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SCREENING OF BIOCHEMICAL PROFILE OF CORD BLOOD IN COMPARISON WITH MATERNAL BLOOD

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ABSTRACT
The cord blood for the project was collected from the hospital. The sample was centrifuged for the serum was collected. The serum was subjected to various qualitative analyses. The analysis includes the determination of the values and different enzymes lide amylase, alkaline phosphatise etc. The hematocrit values were determined and also the level of RBC and WBC. The ESR of the blood was determined. The presence of antibodies was also detected. The difference between cord blood and maternal blood were studied. It was found that they differ in various biochemical parameters like creatinine, urea, uric acid, glucose, albumin, etc.

KEY WORDS: Maternal blood, Cord blood, Biochemical parameters.

INTRODUCTION

Cord blood is the blood remaining in the umbilical cord and placenta after birth. It is rich with stem cell, which are the building blocks of blood and immune system in the body. Stem cells have the ability to become many other types of cell so it is used to repair and for maintenance of body immune system and blood system also originate from stem cells. There are many convincing clinical evidences proving that stem cells from umbilical cord blood extended much further than the blood forming an immune system and that they can differentiate themselves into brain, heart, liver and bone cells. There are three types of cells in the blood stream the red cell which carries oxygen. the white cell fight against infection and platelet which help bleeding. The correct balance between each cell type must be maintained production of blood cell is controlled by natural chemical called growth factors which may be used in Treatment. The tissue which produces the blood cells. It is found within hollow cavities of bones in the body. Bone contains the stem cells from which all blood cells are derived. Examination of the bone marrow is an important part, of the diagnosis of Leukemia. The growth retardation is one of the prominent features of essential fatty acid deficiency in humans. After saponification of the isolated phospholipids, fatty acids were converted to the corresponding fatty acid methyl esters. The phospholipids were isolated by solid d-phase extraction of total lipid extracts on amino prophyl silica-columns Nino and Shaw reported that decreased maternal ascorbic acid has been associated with purgative low birth, pre enclampsia anaemia an premature rupture of the foetal membrane. The ascorbic acid level of babies from the cord was significantly higher that of the maternal serum. The concentration of EFA's was found in red blood cell membrane of cord blood. He also revealed that plasma phospholipids are present in the walls of the Umbilical artery of low birth weight neonates. In premature infants, birth weight was positively associated with the proportions of arachidonic acids and dihomolinolenic cid in plasma triacylglycerols and choline phosphoglycerides. The amino acid status was regarded as a causative factor in the observation growth restriction. Cord blood stem cells may have uses beyond transplantation. "Stem cells have long been considered optimal vehicle for gene therapy" says Wagner. The Umbilical cord plasma essential Fatty acid concentrations are positively with both maternal plasma essential fatty acid concentration and maternal dietary intake. The Diretly after parturition, a blood sample we obtained from the umbilical vein. Plasma we separated from cells by centrifugation nd stored under nitrogen at $80^{\circ}C$. Ethanol is potent modulator of lipid metabolism and is known to alter the fatty acid profiles of many organs. Young et al (2003) reported that the concentration of free amino acid in the maternal blood and umbilical cord blood are similar. The less number of stem cells are present in cord blood. The more immune cells and cord blood is very rich in early stem cells. It has the ability to differentiate into other tissues, organs and blood vessels and they can be used to treat a host of disease. The cord blood contains HSC. These prolierative

cells are about one log fewer in number than can be obtained from bone marrow or peripheral blood HSC donation, but they have greater proliferative and colony forming capacity, and are more responsive to some growth factors. Also because they are more 'naive' than proliferative cells from bone marrow, they seem to produce fewer complications associated with some aspects of HSC transplantation. The cord blood might be useful source of stem cells other than hemopoietic precursors. Reports suggest that not only are mesenchymal and neural precursor cells present but that some cord blood cells, present in extremely low frequently, may have the capacity to develop into many different lineages including cartilage, fat cells, hepatic and cardiac cells. Some transplant centres recommend cord blood collection and storage from siblings born into a family where there is a known genetic disease minable to HSC transplantation. If the cells re HLA-compatible, they may be used for the affected child. if not, they may be useable for a future HLA-compatible sibling. If the newborn child itself develops the disease, its own cord HSC may in future be useable as a vehicle for somatic gene therapy, when these techniques have been fully developed.[1-13]

MATERIALS AND METHODS

All the glass ware soaked overnight in mild detergent solution. Next day they were washed thoroughly in tap water and finally with triple distilled water. After in drying, they were wrapped in paper and sterilized in hot air oven at 180°c for an hour. Experimental specimen: we selected umbilical cord blood as our experimental specimen.

Umbilical cord

Umbilical cord, long flexible cord that allows a fetus to be nourished a sit grows and develops within the uterus, or womb. On one end, the cord attaches to the placenta. It is in the placenta that the blood vessels of the mother and fetus exchange content from each mother's circulatory systems. The umbilical cord contains two large arteries, which deliver oxygen and nutrients to the fetus from the placenta, and one large vein, which carries carbon dioxide and other wastes from the fetus to the placenta transferred to the blood stream, most of these washes are soon eliminated through the mother's excretory system. As the fetus approaches birth's the umbilical cord is about 50cm (20 in) long and has diameter of 1.5cm (0.5). Shortly aver, the cord is clamped in two places and severed between the clamps. The infant is thereby separated from the placenta with the clamps preventing unnecessary loss of blood. The small portion of the cord left attached to the baby's abdomen withers and within a week or ten days it fills off the infant's body. The resulting scar is known as the umbilicus, navel or belly button.

Collection of cord blood

Collecting cord blood is relatively simple procedure. Immediately after a baby is delivered the umbilical cord is lamped and baby is removed from the area. The needle or cannula is usually placed in the umbilical vein and the placenta is gently messaged to aid draying cord blood from the placenta. The blood flows in it by gravity, until it stops, after which the collection is complete. The collection typically takes 2 to 5 minutes. It is painless, risk free procedure for both mother and child. After collection the cord blood was frozen s whole blood system using blood collection bags to minimize the risk of contamination.

HEMETOGRAM

RBC COUNT

The blood was diluted with the RBC diluting fluid. The micropipette with red bead used must be absolutely clean and free from moisture before the blood drawn into it. The blood was drawn up to zero mark of the pipette and the RBC diluting fluid were taken up to 10.5 marks and mixed well. The counting chamber was allowed to sand undisturbed for three to four minutes. The counting chamber was placed on the microscope stage under the high power objective. The ruled area and then the area where the counting is to begin was located ie, Five central squares. The number of RBC counted was multiplied by 10,000 and gives total RBC present/mm.

TOTAL COUNT OF WBC

The blood was diluted with the WBC diluting fluid. The micropipette with white bead used must be absolutely clean and free from moisture before the blood drawn into it. The blood was drawn up to zero mark of the pipette and WBC diluting fluid were taken up to 11 mark and mixed well. The counting chamber and cover slip must be clean and free the dust. The cover slip was arranged over the ruled area of the counting chamber, using a pastuer pipette. The fluid was charged and the chamber was allowed to stand undisturbed for three to four minutes.

The counting was placed on the microscope stage under the low power objective. The ruled area and then the area where the counting is to begin was located i.e., four corner squares. The number of WBC counted was multiplied by 50 and gives total WBC present/mm3.

DIFFERENTIAL LEUCOCYTE COUNT

The differential blood count of the cord blood was made by using the method of Hudson and Hay (1991). Grease free microscopic glass slides were taken. A drop of blood and a thin smear was made. (The spreader slide was kept at 45°angle). The drop was read through the length of the slides and upwards the other end and the spreader was lifted upward. This had given a tougue shaped smear. This smear was allowed to air dry before staining. The slide was placed on the stand in a perfectly horizontal position and Leishman's stain was added completely in the surface. The stain was allowed to stand for 3-5 minutes. A few drops of buffered water were added to the meniscus to the margin. The mixture was left for three minutes. The bottom of the slide was wiped to

remove the dust and stain. The slide was examined microscopically on the oil immersion objective. The ideal position of the slide is the tail end of the smear. The leukocytes were identification and counted as appeared. The movement was shifted gradually laterly and more 100cells counted. The values of different morphological types were expressed as percentage.

Table-1: DIFFERENTIAL LEUCOCYTE COUNT

S. No	Differential count	Cord blood % level	Maternal blood % level
1	Lymphocyte	45	48
2	Eosinophils	10	1
3	Basophils	5	1
4	Neutrophils	40	50

ESTIMATION OF BLOOD HAEMOGLOBIN

To estimate the amount of haemoglobin present in the blood. The graduated tube was cleaned and fills up to the mark with N\10 Hcl. The pipette was clean and allowed to dry completely. The first drop was wiped with cotton and the blood was sucked through the micropipette and transformed into the diluted tube. A little of HCL was sucked up and added in the same tube. The graduated tube was allowed to stand for five minutes and add distilled water drop wise and mixed well 1 with stirrer, until the colour matches with reading on the gram and percentage scale are read. Haemoglobin was also determined colourimetrically using Drabkins solution.

Table-2: Estimation of blood haemoglobin

SI.NO.	Cord blood	Maternal blood
1	8.9 gm%	9gm%

ESTIMATION OF ERYTHROCYTE SEDIMENTATION RATE (ESR)

Blood is sucked in the Westergren's pipette up to 'O' mark. The Westergren pipette is mounted vertically in the stand. Noted the time and allowed the tube to stand for exactly 1 hour. Read the upper level of red cells exactly before one hour. Report the result in terms of mm/after/hr.

Table-3

SI.NO	Time duration	Cord blood	Maternal blood
1	30 minutes	10mm	9mm
2	60 minutes	2mm	18mm

HAEMATOCRIT VALUE (PACKED CELL VOLUME)

Take oxalated blood and mix thoroughly by repeated inversion and fill in wintrob's tube up to 100 mark. Centrifuge at 2500 rpm for 30 minutes. The original column of blood in the tube being 10mm. The volume of packed cells can be read directly as a percentage.

ESTIMATION URIC ACID-CARAWAY METHOD

Serum was deproteinised by tungstric acid. To the test add 3ml of protein free filterate. Then add 1ml sodium carbonate solution. Mix, then add 1ml of phosphotungstic acid. Mix and allow standing for 15 minutes and reading at 650mm.

ESTIMATION OF CREATININE- JAFFS ALKALINE PICRATE METHOD

Pipette out 0.5ml of serum deproteinized by tungstic acid. 3ml of protein Free solution was taken, to this add 0.5ml sodium hydroxide. Keep it in room temperature or 5 minutes. Read at 540nm.

ESTIMATIONOF CALCIUM- CLARK COLLIP METHOD

Serum was precipitated by 4% ammonium oxalate. 3ml of 2% ammonia was added to the precipitate add 2ml of 1 NH2SO4. For the precipitate to dissolve titrate the content in hot condition against 0.01N KMNO4. The end point is the appearance of faint pink colour.

ESTIMATION OF GLUCOSE BY GOD-POD METHOD

Pipette out 1ml of glucose reagent 10µl of serum was added. Mixed well incubate for 5 minutes at 37°c. Read absorbance against the blank.

ESTIMATION OF PRROTEIN BY BIURET METTHOD

Pipette out 1ml of biuret reagent 2ml distilled water and 0.05ml of serum was added. Incubate for 10 minutes at 37°C. Read at 555nm.

ESTIMATION OF ALBUMIN BY BIURET METHOD

Albumin binds with buffered dye reagent. 2ml of distilled water and 0.01ml of serum. Mixed well, Red at 630nm.

ESTIMATION OF INORGANIC PHOSPHROUS BY FISKE AND SUBBAROW METHOD

Serum was deproteinised by 10% TCA. Pipette out 2ml of the supernatant and make up to 5ml with distilled water. 1ml of molybdate liand 0.4ml of ANSA was added kept at room temperature for 5 minutes and read at 680nm.

ESTIMATION OF RNA-ORCINOL METHOD

Acid hydrolysis on RNA release ribose and this in presence of strong acid dehydrates to yield furfural. Orcinol reacts with furfural in the presence of ferric chloride to give a green colour. Pipette out 1ml of serum, 1ml of distilled water and 3ml of orcinol reagent was

added. Heated in boiling water bath for 15 minutes. Green colour was formed read at 665nm.

ESTIMATION OF PHOSPHOLIPID-FISKE-SUBBROW'S METHOD

Serum phospholipids are extracted with ethanolic ether and digested with perchloric acid into liberate inorganic phosphorous. The mount of phpospholipids is then calculated.

SCREENING OF ENZYMES

ALKALINE PHOSPHATASE (ALP)

Pipette 6ml of buffered substrate in 'Test' and incubate for 3 minutes at 37°c. Add 0.3ml of serum mix and continue incubation for 15 minutes. After incubation add 2,7ml of diluted folin-phenol reagent. Mix well and centrifuge pipette 4ml of supernatant add 1ml of 29% NA2CO3. Incubate for 15 minutes at 37°c.The colour was read at 680nm.

ACID PHOSPHATASE

Pipette 6ml of buffered substrate in the 'Test' incubate for 3 minutes at 37°c. Add 0.3ml of serum to the test; mix and continue incubation for 1 hour. After 1 hour add 2.7mlofdiluted folin-phenol reagent. Mix well and centrifuge pipette 4ml of supernatant add 1ml of 20% NA2CO3. Incubate to 15 minutes at 37°c.The colour developed was read at 680nm.

ASPARATATE TRANSAMINASE (AST)

Add 1mlof buffered substrate and 0.2ml of serum to test and incubate the tubes at 37°c for 1 hour. After 1 hour add 2 drops (0.07ml) of aniline citrate reagent and add 1ml dinitro phenyl hydrazine and wait for 20 minutes. Finally add 10ml of 0.4N NAOH. The colour developed was read at 520nm .

AMYLASE

Take 1ml of buffered starch substrate. Add 0.1ml of 1 in 10 diluted serum to the test. Incubate at 37°c for 15 minutes. Add 0.4ml of working water and 0.1ml of serum. Mix well and read immediately at 600nm.

PROTEASE

Mixed 1ml of serum and 1ml of caesin substrate which is prepared in phosphate buffer of pH 6.9. Incubate at 45 °c for 20 minutes.

Table-4 Level of biochemical parameters

SI.NO	Biochemical parameters	Cord blood level	Maternal blood
1	Glucose	8mg/dl	90mg/dl
2	Protein	10.74g/dl	7gm/dl
3	Albumin	5.33G/DL	4gm/dl
4	A/G ratio	.99	1.33
5	Creatinine	1.33mg/dl	0.9mg/dl
6	Uric acid	2.11mg/dl	3mg/dl
7	Urea	4mg/dl	18mg/dl
8	RNA	1.868mg/dl	8.406mg/dl
9	Phospholipids	740mg/dl	340mg/dl

Table-5 shows the result of electrolytes

SI.NO	Electrolytes	Cord blood level	Maternal blood level
1	Calcium	10mg/dl	10mg/dl
2	Inorganic phosphorous	14.2mg/dl	3.5mg/dl

RESULTS AND DISCUSSION

In the present study, there was no significance difference between maternal blood and cord value. However the slight decreases observed in the values of hematogram, biomolecules (urea, uric acid, creatinine, blood urea nitrogen) in the cord blood. But in the maternal blood there was slight increase observed in the values of glucose, protein,ribonucleic acid. There was slight but significant decrease in the PCV (packed cell volume), haemoglobin, MCHV was observed in maternal blood. But moderately higher values of platelets, esinophils and WBC were found in cord blood. The enzyme activity of alkaline phosphatise was higher in cord blood. It may be due to its increased utilization in the formation of bone cells. The activity of other enzymes was found to be lower it may be due to non utility by the fetal (because most of the liver enzymes were active only after the complete functioning of the liver cells). Increased level of antibody gives a clear indication for increased immunity in the maternal blood. The result of present study indicated that need to regulatory monitor the bioactive compounds status of pregnant women thereby making cord blood as values as possible.

Table-6: LEVEL OF ENZYMES

SL.NO.	Enzymes	Cord blood level	Matenal blood
1	Alkaline phosphatase	13.3KA units	9KA units
2	Acid phosphtse	.375 KA units	1.1KA units
3	Aspartate transaminase	7.21 units	12 IU
4	Alanine transaminase	13.05 IU	14 IU
5	Amylase	105.8 I units	276 IU

Table -7 LIPID PROFILE

S. No	Components	Cord blood level	Maternal blood
1	Total Cholesterol	105mg/dl	175mg/dl
2	HDL Cholesterol	26mg/dl	51mg/dl
3	LDL Cholesterol	23mg/dl	61mg/dl
4	Triglycerides	115mg/dl	85mg/dl
5	VLDL Cholesterol	23mg/dl	29mg/dl

Table -8 SHOWS THE LEVEL OF BLOOD CELL COUNTS

S. No	Blood cells	Cord blood level	Maternal blood
1	RBC	4×10^6 Cells/cubicmm	5×10^6
2	WBC	11,200 Cells/cubicmm	8000cells/cubicmm

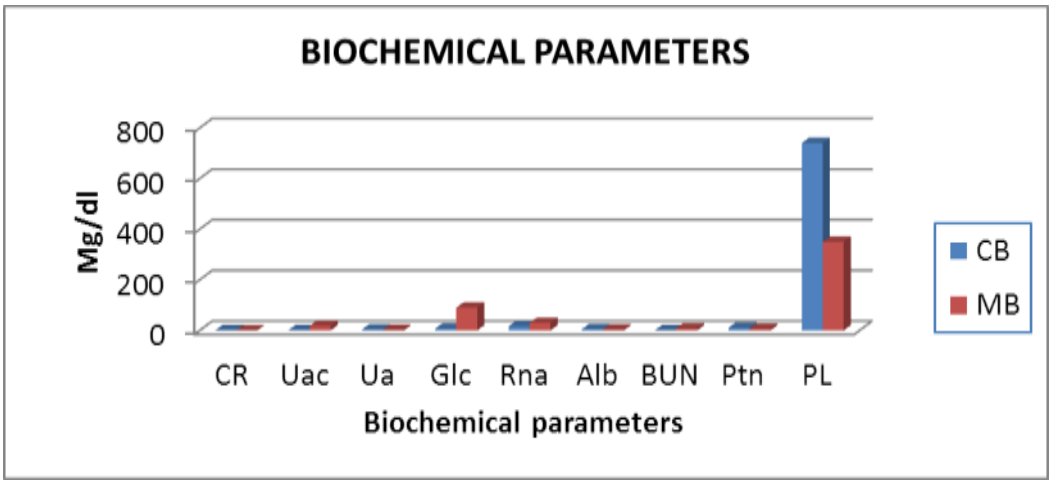
Table-9: Levels of Haematocrits

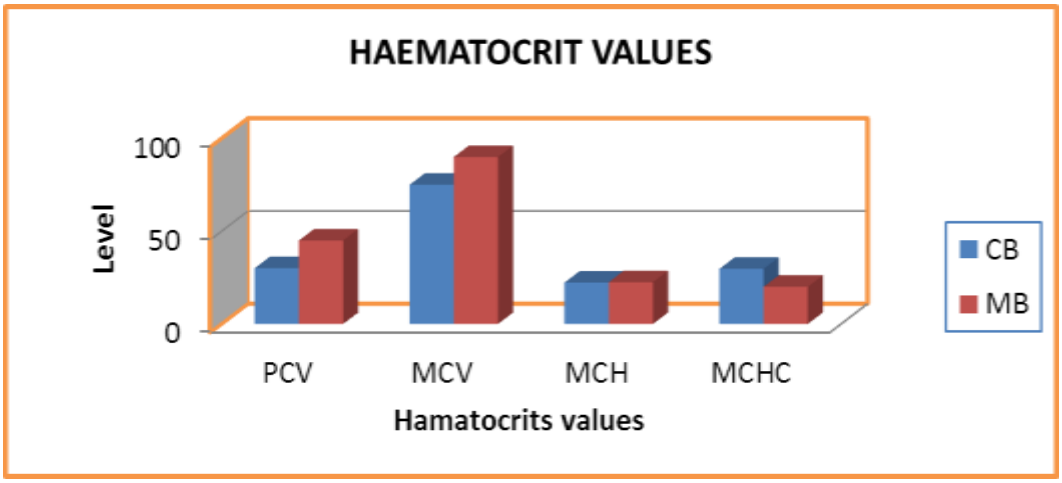
S. NO	Haematocrits	Cord blood level	Maternal blood
1	PVC	30%	45%
2	MCV	7 cubic microns	90 cubic microns
3	MCH	22.5 micro grams	22.micro grams
4	MCHC	29.66%	20%

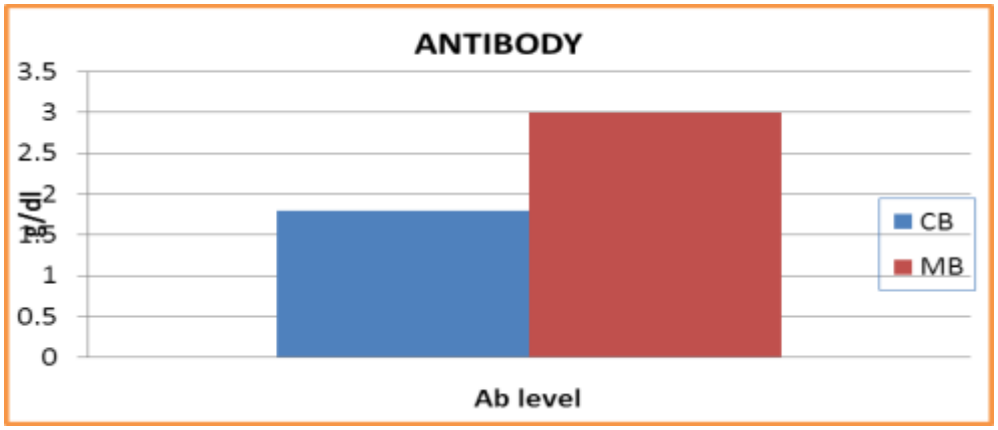
Table- 10: LEVEL OF IMMUNOLOGICAL PARAMETERS

SL.NO.	Immunological parmeters	Cord blood level	Maternal blood
1	Antibody	1.8m/dl	3gm/dl

	CR	Uac	Ua	Glc	Rna	Alb	BUN	Ptn
CB	1.33	2.11	4	8	16.8	5.33	1.868	10.74
MB	0.9	18	3	90	30	4	8.406	7



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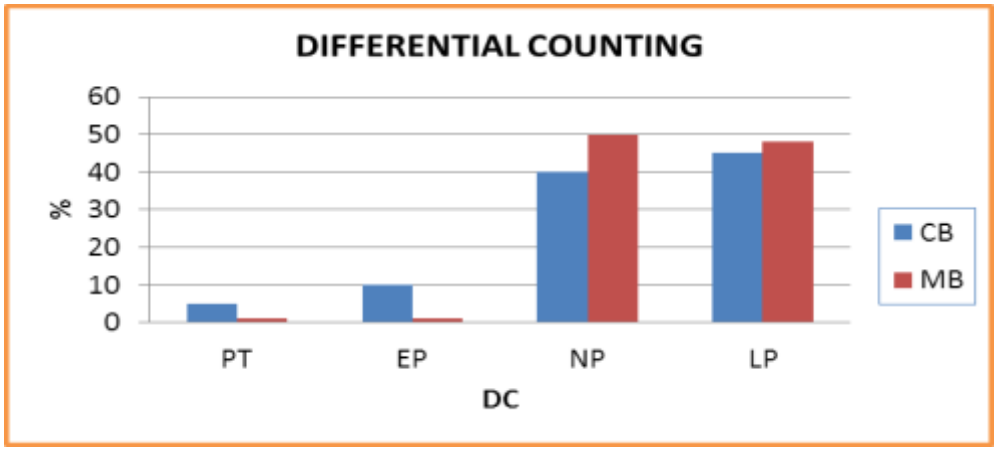
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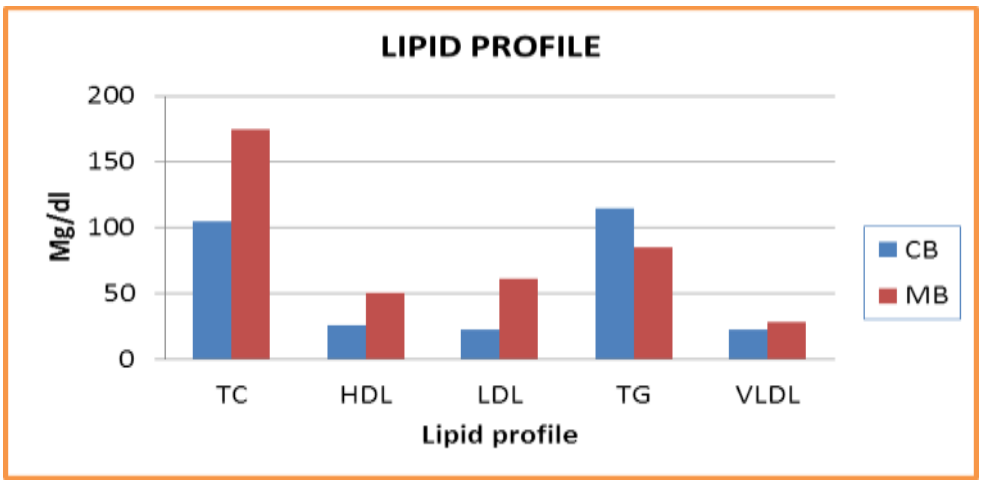
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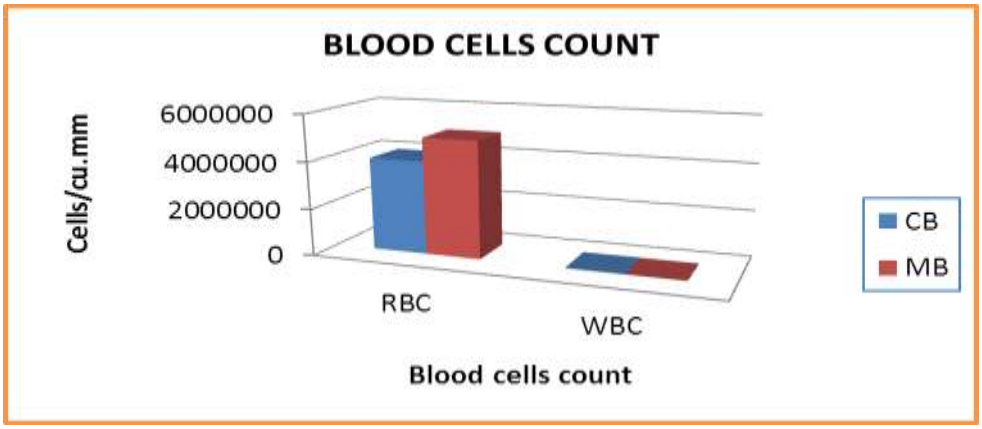
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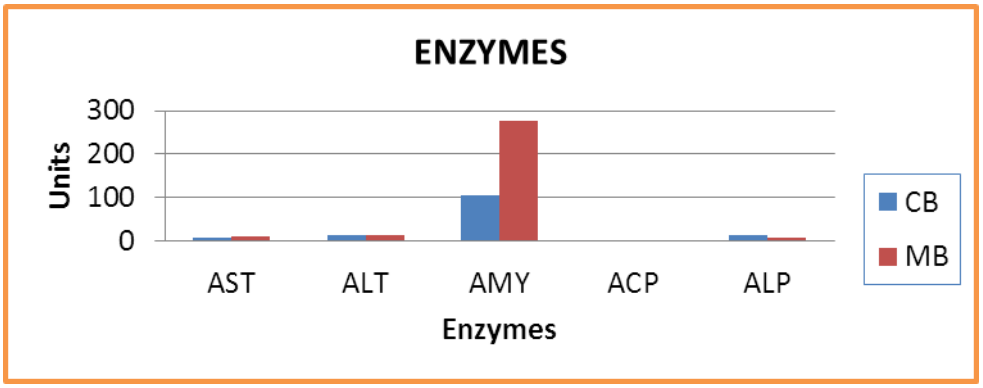

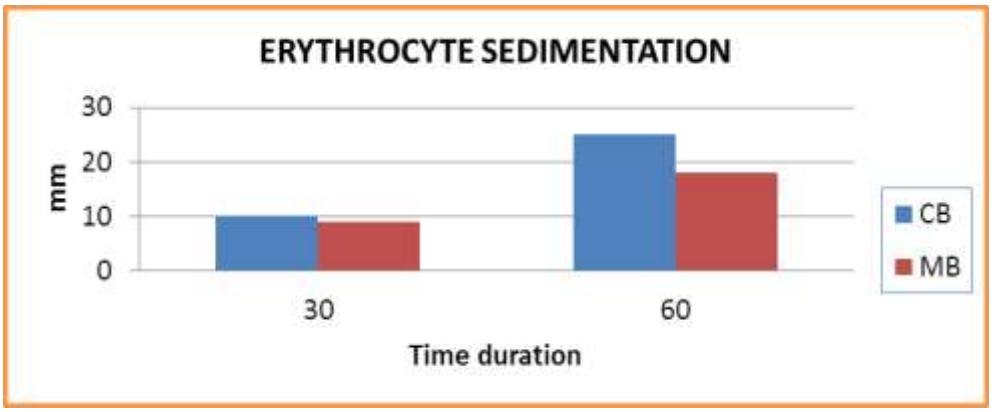

Figure 1-9: various parameters comparisons cord blood and maternal blood.

CONCLUSION

The report on screening of biochemical profile of cord blood in comparison with maternal blood was analysed. The all the haematological parameters like Hb, Total count, differential count and haematocrits and lipid profile, enzyme parameters shows the good difference between the two types of blood. It will very helpful for the medical field.

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