

Immature Platelet Fraction (IPF) in Preeclampsia: A Prospective Comparative Study

Vidushi Kulshrestha, Ediga Asha Jyothi, Tushar Sehgal*, Richa Vatsa, Vatsla Dadhwal

Department of Obstetrics and Gynaecology & *Laboratory Medicine (Haematology), All India Institute of Medical Sciences, New Delhi, India



Introduction

Preeclampsia

- Affects 3-5% of all gestations
- Platelet activation is one of the key pathological events in preeclampsia.
- ↑ in platelet activation & aggregation → physiological fall in platelet count → ↑ platelet turnover (thrombopoiesis).
- These newly released immature platelets are larger, more reactive with higher RNA content & are known as **immature platelets** or **reticulated platelets**.

Immature Platelet Fraction (IPF):

- IPF is the percentage of immature platelets in circulation compared to total number of platelets. (Everett et al, Thromb Haemost. 2014)
- IPF ↑ with increasing thrombopoiesis activity.
- Overall, pathogenesis in preeclampsia is manifested as ↓low platelet count, ↑ MPV and ↑ IPF. (Moraes et al, 2022)
- Considered a sensitive index for rapid platelet destruction which underlies pathogenesis of preeclampsia. (Bernstein et al, Arch Gynecol Obstet 2019)
- Determined by flow cytometry using a proprietary fluorescent dye containing polymethine and oxazine; which penetrate cell membrane staining RNA.
- Recently attracted much attention in preeclampsia because they are easily measurable.
- However, most studies in preeclampsia are either retrospective or cross-sectional. None had prospectively evaluated these indices at more than one-time point.

Hence to explore the utility of IPF in preeclampsia, this prospective study was undertaken.

Aims & Objectives

Primary Objective

- To study the trend of IPF in pregnant patients with preeclampsia at diagnosis and delivery compared to normotensive pregnant controls.

Secondary Objectives

- To correlate IPF with severity of preeclampsia.
- To correlate IPF with composite adverse pregnancy outcomes.

Materials and Methods

- Study Design:** Prospective comparative observational study conducted after taking institute's ethical clearance.
- Subjects (Group-1), n=30: Pregnant women 18-40 years who were diagnosed with preeclampsia as per ACOG definition of preeclampsia.
- Controls (Group-2), n=30: Gestational age matched normotensive women, >18 years

Exclusion criteria (for both groups):

- Platelet disorders (ITP, TTP), aplastic anaemia, anticoagulant therapy, renal and ischemic heart disease, collagen vascular and autoimmune disorders.

Methodology:

- Subjects and controls meeting the inclusion and exclusion criteria were recruited after taking written informed consent.
- Demographic variables were noted, a detailed history and obstetric examination was performed at recruitment.
- In group-1, any severe features were recorded such as blurred vision, upper abdominal pain, epigastric pain, nausea or vomiting.

- Approximately 7-8 mL venous blood sample was drawn. IPF was analyzed by flow cytometry using oxazine dye by Sysmex XN 9000 (Cobe, Japan).

- Samples were taken twice:

- In group-1, at baseline when diagnosis of preeclampsia was made & in group-2 at recruitment.
- Subsequent sampling was repeated on the day of delivery, but before induction of labour in patients who were induced or before shifting patients for caesarean in group-1 and at the time of admission in labour room for controls.

- Additionally, group-1 patients were also subjected to 24-hours urinary protein, hepatic & renal function tests and fundus examination as per routine protocol for preeclampsia.

- All subjects and controls continued to get antenatal care as per hospital protocol.

Outcome Measures:

Primary outcome-

- IPF at diagnosis and delivery in preeclampsia vs. controls

Secondary outcome-

- Correlation of IPF with severity of preeclampsia and composite adverse pregnancy outcomes*

*Composite adverse pregnancy outcomes:

- Maternal: Severe preeclampsia, eclampsia, HELLP syndrome
- Fetal: fetal growth restriction, intrauterine fetal death, prematurity, low birth weight, birth asphyxia

Statistical analysis:

- Data analysis was carried out using statistical package STATA version 14.0.
- Categorical variables were compared by chi-square or fisher exact test. Continuous variables were compared by independent t-test (if parametric) and by Wilcoxon rank sum test (if non-parametric). Subgroup analysis for variables were compared by paired t-test and Wilcoxon-signed rank test. P value less than 0.05 was taken as statistically significant.

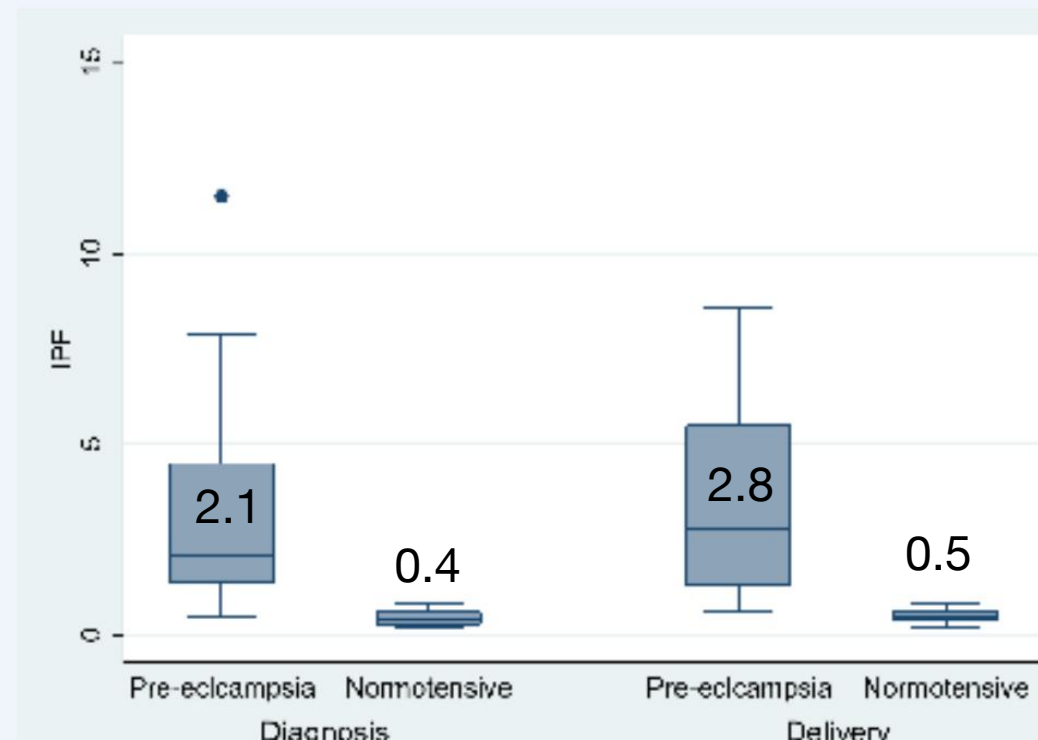
Results

Mean gestational age at diagnosis of preeclampsia was 32.57± 2.61 weeks In group-1; and was 32.54±2.72 weeks at enrolment in group-2 (p 0.97).

Primary outcome measures in group-1 and group-2

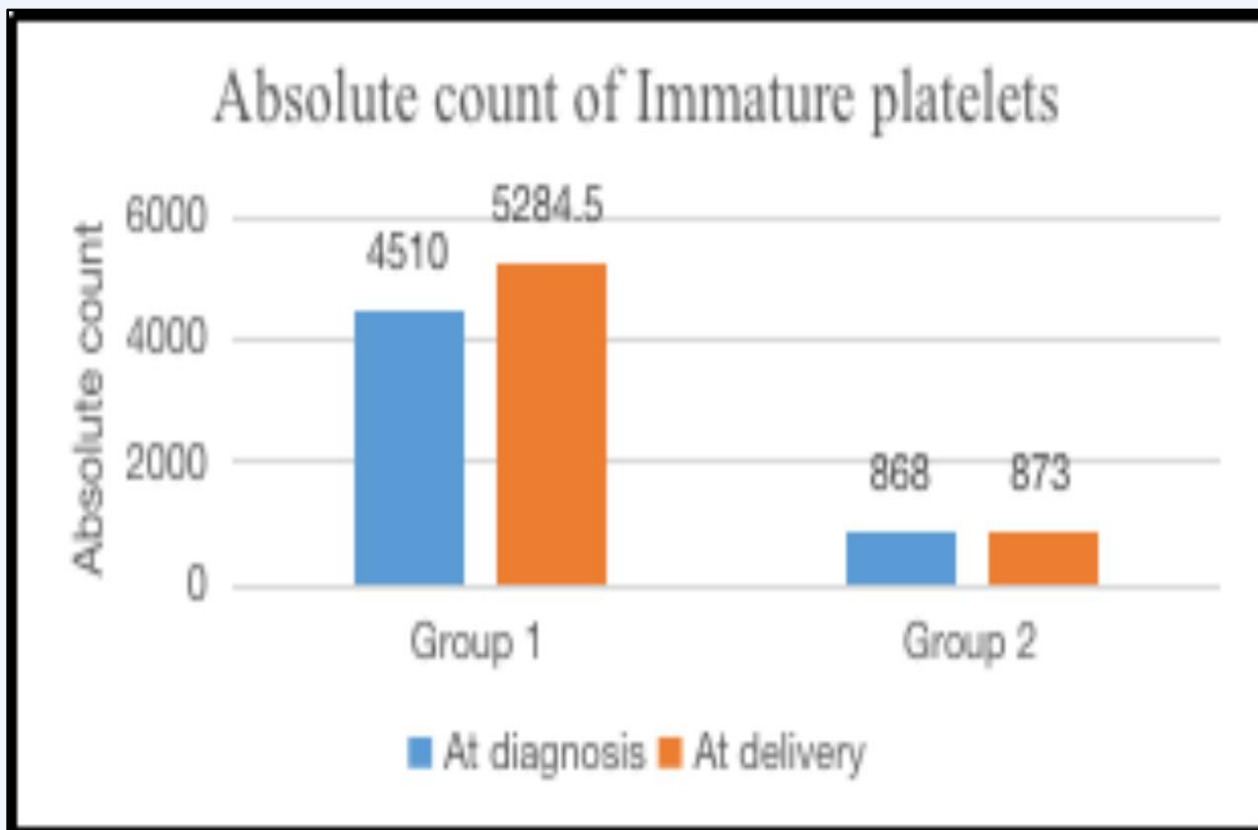
Parameters	Time points	Median (range)		P value*
		Group-1	Group-2	
IPF (%)	At baseline	2.1 (0.5-11.5)	0.4 (0.2-0.8)	0.0001
	At delivery	2.8 (0.6-8.6)	0.5 (0.2-0.8)	0.0001
	P value	0.308	0.375	
	% Change	33.33%	25%	
Absolute IP/μl	At baseline	4510 (1638-21735)	868 (300-1760)	<0.0001
	At delivery	5284.5 (1836-15939)	873 (202-1750)	<0.0001
	P value	0.289	0.853	
	% Change	17.17%	0.57%	

Box whisker plot depicting median (range) of IPF



- IPF was significantly higher in group-1 at both time points.
- 1 patient had extremely high value, 11.5 at diagnosis
- Range was narrow in group-2 both at baseline and delivery.
- Trend of IPF from baseline to delivery: Rise which was not significant in either group.

Absolute count of immature platelets (Absolute IP)



- Absolute IP was also significantly higher in group 1 compared to controls,
- But rise from diagnosis till delivery was not significant

Secondary Outcomes:

- There was no correlation of IPF with composite feto-maternal outcomes.
- ROC analysis was carried out. The analysis showed cut-off value was 0.75 with 93% sensitivity and 97% specificity. Area under curve (AUC) was 0.97 (95% CI: 0.94 – 1.0) with standard error (SE) of 0.05.

Subgroup analysis of IPF in group-1: Overall and according to severity and onset of preeclampsia

Parameters Median (range)	Time points	Group 1 (Overall) N=30	Subgroups according to severity			Subgroups according to onset		
			PE without severe features (N=17)	PE with severe features (N=13)	P value*	Early onset (N=17)	Late onset (N=13)	P value**
IPF (%)	At baseline	2.1 (0.5-11.5)	1.9 (0.5-7.9)	3.3 (0.8-11.5)	0.203	2.1 (0.5-11.5)	2.1 (0.5-7.8)	0.571
	At delivery	2.8 (0.6-8.6)	2.8 (0.6-6.5)	3.8 (1.1-8.6)	0.216	2.9 (0.6-6.5)	2.7 (0.6-8.6)	0.966
	P value	0.308	0.368	0.6		0.635	0.014	
	% Change	33.33%	47.36%	15.15%		38.09%	28.57%	
Absolute IP/μl	At baseline	4510 (1638-21735)	3618 (1638-13179)	5640 (1808-21735)	0.156	5206 (1638-21735)	3810 (1750-13986)	
	At delivery	5284.5 (1836-15939)	5236 (1836-14080)	5832 (2028-15939)	0.196	5236 (1926-11840)	5684 (1836-15939)	0.414
	P value	0.289	0.331	0.649	0.522	0.01		
	% Change	17.17%	44.72%	3.4%	0.57%	49.18%		

*Between PE with and without severe features

**Between early and late onset PE

Discussion

- Though the studies are limited, high IPF values were observed even at normal platelet count.
- It reflects the stage of thrombopoiesis and can be used to distinguish the causes of thrombocytopenia, with it being a more stable parameter than mean platelet volume (MPV).
- Also, IPF was demonstrated as a more stable marker than platelet count or MPV in preeclampsia. (Moraes et al, 2022)
- IPF is derived from routine blood investigations within accessible time; and are less expensive unlike other parameters and biomarkers presently in use for screening, diagnosis and assessing severity of preeclampsia (such as sFLT-1/PLGF ratio, PAPP-A, LDH, soluble endoglin, placental protein-13, HbF or uterine artery doppler); which are expensive, need higher laboratory techniques and are time consuming.

Conclusion

- PF levels were significantly higher in women with preeclampsia than in normotensive controls, both at diagnosis and at delivery; though there was no correlation with severity, onset and feto-maternal outcomes.
- Overall IPF did not change longitudinally from diagnosis to delivery except in late onset preeclampsia.
- IPF is a simple, inexpensive laboratory test and its clinical utility in preeclampsia may be explored in low resource settings.