Placental umbilical cord blood transfusion: A novel method of treatment of patients with malaria in the background of anemia

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Summary

Malaria is an annual killer of over one million people globally and its essential co-morbidity is anemia. Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and WBC counts, hypo-antigenic nature, altered metabolic profile, high affinity for oxygen and the antimalarial effect of cord blood, is an ideal choice in case of malaria with anemia necessitating blood transfusion.

This article presents our experience with 94 units of placental umbilical cord whole blood (52 ml-143 ml, mean 81 ml ± 6.6 ml SD, median 82 ml, mean packed cell volume 48.9 ± 4.1 SD, mean percent hemoglobin concentration 16.4 g/dl ± 1.6 g/dl SD; after collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection). It was collected after lower uterine cesarean section (LUCS) from consenting mothers (from 1 April 1999 to April 2005) and transfused to 39 informed, consenting patients (24 males + 15 females, aged 8-72 yrs, mean 39.4 yrs). Twenty-two patients were infected with Plasmodium falciparum and 17 had plasmodium vivax infection. For inclusion in this study, the patient's percent plasma hemoglobin had to be 8 g/dl or less (the pretransfusion hemoglobin in malaria-infected patients in this series varied from 5.4 g/dl to 7.9 g/dl).

The rise of hemoglobin within 72 hours after two units of freshly collected cord blood transfusion was .5 g/dl to 1.6 g/dl. Each patient received two to six units of freshly collected cord blood transfusion (two units at a time), depending on availability and compatibility. No clinical, immunological or non-immunological reactions have been encountered so far.

Key words: Placental umbilical cord blood transfusion; Safe; Malaria.

Introduction

Malaria, caused by infection with *Plasmodium falciparum*, kills over one million people a year [1]. Anemia due to malaria is a major health problem in endemic areas for young children and pregnant women. This anemia is caused by excess removal of nonparasitized erythrocytes in addition to immune destruction of parasitized red cells, and impaired compensation for this loss by bone marrow dysfunction. The pathogenesis is complex and a predominant mechanism has not been identified. Certain parasite and host characteristics may modify the anemia. Concomitant infections and nutritional deficiencies also contribute to anemia and may interact with the malarial infection. Though Plasmodium falciparum is the predominant cause of anemia and its complications, Plasmodium Vivax can also cause anemia and thrombocytopenia requiring hospitalization.

In order to combat anemia, there are options of concentrated fresh red blood cell (RBC) transfusion or erythropoietin injection, or blood substitutes (oxygen carriers like perflurocarbon compounds, etc.), apart from dietary supplementation of hematinics along with other essential nutrient support needed for proper erythropoiesis.

The real problem lies in the availability of proper screened blood at the nano or molecular level which is a difficult task in major areas of the developing world, apart from the cost and complications of erythropoietin therapy, which has fueled the continued search for an ideal blood substitute.

The placenta is a readily available source of fresh whole blood, if collected aseptically after lower uterine cesarean section (LUCS) and passed through the standard adult blood screening procedure. This placental blood is rich in fetal hemoglobin and has the potential to carry more oxygen than adult hemoglobin to the tissue vol/vol, because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term. The formidable placental barrier is one of the finest biological barriers which protects the baby from infection till term.

This placenta or afterbirth is discarded routinely everywhere, and is actually a cause of environmental pollution in many parts of the developing world (in India alone, there are more than 20 million placentas produced as afterbirth every year) because it attracts natural scavengers and spreads infection unless aseptically treated or incinerated. Centers of excellence in the Western developed world have been doing research on the use of a tiny microscopic fraction of cord blood, i.e., CD 34 stem cells

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only (.01% of the nucleated cells of placental blood). My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology [2, 3].

Whether fetal hemoglobin rich placental umbilical cord whole blood, with its various unique features, can be an emergency and safe substitute for adult whole blood in cases of malaria victims with a percent hemoglobin concentration of less than 8 g/dl, was the main idea behind the present study.

Material and Methods

Ninety-four units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine cesarean section under general or regional anesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kg, or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14 ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4°C, after putting the necessary identification markings. Another sample of cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, and malaria as per standard blood transfusion protocol, which has been reported earlier [4]. The collected cord whole blood was transfused within three days of collection to a malaria victim with anemia after grouping, cross-matching and following the standard adult blood transfusion WHO guidelines for transfusion, strictly adhering to the institutional ethical committee guidelines and the patient consent protocol.

Result and Analysis

We investigated 39 patients (24 males and 15 females, aged 8-72 yrs, mean 39.4 yrs). Twenty-two patients were infected with Plasmodium falciparum and 17 had plasmodium vivax infection. All of them were admitted in medical wards with anemia in the background of confirmed Plasmodium infection. Those with acute blood loss were excluded from the study. Patients were investigated for possible parasitic, bacterial, mycobacterial and nutritional causes of anemia, including bone marrow aspiration, to identify potentially treatable causes. Treating malaria in a free government hospital in Calcutta (India) may be deceptive because of the overlapping of nutritional deficiencies and various infections and infestations in the background of neglect and socioeconomic deprivation. Our experience suggested that presentation of malaria might be tricky from the very beginning in many cases of falciparum infection. The progression of anemia and at times jaundice often correlates with the parasitic load on the host.

Organophilic sequestration of the mature parasite may have a role in the clinical presentation. Only the mature form of the parasite, i.e, schizonts, have the capability to sequester and cause eventual organopathy [5]. The immature forms are generally less sensitive to conventional antimalarials. Again, if there is a predominance of mature forms of falciparum parasites in the peripheral blood, this may be the reflection of a greater sequestered biomass, justifying the possibility of a severe disease [6]. This phenomenon of sequestration is generally absent in benign forms of the disease (P. vivax, P. ovale, P. malariae). Whether this sequestration is due to increased adhesibility of cytoadherent capillaries or loss of vascular tone by the RBC destruction *vis-à-vis* interaction with NO_2, or because of prostaglandin or specific cytokine mediated phenomenon, is under intense speculation.

All cases of falciparum malaria after diagnoses (thick/thin smear parasitic load, immunochromatographic assessment of Falciparum antigen) are admitted to the hospital and observed for 72 hours or longer as a routine. A more aggressive posture in pediatric and geriatric patients is taken, even if there are no features of unconsciousness, jaundice, breathing difficulty or severe anemia at the time of presentation. Even in cases of adults with falciparum infection, a thorough study of the falciparum parasitic load must precede other coexisting disease(s) like diabetes, chronic obstructive pulmonary disease (COPD), arthritis, hepatitis, hypothyroid, ischemic heart disease or any other pre-existing organopathy or diseases necessitating prolonged steroid therapy, which may confuse, complicate and contribute to increased morbidity and mortality in Falciparum malaria.

In the state government hospitals in Calcutta (India), mostly poor patients with limited resources are treated. As we are cognizant of the potential of cord blood, its rich mix of fetal and adult hemoglobin, high platelet and WBC counts, its hypoantigenic nature and altered metabolic profile, high oxygen affinity and the possible antimalarial effect of cord blood, we collected placental umbilical cord whole blood after LUCS from consenting mothers and transfused it to malaria patients with anemia (after collection, the blood was immediately transfused to a screened and consented volunteer waiting for the cord blood transfusion. Sometimes, very rarely, the collected blood is kept in the refrigerator and transfused as early as possible within 72 hours of collection). Ninety-four units of cord blood (52 ml-143 ml, mean 81 ml ± 6.6 ml SD, median 82 ml, mean packed cell volume 48.9 ± 4.1 SD, and a mean hemoglobin concentration of 16.4 g/dl ± 1.6 g/dl SD) were transfused to 39 informed, consenting patients with a percent of plasma hemoglobin of 8 g/dl or less from 1 April 1999 to April 2005. We followed the standard safe transfusion protocol as per the WHO guidelines, and transfused two units at a time to individual patients. The maximum amount a recipient

received was six units of placental blood. The amount of cord blood transfusion depended on the severity of the anemia and availability of compatible and screened cord blood.

The pretransfusion hemoglobin in the malaria-infected patients in this study varied from 5.4 g/dl to 7.9 g/dl. The rise of hemoglobin as estimated 72 hours after two units of cord blood transfusion was .5 g/dl to 1.6 g/dl. There was also an improvement in appetite and a sense of well being in all the cord blood transfusion recipients.

No immunological or non-immunological reactions have been encountered so far. Immediate reactions due to transfusion, viz, fever, chill and rigor, flank pain, back pain, blood in urine, fainting or dizziness, were also not noted in any of the cases. Even late reactions like mild or progressive renal complications were not encountered. Fetomaternal cell traffic has been implicated for the cause of scleroderma in mothers in cases of male babies. In the present series, we did not see any such rare or unusual complication due to neonatal blood transfusion in the adult system.

Discussion

Anemia in malaria may be due to the acute impact of hemolysis or it can have a chronic form of presentation as a result of drugs, diet and socioeconomic status, and its impact on nutrition, subclinical presentation, or because of the immune or carrier state of the disease. In cases of anemia in chronic disease there is an acute or chronic immune activation of a specific cytokine system which helps in shifting the iron from its normal route. The condition has also been termed as "anemia of inflammation" [7]. This condition is immune driven; the cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells – all of which contribute to the pathogenesis of anemia. Erythropoiesis can be affected by disease underlying anemia in chronic disease. It can also be due to proinflammatory cytokines or free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., cobalamin and folic acid), hypersplenism and hemolysis may also contribute to the anemic process affecting diseases like malaria in different presentations. Recent studies have given us some clues on the cause and perpetuation of anemia in malaria and other chronic diseases.

Hepcidin, an iron-regulated acute-phase protein composed of 25 amino acids, has helped to shed light on the relationship of immune response to iron homeostasis and anemia in chronic disease. Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α. Transgenic or constitutive overexpression of hepcidin results in severe iron-deficiency anemia in mice [8].

Blood transfusion is an option to tackle severe life threatening anemia. Another option to tackle anemia is to inject erythropoietin, provided there is no dearth of iron or B12 stores. However, there is little data currently available on the possible effects of erythropoietin on the course of the underlying disease, particularly since erythropoietin can exert additional biologic effects, including interference with the signal-transduction cascade of cytokines [9].

Considering the developing countries' perspective of the health infrastructure as a whole including its poorly trained manpower facilities, inadequate screening and cost incurred for adequate screening of blood for transfusion at the nano level, we tried to solve our own problem through our own resources. In India alone, more than 20 million registered births take place every year. The placenta is a rich source of cord blood (at term there is up to 150 ml of blood in the placental circulation). It also has a unique microenvironment and its sensitization impact on cord blood cells may have a role in umbilical cord blood (UCB) transplantation. Besides intrinsic differences, the hematopoietic stem cell (HSC) sources in UCB cells have had a different set of microenvironmental exposures compared to those of adult marrow or peripheral blood stem (PBS) cells. All HSC sources are influenced by the microenvironment from which they are derived. An example of differences between sources are some of the observed changes in HSC cycle status, gene expression and adhesive and invasive properties induced by mobilization procedures used to generate PBS cells, e.g., granulocyte colony stimulating factor (G-CSF). The placenta is a complex organ that regulates fetomaternal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., G-CSF, c-kit ligand (stem cell factor [SCF]), granulocyte macrophage colony stimulating factor (GM-CSF), Interleukin 15 (IL-15), and others are produced by the placenta. Production of G-CSF by the placenta may be especially relevant in UCB transfusion. G-CSF is produced both by the maternal decidua and the fetal chorionic villi and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation. An experiment has demonstrated that the administration of recombinant human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses [10]. The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each others allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogenic targets including choriocarcinoma cells. Freshly collected cord blood, rich in hemoglobin and growth factors, may have a positive impact on anemia in chronic disease. In sum, though precisely not clear as yet, the functional hypoantigenecity of the cord blood antigen with its complex cytokine interaction may have a positive effect on the correction of anemia in malaria apart from other factors like the role of drugs, parasitic load, nutrition or helminthiasis, or other associated features like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient. All the patients irrespective of their background tolerated the procedure well and there was a sense of well being in most of the cases as mentioned earlier.

Our group of researchers is working on the problem of fetal cell or tissue transplantation in the adult system. We have also been working on the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatric to the geriatric age group for different indications since April 1999. We received a research grant from the Department of Science and Technology of the Govt. of West Bengal, India, and have published several reports [11-16]. We are of the opinion that the growth factor and cytokine filled cord blood collected aseptically from the placenta of consenting mothers, after the birth of a healthy baby, has all the potential to be an effective therapeutic adjuvant for malaria patients with anemia in the underprivileged world. Cord blood is protected from infection as a result of nature's finest biological sieve, i.e., the placenta, and contains 60-80% fetal hemoglobin (which can carry 60% more hemoglobin than adult hemoglobin). Moreover, it has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential, due to its rich cytokine and growth factor content, to play a role in immune response modification in chronic anemia (which we are presently studying).

Conclusion

Severe childhood malarial anemia is commonly treated with blood transfusions. Although transfusion may decrease short-term mortality, the risk of HIV transmission is considerable in Africa and many parts of Asia. Transfusion-associated AIDS accounts for 10% of all cases of AIDS in Africa. The risk of HIV-1 contamination in transfusions continues to exist, even in countries where blood products are screened, because of limitations in test sensitivity, human error, and the window period. Furthermore, 30 African countries do not screen all of their blood products because of resource limitations. Decision analysis should be used to compare survival outcomes of severely anemic patients who receive transfusions against those who do not. Results indicate from African studies that when 5% of the blood supply is HIV-1 contaminated, everyone with a 6.6% or more risk of dying from anemia should be transfused [17]. In this context, it should be noted that the placenta is an unique and formidable biological barrier which protects the conceptus till term. There are many substances like P-glycoprotein, which form a functional barrier between maternal and fetal blood circulation in the placenta, thus protecting the fetus from exposure [18]. Even HIV cannot cross this barrier easily. However, at or near term there is an increase in the fetomaternal bi-directional traffic as some cells may have access to the maternal circulation depending on the viral load pathogenecity of the virus, the maternal immune condition and many more hitherto identified and non identified factors. One investigator group has suggested that the trophoblastic barrier remains uninfected in full-term placentae of HIV-seropositive mothers undergoing antiretroviral therapy. They suggested that in utero HIV transmission, if at all, occurs at the end of gestation through alternative routes, such as chorioamnionitis with leakage of the virus into the amniotic cavity or trophoblast damage [19].

We are of the opinion that the medical fraternity, particularly in the under-resourced world, should use the biological advantage provided by placental cord blood to treat patients infected with malaria contributing to anemia.

In the developed world, umbilical cord blood is now accepted as an alternative source for hematopoietic stem cells for transplantation, especially in children, due to its many practical advantages. It is an alternative source of stem cells and is easil available. Collection of this blood is without any risk for mothers. There is less possibility of infection, particularly cytomegalovirus; reduced risk of GVHD, with easy HLA-matching criteria for donor-recipient selection and UCB banks have been established for related and unrelated UCB transfusions with about 100,000 units currently available [20].

However, these centers use only .01% of the cord blood, i.e., the stem cells only, and discard 99.99%. This rejected blood can improve hemoglobin concentrations of a 7.2 kg child by an amount (30 g/l) equivalent to 10 ml/kg of packed cells. The high oxygen affinity and anti-malarial effect of fetal hemoglobin could increase the value of cord blood for transfusion in malaria-associated anemia [21].

In this preliminary communication of our work with cord blood in malaria patients whom we have followed-up for six years, we have seen that properly screened freshly collected cord blood transfusion is safe in malaria patients from the pediatric to the geriatric age group. It has improved the hemoglobin content of all the patients. This may perhaps be due to the transfusion of the cord blood itself, which as noted is rich in fetal hemoglobin, or the cytokine or growth factor impact of the recipient's bone marrow, which may have antagonized the chronic or inflammatory anemia. The transfusion of hypoantigenic cord blood also did not trigger any immunological or non immunological reaction. This study suggests that the rise in hemoglobin in cases of transfusion of freshly collected umbilical cord blood to a host with severe anemia, could be the result of a positive overall impact in the host's bone marrow due to the rich cytokine and growth factor content of the cord blood, apart from the transfer of cord blood hemoglobin.

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References

[1] Severe falciparum malaria. World Health Organization. Communicable Diseases Cluster. *Trans. R. Soc. Trop. Med. Hyg.,* 2000, 94 (suppl. 1), S1.

[2] Bhattacharya N., Mukherjee K.L., Chettri M.K. *et al.*: "A study report of 174 units of placental umbilical cord whole blood transfusion in 62 patients as a rich source of fetal hemoglobin supply in different indications of blood transfusion". *Clin. Exp. Obstet. Gynecol.,* 2001, *28*, 47.
[3] Bhattacharya N.: "Placental umbilical cord whole blood transfusion". *J. Am. Coll. Surg.,* 2004, *1992*, 347.
[4] 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,* 557.
[5] Silamut K., Phu N.H., Whytti C. *et al.*: "A quantitative analysis of microvascular sequestration of malaria parasite in human brain". *Am. J. Pathol.,* 1999, *155,* 395.
[6] Silamut K., White N.J.: "Relationship of the stage of parasite development in the peripheral blood to the prognosis in severe Falciparum malaria". *Trans. R. Soc. Trop. Med. Hyg.,* 1993, *87,* 436.
[7] Weiss G.: "Pathogenesis and treatment of anaemia of chronic disease". *Blood Rev.*, 2002, 16, 87.
[8] Nemeth E., Rivera S., Gabayan V. *et al.*: "IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin". *J. Clin. Invest.,* 2004, *113*, 1271.
[9] Nicolas G., Bennoun M., Porteu A. *et al.*: "Severe iron deficiency anemia in transgenic mice expressing liver hepcidin". *Proc. Natl. Acad. Sci U S A,* 2002, *99*, 4596.
[10] Jelkmann W.: "Proinflammatory cytokines lowering erythropoietin production". *J. Interferon Cytokine Res.,* 1998, *18,* 555.
[11] McCracken S., Layton J.E., Shorter S.C., Starkey P.M., Barlow D.H., Mardon H.J.: "Expression of granulocyte-colony stimulating factor and its receptor is regulated during the development of the human placenta". *J. Endocrinol.,* 1996, *149*, 249.
[11] Bhattacharya N., Chhetri M.K., Mukherjee K.L. *et al.*: "Can human fetal cortical brain tissue transplant (up to 20 weeks) sustain its metabolic and oxygen requirements in a heterotrophic site outside brain? A study of 12 volunteers with Parkinson's disease". *Clin. Exp. Obstet. Gynecol.*, 2002, *29,* 256.
[12] Bhattacharya N., Chhetri M.K., Mukherjee K.L. *et al.*: "Human fetal adrenal transplant (A possible role in relieving intractable pain in advanced rheumatoid arthritis)". *Clin. Exp. Obstet. Gynecol.*, 2004, *3*, 167.
[13] Bhattacharya N., Mukherjee K.L., Chettri M.K. et al.: "A unique experience with human pre-immune (12 weeks) and hypo-immune (16 weeks) fetal thymus transplant in a vascular subcutaneous axillary fold in patients with advanced cancer (A report of two cases)". *Eur. J. Gynecol. Oncol.,* 2001, *22,* 273.
[14] Bhattacharya N.: "Fetal tissue/organ transplant in HLA randomized adult's vascular subcutaneous axillary fold (A preliminary report of 14 patients)". *Clin. Exp. Obstet. Gynecol.,* 2001, *28*, 233.
[15] Bhattacharya N.: "Fetal cell/tissue therapy in adult disease - A new horizon in regenerative medicine". *Clin. Exp. Obstet. Gynecol.,* 2004, *31,* 167.
[16] Bhattacharya N.: "Placental umbilical cord blood transfusion in transfusion-dependent beta thalassemic patients: a preliminary communication". *Clin. Exp. Obstet. Gynecol.,* 2005, *32,* 102.
[17] Heymann S.J., Brewer T.F.: "The problem of transfusion-associated acquired immunodeficiency syndrome in Africa: a quantitative approach". *Am. J. Infect. Control,* 1992, *20*, 256.
[18] Molsa M., Heikkinen T., Hakkola J., 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,* 118.
[19] Tscherning-Casper C., Papadogiannakis N., Anvret M., Stolpe L., Lindgren S., Bohlin A.B., Albert J., Fenyö E.M.: "The trophoblastic epithelial barrier is not infected in full-term placentae of human immunodeficiency virus-seropositive mothers undergoing antiretroviral therapy". *J. Virol.,* 1999, *73*, 9673.
[20] Chao N.J., Emerson S.G., Weinberg K.I.: "Stem cell transplantation (cord blood transplants)". *Hematology,* 2004 (Am. Soc. Hematol. Educ. Program), 354.
[21] 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,* 678.

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