# Placental umbilical cord blood transfusion: A new method of treatment of patients with diabetes and microalbuminuria in the background of anemia

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**Summary**

Diabetes mellitus is the commonest endocrine disease in all populations and all age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion or impaired insulin action, or both. Anemia is a common accompaniment of diabetes, particularly in those with albuminuria justifying tubulointestitial injury or reduced renal function. There are other additional factors present in diabetes, which may contribute to the development of an increased risk of anemia.

Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and WBC counts, hypo-antigenic nature, altered metabolic profile and high affinity for oxygen, may be an ideal choice for cases of diabetes with severe anemia necessitating blood transfusion.

This article presents my team's experience with 78 units of placental umbilical cord whole blood (from 1 April 1999 to April 2005), collected after lower uterine cesarean section (LUCS) from consenting mothers (56 ml - 138, ml mean 82 ml ± 5.6 ml SD, median 84 ml, mean packed cell volume 49.7 ± 4.2 SD, mean percent hemoglobin concentration 16.6 g/dl ± 1.5 g/dl SD) and transfused to diabetes patients with microalbuminuria and severe anemia necessitating transfusion. After collection, the blood was transfused, in most cases immediately after completion of the essential norms of transfusion. In rare cases, it was kept in the refrigerator and transfused within 72 hours of collection to a suitable recipient.

For inclusion in this study, the patient's percent plasma hemoglobin had to be 8 g/dl or less (the pretransfusion hemoglobin in this series varied from 5.2 g/dl to 7.8 g/dl) in the background of type two diabetes (fasting sugar 200 mg or more), along with features of microalbuminuria (albumin excretion 30-299 mg/g creatinine). This study included 39 informed consenting patients (22 males + 17 females, aged 48-74 yrs, mean 59.6 yrs). The patients were randomized into two groups: Group A (control cases N = 15, males = 8 and females = 7) and Group B (study group N = 24, males = 14 and females = 10).

In Group A the rise of hemoglobin (Hgb) after two units of adult blood transfusion was 1.5 to 1.8 g/dl, as seen after a 72-hour blood sample assessment. The rise of Hgb as noted after 72 hours of two units of freshly collected cord blood transfusion was .6 g/dl to 1.5 g/dl. Each patient received two of four units of freshly collected cord blood transfusion (two units at a time), depending on availability and compatibility. Microalbuminuria was assessed in both groups after one month of treatment with transfusion and other identical support. The mean result was 152 ± 18 m SD of albumin per gram of creatinine excreted through 24-hour urine (pretransfusion mean excretion was 189 ± 16 mg) in Group A and 103 ± 16 mg SD of albumin excretion per gram of creatinine in 24-hour excretion of urine in Group B (pretransfusion mean excretion was 193 ± 21 mg). Univariate analysis using Fisher's exact test was performed for the results of Groups A and B. The difference between Group A and B values and its comparison with the pretransfusion microalbuminuria appeared to be statistically significant ($p <$ less than .003).

We have not encountered any clinical, immunological or non-immunological reaction so far in either group. Fetomaternal cell traffic has been implicated as the cause of scleroderma in mothers delivering male babies. In the present series, we did not see any such rare and unusual complication due to neonatal blood transfusion in the adult system in Group B patients in the six years from the initiation of the study.

*Key words:* Safe; Placental umbilical cord blood transfusion; Diabetic patients with anemia and microalbuminuria.

## Introduction

Anemia is a common accompaniment of diabetes, particularly in patients with albuminuria or reduced renal function. The estimated prevalence of anemia depends on essentially arbitrary criteria used to define the presence or absence of anemia. Anemia in the background of chronic disease is mostly immune driven, where cytokines and cells of the reticuloendothelial system participate in altering iron homeostasis. They also prevent adequate proliferation of erythroid progenitor cells, dysregulation of erythropoietin (EPO) production and sensitivity, leaving aside the problem of the altered life span of red cells [1]. When a patient with a chronic metabolic disease like diabetes presents with anemia, many factors have been suggested as the reason for the onset of anemia. A lower Hgb count is significantly associated with a more rapid decline in the glomerular filtration rate (GFR) [2]. Furthermore, treating anemia early in renal failure has been demonstrated to slow the rate of decline in renal function [3]. One of the most potent causes of suboptimal response to EPO is chronic and overt inflammation, associated with an increased production of cytokines, such as tumor necrosis factor-α, interleukin-1, or interferon-β [4], which might suppress erythrocyte stem cell proliferation [5]. Anemia also has a negative impact on patient survival, and is considered to be an important cardiovascular risk factor associated with renal disease. It appears more likely that

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proteinuria is a marker of tubulointerstitial injury in diabetes [6], perhaps more so than in non-diabetic conditions associated with proteinuria, which is considered to be primarily glomerular in origin. It has been suggested that the widespread use of ACE inhibitors may contribute to anemia in patients with diabetes [7]. Excretion of growth factors in the urine has been implicated in the pathogenesis of tubulointerstitial disease, which characterizes proteinuric renal disease. Understanding the pathogenesis of anemia associated with diabetes and nephropathy may therefore lead to opportunities for developing interventions to optimize outcomes in these patients.

In order to combat severe anemia (Hgb 8 g/dl or less), there are several options: concentrated fresh 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 properly screened blood at the nano or molecular level. This is a difficult task even in most areas of the developing world. Apart from this, the cost and complications of erythropoietin therapy have fuelled the continued search for an ideal blood substitute.

The placenta is a readily available source of fresh whole blood. It can be collected aseptically after lower uterine caesarean section (LUCS) and tested according to 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 after the birth of a healthy newborn at or near term because of its fetal hemoglobin component. The formidable placental barrier is one of the finest biological barricades that protects the baby from infection till term.

This placenta, or the 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. 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 [8, 9]. Whether fetal hemoglobin rich placental umbilical cord whole blood with its various unique features could be an emergency and safe substitute for adult whole blood in cases of diabetes with anemia, with percent hemoglobin concentration of less than 8 g/dl, was the main idea behind the present study.

## Material and Methods

Thirty-nine informed, consenting patients (22 males + 17 females, aged 48-74 yrs, mean 59.6 yrs) were included in the study. The patients were randomized into two groups: Group A (control cases N = 15, males = 8 and females = 7) and Group B (study group N = 24, males = 14 and females = 10). Group A (N = 15) was treated according to the standard regime of act-rapid insulin, ace-inhibitor, combating dyslipidemia, hyperuricosuria, and fresh adult RBC transfusion (2-4 units each) depending on availability. Group B (N = 24) patients were treated with an identical regime but freshly collected cord blood was transfused instead of adult blood (2-4 units each) depending on availability after cross-matching and fulfilling other essential criteria.

Type 2 diabetes patients who were enrolled in the present study were clinically examined and standard indices were recorded from the tested blood, including creatinine, urea, albumin, fasting blood glucose, fasting lipid profile, HbA1c, C-reactive protein and ferritin. Urinary creatinine, urea, albumin, and protein obtained from a 24-hour collection were also recorded. The medical records of these patients showed no evidence of advanced diabetic nephropathy (creatinine clearance ≥ 30 mg/kg/1.7 $m^2$).

For inclusion in the study, the patient's percent of plasma hemoglobin had to be 8 g or less (the pretransfusion hemoglobin in this series varied from 5.2 g/dl to 7.8 g/dl) in the background of Type 2 diabetes (fasting sugar 200 mg or more) along with features of microalbuminuria (albumin excretion 30-299 mg/g creatinine).

Seventy-eight units of human placental umbilical cord blood were collected from consenting mothers aseptically after LUCS under general or regional anesthesia. The collected blood volume varied from 56 ml - 138 ml, mean 82 ml ± 5.6 ml SD, median 84 ml, mean packed cell volume 49.7 ± 4.2 SD, mean percent hemoglobin concentration 16.6 g/dl ± 1.5 g/dl SD. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kg, or if the mother was suffering from any specific disease like hepatitis or HIV, etc., the cord blood collection was abandoned. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4°C, after putting necessary identification markings. Another sample of the 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, on which we have reported earlier [10, 11]. The collected cord whole blood was transfused as early as possible (at the latest, 72 hours after collection), to a diabetic patient with anemia in Group B, after grouping, cross- matching and following the standard adult blood transfusion protocol. There was strict adherence to the institutional ethical committee guidelines and the patient consent protocol in all cases.

## Result and Analysis

Anemia is an important component of diabetic nephropathy. As mentioned earlier, anemia in diabetic patients is the result of diminished erythropoietin production and, to a lesser degree, of increased excretion of erythropoietin in the urine, whereas erythropoietin responsiveness remains unchanged initially. What can be done to treat under-resourced patients with anemia and diabetes who cannot afford to buy erythropoietin or even arrange for fresh whole blood or a RBC component for transfusion?

All the patients in the present study protocol were admitted with anemia in the background of confirmed Type 2 diabetes with microalbuminuria. Those with acute blood loss were excluded from the study. Patients were investigated for possible parasitic, bacterial, mycobacterial and nutritional reasons for their anemic condition, and the studies included bone marrow aspiration (if necessary) to identify potentially treatable causes. Diagnoses of the etiopathogenesis of anemia in a free government hospital in Calcutta (India) may be deceptive because of

the overlapping of nutritional deficiencies and various subclinical infections and infestations in the background of neglect and socioeconomic deprivation. In the state government hospitals in Calcutta, we treat mostly poor patients with limited resources.

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, as well as its high oxygen affinity – placental umbilical cord whole blood after LUCS was collected from consenting mothers and transfused to diabetic patients with anemia. Two units of cord blood were transfused at a time to each patient. The recipient who got the maximum amount received four units of placental cord blood. The amount of transfusion depended on the severity of anemia and the availability of compatible and screened cord blood.

The data showed that in Group A there was an increase in the Hgb count after two units of adult blood transfusion – from 1.5 to 1.8 g/dl. The rise of hemoglobin, as estimated after 72 hours in Group B, after two units of cord blood transfusion, was .5 g/dl to 1.6 g/dl. There was also subjective improvement in appetite and a sense of well being in all the cord blood recipients.

Microalbuminuria was assessed in both groups after one month of treatment with transfusion and other identical support. The mean value of albumin excretion in 24- hour urine was 152 ± 18 mg SD of albumin per gram of creatinine (the pretransfusion mean was 189 ± 16 mg) in Group A and 103 ± 16 mg SD per gram of creatinine in Group B (the pretransfusion mean value was 193 ± 21 mg).

Univariate analysis using Fisher's exact test was performed for the results of Group A and Goup B. The differences in Group A and B values of microalbuminuria appeared to be statistically significant ($p <$ less than .003).

Immediate reactions due to transfusion, i.e., fever, chill, rigor, flank pain, back pain, blood in urine, fainting or dizziness, were also not seen in any of the cases. Even late reactions like mild or progressive renal complications were not encountered in any case in either group.

Fetomaternal cell traffic has been implicated as the cause of scleroderma in mothers delivering 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 in Group B patients.

## Discussion

The most important cause of end-state renal disease is diabetes. It causes a decline in the production or receptor sensitivity or dysregulation of the excretion of erythropoietin. This EPO concentration may provide indirect evidence of the functioning renal status. In some cases of uncontrolled diabetes, there is unexplained anemia which could be due to low serum levels, or blunted response to erythropoietin as a result of interstitial damage, or an abnormal glycosylation of the cytokine system, dysautonomia, or presence of infection only to name a few important causes [12-23]. Anemia is very common in diabetes and potentially contributes to the pathogenesis of different diabetic complications. Independent predictors of anemia in diabetes are transferrin satuation, glomerular filtration rate, sex, albumin excretion rate, glycosylation status, etc. [24]. In case 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" [25]. This condition is immune driven. It could also be due to pro-inflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism, hemolysis, helminthiasis, and malnutrition may all contribute to the anemic process as well. Hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids has helped to shed light on the relationship of the immune response to iron homeostasis and anemia in chronic disease [26, 27].

Blood transfusion is an option to tackle severe life threatening anemia. Another option to tackle anemia is to inject EPO, provided there is no dearth of iron or B12 stores. However, there is little data currently available on the possible effects of EPO on the course of the underlying disease, particularly since it may exert additional biologic effects, including interference with the signal-transduction cascade of cytokines [28].

In the underprivileged world, many patients treated in government hospitals cannot afford to buy EPO to prevent renal deterioration and correct the anemic conditon, and in many situations they cannot even arrange for fresh whole blood or concentrated RBCs. These are the cases which can benefit from cord blood transfusion. Considering the insufficient health infrastructure in developing countries, the poorly trained manpower facilities, inadequate screening and cost of screening of blood at the nano level for transfusion, we tried to solve the 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 has a sensitization impact on cord blood cells. The placenta is a complex organ that regulates maternofetal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., granulocyte colony stimulating factor, (G-CSF), c-kit ligand (stem cell factor [SCF]), and granulocyte macrophage colony stimulating factor (GM-CSF). Interleukin 15 (IL-15), and others are produced by the placenta. 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 [29]. The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each other's allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

Freshly collected cord blood, rich in hemoglobin and growth factors, may have a positive impact on anemia in chronic disease. All the patients, irrespective of their background in our present series, tolerated the procedure well and there was a sense of well being in most of the cases, as mentioned earlier.

The exact etiopathogenesis behind the improvement of the microalbuminuria in the cord blood transfusion (Group B) is not clear, apart from the fact that freshly collected cord blood is rich in its content of many cytokine or growth factors whose individual impact is currently under study. Fetal hemoglobin rich cord blood with its altered viscosity, may have a positive impact on renal perfusion. Cord blood has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential to play a role in immune response modification in chronic anemia (which we are studying at present), due to its rich cytokine and growth factor content.

Our group of researchers is working on the problem of fetal cell or tissue transplant 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 in different indications since 1 April 1999 [30-34].

## Conclusion

Cord blood may be a safer alternative in a contemporary context. Although transfusion of adult blood may decrease short-term mortality, the risk of human immunodeficiency virus (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 [35].

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 forms a functional barrier between maternal and fetal blood circulation in the placenta, thus protecting the fetus from exposure [36]. 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 investigating 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 [37].

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 easy 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. Umbilical cord blood (UCB) banks have been established for related and unrelated UCB transplants with about 100,000 units currently available [38]. The use of cord blood can save lives in cases of severe anemia in under-resourced children of Africa [39] or any other part of the world.

However, these centers which use cord blood for transplantation purposes, use only .01% of the cord blood, i.e., the stem cells only, and discard 99.99%. In this preliminary communication of our work with cord blood in patients whom we have followed-up for six years, we have seen that properly screened freshly collected cord blood transfusion is safe in diabetic patients who are also suffering from anemia. It improved the hemoglobin content of all the patients in our study. This may perhaps be due to the transfusion of the cord blood itself, which as noted, is rich in fetal hemoglobin, or because of the cytokine or growth factor impact on the recipients' bone marrow and kidneys, which may have antagonized the chronic or inflammatory anemia and erythropoietin deficiency, or receptor sensitivity caused by tubulointerstitial injuries in diabetic patients. Proteinuria is not only a major correlate of declining renal function but may also directly lead to disease progression by contributing to tubulointerstitial injury due to the release of inflammatory and vasoactive substances into the interstitium [40]. The enhanced ultrafiltration of growth factors that occurs in proteinuric states has also been implicated as pathogenetically linked to the development of tubulointerstitial disease [41, 42]. The improvement of microalbuminuria in Group B patients may be a result of the positive effect of the pregnancy-specific growth factors and cytokine systems on the renal derangement caused by diabetes. The transfusion of hypoantigenic cord blood also did not trigger any immunological or non-immunological reaction. Hence, it appears that freshly collected cord blood transfusion is not only a transfusion of fetal hemoglobin-rich high oxygen affinity blood, but also an infusion of serum which is rich in pregnancy specific growth factors and cytokines. In diabetic patients with anemia its promise could be immense, not only in under-resourced countries where patients are cash-strapped, but also in developed countries where patients may benefit from the extra potential of cord blood.

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