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STUDY OF CHANGES IN HAEMOGLOBIN LEVEL AND LEUKOCYTE COUNTS IN CORD BLOOD OF NEWBORNS OF HYPERTENSIVE MOTHERS IN AGMC AND GB PANT HOSPITAL

Evalyn Singnarpi¹, Debanjan Bhattacharjee², Jahar Lal Baidya³, S. N. Naithok Jamatia⁴

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ABSTRACT: AIMS AND OBJECTIVES: To observe the change in haemoglobin level and leukocyte counts in the cord blood of neonates born of hypertensive mothers by comparing it with the haemoglobin level and leukocyte counts of cord blood of neonates born of healthy mothers. **MATERIALS AND METHODS:** A Prospective observational study was carried out among thirty newborns of hypertensive mothers and thirty apparently healthy newborns of normotensive mothers in, West Tripura. Haemoglobin level was estimated by Sahli's method and total Leukocyte count (TLC) was done using Improved Neubauer's Counting chamber. Absolute counts were obtained by doing Differential Leukocyte Count (DLC) and the value obtained from TLC. For DLC, slides were stained with Leishman's stain. Statistical analysis was carried out using t-test for the comparison of differences of the means by SPSS 15.0. **RESULTS:** Haemoglobin level was found to be significantly higher in the study group as compared to control group. Leukopenia, neutropenia, lymphopenia and anaemia were found in 10%, 3.3%, 13.3% and 23.3% subjects respectively in the study group. Leukopenia, lymphopenia and anaemia were also found in 6.6%, 10% and 56.6% subjects respectively in the control group. **CONCLUSION:** A significant haematological changes occur in the newborns of hypertensive mothers and further research need to be carried out to find the relation between hypertensive disorders and changes in haematological parameters of the newborns. **KEYWORDS:** Cord blood, Haemoglobin percentage, Leucocytes count, Hypertensive mothers.

INTRODUCTION: Hypertensive disorders of pregnancy affect about 10% of all pregnant women all over the world. It is an important cause of morbidity and mortality among mothers and babies.^[1] Hypertension during pregnancy can be classified into four categories: 1) preeclampsia-eclampsia, 2) chronic hypertension(of any cause) ,3) chronic hypertension with superimposed preeclampsia, and 4) gestational hypertension.^[2] The uteroplacental unit is one of the organs which is affected by capillary damage in hypertensive pregnant women.^[3]

The newborns of hypertensive mothers have significantly higher incidence of somatic growth retardation, low Apgar scores, delayed adaptation, patent ductus arteriosus and gastrointestinal hypomotility and have a spectrum of haematological changes.^[4,5] The haematological changes include polycythaemia, thrombocytopenia, leukopenia, neutropenia.^[3,4,6]

The haemoglobin level was found to be higher in some studies and also lower in the other of the neonates of hypertensive mothers as compared to neonates born of normotensive mothers.^[3,4,5] Total leukocyte count and neutrophil count was found to be lower in this group.^[3,4,5,7] Also monocyte and eosinophil count was found to be less in this group.^[3] Erythropoiesis increase in infants can be due to uteroplacental insufficiency.^[3,8,9]

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This can give rise to polycythemia and can lead to impairment in tissue oxygenation and perfusion and formation of microthrombi.^[6] Neutropenia is well documented in this group.^[3,4,10,11,12,7] This can be associated with infection and sepsis.^[3,10,12] Studies show multiple mechanisms leading to decrease in neutrophil count.^[3,13]

A number of pregnant mothers in the state of Tripura suffer from hypertension. In this study, cord blood was used to assess the Haemoglobin level (Hb) and leukocyte counts including Total Leukocyte Count (TLC) and Absolute Counts for different leukocytes of newborns of hypertensive mothers constituting the study group and compare it with that of newborns of normotensive mothers constituting the control group. The study was performed using simple procedures and instruments to obtain the results from cord blood. The Hb level and leukocyte counts obtained were then compared between the two groups. This study can give an idea of the health status neonates born of hypertensive mothers as well as that of healthy mothers. It can thus help in providing better health care to the pregnant mothers and their neonates.

MATERIALS AND METHODS: Type of study - Prospective observational study.

STUDY PROCEDURE: 2 ml of cord blood anti-coagulated with EDTA was collected from neonates of hypertensive mothers [study group] and of normotensive mothers [control group] respectively after delivery. For each of the samples haemoglobin level and leukocyte counts were done:

1. Haemoglobin level was estimated by Sahli's method.
2. For leukocyte counts, Total leukocyte count and Absolute count for different leukocytes was done.

Total Leukocyte count (TLC) was done using Improved Neubauer's Counting chamber. Absolute counts were obtained by doing Differential Leukocyte Count (DLC) and the value obtained from TLC. For DLC, slides were stained with Leishman's stain.

Sample size and inclusion criteria-Thirty newborns of hypertensive mothers constitute the study group and thirty apparently healthy newborns of normotensive mothers constitute the control group. As majority of mothers come only during the period of delivery and leave after few days, the follow up of subjects cannot be done. So, pregnant mothers whose blood pressure was $\geq 140/90$ mm of Hg were included as hypertensive.

EXCLUSION CRITERIA: Newborns whose mothers had diabetes mellitus, severe anaemia, premature rupture of membranes, perinatal infections, and gestational age <28 weeks were excluded and neonates having severe asphyxia were excluded from the study.

DATA COLLECTION PROCEDURE: Relevant information of the pregnant mothers was collected from hospital records and results obtained from the study of cord blood were recorded.

INSTRUMENTS: For haemoglobin estimation, Sahli's haemoglobinometer were used. For study of leukocyte counts, light microscope and Improved Neubauer's counting chamber were used.

QUALITY CONTROL: For quality control the instruments to be used were checked before doing the study.

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PLAN OF ANALYSIS: The statistical analysis was carried out using t-test for the comparison of differences of the means by SPSS 15.0 for windows.

ETHICAL CONSIDERATIONS: Permission for the study was obtained from the Institutional Ethics Committee of AGMC and GBP Hospital. Consents were taken from the participants by signing in the previously prepared Informed Consent Form.

OBSERVATIONS AND RESULTS: During the study, 30 neonates with gestational age between 37-43 weeks born of mothers with maternal age between 18-33 years were included in the study group and 30 neonates with gestational age between 37 weeks- 41weeks born of mothers with maternal age between 18-34 years were included in the control group. The important factors relating to pregnancy of hypertensive and normotensive mothers are given in Table 1.

A haemoglobin value of less than 13.5 g/dl was considered anaemic and a level more than 22g/dl was considered polycythaemic. A leukocyte count less than 5,000/mm³ was considered leukopenic, a neutrophil count of less than 1,500/mm³ was considered neutropenic and a lymphocyte count less than 3,000/mm³ was considered lymphopenic.^[3,8,13] Hematological parameters of study and control groups were shown in Table 2.

Haemoglobin level were found to be significantly higher in the study group as compared to control group ($p < 0.05$). Total leukocyte count and Absolute counts for neutrophils and lymphocytes and Absolute count for basophils were lower and higher respectively in study group compared to control group but was not significant ($p > 0.05$).

Table 3 shows the state of cytopenic subjects. In relation to cytopenia, 3 male leukopenic subjects, 1 male neutropenic subject, 4 lymphopenic subjects (3 male, 1 female) and 1 neutropenic + lymphopenic subjects were found in the study group. In control group, 2 male leukopenic subjects and 3 lymphopenic subjects (2 male, 1 female) were found.

Pie Chart 1 shows the state of anaemic and non- anaemic subjects of study group. In the study group, 7 subjects (3 males, 4 females) i.e. 23.3% (10% males, 13.3% females) subjects were found to be anaemic. Pie Chart 2 shows state of anaemic subjects of control group. In the control group, 17 subjects (12 males, 5 females) i.e. 56.7% (40% males, 16.7% females) subjects were found to be anaemic. No subjects were found to be polycythaemic both in study group and control group. In the study group, 1 subject was found to be anaemic among the 4 lymphopenic subjects and in the control group, 2 subjects were found to be anaemic among the 3 lymphopenic subjects.

Pie Chart 3 showing the state of anaemic, non-anaemic and cytopenic subjects in the study. In this study, 24 subjects (15 males, 9 females) i.e. 40% (25% males, 15% females) subjects were found to be anaemic. 5(8.3%) subjects (male) were found to be leukopenic. 1 (1.7%) subject (male) was found to be neutropenic. 7 subjects (5 males, 2 females) i.e. 11.6% (8.3% males, 3.3% females) subjects were found to be lymphopenic.

DISCUSSION: In this study, the haemoglobin level of the study group was found to be significantly higher than the control group and was comparable to that of the studies which showed haemoglobin level to be higher in the newborns of hypertensive mothers.^[3,5] But it contradicts the observation in another study where hemoglobin level was found to be lower in the newborns of hypertensive mothers.^[4]

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In this study, leukopenia was found in 10% of subjects in study group. Similar studies conducted by Prakash PL et al^[4] found leukopenia in 63.9%, 45.3% and 39.3% babies of pre-eclamptic, eclamptic and gestational hypertension mothers respectively and 28.5% by Mosayebi Z et al.^[13] Neutropenia was found in 3.3% of newborns of hypertensive mothers. Other studies also found neutropenia in newborns of hypertensive mothers as 58.1% by Bolat A et al,^[3] 17.9%, 30.6% and 27.5% babies of preeclamptic, eclamptic and gestational hypertension mothers respectively by Prakash PL et al^[4] and 37% of babies of pre-eclamptic mothers by Mosayebi Z et al.^[13] Lymphopenia was found in 13.3% in newborns of hypertensive mothers in this study and 35.5% in study by Bolat A et al.^[3]

No polycythaemia were found in the newborns of hypertensive mothers as compared to 8.2% by Onyiruika AN et al.^[7] Anaemia was found in 23.3% of newborns born of hypertensive mothers as compared to 39.2% by Mosayebi Z et al.^[13]

The increase in haemoglobin level in the study group can be due to the hypoxia leading to uteroplacental insufficiency resulting in increase in erythropoiesis. This is supported by the works of Bolat A et al and Sivkumar et al, but is contradictory to the work of Prakash PL et al. But anemia was documented in 23.4% of infants of hypertensive mothers in this study which was found to be similar to those of Mosayebi Z et al and Prakash PL et al. This may be due to a number of reasons- it can be due to ineffective erythropoiesis among the infants of hypertensive mothers because of inhibitory factors produced due to hypertensive disorders. The balance between the response of hypoxia and the inhibitory factors may lead to increase or decrease in erythropoiesis.

It can be also due to nutritional deficiency of the newborns during intrauterine life. Leukopenia, neutropenia and lymphopenia was documented which were supported by other studies and this may be due to inhibitor of neutrophil production.^[9] It was also reported that the balance of erythropoiesis and granulopoiesis shifted to the direction of erythropoiesis, and because of hypoxia and the resulting increase in reticulopoiesis in normal haematopoiesis regulation, no adequate stem cells remained for granulopoiesis and as a result inadequate granulopoiesis was observed and dysgranulopoiesis developed.^[3] Thus in response to hypoxia and inhibitory factors for both erythropoiesis and leukopoiesis produced during hypertensive disorders of pregnancy can lead to changes in haemoglobin level and leukocyte counts.

CONCLUSIONS: In this study as there were significant changes in the haematological parameters of the newborns, examining the cord blood can be done as a tool for screening of the newborns of both hypertensive and normotensive mothers to detect vulnerability to diseases which can result in improvement of health of newborns. Further research work can be done to relate the effect of hypertensive disorders and haematological disturbances in newborns.

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TABLES AND CHARTS:

Parameter	Study group	Control group
Maternal Age (years)^a	23.47±4.25 (18-33)	23.80±3.55 (18-34)
Number of pregnancy^b		
1	24(80)	20(66.7)
2	2(6.7)	5(16.7)
3	1(3.3)	3(10.0)
4	0(0)	2(6.7)
5	2(6.7)	0(0)
6	1(3.3)	0(0)
Parity^a	0.4±1.07(0-4)	0.37±0.55(0-2)
Gestational age(weeks) ^a	38.69±1.99 (37.0-43.0)	38.60±1.63 (37.0-41.42)

Table 1: Pregnancy details of cases and controls

^aValues given as mean±SD (standard deviation) (minimum-maximum)

^bValues are given as numbers (%)

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Parameter	Study group	Control group	P
Hb (gm/dl) ^a	14.53±1.46(11.2-18.2)	13.56±1.93(10-18)	0.033
TLC (per cu. mm.) ^a	10040.00±3534.05 (3600-17000)	12221.67±5793.06 (3900-23000)	0.085
ANC (per cu. mm.) ^a	4398.27±1884.08 (1332-8268)	5504.90±2864.12 (1854-11872)	0.086
ALC (per cu. mm.) ^a	5636.93±2554.38 (2124-12920)	6706.77±3443.66 (2028-16128)	0.177
ABC (per cu. mm.) ^a	4.80±26.29(0-144)	0	0.321

Table 2: Haematological parameters of study and control group

[Hb- Haemoglobin, TLC- Total leukocyte count, ANC- Absolute neutrophil count, ALC- Absolute lymphocyte count, ABC- Absolute basophil count.]

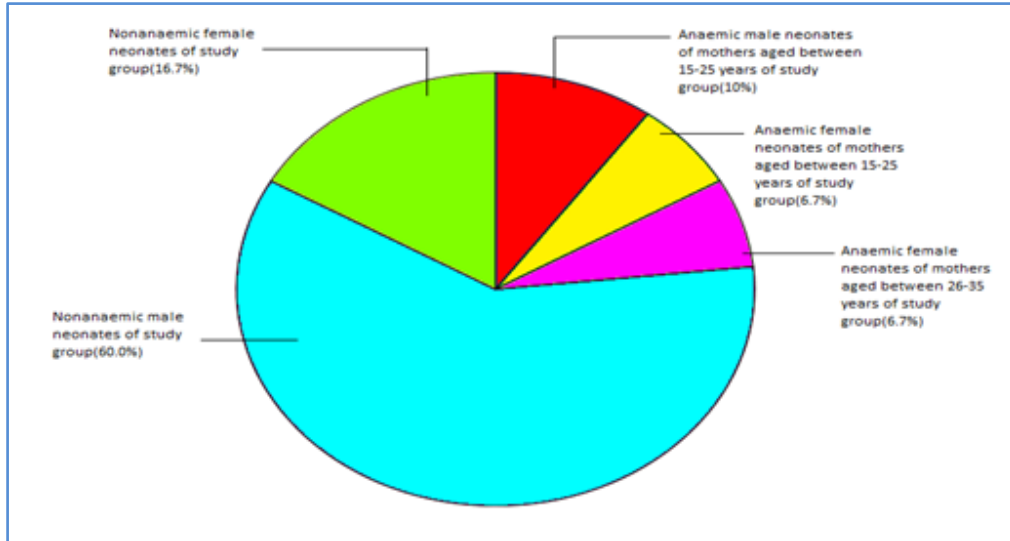
^aValues given as mean±SD (standard deviation) (minimum-maximum)

Parameter	Study group	Control group
Leukopenic (%)	3(10)	2(6.6)
male	3(10)	2(6.6)
female	0(0)	0(0)
Neutropenia (%)	1(3.33)	0(0.00)
male	1(3.33)	0(0.00)
female	0(0.00)	0(0.00)
Lymphopenia(%)	4(13.33)	3(10.00)
male	3(10.00)	2(6.66)
female	1(3.33)	1(3.33)
Lymphopenia+ Neutropenia (%) ^a	1(3.33)	0(0.00)

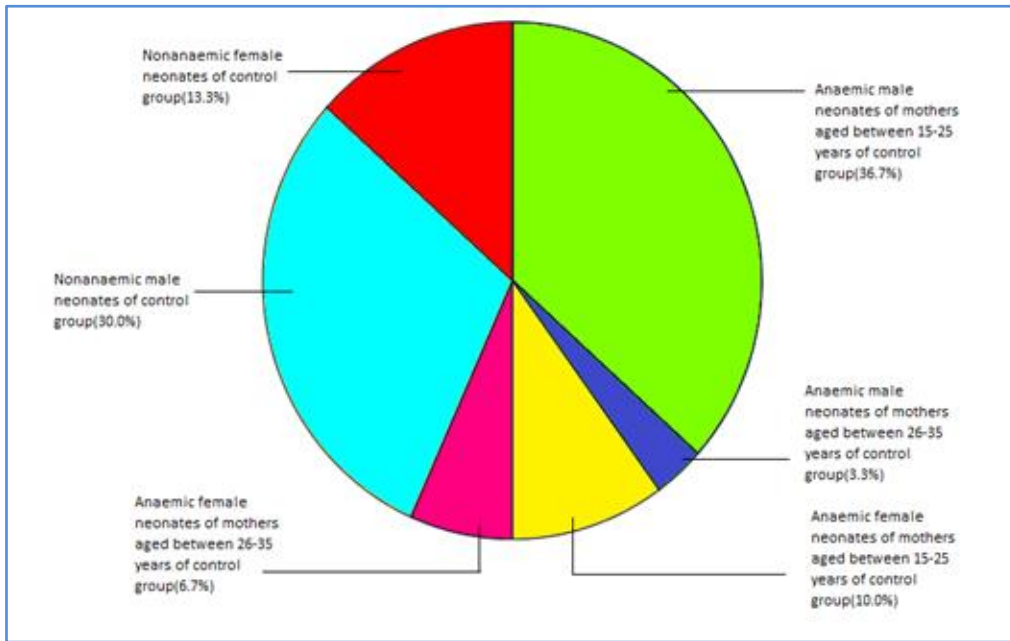
Table 3: Cytopenic subjects in study group and control group

^aLymphopenic+neutropenic subjects were also counted in the neutropenic and lymphopenic groups separately.

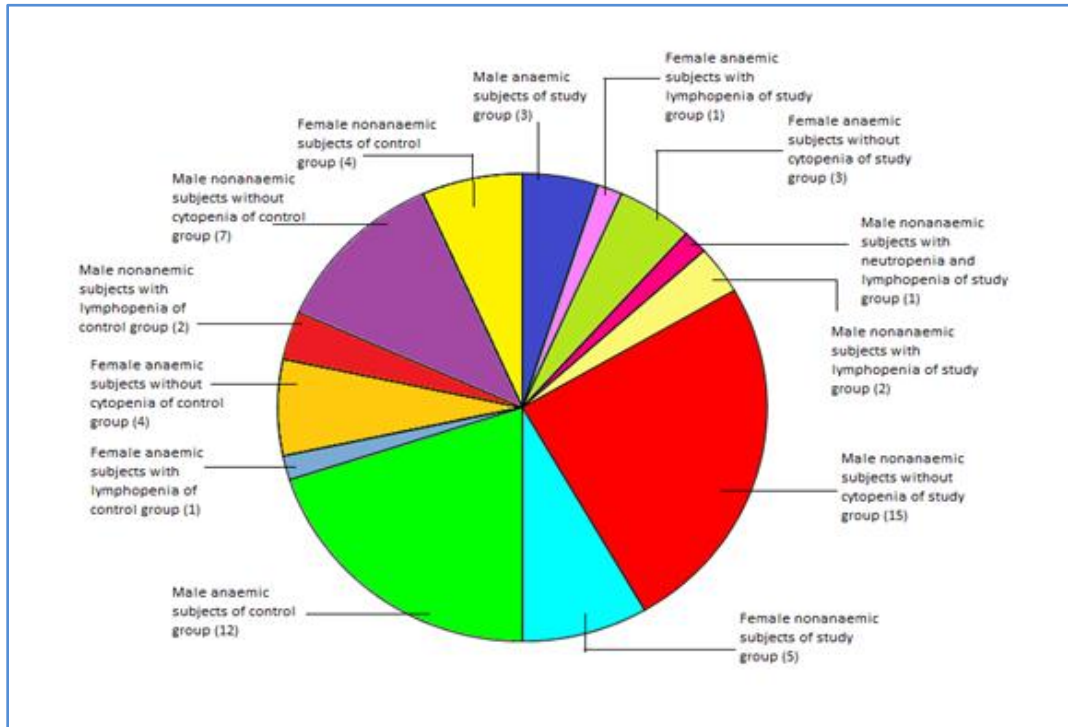
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Pie Chart 1: Anaemic and non-anaemic subjects of Study group



Pie Chart 2: Anaemic and non-anaemic subjects of control group



Pie Chart 3: Anaemic, non-anaemic and cytopenic subjects of study

AUTHORS:

1. Evalyn Singnarpi
2. Debanjan Bhattacharjee
3. Jahar Lal Baidya
4. S. N. Naithok Jamatia

PARTICULARS OF CONTRIBUTORS

1. Associate Professor, Department of Physiology, AGMC & GB Hospital, Agartala, Tripura.
2. Under Graduate Student, Department of Physiology, AGMC & GB Hospital, Agartala, Tripura.
3. Associate Professor, Department of Obstetrics & Gynaecology, AGMC & GB Hospital, Agartala, Tripura.

4. Tutor, Department of Physiology, AGMC & GB Hospital, Agartala, Tripura.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Evalyn Singnarpi,
Associate Professor,
Department of Physiology,
Agartala Government Medical College & GB
Hospital, Agartala, Tripura-799006.
Email: samikache@rediffmail.com

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