12	8,12
D16S539	12,12	9,14	10,11	10,11	10,11	10,11	10,11	10,11	10,11
D2S1338	23,23	18,23	19,23	19,23	19,23	19,23	19,23	19,23	19,23
D19S433	12,14.2	15,15	14,15	14,15	14,15	14,15	14,15	14,15	14,15
vWA	15,18	17,19	16,16	16,16	16,16	16,16	16,16	16,16	16,16
TPOX	9,12	6,8	10,10,	10,10	10,10	10,10	10,10	10,10	10,10
D18S51	12,14	17,20	14,17	14,17	14,17	14,17	14,17	14,17	14,17
D5S818	11,11	10,13	12,12	12,12	12,12	12,12	12,12	12,12	12,12
FGA	24,24	20,24	22,24	22,24	22,24	22,24	22,24	22,24	22,24
Amelogenin									
212 Base X specific	-	-	X,X	X,X	X,X	X,X	X,X	X,X	X,X
218 base Y specific	X,Y	X,Y	-	-	-	-	-	-	-

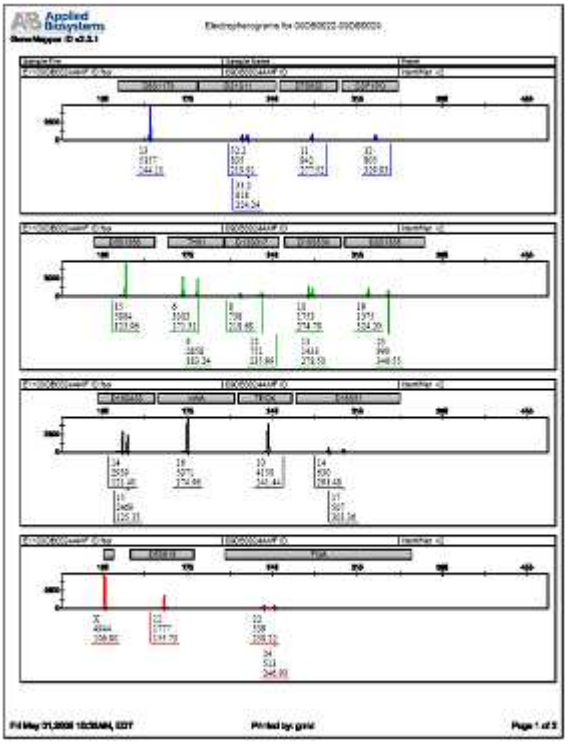
The rest of the samples had similar results



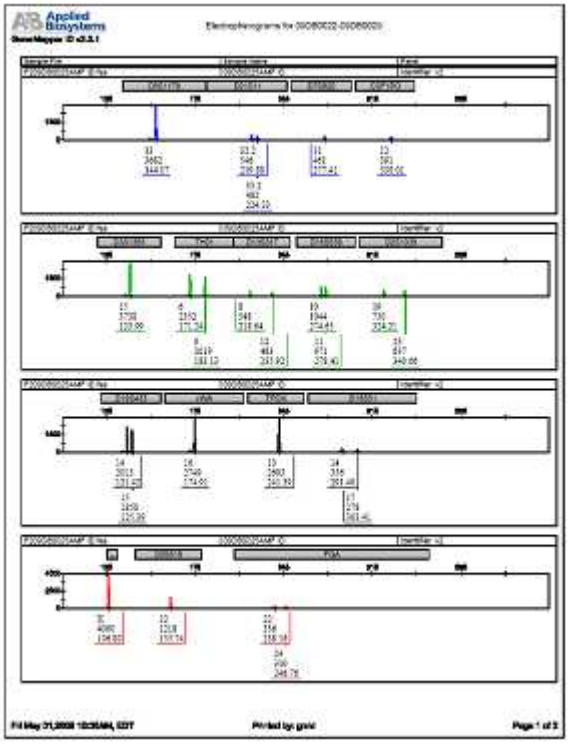
Electropherogram 1



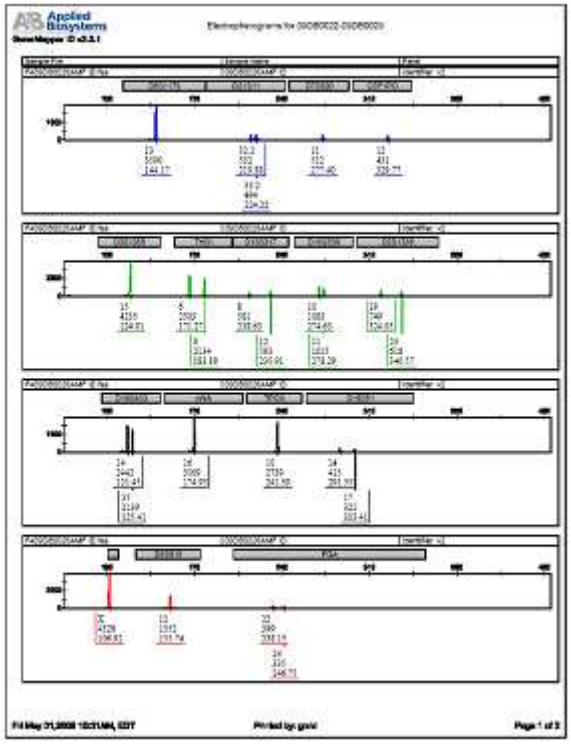
Electropherogram 2



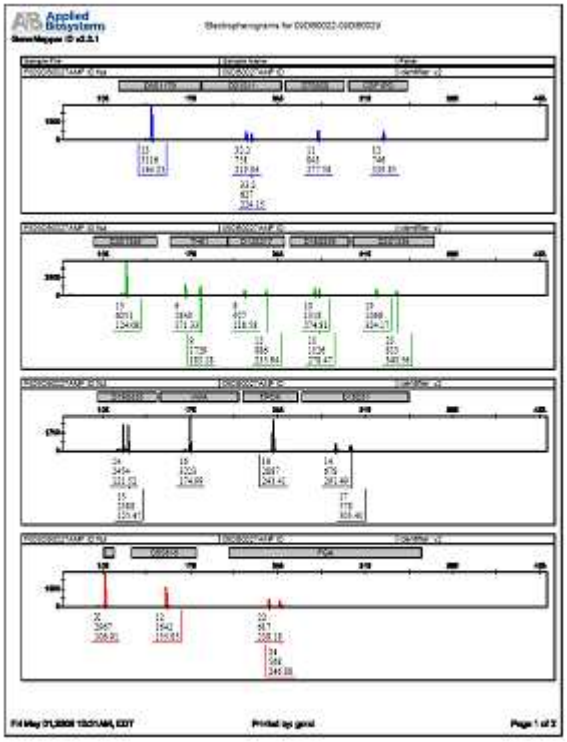
Electropherogram 3



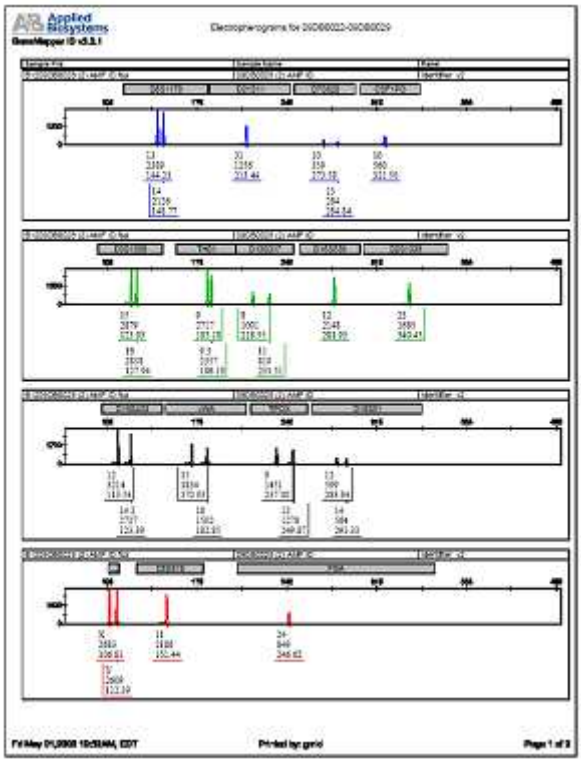
Electropherogram 4



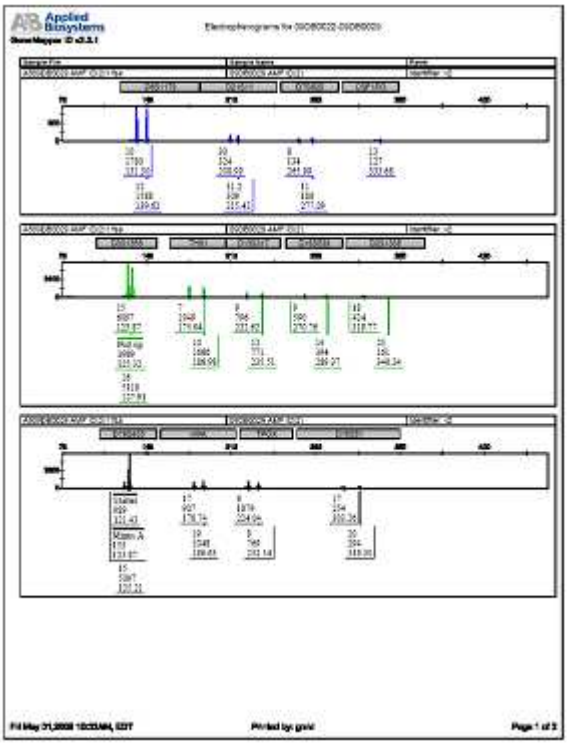
Electropherogram 5



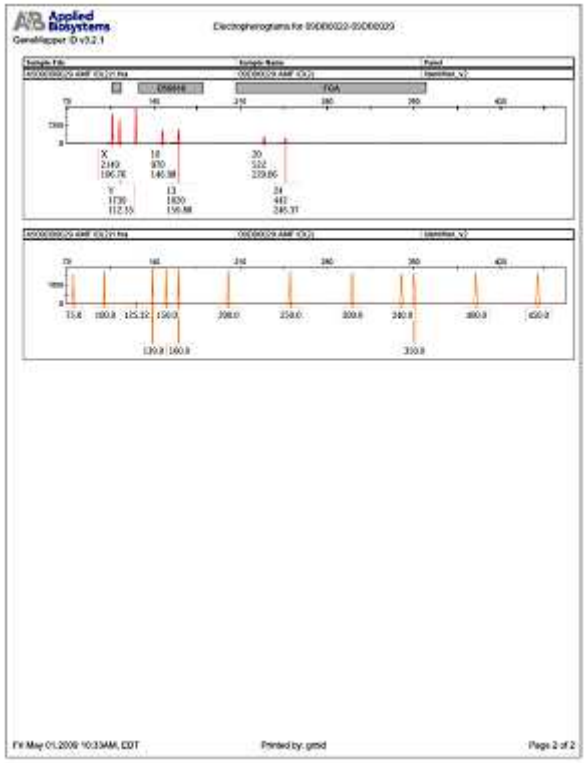
Electropherogram 6



Electropherogram 7



Electropherogram 8A



Electropherogram 8B

Electropherogram: 1,2,3,4,5 and 6 represents the DNA profile of pre-transfusion, immediately after transfusion of donor 1, immediately after transfusion of donor 2,1 hour,12 hour and 24 hour post transfusion samples respectively obtained from recipient's '5'.

Electropherogram: 7 represent the DNA profile of donor 1.

Electropherogram: 8A & 8B represents the DNA profile of donor 2.

DISCUSSIONS

We have performed the PCR- D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin analysis on serial blood samples that were drawn from the transfusion female recipients at various time intervals to study the effect of massive blood transfusion on the DNA typing results using the male whole blood containing sufficient amount of leukocytes. Pre-transfusion blood samples from the female recipients and all the corresponding unfiltered whole blood samples transfusion from male donors were included in the study to obtain reference DNA profiles for comparison.

With the body weight up-to 20 kg, estimated loss up-to 1200ml from1600ml total volume, transfusion of up to 2 units of unfiltered 750ml whole blood with TLC 7200-8600 (starting 20 hours after donation till 26 hrs) and the blood sampling up to 24 hours post transfusion had no effect on the post-transfusion DNA profiles of all the seven recipients studied. The TLC of 7200-8600 in donor's blood ensures the entrance of donors DNA into the recipient's body while transfusion. All the donor samples were successfully typed by PCR technique to ensure the detectable levels of DNA in the unit of blood stored for transfusion. However, no donor STRs and 218 base Y specific band (Amelogenin) alleles were observed in any of the post-transfusion samples collected from female recipients. These fact support our earlier finding that

the DNA pattern generated by STRs, RFLP and PCR based HLADQA1 and Polymarker from recipient are not influenced by blood transfusion using whole blood (Mukherjee and Biswas, 2005; Giroti et al., 2002) and packed red blood cell containing few of leukocytes (Wenk and Chiafari, 1997; Huckenbeck and Rand, 1994; Brauner et al., 1997; Reid et al., 2000). Our results indicate that the male donors DNA are not detectable even on very sensitive ABI PRISM® 310 Genetic analyzer platform using primer for STRs and Y chromosome micro-satellite markers (Amp Fl STR® Identifiler ® PCR Amplification Kit) even in a massively transfused female. On the other hand detection of 0.1% of male DNA against a background of female DNA using primers for the Y chromosome microsatellite marker was reported, but the sensitivity increased up to 0.0001% using nested primers and similarly D1S80 minisatellite locus has been reported as a useful marker for detecting microchimerism with a sensitivity of 0.1% (Sahota et al., 1998) . The detection of Y- chromosome specific DNA following nested PCR in female trauma patients who received blood transfusion from male donors was reported by (Viëtor et al., 2000). Different PCR amplification strategies have been successfully applied for detection of microchimerism in blood (Reed et al., 2001). Earlier studies have demonstrated that less abundant DNA in artificially generated paired mixtures was detectable by PCR-based Short Tandem Repeat (STR) assays even when it represented only 10% of the total DNA within the mixture (Hammond and Caskey, 1992; van Oorschot et al., 1996). Rubocki et al., 2001, reported detection of natural DNA mixtures in fraternal twins using STR analysis. Donatio et al., 2013, recently reported the capability of STRs in detecting mixed blood cell populations where the fraction of the minoritary population is as low as 2.5%.

Why a transfusion effect did not occur in our results even after using unfiltered whole blood for the transfusion can be well understood by information regarding survival kinetics of donor leucocytes in the recipient's circulation and the sensitivity of the assay system.

Firstly the granulocytes which contains about 70% of total white blood cells, suffer loss of function after 24 to 48 hours storage at 4º C (Spivak, 1984). As soon as a donor's blood enters the recipient's circulation the transfused white cells begin to disappear from the recipient's circulation and move into the tissue spaces. It has been demonstrated that donor leucocytes persisted in the circulation for only 30 to 90 minutes after transfusion (White, 1954). Reports are also available for longer leukocyte survival of 30 – 50% at 6 hour and 5-10% at 24 hour (Rosse and Gurney, 1959). Though we have used unfiltered whole blood containing large number of leucocytes, the samples drawn from massive transfused subjects will inevitably contain very few numbers of leucocytes as the transfused leucocytes moved into the tissue space and cleared by the reticulo endothelial system. Even the highly sensitive PCR- initiated STRs analysis that we have used, which was most likely to detect different sources of DNA, did not reveal any alleles corresponding to the transfusion donors in the post-transfusion profiles of the recipients. The techniques used in the present study to detect DNA samples is very sensitive but not sensitive enough to detect very small number of nuclear fragments of the donor present in the post transfusion samples of the recipients after massive transfusion of blood and up to 24-hour post transfusion.

CONCLUSIONS

The present study demonstrate that the PCR- D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and Amelogenin loci analysis techniques on ABI PRISM® 310 Genetic analyzer platform are reliable and sensitive enough to retain specificity to reveal the secrets of individuality even after 75% blood loss and 65% blood volume replacement by transfusion with blood having large number of leucocytes, up to 60-hour post transfusion.

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