

Original Research Article
Utility of placental Umbilical Cord Blood in
autoimmune and degenerative disorders

SHORT TITLE OF PAPER
Utility of placental UCB

ABSTRACT

Background: Umbilical cord blood is whole human blood (60 to 80 ml) that remains in the placenta and umbilical cord after childbirth; generally considered as a medical waste. It is a rich source of stem cells, growth factor, cytokines, etc., and, can be collected, stored and utilized in treatment of incurable diseases.

Aims and objects: The aim of the present study is to establish the fact that placental umbilical cord whole blood is a safe alternative to adult blood and to assess its utility in degenerative and autoimmune disease along with its hematological parameters.

Materials and methods: It is a prospective two year study (From September 2016 to August 2018) of 250 umbilical cord whole blood transfusions in autoimmune and degenerative disorders at Gajra Raja Medical College, Gwalior, India. Follow up of patients was done up to 3 months and data was collected and analyzed statistically by frequency distribution and percentage proportion.

Results: A total of 250 units meeting the inclusion and exclusion criteria were transfused to 99 preregistered patients; Vitiligo 61 (159 transfusions), Thalassemia 15 (30), Retinitis Pigmentosa 9 (23), Geriatric Disorders 9 (24), Aplastic anemia 4 (9) and High Myopia 1 (5). Out of 250 transfusions, in one case (0.4%) adverse event was reported. Outcome of transfusion reveals; In Vitiligo –regimentation in affected area, Thalassemia-reduction in frequency of transfusions, Retinitis

Pigmentosa- improvement in vision area, Geriatric patients- sense of well being, Aplastic anemia- prolonged survival and High Myopia-improvement in vision area.

Conclusion: Umbilical cord blood is safe and genuine alternative of adult blood. It is effective in degenerative and autoimmune diseases. It should not be discarded as medical waste and utilized judiciously in the human well being.

Key Words: Umbilical Cord Blood (UCB), Transfusion, Stem cell, Cytokines and growth factor

INTRODUCTION

The role of the blood and its components is increasing day by day in the allopathic medicine, not only because of its requirement in accidents, surgeries, and anemia cases but also it has a therapeutic value in so many diseases. Till date, there has been no complete substitute for human blood [1]. What so ever on trial as substitutes for blood are not fulfilling the desired parameters? As far as artificial blood is concerned, no truthfully safe and efficient artificial blood product is

currently marketed [2]. Here, we can say that the term “artificial blood” is really a misnomer. The complexity of blood is far too great to allow for absolute duplication in a laboratory [3]. So we are looking for the optional source of human blood; that can be umbilical cord blood (UCB) or cadaveric blood which can be saved and utilized [4]. Previously, it was a general practice that after birth placenta and its cord was discarded as a medical waste [5]. Placenta and its cord has 80 to 120 ml precious human blood, a rich source of stem cells, so it can be saved, stored and used as a replacement of adult whole human blood as well as a source of virgin stem cells [6]. Cord blood collection is done by venipuncture of an umbilical cord and blood flows by gravity into a citrate phosphate dextrose (CPD) bag. Collection of UCB is similar to the manner in which a conventional adult blood donation is collected and it is painless [7]. Human leukocyte antigen (HLA) on umbilical cord blood stem cells is under developed, so it has a better tolerance for HLA mismatching in comparison with adult hematopoietic stem cell [8]. Stem cells in UCB are 0.01% of the total cellular content, whereas, it is 0.1% and 0.001% in bone marrow and peripheral blood respectively. So here it is wise not to discard 99.9 % UCB Cells for the sake of 0.01% stem cells [9]. UCB has higher cellular content as compared to adult human blood. Hemoglobin in UCB is 20g/ cu mm as compared to 14.4g/cu mm in adult blood with 70% fetal hemoglobin, platelets are 750000 against 250000 for each cu mm of adult blood and leukocyte count of 24000 as a detriment to 6500-10,500 cells/ cu mm in adult blood. Due high fetal hemoglobin content of UCB it carries 60 % more oxygen than adult hemoglobin [10].

In 1939, Halbrecht reported his attempt to try human UCB as a blood substitute in Lancet. However, the absence of an anticoagulant like acid citrate dextrose resulted in the formation of clots [11, 12]. For the first time in 1999, researchers at different

Government Hospitals, Kolkata (India) with support and, ethical and scientific clearance from the West Bengal State Government, India, routinely started using UCB as a true transfusion substitute successfully without jeopardizing the patients' safety [13]. This was considered worldwide as a new thought to implement in transfusion medicine. In 2004 again Bhattacharya N transfused 413 units of UCB to 129 notified permitted patients of different age groups suffering from advanced cancer, systemic lupus erythematosus, ankylosing spondylitis, aplastic anemia, rheumatoid arthritis and thalassemia major. In none of the cases, the investigators encountered any case of immunologic or non-immunologic reaction in subsequent follow-up studies [14].

Umbilical cord blood is wealthy in fetal hemo-components and hence can be a perfect and elective source for both autologous and allogenic blood transfusion in new conceived babies [15, 16].

Cord RBCs are unique cells that differ from adult RBCs in membrane composition and biophysical properties [17, 18], hemoglobin structure [17-20], metabolism and enzymatic profile [21,22].

On account of a healthy neonate, UCB does not contain any obtained antibodies. Erythrocyte articulation of Antigen A and B is to extremely low and is regularly around 3% to 7%. For minor blood groups like Kelly and Luther, the antigenic articulation is nearly undetectable [23]. In this way UCB is considered safe for transfusion. Therefore, the present study is designed and carried out to discern the utility of UCB in various diseases and geriatric disorders.

MATERIALS AND METHODS

It is a prospective two year study (September 2016 to August 2018) of 250 UCB transfusions in autoimmune and degenerative disorders (i.e. mainly vitiligo,

109 aplastic anemic, thalassemia, retinitis pigmentosa and other ophthalmic
110 degenerative diseases, and geriatric patients). UCB was collected in CPD bags of
111 100 ml capacity (HLL Life-care Limited) with all aseptic precautions from normal
112 healthy deliveries conducted in Labor Room, Obstetrics and Gynecology
113 Department and transported to blood bank, J. A. Hospital Gwalior. Blood grouping
114 and Rh typing, TTIs status of blood unit and, DCT (Direct Coombs Test) and ICT
115 (Indirect Coombs Test) of blood were done in blood bank. ABO and Rh blood
116 grouping, DCT and ICT of the UCB sample was done by conventional tube
117 methods and gel technology (column agglutination; Tulip Diagnostics). TTIs test
118 of UCB units was done by Elisa Method using Elisa reader (Robonik and Elisa kit
119 of J Mitra & co. Pvt. Ltd). Along with collection of UCB, out of 250 cases, in 100
120 cases we were able to collect 3ml blood from the cord separately in EDTA vial for
121 complete hemogram. Complete hemogram was performed on 5 part hematology
122 analyzer (MINDRAY –MODEL BC-5300).

123 Blood units fit for transfusion, after cross match were transfused to pre registered
124 patients for the study. Patients were registered either on request of clinicians who
125 had an opinion that their patients will be benefited by the transfusion of UCB or to
126 those patients who were willing to have UCB transfusion as a prospective
127 treatment for their illness.

128 **Inclusion criteria:** UCB was collected only from patients who gave legal consent
129 for the procedure. UCB was collected from normal deliveries with a visibly healthy
130 placenta and fulfilling all aseptic criteria.

131 **Exclusion criteria:** UCB from diseased or anemic placenta was not collected.

132 Collected Units positive for HIV I &II, HbsAg, HCV, VDRL and MP were
133 discarded. Unit's positive for ICT and DCT were also discarded. Quantity of blood
134 collected less than 60 ml was unfit for transfusion.

Cord blood was transfused at J.A. Groups of Hospitals and at other hospitals of Gwalior under medical supervision with proper documentation. After completion of transfusion, follow-up of patients were done in accordance with the treating doctor every 15 days up to 3 months.

RESULTS

In the present study, Out of the 269 UCB collected, 19 units were found unfit and discarded, details of discarded units are depicted in Figure 1.

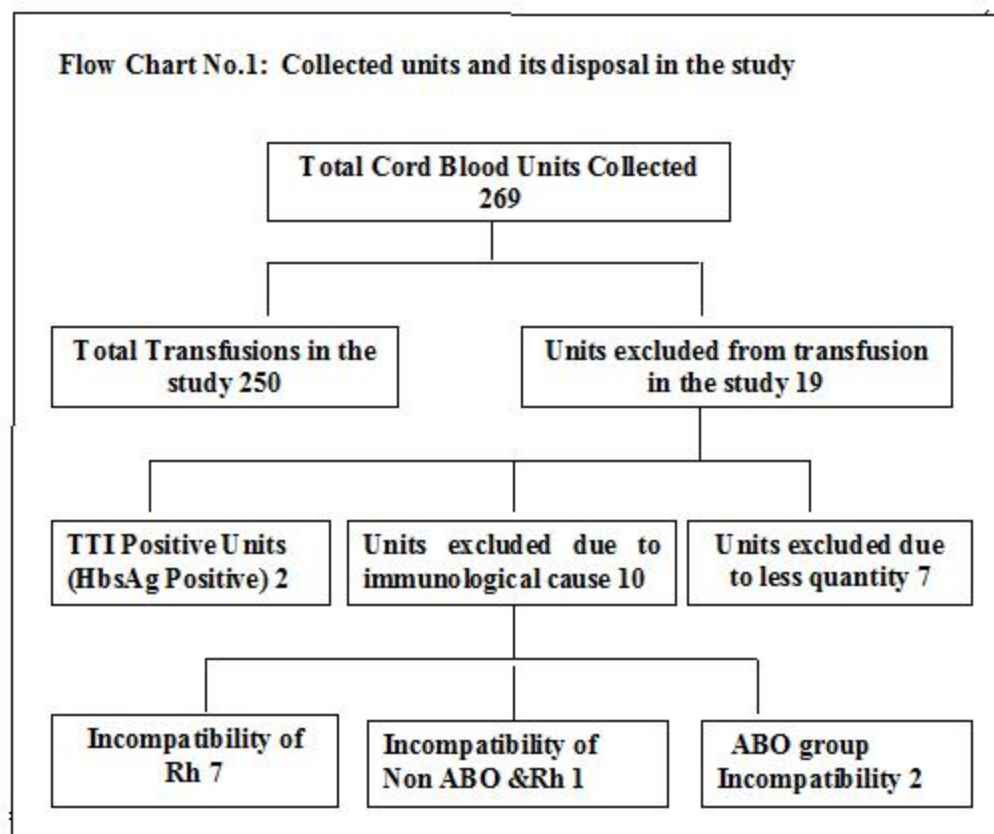


Figure 1. Flowchart depicting the collected and discarded UCB units.

ABO and Rh distribution of UCB units in the study are depicted in Figure 2.

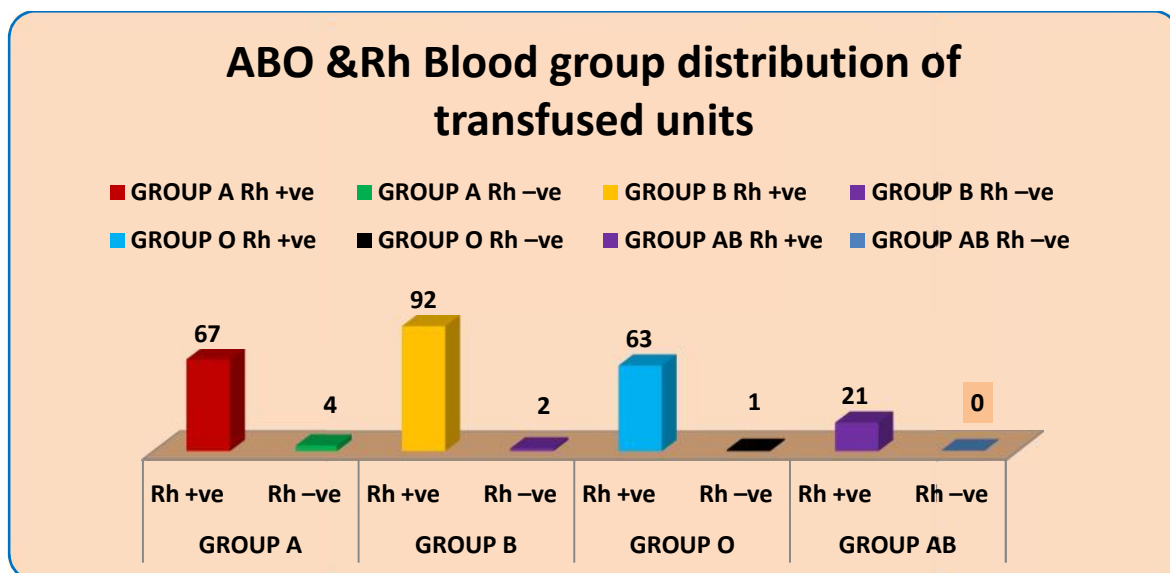


Figure 2: ABO and Rh distribution of UCB units in the study

Out of 250 UCB units accepted for transfusion, 100 randomly selected samples were subjected to hematological analysis. Findings are summarized in Table 1.

Table 1. Hematological parameters of 100 UCB units.

Parameters	Mean $\pm$ Standard Deviation	Range
WBC	$12.0 \pm 2.47 \times 10^3/\text{cu mm}$	$8-17 \times 10^3/\text{cu mm}$
RBC	$4.4 \pm 0.44 \times 10^6/\text{cu mm}$	$3.2-5.9 \times 10^6/\text{cu mm}$
Platelet	$217.0 \pm 59.25 \times 10^3/\text{cu mm}$	$120 \pm 350 \times 10^3/\text{cu mm}$
HGB	$14.20 \pm 0.79 \text{ g/dl}$	$12.63-16.8 \text{ g/dl}$
HCT (%)	$43.23 \% \pm 2.67\%$	$37-50 \%$
MCV (fl)	$98.36 \pm 4.6 \text{ fl}$	$93.4-110.2 \text{ fl}$
MCH (pg)	$38.22 \pm 1.65 \text{ pg}$	$30.2-36 \text{ pg}$
Differential WBC		
Polymorphs	$44.77 \pm 8.27 \%$	$22-60 \%$
Lymphocytes	$47.14 \pm 6.7\%$	$40-72\%$
Monocytes	$4.67 \pm 2.10 \%$	$2-10 \%$
Eosinophils	$3.14 \pm 1.84\%$	$2-10 \%$
Basophils	$1.05 \pm 1.65\%$	$0-6\%$

Quantity of blood varied from 60 to 100 ml with the mean of $70.66 \pm 12.74 \text{SD}$.

Average transfusion per patient in present study was 2.53: minimum one and maximum 5 transfusions.

In the study, total number of patients enrolled was 99 in whom 250 transfusions were given. Age of the patients range was from less than 1 year to 68 years with an average age of $28.25 \pm 20.22 \text{SD}$.

Out of 99 Patients, 52 (52.53 %) were male and 47 (47.47%) were female. ABO and Rh-D distribution of 99 patients in the present study was; A: 20, B: 34, O: 16

199 and AB: 7. Out of total 99 patients Rh-D status of patients; 95 were Rh positive
200 and 4 patients were Rh negative.

201 Transfusion adverse reaction was noted only in 1 case (0.4%). Reaction was febrile
202 non hemolytic transfusion reaction (FNHTR) which was due to platelet and
203 leukocyte content of cord blood. This was managed symptomatically.

204 Disease wise distribution of patients, number of transfusions and clinical outcome
205 is summarized in Table 2.

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220 **Table 2. Disease wise distribution of patients, number of transfusions and**
221 **clinical outcome.**

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<i>Sino.</i>	<i>Diagnosis</i>	<i>No. of Patients</i>	<i>No. of transfusions</i>	<i>Average transfusions /patient</i>	<i>Clinical Outcome</i>
1	Vitiligo	61	159	2.60	Re-pigmentation in affected area • Good response[*] - 30% • Average response[#] - 50% • No response- 20% • Initiation of re-pigmentation in old, chronic, non-responding patients
2	Thalassemia	15	30	2	Reduction in frequency of transfusion • Hemoglobin level of patients increased from 0.5 to 1 gm/dl • Interval between two transfusions increased by 11± 3 days.
3	Aplastic Anemia	4	9	2.25	Prolonged survival UCB transfusion 3 to 4 time / year in 27Y/M • Hb maintained at 8gm/dl platelet count 35-40 x 10³ / cu mm.
4	Retinitis Pigmentosa	9	23	2.55	Improvement in vision area • 20-30% with minimum two transfusions
5	Geriatric Disorders	9	24	2.66	Sense of well being and ride of common geriatric sign & symptoms
6	High Myopia	1	5	5	Improvement in vision area
* 2/3 rd re-pigmentation in affected area, # half re-pigmentation in affected area					

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227 Satisfactory recovery in patients of vitiligo is shown in following Figure 3 & 4.

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Figure 3: (A) Vitiligo mark behind the left Ear. (B) Recovery after First UCB transfusion. (C) Complete recovery after 2nd UCB transfusion



Figure 4: (A) Multiple Vitiligo mark over face. (B) Recovery after First transfusion of UCB. (C) Almost complete recovery after 2nd UCB Transfusion

DISCUSSION

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244 Allogenic UCB transfusion was first reported in the 1930s, before the advent of
245 modern blood transfusion services [11, 12]. Then, most researches and clinical
246 activities relating to UCB have limited up to autologous UCB transfusion in
247 preterm neonates and utility of UCB stem cells in bone marrow transplants.
248 Transfusion of UCB as a therapy for various diseases has not been extensively
249 studied. Use of UCB to ameliorate symptoms in malignancies, autoimmune
250 disorders, anemia, geriatric disorders and leprosy have been reported in few
251 studies, and did not reveal any adverse event due to UCB transfusion [23,
252 24,25,26,27,28].

253 Status of UCB as a safe alternative to whole human adult blood is further
254 strengthened by the results of our study, wherein 0.4% (only one case) of
255 FNHTR was reported, a most commonly encountered adverse event of blood
256 transfusion.

257 Variability has been observed in reporting the hematological parameters of UCB in
258 various studies. Agrawal N in her study of “Transfusion of Placental Umbilical
259 Cord Whole Blood (Rich in Stem Cells) in Transfusion Dependent Patients and to
260 Assess Its Outcome” reported hemoglobin of cord blood varying from 16.2 to 20.2
261 g/dl against the average adult hemoglobin 14.4 g/dl with the average 72 gm % of
262 fetal hemoglobin [4]. Bhattacharya N reported mean hemoglobin concentration
263 18- 12.4 g/dL and 18 - 14.8 g/dl in his two different studies [13, 29]. Noguera NI
264 et al [30] reported hemoglobin levels of 14.4 - 16.6 g/dl. In our study, hemoglobin
265 range was found to be 12.63-16.8 g/dl.

266 Despite the comparable values of hemoglobin in UCB with adult whole blood in
267 our study, it has been found to be effective in thalassemia patients because of
268 presence of fetal hemoglobin in UCB. Of the 15 thalassemic patients in our study,

receiving an average of 2 transfusions/ patient, the hemoglobin level increased from 0.5 to 1.0 gm/dl. Hemoglobin estimation was repeated 15 days after transfusion where there was marked secondary rise in the hemoglobin concentration which may be due to cytokine stimulation effect of cord blood on the host bone marrow. In a similar study of Bhattacharya N on thalassemic patients there was an increase in hemoglobin from 0.6 to 1.2 gm/dl and secondary rise in hemoglobin seen after 7 days of transfusion [31]. Due to higher O₂ carrying capacity of umbilical cord whole blood, less number of transfusions were needed in our patients. This might be helpful in reducing iron overload and preventing heart failure in these chronic transfusion dependent patients.

In the patient group of aplastic anemia (total case- 4) in our study, follow up could be done of 1 case only. The said patient received 4 transfusions of UCB, has a satisfactory recovery from the disease and still in our treatment since 2 years. His Hb is maintained at 8gm/dl and platelet count at 35-40 x 10³ / cu mm with UCB transfusion. Presently he requires approximately two transfusions of UCB or packed RBCs / year for anemia correction. In a study of hematological malignancies done by Bhattacharya N [32], he gave multiple transfusions to six malignant cases and he reported change in CD34 level in peripheral blood. One of their cases showed a frequent steep rise up to 99% and a sustained high level of CD34 with clinical remission of disease. However, we have not included hematological malignancy in our study. From above observation on the aplastic anemia case, it is our presumption that improvement in the patient condition may be either because of stimulation of existing bone marrow by growth factors and cytokines or acceptance of transfused stem cells by host.

Encouraged by results of our study in vitiligo patients, we hypothesize that growth factors, cytokines and some unknown factor/ proteins in the UCB work for regeneration of melanocytes or transfused stem cells richly present in UCB implant

at vitiligo sites and get converted in melanocytes. Dramatic regeneration of melanocytes is seen in 61 vitiligo patients in our study. Repigmentation was observed in 2/3rd to half of the affected area in 80% of patients and initiation of pigmentation was seen in chronic non responders on follow up. Our results strengthen the observations of study conducted at our institute in 2016 by Agarwal N [4]. To best of our knowledge, no one else has transfused UCB to the vitiligo patients as treatment apart from our institute.

Similarly in our 9 case of retinitis pigmentosa which received 23 UCB transfusions with the interval of 1 month all the patients had improvement in their vision by 30% to 40%. There was no other study reported for the same, except for the study of Agawam N who in our institute reported the improvement in one case (the only case in the study) of retinitis pigmentosa after UCB transfusion [4]. We suggest conduction of a study with higher case numbers of vitiligo and retinitis pigmentosa to bring UCB transfusion in forefront as a prospective treatment, since both of these diseases have been deemed incurable:

“There are no cures for vitiligo and retinitis pigmentosa only several supportive options treatment are available” [33, 34]

Due to increasing geriatric population all over the world and an arising need for advent of regenerative medicine, newer modalities are being investigated for a targeted approach. Old age is marked with loss of renewal of stem cell niche in body which in turn affects the tissue repair. Therefore, stem cell therapy has come up as potential candidates for regenerative medicine. Cord blood is richer in stem cells constituting 0.01% of cellular population as compared to adult peripheral blood which has 0.001% of stem cells [31]. Promising results are reported by Jesse Karmazin, an entrepreneur who launched a clinical trial on the potential of “young blood” through his startup **Ambrosia**. He reported that within a month, most

participants “see improvements” from the one-time infusion of a two-liter bagful of plasma [35]. With an intention to alleviate the symptoms of old age, we transfused 24 UCB units to 9 geriatric patients with the average of 2.66 transfusions /patient. All the patients reported satisfactory response with UCB with improvement in lack of interest in work, tiredness, fatigue and general weakness. Control over their geriatric diseases like; asthma, diabetes, thyroid, etc was also achieved. With the results of our study, we hypothesize that UCB proves to be a better substitute to “young blood” to treat geriatric disorders and suggest larger clinical trials or studies to prove the same.

In our study we could enroll only one patient of high myopia, to whom 5 transfusions were given. We observed a subjective finding in the patient; his vision was improved from 6/60 to 6/24 (from 1st row to 3rd row of Snellen chart) in both the eyes. So, we can say there is a hope for high myopic patient by the treatment of UCB transfusion.

The UNICEF estimates that averages of 3.53 lakhs babies are born each day around the world. If per delivery placenta and umbilical cord have on an average 70 ml blood then total blood; $3.53 \text{ lakh} \times 0.07 \text{ liter} = 2.45 \text{ lakh liter}$. This precious human cord blood i.e. 2.45 lakh liter/ day, if not utilized, is a medical waste; so it is not wise to waste the precious human cord blood.

CONCLUSION

Umbilical cord blood is safe and genuine alternative of adult blood. It is effective in

the treatment of degenerative and autoimmune diseases. UCB is also useful in neonates (less quantity of blood is required), chronically transfused patients and a hope for geriatric persons. By our study and previous studies, it is concluded that there is no chance of graft versus host disease by UCB transfusion. Here, we are recommending that it should not be discarded as medical waste and utilized judiciously in the interest of human well being.

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