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Original Article

LABORATORY CHARACTERISTICS OF UMBILICAL CORD BLOOD IN MAKURDI, NORTH-CENTRAL NIGERIA

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Abstract

Umbilical cord blood (UCB) which is useful in supportive and definitive management of inherited and acquired disorders is usually discarded in our environment. We sought to establish reference values for some haematological parameters of UCB in Makurdi, Nigeria to assist clinicians better interpret results of haematological parameters of UCB. This was a prospective quantitative study that involved analyzing UCB of qualified women to determine its complete blood count, fetal haemoglobin concentration, clotting profile and fibrinogen concentration. Reference ranges of these parameters were thereafter calculated using normal distribution method. The effects of maternal and fetal factors on these parameters were assessed using the Student t-test and ANOVA. The mean total white blood count (TWBC) was 12.3±3.7 x 10^9/L. Female births had significantly higher TWBC than male births (13.2 ± 3.3 x 10^9/L vs 11.0 ± 3.8 x 10^9/L, p=0.003). Babies that weighed 3.0-3.5kg also had significantly higher TWBC (18.9x10^9L) than those that weighed 2.4-2.9kg (8.7x10^9/L) p=0.010.Female births had significantly higher haemoglobin concentration (Hb) (13.9g/dl) than male births (11.9g/dl) p=0.001. Similarly, older women aged 32-41 years had significantly higher Hb (13.4g/dl) than those aged 18-24 years (11.6g/dl) p=0.002. Also, women that had more than two children had higher Hb than those who had one or two, (14.7g/dl vs 12.3g/dl) p=0.030. Babies that weighed 3.0-3.5kg at birth also had significantly higher Hb (16.2g/dl) than babies that weighed 2.4-2.9kg (12.3g/dl), p=0.003.The reference values of the haematological parameters of cord blood in our study were similar to what was reported from other developing countries. This study has provided data on haematological parameters of UCB for clinical use in our environment and we recommend routine UCB analysis in order to aid early detection of some inherited and congenital disorder.

***Keywords**: Haematological parameters, Makurdi, Umbilical cord blood*

Introduction

Umbilical cord blood (UCB) is a rich source of haemopoietic stem cells[1] and has been used for stem cell transplantation in the treatment of disorders like leukaemias, lymphomas and some non-haematological conditions.[2] Following the discovery that the numbers of haemopoietic stem cells (HPC) in single UCB collections were within the range associated with successful bone marrow transplant (BMT), the first umbilical cord blood transplantation (UCBT) was performed in France in 1988 in a patient who was affected by Fanconi's anaemia.[3] An analysis of the International cord blood transplant registry showed that between 1988 and 2013 more than 30,000 UCBT has been performed on patients with malignant and non- malignant disorders.[4]Some of the benefits of UCBT include the fact that the haemopoietic stem cells in UCB have an extensive proliferative capacity that exceeded that of bone marrow (BM) HPC.[5] The procedure for UCBT is less sophisticated, cheaper and UCB is readily available. Reactions and rejections are less likely because UCB is immunologically naive.
Aside its transplantation value, cord blood has been used as an alternative to blood transfusion especially in children.[6] In this regard, it can become a major

relieve to shortage of blood for transfusion in our health facilities. In Nigeria, the placenta, which is a rich source of UCB is usually discarded after birth. It has been reported that, up to 150ml of blood can be harvested from the placenta.[7] It therefore means that with a birth rate of about 6.8 million children per annum,[8] about (one billion and twenty million) 1,020,000,000 ml of cord blood is wasted annually.

Though there are many researches and publications on the efficacy and safety of umbilical cord blood for haematopoietic transplantation and transfusion, very little is published of reference ranges of its haematological values.[9] In Nigeria, there are even fewer data on haematological properties of cord blood. We therefore aim to establish the reference values for some haematological parameters of cord blood and the effects of maternal and fetal factors on these parameters in Makurdi, Nigeria in order to assist clinicians especially Neonatologist in making decision on the suitability of cord blood for either transplantation or transfusion and detection of some inherited and congenital disorders like sickle cell disorders, thalassaemias, congenital thrombocytopenias etc.

Materials and Methods

This was a prospective quantitative study that sought to measure some laboratory characteristics of UCB with the aim of establishing reference values for our local laboratory and determining how maternal and fetal factors affect these parameters. Ethical approval for this study was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi. Confidentiality of the participants was achieved by excluding their names and file numbers from the data. The study was carried out at the Benue State University Teaching Hospital, and the General Hospital Northbank, both in Makurdi, North central Nigeria. The Benue State University Teaching Hospital is a tertiary health facility while the General Hospital Northbank is a secondary health facility both in the state capital, Makurdi. These two centres were chosen because they have adequate facilities and manpower to ensure the success of the study and good number of the study population (pregnant women) is readily available at these centres. The sample population comprised of pregnant women resident in Makurdi at the time of the study. The sample size for this study was 180, which is above the recommended sample size of 120 required for reference value calculation.[10] The sample size was increased 180 to boost the power of the study. Only uncomplicated and term pregnancies were included in the study. Pregnancies with any form of complications e.g. pre-eclampsia and pre-term deliveries were excluded.

Consenting pregnant women without any obstetrics complications were recruited in their third trimester during the routine antenatal clinic visits. Systematic random sampling method which involved selecting every third qualified candidate as they present to the Obstetrician was used. Participants consented in writing after adequate informed briefing on the study.

At labor, and within 10 minutes after delivery and separation of the baby from the placenta, 8ml of cord blood was withdrawn from the umbilical cord vein using a 10ml syringe. From this, 3ml of cord blood was dispensed into an Ethylene diamine tetra acetic acid (EDTA) bottle for complete blood count (CBC) and fetal haemoglobin estimation. Four and half (4.5ml) was dispensed into sodium citrate (0.5ml) bottle for prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen concentration estimation.

Maternal weight, baby's weight and gender and mode of delivery were also taken and documented immediately after delivery.

CBC was carried out using Sysmex haematology auto-analyzer (Sysmex Corporation of America) model KX-21N. Fetal haemoglobin estimation was done using the acid–elution cytochemical method described by kleihauer *et al*.[11] These analyses were carried out within 24 hours of sample collection. The PT, APTT were determined manually according to procedure described by Lafan *et al*,[12] while the fibrinogen levels were determined by the Clauss assay method.

Data obtained from these analyses were analyzed using the statistical package for social sciences version 19. Reference values were determined using the method described by Ozarda *et al*,[13] Ceriotti *et al*,[14] and International Federation of Clinical Chemistry (IFCC) Expert Panel on Reference[15] and expressed as a mean and standard deviation. The effects of maternal and fetal factors on these parameters were assessed using the Student t-test and the one way ANOVA.

Results

Umbilical cord bloods from 180 participants were analyzed. The participants were aged 18 years to 42 years with an average age of 30.3 ± 4.2 years and weight of 64±19kg. Majority (51.1%) was civil servants and gravida 3 (39.4%). The average birth weight of the babies was 3.0 ± 0.3 kg. Male births comprised 38.3% while female births were 61.7%. The demographic characteristics of the participants and the babies are shown in table 1.

Table 1: Demographic characteristics of the mothers and babies

Parameter	Number (n)	Percentage (%)
Maternal Age(years)		
18-24	22	12.2
25-31	88	48.9
32-42	70	38.9
Maternal weight(kg)		
45-70	97	53.9
71-96	83	46.1
Occupation		
Student	45	25.0
Trader	22	12.2
C/servant	92	51.1
H/wife	21	11.7
Parity		
P0	44	24.4
P1	22	12.2
P2	71	39.4
>P2	43	24.0
Baby's gender		
Male	69	38.3
Female	111	61.7
Baby's weight(kg)		
2.4-2.9	87	48.3
3.0-3.5	93	51.7
Mode of delivery		
V/delivery	163	90.6
C/S	17	9.4

Key: C/servant-Civil servant, P0-Para 0, P1-Para 1, P2- Para 2, V/delivery-Vaginal delivery, C/S-Caesarian section

The mean total white blood count (TWBC) was 12.3±3.7 x 10^9/L. Female births had significantly higher TWBC than male births (13.2 ± 3.3 x 10^9/L vs 11.0 ± 3.8 x 10^9/L, p=0.003). Babies that weighed 3.0-3.5kg also had significantly higher TWBC (18.9x10^9L) than those that weigh 2.4-2.9kg (8.7x10^9/L) p=0.010. The values of three-part white blood cell differential were lymphocyte of 38.4 ± 10.2%, neutrophil of 53.9 ±9.3% and mixed of 7.6 ± 6.0%.
The haemoglobin concentration was significantly higher in females (13.9 ± 1.7g/dl vs 11.9 ± 0.6g/dl) p=0.005. The average fetal haemoglobin (HbF) level was 98.4 ± 2.0%. The average Mean cell volume (MCV) was 104.7 ± 11.4fl, while the Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were 32.9 ± 2.8pg and 31.5 ± 1.3g/dL respectively.
The platelet count for males was 196.5 ± 131 x 10^9/L and 224.8 ± 81.4 x 10^9/L for females (p=0.089). The platelet distribution width (PDW) and Mean platelet volume (MPV) were 16.2 ± 3.6fL and 10.3 ± 1.1 fL respectively.
The clotting profile showed a mean prothrombin time (PT) of 12.9 ± 3.7 seconds, activated partial thromboplastin time (APTT) of 44.9 ± 7.2 seconds and mean fibrinogen level of 262.6 ± 53.2mg/kg. The reference value of some haematological parameters of UCB in male and female births is as shown in table 2.

Table 2: Reference values of some haematological parameters of cord blood

Parameter	Male	Female	Average
TWBC (x10^9/L)	11.0±3.8	13.2±3.3	12.3±3.7
Hb (g/dL)	11.9±0.6	13.9±1.6	13.1±1.6
MCV (fL)	98.2±7.6	109.3±11.5	104.7±11.4
MCH (pg)	31.5±2.4	33.9±2.7	32.8±2.8
MCHC (g/dL)	32.0±1.2	31.1±1.2	31.5±1.3
Platelet (x10^9/L)	196.5±131.6	224.8±81.5	212.9±106.1
PDW (fL)	18.1±2.0	14.9±3.8	16.2±3.6
MPV (fL)	10.4±0.9	14.9±3.8	10.4±1.1
HbF(%)	98.0±1.1	98.8±2.4	98.4±2.0
PT (sec)	14.2±5.0	11.9±1.8	12.9±3.7
APTT (sec)	45.7±8.3	44.4±7.16	44.9±7.2
Fibrinogen(mg/kg)	315.8±45.1	209.4±20.2	262±53

Key: TWBC-Total white blood cell count, Hb-Haemoglobin concentration,MCV-Mean cell volume, MCH-Mean cell volume, MCHC-Mean corpuscular haemoglobin concentration, PDW-Platelet distribution width, MPV-Mean platelet volume, HbF-Fetal haemoglobin, PT-Prothrombin time, APTT Activated partial thromboplastin time.

Table 3 shows that female births had significantly higher haemoglobin concentration (Hb) (13.9g/dl) than male births (11.9g/dl) p=0.001. Similarly, older women aged 32-41 years had significantly higher Hb (13.4g/dl) than younger ones aged 18-24 years (11.6g/dl) p=0.002. Also, women that have had more than two children (>P2) had higher Hb than those who had one or two, (14.7g/dl vs 12.3g/dl) p=0.030. Babies that weighed 3.0-3.5kg at birth had significantly higher Hb (16.2g/dl) than those that weighed 2.4-2.9kg (12.3g/dl), p=0.003.
Total white blood cell count (TWBC) was significantly higher in female births 13.1x10^9/L and in those that weighed 3.0-3.5kg (18.9x10^9L) than in male births (11.1x10^9/L) and in those that weighed 2.4-2.9kg (8.7x10^9/L).
The platelet count was not affected by either maternal or fetal factors.

Table 3: Impact of maternal and fetal factors on the haematological parameters of cord blood

Parameters(n)	TWBC ($x10^9$/L)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelet ($x10^9$/L)	PDW (fL)	MPV (fL)	HbF	PT (sec)	APTT (sec)	Fibrinogen(mg/kg)
Maternal Age(yrs)												
18-24	8.9	11.6	95.8	32.5	33.9	382.0	19.9	9.0	96.0	20.0	50.0	181.3
25-31	10.9	13.1	107.8	32.9	30.6	232.6	16.2	10.7	98.2	11.9	43.2	230.4
32-41	14.6	13.4	102.8	32.9	32.0	212.9	15.5	10.2	99.2	12.6	46.0	220.5
p-value	***0.546***	***0.002****	***0.912***	***0.237***	***0.832***	***0.736***	***0.556***	***0.409***	***0.987***	***0.213***	***0.398***	***0.785***
Maternal weight(kg)												
45-70	10.5	12.4	105.6	31.9	31.4	286.7	16.9	10.5	98.7	14.6	48.3	231.6
71-96	13.3	14.5	106.6	32.7	32.8	243.2	18.5	10.2	98.6	12.3	47.8	198.6
p-value	*0.350*	*0.981*	*0.807*	*0.656*	*0.089*	*0.897*	*0.767*	*0.675*	*0.989*	*0.657*	*0.454*	*0.125*
Occupation												
Student	9.5	12.6	111.8	34.7	31.3	220.8	18.6	10.3	98.0	14.7	42.9	330.2
Business	13.8	15.0	107.4	34.9	32.5	251.0	10.0	8.8	100.0	10.0	35.0	230.4
C/servant	13.3	12.9	99.5	31.3	31.4	200.0	15.9	10.7	98.2	12.4	47.3	193.7
H/wife	12.0	14.0	116.9	36.4	31.1	242.0	17.5	9.9	100.0	14.0	44.0	320.5
p-value	***0.453***	***0.080***	***0.709***	***0.933***	***0.854***	***0.780***	***0.090***	***0.073***	***0.998***	***0.737***	***0.689***	***0.205***
Parity												
P0	12.3	13.2	99.9	32.5	32.5	229.5	19.4	10.1	98.0	15.5	51.5	160.3
P1	8.7	12.3	107.8	33.1	30.7	47.0	19.2	10.8	98.0	10.0	34.0	194.5
P2	11.1	12.2	106.2	32.8	31.1	241.1	15.5	10.1	97.9	11.9	47.9	185.4
>P2	15.9	14.7	105.2	32.2	32.2	199.9	14.3	11.1	99.2	12.0	40.9	220.5
p-value	***0.409***	***0.030****	***0.998***	***0.877***	***0.766***	***0.089***	***0.333***	***0.988***	***0.777***	***0.083***	***0.993***	***0.309***
Baby's gender												
Male	11.1	11.9	98.3	31.5	32.0	196.5	18.1	10.4	98.0	14.2	45.7	315.8
Female	13.1	13.9	109.3	33.9	31.1	224.8	14.9	10.4	98.0	12.0	44.4	209.4
p-value	***0.005****	***0.001****	***0.065***	***0.098***	***0.887***	***0.089***	***0.098***	***0.100***	***0.102***	***0.071***	***0.985***	***0.122***
Baby's weight (kg)												
2.4-2.9	8.7	12.3	107.8	33.1	30.7	229.4	19.2	10.8	98.0	10.0	34.0	302.4
3.0-3.5	18.9	16.2	110.2	32.3	29.3	205.0	19.2	12.8	99.5	10.0	44.0	198.9
p-value	***0.010****	***0.003****	***0.085***	***0.980***	***0.888***	***0.754***	***0.101***	***0.812***	***0.122***	***0.100***	***0.232***	***0.984***
M/delivery												
V/delivery	10.4	12.7	103.9	32.0	30.9	244.7	15.1	10.5	97.9	12.2	47.6	248.5
CS	11.1	12.5	98.1	32.3	33.0	288.5	15.7	9.3	97.5	17.0	44.0	320.3
p-value	*0.987*	*0.109*	*0.080*	*0.910*	*0.346*	*0.897*	*0.121*	*0.968*	*0.109*	*0.087*	*0.433*	*0.832*

Discussion

Umbilical Cord blood (UCB) is a rich source of haemopoietic stem cell for transplantation.[16] It is a good source of blood sample specimen for investigation for the neonate,[17] and can be used as a substitute for blood transfusion especially in children. Its analysis is widely accepted for generating information on genetic defects, haematological disorders and infections in neonates. Some of these disorders include sickle cell disorders, thalassaemias, thrombocytopenias, leukocytosis etc. It is therefore imperative for centers to have local reference values for UCB parameters to facilitate proper interpretation of results of UCB analyses.

The mean total white blood count (TWBC) from our study was slightly higher than what was reported in Lagos,[18] Greece[19] and in an urban city in India,[20] but less than the value recorded from a study in Karachi.[21] Variations in TWBC may result from factors such as prevalence of infection in the study area, maternal health status and the method used for analysis. These geographical variations in the blood count require that centres should establish their reference values to aid interpretation of local results. The methodology for determining the TWBC analysis in UCB is one critical factor that affects the results. For methods that do not differentiate nucleated red blood cells from white blood cells, it is necessary to carry out correction of the TWBC to obtain the actual count. In our study,

correction of the TWBC was done. This could account for the lower value we got when compared with the report from Karachi. In our study, we noted that Female births had significantly higher TWBC than males, this finding is similar to what Bain[22] reported in a related study but Adediran *et al*,[21] in Lagos did not record any significant gender disparity for this parameter.[23]The reason for the gender variations in TWBC was not easily evident and was not explained by previous studies.

The average haemoglobin concentration from this study is similar to the value from Port Harcourt[18] in Nigeria, but lower than the value reported by Ritushri *et al*,[24] in Berhampur, India. This reflects the fact that haemoglobin concentration varies with race and geographical location as has been established. The haemoglobin concentration in female births in our study was higher than what was reported by Adediran *et al*,[21] and Ritushri *et al.* Although gender is one of the factors that influence Hb concentration in new born,[25] the time of clamping of the umbilical cord is a major contributor to the Hb concentration of the UCB. In our study, the cord was clamped as soon as the baby was delivered, usually in less than 5 minutes.

The red cell indices (MCV, MCH and MCHC), fetal haemoglobin and platelet parameters (platelet count, PDW and MPV) were within the ranges reported in other studies.[18-24]An abnormally low MCV and MCH may be an indication of a genetic disorder like thalassemia and sickle cell disorders. Equally a very low UCB platelet may indicate a congenital platelet disorder like thrombocytopenia associated with absent radii. So the assessment of MCV, MCH and platelet in umbilical cord blood may serve as a screening test for some of these haematological disorders.

The results of the clotting profile (PT, APTT and Fibrinogen level) from our study were in agreement with reports from other studies.[26-27]

Our study revealed that female births, older mothers, increased parity (P>2) and babies that weighed more than 3kg had significantly higher haemoglobin concentration. The higher haemoglobin concentration noted in female births is opposite to what is obtainable in adults where males have higher haemoglobin concentrations. The reason for the higher haemoglobin concentration in adult males compared to adult females can be attributed largely to the influence of androgenic hormones on erythropoiesis. The androgenic hormone influence is absent in neonate and this may contribute to the higher haemoglobin concentrations noted in female births. Similar influence of baby's weight on umbilical cord Hb concentration was reported by other studies.[22,28] However, Vora *et al*,[29] Borna *et al*,[30] and Gadhia *et al*,[31] reported that all UCB parameters decrease with increasing parity of the women.The decrease in UCB parameters in multiparous women may be linked with deficiency of vitamins and other essential elements necessary for haemopoiesis associated with increasing parity. This has been one of the reasons for strong advocacy for family planning in our society.

Conclusion

We have been able to establish baseline reference values for interpretation of some haematological parameters of umbilical cord blood in our centre. Our values were largely similar to reports from other centers in our country. In interpreting these results, the influence of gender of the baby, maternal age and parity should be taken into consideration.

We recommend routine haematological analysis of umbilical cord blood to enable Pediatricians pick up some inherited and congenital disorders early.

Limitations

We could not carry out biochemical and microbiological investigations on the UCB due to scarcity of funds.

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