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CD34 positive stem cells recovered from cord blood remain viable after six months of cryoprotective storage process

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Cord blood can be used as an alternative source for bone marrow transplantation and its use is developing into a new field of treatment for patients presenting with haematological disorders, immunological defects and specific genetic diseases; including haemoglobinopathies. The aim was to assess the viability of frozen cord blood as a source of HSC which may be suitable for transplantation. Blood specimens were obtained from umbilical cords of 30 consenting mothers and dispensed into 5 cryovials with glycerine for freezing at -20°C; while quantitative assay was carried out on a fresh citrated sample by immunophenotyping using CD34 as marker of HSC. Partec Cyflow cube 6 was used to measure viable cells after labelling the cells with specific fluorochrome/antibody obtained from Sysmex Partec. A repeat quantification was carried out at one month interval for 5 consecutive months and results generated were analysed using T- independent test. The mean ± standard error of mean (SEM) for the 6 consecutive counts were 20,798±2750, 19849±2691, 19223±2637, 18363±2582, 17052±2583 and 16184±2423. The p values obtained when the cryoprotected samples were compared to the baseline were 0.806, 0.681, 0.521, 0.325 and 0.213; reflecting that subsequent counts were insignificantly different from the baseline count. Thus, it is a safe alternative in resource-poor setting to store stem cells in a cryoprotective agent and freeze at -20°C for up to 6 months, without significant depreciation in viability. This alternative should be explored and further researches should be conducted with possibility of extending the number of months.

Key words: CD34+ cells, immunophenotyping, stem cells, cryopreservation.

INTRODUCTION

Bone marrow was the source of stem cell used for transplantation by a team led by Thomas E D at the Fred Hutchinson Cancer Research Center from the 1950s through the 1970s. The team pioneered stem cells

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transplantation and her work was later recognized with a Nobel Prize in Physiology awarded to the team lead. Thomas' work showed that bone marrow cells infused intravenously could repopulate the bone marrow and produce new blood cells of all lineages (Bashour et al., 1980).

The first physician to perform a successful human bone marrow transplant on a disease aside cancer was Robert A. Good at the University of Minnesota in 1968. Seven years after, John Kersey also of the same university performed the first successful bone marrow transplant to cure lymphoma. His patient, a 16-year-old-boy, was documented to be the longest-living lymphoma transplant survivor (Chan et al., 2001).

Although, the haemopoietic stem cells (HSCs) can be frozen for prolonged periods without damaging too many cells, for allogeneic transplantation procedures, fresh HSCs are preferred in order to avoid cell loss that might occur during the freezing and thawing process (Ishihara et al., 1976).

Allogeneic cord blood is stored frozen at a cord blood bank because it is only obtainable during delivery; cord blood being a potent source of HSCs (Bakry et al., 2008). To preserve HSCs, a cryoprotective agent like glycerol must be added, and the cells must be cooled very slowly in a controlled-rate freezer to prevent osmotic cellular injury during ice crystal formation. HSC may be stored for years in a cryofreezer or liquid nitrogen tank (Zhao and Mazzone, 2010).

Cord blood as a potential source of primitive HSCs is available in advanced countries of the world, for clinical application to reconstitute the hematopoietic system in affected individuals requiring treatment. Cord blood can be used as an alternative source for bone marrow transplantation and its use is developing into a new field of treatment for patients presenting with haematological disorders, immunological defects and specific genetic diseases; including haemoglobinopathies (Eapen et al., 2007; Frangoul et al., 2010; Prasad et al., 2008; Wagner et al., 2002).

A total of 50,417 hematopoietic stem cell transplants were reported to have taken place worldwide in 2006, according to a global survey of 1327 centers in 71 countries conducted by the Worldwide Network for Blood and Marrow Transplantation. Of these, 28,901 (57.3%) were autologous, 11,928 (23.7%) from related donors and 9,588 (19.0%) from unrelated donors; giving rise to a cumulative of 21,516 (42.7%) for allogeneic transplantation cases. In 2014, stem cell products provided for unrelated transplantation worldwide had increased to 20,604. Of these, 4,149 (20.1%) were from bone marrow donations; 12,506 (60.7%) from peripheral blood stem cell donations; and 3,949 (19.2%) from cord blood units (Wore et al., 2015).

Umbilical cord blood is obtained when a mother consents and donates her infant's umbilical cord and placenta after birth. Cord blood has a higher concentration of HSCs than is normally found in adult blood. However, the small quantity of blood obtained from an umbilical cord (typically about 50 mL) makes it more suitable for transplantation into small children than into adults. Newer techniques using *ex-vivo* expansion of cord blood units or the use of two cord blood units from different donors allow cord blood transplants to be used in adults (Arien-Zakay et al., 2010).

In Nigeria, so many sufferers from genetic disorders like sickle cell diseases and haematological malignancies are patronizing quacks and herbal homes because of the fact that they have no hope of 'total cure'. An inquiry into the feasibility of using cord blood as a potent source of HSCs will be of help in future allogeneic stem cell transplantation in Nigeria.

This work aimed at assessing the viability of cord blood obtained from Osogbo and stored frozen as a source of HSCs, which may be suitable for transplantation.

METHODOLOGY

Study area

This study was carried out in Osun State in the southwestern part of Nigeria. The study sites included Ladoke Akintola University Teaching Hospital, Idiseke; State Hospital, Asubiaro; and Comprehensive Health Center, Kooda, Ilobu; all in Osun State. The health institutions chosen represent tertiary, secondary and primary health care levels, respectively. The comprehensive health center is located in a rural community while the other hospitals are located in Osogbo, an urban city in Osun State.

Protocol

The study was a longitudinal study of cord blood samples collected after delivery from 10 volunteers per study site, translating to 30 samples. The cord blood samples were harvested from the cord and placenta in aliquot of 2 ml, in 6 microvials. A vial was analysed less than 2 h after sample collection while the remaining 5 vials were mixed with glycerol, 2 ml per vial too, then frozen at -20°C, to achieve cryoprotection. Quantitative assay was carried out by immunophenotyping using CD34 as a marker of the HSC and all previously frozen samples were washed four times in phosphate buffer saline (PBS) before making 50% cells suspension in PBS for immunophenotyping. Partec Cyflow cube 6 was used to measure viable cells after labelling the cells with specific anti-CD34/PE obtained from Sysmex-Partec, Germany. These cells were earlier treated with leukocyte fixation solution and erythrocytes lysis solution. All reagents were supplied by Sysmex-Partec from Germany. A repeat quantification was carried out at one month interval for the 5 consecutive months and results generated were documented accordingly.

The data generated were coded, entered, validated and analysed using Statistical Package for Social Science (SPSS) version 20.0. The mean and standard error of mean generated per group were computed and compared to determine significant difference, using T independent test. P values below 0.05 were considered significant.

RESULTS

The mothers who consented to participate in the study

Table 1. Blood groups and parity of mothers who consented to using their cord blood.

Parameter	Frequency	%
Blood group		
A+	9	30.0
AB−	1	3.3
B−	1	3.3
B+	10	33.3
O−	1	3.3
O+	8	26.7
Parity		
Primigravida	9	30
Multigravida	21	70
Total	30	100.0

Table 2. Blood group distribution of babies.

Blood group	Frequency	%
A +	5	16.7
AB −	2	6.7
AB +	2	6.7
B −	1	3.3
B +	6	20.0
O +	14	46.7
Total	30	100.0

Table 3. CD34+ cells counts across months of storage (n=30) and p values from T independent test.

Month	Mean	Std. error of mean	p value
Baseline	20798.6	2750.29	Not applicable
1 month	19849.9	2691.61	0.806
2 months	19223.4	2637.17	0.681
3 months	18363.2	2582.20	0.521
4 months	17052.0	2583.33	0.325
5 months	16184.4	2423.36	0.213

were 30 in number; 21 (70%) were multigravida, while 9 (30%) were primigravida. The participants' ABO and Rh blood types B Rh 'D' positive having 10 (33.33%), being the highest frequency in this categorisation (Table 1). However, group O Rh 'D' positive has the highest frequency of 14 (46.67%) among the babies (Table 2).

The mean ± SEM for the 6 consecutive counts were 20,798±2,750, 19,849±2,691, 19,223±2,637, 18363±2582, 17052±2583 and 16184±2423. The p values obtained when the cryoprotected samples were compared to the baseline using T independent test were 0.806, 0.681, 0.521, 0.325 and 0.213 reflecting that all values, though descending, were insignificantly different from the baseline count (Table 3).

DISCUSSION

More than 2 decades ago, advanced nations of the world considered umbilical cord as an alternative source of stem cells for clinical application and its suitability are being reviewed since then (Thompson, 1995). A prominent limitation for Nigeria to key into the technology includes non-availability of required equipments for freezing blood and blood related products at ultra low temperature (example include nitrogen tank) in its blood

storage facilities coupled with limited awareness on the relevance of the technology to our practice in hospital services. Thus, stem cell banking is not pronounced in Nigeria till now.

Incidentally, the umbilical cords and placenta are collected and wasted by Yoruba speaking Nigerian parents for cultural purposes (Joseph, 2014). The potentiality of making use of this rich resource being a source of HSC is dashed by inability to run cord blood banking which is mostly hinged on lack of facility to achieve viability during storage.

Now that it has been established that a safe alternative in resource-poor setting is to store stem cells in a cryoprotective agent like glycerine and freeze at -20°C for up to 6 months, it is a responsibility to create awareness among parents on usefulness of cord blood for the babies and other related or unrelated potential recipients. It is equally important that larger numbers of samples are assessed for longer period of 2 years or more.

This work was delimited by limited resources since the work was sponsored by the researchers only, without funding from any other source within and outside Nigeria.

Conclusion

Cord blood stored frozen in the conventional domestic freezer (-20°C) remain a good source of HSC, up to 6 months of storage. Inadequacies in health care delivery system in Nigeria contribute to the practice of medical tourism which is now being allegedly abused by political office holders. Hence, policy formulation on the menace can only be appropriate if health care delivery systems in Nigeria are strengthened. One of such practices that can strengthen the systems is establishment of standard cord blood banking system which requires step-wise procedure. Sustainability is key and most achievable through the use of realistically available facilities for freezing in most centers. Further efforts are recommended to assess the viability of frozen cord blood in freezers and liquid nitrogen, using larger sample size.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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