A Study of Hematological Parameters related to Platelets in Normal Pregnancy and Pregnancy Induced Hypertension

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Abstract: Objective: To compare hematological parameters in relationship with platelets in normal pregnancy and Pregnancy induced hypertension. Methods: Bleeding time (Duke’s method), Clotting time (Lee and white method), And Platelet count direct method (Breecher krounkit method), Assay of PT/APTT. Results: Comparative decrease in platelet count as the pressure rises in PIH. There is prolongation of PT and APTT compared to normal pregnant women. The thrombocytopenia (which is physiological in gestation) becomes critical when the platelet function activity is affected and platelet count falls below 1Lakh/cm³ (calling for intervention, alarming as count <60,000/cm³ disseminated intravascular coagulation), imminent danger of coagulopathy seen. Conclusion: Hypertensive disorders contribute to the second leading cause of maternal mortality. The hypertensive disorders of pregnancy predispose women to acute or chronic uteroplacental insufficiency resulting in ante or intrapartum hypoxia that may lead to fetal death, intra growth retardation, and or pre-term delivery. Hence we must aim directly towards prevention of seizures and reduction in blood pressure. Expectant mother should be monitored carefully during gestation period, by monitoring the coagulation profile with DBF. Absolute platelet count is an indicator to assess PIH.

Keywords: Pregnancy induced hypertension, Hematological Parameters, Absolute platelet count, Normal Pregnancy, Pregnancy Induced Hypertension (PIH)

1. Introduction

Pregnancy is a boon to motherhood, a cherished desire for every woman is associated with many physiological changes which only help in bringing out the offspring into the world in healthy rosy condition. The physiological changes range from quantitative and qualitative changes. All the changes are physiological, they become normal after delivery. To begin with hypertension the basic cause could be changes in blood volume or vasoconstriction, anemia or poor socioeconomic status. Whatever be the underlying cause if we can catch the early bird, probably an insight is taken up in the study of hematological parameters during pregnancy induced hypertension. During pregnancy, plasma volume increases from the non-pregnant level of 2600 ml to about 3800ml. This increase occurs early in pregnancy and there is not much further change after 32 weeks’ gestation. The red cell mass also increases from a non-pregnant level of 1400 ml to 1650 -1800 ml depending on whether iron supplementation has been given. The increase in red cell mass occurs steadily until term. However, since plasma volume increases proportionately more than red cell mass, the haematocrit and hemoglobin concentration fall during pregnancy. Hemoglobin level of 10.5g/l would not be unusual in a healthy pregnancy. Cardiac output increases by about 40% but heart rate increases by only about 10% from 80b.p.m to 90b.p.m. during pregnancy. Therefore, there must be an associated increase in stroke volume. The increase in cardiac output is more than is necessary to distribute the extra 30-50 ml of oxygen consumed per minute in pregnancy. The marked rise in cardiac output which occurs in pregnancy does not cause a rise in blood pressure, unless a pathological process such as pre-eclampsia occurs. Therefore, there must be a decrease in total peripheral resistance, and this vasodilatation accommodates the increased blood flow to the uterus, kidney, skin and other organs. The decreased peripheral vascular resistance does not always keep strictly in proportion with the increase in cardiac output and during the middle of pregnancy from, perhaps, 8 to 36 weeks, the systolic blood pressure may fall by up to 5 mmHg, and the diastolic blood pressure by up to 10 mmHg, because the peripheral resistance falls by more than cardiac output rises. Other factors affecting blood pressure are posture and uterine contractions, which act via the changes in cardiac output. Out of all hematological changes that occur in pre-eclampsia and eclampsia, thrombocytopenia is the most common hematological abnormality found. The other tests Prothrombin test, partial thromboplastin time (PTT), fibrinectin level, decrease anti-thrombin III level, decrease in alpha 2 antitrypsin, increase insoluble forms like tyrosine kinase concentration, decrease in circulating free PIGF (placental growth factor) and VEGF (vascular endothelial growth factor). The degree of thrombocytopenia increases with severity of disease and the incidence of thrombocytopenia depend on the severity of the disease process. Lower the platelet count greater the maternal and fetal morbidity and mortality. Overt thrombocytopenia defined by platelet count <1 Lakh/mm³ indicates severity of disease process where in most cases delivery is indicated because platelet number continues to decrease after that HELLP syndrome (Hemolysis,Elevated liver enzymes, low platelet count) having platelet count <1 lakh/mm³ shows poor fetal outcome. It occurs in 2-12% women with severe pre-eclampsia or eclampsia. Early assessment of severity of PIH is necessary to prevent complications like HELLP syndrome and increased maternal and fetal morbidity and mortality. So this study was undertaken to assess the severity...
of PIH by a method that is rapid, cheaper and can be used in routine monitoring. Hypertensive disorders contribute the second leading cause of maternal mortality (Fogsi study 1987). The fetal outcome is directly proportional to the quality of obstetric care in the community. Severe hypertension in early pregnancy increases the perinatal mortality by 10-20%. Hypertensive disorders constitute 12% of all perinatal deaths in the community. The hypertensive disorders of pregnancy, constitute the most widely studied, discussed and analyzed condition because of the fact that they adversely affect the fetus. Hypertensive disorders of pregnancy can cause asymmetrical IUGR, and also symmetrical IUGR, thereby in later case, compromising the intellectual abilities in future of the child. The hypertensive disorders of pregnancy predispose women to acute or chronic uteroplacental insufficiency resulting in ante or intrapartum hypoxia that may lead to fetal death, intra uterine growth retardation, and or pre -term delivery.

2. Materials and Methods

The subjects are normotensive pregnant women and pregnancy induced hypertensive patients of Malla Reddy Narayana Multispecialty Hospital, Hyderabad. The subjects for control group are the normotensive pregnant women attended the O.P. at Malla Reddy Narayana Multispecialty Hospital, Hyderabad.

Inclusion criteria for selection:

Control Group:
- In the age group of 20 -25 years.
- All are primi-gravida.

For study group:
- In the age group of 20 -25 years.
- All are primi-gravida with known case of pregnancy induced hypertension.
- All the patients are admitted cases at Malla Reddy Narayana Multispecialty Hospital, Hyderabad.

Exclusion criteria for both control and study group:
- Known case of Hypertension.
- Known case of Diabetes.
- Known case of Asthma
- Known case of Epilepsy

This case control study was conducted at Malla Reddy Narayana Multispecialty Hospital, Hyderabad. Informed consent was taken from the patients for inclusion in this study. Cases comprised of Primi-gravida at 3rd trimester, with known cases of Pregnancy Induced Hypertension admitted in hospital for safe institutional delivery. The control group are the women primi-gravida who have attended the regular O.P. and I.P. at Malla Reddy Narayana Multispecialty Hospital, Hyderabad. 5ml of blood anti-coagulated with EDTA was collected and various hematological parameters were studied. These included Hemoglobin, total and differential counts, Platelet count, red cell indices like, PVC, MCV, MCH, MCHC and B.T. CT Prothrombin and APTT. Maternal details like age, parity, immunization status gestation age, onset of symptoms, blood pressure recordings and presence of seizes were noted. Statistical analysis was carried out to see the relationship between the normotensive pregnant women and the pregnancy induced hypertension women. P. value of < 0.05 was taken as significant. All the information is recorded in case sheet proforma and later analyzed.

Methods

Bleeding time (Duke’s method)
Clotting time (Lee and white method)
Platelet count direct method (Breecher kronkite method).
Assay of PT/APTT: These were done by materials and methods marketed by tulip diagnostics, pvt. ltd., Goa, India, distributions of reagents/kits of diagnostics stage in India

3. Analysis of the Study

The mean SBP in control group is 109.67 and in study group is 138. The estimated P value for SBP in control group and study group is < 0.001. Thus there is a significant increase in SBP in study group 1 as compared to control group. The mean for SBP in control group is 109.67 and study group 2 is 154 as shown in table 2. The estimated P value for SBP in control group and study group 2 is < 0.001. Thus there is a significant increase in SBP in study group 2 compared to control group. The mean for SBP in study group 1 is 138 and study group 2 is 154. The estimated P value for SBP in study group 1 and study group 2 is < 0.001. Thus there is significant increase in SBP in study group 2 compared to study group 1. The mean for DBP in control group is 71.00 and study group 1 is 94. The estimated P value for DBP in control group and study group 1 is < 0.001. Thus there is significant increase in DBP in study group 2 compared to control group. The mean for DBP in control group is 71.00 and study group 2 is 112. The estimated P value for DBP in control group and study group 2 is < 0.001. Thus there is significant increase in DBP in study group 2 compared to control group. The mean for DBP in study group 1 is 94 and study group 2 is 112. The estimated P value for DBP in study group 1 and study group 2 is < 0.001. Thus there is significant increase in DBP in study group 2 compared to study group 1. There is no significance for Hb%, BT, CT in this study. There is no significance for platelet count between control group and study group 1. The mean for platelet count in control group 1 is 2.21 and study group 2 is 1.67. The estimated P value for platelet count in control group and study group 2 is < 0.001. Thus there is significant decrease in platelet count in study group 2 compared to study group 1. The mean for platelet count in study group 2 is 2.13 and study group 2 is 1.67. The estimated P value for platelet count in study group 1 and study group 2 is < 0.001. Thus there is significant decrease in platelet count in study group 2 compared to study group 1. There is no significance for Prothrombin time between control group and study group 1. The mean for Prothrombin time in control group is 14.7 and study group 2 is 15.86. The estimated P value for Prothrombin time in control group and study group 2 is < 0.001. Thus there is significant decrease in platelet count in study group 2 compared to study group 1. The mean for Prothrombin time in study group 1 is 14.73 and study group 2 is 15.86. The estimated P value for Prothrombin time in study group 1 and study group 2 is < 0.001. Thus there is significant decrease in platelet count in study group 2.
compared to study group 1. There is no significance for APTT in control group and study group 1. The mean for APTT in control group 1 is 25.33 and study group 2 is 27.2. The estimated P value for APTT in control group and study group 2 is <0.001. Thus there is significant decrease in platelet count in study group 2 compared to control group. The mean for APTT in study group 1 is 25.4 and study group 2 is 27.2. The estimated P value for APTT in control group and study group 2 is <0.001. Thus there is significant decrease in platelet count in study group 2 compared to control group. There is no significance for INR of PT in control group and study group 1. The mean for INR of control group 1 is 1.0744 and study group 2 is 1.266. The estimated P value for APTT in study group 1 is 1.032467 and study group 2 is <0.001. Thus there is significant decrease in platelet count in study group 2 compared to control group. The mean for INR of study group 1 is 1.12393 and study group 2 is 1.266. The estimated P value for INR of Prothrombin time in study group 1 and study group 2 is <0.001. Thus there is significant decrease in platelet count in study group 2 compared to control group. There is no significance for INR of APTT in control group and study group 1. The mean for INR of APTT study group 1 is 1.056467 and study group 2 is 1.191733. The estimated P value for INR of APTT in control group and study group 2 is <0.001. Thus there is significant decrease in platelet count in study group 2 compared to control group. The mean for INR of APTT study group 1 is 1.032467 and study group 2 is 1.191733. The estimated P value for INR of APTT in study group 1 and study group 2 is <0.001. Thus there is significant increase in INR of APTT in study group 2 compared to study group 1. The INR of total controls and cases (30) in number was analysed. The results were significant p< 0.001. (Both Prothrombin Time and APTT)

Prothrombin Time

Control mean 1.0638 cases...1.19496 P <.001 Result Significant APTT. Control Mean– 0.994033 cases. 1.1121 p< 0.001 Result significant. Correlation of DBP with platelet count, PT and APTT in study group 1. From the correlation study we observe that in study group 1 DBP with platelet count, PT and APTT are negatively correlated (shown in figure), we mean that DBP increases slightly on the other hand platelet count, PT and APTT decreases. Correlation of DBP with platelet count, PT and APTT in study group 2. From the correlation study we observe that in study group 2 DBP with platelet count, PT and APTT are positively correlated (shown in figure), we mean that DBP increases on the other hand platelet count decreases slightly, PT and APTT (test) increases in the same direction.

ANOVA Study of single factor of platelet count in control group, study group1 and study group2. There is significance of platelet count between groups and within groups as the P value is <0.0001.

ANOVA single factor for platelet count in control group, study group1 and study group 2. There is significance of PT between groups and within groups as the P value is <0.001. ANOVA single factor for APTT in control group, study group and study group 2. There is significance for APTT estimation between groups and within groups as the P value is <0.0001.

4. Discussion

Study of analysis of hematological parameters between normal pregnancy and pregnancy induced hypertension, which was conducted at Malla Reddy Narayana Multispeciality Hospital, Hyderabad. We have taken thirty normal pregnant women as control group and thirty pregnancy induced patients as study group. These patients are grouped into two depending on blood pressure (DBP).

Study group I with diastolic blood pressure between 90-100 mmHg and Study group II with diastolic blood pressure above100mmHg. We have recorded blood pressure and Hemoglobin percentage, blood grouping, bleeding time, clotting time, platelet count, Prothrombin time and APTT. Average age of patients between 20-25years, with gestational age of 36-40 weeks and all are primiparous women. We have studied PIH in primiparous women because the incidence of PIH is 4.5 times more common. According to Jun Zhang2, Jonathan Zeister, Maureen C. Hatch and Gertrude Berkowitz30, Study on Epidemiology of Pregnancy - Induced Hypertension, the incidence of PIH in nulliparous women is 4 -5 times higher than that in multipara. PIH occurs mainly in primiparous woman (85%) who have a 4-5 times higher risk than multi-parous women. Zuspan FP 11. New concepts in the understanding of hypertensive diseases during pregnancy, an overview of Clinical Perinatal 1991. According to his studies antiphospholipid antibodies (APAS) have been linked to adverse maternal and fetal sequelae, such as recurrent fetal loss, fetal growth retardation, PIH, thrombo-embolism, and thrombocytopenia. Lockwood,CJ, R and JH, the immunology and obstetrical consequences of antiphospholipid antibodies, obstetric gynaecological survey 1994. The ultimate goal is to prevent eclampsia, because a sizable proportion of PIH is probably due to chronic renal disease or latent hypertension according to Fisher KA, Luger A. S. Pargo BH, et al 5 and Hypertension in pregnancy, Medicine 1981. In Aberdeen the incidence in primigravidae has fluctuated between 2% and 7.7 % since 1950. In the same study the incidence in multiparae was 0.8 - 2.6% (Campbell & Mac Gillivray 1999) 3. In our study we have observed that there is no alteration in the bleeding time, clotting time but there are changes in coagulation factors and activators of intrinsic system and extrinsic system.

Bleeding Time: Normal values range between 1-5 minutes these values did not show any significant variations statistically between normal pregnancy and PIH, both mild and severe categories. This finding is consistent with Agarwal 2 and Buradhkar, 1978 2. However, bleeding time was significantly prolonged in association with reduced platelet counts ranging between 1 Lakh /cmm and 1.5 Lakh/cmm in the eclampsia group. This finding can be explained on the basis that the platelets are consumed in the formation of blood clots.

Clotting Time: Normal values were found to be between 3-6 minutes in this study. There was no statistically significant variation when the values were compared among all the study groups. This is consistent with the findings of Agarwal & Buradhkar, 1978.

Platelet Count: In our study thrombocytopenia was significantly revealed. Our results correlate with other works.
too. Gestational thrombocytopenia is not a pathological process. It is the most common cause of thrombocytopenia during pregnancy and occurs in 5-8% of all pregnant women. The platelet counts are in the lower range of normal and can be as low as 100 x 10^9/L. The quantitative decrease in platelets is balance d by enhanced platelet activity. Women with this disorder are not at risk of bleeding. It is a diagnosis of exclusion. S. Mohapatra et al 12, studied on platelet estimation: Its prognostic value is pregnancy induced Hypertension. Thrombocytopenia is an associated phenomenon of pregnancy induced hypertension (PIH). But the accurate count of platelets either by manual, (direct or indirect methods) or by automated cell counters is not feasible for all patients at all hospitals. Mohapatra 12 study group have adopted the method of platelet estimation, not platelet count as an alternative procedure to estimate the degree of thrombocytopenia in patients with PIH cases. We have observed thrombocytopenia (Significance level P value < 0.001) and recorded APTT and Pro thrombin levels. Out of all hematological changes that occur in pre-eclampsia and eclampsia thrombocytopenia is the most common hematological abnormality found. The degree of thrombocytopenia increases with severity of disease and the incidence of thrombocytopenia depend on the severity of disease process. Lower the platelet count, greater are maternal and fetal morbidity and mortality. In our study platelet count is significant with p value < 0.001 between control group and study group I and II. Profound d changes in the coagulation and fibrinolytic system occur during normal pregnancy causing a hypercoagulable state. M. Srivastava, S. Bali, J. Pandey, B. Nayer, V.H. Talib 9 studied on Pregnancy Induced Hypertension and anti-thrombin –III levels. There is a distinct possibility of accentuation of the hypercoagulable state of pregnancy during eclampsia and pre-eclampsia. The study group included 119 women with PIH in third trimester of pregnancy. Criteria for selection were elevation of systolic blood pressure, fulfilling these criteria with or without edema or proteinuria after 20 weeks of pregnancy were included in the study. The following tests were carried out on all samples, Prothrombin time (PT), Activated partial thromboplastin time (APTT), all were prolonged. In Srivastava et al 1 study APTT increased, fibrin degradation products increased, Prothrombin time and thrombin time increased and antithrombin III decreased. This hypercoagulability is a result of an increase in activated blood coagulation factors, activation of platelets or decrease in the level of blood coagulation inhibitors. AT- III can be a useful marker for severity of disease and help in clinical decision making for the obstetrician. Severity of PIH and thrombocytopenia observed are closely related which indicates that thrombocytopenia is directly proportional to the severity of PIH.

<table>
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<tr>
<th>No. (Mean)</th>
<th>Platelet (L/cmm)</th>
<th>APTT (Sec)</th>
<th>PT(Sec)</th>
<th>BT (Sec)</th>
<th>CT (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Group –I</td>
<td>2.13</td>
<td>25.40</td>
<td>14.733</td>
<td>1.33</td>
<td>3.41</td>
</tr>
<tr>
<td>Study Group –II</td>
<td>1.68</td>
<td>27.2</td>
<td>15.86</td>
<td>1.39</td>
<td>3.47</td>
</tr>
<tr>
<td>Significance p-value</td>
<td>Significant</td>
<td>&lt;0.001</td>
<td>Significant</td>
<td>Not Significant</td>
<td>Not Significant</td>
</tr>
<tr>
<td>Between Control and Study Group –I (C:S1)</td>
<td></td>
<td>Not significant</td>
<td>Not significant</td>
<td>Significant</td>
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<td></td>
<td>=0.05</td>
<td>&gt;0.05</td>
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<td>&lt;0.001</td>
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<td>Between Control and Study Group –II (C:S2)</td>
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<td>=0.05</td>
<td>&gt;0.05</td>
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<td>Between</td>
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A Vincelot1, N. Nathan, D. Collet, Y. Mehaddi, P. Grand champ and A. Julia studied on platelet function during pregnancy: an evaluation using the PFA -100 analyzer. They have seen there is correlation of thrombocytopenia and also studied platelet function. The only tests of platelet function are bleeding time and platelet number. Bleeding time lacks sensitivity and specificity but the PFA-100, an in vitro analyzer of platelet function may be of value. This study aimed to evaluate any correlation between platelet number and function using the PFA-100 in pregnant women. This study was correlating with platelet function. They studied patients in four groups. They concluded that platelet function activity was preserved until platelets fall <60,000/cmm in pregnancy induced thrombocytopenia because thrombocytopenia is a common physiological phenomenon in gestation as mentioned earlier. In PIH with thrombocytopenia or pre-e clampsia platelet function becomes abnormal when the count is less than 1 Lakh/cmm. The study conducted on coagulation profile in pregnancy induced hypertension at Govt. medical College Nagpur; they found that platelet count, PT, TT shown significance. Platelet count has dropped, PTT and PT were prolonged as in our study. They concluded that abnormalities pertain to coagulation parameters interfere with intravascular coagulation. Platelet count PTT, TT have predictive value in detecting DIC in PIH, and these parameters show more abnormal result with increasing severity of PIH. Zhonghua Fu Chan Ke ZA Zhi, 200510 has done a study like ours. They studied on the variation of platelet function in pregnancy induced hypertension and gestational diabetes mellitus. Their study is on platelet activity and function in pregnancy induced hypertension and gestational diabetes mellitus (GDM). Their conclusion was platelet count in PIH was lower than that of control (P < 0.01) but there was no significant difference between GDM and controls. Their conclusion was platelet activity is enhanced in PIH and GDM and may play an important role in the pathogenesis and development of the two diseases. S. Narayan, S. Kumari, S. Mangwana, K.B. Logani, M.Kabra13 studied on consumption coagulopathy in Neonates born to mothers with pregnancy induced hypertension. The present study showed statistically significant prolongation of PT, APTT and TT in neonates born to PIH mothers as compared to those born to normotensive mothers, the derangements were more pronounced in neonates of severe PIH as compared to neonates of mild PIH mother especially in preterm babies.

Prothrombin time:- Prothrombin time in our group, there is significance. Our findings do not similar to the findings of Ezra C. Davidson et al 14, in their study on coagulation studies in hypertensive Toxaemias 1972, did not found PT increased significantly. Prothrombin time is done by Liquiplastin

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method, Tulip Diagnostics (P) LTD. The normal value is 10-14 seconds. APTT acts at extrinsic pathway and PT is at intrinsic pathway. Most important is measurement of INR was done, is now, standardised method. Prothrombin time increased in vitamin K deficiency, increased consumption of platelets in DIC. Each manufacturer assigns on ISI (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an internationally standardised sample. The ISI is usually between 1.0 and 2.0. The INR is the ratio of a patient’s Prothrombin time to a normal (Control) sample, raised to the power of the ISI value for the analytical system used. The Prothrombin time is the time taken for plasma to clot after addition of tissue factor (Obtained from animals). This measures the quality of the extrinsic pathway (as well as the common pathway) of coagulation. A high INR level such as INR= 5 indicates that there is a high chance of bleeding, where as if the INR= 0.5 then there is a high chance of having a clot. Normal range for a healthy person is 0.9–1.3, and for people on warfarin therapy, 2.0 - 3.0 although the target INR may be higher in particular situations, such as for those with a mechanical heart valve, or bridging warfarin with a low molecular weight heparin.

APTT: Activated partial thromboplastin time normal values ranged between 22-34 seconds with LIQUICELIN-E method manufacture of TULIP diagnostics P (LTD), were found to be statistically highly significant among normal pregnancy and mild and severe PIH. APTT values were prolonged in the PIH patients. This finding is constant with those of Ezra C. Davidson et al. 1972. These changes of low platelet count, prolongation of PT and APTT reflect a picture of utilization of clotting factors due to mild intravascular coagulation. APTT is a performance indicator measuring the efficacy of both the "Intrinsic" and common coagulation pathways. It is used in conjunction with the Prothrombin time (PT) which measures the extrinsic pathway. In our study the comparison between control and Study group I and control and Study group II were proved highly significant in both these groups P <0.001. The comparison in between study groups also showed significance p. value <0.001. We thereby can interpret that there is direct correlation between severity of PIH and PT and APTT, increasing DBP, increase in APTT & PT. As statistical results shown with increase in PIH, there is fall in platelet count.

5. Summary

Hypertensive disorders contribute to the second leading cause of maternal mortality. The hypertensive disorders of pregnancy predispose women to acute or chronic uteroplacental insufficiency resulting in ante or intrapartum hypoxia that may lead to fetal death, intra growth retardation, and or pre-term delivery. Hence we must aim at symptomatic and empirical management directly towards prevention of seizures and reduction in blood pressure. Our study has shown that there is a comparative decrease in platelet count as the pressure raising in PIH. There is prolongation of PT and APTT compared to normal pregnant women. The thrombocytopenia (which is physiological in gestation) becomes critical when the platelet function activity is affected and platelet count falls below 1Lakh/c mm (calling for intervention, alarming as count <60,000/cmm disseminated intravascular coagulation), imminent danger of coagulopathy seen. Expectant mother should be monitored carefully during gestation period, by monitoring the coagulation profile with diastolic blood pressure. Absolute platelet count is an indicator to assess pregnancy induced hypertension. Thus there is need for early recognition of the disease, prevention of further progress of disease to eclampsia and to reduce the fetal morbidity and mortality.

### References

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