

Cord Blood Banking: An Indian Perspective

Verma V^{1*}, Tabassum N¹, Yadav C.B¹, Kumar M², Singh A.K³, Singh M.P¹, Kumar A⁴, Singh B⁵ and Gautam S.K⁶

¹Centre of Biotechnology, Nehru Science Complex, University of Allahabad, Allahabad, India

²Department of Microbiology and Immunology, National Institute of Nutrition, Hyderabad, India

³Department of Biochemistry, Institute of Science, Banaras Hindu University, India

⁴Department of Zoology, MLK Post Graduate College, Balrampur, India

⁵Indian Veterinary Research Institute, Regional Station Palampur, India

⁶Department of Biotechnology, Kurukshetra University, Kurukshetra, India

Abstract

Cord blood transplantation offers several significant advantages over bone marrow transplantation such as reduced graft versus host diseases and low vulnerability of rejection if human leukocyte antigen (HLA) is mismatched. However, the probability of a successful engraftment is reduced in case of cord blood transplantation since the volume of haematopoietic stem cells isolated from cord blood is insufficient for adults. Perceiving the clinical potential of cord blood many public and private banks sprouted up in different parts of the world to store this biological entity for future use. While public blood banks use the stored blood for the benefits of common people, the private enterprises are set up for autologous applications. The hike in the number of private banks is however questioned since various data have suggested that cord blood transplantation has been insignificant for autologous use and making money out of people's anxiety and incognizance is not only unethical but also treacherous. In this review we will discuss the basics of cord blood transplantation and the perspective of stem cell research in developing nations like India.

Keywords: Umbilical cord blood transplantation; Stem cells; Human leukocyte antigen; Private cord blood banks; Paediatrics

Abbreviations: iPSCs: Induced Pluripotent Stem Cells; UCB: Umbilical Cord Blood; FACS: Fluorescence Activated Cell Sorting; HLA: Human Leukocyte Antigen; BMT: Bone Marrow Transplantation; CBT: Cord Blood Transplantation; GVL: Graft versus Leukaemia; ICMR: Indian Council of Medical Research; DBT: Department of Biotechnology; DCGI: Drug Controller General of India

Introduction

Stem cells characterized by the potential of self-renewal and differentiation have so far shown promising results in the treatment of both malignant and non-malignant disorders. The culture of stem cells and their induced proliferation to give rise to a particular lineage of cells is thought to make a paradigm shift in the approach towards treatment of acute disorders like myocardial infarction. The reprogramming of stem cells to the pluripotent state by overexpression of several transcription factors such as SOX2, OCT4, NANOG, MYC, LIN28 [1] has been a breakthrough in the field of regenerative medicine however the concern is regarding the source of somatic cells for reprogramming since most of iPSC lines described so far has been isolated from skin fibroblasts and other cell types that require harvesting via surgical intervention [2]. Bone marrow transplantation although successful to some extent also required collection of stem cells through similar invasive and painful procedures. In contrast to above procedures the isolation of cells from cord blood has shown promising alternative since the endothelial cells derived from cord blood has now been successfully reprogrammed to iPSCs [3] and cord blood transplantation has offered several significant advantages over bone marrow transplants.

Umbilical cord connects the foetus to the placenta and mediates the supply of oxygen and nutrients to developing foetus. It is approximately 50-70 cm long and 2 cm in diameter, and is known to mediate the fetoplacental circulation and has its origin from the same zygote which gives rise to the foetus [4]. It is composed of three blood vessels which are embedded in a gelatinous substance called as Wharton's jelly and surrounded by amnion [5]. Two umbilical arteries carry the deoxygenated blood from the foetus to the placenta whereas an umbilical

vein carries the oxygen rich blood from the placenta to the foetus helping in its growth and development [6]. Soon after a baby is born the umbilical cord is clamped while previously it used to be discarded. But nowadays with the discovery of cord blood transplantation and its potential use in therapy there is growing concern about the storage of the cord blood and hence what had been a biological waste so far is now playing the role of saviour of human life.

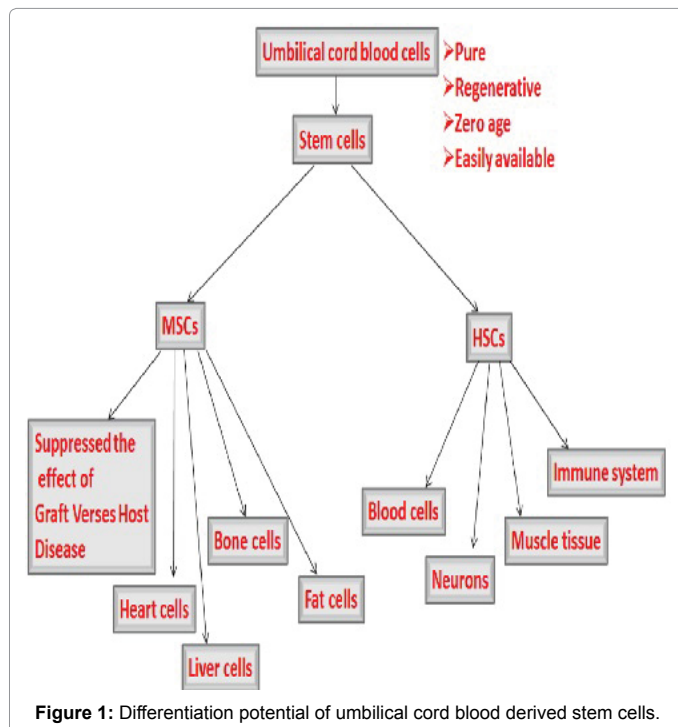
Cord blood cells isolated from the clamped umbilical cord differ from those of bone marrow and peripheral blood in composition, number as well as properties. Cord blood is a rich source of haematopoietic stem cells [7]. These cells have both the properties of multi-potency that is the ability to differentiate into both the myeloid as well as lymphoid lineage of cells and self-renewal (the ability to give rise to identical daughter cells without differentiation). Different types of cord blood cells are also characterized by long telomere in their DNA as compared to their counterparts in bone marrow and peripheral blood and this allows a longer time of haematopoiesis [8]. The isolation of hematopoietic stem cells (HSCs) from umbilical cord blood (UCB) was carried out using density gradient centrifugation, CD34 immuno-magnetic separation as well as by fluorescence activated cell sorting (FACS) [9]. These cells are now used to treat various types of malignancies like leukaemia and autoimmune disorders [10]. While HSCs have long been used in the clinical therapies and are successfully isolated from cord blood, UCB also contains mesenchymal stem cells which are capable of differentiating into cells of different connective tissue lineage such as bone, cartilage and adipose tissue [11] (Figure 1). Initially it is identified

***Corresponding author:** Verma V, Centre of Biotechnology, University of Allahabad, Allahabad 211002, Uttar Pradesh, India, E-mail: verma29@gmail.com

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in the bone marrow and the potential of differentiation of these cells decreases with age [12]. However, till date very little information is available regarding isolation and characterization of MSCs from cord blood and it is a laborious and time consuming process that results in the low yield of cells [13]. Many private and public cord blood banks have been developed around various corners of the world to store and harvest the therapeutic potential of these cells. While cord blood units stored in public banks are accessible to mass population and are not for profit seeking, blood at private banks are for autologous use and they charge money in lieu of storage. Cord blood banks in India are still in a stage of infancy. To name only 14 private and 4 public banks are in operation so far and still the total number of cord blood transplants has not touched the century mark. Being the home of second largest population of the world India has an immense potential in the field of cord blood storage and for this the development of public banks is to be fostered. India is one of the biggest potential UCB repository.

Cord Blood Transplantation

In the process of transplantation person's defective stem cell is replaced by normal one. These normal stem cells could be isolated from bone marrow, peripheral blood and cord blood. The first autologous stem cell transplantation was undertaken by Dr. E. Donnall Thomas in 1957. It was after 32 years in 1989 that professor Gluckman for the first time reported UCB transplantation in a male child suffering from Fanconi's anaemia using cord blood isolated from his human leukocyte antigen (HLA) matched sister who has not been affected by Fanconi's mutation [14]. The transplantation was found to be successful without any graft versus host disease and still the patient is alive. Since then thousands of UCB transplantations have been clinically performed. When compared to other two alternatives that is bone marrow and peripheral blood transplantation, it was found that cells isolated from cord blood offered several advantages such as the cells with a CD34⁺ and CD38⁻ phenotype are found in G₀ phase and have a greater proliferative response to cytokines and are found to be less dependent

on the stromal cells than the corresponding cells in the bone marrow and peripheral blood [15,16]. On the CD34⁺ cells of the umbilical cord blood, as compared with bone marrow and peripheral blood, there is higher expression of proteoglycan and other adhesion molecules of integrin group such as CD49d or CD49f, but there is lower expression of CD11 and CD18 [17]. Integrin molecules stop neutrophils which undergo skeletal changes and migrate through the vascular lumen into the extra-vascular space by adhering to intracellular adhesion molecules (ICAMs). Cord blood cells are also characterised by long telomeric DNA in comparison to their analogues from peripheral blood bone marrow, which allows these cells to undergo haematopoiesis for a longer period of time [18]. These cells are less vulnerable to graft versus host disease (in this disease the immune cells that have resulted from transplantation recognise the host cell as foreign and subsequently kill it) because of immaturity of immune system at the time of birth and decreased potential of alloreactive lymphocytes [19]. As a consequence of this immaturity of immune cells the necessity of human leukocyte antigen (proteins on the surface of cell that regulates immune response) to be a perfect match is somewhat reduced. Also graft versus leukaemia effect (effect mediated by allogeneic lymphocytes against leukaemia after allogeneic transplantation of HSCs) is maintained. CD34⁺ cells of the cord blood are more resistant to Pyronin Y staining as compared to the bone marrow cells because under childbirth stress condition CD34⁺ cells in cord blood are triggered by many cytokines, and therefore, are less sensitive to the feasible toxic environmental substances [20]. Myeloid progenitor cells derived from UCB are relatively chemo-resistant. This adds to the advantage of UCB transplantation since transplantation of blood cells is often combined with severe chemo therapies and this chemo-resistant UCB cells with chemotherapy will avoid the relapse of disease that is sometimes observed after haematopoietic malignancy [21]. Because cord blood units are obtained from the umbilical cord there is no issue of pain and risk to mother and foetus.

Despite of all the above advantages there is some hindrance to the large scale use of UCB as seen in larger engraftment time. Two factors based on which engraftment success is valued are the recovery of neutrophils and platelets production [22] and it has been seen that neutrophil recovery takes 22 to 27 days as opposed to just 18 days for unrelated bone marrow transplantation (BMT). Also it took almost 60 days for platelets recovery in case of cord blood transplantation (CBT) as compared to 29 days in case of BMT [23]. Increase in the dose of transfused cells certainly shortens the neutrophil recovery time but this may not be a realistic solution as there is low availability of UCB cells [24]. Also, since a limited amount of cord blood is collected from cord blood, there is almost 10% reduction in the amount of useful stem cells that are isolated from the cord blood as compared to bone marrow [25]. However this obstacle could be removed using two HLA matched units at the same time.

Cord Blood Banking

Perceiving the potential and application of cord blood in therapy many public and private cord blood banks had sprouted in different parts of the world. Cord blood, a source of potentially lifesaving cells, is harvested without any significant health risk to the mother and the new born baby from either the delivered placenta or during the third stage of labor [26]. The task of collection is performed in sterile environment where the umbilical vein is punctured with a needle attached to a sterile, closed system collection bags containing citrate phosphate dextrose or heparin anticoagulant which is positioned lower than the placenta and blood flows from the placenta through the cord to the bags. The collected units are labelled and then shipped to cord blood

Location	Number of Banks	Facilitating Organisation
Gurugram	4	Cryobank International, Life Cell International, Unistem Biosciences, Totipotent RX cell therapy
Maharashtra	3	Reliance Life Sciences, Ree Laboratories, Regenerative Medical Services.
Karnataka and Gujarat	4	Narayana Hrudayalaya, Cryo Save India, Stemcyte India, Best well Care Management Services.
Tamil Nadu, Andhra Pradesh, West Bengal	3	Life Cell international, Pathcare labs, Cord Life Sciences.

Table 1: Umbilical cord blood banks in India.

banks. The first public bank was set up in New York (USA) in the year 1992 [27] and now there are about 225,000 cord blood units frozen in 38 public cord blood banks in 25 countries [28]. However, the entire process of collection, processing, testing and cryopreservation of cord blood is quite cumbersome and non-regulated and this leads to a loss of 10 to 20 percent of the initially collected blood volume and cell counts [29]. The public banks are also called as not for profit banks since they do not ask for money in lieu of storage and they store the cord blood so that it is available to suitably matched recipients in different parts of the world. While public cord blood banks are appreciated there is a concern on the hike in the number of private blood banks. These banks ask for high financial assistance in lieu of storage of cord blood and allow it to be used only for autologous and allogenic transplantation if need arises in future. In contrast to their suggestion and promises recent data have shown that the role of autologous transplantation has not only been restricted due to the proven effect of graft versus leukaemia (GVL) by allogenic transplantation but also autologous CB offers no known clinical advantages over the stem cells isolated from bone marrow [30]. As far as the mathematics of autologous transplantation is concerned the probability of a family to use its own stored CB is almost 1:400 to 1:200,000 [31] and in case such a circumstance arises in future the bone marrow derived cells of a sibling could offer the same effect. Autologous cord blood cells are non-usable for treatment of cancerous malignancies such as leukaemia since the genetic mutation that gives rise to cancer already exists in the DNA of cord blood [32]. It is also an area of research and hence doubts arise that for how long these cells will maintain their efficacy and usefulness after being frozen. So private banks are just exploiting people out of their anxiety and incognizance which is not only unethical but also treacherous.

Indian Perspective on Umbilical Cord Blood Stem Cells

With a crude birth rate of 21.8 per 1000 people and home to 15% of the world population India would be one of the largest collectors of umbilical cord blood in the world. Three public banks have been established in India so far – Relicord, Jeevan cord and Stemcyte collectively having 5000 units [33]. According to health ministry of India there are 14 approved private umbilical cord blood banks operating in the country and a total of 29,993 bio-specimens have been collected from 24,533 patients in the hospitals till February 2015 (Table 1).

World Bank reports suggest that India is one of the highest ranking countries in the world as far as the number of malnourished children is concerned and according to the report of Global Hunger Index 2015, India is ranked 20th with a serious hunger situation. So a country where people are suffering from malnutrition, poverty, hunger and illiteracy, storage of cord blood at private banks which ask for a high rate just seems to be unrealistic. There is also a lack of proper training among nurses and sterile environment for the collection of the blood in hospitals and hence the chance of cord blood unit getting contaminated and hence

non-viability is quite alarming. Though public banks have the potential to play a significant role in public welfare but since their number is low they have not been so promising and this has been the major hindrance so far since most of the deliveries occur at public hospitals.

In 2016, researchers did a survey about the awareness of UCB banking and found that the general understanding of banking was poor in India. Only 26.5% women knew exactly what the UCB stem cell banking is, 31% knew about private cord blood banking while only 16% were aware about public cord blood banking [34]. They also found that the awareness of cord blood banking depends on the level of education. Another group of researchers surveyed the lay persons and doctors and found that 58% doctors and 82% lay persons were unaware about the UCB transplantation. Besides this lack of knowledge, 40% doctors and 69% persons from the population were willing for UCB banking for their child. This study demonstrated that the obstetricians and the pediatricians should take a central role in providing the correct information to the parents which helps them in taking a correct decision [35].

Regulatory Bodies

There are 3 major regulatory bodies, which are responsible for formulating policies in cord blood banking sector. These are Indian Council of Medical Research (ICMR), Department of Biotechnology (DBT) and Drug Controller General of India (DCGI). They issue guidelines on UCB banking facilities, which facilitate stem cell research via public- private partnership. Also they encourage public cord blood banking to extend affordable treatment and regulate travel and tourism sector. In July 2006, draft guidelines for stem cell research including guidelines for cord blood stem cell banking were formulated and submitted by ICMR and DBT. On July16, 2007 a joint committee of ICMR and DBT was organized to discuss the Annexure on “Laboratory standards for cell collection and processing for clinical use” [36].

Medical Tourism in India

‘Medical Tourism’ refers to patients going to a different country for an either urgent or elective medical procedure. In India, medical tourism is growing at the rate of 30% in a year and expected to generate revenues of Rs.100 billion by 2012 as per the study conducted by CII and McKinsey [37]. Patients from the western world have started looking for life saving treatments in low wage countries especially in India. New England Journal of medicine named this new breed of growing patients as ‘Medical Refugees’. They use low cost destinations like India for life saving surgeries [38]. Thus, with established edge in surgical treatments, India might play an important role in one of the world’s fastest growing industries - ‘Medical Tourism’ particularly stem cell therapy. However, creation from stem cell is considered to be age old process in Ancient India. In Adi parva, one of the chapters of Mahabharata (one of the two major Sanskrit epics of ancient India), it is depicted that the antagonists, the hundred Kaurava brothers were created from piece of a flesh delivered by their mother. A sage then divided the flesh into hundred parts and then incubated and treated with herbs and ghee. Out of which the Kauravas were born. BG Matapurkar, a pioneer in adult stem cell research has inferred this episode as evidence of prehistoric Indian acquaintance with stem cell technology [39]. Maybe stem cell research was altogether a lost science of ancient India.

Conclusion

Due to high birth rate as well as genetic diversity, India has a great prospect for UCB banking. A very few number of UCB transplants have been performed in India so far due to high rate and inadequate number

of accessibility of UCB units. But with the existence of three public and seven private UCB banks in India, transplantation will get better in the coming years. Cord blood stem cells are being used in the treatment of 40 medical conditions with over 72 potential disease targets.

Future Prospective

Clinical use of UCB has increased over the past several years and this has led to the development of entire UCB banking industry. UCB storage in India needs to build up public private partnership relation where UCB can be stored economically. Many private banks keep on growing in India because most of the families store UCB in private banks with promising advantages in degenerative disorders in the future. To increase the number of transplantations in India, full participation and considerable investment by the Government is essential.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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