Original article

Study of genetic variability of umbilical cord blood using RAPD assay

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Abstract

Background*:** The genetic variability of Umbilical Cord Blood (UCB) is most important for newborn screening, therapeutic possibility of haematological disorders as well as for the establishment of cord blood banking and stem cell research. ***Method*:** Genetic variability of umbilical cord blood (UCB) of 22 human subjects was evaluated first time by applying Random Amplified Polymorphic DNA (RAPD) assay using six decamar primers (B-14, OPB-05, OPB-08, OPB-12, OPB-19 and UBC-122). ***Result: A total number of bands were recorded 312 from 116 polymorphic loci and single monomorphic locus. All the markers showed highest polymorphism (100%) except the primer OPB 08 (92.31%) among tested individuals. The genetic distance was observed with highest 1.0 and lowest 0.72 respectively whereas mean genetic distance was recorded 0.90. Considering Shannon-Wiener index average diversity was recorded 0.139365. The mean Nei genetic similarity was found 0.17 which was found opposition to genetic distances. A phylogenetic relationship among the individual subjects was also observed between the linkage distances of 11 to 27 with 8 clades, 3 subclusters and a cluster. In addition, average allele frequency p and q was observed 0.08156 and 0.948751 respectively whereas highest intra locus gene diversity and average gene diversity were found 3.323817 and 0.144572 respectively. ***Conclusion***: Considering different parameters, higher genetic variability was found among the experimental subjects, probably due to the mixture DNA of parents and newborn.

Keywords: Genetic variability; umbilical cord blood; cell, RAPD

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Introduction

Patterns of genetic diversity in human populations constitute an important foundation for many areas of research in human genetics and these include genetic testing and screening, selective breeding, population control, sterilization, selective abortion, embryo testing and selection, sperm donation, egg donation, embryo donation, surrogate pregnancy, fertility drugs, contraception, cloning embryos and germ line or somatic cell manipulation etc.[1,2] Cord blood banking involves the collection, processing, testing, banking, registration, selection and release of cord blood unit under strict quality-controlled conditions for ultimate transplantation.

The genetic variability of Umbilical Cord Blood (UCB) is most important for the therapeutic possibility for patients with a wide range of haematological disorders, especially in children. Therefore, it is necessary to know the genetic variability of human subjects to ensure the possible

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use of cord blood or cord blood cells for therapeutic uses especially for new born or for the establishment of cord blood banking. Though lots of researches has been conducted on genetic diseases throughout the world contrary limited researches have been found in medical genetics in Bangladesh[3], and very limited research is conducted in Bangladesh using cord blood genetics [4,5,6,7]. In this study, a first-time research of RAPD genotyping was considered using UCB of human subjects as RAPD technique is cost effective, less time consuming and do not need to know prior genetic knowledge. The specific objectives of this research are to assess the genetic variability of umbilical cord blood based on RAPD technique and to observe genetic relationship among individual subjects.

Materials and Methods

Sample collection and preservation

The research work was conducted in different laboratory of the Department of Genetic Engineering and Biotechnology (GEB) at Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh. UCB samples were collected just after delivery directly from the labor room and Operation Theater room by the permission of Department of Obstetrics and Gynae at Jalalabad Ragib Rabeya Medical College, Sylhet. 5 ml of UCB from each cord was dropped into sterile vial containing 50 µl (10 µl/ml blood) of 10% solution of potassium EDTA (K_2EDTA). The vials with UCB were preserved at –20°C until the extraction of DNA.

DNA extraction

Genomic DNA was extracted from the UCB samples through the modified phenol-chloroform protocol designed by Roe *et al*.[8]. The quality of extracted DNA was checked by agarose gel electrophoresis using 0.8% agarose. The gel was viewed and taken photograph using gel documentation system. A good quality DNA bands were observed from each sample when compared with 1kb plus DNA ladder (Promega).

PCR amplification

In this experiment, six decamer RAPD primers such as OPB-05 (5'- TGCGCCCTTC-3'), OPB-08 (5'- GTCCACACGG-3'), OPB-12 (5'- CCTTGACGCA-3'), OPB-19 (5'- ACCCCCGAAG-3'), B-14 (5'- TCCGCTCTGG-3') and UBC-122 (5'- GTAGACGAGC-3') were selected from Operon Technologies Inc. USA for studying genetic variability. PCR reactions were performed for each sample for each primer in a 15 µl reaction mixture containing 7.5 µl of master mix (Promega Hot Start), 2 µl of primer, 2µl of template DNA and 3.5 µl nuclease free waters. PCR reaction was conducted for pre heating at 94˚C for 3 minutes, denaturation at 94ºC for 1 minutes for all primer except UBC-122 denaturation at 94ºC for 45 sec; annealing temperature for this PCR was about 27˚C (for B-14, OPB-12, UBC-122)/29ºC (for OPB-05, OPB-08, OPB-19) in 1 minute and 2 minutes for all primers except for UBC-122 at 1.5 minutes elongation or extension at 72˚C. A final step of 7 minutes at 72ºC except for the primer UBC-122 for 5 minutes at 72ºC was added to allow complete extension of the amplified fragments. The PCR was run for 35 cycles for primer B-14, OPB-12, UBC-122 and 40 cycles for primer OPB-05, OPB-08, OPB-19. PCR products were checked by electrophoresis on 1.2% agarose with 4µl DNA where 1kb plus (gene ruler) ladder was used to compare the migration of DNA. This gel was then placed in gel documentation system and photograph was taken by digital camera (Nikon Cool Pix P100 26X Zoom 10.3 MP). Good quality RAPD bands were revealed by all the primers.

Genetic Analysis

Using different software and equations, RAPD data of this experiment was interpreted. Molecular weight of bands was measured by using the software of AlphaEaseFC 4.0 and according to banding location DNA data was scored. Genetic distance was analyzed by the equation of D = 1- Nxy / Nx+Ny- Nxy where, D is the genetic distance between sample x and y, Nxy is the number of band shared by sample x and y, Nx is the number of bands in sample x and Ny is the number of bands in sample y. Shannon-Wiener Index among individuals was calculated with the following formula

$$HI = -\sum_{i=1}^{S} (Pi)(\ln Pi)$$

Where, HI is symbol for diversity in a sample of S species or kinds, S is the number of species in the sample, Pi is relative abundance of i^{th} species or kind measure (P1= Si/N), N is total number of individuals of all kind, Si is the number of individuals of i^{th} species and I is shannon information index. Nei's genetic similarity among individuals was calculated using pair wise comparison by the formula of F= 2Nxy/ Nx+Ny where, F is the Nei's genetic similarity, Nxy

is the number of shared band between X and Y, Nx is the number of band in X and Ny is the number of band in Y. Linkgae distance was measured using new.sta software and based on linkage distance intra-individual genetic relationship was analyzed through dendogram by using software "Statistica". Allele and genotype frequency was calculated by using Hardy-Weinberg equilibrium [$(p_+q)^2 = p^2 _+ 2pq _+ q^2 =1$ or p = 1-q].

Ethics clearance

The samples were collected by the approval of the Graduate Research Ethics Committee (Headed by the Dean of the School of Life Sciences), Shahjalal University of Science and Technology, Sylhet with the consent of the Director of Jalalabad Ragib Rabeya Medical College, Sylhet, Bangladesh.

Results

DNA profiling and polymorphism

Six RAPD primers were used to assess the genetic variability of UCB sample among 22 individuals of human subject. DNA profiling and data scoring were studied separately for each primer. The size of amplified products was measured and DNA bands were compared with 1 kb plus ladder which was ranged from 75 bp to 20,000 bp (GeneRulerTM). Each amplified DNA band was defined by the presence (1) or absence (0) of bands at particular positions on the gel. According to the banding of DNA, few numbers of bands were revealed by all the primers at different lengths of DNA. Total 312 bands were detected among 22 individuals of human subjects within 116 loci where only one monomorphic locus was detected by the primer OPB 08 (Table 1). The highest number of bands (77) amplified by the primer OPB-05 and the lowest number of bands (37) amplified by the primer UBC-122. All the markers showed highest polymorphism (100%) except the primer OPB 08 (92.31%) among the tested individuals. The highest number of bands (3.5) per individual was amplified from the primer OPB-05 and the lowest number of bands (1.68) per individual was amplified by the primer UBC-122.

Table 1: RAPD banding summary of 22 human subjects

Primers	SD	TB	PL	ML	% PL	BS
B-14	337-2088	40	16	-	100	1.82
OPB-05	288-4000	77	30	-	100	3.5
OPB-08	251-1135	44	12	1	92.31	2
OPB-12	440-4864	44	15	-	100	2
OPB-19	579-1732	70	26	-	100	3.18
UBC-122	251-3043	37	17	-	100	1.68
Total		312	116			
Average		52	19.33		98.72	

SD= Size of DNA (bp), TB= Total Number of DNA Bands, PL= Number of polymorphic Loci, ML= Monomorphic locus, BS= Number of bands per sample

Assessment of genetic variability

In this study, lowest and highest genetic distances were found 0.72 and 1.00 among the experimental individuals whereas mean genetic distance was recorded 0.90, indicating higher genetic distance diversity was recorded in the experimental subjects (Table 2).

Table 2: Genetic distance among individuals of human subjects

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	---	0.782	0.97	0.88	0.96	0.96	0.96	1	0.90	0.96	0.97	0.9	0.93	0.93	0.96	0.95	0.96	1	0.96	1	0.92	0.93
2		---	0.73	0.84	0.96	0.84	0.96	1	0.86	0.96	0.97	0.91	0.97	0.94	0.92	1	1	0.97	1	1	0.93	0.93
3			---	0.77	0.84	0.86	0.93	0.89	0.96	0.97	0.97	1	0.97	0.94	0.93	1	1	0.91	1	0.94	0.97	1
4				---	0.8	0.83	0.96	0.92	0.96	1	1	0.95	0.89	0.97	0.96	1	0.96	0.97	1	0.94	1	0.93
5					---	0.86	0.77	0.9	0.95	0.9	0.88	1	0.92	1	1	0.95	1	0.96	0.95	1	0.96	0.88
6						---	0.8	0.92	0.85	0.76	0.81	0.95	0.96	1	1	0.95	1	0.97	0.94	0.97	0.93	1
7							---	0.8	0.83	0.79	0.55	0.75	0.96	0.88	1	0.94	1	0.97	0.95	1	0.87	0.96
8								---	0.95	0.75	0.89	0.95	0.97	0.85	0.87	1	1	0.93	1	0.97	0.96	0.93
9									---	0.9	0.88	0.73	0.92	0.96	0.95	0.94	1	0.96	0.9	0.96	0.95	0.92
10										---	0.85	0.95	1	0.96	1	1	0.95	0.96	1	1	0.88	0.93

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
11											---	0.77	0.87	0.91	0.93	0.88	1	0.94	0.93	0.97	0.9	1
12												---	0.91	0.83	0.9	0.94	1	1	0.95	0.96	0.95	0.96
13													---	0.88	0.8	0.91	0.89	1	0.8	0.91	0.90	0.90
14														---	0.77	0.88	0.93	0.88	0.86	0.97	0.94	1
15															---	0.84	0.81	0.86	0.92	0.93	0.92	0.93
16																---	0.952	0.88	0.84	0.92	0.96	0.96
17																	---	0.67	0.81	0.90	0.88	0.89
18																		---	0.90	0.91	0.94	0.94
19																			---	0.90	0.96	0.93
20																				---	0.83	0.84
21																					---	0.72
22																						---

Considering Shannon-Wiener Index, low and high values of Shannon-Wiener Index were observed 0.10228 and 0.17043 and in an average it was found 0.139365.

Measurement of genetic similarity

In this study, Nei genetic similarity was observed among 22 individuals of human subject whereas highest and lowest genetic similarities were found 0.62 (62%) and 0.00 (0%) and in average 0.17 which was opposition to genetic distances, indicating higher genetic variability (Table 3).

Table 3: Nei's genetic similarity among individuals of human subject

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	-	0.36	0.07	0.22	0.08	0.07	0.08	0.00	0.17	0.08	0.07	0.18	0.13	0.13	0.15	0.09	0.08	0.00	0.08	0.00	0.14	0.13
2		--	0.42	0.28	0.08	0.28	0.08	0.00	0.24	0.07	0.06	0.17	0.06	0.12	0.14	0.00	0.00	0.06	0.00	0.00	0.13	0.13
3			--	0.38	0.28	0.25	0.14	0.19	0.07	0.07	0.06	0.00	0.06	0.11	0.13	0.00	0.00	0.16	0.00	0.11	0.06	0.00
4				--	0.32	0.29	0.08	0.15	0.08	0.00	0.00	0.09	0.19	0.12	0.07	0.00	0.07	0.06	0.00	0.12	0.00	0.13
5					--	0.24	0.36	0.17	0.10	0.17	0.21	0.00	0.14	0.00	0.00	0.10	0.00	0.07	0.08	0.00	0.08	0.21
6						--	0.32	0.15	0.25	0.39	0.31	0.09	0.07	0.00	0.00	0.09	0.00	0.06	0.07	0.06	0.14	0.00
7							--	0.33	0.29	0.35	0.62	0.40	0.07	0.20	0.00	0.10	0.00	0.07	0.08	0.00	0.23	0.07
8								--	0.09	0.40	0.19	0.09	0.07	0.25	0.23	0.00	0.00	0.13	0.00	0.06	0.07	0.13
9									--	0.18	0.21	0.42	0.15	0.07	0.09	0.11	0.00	0.08	0.17	0.07	0.08	0.15
10										--	0.27	0.10	0.00	0.10	0.00	0.00	0.08	0.07	0.00	0.00	0.22	0.14
11											--	0.37	0.23	0.16	0.13	0.22	0.00	0.11	0.13	0.05	0.18	0.00
12												--	0.15	0.29	0.18	0.11	0.00	0.00	0.09	0.07	0.08	0.08
13													--	0.22	0.33	0.15	0.20	0.00	0.33	0.17	0.19	0.18
14														--	0.38	0.21	0.13	0.21	0.25	0.05	0.12	0.00
15															--	0.27	0.31	0.25	0.15	0.13	0.14	0.13
16																--	0.09	0.21	0.27	0.14	0.08	0.08

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
17																	--	0.50	0.31	0.19	0.21	0.20
18																		--	0.19	0.16	0.12	0.11
19																			--	0.19	0.07	0.13
20																				--	0.29	0.28
21																					--	0.44
22																						--

Genetic relationship among experimental subjects

The phylogenetic relationships among 22 individuals of human subject genotypes were considered for the present study using UPGMA (Unweighted Pair Group Method of Arithmetic Means) (Figure 1). The UPGMA clustering system generated 8 clades with different linkage distances. A subcluster was made considering clade 1 and 2 with direct joining of individual 3. Second subcluster was created using clade 3, 4 and 5 with connection of individual 6. Clade 6 and 7 formed subcluster 3 with the connection of individual 19, 13 and 14. Finally a cluster was made with inter connection of subcluster 1, 2 and 3 as well as direct associated with individual 20 and clade 8.

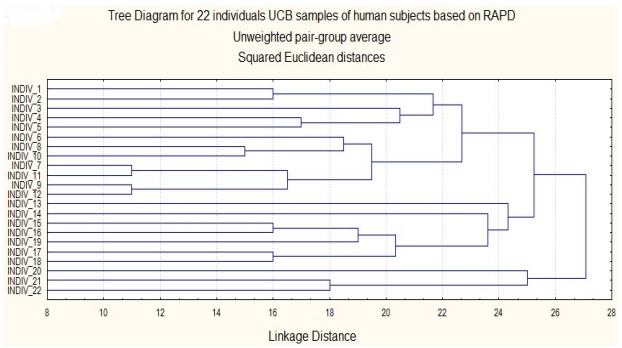

Figure 1. Tree diagram of linkage distance among 22 individuals of human subject

Allele frequency and gene diversity

According to Hardy-Weinberg formula genotype frequency, allele frequency, intra-locus gene and average gene diversity were measured from the data of six RAPD primer whereas intra-locus gene diversity were recorded 1.721631, 3.323817, 1.87943, 1.887156, 3.048614 and 1.621173 respectively and average gene diversity were observed 0.107602, 0.110794, 0.144572, 0.12581, 0.117254 and 0.095363 respectively. Among the six primers highest and lowest intra locus gene diversity was observed in primer OPB-05 (3.323817) and UBC-122 (1.621173) respectively. The highest and lowest average gene diversity was recorded 0.144572 in primer OPB-08 and 0.095363 in primer UBC-122 respectively. Genotype frequencies like p_1 and p_2 highest value observed in primer OPB-08 (0.153846) and UBC-122 respectively. Primer OPB-08 also showed highest value of allele frequency p (0.08156) and UBC-122 showed highest value of q (0.948751).

Discussion

Recently cord blood is being used generally for new born screening. In addition, formation of blood bank and blood transfusion is being performed using cord blood through stem cell identification, isolation, culture *in vitro*. In those case, genetic status of cord blood is most important while a mixture of genes available in cord blood that comes from parents and new born. Therefore, in this experiment, genetic variability was observed using umbilical cord blood among 22 human subjects. Newborn screening is mainly considered by using collected blood through heel prick of the newborn[9], however, in case of a developing country like Bangladesh, most of the people are not concerned about newborn screening immediately after birth while newborn screening has been established in recent years[10]. Though newborn screening has been practiced now a days, the parents are unwilling to give blood from heel prick or vein of the newborn. Considering this circumstance, this experiment was done using umbilical cord blood which is considered as waste materials after delivery.

In this experiment, six RAPD primers have been used to compare genetic variability among 22 UCB samples with their genetic relationship. Six selected random primers generated 312 bands ranging from 251 to 4864 bp, corresponding to an average of 52 bands per primer. Of these bands, 98.72% (116 in total) were from polymorphic loci among 22 individuals and the total numbers of bands produced from polymorphic loci by each primer were different.

The highest number of bands was produced by OPB-05. All the primers revealed 116 polymorphic loci and highest level of polymorphic loci found 30 in OPB-05 primer. Though RAPD assay is not available for cord blood genetic research, however, genetic diversity of umbilical cord blood unit was performed for transplant of the national centre of blood transfusion (Mexico) where only HLA gene diversity was analyzed[11].

In this experiment, genetic distance found lower to higher (0.72 to 1) which was supported by another research done in Mexico where they found genetic distance in Morelos city was 0.0084 and they got Hardy-Weinberg p value 0.2674 to 0.9674[11] which was also comparable with this primers allele frequency (p value). In Mexico this study of UCB sample analysis has been done to produce an effective UCB bank which is also needed in our country to treat different cord blood disorders like thalassemia, sickle cell anaemia etc.

Genetic characterization of the HLA system is needed in the selection study of donors/receptors in hematopoietic transplants. The HLA genotype frequencies data were used to estimate genetic distance between the top five populations analyzed in the study of Mexico City[12]. These genetic distances were used to perform a Neighbor-Joining[13], Dendogram, using PHYLIP software v.3.6[14,15], comprising the programs GENDIST and NEIGHBOR. In this experiment the genetic distance, squared euclidean distance (linkage distance), similarity and dendogram were made by using "Statistica." It was trying to determine similar things for greater Sylhet region of Bangladesh because patients come in this hospital from various areas of this region. In this study genotype frequencies p_1 and p_2 highest value observed in primer OPB-08 (0.153846) and UBC-122 respectively. Primer OPB-08 also showed highest value of allele frequency p (0.08156) and UBC-122 showed highest value of q (0.948751) which was significantly comparable to Bello-López *et al*.[11] whereas genetic distances analysis showed that the top five populations analyzed. Allelic groups obtained by medium resolution techniques do not identify distance distributions, allele discrimination within a subgroup, even functional or structural variation which was significantly different from each other; however, in the present study it was also similarly found.

From these data, though the genetic distance value showed higher genetic diversity whereas only twenty-two individuals were studied but bands with very limited polymorphism were found in the gel by six arbitrary RAPD primers. However, this finding could be considered as base line study of genetic variability of cord blood and it should also be considered comparative genetic study of parents and new born.

Conclusion

RAPD based genetic variability was analyzed using UCB samples of human subjects which may help us to resolve many diseases. With proven therapeutic effect in treating over 40 diseases, umbilical cord blood is a valuable, non-controversial source of stem cells. Certain blood disorders such as sickle cell anemia, thalassemia, bone marrow failure syndromes, cancers and other genetic diseases can be treated by using UCB. To date, nearly 8,000 cord blood transplants have been performed world wide[16]. The goal is to make unrelated donor cord blood transplantation for different diseases that stand to benefit from this approach through measuring genetic distance and similarity. If this work expanded over the country, it may help us to treat genetic diseases.

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Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh.

Conflict of interest

The authors do not have any competing interests.

Authors's contribution

Data gathering and idea owner of this study: Miah, M. F., Rahman, M. S. E., Mahmud, M. G. R.

Study design: Miah, M. F., Rahman, M. S. E., Mahmud, M. G. R.

Data gathering: Rahman, M. S. E., Chowdhury, S. F., Mahmud, M. G. R.

Writing and submitting manuscript: Miah, M. F., Rahman, M. S. E., Chowdhury, S. F.

Editing and approval of final draft: Miah, M. F., Rahman, M. S. E., Chowdhury, S. F.

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