

Original Research Article

Significance of Relationship between Platelet Indices and Hyperlipidemia in Predicting Ischaemic Events

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Abstract: Atherosclerosis begins with damage to the endothelium. It's caused by high blood pressure, smoking, or high cholesterol. That damage leads to the formation of plaque. When bad cholesterol, or LDL, crosses the damaged endothelium, the cholesterol enters the wall of the artery. That causes your white blood cells to stream in to digest the LDL. Over years, cholesterol and cells become plaque in the wall of the artery. This was a prospective case control study conducted between June to August 2016 in the Department of Pathology in collaboration with Department of Cardiology, General medicine and Department of Biochemistry of Prathima Institute of Medical Sciences, Karimnagar. The study and control groups were found to be age and sex matched statistically. Hence, no bias in the results was observed due to age and sex in the results. Out of the total 80 cases of hyperlipidemia, 47 were males and 33 were females. The patients fell into the age group between 23 to 83 years. Mean age in study group - 53.65 years and in control group - 56.35. All three PVI were significantly higher in hyperlipidemic cases than the normolipidemic controls (p-value <0.05). Isolated hyperlipidemia showed no significant difference in platelet parameters than controls (p-value >0.05). Hyperlipidemia associated with other diseases like diabetes, hypertension and CAD showed significantly lower platelet counts and significantly higher platelet indices (p-value <0.05) than controls.

Keywords: Hyperlipidemia, Platelet indices, ischemia

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease that plagues humankind for millennia. Carl von Rokitansky and Rudolf Virchow controversially explained the concept of inflammatory causes in the middle of the 19th century [1]. Hyperlipidemia, very closely associated to atherosclerosis, is a major acquired risk factor, where chronic hypercholesterolemia plays a role in endothelial dysfunction [2]. Dyslipidemia or Hyperlipidemia can also be described as elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). Increased Low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk. But these well-known risk factors are not enough for complete risk evaluation and formulation of treatment strategies in clinical practice [3]. Moreover many studies have concluded that it has a common association with diabetes mellitus, obesity and hypertension [4- 7]. These

conditions increase the risk of ischaemic events in dyslipidemic patients.

Platelets, are now gaining interest as inflammatory cells contributing to pathophysiology of atherothrombosis [8, 9]. One of the contribution theories states hyperlipidemia increases the cholesterol content in platelets and enhances their reactivity. Hyperactive platelets may contribute to accelerated atherogenesis associated with CAD and stroke [10]. This hyperactivity of platelets manifests as alterations in platelet volume indices. Automated cell counters have made the platelet count (PC) and the platelet volume indices (PVI) routinely available in most clinical laboratories at no additional cost. Larger platelets are considered to be metabolically, enzymatically and functionally more active than the smaller platelets [11]. These produce more thromboxane B2 than platelets in normal steady state function and are hemostatically

more active and hence have more thrombogenic potential [12].

AIMS AND OBJECTIVES

1. The aim of study was to compare variables of platelet count and platelet volume indices (MPV, PDW, P-LCR) in the study group and the control group.
2. Compare the platelet indices of patients with isolated hyperlipidemia to those with associated diseases like atherosclerosis, diabetes and hypertension.
3. Study the alterations in PVI which would help in formulating treatment and prevention strategy of ischaemic events.

MATERIALS & METHODS

Study Design and Set Up:

This was a prospective case control study conducted between June to August 2016 in the Department of Pathology in collaboration with Department of Cardiology, General medicine and Department of Biochemistry of Prathima institute of medical sciences, Karimnagar. A total of 80 cases (Fig1) with deranged lipid profile was traced from department of biochemistry, cardiology and medicine outpatient clinics as well as admitted patients. The exclusion criteria were pregnant women, patients on anti-platelet therapy, anemia and infections.

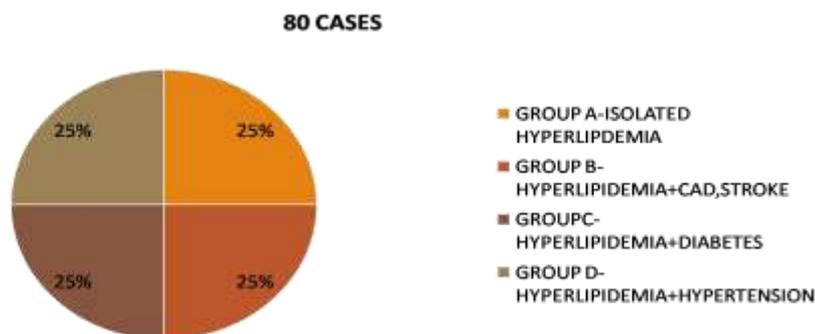


Fig 1: Distribution of study group

Selection of hyperlipidemia cases was done based National Cholesterol Education Programme, ATP III evidence-based guidelines 2001 for cholesterol testing and management:

- Total Cholesterol: ≥ 240 mg/dl
- Total Triglyceride: ≥ 200 mg/dl
- LDL levels : ≥ 160 mg/dl

Criteria for selection of hypertensive cases with hyperlipidemia - Above stage 1 hypertension that is systolic BP >140 mmHg and/or diastolic >90 mmHg were taken. Criteria for selection of diabetic cases with hyperlipidemia - Anti Diabetic Association Criteria of Fasting Blood Glucose >126 mg/dl. Patients who had multiple diseases were placed in more than one group. Patients with hyperlipidemia in spite on lipid lowering drugs were also included.

Control group:

Control group consisted of 20 age and sex matched normal subjects attending the hospital outpatient clinics of cardiac and medicine for a general health checkup or some unrelated complaints.

Selection criteria for the control group were:

1. Lipid profile was within normal range on recent laboratory reports.
2. No atherosclerotic disease, Diabetes mellitus or hypertension.

2 ml blood sample was collected in EDTA coated tubes from the antecubital vein by a clean puncture avoiding bubbles and froth. The samples were run within two to six hours of venepuncture using the analyzer to avoid time related artefactual changes. Samples were analyzed by the SWELAB alfa, basic model. Three part differential automated hematology analyzer for obtaining the platelet parameters – PVI (e.g. MPV, PDW and P-LCR) and Platelet Count.

PLATELET PARAMETER	REFERENCE RANGE
PLATELET COUNT	1.5-4.5 x 10 ⁹ mm ³
MEAN PLATELET VOLUME(MPV)	7.2-11 fl
PLATELET DISTRIBUTION WIDTH(PDW)	9.3-14 fl
PLATELET LARGE CELL RATIO (P-LCR)	11-66%

Data was collected along with lipid profile parameters and the statistical tests applied on the samples were mean, standard deviation, independent sample t test, ANOVA test, Pearson’s correlation coefficient (r). Significance is assessed at 5 % level of significance and 95% confidence interval.

Assumptions

- Dependent variables are normally distributed.

- Samples drawn from the population are random.

RESULTS

The study and control groups were found to be age and sex matched statistically. Hence, no bias in the results was observed due to age and sex in the results. Out of the total 80 cases of hyperlipidemia, 47 were males and 33 were females. The patients fell into the age group between 23 to 83 years. Mean age in study group - 53.65 years and in control group - 56.35. (Fig2)

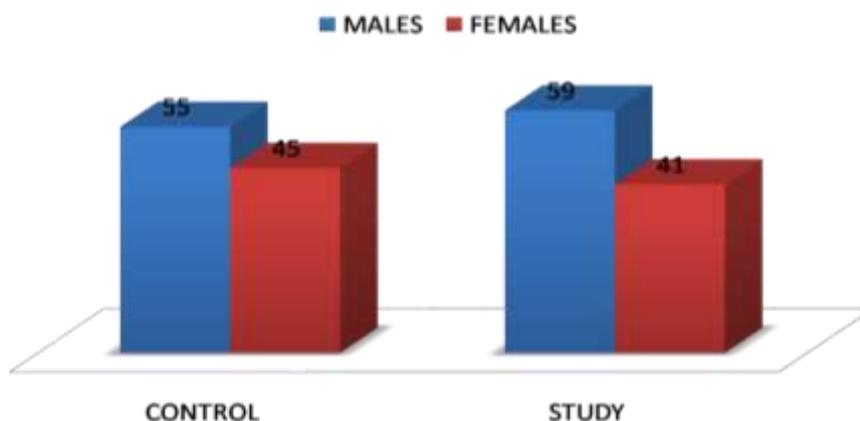


Fig 2: Age and Sex distribution

Mean MPV (8.51±1.09), PDW (13.63 ± 2.43) and P-LCR (19.15 ± 3.44) of cases were higher than the controls (mean MPV = 7.24 ±1.02; mean PDW= 11.3 ± 0.92, mean P-LCR = 13.41 ± 2.38) as shown in Table-1. So, all 3 studied PVI were found to be significantly

higher (p value = <0.0001) in the hyperlipidemia patients than the normolipidemic patients. Platelet count of study group was significantly lower than the control group .Table 1(p value = 0.0007)

Table1: Mean PC, MPV, PDW and P-LCR of cases against control

Parameters	Group	No. of study cases	Mean± SD	p- value
PC	STUDY	80	2.14 ± 0.50	=0.0007
	CONTROL	20	2.58 ± 0.50	
MPV	STUDY	80	8.51 ± 1.09	<0.0001
	CONTROL	20	7.24 ± 1.02	
PDW	STUDY	80	13.63 ± 2.43	<0.0001
	CONTROL	20	11.3 ± 0.92	
P-LCR	STUDY	80	19.15 ± 3.44	<0.0001
	CONTROL	20	13.41 ± 2.38	

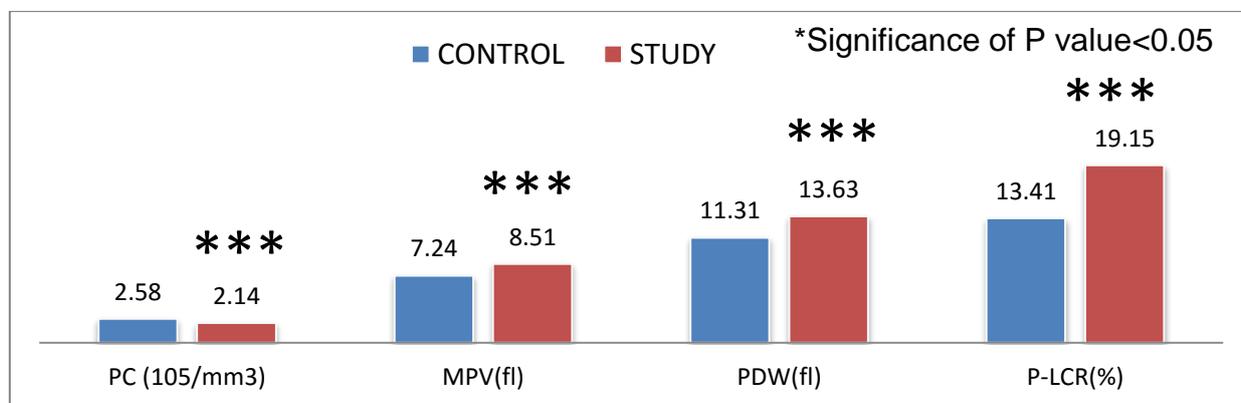


Fig 3: platelet count and platelet volume indices in study and control group

On comparing platelet parameters in different groups vs. control group. It was found that all three PVI were significantly higher in groups B, C and D than the

control group. There was no significant difference in these indices in group A and the control (Table 2)

Table 2: MPV, PDW, & P-LCR in groups A – D against that of the controls

GROUP	No.of. patients	MPV		PDW		P-LCR	
		(Mean± SD)	p-value	(Mean± SD)	p-value	(Mean± SD)	p-value
CONTROL	20	7.24 ± 1.02		11.31 ± 0.92		13.41 ± 2.38	
A	20	7.48 ± 1.23	0.590	13.03 ± 3.03	0.019	14.78 ± 0.02	0.349
B	20	9.15 ± 0.94	< 0.0001	14.38 ± 3.09	< 0.0001	21.13 ± 6.51	< 0.0001
C	20	8.94 ± 1.06	< 0.0001	14.07 ± 2.68	=0.0001	18.8 ± 5.13	=0.0001
D	20	8.61 ± 1.04	=0.0002	13.56 ± 2.79	< 0.001	18.69 ± 4.90	=0.0001

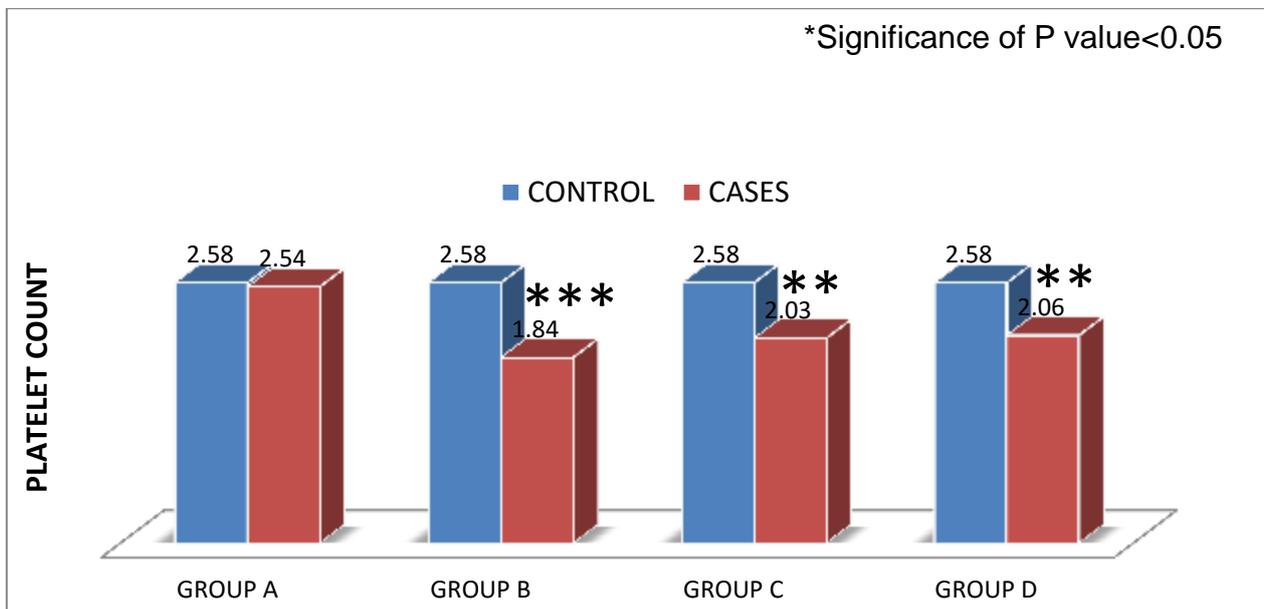


Fig 4: Platelet Count in Study and Control Group

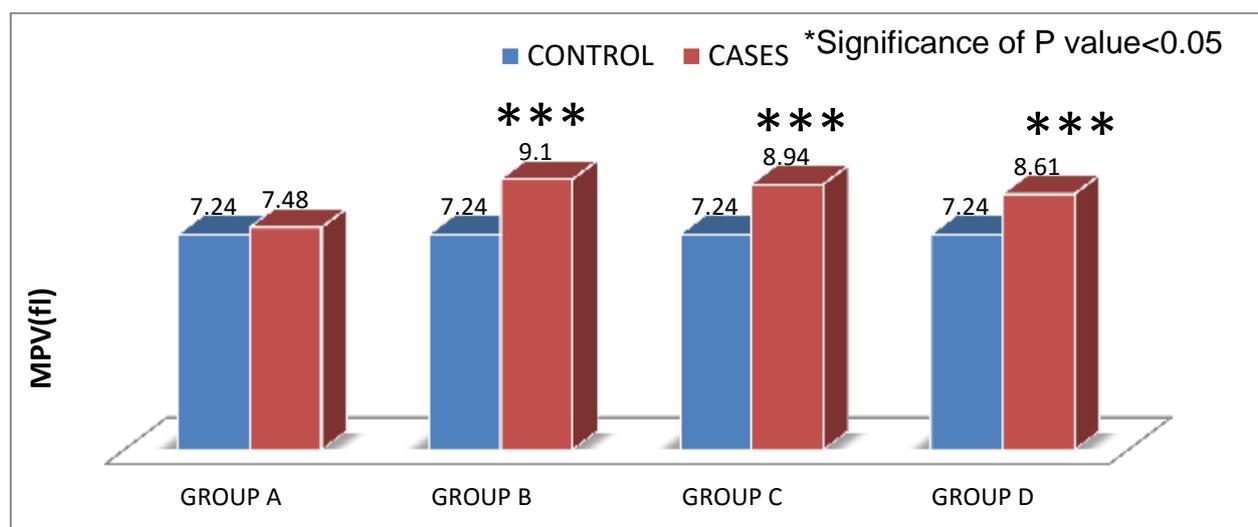


Fig 5: Mean Platelet Volume in Study and Control Groups

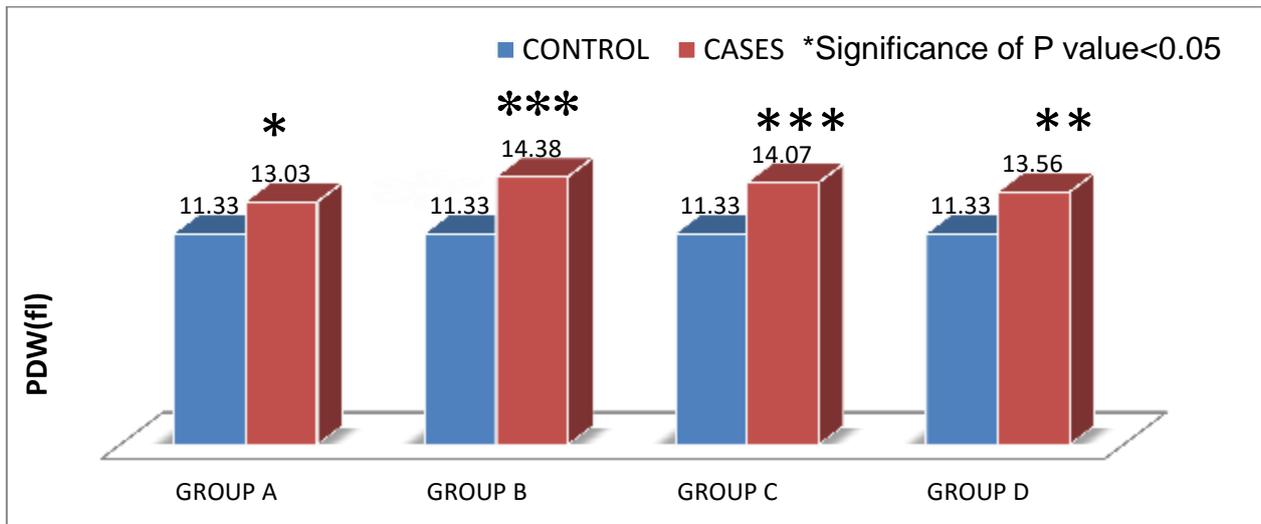


Fig 6: Platelet Distribution Width in Study And Control Group

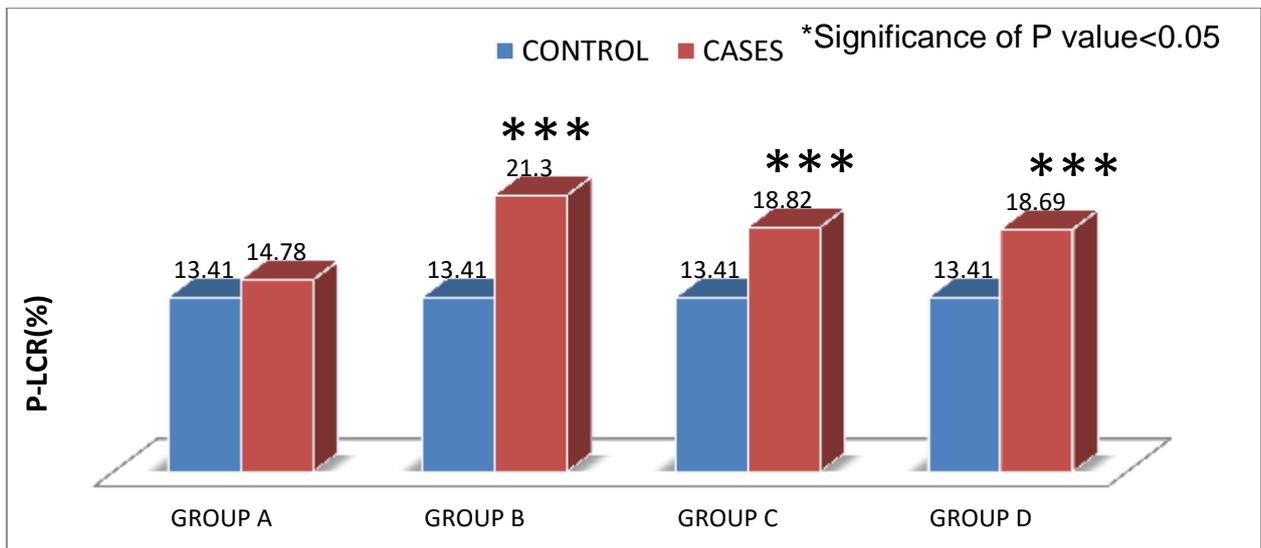


Fig 7: platelet large cell ratio in study and control groups

So, Hyperlipidemia associated with other diseases had significantly higher PVI than Isolated hyperlipidemia.

Correlation of PC and PVI with the severity of hyperlipidemia was done:

- Poorly positive correlation was seen between PVI and severity of hypercholesterolemia.(Table. 3)

- MPV, PDW and P-LCR showed a poorly positive correlation with serum total cholesterol levels.
- No correlation with triglyceride levels (p-value > 0.05) and LDL levels (p-value > 0.05)
- Platelet numbers correlated negatively with MPV, PDW and P-LCR.

Table 3: Correlation between TC, PC and PVI

TC	PVI	Correlation coefficient(r)	p- value
TC	MPV	0.200	0.0002
TC	PDW	0.350	0.0001
TC	P-LCR	0.289	0.690

PC	PVI	Correlation coefficient(r)	p- value
PC	MPV	-0.3	< 0.001
PC	PDW	-0.4	< 0.001
PC	P-LCR	-0.2	< 0.001

DISCUSSION

Role of hyperlipidemia and platelets in thromboembolic events is well-known and various platelet volume indices have been studied in these conditions. Samples collected in EDTA coated tubes were run in Swelab automated coulter, within 2 – 6 hours after venepuncture to avoid artefactual changes in platelet size as proposed by *Thompson et al.*; [13]. This case control study was conducted with both age and sex matched controls to avoid any bias in our results [14-16]. Patients with persistent hyperlipidemia even after treatment were also included based on the study of *Fuchs J et al.*; [17]. The results obtained in study group were similar to study done by *Grotto et al.*; [18].

All three PVI – MPV, PDW & P-LCR were significantly higher (p-value <0.05) in the study group than the controls, i.e. hyperlipidemic patients had significantly higher MPV, PDW and P-LCR than the normolipidemic patients. It is observed that hyperlipidemia is widely associated with many diseases so we decided to divide our study group of hyperlipidemia into further sub-groups- groups A – D based on the presence of the associated diseases.

Group A (n = 20; 25%) comprising of hyperlipidemic patients without any other associated disease, had no significant difference (p > 0.05) in their platelet count, MPV and P-LCR (p-value – 0.429, 0.590, 0.349 respectively) against the controls except for PDW(p-value0.019). A similar study using the same platelet volume indices in dyslipidemic patients done by *Grotto et al.*; found that MPV, PDW and P-LCR were significantly higher in dyslipidemic patients

than in controls (P < 0.00001) [18]. This is in discordance with our study if we take into account the results of group-A patients having hyperlipidemia only as in our study, this group had raised PDW than the control group. *Ravindran et al.*; [19] studied PC, PDW and Plateletcrit in hypercholesterolemic patients and found that there was no significant difference in platelet counts between the healthy controls and the hyperlipidemic patients and an increase in PDW only in patients who had hyperlipidemia associated with CAD. They emphasized that existence of more than one risk factor is found to have a significant effect on the platelet hyper-responsiveness.

Our group-B patients (n = 20; 25%), had Coronary Artery Disease and Cerebro Vascular Accidents along with hyperlipidemia. In our study platelet count was significantly lower (p value (p<0.001) and all the PVI (MPV, PDW, P-LCR) (p<0.0001) were significantly higher in this group than the normal controls. This study was similar to other studies which also found a decreased platelet count in AMI patients as compared to the controls [12, 20, 21].

Group-C patients (n = 20; 25%) in our study had diabetes mellitus along with hyperlipidemia and it was found that these patients also had significantly lower platelet count (p value= (p<0.001) and higher MPV, PDW and P-LCR (p<0.0001) than the control group. Our results were similar to a very recent study done by *Jindal et al.*; who also found all these three platelet volume indices higher in the diabetic patients compared to the controls [22]. This strengthens the hypothesis that hyperglycemia contributes to heightened

platelet reactivity directly as well as through glycation of platelet proteins and hence results in an increase in platelet volume indices.

Group-D patients (n = 20; 25%) in our study had hyperlipidemia in association with hypertension. We found that all three PVI; MPV, PDW and P-LCR were significantly higher in this group as compared to the controls (Table- 2; p value <0.0002, 0.001, 0.0001 respectively) with a significant difference in the PC (p<0.001) between the two groups. The results of this study group were similar to the study conducted by Halil Ibrahim Onder *et al.*; [23]. These findings concluded that the patients who were having hyperlipidemia associated with other diseases had significantly higher PVI i.e. larger platelets than the normolipidemic patients.

An attempt was also made to correlate these platelet volume indices with the severity of hyperlipidemia. For this, PC and PVI values were correlated with the increasing values of total cholesterol and triglyceride levels. It was found that all 3 PVI had a poor positive correlation with increasing total cholesterol; however no correlation was seen with increasing triglyceride. This means that platelet size was directly proportional to the severity of hyperlipidemia (hypercholesterolemia).

A previous study conducted by Khemka *et al.*; [24] divided the study group into similar groups and found the PVI to be higher in dyslipidemic patients with associated diseases but no significant change in platelet count by which it differs from the present study. We had certain limitations and constraints in this study. Although we took a sample size of 100 patients in this study, the division of them into subgroups gave us a very small sample size for each sub- group which might be the reason of a different result in platelet count. So, further such studies with larger sample size may help to remove such discrepancies.

CONCLUSION

All three PVI were significantly higher in hyperlipidemic cases than the normolipidemic controls (p-value <0.05). Isolated hyperlipidemia showed no significant difference in platelet parameters than controls (p-value >0.05). Hyperlipidemia associated with other diseases like diabetes, hypertension and CAD showed significantly lower platelet counts and significantly higher platelet indices (p-value <0.05) than controls. This strengthens the hypothesis that hyperglycemia in diabetes contributes to heightened platelet reactivity directly as well as through glycation

of platelet proteins and hence results in an increase in platelet volume indices.

A positive correlation of platelet indices with increasing cholesterol levels states thrombogenic potential of Hyperlipidemia. Hyperlipidemia when associated with other diseases brings the platelets in a prothrombotic active state as reflected by the changes in its PVI and PC. PVI estimation is an early, economical and rapid procedure and should be advised specifically in dyslipidemic patients to start the platelet inhibition therapy along with statins to prevent CAD or stroke. Further studies should be conducted on a larger sample size to prove this hypothesis.

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