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Identification and quantification of Chimerism in bone marrow transplants leukemic patients

Majeed A. Sabbah[1], Halah Kh. Ibrahim Al-Sammarraie[1], Mohammed M. Al-zubaidi[1], Hannan Kh. Mahmood[2], and Khalifa M. Salih[2], Qasim Sh. Al-Mayah[3], Ala Hazim Bader[4]

1 Forensic DNA Center/ Al-Nahrain University[1]/ Paternity and Kinship Division.
2 Living investigation Department, Medico-Legal Directorate, Ministry of Health[2] /Medical Research unit.
3 College of Medicine- Al-Nahrain University/ Baghdad, Iraq.
4 Kamal AL-Al-Sammarraie , Infertility Center./ Ministry of health .

Abstract:

Quantification of chimerism after bone marrow transplantation is essential for evaluation the successful of engraftment after allogenic stem cells transplantation. The aim of this work is to use STR typing test for identification and quantification the chimerism in bone marrow transplant patients. Two patients subjected to bone marrow transplantation were analyzed. DNA extracted from patients (recipient) blood, cheek swabs and donor cheek swabs. Quantifiler Real time PCR kit was used for DNA quantification. Powerplex21 kit was used for amplification STR loci. STR profiling was performed by Genetic analyzer. DNA extraction and quantification were successfully performed with suitable quantity and purity. STR loci fully amplified and analyzed. In both cases recipient and Donor shared alleles with no DNA mixtures. Few loci showed Type 3 chimerism. In conclusion the results showed that the test successfully identify used for identification and quantification of chimerism in recipient patients.

List of abbreviation: DNA= Deoxyribonucleic acid, STR- short tandem repeat, SCT= stem cells transplantation.

Key words: *Chimerism, Transplantation, STR, Engraftment.*

Introduction:

Allogeneic hematopoietic stem cells transplantation (SCT) is one of the curative methods established for malignant and non-malignant patients (1). Artificial chimerism results from transfused blood stem cells of intrauterine transfusion or allogenic bone marrow transplantation as well as other organ transplantations (2).

The presence of donor cells in recipient blood is called complete chimerism while presence of donor and recipient cells in recipient blood called mixed chimerism (3). Identification and quantification of chimerism is important procedure for evaluation the successful of transplantation (4,5). Many techniques were established for chimerism analysis such as cytogenetics (6), real time PCR (7), variable tandem repeats (8), single nucleotide polymorphism (9), but the short tandem repeats (STR) is mostly used due to its accuracy and reproducibility (10).

Short tandem repeat (STR) genetic loci are highly polymorphic repeat sequences(11), this feature gave its importance for human identification and it's considered as the most informative genetic markers for providing high degree of discrimination in various forensic and court issues (12). Commercial STR kits that usually used for forensic applications were evaluated for chimerism quantification (13). STR locus must have different alleles in-patient and donor in order to be informative, for that the informativeness is differ among related and unrelated individuals (14).

Technical recommendations and quantification for the analysis of informative and non-informative loci were described in many studies (13, 15). In Iraq it may be the first study were conducted to analyze chimerism using STR method. The aim of this work is identify and if available qualitative chimerism analysis of informative loci in two Iraqi bone marrow transplant patients.

Corresponding Address:
Majeed A. Sabbah
Forensic DNA Center/ Al-Nahrain University1/ Paternity and Kinship Division..
Email: majeedbio@gmail.com

Materials and methods:

patients:

Two cases were analyzed, the first, patient with leukemia had bone marrow transplantation two years ago. The second, patient with leukemia had bone marrow transplantation one year ago.

Samples and DNA extraction:

Phenol-chloroform protocol (organic solvent) (16,17) was used for DNA extraction from blood and cheek samples.

DNA quantification:

Real time PCR Quantifiler Kit (Applied Biosystems) was used for DNA quantification in Fast 7500 real Time PCR machine (Applied Biosystems) according to manufacture instructions.

PCR amplification:

Powerplex21 kit (Promega) was used for amplification 21 STR loci in Veriti thermal cycler (Applied Biosystems) according to manufacture instructions.

STR profiling:

Genetic analyzer 3130XL cycler (Applied Biosystems) was used for profiling of STR fragments according to powerplex21 kit analysis parameters.

Results and discussion:

Organic method was used for DNA extraction from blood and cheek samples. The results showed that the DNA was intact as seen by agarose gel electrophoresis and in good purity (1.6-1.8) as determined by Nanodrop. The quantity of extracted DNA was suitable for analysis by genetic analyzer which require more than 1ng (18).

Twenty one STR loci were PCR amplified successfully then analyzed by genetic analyzer. Table 1 and 2 shows the STR loci alleles with their relative fluorescence units (RFU) of the two patients respectively. Figure 1 showed the STR profile of the patient two bloods STR loci with RFUs. Quantitation of chimerism using STR requires consideration to several factors (13). STR profiles examined for the presence of mixtures (more than two peaks for heterozygous and two alleles for homozygous), the results showed no mixtures and as expected the shared equally between donor and recipients since they are brothers for both cases. Following that, informative loci that can be used for quantification the chimerism examined. There are three types of informative loci, type 1 (fully informative), type 2 (informative), and type 3 (13). Our results showed that for patient 1 there was no informative loci, and for patient 2 There are three loci with type3 (D3S1358, D6S1043, TPOX). By application the equation for type 3 {% Chimerism= (C/[(A-C/2)+C)* 100} for the three loci the results was not accurate since the % chimerism were (96%, 96.1%, and 88.4% for D3S1358, D6S1043, TPOX respectively with the mean 93.5%). Quantification chimerism among the TPOX and other two loci showed some variation. It was indicated previously that type 3 may prone to error due the co-migration of alleles at the same location. The low number of informative loci in this study (three loci is the minimum acceptable loci) is due to relatedness of recipient and donor (13). Many factors affect the successful of the test, recipient and donor loci, quantity of DNA and DNA profile generated without stutters (15). Future studies are recommended for evaluation different STR kits in this approach with different loci in order to exclude the similarities in loci alleles which affect the informativeness of lici in Chimerism quantification. The results showed that the test successfully identify used for identification and quantification of chimerism in recipient patients.

Table (1): Alleles and relative fluorescence units (RFU) of recipients and donors STR analysis.

Locus		Recipient 1 (Cheek (swab		(Recipient 1 (Blood		(Donor1 (Cheek swab	
D3S1358	Allele	17	18	17	18	17	18
	RFU	4934	4232	4501	3718	8884	8773
D1S1656	Allele	15.3	16	15.3	16	15.3	16
	RFU	2324	1580	1540	1861	3890	3592
D6S1043	Allele	12	20	12	12	12	12
	RFU	8578	3178	13959	13959	23901	23901
D13S317	Allele	12	13	12	12	12	12
	RFU	7339	3812	15619	15619	20281	20281
Penta E	Allele	8	12	12	13	12	13
	RFU	8443	9022	12104	11341	16002	14893
D16S539	Allele	10	11	11	12	11	12
	RFU	5157	6279	5844	6402	13111	13693
D18S51	Allele	**16**	**19**	**12**	**13**	**12**	**13**
	RFU	3021	2621	3647	3495	7546	7458
D2S1338	Allele	19	23	23	24	23	24
	RFU	5029	6787	6915	5678	10637	9800

CSF1PO	Allele	10	12	11	12	11	12
	RFU	4717	5517	7923	7161	10476	10195
Penta D	Allele	14	14	14	14	14	14
	RFU	13932	13932	14389	14389	22155	22155
TH01	Allele	6	9.3	6	9.3	6	9.3
	RFU	2172	1852	1641	1115	3320	2922
vWA	Allele	16	17	16	17	16	17
	RFU	5043	5112	5207	5225	9637	9673
D21S11	Allele	29	30	28	29	28	29
	RFU	1226	1102	1071	1076	1635	1912
D7S820	Allele	10	12	8	10	8	10
	RFU	2474	1347	2159	1435	3732	3511
D5S818	Allele	11	12	12	12	11	12
	RFU	1202	793	720	720	1041	1172
TPOX	Allele	8	9	8	9	8	9
	RFU	1431	1328	1275	1288	2348	2286
D8S1179	Allele	10	12	10	16	10	16
	RFU	2012	1415	1379	1184	2549	2219
D12S391	Allele	23	24	15	21	23	24
	RFU	268	266	290	241	469	456
D19S433	Allele	12	14	12	14	12	14
	RFU	510	680	316	358	980	889
FGA	Allele	20	25	20	21	20	21
	RFU	620	394	390	414	797	692
Xx	Allele	x	X	x	Y	X	Y
	RFU	10270	10270	6943	6950	13510	11914

Table (2): Alleles and relative fluorescence units (RFU) of recipients and donors STR analysis.

Locus		**(Recipient 2 (Cheek swab**		**(Recipient 2 (Blood**		**(Donor2 (Cheek swab**	
D3S1358	Allele	15	15	15	18	15	18
	RFU	10194	10194	7605	7502	11665	11120
D1S1656	Allele	15	16	15	16	15	16
	RFU	2813	2751	3353	2750	4872	4376
D6S1043	Allele	20	20	12	20	12	20
	RFU	15235	15235	8677	8763	15057	14324
D13S317	Allele	8	9	8	8	8	8
	RFU	12081	6749	20313	20313	30827	
Penta E	Allele	12	16	12	12	12	12
	RFU	16791	10949	31651	31651	40272	
D16S539	Allele	13	13	13	13	13	13
	RFU	17323	17323	17813	17813	26018	
D18S51	Allele	**14**	**18**	**14**	**16**	**14**	**16**
	RFU	6202	4184	5540	5715	9662	9353

D2S1338	Allele	17	18	17	21	17	21
	RFU	9533	7145	10398	10008	14113	15172
CSF1PO	Allele	11	13	11	13	11	13
	RFU	8884	9527	8637	9232	12965	12671
Penta D	Allele	8	13	8	13	8	13
	RFU	11482	10522	11877	15962	19004	19774
TH01	Allele	9	9.3	7	9	7	9
	RFU	2262	1674	2301	2913	4397	4244
vWA	Allele	18	19	18	19	18	19
	RFU	10563	9985	8088	7579	10323	10216
D21S11	Allele	30	32	30	32	30	32
	RFU	1324	1249	1675	1929	2335	2142
D7S820	Allele	10	11	10	11	10	11
	RFU	3166	2959	3405	2890	4290	4340
D5S818	Allele	11	13	11	13	11	13
	RFU	1093	1062	1397	1397	1909	1812
TPOX	Allele	8	8	8	9	8	9
	RFU	4272	4272	2641	2615	3006	3386
D8S1179	Allele	13	14	11	14	11	14
	RFU	1178	1377	2354	2050	2750	2548
D12S391	Allele	18	20	18	20	18	20
	RFU	229	357	414	370	679	775
D19S433	Allele	13.2	14	13.2	14	31.2	14
	RFU	484	488	512	501	1095	990
FGA	Allele	21	23	21	24	21	24
	RFU	555	398	895	757	1014	983
Xx	Allele	x	Y	X	Y	X	Y
	RFU	7892	8102	9672	10698	15621	14405

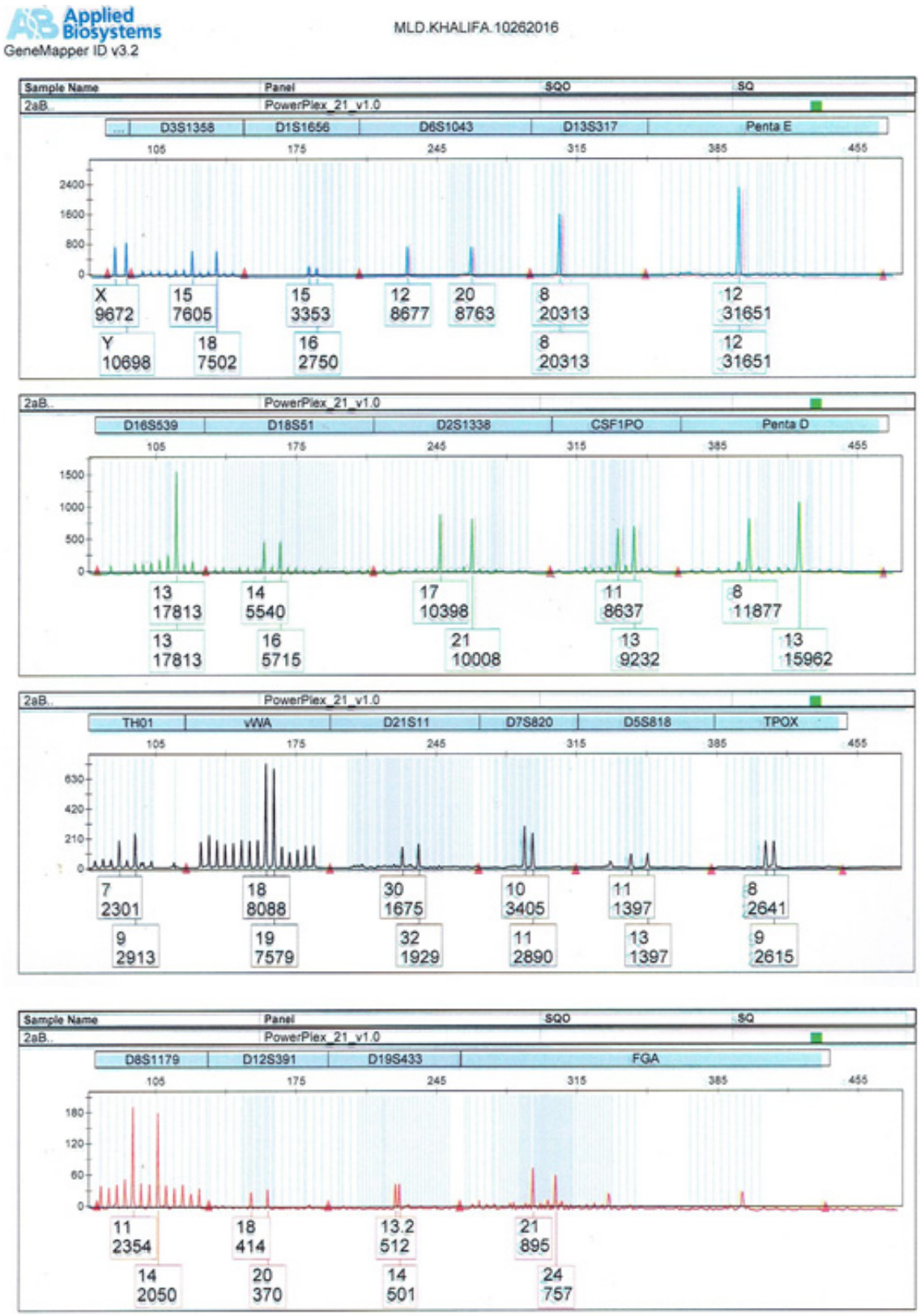

Fig 1: Electropherogram STR profile of recipient 2 blood.

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تحديد وتقدير كمية الكايميرا لمرضى نقل نخاع العظم المصابين بابيضاض الدم الحاد

مجيد ارشيد سباح[1]، هالة خالد ابراهيم[1]، محمد مهدي الزبيدي[1]، حنان خليل[2]، خليفة صالح[2]، قاسم شرهان المياح[3]، الاء حازم بدر[4]

1 مركز الدنا العدلي للبحث والتدريب/ جامعة النهرين
2 دائرة الطب العدلي/ وزارة الصحة
3 كلية الطب/ جامعة النهرين
4 مركز العقم/ مستشفى كمال السامرائي/ وزارة الصحة

الخلاصه:

ان تقدير كمية الكايميرا (Chimerism) بعد عملية نقل النخاع العظمي مهمة لتقييم نجاح النقل بعد نقل الخلايا الجذعية . يهدف البحث الى استخدام فحص STR Typing لتحديد وحساب كمية الكايميرا لمريضين مصابين بمرض ابيضاض الدم الحاد المنقول لهم نخاع العظم . تم عزل وتنقية الحمض النووي من دم المريض (المستلم) ومن الخلايا الظهارية المبطنة للفم . وكذلك من الخلايا الظهارية المبطنة للفم للشخص الواهب. تم قياس كمية الحمض النووي باستخدام تقنية تفاعل البلمرة اللحظي. وتم تضخيم مواقع ال (STR)باستخدام العدة Powerplex 21. تم عزل وتحديد كمية الحمض النووي بنجاح. كذلك تم تضخييم وتحليل المواقع الوراثية بشكل كامل . لوحظ وجود اليلات مشتركة مع عدم وجود اي خليط للواهب والمستلم . اظهرت بعض المواقع النوع الثالث للكايميرا . اظهرت الدراسة نجاح تحديد وتقدير كمية الكايميرا للمرضى .