

## Evaluation of hematological profile of cord blood and placental histopathology in neonates with perinatal asphyxia.

Feroz Alam, Mehar Aziz, S. Manazir Ali\*, Seema Hakim\*\*, Nishat Afroz

Department of Pathology, Pediatrics\* and Obstetrics & Gynaecology\*\*, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh 202002, U.P, India.

**Keywords:** Hematological profile, cord blood, placental, neonates, perinatal, asphyxia

*Accepted February 23 2012*

### Introduction

Perinatal asphyxia (derived from Greek word *a-sphyxos* meaning born without an evident pulse) is one of the most important causes of fetal distress [1]. Perinatal asphyxia remains a significant cause of perinatal morbidity and mortality the world over, and is known to complicate upto 5 – 10% of all deliveries [2]. A wide variety of maternal, fetal and labor conditions give rise to perinatal asphyxia. Diagnosing perinatal asphyxia is important because of the potentially avoidable nature of the lesions, but due to the complex pathophysiological mechanisms involved in causing perinatal asphyxia, an early diagnosis becomes difficult. A wide variety of clinical parameters and laboratory tests are employed to diagnose perinatal asphyxia, like Apgar's score, umbilical arterial acidemia/base excess, intrapartum electronic fetal monitoring, fetal scalp pH measurement and presence of meconium in amniotic fluid. However, to date no single marker of perinatal asphyxia has shown good predictive efficacy and only a combination of various indices can help in an early diagnosis of perinatal asphyxia. Recently nucleated red blood cells (nRBC's) have been reported as a marker of perinatal asphyxia [3].

### Material and Methods

This study was carried out prospectively in Department of Pathology in collaboration with Department of Obstetrics and Gynecology and Neonatal Intensive Care Unit (NICU), of Department of Pediatrics.

Detailed antenatal and perinatal history was taken, and all the newborns were examined to assess the 5 minute Apgar's score. The study was carried out on 50 neonates clinically suspicious of having asphyxia (study group), while 15 healthy neonates were taken as control. Singleton, term ( $\geq 37$  weeks of gestation) neonates who were vaginally delivered with no maternal and neonatal complications during and after delivery having a 5 minute Apgar's score 9-10, an umbilical artery pH  $> 7.20$  were

Many acute and chronic diseases cause increase in the number of circulating nRBC's, such as premature delivery, maternal diabetes mellitus, anemia in pregnancy [4], pregnancy induced hypertension. Fetal causes can be growth retardation (SGA), congenital heart diseases and premature delivery [5].

In tropical countries like India, the cause of neonatal asphyxia may also be due to poor maternal health. In such countries nutritional state of the mother during pregnancy, deficiency diseases, parasitic infestations, pregnancy induced hypertension (PIH) diabetes mellitus are more prevalent. Most patients are inadequately treated because of lack of proper basic healthcare facilities, their absence during follow-up and non-compliance to the health care advice. Placental insufficiency is also very common in rural population because of illiteracy, traditional habits, taboos, poor personal hygiene, excess physical work, low socio-economic status and non availability of nutritional supplements necessary during pregnancy. This study was carried out with an aim of correlating hematological profile of neonates cord blood with placental lesions in cases of perinatal asphyxia, with special emphasis on detailed gross and histopathological examination of placenta.

included in control group whereas neonates in study group were those who had a pH  $< 7.20$ , 5 minute Apgar's score of  $< 6$ , and/or a variability in Fetal heart rate (FHR). The study group was further subdivided into acute fetal distress (AFD-37 cases) and chronic fetal distress (CFD-13 cases). Mothers of neonates in the AFD group had a normal antenatal period and only just before or during delivery, the acute fetal distress (determined by FHR tracings) developed, because of various reasons such as cord occlusion, hypertonic uterus, premature rupture of membranes, abnormal presentation or prolonged delivery etc. In the CFD group there were women known to have disorders of placental insufficiency (pregnancy induced hypertension and diabetes) and showing the signs of chronic fetal hypoxia, such as abnormal fetal heart rate tracings during antenatal period, intra-uterine growth re-

tardation(IUGR),oligohydramnios etc. The criteria of pH, Apgar's score & FHR variability were only strictly followed in cases of acute fetal distress group and not in chronic fetal distress group.

At delivery the umbilical cord was double clamped and 2 ml of umbilical venous blood was collected and hematological tests were performed including hemoglobin estimation, total leukocyte count, nRBC count / 100 WBC's (cord blood smear examination) and nRBC count/cumm of blood. pH estimation was done in 2 ml umbilical arterial blood collected in preheparanized syringe. The pH analysis was done by arterial blood gas analyzer (ESCHWEILER,COMBISYS II)

Placenta was collected in formalin from labor room and later processed for histopathological examination. After gross examination four sections were taken routinely(1cord,1membranes,2parenchyma) and additional sections were taken from any grossly visible lesion if present. The sections were stained routinely with Haematoxylin & Eosin , Reticulin and van-Gieson stain.

All the statistical analysis was done on software Statistical Package for Social Sciences (SPSS) version 10.Students t – test was used to compare statistically the

value of nRBC's/100 WBC's between the control and both the acute fetal distress group and chronic fetal distress group separately ,and a value of  $p<0.001$  was taken as significant.

## Results

### Cord blood nRBC's count

In the present study  $2.93 \pm 1.10$  nRBC's/100WBC's or differently expressed  $288.33 \pm 76.64$  nRBC's/cumm of blood were found in control group and these values were well within normal limits. In the AFD group  $20.00 \pm 12.92$  nRBC's/100WBC's or  $2221.78 \pm 1407.24$  nRBC's/cumm of blood were found, and these values were significantly higher ( $p<0.001$ ) than the values of control group. In the CFD group (known to have chronic utero-placental insufficiency)  $25.64 \pm 15.28$  nRBC's/-100WBC's or  $3219.00 \pm 2449.61$  nRBC's/cumm of blood were found, and these values were also significantly higher( $p<0.001$ ) than control group. Thus both AFD & CFD cases exhibited increased nRBC count,but no statistically significant difference was seen between the AFD and CFD groups ( $p>0.01$ ).

**Table 1.** Showing Values of different hematological indices and statistical Significance in cases and controls

Group	Hemoglobin (gm/dl) Mean $\pm$ S.D	TLC (per cumm of blood) Mean $\pm$ S.D	nRBC/100WBC Mean $\pm$ S.D	nRBC/cumm of blood Mean $\pm$ S.D
Control (n=15)	17.33 $\pm$ 1.65	10400.00 $\pm$ 2904.43	2.93 $\pm$ 1.10	288.33 $\pm$ 76.64
Acute fetal distress (AFD,n=37)	16.45 $\pm$ 2.57	11640.54 $\pm$ 3629.39	20.00 $\pm$ 12.92	2221.78 $\pm$ 1407.24
Chronic fetal distress (CFD,n=13)	16.43 $\pm$ 3.03	12118.18 $\pm$ 2998.94	25.64 $\pm$ 15.28	3219.00 $\pm$ 2449.61
AFD:Control	–	–	$p<0.001$ (significant)	–
CFD:Control	–	–	$p<0.001$ (significant)	–
CFD:AFD	–	–	$p>0.1$ (not significant)	–

**Table 2.** Showing results of histopathological lesions of placenta in control and cases

Group	Infarct	Fibrosis	Fibrinoid necrosis	Increased syncytial knots	Increased villous vascularity	Thrombosis of fetal ves- sels	Intervillous thrombus+fibrin plugs	Intervillous oedema
Control (n = 15)	1	2	-	1	-	-	1	-
AFD (n = 37)	4						;	4
CFD (n = 13)	9	8	11	11	10	5	4	11

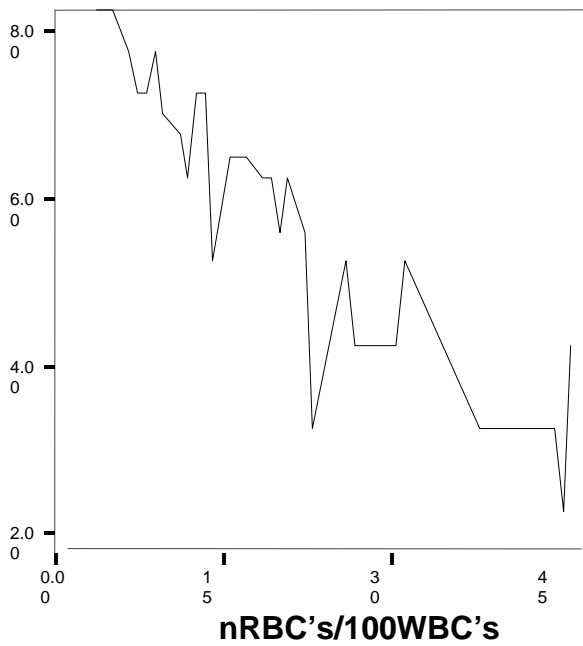


Figure 1. Showing near inverse correlation between nRBC's and 5-min APGAR'S score

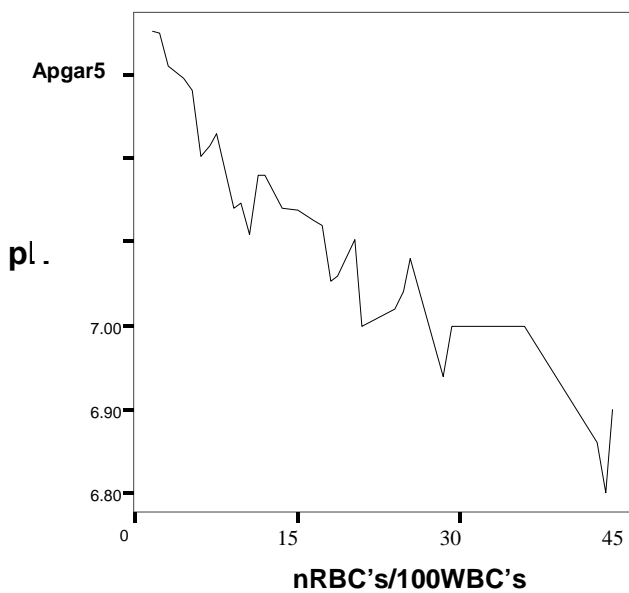


Figure 2. Showing near inverse correlation between nRBC's and Umbilical arterial pH

Table I shows the Mean  $\pm$  S.D (standard deviation) of various hematological parameters (hemoglobin, total leucocyte count - TLC, nRBC's/ 100WBC's and nRBC's/cumm of blood) in AFD and CFD groups. The correlation between 5 min – Apgar's score and nRBC's/100 WBC's (of both control and study group),

was  $r = - 0.90$ ,  $t = 16.5$  and  $p < 0.001$ , thus 5 min Apgar's score and nRBC count were significantly correlated. The graph in the Figure1, shows the variation of nRBC's/100 WBC's with 5 min – Apgar's score. The correlation between umbilical arterial pH and nRBC's/100 WBC's (of both study and control group), was  $r = -0.91$ ,  $t = 16.5$  and  $p < 0.001$ , thus umbilical arterial pH and nRBC count were significantly correlated. The graph in the Figure2 shows the variation of nRBC's/100 WBC's with umbilical arterial pH.

No statistically significant relationship was seen between nRBC count and sex, mode of delivery and hemoglobin concentration of the neonate.

### Observations of Placental Examination

The placentas of study and control group were examined grossly and microscopically .On gross examination, the placenta of CFD group were found to be slightly lower in weight and in gross measurement in comparison to the control group. No significant difference in weight/-measurement was seen on gross examination of placenta of AFD and control groups.

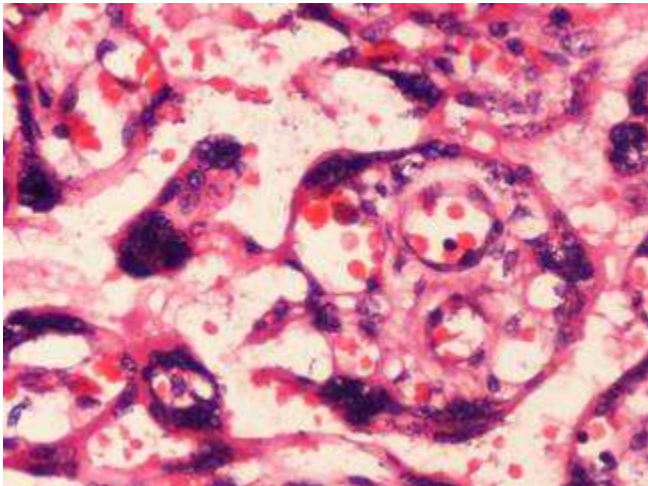
Histopathological examination of placenta was also done in all the cases of study and control group, placenta of neonates in AFD group showed fewer pathological changes, whereas these changes were marked in CFD group(as enumerated in table II ),all pointing towards the chronic utero-placental insufficiency thereby leading to fetal nutrient and oxygen deprivation.

The most notable findings of histopathological examination were, infarction of villi (Fig. 1), increased syncytial knots, increased villous vascularity (Fig. 2) intervillous thrombus and fibrin plugs (Fig. 3), intervillous edema, fibrosis and prominent fibrinoid necrosis of the villi (Fig. 4).

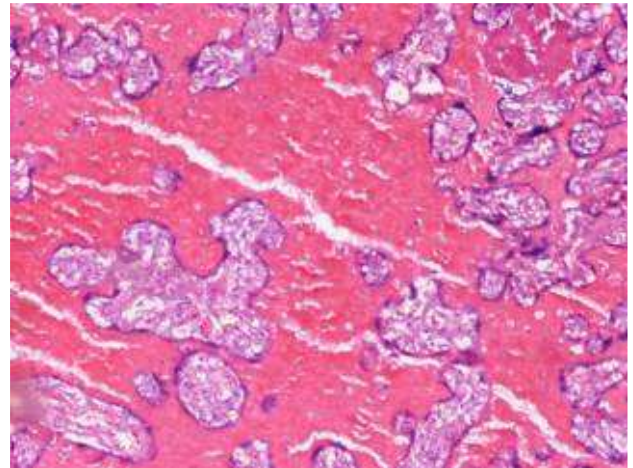
---

The study was a purely institutional work and no grants were received from anywhere.

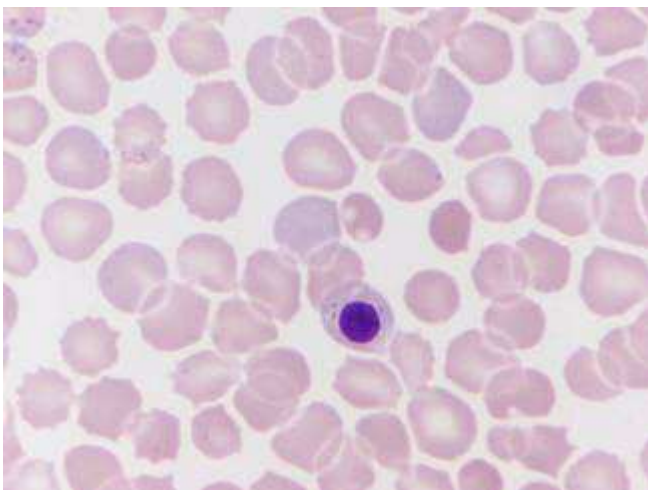
---



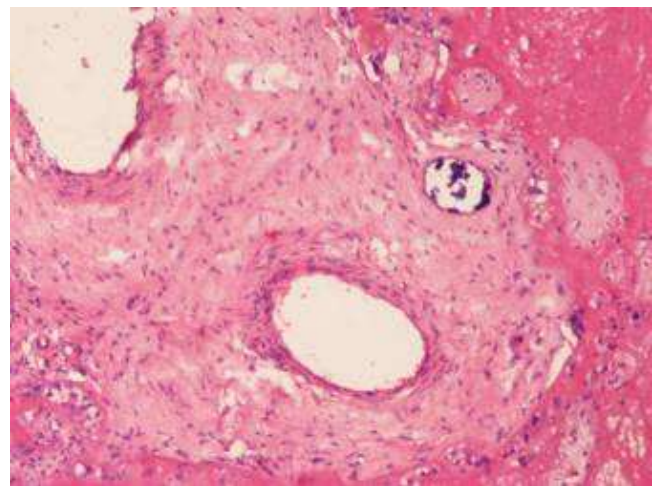
**Figure 3.** Increased syncytial knots (x400 H&E)



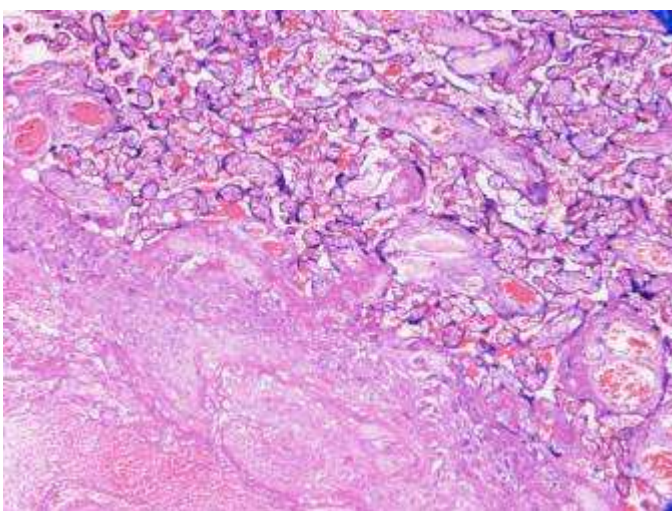
**Figure 6.** Intervillous thrombus (x100 H&E)



**Figure 4.** nRBC and polychromatic cells (Leishman's x100)



**Figure 7.** Fibrosis of villi and calcification(x100 H&E)



**Figure 5.** Infraction (x40 H&E)

## Discussion

In normal newborns less than 10 nRBC's are found per 100 white blood cells (WBC's) or about 500 nRBC's/cumm of blood are within normal limit [6]. Utero-placental insufficiency leading to hypoxia is a very significant cause. Fox commented that the number of nRBC's is "a rough guide to the degree of oxygen deprivation" [3]. Hypoxia can be acute or chronic, however the mechanism leading to raised nRBC's in both the conditions may be different [8].

In the largest study on nRBC's Hanlon-Lundberg [5] found 9 cases out of 1112 having >100 nRBC's/100 WBC's and said that these cases were *enigmatic* because 8 cases out of these 9 had an uneventful antepartum, intrapartum and neonatal courses, they mentioned that no reason was found for such marked elevations. One of the case was born to a diabetic mother & was admitted to

NICU for treatment of ABO incompatibility. According to Hermansen [6] 1-2% of apparently normal newborns have idiopathic increase in nRBC's, he also quoted that Naeye & Localio reported 2.4% of such outliers and Green & Mimouni found 5% of such outliers. In all these studies such markedly increased levels of nRBC's were said to be enigmatic/idiopathic. With due respect to all these experienced researchers all of them turned a blind eye to such markedly increased levels, but the question was that if nRBC's reflects asphyxia then how these neonates with such markedly increased levels had no signs/symptoms of fetal distress??. The studies become self contradictory!!

However in the present study in few cases there was marked raised nRBCs which may be because of chronic uteroplacental insufficiency.

In the last decade so many researches have been done to prove that nRBC's are a very reliable marker of perinatal asphyxia and all of them showed an inverse relationship between nRBC's and so called direct markers of asphyxia (pH & Apgar's score), but not a single study showed that the relationship is exactly linear (which theoretically should be according to the results of all these studies), then what was preventing the relationship to become exactly linear[9]? There definitely was a very significant cause and which was continuously being missed.

A significant conclusion is the **role of placenta** in the newborns health/disease status, and this important fact was continuously being missed.

These newborns must be having chronic utero-placental insufficiency, since the insufficiency in such cases is of very long duration, the fetus adapts to this long duration of placental insufficiency and shows no signs/symptoms of distress, but the fetus doesn't fight with this severe distress by dangerous mechanisms (increasing acidosis & FHR variability - this is the reason for normal pH & Apgar score) instead the fetus moves into a safer mode and develops another mechanism to fight this long duration and severe insufficiency (which is effective in protecting and rescuing the fetus), by raising its capacity to produce erythrocytes and also releases less mature RBC's (the nRBC's) into circulation to overcome this severe chronic hypoxia. This mechanism is mediated via erythropoietin and has already been proved by Ferber in 2004 [10]. Later in 2005 Ferber [11] found that IL-6 is the mediator that is responsible for increased nRBC's in cases of acute asphyxia.

Again with due regards to all and especially Mr Hanlon & Lundberg [5], they themselves solved these puzzles in their study where they found that one of those enigmatic neonates was born to a diabetic mother (the disease is well known to cause utero-placental insufficiency) and was

treated in NICU for ABO incompatibility. It is a very well known fact that ABO mismatch in newborns causes only mild hemolysis and this mild hemolysis cannot cause nRBC's to rise to the extent >100/100 WBC's. So, the real culprit was maternal diabetes which caused chronic utero-placental insufficiency and placed the neonate into that enigmatic group.

According to Browne and Veall the maternal utero-placental blood flow is decreased in pre-eclampsia [13]. According to Thompson fetal hypoxia is not uncommon near term and accordingly it may lead to fetal distress and fetal death [14]. Naeye and Friedman calculated that 70% of the excess fetal deaths in women with hypertension are due to large placental infarcts and markedly small placental size [15]. Budliger studied 95 placentas and found that infarcts were slightly more common and larger in PIH than in controls [16]. Wentworth examined 679 placentas and found infarcts in 67% cases of severe toxemia [17]. Wallenburg also found a significant increase of infarcts in toxemia [18].

Tenney and Parker [19] emphasized that the increased budding of placental syncytium is characteristic of pre-eclampsia. It shows as bunching of syncytial cytoplasm and agglomeration of nuclei, which produces characteristic knots at the villous surface. Although some such knots are normally found in preterm and term placentas, the number of these syncytial knots is found to be much increased in cases of toxemia. Tominaga and Page attributed these morphologic changes in placenta to hypoxia [20].

Similar to the observation in our study Mathews and Masodkar also observed that fibrinoid necrosis was more common in pre-eclampsia [21,22].

Similarly fibrosis, thrombosis of fetal vessels, intervillous thrombus and fibrin plugs, have all been documented to get increased in cases of neonatal asphyxia [23].

Thus the findings of this study may have important implications in determining the etiology and pathological lesions leading to the compromised state of the neonate (acute or chronic) and placental study should never be undermined in compromised newborns.

Hence, it could be summarized from the above mentioned discussion that few nRBC's are present in normal newborns and an increase in nRBC count in cord blood is highly suggestive of perinatal asphyxia (acute or chronic), and are even much more specific than Apgar score & pH (especially in chronic asphyxia). A significant fact which is missed in all the studies on nRBC's before, is that placental lesions play a very vital role in the development of chronic asphyxia and this change in histomorphology of placenta is responsible for marked increase in

nRBC count in chronic asphyxia. Nevertheless a simple, cheap, rapid and non-invasive test of nRBC count, obtained from an otherwise discarded sample of cord blood provides valuable information about the well being of the newborn, and correlates well with Apgar score, umbilical arterial pH (in acute fetal distress) and placental histomorphology (especially in chronic fetal distress). Thus this study may be helpful in post natal monitoring and management of maternal and neonatal health, planning and managing subsequent pregnancies, and preventing perinatal asphyxia in future pregnancies if any specific cause/lesion is found in the placenta itself.

## References

1. Steer PJ, Danielian P. Fetal distress in labour. In James, Steer, Weiner, Gonik : High risk pregnancy III<sup>rd</sup> edition W. B Saunders 2005 ; pp 1450-1471.
2. Low JA. The role of blood gas and acid base assessment in the diagnosis of intrapartum fetal asphyxia. Am J Obstet Gynecol 1988; 159: 1235-1240.
3. Phelan JP, Ahn MO, Korst LM, Martin GI. Nucleated red blood cells - A marker for fetal asphyxia? Am J Obstet Gynecol 1995; 173: 1380-1384.
4. Saracoglu F, Sahin I, Eser E, Gol K, Turkkani B. Nucleated red blood cells as a marker in acute and chronic fetal asphyxia. Int. J Gynaecol Obstet 2000; 71: 113-118.
5. Anderson GW: Studies on the nucleated red cell count in the chorionic capillaries and the cord blood of various ages of pregnancy. Am J. Obstet Gynecol 1941; 42: 1-14.
6. Hermansen M C: Nucleated red blood cells in the fetus and newborn. Arch Dis Child Fetal Neonatal Ed 2001; 84 : F211-F215
7. Hanlon-Lundberg KM, Kirby RS, Gandhi S, Broekhuizen FF. Nucleated red blood cells in cord blood of singleton term neonates. Am J Obstet Gynaecol 1997; 176: 1149-1156.
8. Ferber A, Frindel Z, Brenner AW, Minior VK, Divon MY.: Are elevated fetal nucleated red blood cell counts an indirect reflection of enhanced erythropoietin activity? Am J Obstet Gynaecol 2004; 190: 1473- 75(s).
9. Ferber A, Minior VK, Bornstein E, Divon MY. Fetal "nonreassuring status" is associated with elevation of nucleated red blood cell counts and interleukin-6 Am J Obstet Gynaecol 2005; 192: 1427-1479(s).
10. Ghosh B, Mittal S, Kumar S, Dadhval V. Prediction of perinatal asphyxia with nucleated red blood cells in cord blood of newborns. Int J Gynecol Obstet 2003 ; 81: 267-271.
11. Ferns SJ, Bhat BV, Basu D. Value of nucleated red blood cells in Predicting severity and outcome of Perinatal asphyxia. Indian J Pathol Microbiol. 2004; 47(4): 503-505.
12. Benirschke K. The placenta: how to examine it and what you can learn? Contemp Obstet and Gynaecol. 1981; 17: 117-119.
13. Browne JCM, Veall N. The maternal blood flow in normotensive and hypertensive women. J Obstet Gynaecol of British Empire 1953; 60: 141-147.
14. Thomson AM, Billewicz WZ, Hytten FE. Placenta in relation to birth weight. J. Obstet Gynaecol of British Commonwealth. 1969; 76: 865-872.
15. Naeye RL, Friedman EA: Causes of perinatal death associated with gestational hypertension and proteinuria. Am J. Obst and Gynecol 1979; 133: 8-11.
16. Budliger H. Plazentarveränderungen undirher Beziehung Zur Spattoxikose und Perinatalen Kindlichen Sterblichkeit. Fortschr Geburtshilfe Gynakol 1964 17 : 86-110.
17. Wentworth P. Placental infarction and toxemia of pregnancy. Am J Obstet Gynecol 1967; 99: 318 – 326.
18. Wallenberg HCS: Über den Zusammenhang Zwischen Spatgestose und Placentarinfarkt. Arch Gynakol 1969; 208: 80 -90.
19. Tenney B, Parker F. The Placenta in toxemia of pregnancy. Am J Obstet Gynecol 1940; 39: 100-1005.
20. Tominaga T, Page EW: Accommodation of the human placenta to hypoxia. Am J Obstet Gynecol 1966; 84: 679-691.
21. Mathews R, Aikat M, Aikat BK : Morphological study of placenta in abnormal pregnancies. Indian J Path Bact 1973; pp 15-24.
22. Masodkar AR, Kalamkar LR, Patki DS. Histopathology of placenta and its correlation with fetal outcome. J. Obst. Gynaecol India 1985; 35 : 294.

## Correspondence to:

S. Manazir Ali  
 Department of Pediatrics  
 Jawahar Lal Nehru Medical College and Hospital  
 Aligarh Muslim University  
 Aligarh 202002 (U.P.)  
 India

