Cord Blood in Transfusion Medicine: The Latest Miracle in Transfusion Science

Blood transfusions are sometimes essential to save life. However, many still die all over the world, particularly in resource-constrained countries, because of an inadequate supply of safe blood and blood products for transfusion. Half a million women still die at childbirth from pregnancy-related complications with haemorrhage accounting for 25% of such complications; it is the most common cause of maternal death. Further, malnutrition, thalassaemia, and severe anaemia are prevalent diseases in children, especially in the developing world, as also in adults, which require blood transfusion. Globally over 80 million units of blood are collected every year, but only 39% of this is collected in the developing world which includes 82% of the global population, thus leaving a wide gap between supply and demand. Interestingly, cord blood could help fill this gap, and additionally add benefits yet unthought of. This paper examines the potentials of cord blood with the objective of exciting the medical community to further research.

There are about 100 million births in the world annually, conservatively speaking. In India alone, there are more than 20 million births per annum, which means that over 20 million placentas are used for the growth of the foetus and later discarded every year. One of the products of the placenta is cord blood; it has immense potentials. The placenta is a complex organ that regulates foetomaternal interactions. Many cytokines that influence the lymphohaematopoietic environment are produced in the placenta in abundance. Therefore, placental umbilical cord blood contains beneficial substances over and above that of normal adult blood. Further, because the foetus grows into a neonate within the safe confines of the womb where the placenta plays an important role in ensuring security, placental cord blood may be assumed as safe due to the molecular screening intrinsic to the functional barrier of a healthy placenta. An estimated 8,785,000 l of cord blood is produced globally per year if an average of 84-90 ml/placenta collection is assumed. Our group of medical scientists and clinicians, with assistance from the department of science and technology, Government of West Bengal, transfused ABO screened and HLA matched randomised foetal blood in cases of anaemia resulting from malaria, diabetes, thalassaemia, leprosy, rheumatoid arthritis, tuberculosis, malignancy, AIDS, only to name a few diseases that can cause anaemia and found it not only to be safe but perhaps providing additional benefits that need further study[1-12].

In parts of the world where research is ongoing, a microscopic section of cord blood's mononuclear cells (0.01% nucleated cells) is used for transplantation purposes, while the rest ie, 99.99% is discarded. However, the discarded part also has many potential uses. The blood volume of a foetus at term is 80-85 ml/kg on an average. The placental vessel at term contains approximately 150 ml of cord blood. Cord blood contains three types of haemoglobin, haemoglobin F (foetal) [HbF], haemoglobin A (HbA) and HbA2, of which HbF constitutes the major fraction. HbA accounts for 15-40% and HbA2 is present only in trace amounts at birth. It may be noted that HbF, which is the major component, has a greater oxygen binding affinity than HbA.

The use of haematopoietic stem cells from cord blood is now well documented. These are harvested in many laboratories globally and stored in cord blood banks. But apart from haematopoetic stem cells, cord blood also contains potent angiogenesis stimulating cells. $CD34^+$ $CD11b^+$ fraction of cells, which is approximately half of the $CD34^+$ fraction of cord blood, have been demonstrated to possess the ability to differentiate into functional endothelial cells *in vitro* and

in vivo[13]. In addition, there are some mesenchymal stem cells (MSCs) in the cord blood which are classically defined as adherent to plastic and expressing a non-haematopoietic cell surface phenotype, consisting of $CD34^-$, $CD45^-$, $HLA\text{-}DR^-$, while possessing markers such as STRO1, VCAM, CD13, CD29, CD44, CD90, CD105, and SH3[14]. In addition cord blood cells with markers and activities resembling embryonic stem cells have been found[15]. Investigators have identified a population of $CD34^-$ cells expressing OCT4, Nanog, SSEA3 and SSEA4, which could differentiate into cells of the mesoderm, ectoderm and endoderm lineages[15].

The first widespread use of cord blood as a stem cell source was in the treatment of paediatric haematological malignancies after myeloablative conditioning. Outside the area of oncology, the clinical utility of cord blood has been demonstrated in various areas ranging from reconstitution of defective immune systems to correcting congenital haematological abnormalities, to inducing angiogenesis. Additionally, experiments are ongoing on its regeneration potential which may have long term consequences for treatment of a variety of intractable diseases[16-18]. It is therefore important to mention how cord blood is different from adult blood and why it may serve as a blood substitute with additional benefits in certain disease conditions.

The red cell collected from the newborn's cord blood differs from the adult RBC in that there is an increase of the immunoreactive myosin in the red cell membrane[19] and the total value of lipid, phospholipid and cholesterol are more in the cord blood red cell than in adult RBC[20]. Even the antigen expression of cord blood RBC differs from the adult RBC. There are also fundamental metabolic differences in the cord blood and the adult blood, for example, the activities of phosphoglycerate kinase, enolase, glyceraldehyde -3-phosphate dehydrogenase, glucose phosphate isomerase, etc, of the Embden-Meyerhof pathway are definitely increased in cord blood[21] and even the non-glycolytic enzymes like carbonic anhydrase and acetylcholine esterase is distinctly different from the adult blood[22]. These differences are significant because of the possibilities of better tolerance and additional benefits.

It is important to remember that foetal haemoglobin serves the foetus well during its term in the womb producing a healthy newborn at the end. It is well-known that most types of normal haemoglobin, including haemoglobin A, haemoglobin A2, as well as haemoglobin F, are tetramers composed of four protein subunits and four haem prosthetic groups. Whereas adult haemoglobin is composed of two α (alpha) and two β (beta) sub-units, foetal hemoglobin is composed of two α sub-units and two γ (gamma) sub-units and is commonly denoted as $\alpha 2\gamma 2$. Because of its presence in foetal hemoglobin, the γ sub-unit is commonly called the "foetal" haemoglobin sub-unit.

The gamma sub-unit is encoded on chromosome 11, as is the beta sub-unit. There are two similar copies of the gamma subunit gene: γG which has a glycine at position 136, and γA which has an alanine. The gene that codes for the alpha sub-unit is located on chromosome 16 and is also present in duplicate. Foetal haemoglobin has greater affinity for oxygen than adult haemoglobin. The P50 value (ie, the partial pressure of oxygen at which the protein is 50% saturated) for foetal haemoglobin is lower than adult haemoglobin. The P50 value of foetal haemoglobin is roughly 19 mm Hg, whereas adult haemoglobin is approximately 26.8 mm Hg. As a result, the "oxygen saturation curve", which plots per cent saturation *versus* pO2, is left-shifted for foetal haemoglobin as compared to adult haemoglobin. This greater affinity for oxygen is explained by the lack of foetal haemoglobin's interaction with 2,3-bisphosphoglycerate (2,3-BPG or 2,3-DPG). In adult red blood cells, this substance decreases the affinity of haemoglobin for oxygen. This 2,3-BPG is also present in foetal red blood cells but interacts less efficiently with foetal haemoglobin than adult haemoglobin[23]. Ultimately,

foetal haemoglobin has greater oxygen carrying capacity than adult blood, and this has therapeutic potential in various diseases.

The word 'potential' has been used several times in this chapter and that is because of the limited nature of clinical experimentation because of existing regulations and ethical debates. But there is no doubt, given the cellular and acellular constituents of cord blood that there is great potential for clinical applications. Recent advances in biology and medicine have introduced new technologies to study the mechanisms of genetic switching of the haemoglobin chain from alpha to beta during human foetal development, the site of haematopoiesis during foetal development, and its change from yolk sac eventually to bone marrow. This raises a number of pertinent questions: Do the developing haematopoietic stem cells come from the same origin or from different sites during the shift of the place off its synthesis and turnover? What is the exact role of the stroma as a haematopoietic organ, and what is its interaction with haematopoietic progenitor cells? Why do haematopoietic stem cells home to a particular site in cases of amphibians, birds, and mammals? Knowledge and understanding of these issues may lead to the development of animal models, successful therapies, and novel methods to treat intractable diseases.

In the future, medical research can provide answers to some, if not all, these questions. It is only through understanding hitherto unexplored areas that new cures may be discovered. We invite investigators to contribute to the understanding of the molecular mechanisms underlying the immunomodulation capability of cord blood, as well as the development of strategies to use this immunomodulation in clinical practice, and the evaluation of outcomes of the new modalities for the characterisation of the components of foetal blood and the placenta, their use in therapy and in means for measuring outcomes from treatment trials. Pregnancy cytokines provide new insights into understanding the expression of different antigens, their presence or absence. Regulations using animal models, like human haematopoiesis in animal hosts following xenograft in severe combined immunodeficiency (SCID) mouse system and foetal sheep systems, also appear promising. Similarly, the therapeutic potential of nucleated RBC, colony forming unit (CFU), Gower 1 and 2 haemoglobin collected from the developing human foetus, if applied in human and animal system to treat refractory anaemia, may have fruitful clinical implication for futuristic medicine. Future research may focus on the following aspects in particular:

(a) Autologous and allogeneic cord blood transfusion from the paediatric to the geriatric group.

(b) Serum constituent characterisation therapy using cord blood serum viz, cord blood biomarkers, IL1, IL6, and IL8 are selectively associated with foetal infection. These markers may be clinically useful indicators of extensive intra-uterine infection associated with poor neonatal outcome.

(c) Role of foetal blood in suppression of inflammation and role of cytokines like IL3, granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), and GM-CSF in immunomodulation.

(d) The mechanism of inflammation of the growing foetal blood using the animal model or some other model.

(e) Role of modifiers of inflammation contained (regulatory T cells) in foetal blood.

(f) Emergency use of foetal blood in nuclear radiation disasters keeping in mind its regeneration potential.

(g) Use of foetal blood in different indications as blood substitute when there is more demand than supply of blood for transfusion (for example in emergencies like earthquakes, tsunamis, others).

(h) Use of serum from cord blood for any purpose viz, cord blood serum to treat corneal xerosis and ulceration.

(i) Antigen expression and metabolic differences of cord blood and adult blood with special reference to clinical implications.

(j) Comparison of cord blood preservation and its functional variation with adult blood.

(k) Adverse outcomes after cord blood transfusion or its constituent therapy… if it happens, how to prevent and combat the situation.

(l) Prophylactic use of leucocyte-reduced components to prevent primary human leucocyte antigen (HLA) alloimmunisation, which may be a principle cause of refractoriness to platelet transfusion; the effectiveness of leucocyte-reduced components in the prevention of cytomegalovirus (CMV)[24].

Our group of medical scientists and clinicians conducted over 1000 cord blood transfusions with safe outcomes in all cases, as indicated in our published studies, from 1999 till recent times in children and adults for various indications[25]. Not a single case of immediate or delayed immunological or non-immunological reaction was reported. All transfusions were duly screened and approved by the institutional ethical committee and consent was obtained from the patient/guardian and the donor's guardian. We suggest that the medical fraternity, globally, should use cord blood, a precious gift of nature, which is free from infection, hypo-antigenic in nature, has an altered metabolic profile, is enriched with growth factors and cytokine filled plasma and has a potential higher oxygen carrying capacity than for adult blood, as an emergency source of blood transfusion for the management of anaemia of any aetiology. Further, some of the questions raised in this paper should be investigated, following all ethical standards, so that the full potentials of cord blood may be scientifically investigated and benefits extracted.

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