SHORT TANDEM REPEAT TECHNOLOGY HAS DIVERSE APPLICATIONS: INDIVIDUAL IDENTIFICATION, PHYLOGENETIC RECONSTRUCTION AND CHIMERISM BASED POST HAEMATOPOIETIC STEM CELL TRANSPLANTATION GRAFT MONITORING

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ABSTRACT

BACKGROUND: *Short Tandem Repeat (STR) loci are widely considered to be effective for variety of applications including forensic applications, phylogenetic reconstruction and chimerism based post Haematopoietic Stem Cell Transplantation (HSCT) graft monitoring. For each application, specific sets of STR loci are used.* ***AIMS:*** *In the present study, we have attempted to use same set of STR loci for varied purposes based on their efficacy and informativity.* ***SETTINGS AND DESIGN:*** *Population and patient based study.* ***MATERIALS AND METHODS:*** *We have analyzed 5 STR loci – vWA, Tho1, FES, F13 and TPOX in 1000 North Indians. All five markers were also analyzed for chimerism based graft monitoring after HSCT in 42 HLA matched pair of patient-donor to predict the outcome of transplantation.* ***STATISTICAL ANALYSIS:*** *The analysis was done for Hardy Weinberg equilibrium (HWE), Heterozygosity, Polymorphism information content (PIC) and Power of Exclusion and Phylogenetic assessment.* ***RESULTS AND CONCLUSIONS:*** *High allelic variability in term of Heterozygosity (0.68-0.76), PIC (0.66-0.74) and high Power of exclusion (0.28-0.38) indicating high forensic utility. The ensuing PC plots finely resolved three basal clusters corresponding to three geo-ethnic groups of African, Orientals, and Caucasians. In post HSCT chimerism analysis, it was found that together these markers were informative in 38 pairs (98%) and were able to predict the chimerism status successfully. There is a possibility that these STR loci along with forensic and phylogenetic importance, can predict the outcome of HSCT successfully.*

KEY WORDS: *Short Tandem Repeat, Chimerism, Phylogeny, Haematopoietic Stem Cell transplantation, Forensics*

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INTRODUCTION

Two randomly selected humans are genetically about 99.9% identical [1] while remaining fraction of 0.1% confers the element of uniqueness to every human. This DNA fraction, which is an important tool to study human diversity, includes Short and variable number of tandem repeat (STRs and VNTRs),[2,3] single nucleotide polymorphism (SNPs)[4] and insertions of reiterated elements (Polymorphic *Alu* Insertions- PAIs).[5]

The STRs are one of the most polymorphic markers [6] and are made up of the tandem repeats of sequences ranging from 2-6 base pairs.[7] These STR show wide and uniform distribution throughout the genome, PCR feasible short sequence length and high level of relatively stable polymorphism. These varied properties of STR loci decide their suitability to a particular application. For forensic applications, STR loci should possess numerous observed alleles, high level of heterozygosity, high polymorphism information content (PIC) and high power of exclusion.[8] On the contrary, STR loci that have substantial, lower allelic counts and carries signature alleles for specific population are preferred for phylogenetic analyses.[2,3] In Chimerism based post Haematopoietic stem cell transplantation (HSCT) graft-monitoring analysis, the criterion of selection is informativity of STR loci i.e. having different allelic profiles in-patient and donor.[9]

Over past two decades, allogenic HSCT has become the treatment of choice for patients suffering from malignant and non-malignant hematological disorders. Allogenic HSCT has been effective in reconstitution of normal haemotopoiesis in these patients and have preferred therapeutic option, primarily because of its intrinsic graft versus leukemia (GVL) effect.[10] Chimerism in allogenic HSCT is an important state, which develops after engraftment, and it is an important criterion to know about the outcomes like disease relapse, graft rejection or Graft versus host disease (GvHD).[9-11]

Present study has been carried out to explore the utility of STRs in diverse applications of forensic analysis, phylogenetic reconstruction and Chimerism based graft monitoring. Various researchers in the recent past have done similar studies but they have used different sets of markers for different purposes. We have focused on the fact that can same set of STR loci could be used for different applications. For this purpose, we have analyzed five STR loci in 1000 random North Indians and criterion mandatory for a good forensic system has been analyzed. Five STR loci studied are Tho1, vWA, FES. F13 and TPOX. We have compiled a geographically targeted and racially diverse set of 20-population database from forensic literature to reconstruct the phylogenetic relationships between different population groups based on the allele frequency data of the five STRs.[12] Finally, same loci have been analyzed in 42 HLA matched pairs of patient-donor to assess the informativity and success in predicting graft status after HSCT.

MATERIALS AND METHODS

A total of 1000 unrelated individuals were randomly selected with the help of regional addresses and random numbers list generated with the help of computer. Samples were collected from the different collection sites of Uttar Pradesh. Whole blood obtained by venipuncture was collected in EDTA (Merk)

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vacutainer tubes. Three-generation pedigree charts were prepared to assure un-relatedness in all the samples. The ethical committee of the institute approved the study and blood samples were taken after obtaining informed consent from the subjects.

DNA was extracted as described by Comey et. al, 1994.[13] All the samples were analyzed for five STR loci, which were detected by PCR amplification using flanking primers described by Perez-Lezaun et al. 1999.[14] The amplified product was separated and detected on 9% Poly acryl amide gel (PAGE) using silver staining.

Allele frequencies were calculated by a simple gene count method. Three separate analyses were executed, each based upon the allelic frequency distribution of the five STR. At first, Heterozygosity, Hardy-Weinberg equilibrium (HWE), PIC and power of exclusion were calculated using CERVUS. Secondly, principal components (PC) of the population gene differences were computed and first two PC were plotted by POPSTR.

Total 84 individuals i.e. 42 pair of HLA matched patient and donor, who have been referred to Department of Hematology of our institute have been selected. Patients were suffering from various malignant and non-malignant disorders. 15 patients have undergone HSCT while others are getting myelo/non-myeloablative chemo-radio therapies and different conditioning regimens. All the 42 patient-donor pairs were analyzed for five STR loci. Informative marker - one at which patient and donor have at least one different allele were selected and were then used to establish chimerism status after HSCT in 15 patients after every 14th day of HSCT till three months and then, every month afterward. DNA of donor and patient before HSCT were mixed in a series of varying concentration ranging from 100% donor to 10%, 25%, 50%, 75% and 100% patient's DNA. These mixed DNA samples were amplified for each marker to quantify the presence of recipient's cells.

RESULTS

Analysis of five STR loci i.e. Tho1, vWA, FES, F13 and TPOX has revealed high level of diversity among North Indians. Total 7-8 alleles were found (7 each for Tho1, vWA, F13 and TPOX and 8 for FES). All the loci were in HWE. High allelic variability was depicted by high-observed heterozygosity (0.68 at Tho1-0.76 at vWA), high PIC (0.66 at F13-0.74 at Tho1) and high power of exclusion (0.28 at F13-0.38 at Tho1). All these criterions are shown in Table 1 and are indicative of the fact that these STR loci are highly informative tool for forensic applications.

42 HLA matched patient-donor pair analyzed for five STR loci provided additional proof of diverse and wider applications of these STR loci. VWA was found to be most informative of all (62%), followed by Tho1 and FES (55% each), TPOX (52%) and F13 (44%). When considered together, then a combination of the five STR loci was found informative in 98% of the cases. More interestingly, a combination of Tho1, TPOX and vWA was found to be informative in 80% of the pairs while a combination of Tho1, FES and vWA was found

Table 1: Analysis of different statistical criterion mendatory for a STR loci to be an effective forensic loci

	Tho1	*vWA*	*FES*	*F13*	*TPOX*
Total no. of observed alleles	7	7	8	7	7
Observ. Heterozygosity	0.680	0.760	0.760	0.740	0.730
Polymorphism Information Content	0.738	0.714	0.725	0.660	0.669
Power of exclusion	0.381	0.354	0.371	0.283	0.308
Average heterozygosity			0.734		
Mean Polymorphism Information Content			0.700		
Total exclusionary power			0.875		

to be informative in 70% of the families. Informative and non-informative markers are shown in Figure 1a.

Out of these 42 pairs, 15 have undergone HSCT, and graft monitoring has been carried out successfully in all the patients post HSCT and is summarized in Table 2. Three of the patients revealed Complete Chimerism (CC-only donor's cell) from day 14th onwards; seven showed mixed chimerism (MC -both donor and autologous cells) for first three months and then later developed CC. Three are still persisting with MC while two exhibited hematological relapse. We have been able to detect upto 10-20% of the recipient's cell in case of MC. Different states of chimerism detected are depicted in Figure 1b.

Based on the allele frequency distribution of

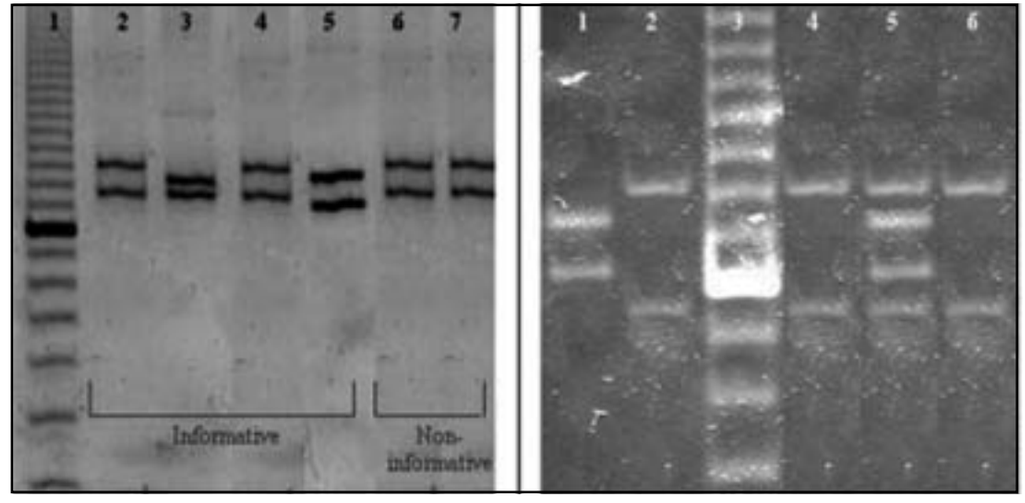

Figure 1(a): Lane1, DNA ladder, Lane 2,4,6: patient; Lane 3,5,7: Donor.

Figure 1(b): Chimerism analysis done with TPOX after HSCT to monitor graft status. Lane 1, Patient before HSCT, Lane 2: Donor before HSCT; Lane 3: DNA ladder, Lane 4: 1st sample (14 days) of patient after HSCT showing Complete chimerism; Lane 5: 4th sample (120 days) of patient after HSCT showing Mixed chimerism; Lane 6: 8th saple (210 days) of patient after DLI has been given again showing Complete chimerism.

Figure 1: Different STR genotypes revealing informativity and non-informativity in patient donor pairs and detection of chimerism status after HSCT and DLI has been done.

Table 2: Chimerism profile of 15 patients analyzed for five STR loci

Patient No.	*Chimerism status after in samples at different time intervals after HSCT**																											
	14th Day			*30th Day*			*45th Day*			*60th Day*			*3 months*			*4 months*			*6 months*			*12 months*			*15 months*			*18 months*		
	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D	S	P	D
1	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-									
2	**M**	40	60	**M**	40	60	**M**	50	50	**M**	50	50																		
3	**M**	70	30	**M**	70	30	**M**	70	30	**M**	80	20	**M**	80	20	**C**	100	-												
4	**M**	70	30	**M**	70	30	**M**	70	30	**M**	80	20	**M**	80	20	**C**	100	-	**C**	100	-									
5	**M**	50	50	**M**	70	30	**M**	70	30	**M**	80	20	**M**	80	20															
6	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-												
7	**M**	70	30	**M**	80	20	**M**	70	30	**M**	80	20	**M**	80	20	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-			
8	**M**	80	20	**M**	80	20	**M**	80	20	**M**	80	20	**M**	80	20	**M**	100	-	**C**	100	-									
9	**M**	50	50	**M**	50	50	**M**	70	30	**M**	70	30	**M**	80	20	**C**	100	-	**C**	100	-	**C**	100	-						
10	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-	**C**	100	-												
11	**C**	100	-	**M**	70	30	**M**	20	80	**M**	10	90	**H**	-	100															
12	**M**	50	50	**M**	70	30	**M**	70	30	**M**	80	20	**M**	80	20	**C**	100	-												
13	**M**	50	50	**M**	50	50	**M**	50	50	**M**	50	50	**M**	60	40															
14	**M**	50	50	**M**	30	70	**M**	30	70	**M**	30	70	**M**	10	90	**H**	-	100												
15	**M**	80	20	**M**	80	20	**M**	80	20	**M**	80	20	**M**	80	20	**C**	100	-	**C**	100	-									

* S = Chimerism status, P = % patients own cell (DNA), D = % donors cell (DNA), C = Complete chimerism, M = Mixed Chimerism, H = Hematological relapse

the five STR loci, phylogenetic analysis has been carried out among 21 populations (One studied population and 20 from compiled database). First and second PC that together constitutes 59.8% of the total variability (PC1-34.4% and PC2-24.4%) were plotted and presented in Figure 2. A patristic and significant separation of Africans, Caucasians and Oriental was clearly visible. North Indian population clustered with Caucasians near USA-Caucasians, Germans and Arabs.

DISCUSSION

Present study revealed that substantial and meaningful information was obtained in all the fields- forensics, phylogenetic assessment and chimerism analysis, when this set of five STR loci was analyzed together. Data generated on analysis of five STR loci- Tho1, vWA, FES, F13 and TPOX has exhibited high allelic variability, high average observed Heterozygosity (0.734), high PIC (total. 0.70) and high power of exclusion (total 0.875). All these statistical measurements are indicative that these STR loci have high forensic potential. Moreover, high heterogeneity and diversity of North Indian population has further increased the sensitivity and resolving power of these STR loci.

Figure 2: PC plot analysis based on allele frequency differences

Other interesting finding is that these forensic STR loci are powerful enough to provide fine resolution for the reconstruction of recent human evolutionary history. Principal component plot (PC-plot) depict strong ethnical partitioning of African, Caucasians and Orientals and deciphered the phylogenetic information about North Indian population which is in accordance with those derived from other markers as well as historical evidences of origin of present day north Indian populations.[15-17] The PC plot depicted a strong ethnical phylogeny indicating that high heterozygosity and/or numerous observed alleles do not necessarily interfere with the phylogenetic information content of the locus, provided that frequency distribution of the populations are significantly different.

Most important finding was success of these STR loci in post transplant monitoring by Chimerism analysis. The main goal of post-transplantation monitoring is to predict the negative events like disease relapse, graft rejection and GvHD in order to set up the relevant preventive therapeutics. In this context, chimerism analysis is beyond doubt an important method in monitoring post-HSCT outcome. In fact, several previous workers have suggested that an accurate quantitative analysis of chimerism kinetics would permit early differentiation between the absence of engraftment and a delay in engraftment as well as early detection of patients with a high risk of GvHD or those liable to relapse. The chimerism detection technique, therefore,

should be selected with extreme care, in regard to type of disease, therapies and regimens used; so that exact information could be obtained. For all these clinical applications, the optimal methodological approach needs to be informative, sensitive and quantitatively accurate.

In most of the bone marrow transplant centers in India, STR kits are being used which are quite expensive, however, no one has investigated into the fact that which of the STR loci appears more informative and cost effective. In sex mismatched HSCTs, Y and X-chromosome specific markers, Amelogenin loci and dual color Fluorescent in situ hybridization (FISH) has been frequently used.[18,19] The STRs/VNTRs analysis is regarded as the most sensitive and rapid method for determining the chimerism status. Moreover, STR/VNTR genotyping is independent of HLA mismatching or sex mismatching and can be used immediately after transplant when very few cells are available for analysis.[10]

Five STR analyzed in the present study not only shows high rate of informativity but also successfully monitored graft status after HSCT in 15 patients. Moreover, upto 10% of recipients DNA have been detected in two of the patients exhibiting MC despite of the fact that this sensitivity is based on comparison with mixed DNA samples. Such an analysis if carried out using Quantitative PCR could be of great importance in the light of latest therapeutic strategies of non-myeloablative stem cell HSCT, donor's lymphocyte infusion etc., when an early diagnosis is an important aspect of prognosis.[10,11]

Our results indicate that these STRs along with their reputed importance in individual identification are powerful enough to reconstruct the human phylogenies and also to predict the outcome of HSCT by chimerism based careful graft monitoring.

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