Umbilical Cord blood transfusion: A new therapeutic tool in Modern Haematology

The process by which allogeneic blood is collected from the umbilical cord vein after its clamping from the lower uterine caesarean section (LUCS)/Vaginal delivery, and is administered after screening for transfusion transmitted disease like HIV 1& 2, Hepatitis B & C, Malaria , Syphilis etc and ABO/Rh group matching of the donor and the recipient, and then intravenously passing it to the recipient after proper informed consent and ethical clearance from the Institute ,for therapeutic benefit is the standard practice for the umbilical cord blood transfusion.

Clinical research pertaining to 0.01% of the cord blood components known as haematopoietic stem Cell (CD34) is already an established area in the field of cord blood stem cell therapy since the first successful transplantation of cord blood derived haematopoietic stem cells in a patient with Fanconi's anaemia in 1989.(1) However, in 1999, for the first time, researchers from Calcutta unlocked the true potentialities of the rest ,often, discarded 99.9% of cord blood and its components.(2) It has been seen that patients with severe anaemia, renal failure and other conditions of low cardio-respiratory reserve with tissue hypoxia conditions in different age groups can be benefitted from cord blood transfusion. (2) Growth factors and cytokines present in the cord blood plasma can be of potential therapeutic use in wound healing with different background disease also. (2)

Contents:

A new blood resource with an edge:

A report by the WHO has revealed that there is about 500,000 pregnancy related deaths globally, of which 99% of these occur in rural areas of developing countries. (2, 3) Around 25% of maternal deaths are due to severe bleeding. (2) Also annually around 2.7 million newborn babies die primarily due to anaemia, underweight, and malnutrition. (3, 4) It is estimated that around 80% of infants weighing less than 1500 g can receive at least one unit of red blood transfusion. In such cases, umbilical cord which is rich in fetal haemocomponents can be an ideal and alternative source for both autologous and allogeneic blood transfusion in neonates. (5, 6, 7, 8, 9, 10)

Roughly 80 million of adult blood is collected every year, but the tragedy is that only 39% of this is collected in the developing world to meet the requirements for an astounding 82% of the global population residing in these poor countries. (4)

Human umbilical cord blood containing an average of 150 ml of blood rich in different favourable blood components like much higher Haemoglobin than adult blood. The cord blood Hb is anywhere between 18 to 22 grams per decilitre whereas in case of adults both men and women it ranges from 12 to 17.5 grams per decilitre (g/dl). Cord blood has 70 percent fetal haemoglobin which has potentialities to carry 60 percent more oxygen than adult Haemoglobin, Platelet count of cord blood is 7,50,000 per microlitre against adult blood which is 2,50,000 per microlitre of blood, WBC in cord blood is 24000 per microlitre as against only 4500-10,000 per microlitre in case of an adult blood, however the scientific community never appreciated its potentialities in different clinical conditions and mostly this bloods are often thrown away as a trash material from the labour room Operation theatre. (11) With over more than 100 million births every year, around 10 billion of human umbilical cord blood can be collected roughly. Since 1999, the immense therapeutic and safety potentialities of human whole cord blood transfusion in cases with various aetiologies are well investigated and documented. (12)

History of whole Umbilical Cord blood transfusion:

In 1939, Halbrecht reported his attempt to try human umbilical cord blood as a blood substitute in Lancet. (13, 14) However, the absence of an anticoagulant like acid citrate dextrose resulted in the formation of clots. (13, 14) Also the concept of screening of blood for different transfusion-related transmitted infectious diseases was not prevalent at that time and it seriously hindered the endeavour for a safe cord blood transfusion. (13, 14)

For the first time in 1999, researchers at different Govt Hospital in the city of Calcutta(India) routinely started using this blood as a true transfusion substitute with support, ethical and scientific clearance from the West Bengal State Government, India, (15) successfully without jeopardising the patients' safety. This was hailed globally as a new therapeutic tool in transfusion medicine. Prof Elaine Gluckman in her visit to Calcutta inspected and reported this in Lancet. She appreciated this transfusion of freshly collected, thoroughly screened cord blood in more than 1000 anaemic patients suffering from malaria, tuberculosis, cancer, beta thalassemia, leprosy, HIV, diabetes and arthritis after obtaining ethical committee clearance and patient informed consent. (11, 16, 17, 18, 19, 20, 21, 22, 23)All cord blood transfusion was conducted strictly following national and international guidelines. Its potentiality of use in other resource restricted countries in Asia and Africa should be stressed.

Another group from the UK in 2003, similarly reported the safe use of cord blood transfusion in many sub-Saharan children who do not have access to adult blood transfusion and suffering from severe anaemia. This group observed the rise in Hb from baseline level in 21% of the children post-transfusion of whole umbilical cord blood. (24)

In 2004,a study published by the American college of surgeons reported the safe and effective transfusion of 413 units of whole cord blood to 129 informed consent patients which included 54 men and 75 women of different age group suffering from advanced cancer, systemic lupus erythematosus, ankylosing spondylitis, aplastic anaemia, rheumatoid arthritis and thalassemia major.(25) In none of the cases, the investigators encountered any single case of immunologic or non-immunologic reaction in subsequent follow-up studies. (25)

Cord Blood and Adult Blood difference:

The erythrocytes collected from the cord blood and the adult blood differs in many important ways. The cord blood red cell membranes are more immune-reactive than adult blood (26) including the total value of lipids, lipid phosphorus and cholesterol. (27) Even the antigenic expression of cord blood is much lower when compared to adult blood. Major Blood group A, B, S and Lutheran antigens are expressed in extremely lesser amount with no expression of Lewis antigen.(28)

Further differences in metabolism like the activities of phosphoglycrate kinase, enolase, glyceraldehydes-3-phosphate dehydrogenase and glucose phosphate isomerase of the Embden Meyerhoff also exists. Cord blood has an increased level of these enzymes when compared to adult blood. Even there is a distinct difference existing between cord blood and adult blood in the non glycolytic pathways involving carbonic anhydrase and acetycholine esterase enzymes.(29,30)

Safety and Efficacy of whole Umbilical cord blood transfusion:

For the last 7 decades, there has been a global attempt to find a genuine blood substitute. (4, 31). An estimated 13 million units of blood worldwide are not tested positive for HIV, Hepatitis virus in developing countries. Further in countries where there is an acute shortage of blood supply, 80% of the blood comes from paid or replacement donors like friends, families or acquaintances even when the infected population is extremely high. (32) In fact, a case from Kenya has been reported where 25% of adult blood deemed safe earlier was transfused into patients only to be found later that these were infected with HIV. (33)

In the Sub-Saharan region and other south East Asian countries where poverty is rampant and affordability is a big issue, adult blood is screened by ELISA method following WHO guidelines. In the west PCR method for molecular level of blood, screening is normally followed. ELISA method of screening blood is much cheaper than PCR screening and can be afforded by many patients in poor countries. (13) However, ELISA normally does screening at the macro or cellular level and therefore a risk of transfusion-related transmitted diseases always exists because of the existence of window period in diseases like Hepatitis C, HIV. (13,34) Cord blood is selectively screened by the blood-placental barrier which is often regarded as nature's most formidable screening method, therefore, reducing the chances of vertical transmission of diseases like bacteria, viruses and other pathogenic substances from the mother to the fetus. (35)

According to Wintrobe in 2003, the ideal substitute for blood transfusion is the transfusion of blood itself. (13) A true blood substitute should have three important properties like i) haemoglobin carrying sufficient oxygen , ii) essential blood components like platelets, cytokines, anti-clotting factors and iii) white blood cells to confer immunity and fight against infections. (13) The current generation of blood substitutes can transport oxygen to tissues and there are agents to replace platelets, coagulation factors, and its various other combinations, including cases of human and bovine haemoglobin based oxygen carrier. (2,

36) However all are still under intense clinical observation and some of them failed to pass the FDA Phase III trials. (2)

The legion in the field of cord blood transplantation, Prof. Elaine Gluckman, reported in the February 2015 Edition of Lancet Haematology that a promising and large-scale study was conducted in Calcutta, India, where more than 1000 units of whole umbilical cord blood were safely and efficiently transfused in children, adults and geriatric age groups for various indications. (37)

Safe transfusion of blood and blood related products has aided many of the advanced modern surgeries worldwide. (38) A reliable supply of safe blood is essential to improve health standards at several levels, especially among women and children, and particularly in the poorer sections of the society anywhere in the world. (39)

Advantages of Cord blood transfusion:

Umbilical cord blood is an extremely convenient and a rich source for haematopoietic, mesenchymal and other stem cells. Also, stem cell collection from cord blood does not require any invasive procedures unlike from the bone marrow or fat tissue.

Higher haemoglobin and oxygen content:

As mentioned earlier, cord blood possesses higher haemoglobin content than adult blood with high oxygen binding capacity due to the presence of high concentration of fetal Haemoglobin (HbF, normally between 50-85 %) in term placentas. The haemoglobin content of cord blood is anywhere between 17.6% to 22%. (39, 40)This HbF has the potentiality to carry 50-60% of more oxygen than adult blood due to the low concentration of 2-3 diphosphoglycerate. (11)The oxygen tension in cord blood or PO2 is 50% saturated and is 6-8 mm of Hg lower than the adult blood thereby resulting in a more left shift in the oxygen dissociation curve because of the poor binding capacity of 2, 3 –diphosphoglycerate with HbF. (11, 41, 42) Also, the platelet and leukocyte content in cord blood is high when compared with adult blood. Further, a rich concentration of cytokine and growth factors are also present in the cord blood plasma.

Hypoantigenicity of Cord Blood:

In the case of a healthy neonate, umbilical cord blood does not contain any acquired antibodies and erythrocytes express extremely low erythrocytic antigens A and B which is around 3% to 7%. (39)This erythrocytic antigen expression is further low for minor blood groups like Kelly and Luther. (39) This hypo antigenicity of cord blood might be due to the fact that the fetus till term remains inside the mother's womb which can be considered to be a sterile or an immunologically privileged site. It is after the birth of the baby that the immune system undergoes more maturation and other complex developmental procedures along with its full expression as the baby gets exposed to different antigens. (43, 44)

Sterile nature of Cord blood:

Cord blood aseptically collected from healthy newborn babies and properly screened is extremely pure and free from any bacterial, viral and pathogenic contamination. The blood of a healthy newborn is always sterile than the adult blood because of the presence of a P-glycoprotein functional barrier between the mother and the fetus in the placenta, also known as the blood placental barrier. (11, 45, 46) Even HIV cannot have a vertical transmission easily from the mother to the fetus except at or near term when the blood placental barrier gets dilated thereby increasing the feto maternal exchange. (11, 40, 41) It has been reported by a group of investigators that the trophoblastic barrier remains unaffected in full term placentas in cases of HIV seropositive mothers undergoing anti retroviral therapy. (11, 45, 46)

Superior engraftment properties of cord blood CD34 cells:

Cord blood derived haematopoietic stem cells have shown to possess superior properties unlike bone marrow derived. Cultures of cord blood-derived CD34 positive cells have a doubling time every 7 to 10 days which is many folds greater than bone marrow-derived CD34 positive stem cells. (47) This greater proliferative capacity of cord blood can be attributed to the longer telomere length of these cells. (47) Some studies have also shown the synthesis of increased amounts of anti-inflammatory cytokine-like IL-10 which plays a major role in down-regulation of GVHD. (11).Secondly, human umbilical cord blood derived CD34 positive myeloid progenitor stem cells have shown to possess delayed but long-term engraftment potential with a higher rate of engraftment and better bone marrow reconstitution compared to adult bone marrow-derived CD34 cells. (43, 44)

Rise in CD34 positive cells in the peripheral blood post cord blood transfusion:

An interesting observation made by the research group in Calcutta following HLA randomized cord blood transfusion in all cases of anaemia in the background of different diseases was the increase in the peripheral count of CD34 positive cells in flow cytometric analysis without any clinical graft versus host reaction and without the support of any immunosuppressant or growth factors. (11, 21) This phenomenon can be due to the transfusion-induced transplantation effect in the host bone marrow by the transfusion of cord blood. Apart from CD 34 positive stem cells, the human umbilical cord blood also possesses a unique microenvironment containing mesenchymal and ectodermal stem cells and a cocktail of cytokines and growth factors. (11, 48) A certain degree of chimerism is required for proper functioning of the bone marrow and this is best achieved by the presence of self-renewal capable stem cells in

the cord blood which post cord blood transfusion can aid in the engraftment of these cells in the host's bone marrow. (11, 48) This property of chimera is supposed to be an important factor behind the rise of peripheral CD34 positive cell count. Normally the endogenous release of CD34 positive haematopoietic stem cells in the peripheral blood is aided by the administration of Granulocyte- Colony Stimulating Factor (G-CSF) or Valproic Acid. However researchers at The School of Tropical Medicine, Calcutta observed a noticeable rise in the peripheral CD 34 positive haematopoietic stem cells post-transfusion without any prior administration of external stimulating growth factors. (11, 21) Other combination of factors like a high concentration of mononuclear cells, altered viscosity, hypoantigenicity and altered metabolic profile might facilitate the bone marrow to combat any chronic deficiency by protecting the organs.

Migration and homing of stem cells after cord blood transfusion:

Normally the general well being of the patients also increases from baseline pos- transfusion of cord blood. This might be due to the selective homing and migration of some predominantly healthy fetal CD34 positive stem cells to the organs or tissues where there is a stem cell demand via up-regulation of the chemokine pathway and receptors. (39) These CD34 positive cells might reside for a long time and integrate, mature and transdifferentiate with the host tissue-specific stem cell niche and later help in the secretion of specific growth factors and other essential cytokines thereby improving the function and efficacy of the organ or tissue. This method of selective migration, homing and engraftment of CD 34 positive cells might attribute towards the overall improvement of general health in patients and can also explain the phenomenon of the initial rise and fall of CD34 positive cells in the peripheral blood circulation. (39)However, the above phenomenon is still under intense investigation and further molecular studies will reveal the ideal mechanism behind this rise in CD 34 cells in the peripheral blood post transfusion. (39)

Potentialities of Cord Blood transfusion:

Potentialities of cord blood transfusion are immense due to its basic important properties like correction of anaemia, long-term bone marrow reconstitution in cases of leukaemia or bone marrow aplasia and a possible source for peri-operative blood transfusion including differentiation potentialities. (40) Based on the 2000 study of Global Burden of Disease by WHO, brain diseases account for a third of all disabilities in Europe and are likely to increase in the coming years. (49) In such cases due to the high oxygen content of the fetal haemoglobin in the cord blood, reperfusion therapies using cord blood transfusion can be initiated. Pre-clinical studies using umbilical cord blood transfusion has shown evidence of neovascularizisation, reduction of neurotoxicity and cell excitotoxicity in the brain. (50)

Intravenous transfusion of umbilical cord blood in neurodegenerative animal models has shown to reduce the mRNA and protein expression of inflammatory molecules. (50)

Further advances in cord blood transfusion include two clinical studies and a case report on autologous transfusion in neonates suffering from a congenital anomaly. (51, 52, 53, 54) Due to its high platelet rich content, whole umbilical cord blood transfusion has the possibility of treating severe thrombocytopenia in patients suffering from dengue.

Alternative to adult blood transfusion:

Cord blood is easily available and can serve as an alternative to blood and transfusion emergencies especially during wars and natural calamities. (18) It can be also used in geriatric age groups for increasing immune competence and combating anaemia. (2) Removing the 0.01 % of the nucleated cells, the rest 99.99% of umbilical cord blood has been used in elderly patients to combat anaemia successfully. (39)

Transfusion versus transplantation in cord blood therapy:

There are two aspects of cord blood stem cell therapy, transplantation, and transfusion.

While cord blood transplantation is common, transfusion of cord blood has started successfully since 1999. Normally cord blood-derived haematopoietic stem cell transplantation for bone marrow reconstitution requires radiotherapy combined with chemotherapy to destroy the faulty bone marrow system of the host and replace it with a new allogeneic one. This process is normally associated with high mortality and morbidity rate. However, such is not the case for whole umbilical cord blood transfusion due to its unique hypo antigenicity where neither immune suppressants like radiation nor chemotherapy are required. (55)

Also the superior property of long-term engraftment of cord blood derived haematopoietic stem cells, these cells can be banked in order to reconstitute the bone marrow in an event of a nuclear disaster or an attack.(56, 57)

References:

1. Gluckman E, Broxmeyer HE, Auerbacm AD, et al. Haematopoietic reconstitution in a patient with Fanconi's anaemia by means of umbilical cordfrom an HLA-identical sibling. N Engl J Med. 1989; 321:1174-1178.

2. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Preface, page no. Viii.

3. http://www.who.int/mediacentre/factsheets/fs348/en

4. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Preface, page no. xxvi.

5. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy specific biological substances, Springer London Dordrecht Heidelberg New York, Springer-Verlag London Limited 2011,Chapter no: 6, Autologus Placental blood transfusion for the therapy of anaemic neonates, Thomas Brune, F.Louwen, C.Troeger, W.Holzgreve and H.S.P. Garritsen.

6. Strauss RG. Autologous transfusions for neonates using placental blood; a cautionary note. Am J Dis Child. 1992; 146: 21-22.

7. Strauss RG. Blood banking issues pertaining to neonatal red blood cell transfusions. Transfus Sci. 1999;21:7-19.

8. Roberts I. Management of neonatal anaemia: the role of erythropoietin. Rila publications Ltd. CME Bull Haematol. 1997;1 (1):5-7.

9. Eichler H, Schaible T, Richter E, et al. Cord blood as a source of autologous erythrocytes for transfusion to preterm infants. Transfusion. 2000; 40:1111-1117.

10. Surbek DV, Glanzmann R, Senn H-P, et al. Can cord blood be used for autologous transfusion in preterm neonates? Eur J Pediatr. 2000; 159:790-791.

11. Niranjan Bhattacharya, Prof. Phillip Stubblefield. Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009) Chapter: 10, Page no. 227-257, Placental Umbilical Cord Whole Blood Transfusion: A True Blood Substitute to combat Anaemia in the background of chronic disease-A study report. (1999-2006)

12. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Preface, page no. ix.

13. Niranjan Bhattacharya, Phillip Stubblefield (Editors).Regenerative Medicine Using Pregnancy-Specific Biological Substances, Springer London Dordrecht Heidelberg New York, Springer-Verlag London Limited 2011. Chapter No. 11, Placental Umbilical cord blood as a true substitute with an Edge, Niranjan Bhattacharya. Page Nos. 103-109.

14. Halbrecht J. Fresh and stored placental blood. Lancet. 1939; 2:1263. doi: 10.1016/S0140-6736(00)74023-2.

15. Niranjan Bhattacharya, Placental umbilical cord whole blood transfusion—A safe and genuine blood substitute for patients of the under-resourced areas of this country at emergency http://www.stmkolkata.org/rmts/research/PlacentalUmbilicalCordWholeBloodTransfusion.pdf

16. Bhattacharya N. Placental umbilical cord blood transfusion: A novel method of treatment of patients with malaria in the background of anaemia. Clin Exp Obstet Gynecol. 2006; 33(1):39-43.

17. Bhattacharya N Clin Exp Obstet Gynecol. 2005; 32(2):102-6.Placental umbilical cord blood transfusion in transfusion-dependent beta thalassemic patients: a preliminary communication.

18. Bhattacharya N, Placental umbilical cord blood transfusion: a new method of treatment of patients with diabetes and microalbuminuria in the background of anaemia. (Clin Exp Obstet Gynecol. 2006; 33(3):164-8.

19. Bhattacharya N, Transient spontaneous engraftment of CD34 hematopoietic cord blood stem cells as seen in peripheral blood: treatment of leprosy patients with anaemia by placental umbilical cord whole blood transfusion, Clin Exp Obstet Gynecol. 2006; 33(3):159-63.

20. Bhattacharya N, A preliminary report of 123 units of placental umbilical cord whole blood transfusion in HIV-positive patients with anaemia and emaciation. Clin Exp Obstet Gynecol. 2006; 33(2):117-21.

21. Bhattacharya N, Placental umbilical cord whole blood transfusion to combat anaemia in the background of tuberculosis and emaciation and its potential role as an immuno-adjuvant therapy for the under-resourced people of the world, Clin Exp Obstet Gynecol. 2006;33(2):99-104.

22. Bhattacharya N, A study of placental umbilical cord whole blood transfusion in 72 patients with anaemia and emaciation in the background of cancer.Eur J Gynaecol Oncol. 2006; 27(2):155-61.

23. Bhattacharya N, Placental umbilical cord whole blood transfusion to combat anaemia in the background of advanced rheumatoid arthritis and emaciation and its potential role as immunoadjuvant therapy, Clin Exp Obstet Gynecol. 2006;33(1):28-33.

24. Hassall O, Bedu-Addo G, Adarkwa M, Danso K, Bates I. Umbilical-cord blood for transfusion in children with severe anaemia in under-resourced countries. Lancet. 2003; 361(9358):678-679.

25. Bhattacharya N. Placental umbilical cord whole blood transfusion: a safe and genuine blood substitute for patients of the under-resourced world at emergency. J Am Coll Surg. 2005; 200(4):557-563.

26. Myosin in Adult and Neonatal Human Erythrocyte Membranes 1668 Blood, Vol 67. No 6 (June), 1986: pp 1668-1674 By Lisa M. Matovcik, Ute Gr#{246}schel-Stewart, and Stanley L. Schrier, Blood, Vol 67. No 6 (June), 1986: pp 1668-1674.)

27. Erythrocyte Lipids in the Neonate, R.G.NEERHOUT, Pediat. Res. 2: 172-178 (1968

28. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy specific biological substances, Springer-Verlag London Limited 2009, Chapter no: 5, Umbilical cord blood and its therapeutic potentialities, Patricia Pranke and Tor onsten.

29. John P.Greer: Editor, Wintrobe's clinical haematology, volume no.1 , page no:1250, Part iv: Disorders of red Cells, Section 5: Other RFed Cell Disorders.page no: 1250,

30. Blood Cells as a Tissue: Proceedings of a Conference held at The Lankenau Hospital October 30-31, 1969 1970th Edition by William L. Holmes (Author)

31. Amberson WR, Mulder AG, Steggerda FR, et al. Mammalian life without red blood corpuscles. Science 1933; 78:106–7.

32. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Introduction, page no. ix.

33. George Kassianos, Immunization: Childhood & Travel health. Page no: 286, Chapter: 50, Wiley Publishers, April 15, 1998.

34. Zinab OA, Hock TT, AbdelHamid, Rozline Hassan. Significance of RT-PCR over ELISA Technique for the Detection of HCV in Blood Donor. Biohealth Science Bulletin 2009, 1(2), 53 – 56.

35. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009)Prof. Niranjan Bhattacharya, Chapter no: 13, Early reports on the prognostic implications and immunotherapeutic potentials of CD34 rich cord whole blood transfusion in advanced breast cancer with severe anemia. Page no: 123.

36. T. Standl, M.-A Burmeister, E.-P Horn, S.Wilhelm, W.T.Knoefel, J. Schulte AM Esch.Bovine haemoglobin based oxygen carrier for patients undergoing haemodilution before liver resection. British journal f anaesthesia, 1998, 80: 189-194.

37. Umbilical cord blood transfusions in low-income countries, Elaine Gluckman, Published Online: 13 February 2015.

38. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Prof. Niranjan Bhattacharya, Perspectives on Cord Blood Transfusion, Introduction, xxvi.

39. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy specific biological substances, Springer-Verlag London Limited 2009, Chapter no: 5, Umbilical cord blood and its therapeutic potentialities, Patricia Pranke and Tor onsten.

40. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Chapter no.13, Umbilical cord blood Transfusion-A Clinical overview, Prof. Dr. Himansu Kumar Basu, page no:290

41. Delivoria - Pahadopoulos M, Roncevic NP, Oski FA, Postnatal changes in oxygen transport of term premature and sick infants: The role of red cell 2, 3 -diphosphoglycerate and adult hemoglobin, Pediatr. Res., 5: 235-245, 1971.

42. Killmartin JV, Interaction of hemoglobin with proteins CO2 & 2,3 diphosphoglycerate, Brit. Med. Bull, 32 : 209-212, 1976.

43. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield. Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009) Chapter: 4, Ex vivo expansion of Cord Blood. Ian K. McNiece & Elizabeth J.Shpall.

44. Laughlin MJ, Barker J, Bambach B, et al. Hematopoietic engraftment and survival in adult recipients of umbilical cord blood from unrelated donors. N Engl J Med. 2001; 344(24):1815-1822.

45. Molsa M, Heikkinen T, Hakala K, Wallerman O, Wadelius M, Wadelius C, Laine K. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005; 78(2):118–22.

46. Tscherning-Casper C, Papadogiannakis N, Anvert M, Stolpe L, Lindgern S, Bohlin AB, Albert J, Fenyo EM. Trophoblastic epithelial barrier is not infected in full term placentae of human immunodeficiency virus-seropositive mothers undergoing antiretroviral therapy. J Virol. 1999; 73 (11):9673–8.

47. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Introduction, page no. xxiv.

48. McCracken S, Layton JE, Shorter SC, Starkey PM, Barlow DH, Mardon HJ. Expression of Granulocyte-colony stimulating factor and its receptor is regulated during the development of the human placenta. J Endocrinol. 1996; 149:249–58.

49. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Introduction, page no. xxvii.

50. Prof. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy specific biological substances, Springer London Dordrecht Heidelberg New York, Springer-

Verlag London Limited 2011. Chapter 17: Placental umbilical cord blood transfusion for stem cell therapy in Neurological Diseases. Page no 172-173.

51. Imura K, Kawahara H, Kitayama Y, et al. Usefulness of cord-blood harvesting for autologus transfusion in surgical newborns with antenatal diagnosis of congenital anomalies. J Pediatr Surg. 2001; 36:851-854.

52. Taguchi T, Suita S, Nakamura M, et al. The efficacy of autologous cord-blood transfusions in neonatal surgical patients. J Pediatr Surg. 2003;38(4):604-607Prof.

53. Hosono S, Mugishima H, Nakano Y, et al. Autologous cord blood transfusion in an infant with a huge sacrococcygeal teratoma. J Perinat Med. 2004;32(2):187-189.

54. Prof. Niranjan Bhattacharya, Prof. Phillip Stubblefield: Editors, Frontiers of cord blood Science, Springer; 2009 edition (5 March 2009), Chapter no.8: Clinical experience of cord blood Autologus transfusion: Shigeharu Hosono, page numbers: 75-81.

55. Niranjan Bhattacharya and Prof. Phillip Stubblefield: Editors, Regenerative Medicine using Pregnancy specific biological substances, Springer London Dordrecht Heidelberg New York, Springer-Verlag London Limited 2011.Chapter no.16 , Possibilities of Using Cord Blood for Improving the Biocompatibility of Implants. K.Kaladhar and Chandra P.Sharma.page: 319-321.

56. Indumathi Somasundaram EditorStem Cell Therapy for Organ Failure, Chapter No: 12, Placental Umbilical cord whole blood and nuclear disaster management: A Possibility, Niranjan Bhattacharya and Sanjukta Bhattacharya, page nos. 167-170. Springer India ,Edition no: 1, 2014,. ISBN 978-81-322-2109-8.

57. H Nagayama, K Misawa , H Tanaka, J Ooi , T Iseki , A Tojo , K Tani , Y Yamada , H Kodo , TA Takahashi , N Yamashita , S Shimazaki and S Asano (February 2002). "Transient hematopoietic stem cell rescue using umbilical cord blood for a lethally irradiated nuclear accident victim" (PDF). Bone Marrow Transplantation **29** (3): 197–204.