A Study on Fibrinogen Levels and Platelet Count in Pregnancy Induced Hypertention

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Abstract- Pregnancy is a physiological process in women but it may be associated with certain risks to the health and life of both the mother and child. Hypertensive disorders complicating pregnancy are common and form one of the deadly triad along with hemorrhage and infection that results in maternal and perinatal morbidity and mortality. Pregnancy can induce hypertension in normotensive women or aggravate already existing hypertension or appears for the first time during pregnancy. The identification of this clinical entity and effective management play a significant role in the outcome of pregnancy, both for the mother and the baby. In the developing countries with uncared pregnancy, this entity on many occasions remains undetected till major complications supervene.

In modern obstetric practice, hypertensive disorders of pregnancy are understood to encompass a clinical spectrum of abnormalities ranging from minimal elevation in blood pressure to severe hypertension with multiorgan dysfunction [1].

This study is aimed to evaluate fibrinogen levels and platelet count in hypertensive disorders of pregnancy.Changes in coagulation profile that occur in normal pregnancy includes the biochemical adaptation especially the hematological changes that occur in response to pregnancy are profound.The levels of several blood coagulation factors are increased during pregnancy.Plasma fibrinogen increases about 65% late in pregnancy.The increase in fibrinogen concentration contributes significantly to the striking increase in ESR.There is moderate decrease in platelet count as pregnancy progresses.Hence it is useful to evaluate these biochemical markers to prevent the complications like pre-eclampsia,eclampsia, abruption placenta,intrauterine infection etc.

Index Terms- fibrinogen levels,platelet count,pre-eclampsia,eclampsia

I. INTRODUCTION

Hypertensive disorders during pregnancy are associated with high maternofoetal mortality and morbidity in both underdeveloped and developed countries [2]. Approximately 70% of hypertensive disorders are due to gestational hypertension this condition is called pre eclampsia [3].Pre eclampsia/eclampsia has been called the "**Disease of Theories**". Although it is a relatively common entity and has been the subject of a large body of research, the search for inciting agent and a unifying pathophysiological mechanism has generated more questions than answers. Even the definition of a disease has been a source of controversy. Nevertheless, research has yielded a great deal of information that has markedly improved maternal and foetal outcomes, and work continues to enhance efforts at prevention of this often devastating condition [1].

Foetal neonatal jeopardy results primarily from compromised placental perfusion and the need for preterm delivery in severe cases. In developed countries, up to 25%of all prenatal deaths are attributable to hypertensive disorders of pregnancy, the major maternal hazards are the consequences of severe hypertension, grand mal seizures and damage to other end organs. In many areas of the world, hypertensive disorders of pregnancy are not the most common cause of maternal death because with modern management, preeclampsia can be ameliorated and eclampsia largely prevented [1].

One way to reduce the impact of arterial hypertension on maternal mortality is to establish the correct diagnosis of hypertensive disorders of pregnancy, and to proceed with an early intervention when it is diagnosed. The clinical signs are considered to be a late manifestation of a disease that has been present since the first trimester of gestation due to "Diagnostic delay". Many tests have attempted to establish the diagnosis of preeclampsia as early as possible, often even before the patient present arterial hypertension. Tests reported for the early diagnosis of hypertensive disorders are Doppler ultrasound assessment of maternal and foetal circulation, uric acid concentration, the supine pressure test, the angiotensin test, microalbuminuria, plasma fibronectin concentration, plasma antithrombin activity, calciuria, prothrombin time, platelet count, fibrinogen levels, APTT and other tests, all of which are of debatable efficacy and practicality[4].

How pregnancy incites or aggravates hypertension remains unsolved despite decades of intensive research. Indeed, hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics. This study is aimed to evaluate some biochemical markers in hypertensive disorders of pregnancy which includes:

1. Platelet count
2. Fibrinogen levels.

Changes in coagulation profile that occur in normal pregnancy includes the biochemical adaptation especially the haematological changes that occurs in response to pregnancy are profound the levels of several blood coagulation factors are increased during pregnancy.

Plasma fibrinogen increases about 65% late in pregnancy. The increase in fibrinogen concentration contributes significantly to the striking increase in ESR. Other clotting factors that increase appreciable during normal pregnancy are factors- VII, VIII.IX & X. prothrombin and factors V and XII do not change. Whereas factors XI and XIII decrease slightly. There is moderate decrease in platelet count as pregnancy progresses.

Disseminated Intravascular Coagulation (DIC) is always a secondary phenomenon trigger by specific obstetrics complications which are listed below [5]:

- Preeclampsia
- Eclampsia
- Abruptio placenta
- Intrauterine infection
- Retained dead foetus
- Placenta accrete
- Hydatidiform mole
- Prolonged shock
- Amniotic fluid embolism.

Hence it is useful to evaluate these biochemical markers to prevent the above cited complications.

II. Aims and Objectives

The present study was undertaken at Department of Biochemistry, SVS
Medical College and Hospital, the following are aims.

A. To study the following parameters in antenatal women after 20 weeks of pregnancy :
   1. Fibrinogen levels
   2. Platelet count

B. To find out any alterations in the above parameters in pregnancy induced hypertension.

C. To compare the above biochemical parameters between the test group and control group.

D. To correlate the outcome of pregnancy with the above biochemical parameters.

III. Materials and Methods

The present study is carried out in the Department Of Biochemistry, SVS Medical College Mahboobnagar. All the subjects included in the study are admitted in Department Of Obstetrics And Gynaecology, SVS Medical College Mahaboobnagar during the year 2010-2011.

A total number of 20 normal antenatal women without PIH are included in control group and a total number of 20 diagnosed cases of PIH are taken as test group. The biochemical parameters of test group is estimated and compared with those of control group. The test group is selected on the basis of vital signs like blood pressure more than 140/90 mmHg and clinical features like oedema, headache, vomiting, epigastric pain, convulsions etc.

The following biochemical parameters are included in present study.
1.Fibrinogen
2.Platelet count

Cases-20 in number (test group)
Inclusion criteria:

1. Age - 18-35years.
2. Antenatal women with hypertension with or without proteinuria, pre-eclampsia and eclampsia.

Exclusion criteria:

1. History of chronic hypertension before completion of 20 weeks of pregnancy.
2. History of Diabetes.

Control – 20 in number
Inclusion criteria
Age group: 18-35 years
Antenatal women without hypertension/pre eclampsia/eclampsia.

Exclusion criteria
Patients with cardiovascular/renal/hepatic complications are excluded from the study.

Method of collection of data
Case history and physical examination findings of both cases and controls are obtained.

Sample collection for the estimation of Fibrinogen:

- Venous blood without undue stasis is collected from peripheral vein by vene puncture under aseptic conditions.
- This blood is transferred into an anticoagulated tubes without delaying the mixing of blood with anticoagulant.
- Exactly 9 parts of freshly collected blood is mixed with 1 part of trisodium citrate (0.11 mol/litre).
- The sample is centrifuged for 15minutes at 3000 rpm and the plasma is separated.
- The separated plasma is transferred in a clean and dry test tube and is tested within 2-3 hours of blood collection.

Instruments used

- Centrifuge machine
- Semi-auto analyser (hemostar, tulip group)

Additional material required

- 10 x 75 mm glass test tubes, pipettes, sample cups, magnet rod.

Sample collection for estimation of platelet count

- Venous blood without undue stasis is collected from peripheral vein by vene puncture under aseptic conditions.

- K2 EDTA is used as an anti coagulant to prepare the anticoagulant blood sample (the dose of K2 EDTA is 1.5 mg/ml blood).
- Anticoagulant blood is mixed up.

Instrument used.

- Auto-analyser (cell counter)

Statistical analysis

Mean and standard deviation of all variables are calculated. The statistical significance is assured using students T-test. P values less than 0.05 are considered significant.

IV. ESTIMATION OF FIBRINOGEN LEVELS

AIM : Determination of fibrinogen level

PRINCIPLE : The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

REAGENT : FIBROQUANT™ kit contains:

1. **Thrombin reagent,** which is a lyophilized preparation from bovine source ~ 50 NIH units per vial.
2. **Fibrinogen calibrator,** which is a lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on a mg/dl basis (refer FIBROQUANT™ graph paper supplied with each kit for the value of each lot).
3. **Owren's buffer,** ready to use (pH 7.35).

V. PROCEDURE

1. A 1:10 dilution of plasma specimen with Owren's buffer solution is prepared.
2. To a 10 x 75 mm test tube at 370C 200µl of 1:10 dilution of plasma sample to be tested is added.
3. Incubated at 370C for one minute.
4. To the test tube 100µl of FIBROQUANT™ thrombin reagent (prewarmed at 370C for one minute) is added and timer is started in hemostar semiautoanalyser simultaneously.
5. The timer is stopped at the first appearance of the fibrin web, as the gel clot begins to form and the time is recorded in seconds.
6. Finally the time obtained in seconds is compared with the fibroquant calibration curve.

INTERPRETATION OF RESULTS

The fibrinogen concentration can be read off directly by interpolating the mean clotting time obtained at 1:10 dilution of the sample, from the calibration curve plotted on the graph paper provided with the FIBROQUANT™ kit for fibrinogen concentration.

NORMAL VALUE

150 - 400 mg/dl.

ESTIMATION OF PLATELET COUNT:

AIM : Determination of platelet count by auto analyser (cell counter)

PRINCIPLE : platelets are counted and sized by the impedance method, as shown in figure. This method is based on the measurement of changes in electrical resistance produced by a particle, which in this case is a blood cell, suspended in conductive diluents as it passes through an aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulses generated indicates the number of particles that pass through the aperture. The amplitude of each pulse is proportional to the volume of each particle. Each particle is amplified and compared to the internal reference voltage channels, which only accepts the pulses of certain amplitude. If the pulse generated is above the platelet lower threshold, it is counted as a platelet.

PROCEDURE

1. The anticoagulant blood sample is mixed up.
2. The sample is placed under the sample probe.
3. Aspirate key is pressed to start the analyses and the results are displayed on the screen is recorded.

NORMAL VALUE

1.5 to3 lakhs/cumm

VI. SUMMARY OF RESULTS

Sl.No	Investigation	Statistical Parameter	Control subjects	Test group
1	Systolic Blood Pressure(mmHg)	Mean	109.5	154
		SD	11.46	11.88
		SEM	2.56	2.66
		t-test	12.05	
		p-Value	<0.0001	
			Control subjects	**Test group**
2	Diastolic Blood Pressure (mmHg)	Mean	71.0	107.5
		SD	8.52	7.86
		SEM	1.9051	1.7575
		t-test	14.08	
		p-Value	<0.0001	

5	Fibrinogen(mg/dl)	Mean	276.75	346.5
		SD	37.31	64.16
		SEM	8.34	14.35
		t-test	4.20	
		p-Value	<0.002	
			Control subjects	**Test group**
6.	Platelet Count (Lacs/cumm)	Mean	2.76	1.86
		SD	0.42	0.29
		SEM	0.09	0.06
		t-test	7.74	
		p-Value	<0.01	

SD : Standard Deviation
SEM: Standard Error of Mean

VII. RESULTS

In the present study the biochemical parameters like Fibrinogen levels and Platelet count were estimated in 20 controls and 20 cases in normotensive and hypertensive pregnant women respectively.

Above table shows the biochemical profile in cases and controls.

I. The mean and standard deviation of systolic blood pressure (mmHg) in controls is 109.5 ± 11.46 as compared to 154 ± 11.88 in test group. The difference is statistically significant, as shown in chart no: 01.

The mean and standard deviation of diastolic blood pressure (mmHg) in controls is 71 ± 8.52 as compared to 107.5 ± 7.86 in test group. The difference is statistically significant, as shown in chart no:

The mean and standard deviation of Fibrinogen levels (mg/dl) in controls is 276.75 ± 37.31 as compared to 346.5 ± 64.16 in test group. The difference is statistically significant, as shown in chart no: 05.

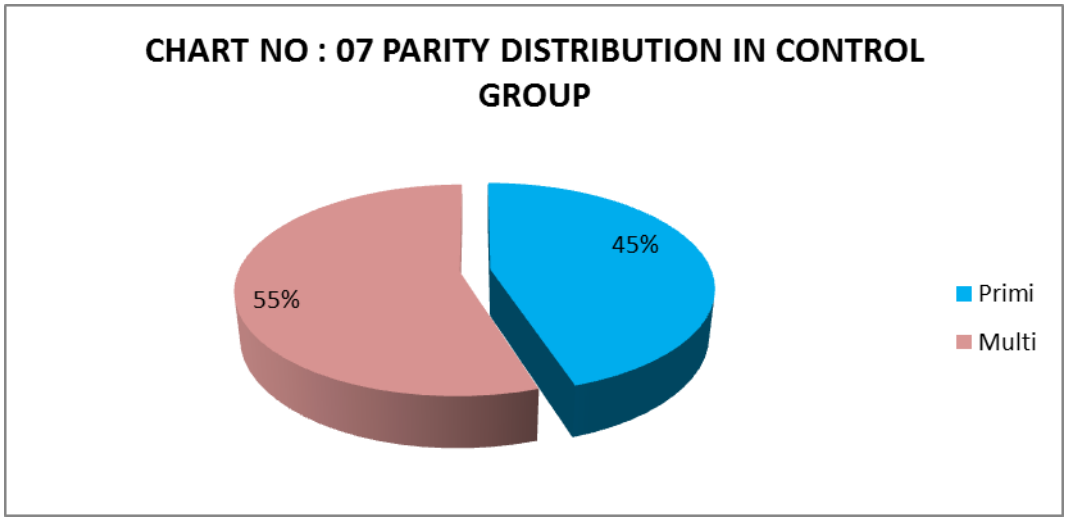
II. The mean and standard deviation of Platelet count (lakhs/cu mm) in controls is 2.76 ± 0.42 as compared to 1.86 ± 0.29 in test group. The difference is statistically significant, as shown in chart no: 06.

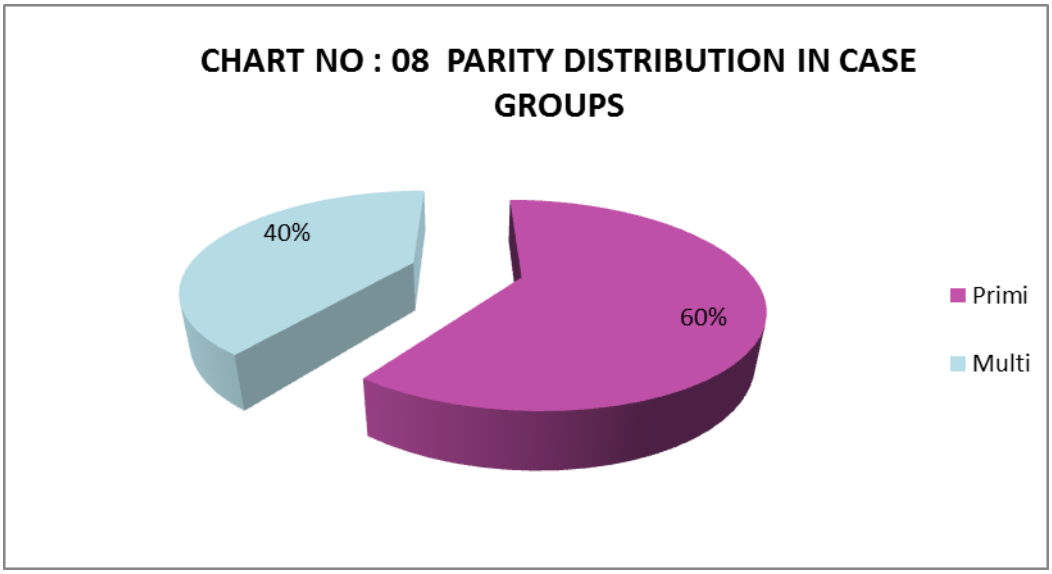
III. The mean and standard deviation of age distribution in controls is 24.55 ± 4.86 as compared to 25.45 ± 4.02 in test group, as shown in table no :05 & 06 and chart no: 09 & 10.

IV. From table IV and chart no: 07 & 08, the parity of the two groups was similar. The percentage composition of primigravidae and multigravidae within the group and between the two groups were similar.

showing parity distribution

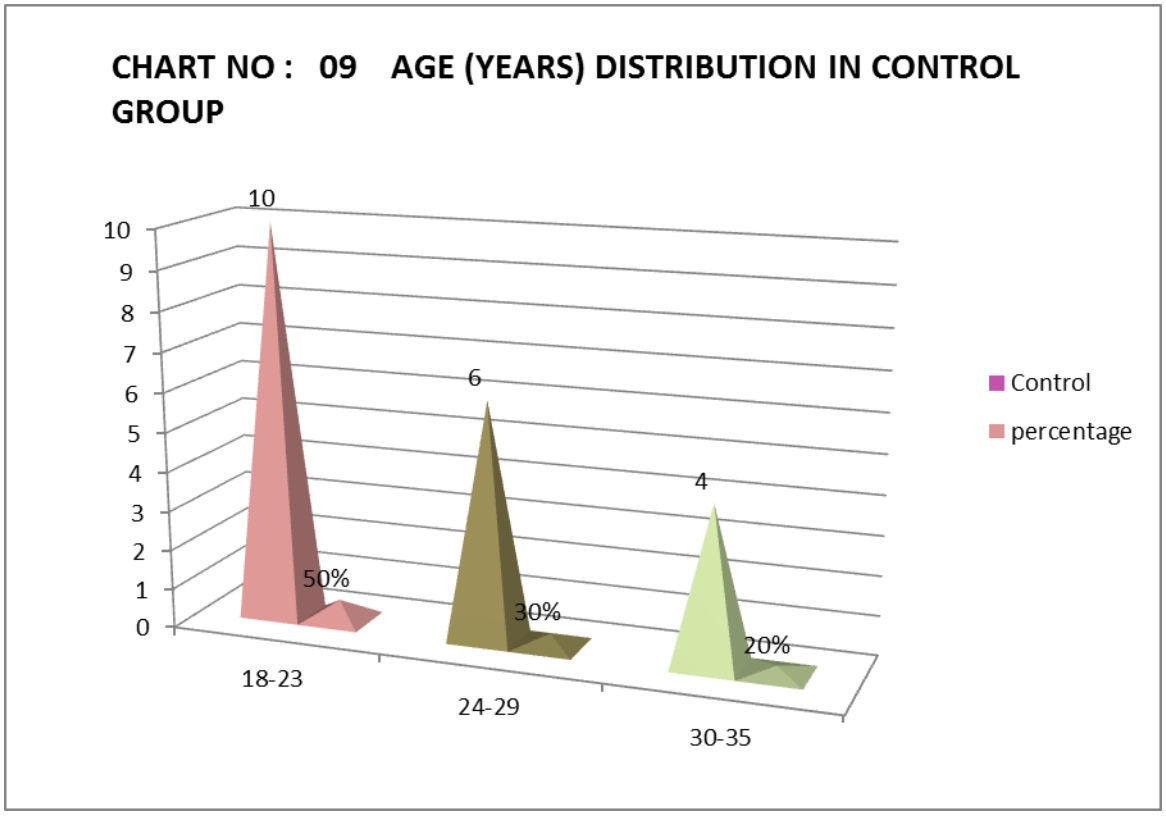
Parity distribution	Control		Cases	
	Number	Percentage	Number	percentage
Primi	09	45%	12	60%
Multi	11	55%	08	40%
Total	20	100	20	100





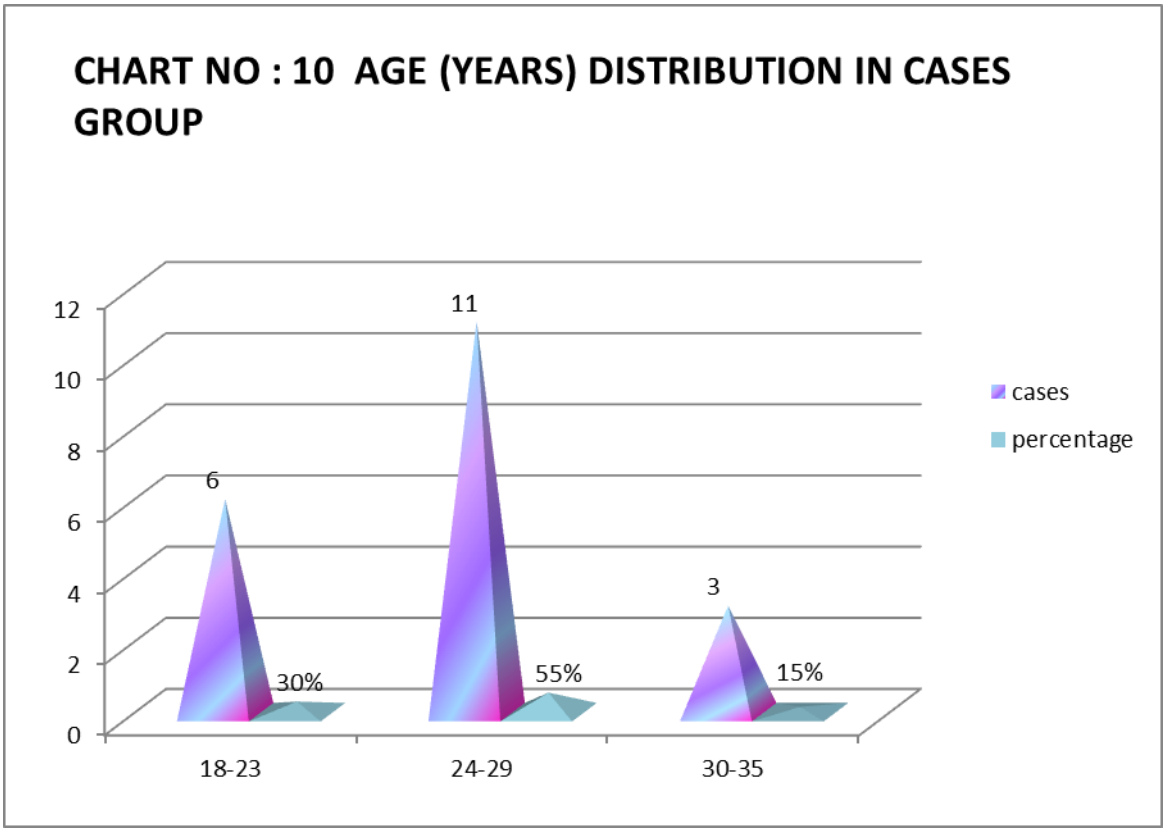
showing the age distribution of control group

Age in years	No: of control subjects	Percentage
18-23	10	50%
24-29	06	30%
30-35	04	20%
Total	20	100%
The mean age was 24.55 years with a standard deviation of 4.86 years.		



showing the age distribution of cases group

Age in years	No: of control subjects	Percentage
18-23	06	30%
24-29	11	55%
30-35	03	15%
Total	20	100%
The mean age was 25.45 years with a standard deviation of 4.02 years.		



VIII. Discussion

Preeclampsia is a serious and life threatening complication in pregnant women. It is a pregnancy specific disorder which rates among one of the major causes of maternal and foetal morbidity and mortality.

More number of cases are seen in underdeveloped and developing countries due to late diagnosis and inadequate antenatal services.

In the present study the biochemical parameters like Fibrinogen Levels And Platelet Count are studied in normotensive and hypertensive(PIH)pregnant women.

The results of the present study are discussed under two groups:

1. Control subjects (normal pregnant women).
2. pregnancy-induced hypertension subjects as cases.

Control group:

A total number of 20 normotensive pregnant women were studied. The age group of these subjects ranges from 18-35 years. All these subjects are normotensive and healthy pregnant women. The results of estimation of Activated Partial Thromboplastin time, Prothrombin time Fibrinogen level and Platelet count are within normal limits.

Case group:

A total number of 20 cases of PIH have been studied. The age group of these subjects ranges from 18-35 years.

All cases showed classical triad of hypertension, proteinuria and oedema. In addition to above symptoms the eclampsia patients are presented with convulsions.

There is significant increase in plasma fibrinogen levels in cases when compared with normal pregnant women, the levels are within normal range and the difference is statistically significant.

There is significant decrease in platelet count in cases when compared with normal pregnant women, the levels are within normal range and the difference is statistically significant.

Biochemical changes consistent with intravascular coagulation and less often erythrocyte destruction may complicate preeclampsia and especially eclampsia. Changes in coagulation profile that occur in normal pregnancy includes the biochemical adaptation especially the haematological changes that occur in response to pregnancy are profound. The levels of several blood coagulation factors are increased during pregnancy.

Plasma fibrinogen increases about 65% late in pregnancy. The increase concentration contributes significantly to the striking increase in ESR. Other clotting factors that increase appreciably during normal pregnancy are factors- VII, VIII, IX, X. Prothrombin and factors- V, XII do not change. Whereas factors- XI and XIII decreases slightly. There is moderate decrease in platelet count as pregnancy progresses.

Maternal thrombocytopenia can be induced acutely by preeclampsia, eclampsia. Overt thrombocytopenia defined by platelet count less than 1,00,000/µl is an ominous sign.

Platelet aggregation is increase in preeclamptic women. Immunological processes or simply platelet deposition at sites of endothelial damage may be the cause. Thrombocytopenia in such disorders is associated with a prolonged bleeding time. The clinical significance of thrombocytopenia in addition to obvious impairment in coagulation is that it reflect the severity of pathological process. The lower the platelet count, the greater are

maternal and foetal mortality and morbidity. The addition of elevated liver enzymes to this clinical picture is even more ominous and a combination of events is referred to as HELLP syndrome [Haemolysis, Elevated Liver Enzymes and Low Platelet Count].

Pregnancy outcome:The outcome of pregnancy in the present study among 20 control subjects 9 delivered normally and in remaining 11 subjects the delivery outcome is not known. Where as in 20 cases, 5 subjects delivered normally, 2 subjects underwent LSCS, in 7 cases ELSCS was done, 2 subjects had foetus with IUGR and 4 with IUD.

IX. Conclusion

A comparative study was done between a normal pregnant women and women with PIH on the levels of Fibrinogen level and platelet count in blood.

The patients were clinically diagnosed based upon history, clinical symptoms, signs and levels of blood pressure.

In all the blood samples obtained from controls and cases fibrinogen levels and platelet count were estimated by standard methods. For this study the results showed a significant rise in fibrinogen levels whereas decrease levels of platelet count I in cases with PIH. But in the control subjects the values for all above paremeters are within normal limits.

From this study it is concluded that estimation of these biochemical parameters plays an important role in the diagnosis of PIH and the evaluation of risk factors, early detection and effective antenatal services, prompt and proper management will decrease the materno-foetal mortality, morbidity and also perinatal mortality.

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