Placental umbilical cord whole blood transfusion—A safe and genuine blood substitute for patients of the under-resourced ares of this country at emergency

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Introduction:

In the animal kingdom swallowing the afterbirth by the mother is a general norm. Nature appears to have provided this precious wisdom to some of its creatures. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). But humans do not seem to know how to use this precious afterbirth, which has protected and nurtured the baby for so long in the womb. Of late, however, since 1989, (**Ref 1, 2**) global consciousness is increasing on the use of umbilical cord blood stem cells as an easily available source of hematopoietic stem cells for bone marrow transplantation. These fetal stem cells (CD 34) cause less graft *vs.* host reactions after transplantation. Recognition of this potentiality in the scientific world has resulted in the collection and harvesting of these cord blood stem cells in many laboratories all over the world. But these hematopoietic stem cells constitute only .01 percent of the nucleated cells of the cord blood .The rest, that is, 99.99 percent of the cord blood is wasted. This wasted precious gift of Mother Nature is rich in fetal hemoglobin, growth factors and other cytokine filled plasma, and is moreover protected in the infection free environment inside the placenta in case of a healthy newborn.

Through evolution, the oxygen free carrying pigment in invertebrates became intracellular for better tissue perfusion in response to growth and metabolic demands from lower vertebrates to higher vertebrates. By incorporating the

hemoglobin inside the RBC rather than carrying oxygen in plasma alone, the evolutionary process increased by about 100 times the oxygen carrying capacity of higher vertebrates. If hemoglobin is extracellular and intravascular, it may exert 5 times more osmotic pressure than plasma protein. As a result, water will be drawn from the tissue space and load the intravascular compartment. By inclusion of the hemoglobin inside the cell, the viscosity of the blood remains low, water is not drawn from the tissue space and the flow of blood with a large protein content is made possible. In addition, RBC membranes contain enzymes that protect the hemoglobin from degradation and allow it to work for three months at a stretch. A lack of enzymes in the membrane free hemoglobin leaves it at risk from oxidative damage by the haem molecule **(Ref 3)** Furthermore, free hemoglobin is always subject to oxidative denaturation apart from acting as a trigger for hypertensive episodes.

In the global search for a suitable hemoglobin based oxygen carrier from human RBC to bovine RBC, or its chemically or genetically modified form, or even from sea creatures (Arenicola Merina), i.e., sea worm, the hemoglobin has been extracted **(Ref 4)** for its potential human use. Animal hemoglobin can trigger allergic reactions and can even damage the kidneys.

Adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2 (1 Gm hemoglobin binds with 1.39 ml of oxygen). Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin **(Ref 5)** of which 20 percent belongs to the adult hemoglobin type (3.36 gms) and 80 percent belongs to the fetal hemoglobin type (13.44gms) **.** The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other feto-maternal factors. Fetal hemoglobin has the potentiality to carry upto 50 percent more oxygen than adult hemoglobin **(Ref 6) ,** i.e., 1Gm of fetal hemoglobin may carry upto 2.08 ml of oxygen. If we simply calculate theoretically the oxygen

carrying potentiality of 100ml of cord blood taking into account of its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin) and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin), it would be around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes, (a) concentration of hydrogen ion, (b) carbondioxide concentration in the blood), (c) body temperature, (d) 2-3 diphosphoglycerate concentration only, to name a few**.**

Whether fetal hemoglobin rich placental umbilical cord whole blood which has the potentiality to carry more oxygen to the tissue Vol/Vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term, and whether it could be an emergency and safe substitute for adult whole blood, was the main idea behind our project, which we submitted to the Dept. of Science and Technology, Govt. of West Bengal, Salt Lake, Calcutta in January 1999. The project was subsequently sanctioned vide order No 495/ST/P/S& T9G-1099 dated 25/3/1999.

Material and Methods:

413 units of human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anaesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kgs., 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 anaesthetist 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 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. A second bag was used if the collection exceeds or nears 100ml and a second prick was made at a proximal region after using a clamp at the first site of prick. The blood flows by gravity and generally within a minute 90 percent of the collection is over and within 2 minutes, in most of the cases, the blood flow ceases completely due to clot formation. In case of any confusion about the condition of the baby, the decision was immediately taken to preserve the blood in consultation with the paediatrician for future use by the baby, or stamped "Unsafe for transfusion", and no risk or chance whatsoever was taken for the eventual recipient of the blood.

When the collection was complete blood bag tubing was closed, sealed, and stored at 1-4 degree centigrade, 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, malaria as per standard blood transfusion protocol.,which we reported earlier **(7)** .

Osmotic fragility study with .45 percent NaCl (N=40) at 4 ° centigrade, 35° and 40° with a time gap of 24 hours, 48 hours, 7 days and 14 days along with oxyhemoglobin (mmole/ml) and plasma hemoglobin (mg/ml) assessment in identical schedule showed that the cord blood was reasonably stable at room temperature. In case of any confusion/contamination, the culture was put aside for identification of the pathogen if any, through appropriate protocol, and the sample was stamped unfit for transfusion.

In the present series, the collection of the blood varied from 50ml -146 ml mean 86 m1$\pm$7.6 ml SD, median 80 ml, mean packed cell volume 48 $\pm$ 4.1 SD, mean hemoglobin

concentration 16.2 Gm percent $\pm$ 1.8 Gm percent SD. After collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of

collection. Donation of the cord blood to the recipient followed the strict guidelines of the human ethical committee of the Hospital headed by an emeritus Professor of Medicine. As a rule, the volunteer who wishes to enroll for the cord blood transfusion programme, must have a hemoglobin count which is below 8 Gm percent. Patients with cancer or some critical illness were given a priority. Before the umbilical cord blood transfusion, a thorough clinical examination of the recipient was done, including the proper monitoring of the BP/Pulse /Respiration and other cardinal and presenting features. Then pre- transfusion, a little blood was drawn from the prospective recipient of cord blood for Blood grouping, Hb/ Tc/ Dc/ ESR/ Platelet/ Coombs test, C-Reactive protein, Urea, Creatinine, Bilirubin and other investigations as per the requirements of the case. For example, Hb electrophoresis was done in case of thalassemia, before and after the transfusion was undertaken to see the impact of transfusion. A little blood was redrawn from the same patient who received cord blood transfusion, after 24 hours, 72 hours, 7days, 1month, 2 months. 3 months and subsequently, clinical follow-up continued at the OPD from time to time, to study the effect of transfusion and adverse reactions if any.

Actual transfusion procedure started after necessary grouping and cross-matching of the specimens and checking the identity of the patient. The cord blood was transfused by a blood transfusion set containing a filter (230 um). For the initial 15 minutes or so the patient was carefully observed to see if there was any transfusion reaction. Thereafter, if all went well, the transfusion rate was increased till it was completed.

Result and Analysis

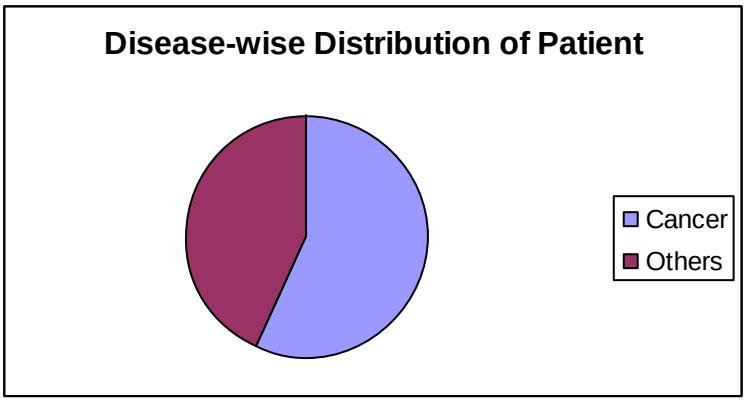


Fig (1) depicts the disease-wise distribution of the patients. Out of 129 cases, 56.58 percent patients were suffering from cancer, and the rest, i.e., 43.42 percent, of the patients were suffering from other diseases.

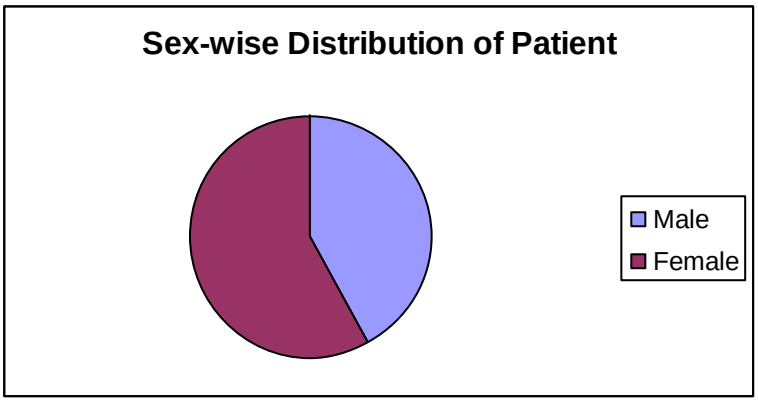


Fig (2) narrates the sex-wise distribution of the 129 cases who were enrolled in the present trial. 41.86 percent participants were male and the rest (58.14 percent) participants were female.

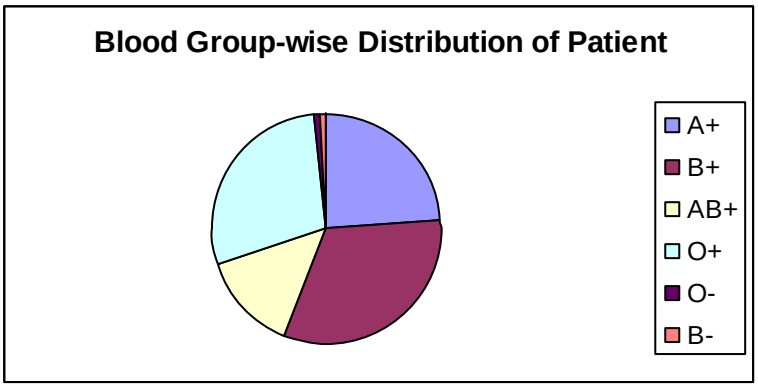


Fig (3) shows the blood group distribution of the patients. In the present series, 24.03 percent belonged to blood group A+, 31.78 percent belonged to blood group B+, 13.95 percent patients belonged to blood group AB+, 28.68 percent patients belonged to blood group O+, .77 percent patients belonged to O- and B- groups each.

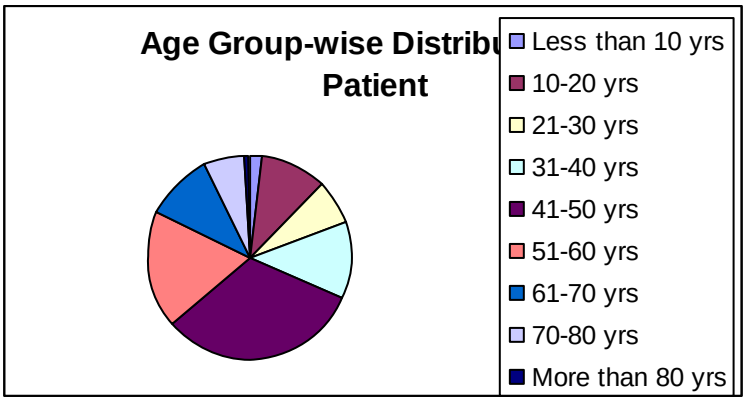
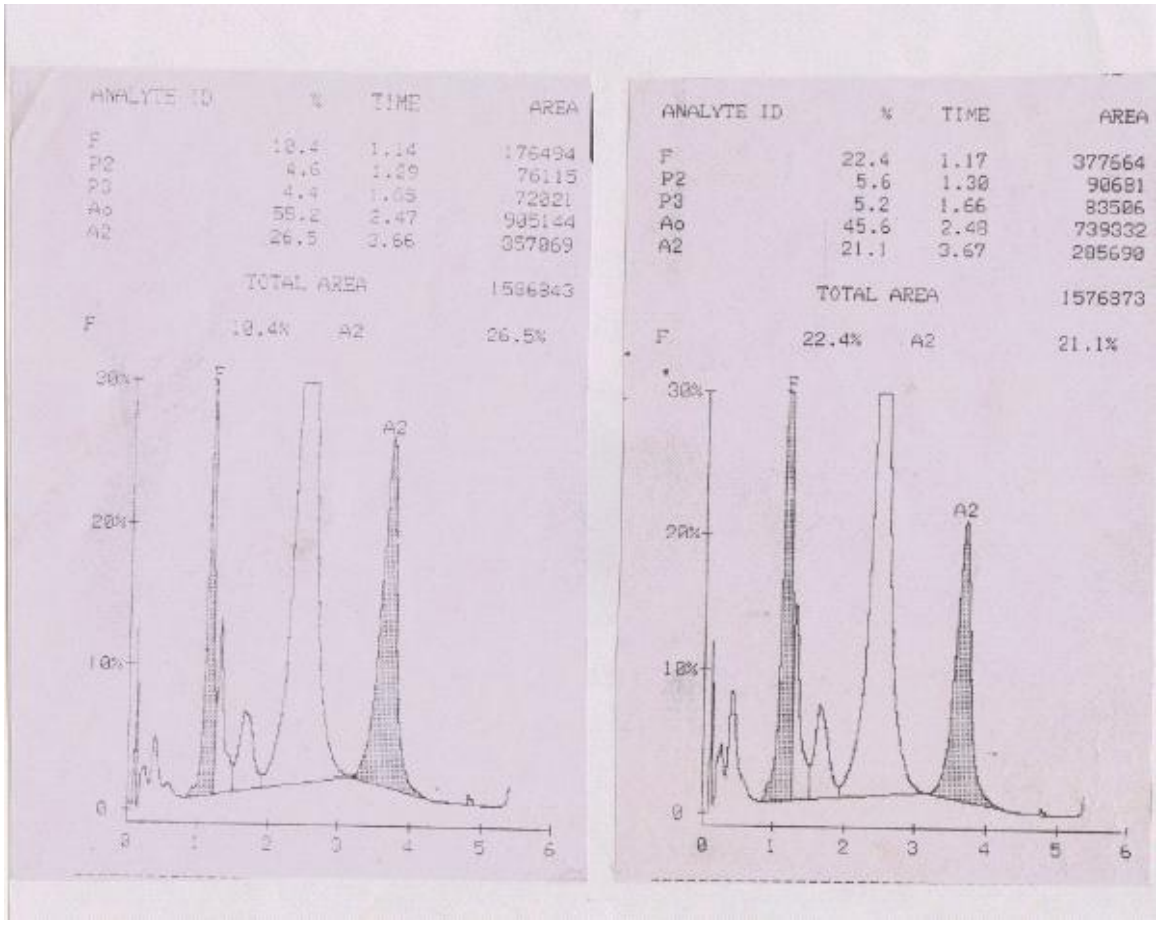


Fig (4) depicts the age group distribution of the patients. Of the 129 patients who enrolled for the present cord blood transfusion protocol, the majority belonged to the 10 – 60 years age group, i.e., 79.86 percent. Patients below 10 years of age constituted 2.32 percent cases. Patients above 60 years, made up 17.82 percent of the cases.

This graph represents the hemoglobin electrophoresis results of a 2 year old boy with thalassemia major who received a single unit of placental umbilical cord whole blood transfusion which resulted in a rise of fetal hemoglobin from 10.4 percent to 22.4 percent.



ANALYTE ID	%	TIME	AREA
F	10.4	1.14	176494
P2	4.6	1.29	76115
P3	4.4	1.65	72821
Ao	55.2	2.47	905144
A2	26.5	3.66	357869
TOTAL AREA			1596343

F 10.4% A2 26.5%

ANALYTE ID	%	TIME	AREA
F	22.4	1.17	377564
P2	5.6	1.30	90681
P3	5.2	1.66	83506
Ao	45.6	2.48	739332
A2	21.1	3.67	285690
TOTAL AREA			1576373

F 22.4% A2 21.1%

List of the patients who received cord blood transfusion under the present series

Sl. No.	Name, Age & Sex	Blood Group	Primary background disease apart from anemia,i.e.Hb 8Gm percent or low	Transfusion of UCB:No of Units	Immediate reaction ,viz,fever,chill and rigor,flank pain,back pain, blood in urine,fainting or dizziness	Late reactions like mild or progression to kidney failure ,shock or delayed anemia	Complications:like mild to moderate discomfort, anemia,shock,acute renal shutdown,lung dysfunction	Unexpected/Unusual Complication	Unknown complication and rare complication ? autoimmune disease or scleroderma due to microchimerism etc with follow up till date
1	AC 35yrs. F	A+ve	Ca-breast stage III	14 units 1st – 23.04.99 last – 09.05.01	Nil	Nil	Nil	Nil	Nil
2	BS 54 yrs M	A+ve	Fibro sarcoma breast stage IV &	5 units 1st – 25.04.99 last –	Nil	Nil	Nil	Nil	Nil

			diabetes	01.08.99					
3	SB 65 yrs M	B+ve	Ca-tonsil stage-II	1 unit 12.05.99	Nil	Nil	Nil	Nil	Nil
4	AP 16yrs M	B+ve	Non hodgkin's lymphoma	8 units 1st – 11.05.99 last – 13.07.00	Nil	Nil	Nil	Nil	Nil
5	GC 42yrs F	B+ve	Ca-breast Stage III	7 units 1st – 17.05.99 last – 22.05.99	Nil	Nil	Nil	Nil	Nil
6	GB 35yrs M	O+ve	Ankylosing spondylitis	6 units 1st – 30.05.99 last – 21.08.99	Nil	Nil	Nil	Nil	Nil
7	KD 40yrs F	A+ve	Ca-breast Stage IV	1 unit 23.06.99	Nil	Nil	Nil	Nil	Nil
8	SD 30yrs F	B+ve	Ca-breast Stage IV	7 units 1st – 04.08.99 last – 06.12.99	Nil	Nil	Nil	Nil	Nil
9	TKM 12yrs M	A+ve	Thalassemia Major	7 units 1st – 08.08.99 last – 24.05.01	Nil	Nil	Nil	Nil	Nil
10	GB	O+v	BPH	1 unit	Nil	Nil	Nil	Nil	Nil

	71 yrs M	e		21.08.99					
11	SR 18 yrs F	AB+ve	Osteomylitis Foot	1 unit 21.08.99	Nil	Nil	Nil	Nil	Nil
12	AG 78 yrs F	O+ve	Ca-larynx Stage IV	6 units 1st – 28.08.99 last – 21.02.00	Nil	Nil	Nil	Nil	Nil
13	GD 75 yrs M	O+ve	BPH	1 unit 31.08.99	Nil	Nil	Nil	Nil	Nil
14	AD 30yrs F	O+ve	Arthritis deformity	4 units 1st – 18.02.99 last – 12.09.99	Nil	Nil	Nil	Nil	Nil
15	SMD 60yrs M	AB+ve	Ca-prostate	1 unit 12.09.99	Nil	Nil	Nil	Nil	Nil
16	AD 45 yrs F	B+ve	Ca-cervix Stage II	9 units 1st – 16.09.99 last – 24.07.00	Nil	Nil	Nil	Nil	Nil
17	SK 55yrs F	A+ve	Ca-colon Stage IV	9 units 1st – 30.09.99 last – 31.05.00	Nil	Nil	Nil	Nil	Nil
18	BDG 50yrs F	B+ve	Ca-breast Stage II	5 units 1st – 03.10.99 last –	Nil	Nil	Nil	Nil	Nil

				03.01.00					
19	AM 60yrs F	AB+ve	Calcaneal #	I unit 25.11.99	Nil	Nil	Nil	Nil	Nil
20	PB 55yrs M	A+ve	Ca-breast Stage IV	I unit 27.01.00	Nil	Nil	Nil	Nil	Nil
21	SC 45yrs F	B+ve	Leucoplakia-Vulva	2 units 1st – 08.02.00 last – 08.02.00	Nil	Nil	Nil	Nil	Nil
22	JM 60yrs F	B+ve	Prolapse-Uterus	I unit 17.02.00	Nil	Nil	Nil	Nil	Nil
23	RT 13 2 yrs M	B+ve	Thalassemia major	1 unit 12.02.00	Nil	Nil	Nil	Nil	Nil
24	EC 52yrs M	O+ve	Ca-ovary metastisis to lung & lever	2 units 1st – 13.02.00 last – 21.02.00	Nil	Nil	Nil	Nil	Nil

25	MK 45yrs M	O+ve	Osteo sarcoma – right hand	5 units 1st – 18.02.00 last – 27.06.00	Nil	Nil	Nil	Nil
26	AS 50yrs M	AB+ve	Thymoma	1 unit 08.03.00	Nil	Nil	Nil	Nil
27	SD 50yrs F	AB+ve	Ca-ovary Stage IV	2 units 1st – 14.03.00 last – 18.04.00	Nil	Nil	Nil	Nil
2	PR	A+ve	Ca-cervix	2 units	Nil	Nil	Nil	Nil

8	55yrs F		Stage IV	1st – 18.04.00 last – 17.05.00				
29	BC 62yrs M	A+ve	Bleeding P/R	I unit 20.04.00	Nil	Nil	Nil	Nil
30	KJ 32yrs F	A+ve	Osteosarcoma leg	6 units 1 unit – 20.04.00 last – 27.06.00	Nil	Nil	Nil	Nil
31	AR 26yrs F	A+ve	SLE	1 unit 15.03.00	Nil	Nil	Nil	Nil
32	SK 37yrs M	O+ve	Ca-penis Stage II	I unit 27.03.00	Nil	Nil	Nil	Nil
33	DCS 51yrs M	O+ve	Metastatic neck gland Stage IV	15 units 1 st UNIT 28.11.99 last – 2.6.2000	Nil	Nil	Nil	Nil
34	RP 46yrs M	AB+ve	Ca-stomach Stage IV	1 unit 07.04.00	Nil	Nil	Nil	Nil
35	NKG 53yrs M	B+ve	Bronchogenic carcinoma Stage IV	5 units 1ST unit – 13.04.00 Last-09.05.2000	Nil	Nil	Nil	Nil
36	SG 60yrs M	B+ve	Ca-prostate Stage IV	1 unit 25.04.00	Nil	Nil	Nil	Nil
37	UP 35yrs F	B+ve	Ca-cervix Stage IV	1 unit 14.05.00	Nil	Nil	Nil	Nil
38	AM 72yrs M	O+ve	BPH	1 unit 17.05.00	Nil	Nil	Nil	Nil
39	AH 60yrs M	O+ve	Pylonic stenosis	1 unit 28.05.00	Nil	Nil	Nil	Nil
40	RDS 40yrs F	O+ve	Bronchiectasis	1 unit 28.05.00	Nil	Nil	Nil	Nil
41	SD 50yrs F	O+ve	Intestinal obstruction……	1 unit 31.05.00	Nil	Nil	Nil	Nil
42	KD 28yrs F	A+ve	Severe anemia	2 units 1st –	Nil	Nil	Nil	Nil

				07.06.00 Last- 12.06.00				
43	MB 42yrs M	O+ve	Ca-Lung with bone metastasis Stage IV	5 units 1st – 22.06.00 Last-***	Nil	Nil	Nil	Nil
44	AB 28yrs F	O+ve	Bleeding P/R in the background of cirrhosis	7 units 1st 22.06.00 Lat- 27.0600	Nil	Nil	Nil	Nil
45	LKM 66 yrs F	AB+ve	AdenocarcinomaUterus	3 units 1st – 01.07.00 Last-	Nil	Nil	Nil	Nil
46	KNH 47yrs F	O+ve	Recurrent peptic ulcer with peptic perforation	4 units 1st- 01.07.00 Last – 27.07.00	Nil	Nil	Nil	Nil
47	RCC 53yrs M	B+ve	Ca-Tongue Stage II	1 unit 02.07.00	Nil	Nil	Nil	Nil
48	MD 17 yrs F	O+ve	Neurocysticercosis	1 unit 11.07.00	Nil	Nil	Nil	Nil
49	SF 27 yrs F	O+ve	Appendicitis	1 unit 12.07.00	Nil	Nil	Nil	Nil
50	RD 47 yrs F	A+ve	Severe anemia & diabetes	3 units 1st - 11.07.00 Last-	Nil	Nil	Nil	Nil

51.	LP 17 yrs F	AB+ve	Severe anemia	7 units 1st 17.07.00 Last- 02.08.00	Nil	Nil	Nil	Nil
52.	NGD 57 yrs M	B+ve	Cholelithiasis	1 unit 17.07.00	Nil	Nil	Nil	Nil

53.	RD 15 yrs F	O+ve	Rheumatoid arthritis and regular fever	1 unit 08.07.00	Nil	Nil	Nil	Nil
54	RC 45 yrs F	O+ve	Ca-Breast Stage II	7 units 1st 14..07.00 Last-17.07.00	Nil	Nil	Nil	Nil
55	SD 47 yrs F	A+ve	Bronchogenic Ca Stage IV	1 unit 19.07.00	Nil	Nil	Nil	Nil
56	PC 78 yrs M	B+ve	Ca-Tongue Stage II	1 unit 19.07.00	Nil	Nil	Nil	Nil
57	AG 35 yrs F	B+ve	Tumor (Rt)Breast	1unit 23.07.00	Nil	Nil	Nil	Nil
58.	SD 45 yrs M	A+Ve	Ca-Lung Stage IV with poor GC	1 unit 02.08.00	Nil	Nil	Nil	Nil
59	KD 8 yrs F	A+ve	Thalassemia Major	16 units 1st 07.08.00 Last-03.01.02	Nil	Nil	Nil	Nil
60	AG 47 yrs F	AB+ve	Thalassemia Minor with acute appendicitis	1 unit 09.08.00	Nil	Nil	Nil	Nil
61.	DS 14 yrs F	O+ve	Burkitts Tumour	7 units lst-02.04.01 Last-14.05.01	Nil	Nil	Nil	Nil
62	KP 60 yrs M	B+ve	Ca-stomach Stage IV	7 units lst-13.04.01 Last-14.05.01	Nil	Nil	Nil	Nil
6	BB 52	B+ve	Ca-	9 units	Nil	Nil	Nil	Nil

3	yrs M		hypophorynx	lst-26.04.01 Last-11.12.01				
64	GB 32 yrs F	B+ve	Ca-Breast Stage IV	33 units lst-12.08.99 Last-10.10.02	Nil	Nil	Nil	Nil
65	AC 45 yrs F	AB+ve	Ca-Breast Stage III with background of Elisa Tb+ve	2 units 1st-01.05.01 Last 16.05.01	Nil	Nil	Nil	Nil
66	SC 18 yrs F	B+ve	Vaginal atresis with severe anemia	1 units 03.05.01	Nil	Nil	Nil	Nil
67	BP 45 yrs F	O+ve	Ca-breast Stage IV	5 units lst-03.05.01 Last-07.07.01	Nil	Nil	Nil	Nil
68	PC 52 yrs M	O+ve	Ca-head pancreas	5 units lst-14.05.01 Last-04.07.01	Nil	Nil	Nil	Nil
69	TM 42 yrs M	AB+ve	Non-healing ulcer in a case of Elesis tb+ve	1m unit 16.05.01	Nil	Nil	Nil	Nil
70	KG 48 yrs F	AB+ve	Severe anemia with rectal prolapse grade III	1 unit 25.05.01	Nil	Nil	Nil	Nil
71	NN 52 yrs F	B+ve	Ca-breast Stage IV	3 units lst-24.05.01 Last-31.03.02	Nil	Nil	Nil	Nil

72	NP 52 yrs M	B+ve	Ca-Larynx Stage IV	1 unit 08.06.01	Nil	Nil	Nil	Nil

73	BM 48yrs <	O+ve	Aplastic anaemia	23 units lst 15.06.01 Last 12.02.02	Nil	Nil	Nil	Nil
74	KB 42 yrs F	A+ve	Rheumatoid arthritis with deformity	1 unit 21.06.01	Nil	Nil	Nil	Nil
75	GD 45 yrs F	A+ve	CA-breast (post operative)	7 units 1st-27.06.01 Last 12.07.01	Nil	Nil	Nil	Nil
76	SK 62 yrs F	O+ve	Prolapsed piles with severe anaemia (post operative case)	1 unit 02.07.01	Nil	Nil	Nil	Nil
77	MG 45 yrs F	AB+ve	Bleeding fibroid with severe anaemia (post operative case)	1 unit 02.07.01	Nil	Nil	Nil	Nil
78	BP 20 yrs F	B-ve	Post ……. syndrome in the background hypothyroidism and elisa Tb+ve	1 unit 02.07.01	Nil	Nil	Nil	Nil
79	KD 28 yrs.F	AB+ve	Post operative phase of Cholecyst	1 unit 13.07.01	Nil	Nil	Nil	Nil

			ectomy with anaemia					
80	DK 33 yrs F	B+ve	Ca-breast (post operative)	2 units lst-16.07.01 Last-17.07.01	Nil	Nil	Nil	Nil
81.	IP 42 yrs M	B+ve	Ca-lower end of oesophagus	4 units 1st-18.07.01 Last-07.08.01	Nil	Nil	Nil	Nil
82	AH 35 yrs M	A+ve	Acute Leukaemia+++	2 units lst 20.07.01 Last-20.07.01	Nil	Nil	Nil	Nil
83	SB 6 yrs M	O+ve	Thalassaem-ia major	6 units 1st 28.07.01 Last - 14.08.01	Nil	Nil	Nil	Nil
84	KCP 38yrs M	B+ve	Myolodysp-castric syndrome	5 units lst 28.07.01 Last-15.09.01	Nil	Nil	Nil	Nil
85	AB 45 yrs F	A+ve	Ca-breast (post operative	5 units 22.08.01 Last-07.05.02	Nil	Nil	Nil	Nil
86	PNP 65yrs M	O+ve	Ca- lung with metastasis in the brain and bones	3 units lst-23.08.01 Last-25.08.01	Nil	Nil	Nil	Nil
87	SG 37 yrs F	A+ve	Lucs (post operative) UCB given to mother	1 unit 27.08.01	Nil	Nil	Nil	Nil
88	PG 35 yrs F	O+ve	Myomectomy	7 units lst-03.09.01	Nil	Nil	Nil	Nil

				Last-05.09.01				
89	PM 25 yrs F	B+ve	Recurrent spontaneous abortion	3 units lst-05.09.01 Last-08.09.01	Nil	Nil	Nil	Nil
90	NN 62 yrs M	A+ve	Ca-lung with tuberculosis	3 units lst-18.09.01 Last-25.09.01	Nil	Nil	Nil	Nil
91	BS 52 yrs F	O+ve	Ca-breast (follow up on chemotherapy)	10 units lst-18.09.01 Last-30.09.02	Nil	Nil	Nil	Nil
92	SS 72yrs M	A+ve	Cerebral atrophy with hypertension	1 unit 21.09.01	Nil	Nil	Nil	Nil
93	MS 25 yrs F	B+ve	SLE	6 units lst-07.09.99 Last-18.10.01	Nil	Nil	Nil	Nil
94	AC 45 yrs M	O+ve	Chr. Cholecyiltitis with anaemia	1 unit 08.11.01	Nil	Nil	Nil	Nil
95	NCD 62 yrs M	B+ve	Fistula in Ano	1 unit 21.12.01	Nil	Nil	Nil	Nil
96	SK 18 yrs F	B+ve	Appendicectomy	1 unit 03.05.01	Nil	Nil	Nil	Nil
97	Pm 62 yrs F	B+ve	Rheumatoid Arthiritis	1 unit 31.05.01	Nil	Nil	Nil	Nil
98	AG 20 yrs M	B+ve	Rectal Polyp with anaemia	7 units lst-30.01.02 Lat-01.02.02	Nil	Nil	Nil	Nil
99	BPD 65 yrs F	B+ve	Ca-lower end of Oesophag	4 units 1st-29.11.01	Nil	Nil	Nil	Nil

			ur	Last-03.12.01				
100	ST 55 yrs F	B+ve	Ca-Cheek Stage IV	4 units lst – 10.11.01 Last-09.02.02	Nil	Nil	Nil	Nil
101	JD 47 yrs F	B+ve	Ca-breast with recurrence	6 units lst-27.01.01 Last-11.02.02	Nil	Nil	Nil	Nil
102	SKR 47 yrs F	A+ve	Ca-kidney with metastatic to lung bone	6 units lst 01.02.02 Last-04.04.02	Nil	Nil	Nil	Nil
103	GD 65 yrs F	O+ve	Ca-ovary Stage IV	4 units lst – 01.02.02 Last-11.02.02	Nil	Nil	Nil	Nil
104	SR 62 yrs F	O+ve	Ca-cheek	1 unit 27.11.01	Nil	Nil	Nil	Nil
105	NM 54 yrs M	A+ve	Ca-lung	2 units lst-02.03.02 Last-05-03-02	Nil	Nil	Nil	Nil
106	GM 46 yrs M	B+ve	Ca-Cx Stage IV	4 units lst-05.03.02 Last-02.05.02	Nil	Nil	Nil	Nil
107	RD 46 yrs M	B+ve	Verrucuous Carcinoma lip	7 units lst-12.03.02 Last-13.03.02	Nil	Nil	Nil	Nil
108	GC46 yrs M	O+ve	Ca base of tongue	7 units lst-08.03.02 Last-04.04.02	Nil	Nil	Nil	Nil
1	SR 49	A+ve	Ca-	7 units	Nil	Nil	Nil	Nil

09	yrs M		stomach	lst-11.03.02 Last-15.06.02				
110.	SD 45yrs M	O-ve	Leucoplakia cheek	1 unit 01.04.02	Nil	Nil	Nil	Nil
111	AG 76 yrs F	O+ve	Ca-LARYNX	I unit 19.04.02	Nil	Nil	Nil	Nil
112	PR 60 yrs F	A+ve	Ca-Cx Stage IV	1 unit 11.05.02	Nil	Nil	Nil	Nil
113	CM 44 yrs F	A+ve	Ca breast	1 unit 11.05.02	Nil	Nil	Nil	Nil
114	PM 14 yrs F	O+ve	Acute lymphoblastic leukaomia	8 units lst-18.05.02 Last-08.10.02	Nil	Nil	Nil	Nil
115	KC 45 yrs F	B+ve	Ca Larynx	7 units lst-07.06.02 Last-08.10.02	Nil	Nil	Nil	Nil
116	AB 45 yrs F	B+ve	Ca-breast	1 unit 15.06.02	Nil	Nil	Nil	Nil
117	NT 55 yrs M	B+ve	Ca-oropharynx	7 units lst-20.06.02 Last-21.06.02	Nil	Nil	Nil	Nil
118	DS 65 yrs F	A+ve	Ca-gall bladder with metastasis	1 unit 01.07.02	Nil	Nil	Nil	Nil
119	GK 47 yrs F	AB+ve	Ca-breast Stage IV	1 unit 09.08.02	Nil	Nil	Nil	Nil

12	CB 46 yrs F	B+ve	Ca-breast	7 units lst-	Nil	Nil	Nil	Nil

0				05.10.02 Last- 05.10.02				
121	MD 86 yrs F	A+ve	Ca-breast	2 units lst- 05.10.02 Last-	Nil	Nil	Nil	Nil
122	MH 45 yrs M	B+ve	Ca-bladder	1 unit 31.10.02	Nil	Nil	Nil	Nil
123	SB 72 yrs F	A+ve	Severe anaemia with Kochs with hypothyroidism with bilateral ovarian tumour	1unit 21.03.02	Nil	Nil	Nil	Nil
124	PM 35 yrs F	AB+ ve	Hepatitis with severe anaemia	1 unit 11.03.02	Nil	Nil	Nil	Nil
125	TN 47 yrs M	AB+ ve	Severe anaemia with cholecystitis (post operative) in the background of diabetes	1 unit 19.04.02	Nil	Nil	Nil	Nil
126	NH 70 yrs M	AB+ ve	Anaemia and prostate enlargement	1 unit 05.05.02	Nil	Nil	Nil	Nil
127	TP 1 3 yrs M	O+v e	Hb E diseases	1 unit 05.05.02	Nil	Nil	Nil	Nil
128	PM 50 yrs M	A+ve	Intestenal kochs with	2 units lst- 20.06.02	Nil	Nil	Nil	Nil

			anaemia	Last-				
129	SS 18 yrs F	O+ve	Pregnancy anaemia UCB given to mother	7 units lst-09.10.02 Last-	Nil	Nil	Nil	Nil

Study results on the stability of cord blood at temperature and time

Mean Fragility (% hemolysis in 0.45% NaCl) with Standard deviation (N= 40)

Temp	TIME			
	24hr	48hr	7 days	14 days
4^0C	12.6 ±3.4	32.9 ± 4.3	45.6 ± 2.8	82.5 ±4.6
35^0C	16.6 ± 2.7	20.4 ± 4.3	53.5 ± 3.9	100
40^0C	45.0 ± 6.4	77.5 ± 3.8	92.6 ± 4.8	100

Mean Oxyhemoglobin (mmole/ml) with Standard deviation (N –34)

Temp	TIME			
	24hr	48hr	7 days	14 days
4^0C	0.32± .12	0.31± .14	0.27±.05	0.26±.12
35^0C	0.31±.06	0.29±.03	0.24±.06	-
40^0C	0.16±	0.09±.01	-	-

Mean Plasma hemoglobin (mg/ml) with Standard Deviation (N –36)

Temp	TIME			
	24hr	48hr	7 days	14 days
4^0C	6.08± .87	6.35±.78	7.04±.89	9.69±1.7
35^0C	4.49±.54	7.65±.86	10.0±2.3	-
40^0C	10.3±1.6	13.3±2.4	-	-

In the present series we are presenting the data of 413 units of aseptically collected cord blood from consenting mothers undergoing LUCS from 1st April 1999. Transfusion services and effective follow up have been continued in the outpatient department to date. The blood was transfused to 129 informed consenting volunteers after the cases were passed through the institution based ethical committee. The list included 54 male and 75 female patients. The age of the patients varied from 2 years to 86 years. 73 patients (56.58 percent) were suffering from advanced cancer and 56 (43.42 percent) patients suffered from

other diseases. Three pediatric patients were less than 10 years old and one patient was more than 80 years old. 22 patients (17.05 percent) were within 60-80 years. The majority of the patients (50.38 percent) were within the 40 to 60 years age group. B+ was the commonest blood group (31.78 percent), followed by O+ (28.68 percent), then A+ (24.03 percent), and lastly AB+ (13.95 percent). O- and B- blood was transfused to one patient each. 33 units (highest) of cord blood was transfused to a patient with advanced cancer with hemoptysis (10 units of cord blood was transfused at a time), followed by 23 units to a patient with aplastic anemia (9 units of cord blood at a time), 16 units in a case of transfusion dependent thalassemic syndrome (8 units of cord blood at a time), followed by 15 units to a patient suffering from stage IV cancer with metachronous metastasis (7 units of cord blood at a time). Among others, those who received more than three units at a time included one patient who was given a transfusion of 10 units of cord blood; 3 patients received 9 units each, 2 patients received 8 units each, 18 patients received 7 units each. In addition, 7 patients received 6 units and 7 patients received 5 units of umbilical cord whole blood transfusion. All patients presenting with anemia (8 Gm hemoglobin or less) and distress, be it in the background of Ankylosing spondylitis, lupus erythematosus, rheumatoid arthritis, aplastic anemia, thalassemia major, to bleeding per rectum or hemoptysis due to malignancy, responded clinically with cord blood transfusion.

The blood volume of a term fetus is approximately 80 - 85 ml/kg **(,8).** The placental vessel at term contains approximately 150 ml of blood **(Ref 9**). The cord blood contains three types of hemoglobin, HbF, HbA, HbA2, of which HbF constitutes the major fraction (50-85 percent) **(Ref 10).** HbA accounts for 15 - 40 percent of hemoglobin and HbA2 is present only in trace amounts at birth **(Ref 11)**. HbF has a greater oxygen affinity than HbA **(Ref 12).** The oxygen tension at which the hemoglobin of the cord blood is 50 percent saturated is 19-20 mm of Hg, 6-8 mm Hg lower than that of normal adult blood. This shift to the left of the hemoglobin oxygen dissolution curve results from poor binding of the 2-3 diphosphoglycerate by HbF **(Ref 13, 14).** The potential complications of blood

transfusion therapy can be grossly divided under two headings, immunological and non-immunological reactions **(Ref 15**). The immunological reactions are related to the stimulation of antibody production by the foreign alloantigens by the different components of transfusion, e.g, RBC, leucocytes, platelets and plasma proteins. Alloimmunizations may lead to immunological reactions in case of future stimulation by a similar antigen. The commonly encountered immunological reactions are haemolytic reactions due to red cell incompatibility. Febrile or pulmonary reactions are related to antigens of leucocytes and platelets. Allergic and anaphylactoid reactions are related to antibodies and it is only very rarely that we can see graft *vs* host reactions due to engraftment of the transfused lymphocytes in case of immunosuppression. The commonly encountered non-immunological reactions are because of physical or chemical properties of the transfused blood /blood products due to bacterial or viral contamination or the circulatory load.

During our experience of transfusion of 413 units of cord blood over the last five years, we have not encountered a single episode of immunological or non-immunological reaction so far. Fetal hemoglobin can carry more oxygen than the mothers blood and there is a potential advantage of the fetal hemoglobin (Bohr's effect) by which it can carry more oxygen at low PCO2 than at high PCO2 **(Ref 16**). Another potent advantage of cord blood transfusion which has therapeutic implication, is the rich cytokine and growth factor filled plasma in the cord blood, which eventually has a positive effect on distressed and emaciated patients. On the basis of our experiences, we can say that cord blood transfusion is safe and can be used in hours of crisis from the pediatric to the geriatric age groups, as an alternative to adult whole blood transfusion, not as an inferior method of transfusion but as an effective supplementation of blood, which has no transfusion related hazards detected so far.

Discussion : Continuous supply of donated blood is vital for the practice of modern medicine, but due to an ever increasing worry over blood borne diseases like HIV, hepatitis or bovine spongyform encephalitis in certain areas, has fuelled

the search for an alternative source for blood transfusion. Moreover, with the current global war against terrorism and other conflicts, the research to develop an ideal blood substitute has received a real boost. This has implications for not only the trauma and emergency surgeons, but the medical fraternity as a whole. Trauma surgeons, perhaps more than any other health care provider, are the first to recognize the urgency of a real blood substitute without jeopardizing the safety aspect of such a transfusion. The current generation of blood substitutes are passing through US Food and Drug Administration (FDA) Phase –III clinical testing. These include RBC substitutes to provide the respiratory functions of hemoglobin, platelet substitutes and coagulation factors. **(Ref 17)** The most promising among the RBC substitutes, as mentioned earlier, is the hemoglobin extracted from the lysis of the RBC from human or bovine sources, or a chemically modified hemoglobin or a genetically engineered hemoglobin molecule. Although these hemoglobin based oxygen carriers have an intrinsic advantage of universal compatibility and storability at room temperature, because of the high cost involved, these would be simply unacceptable to the developing world in particular. Moreover, there are also specific problems of hypertensive impact, gastric irritability and unexplained deaths as reported in a trauma trial on the treatment of severe hemorrhagic shock. **(Ref 18)**

The other hemoglobin substitutes with lesser importance include perflurocarbons, i.e., fluorine substituted with linear or cyclic carbon atoms with high oxygen carrying capacity, and liposome encapsulated hemoglobin **(Ref 19).**
Transfusion of adult blood is never a zero risk event anywhere in the world. Risks associated with adult blood transfusion include transmission of HIV (1 & 2), hepatitis B, C, A, G, Parovirus 19, specially in case of pregnancy, hemolytic anemia and immunocompromised background, apart from the possibility of transfusion of syphilis, kalaazar, malaria (in the developing world), unless the blood is thoroughly screened as per WHO and country specific guidelines. There are also problems of rare blood groups which are not screened normally but have the potentiality to trigger hemolytic reactions . There are many other

reasons of transfusion specific acute or delayed immunological and non immunological reactions, contamination problems with platelet, RBC, etc. Very rarely, there could be an incidence of transfusion induced lung, liver or kidney injury. Lastly, there could also be problems due to immunomodulation. **(Ref 20).** Newly identified, but well known, potential risk factors include the possibility of the transmission of Creutzfeldt Jakob disease in its classical or varient form, even after leucodepletion (lymphocytes are possible source of transmission of infection) as reported in an editorial article in BMJ **(Ref 21).**

Attempts are being made by scientists and clinicians all over the world to make blood transfusions safer through stricter vigilance, emphasis on fewer transfusions and more conservation, preoperative autologous donation, stimulation of erythropoiesis, option for preoperative normovolumic hemodilution, attempts at intraopereative and post operative recovery of blood, inactivation of microbes in the platelet units, use of plasma with reduced viral activity and finally, the use of red cell substitutes **(Ref 22).** However, in spite of all these attempted maneuvers by clinicians, the risk of transfusions is not on the wane.

After our experience with 413 units of cord blood transfusion, we wish to affirm our faith in this safe transfusion protocol because we did not encounter a single case of immunological or non immunological reaction so far in any of our patients, even after the transfusion of 1unit to 33 units (2838 ml on the basis of mean volume calculation) of cord blood to the same patient (with 10 units [mean 86x10 = 860 ml] of cord blood transfusion at a time) in different indications of blood transfusion from the paediatric to the geriatric age group (2 yrs to 86 yrs) in the common background of anemia with malignant or autoimmune or traumatic (surgical or non surgical), infective or congenital background disease (as in case of thalassemia). Our experience suggests that this placental cord blood transfusion could be an unique untapped source of fresh, infection free whole blood, if collected aseptically after the birth of healthy newborns from consenting mothers, and it has all the potentialities to be a ready replacement for blood loss .

In this connection it is worth mentioning another recent collaborative work of the University of Liverpool, U.K., and Komfo Anokye Teaching Hospital at Kumashi, Ghana, on the use of placental umbilical cord blood. They reported a substantial decrease in the mortality of children in sub-Saharan Africa suffering from severe anemia after falciparum infection, with the use of cord blood. **(Ref 23, 24).**

Conclusion:

In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood**. (Ref 25)**

An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or replacement donors (family friends or acquaintances) even when the infected population is high. **(Ref 26)**

For the last 70 years since the publication of the report of Amberson, **(Ref 27**) there have been global attempts to find a genuine blood substitute. Fetal hemoglobin is a natural stress response to hemoglobin synthesis which we try to preserve and augment in case of thalassemia by providing hydoxyurea or other similar drug supports. Other conditions like pregnancy, diabetes, thyroid disease, or anti-epileptic drug therapy, can also increase the fetal hemoglobin concentration. This fetal hemoglobin, with its abundant source, i.e., the placenta (in India alone, there are more than 20 million placentas produced as afterbirth every year), is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated, or incinerated. The western or the developed world has been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (.01 percent of the nucleated cells of the placental blood). My team of doctors has been successfully transfusing this blood as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any aetiology ,i.e., from surgery to medicine from HIV,

thalassemia to leprosy or from advanced cancer to patients with a crippling polyarthritis, etc since 1999 **(Ref 28-35).**. We have applied for a global patent on the use of cord blood in these areas.

In fine to combat the emergency requirement of blood in natural or man made disaster management, ie,civil or military due to current waging war against terrorism ,this precious hypoimmune fetal cells **(36-39)** with altered metabolic profile is a gift of the nature, entrapped inside the placenta which could be readily available source of blood not only in the underresourced countries in the world but in case of the genuine need for blood substitute anywhere in the world at crisis.

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Abstract

The term blood substitute is actually a misnomer because only a part of the total functions of the blood is replaced by any available so called substitute, i.e., oxygen delivery and volume expansion only. Therefore, a more accurate term should be red cell substitute. On the other hand, cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and WBC count, and a plasma filled with cytokine and growth factors, as well as its hypoantigenic nature and altered metabolic profile, has all the potentialities of a real and safe alternative to adult blood during emergencies due to any aetiology of blood loss. It can also prevent ischemia and eventual hypoxic triggered organ failure syndromes.

Our experience of 413 units (50ml -146 ml mean 86 m1±7.6 ml SD, median 80 ml, mean packed cell volume 48 ± 4.1 SD, mean hemoglobin concentration 16.2 Gm percent ± 1.8 Gm percent SD. After collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection) of placental umbilical cord whole blood collection after LUCS from consenting mothers and transfusing the same to 129 informed consented patients after passing through the institutional ethical committee from ist April 1999 till date produced positive results which we want to narrate here. The list included 54 male and 75 female patients. The age of the patients varied from 2 years to 86 years. 73 patients (56.58 percent) were suffering from advanced cancer and 56 (43.42 percent) patients suffered from other diseases. Three pediatric patients were less than 10 years old and one patient was more than 80 years old. 22 patients (17.05 percent) were within 60-80 years. The majority of the patients (50.38 percent) were within the 40 to 60 years age group. B+ was the commonest blood group (31.78 percent), followed by O+ (28.68 percent), then A+ (24.03 percent), and lastly AB+ (13.95 percent). O- and B- blood was transfused to one patient each. 33 units (highest) of cord blood was transfused to a patient with advanced cancer with hemoptysis (10 units of cord blood was transfused at a time), followed by 23 units to a patient with aplastic anemia (9

units of cord blood at a time), 16 units in a case of transfusion dependent thalassemic syndrome (8 units of cord blood at a time). We did not encounter a single case of immunological or non immunological reaction so far.

In case of a disaster or a battlefield scenario, it has been noted that a majority of victims die mainly due to hemorrhagic shock. However, one third of combat casualties may be salvageable if there is intervention with early blood transfusion. No country is safe from man-made or natural disasters. The 9/11 tragedy has shown that any country can be the target of a massive terrorist strike. In all these instances, one common life-saving requirement is blood,.We suggest the medical faternity to use this precious gift of nature ,which is free from infection ,hypoantigenic with altered metabolic profile,filled with growth factor and cytokine filled plasma with potentialities of higher oxygen carrying capacity than the adult blood ,as an emergency source of blood for the management of disaster or crisis of blood anywhere in the world.

Content: Umbilical cord whole blood transfusion is safe ,with altered metabolic profile and hypoantigemnic nature,has the potentialities to carry more oxygen than adult blood and does not trigger any immunological or nonimmunological reactions,therefore posseses all the goodness of a genuine blood substitute to be used in emergency.

Conflict of interest

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