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"The Modulation of Platelets' Activity is Associated with Early Onset of Anxiety Traits in Adolescents"

Kalpna Verma[1], Dileep K Verma[2], Anupma Verma[1], Vandana Ranjan[1,*]

1 – Department of Biochemistry, Dr. Rammanohar Lohia Avadh University, Ayodhya – 224001, UP, India

2 – Department of Psychiatry, Maharshi Vashishtha Autonomous State Medical College and OPEC Hospital, Basti – 272124, UP, India

'*' – Corresponding author – ranjanrml@gmail.com , vandanaranjan@rmlau.ac.in,

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Abstract

Platelets, which share serotonergic pathways with the CNS, offer a unique insight into peripheral responses during anxious behaviour processing. Serotonin, released from active platelets, is known for playing a central role in autonomic and emotional regulation of complex biochemical pathways including those associated with undesired behavioural outcomes of clinical significance, as in the case of anxiety.

Objective: To investigate if the alterations in platelets' activity has a correlation with anxiety traits expressions which can be used as a clinical biomarker for its timely identification and diagnosis at an early age of adolescent.

Design & methods: A total of 268 teenagers aged 13–19 years, included in this study were first, screened through self-assessment questionnaire based on psychological scales (GAD-7, BFNE, and PSS-10) to be grouped under control and test group, followed by analysis of platelets' activity- parameters from their blood samples.

Result: In the test group subjects, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) parameters were significantly elevated, while Platelet Count (PLT) was found to be decreased, as compared to control group. The results for each parameter were found independent of gender factor.

Conclusion: The study strongly suggests an active involvement of serotonergic platelets in the pathways of stress & anxious behaviour processing which might contribute to the development of anxiety disorders at an early age of adolescence. These findings support the merit of the concept that 'a combinatorial modulation in platelets' indices is a potential clinical biomarker to diagnose early-stage anxiety traits in adolescents.

1. Background:

The inappropriate and persistent response of anxiety is considered as a behavioural disorder of clinical as well as social concern. The serotonin, a neurotransmitter, as known for its role in autonomic functions, motor activity, cognition, and various processes associated with emotions such as anger, affection, and mood regulation, is suggested as a sync between CNS and peripheral responses during anxious behaviour processing via altered platelets' activity [6,31,32]. Neuroscientific investigations on serotonergic platelets' activities in anxiety and related disorders have provided valuable insight for this sync [57,15,43,54]. Various parameters including serotonin, Monoamine oxidase (MAO), BDNF, G- protein-coupled receptors, and second messenger systems like cAMP, IP3, DAG, etc., have been found

common between platelets and CNS under biochemical/neural processing of anxiety and depression [23,58,46,47]. The changes in critical parameters, such as blood 5-HT levels, the amount of 5-HT stored in platelets, and the expression of α_2, β_2-adrenoceptors, and 5-HT$_2$A receptors on the platelet surface, during anxiety, have had been a focus of research [2,14]; yet, easily accessible markers such as platelets activation parameters are an underexplored area.

Platelets' activation comes into play, classically, to keep intact the integrity of vascular tissues to maintain homeostasis while anticipating any possible upcoming trauma [8]. Apart from this primary physiological role, as reported in the recent research literature, some other platelets activities confirm its crosstalk with immune response and neuronal activities which have been suggested in behavioural modulation [27,13]. Similar platelets' activities were reported for increased risk of coronary comorbidities in patients of anxiety and depression [12,13]. Serotonin is a key modulator to alter platelets activities in the periphery as well as neuronal activities in CNS [6, 49]. The chronic stress may result in disproportionate activation or dysregulation of platelets' activation mediated by serotonin and may be an underlying mechanism for pathological onset of undesirable persistent changes in behaviour including anxiety and depression disorders [8,34,26].

The common platelet indices, examined in the present study, have provided valuable insights, showing significant associations with both – non-pathological and pathological ranges and have also been reported in anxiety disorders from previous research [57,19,39]. Increased expression of SERT (serotonin transporter) on the surface of platelets following platelets' activation [29,58,11] leads to enhanced serotonin uptake, resulting in decreased peripheral serotonin levels [50,28,40,7] which has been associated with depression and anxiety. Successive to this, enhanced serotonergic activity stimulates further platelet activation via 5-HT$_2$A receptor (12,27,2,42), leading to increased platelet size and consequently elevated mean platelet volume (MPV) [18,22,10,18,10]. The elevated production of activated platelets also contributes to an increased platelet distribution width (PDW) [44,9]. Moreover, chronic stress or inflammatory conditions may cause hyperactivation and aggregation of platelets due to altered serotonin function, potentially resulting in a decreased platelet count (PLT) and plateletcrit (PCT) because of increased consumption or destruction of platelets [57, 8, 9, 13,48]. Therefore, the aims of this study include, evaluating platelet indices such as MPV, PDW, PCT and PLT in adolescent patients with anxiety to establish a preliminary biochemical/clinical screening tool and/or as a promising biomarker towards diagnosis of onsetting anxiety traits at an early age like adolescence [33,36,53,25,41].

Research till date has been found to be focused on the adult population while studies have reported rising prevalence of anxiety in the younger population too; yet clinical insights are too limited (5,18,2,37,10,18) and scientific investigations are negligible [54]. The anxiety and depression in teenagers and its correlation with platelet activities is investigated for its underlying pathologic etiology of developing these disorders which would be instrumental in designing diagnostic tools and preventive measures [34]. This study concluded that the platelets' activities, known for their role in maintaining 5HT amount in periphery, are significant contributor to the underlying physiological mechanism for development of anxiety at an early age of adolescence. Platelet indices are significant to be considered as potential peripheral biomarkers for psychological conditions like anxiety, depression, and stress due to their role in inflammation and serotonergic activity [55,3,17,33,56,27].

2. Materials

2.1 Sample

268 teenagers including 134 males and 134 females (age range: 13-19 years), volunteered for this study, following psychological evaluation, and psychiatric consultation they were assessed for prevalence of anxiety as per the classification in the Diagnostic and Statistical Manual of Mental Disorders V (DSM- V) of the American Psychiatric Association. The subjects/participants (volunteers) were assessed using a self-assessment questionnaire (GBP questionnaire) for **G**eneralized Anxiety Disorder-7 (GAD-7), **B**rief Fear of Negative Evaluation (BFNE), and **P**erceived Stress Scale -10 (PSS-10) anxiety scales as per DSM-V [24,20]. The questionnaire, based on DSM-V, was administered with the information about the study. The written consent for participation in the study was obtained from all participants and/or from their parents or legal guardians as per ethical guidelines stated by IEC. Based on the analysis of questionnaire data, the subjects were divided into two groups, Control (Healthy) and Test (Pathological/presence of confirmed anxiety traits) group. The subjects did not have concomitant diseases (screened by clinical history, physical examination, and blood tests), and none of the volunteers were taking drugs (including anxiolytic medications or psychotropic drugs). Blood samples were obtained from all the volunteer participants.

2.2 Analytical Tool

I. The DSM-V manual of the American Psychiatric Association was referred to create a standard questionnaire for GAD-7, BFNE-12, and PSS-10 anxiety scales [16,38,20,4,30].

II. ABX Pentra XL 80 haematology analyser was used for complete blood count (CBC) including platelet indices (PLT, MPV, PDW, PCT).

2.3 Statistical Tools

All the collected clinical data was sorted as a database using the Microsoft Excel utility for statistical analysis. The advanced statistical analysis was carried out using IBM SPSS 30.0 Statistics software.

3. Methods

This study was a cross-sectional study conducted in the selected hospitals of Ayodhya, Basti and nearby districts in Uttar Pradesh province of India. In the first part of this study, the severity of depression and GAD were assessed by GAD-7, PSS-10 & BFNE anxiety scales [24]. A total of 268 subjects include 168 subjects (test group) with generalized anxiety disorders without a previous history of drug intake and 100 healthy subjects (control group). Their self-assessed reports have confirmed the clinical diagnosis given by a professional psychiatrist. All individuals were in age range of 13-19 years, and both genders were included in the study. The test group comprises of 84 males and 84 females while the control group included 50 males and 50 females.

The patients with the following conditions were excluded from both the study groups which might affect platelets indices (PLT, MPV, PCT, PDW) level i.e. those with comorbid mental illness (panic disorder, PTSD, schizophrenia, OCD, etc.); those with comorbid physical illness (CKD, Cancer, Diabetes, Coronary Heart Disease etc.); patients on antiplatelet drugs like- aspirin, clopidogrel, ticlopidine, prasugrel, abciximab, etc. and anti-inflammatory drugs like furosemide, penicillin, quinidine, quinine, ranitidine, sulphonamides, linezolid, etc. which cause thrombocytopenia [40].

Self-assessment data was obtained by GBP questionnaires, followed by blood sample collection and its analysis for biological parameters under this study. The haematological parameters were measured for Platelet count (PLT), Mean Platelet Volume (MPV), Plateletcrit (PCT), and Platelet Distribution Width (PDW). Blood samples were collected into 5 ml anticoagulant tubes with EDTA as anticoagulant, and the samples were analysed using an automated haematology analyser ABX Pentra XL 80. The data for these analytical parameters were collected

over a period of 18 months, from February 2023 to August, 2024.

The statistical analysis of biochemical data was performed using the Computational software programs (IBM SPSS for Windows, version 30.0. Chicago, SPSS Inc.) and Microsoft Excel. Results on continuous measurements are presented as mean ± standard deviation and are compared using Analysis of Variance. Discrete data is expressed as numbers (%). Pearson's correlation coefficient (r) was used to measure the associations among continuous variables. For all analyses, the statistical significance was fixed at 5% level (p value <0.05).

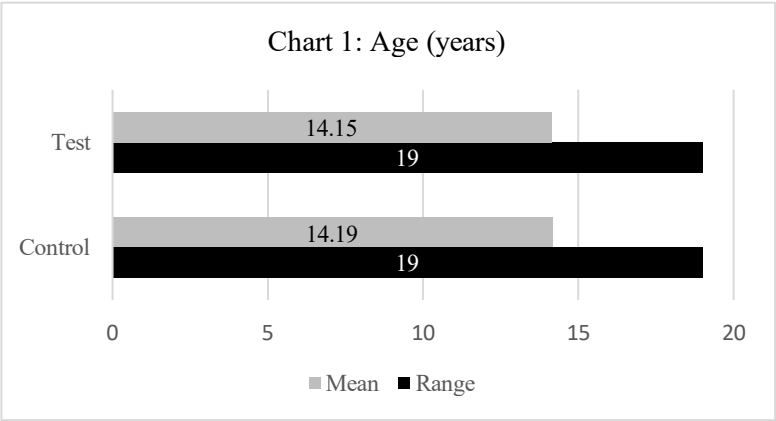
4. Results

4.1. The Age Factor

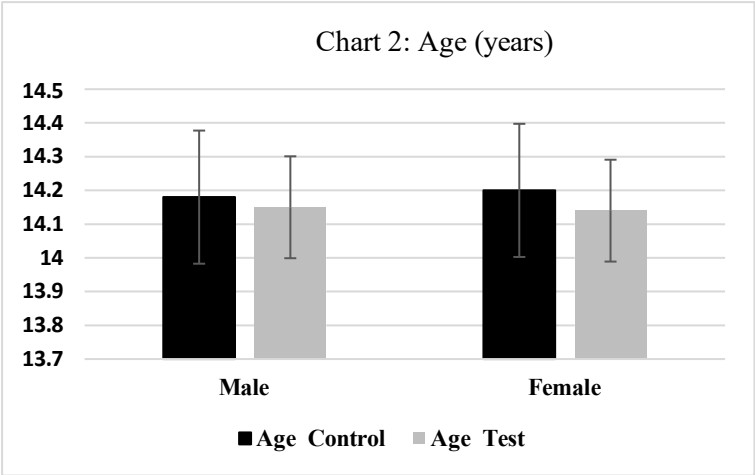
The target population under this study was adolescents. The mean age for the test group and the control group is 14.15 ± 1.38 and 14.19 ± 1.4, respectively. The sample size includes the control and test groups of 100 and 168 individuals, respectively with significant consistency of statistical parameters within & between the two groups (Table 1; p >> 0.05) hence, there were no significant differences in age of participating volunteers from that of highest and lowest of age-range (chart 1). Also, there was no significant variation when observe gender wise (chart 2). Hence, the sample groups included in the study were considered satisfactory towards consistency of sample suitability for age factor.

Table 1: **Descriptive statistics of Age Between Test and Control Groups:**

Age (in years)	Control	Test	Consistency of Age in sample sizes
	100 (50 males+50 females)	**168 (84 male+84 females)**	**p-value**
Mean	14.19	14.15	**0.816**
Median	14.00	14.00	
Mode	13	13	
Std. Deviation	1.426	1.387	



Graph 1: Mean and age range of Age among control and test group (from Table 1)



Graph 2: Gender- wise distribution of Age among control and test group

4.2. The Platelets indices as investigated in the total population under study

The platelet indices PLT, MPV, PCT, and PDW were analysed for all the 268 samples selected under the control and test groups. SPSS analysis (Table 2) found that the mean values of these indices were found within normal biological reference range except for PCT. This data was inclusive of both genders; while gender wise distribution of these indices was also found to follow the same trend i.e., PLT, MPV and PDW lie in the normal biological reference range, but PCT falls abnormally on the higher side for both the genders; hence, no gender bias was observed for abnormal PCT as shown in Table 3. The p-value for each parameter has reflected highly significant data following the statistical analysis ($p < 0.001$) which confirms the reliability of the data set as the mean value perfectly represents the whole sample size. The details are analysed & discussed further in the following sections.

Table 2: Summary of Platelet Indices for the Entire Sample

Total Population (Control + Test)			
Platelet Indices	**Reference range**	**Mean±SD**	**Min-Max**
PLT (Lac cells/mm3)	1.5-4	2.18±0.69	0.54-3.7
MPV (fl)	7.2-11.7	14.03±3.32	8.03-17.6
PCT (%)	0.22-0.24	0.27±0.06	0.14-0.61
PDW (%)	9.1-22.5	19.19±5.8	9.08-27.4

Table 3: Summary of Platelet Indices among gender wise distribution for the Entire Sample

Total Population (Control + Test)					
Platelet Indices	**Male**		**Female**		
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**p-value**
PLT	2.14±0.70	0.54-3.65	2.22±0.67	0.77-3.67	**0.316**

MPV	14.07±3.40	8.12-17.6	13.99±3.23	8.03-7.1	**0.850**
PCT	0.27±0.06	0.14-0.61	0.26±0.05	0.15-0.48	**0.160**
PDW	19.17±5.9	9.25-25.9	19.21±5.80	9.08-27.4	**0.956**

The high p-value is indicative of no significant variation in these indices among gender wise distribution of statistical data; in other words, the values of these parameters were found highly consistent in the age group under this study.

4.3. Platelet Count (PLT)

i. The mean Platelet count, PLT for the sample size (control + test groups) of 268 sample population was found at 2.18±0.69 which falls within the normal clinical reference range i.e. 1.5-4 Lac cells/mm3 (Table 2), reflecting the high consistency of data in the whole population under study.

ii. The Table 4 presents experimental-group wise distribution of platelet indices. For the control group, PLT was found 2.78±0.49 showing lesser std. deviation as compared to the total sample size. The Test group with mean PLT value of 1.83±0.53 (p-value <0.01) exhibited lower PLT than the control group suggesting an association of clinical condition of anxiety with that of changes in PLT value.

iii. Though within the normal range, the deviation of ± 0.53 from the mean value of 1.83 reflects a significant number of observations at the borderline with the lower value of the normal reference range for the PLT, which strengthen the observed fact that anxiety have negative effect on PLT value in the test group. Also, this difference in mean PLT value, between the control and test groups was found significant ($p < 0.05$) (Table 4) which further strengthens the finding that negative modulation of PLT has association with existence of anxiety traits .

iv. Upon comparing the gender-wise distribution of PLT values at four levels- the whole sample size, within control sample size, within test sample size and between the control and test samples, the distinguishable differences were observed.

In the whole sample size, females have slightly higher mean PLT values (2.22±0.67) than males (2.14±0.70) with significance ($p < 0.001$) (Table 3). From data presented in table 3, it is visible that the difference in mean value between male and female is not much apart, but low standard deviation contributes to its high significance and the reliability of the observed difference in the sample size.

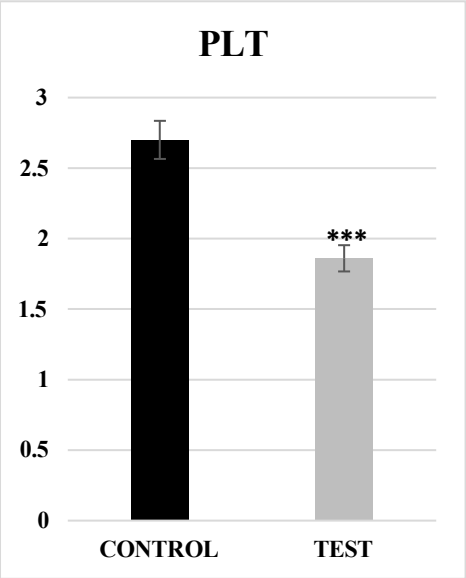
Within the control group, the same trend was observed i.e., males have slightly less mean platelet count (2.74±0.51) as compared to females (2.81±0.46), but this difference was not found significant ($p >> 0.05$) (Table 5). Gender has no significant effect on PLT parameters in healthy subjects as presented in Table 3.

Under the test group, the trend was observed as repetition of control group i.e., males of the test group had slightly less mean platelet count (1.78±0.53) when compared with females (1.88±0.53) with no significant difference ($p >> 0.05$) so, no gender bias found noticeable (Table 6). This means populations of the same age are affected in the same way by the anxiety traits irrespective of gender.

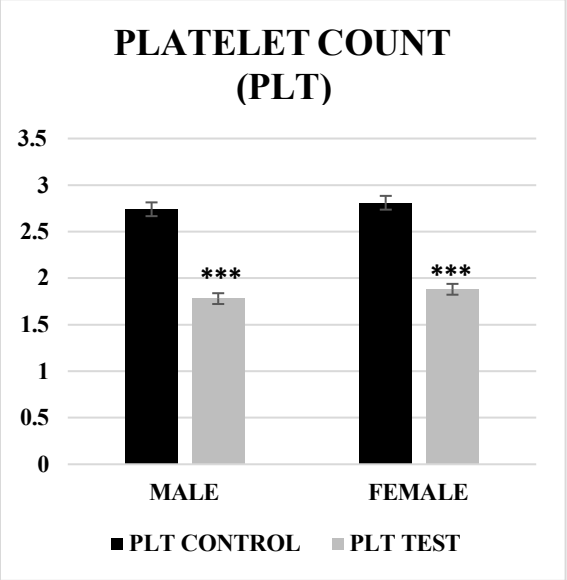
v. The subjects under two experimental groups were compared for the same gender. The males under test and control groups showed that there was a decrease in mean PLT value (1.78±0.53) in the test group as compared with the control group (2.74±0.51) and this decrease was found to be significant ($p < 0.05$) (Table 7). This finding is collinear with that of mentioned in point ii & iii above. It suggests that the anxiety traits have significant association with the alteration of

the PLT index in male teen-age population.

vi. Again, in comparison between control and test groups of females, the test group females too, were observed with a decrease in mean PLT value (1.88±0.53) as compared with the control group females (2.81±0.46) significantly ($p < 0.05$) (Table 8). This observation strongly suggests that anxiety traits have significant pathological association with PLT index in the female teenage population too, which is a suggested confirmation of no-gender bias for the development of pathogenic anxiety traits associated with decrease in PLT value.



Graph 3: Lower Platelet Levels Observed in the test Group (from table 4)



Graph 4: Gender-Based Variations in Platelet Count Among Test and Control Subjects (from table 5 & 6)

Table 4: Mean Values of Platelet Indices in Control and Test Groups

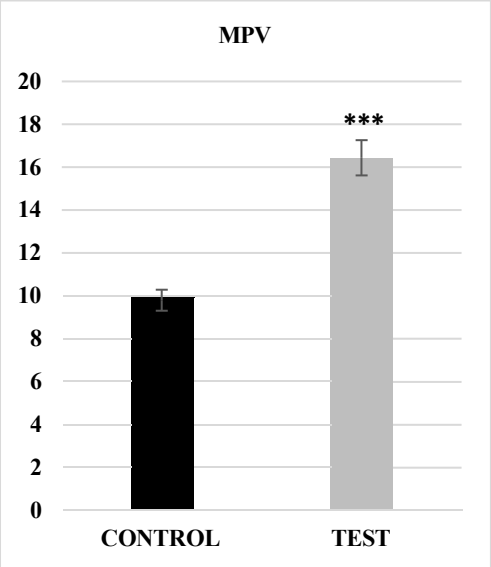
Platelet Indices	Control (Male + Female)		Test (Male + Female)		
	Mean±SD	Min-Max	Mean±SD	Min-Max	p-value
PLT	2.78±0.49	1.72-3.67	1.83±0.53	0.54-2.95	**0.001**
MPV	9.76±1.04	8.03-11.68	16.64±0.46	15.8-17.6	**0.001**
PCT	0.22±0.0007	0.22-0.24	0.30±0.06	0.14-0.61	**0.0001**
PDW	11.8±1.70	9.08-15.38	23.55±1.43	16.3-27.4	**0.004**

4.4. Mean Platelet Volume (MPV)

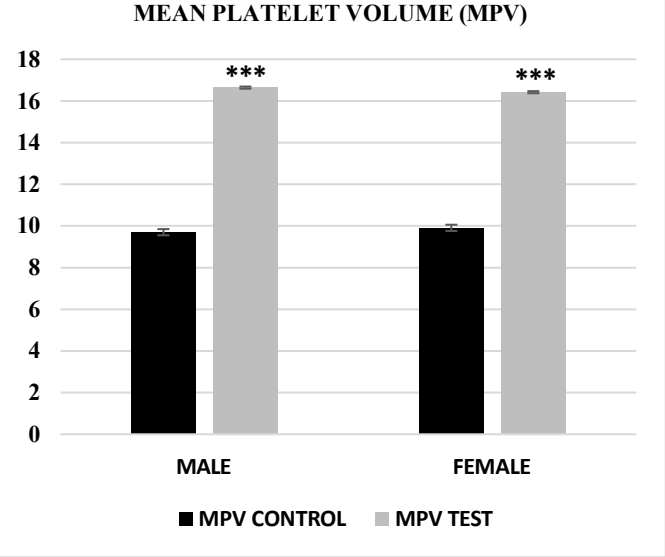
i. The average MPV for the entire sample size of 268 participants, including control and test group, was found 14.03±3.32 (Table 2), which falls within the normal range (7.2-11.7 fl) but on the higher side. For the control group, the average MPV was found at the lower side of the normal range (9.76±1.04), while for the test group, it was found above the normal range (16.64±0.466). The test group showed a significant higher MPV value as compared to the control group ($p < 0.05$) (Table 4). This finding strongly suggests that prevalence of anxiety traits affect the Mean Platelets' Volume indicating higher platelets activity.

ii. When comparing average MPV distribution gender wise across the sample-population (including test and control), though, the males had a slightly higher average MPV (14.07±3.40) than females (13.99±3.23) (Table 3), but with no significant difference. On the other hand within the control group, males had a slightly lower MPV (9.7±1.04) compared to females (9.91±0.46) (Table 5) ($p >> 0.05$) and the same trend was observed in the test group too in which males had slightly lower MPV (16.42±0.34) than females (16.64±0.46) (Table 6) this difference was not found to be significant ($p >> 0.05$) (Table 5 & 6). This reflects that there was no gender bias on the MPV index too, among the healthy and anxious teenage population.

iii. Comparing males in the test and control groups, the test group males have shown a significantly increased MPV value (16.64±0.46) as compared to the control group males (9.7±1.04) ($p < 0.05$) (Table 7). Similarly, for females, the test group showed a significantly raised MPV value (16.42±0.34) when compared with the females of the control group (9.91±0.46) at a significant level ($p < 0.05$) (Table 8). It clearly reflects that anxiety traits are associated with increased MPV value in both male and female i.e., without a gender bias.



Graph 5: Increased MPV observed in the Test subjects (from table 4)



Graph 6: Gender-Based Variations in MPV Among Test and Control Subjects (from table 5 & 6 and 7 & 8)

Table 5: Statistical Gender-Wise Comparison of Platelet Indices Among Control Groups

Control Group					
Platelet Indices	**Male**		**Female**		
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**	**p-value**
PLT	2.74±0.51	1.82-3.67	2.81±0.46	1.72-3.67	**0.576**
MPV	9.7±1.04	8.12-11.55	9.91±0.46	8.03-11.68	**0.331**
PCT	0.22±0.007	0.22-0.24	0.22±0.007	0.22-0.24	**0.534**
PDW	11.75±1.80	9.25-15.38	12±1.58	9.08-14.92	**0.654**

Table 6: Statistical gender wise Comparison of all Platelet Indices among Test Groups

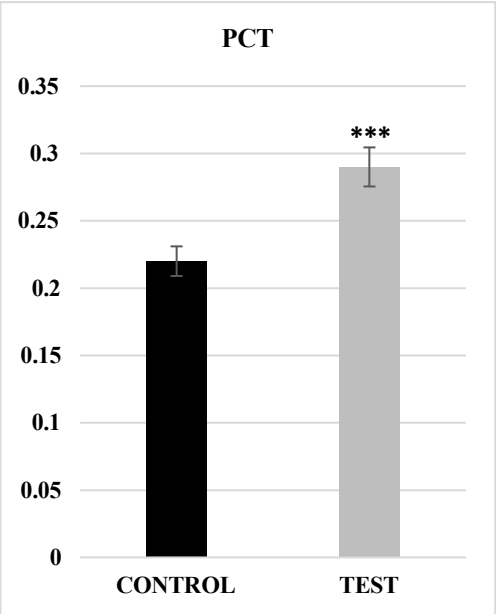
Test Group					
Platelet Indices	**Male**	**Min-Max**	**Female**	**Min-Max**	
	Mean±SD		**Mean±SD**		**p-value**
PLT	1.78±0.53	0.54-2.87	1.88±0.53	0.77-2.95	**0.717**
MPV	16.42±0.34	15.8-17.6	16.64±0.46	15.8-17.1	**0.717**
PCT	0.30±0.06	0.14-0.61	0.28±0.06	0.15-0.48	**0.177**
PDW	23.60±1.10	21.4-25.9	23.51±1.71	16.3-27.4	**0.294**

4.5. Plateletcrit (PCT)

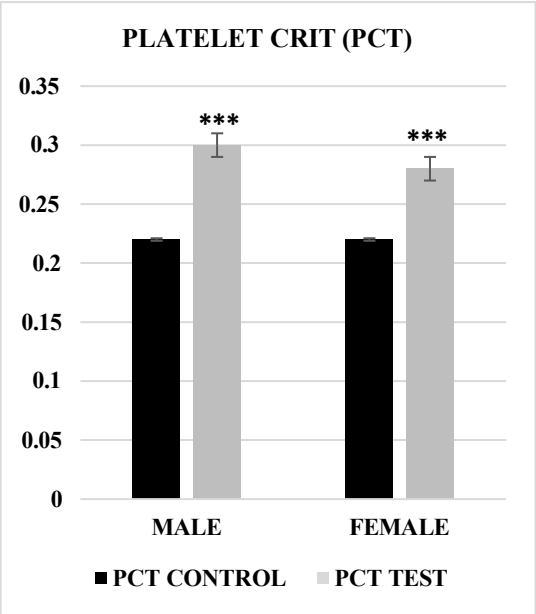
i. The mean PCT value for the total sample size (including males and females from both groups) was 0.27±0.06 (Table 2), which was higher than the top value of normal range (0.22-0.24%). while the control group had an average PCT of 0.22±0.007, within normal range, the test group was recorded with an increase in mean PCT value of 0.30±0.06 which was found highly significant ($p < 0.005$) (Table 4).

ii. In terms of gender-specific PCT distribution across the entire sample size, males showed a slightly higher yet significant mean PCT (0.27±0.06) than females (0.26±0.05) ($p<0.05$) (Table 3). But, within the control group, there was no significant difference ($p >> 0.05$) between the two genders with a similar PCT value (0.22±0.007) (Table 5). In test group (Table 6), mean PCT of 0.30±0.06 & 0.28±0.06 was recorded for males and females respectively, which too, did not show any significant difference ($p >> 0.05$) based on gender; both had abnormally higher value of PCT, above the biological reference range, means both genders have been affected in same way. This increase in PCT in the test group as compared to the control group was found highly significant ($p << 0.05$) for both gender (Table 7 & 8).

iii. The test group males exhibited a significantly elevated PCT (0.30±0.065) as compared to the control group males (0.22±0.007) ($p << 0.05$) (Table 7) and test group females showed a notably increased PCT (0.28±0.061) than the control group (0.22±0.007) females, which was highly significant ($p << 0.05$) (Table 8). These observations confirm that the teenage population, independent of gender, get affected by anxiety induced alterations in PCT values and higher abnormal value of PCT is associated with prevalence of anxiety traits in adolescent population.



Graph 7: Increased PCT observed in the Test subjects (from table 4)



Graph 8: Gender-Based Variations in PCT Among Test and Control Subjects (from table 5 & 6 and 7 & 8)

Table 7: Descriptive Statistics of Platelet Indices in Male Participants

Total male population					
Platelet Indices	**Control**	**Min-Max**	**Test**	**Min-Max**	**p-value**
	Mean±SD		**Mean±SD**		
PLT	2.74±0.51	1.82-3.65	1.78±0.53	0.54-2.87	0.001
MPV	9.7±1.04	8.12-11.55	16.64±0.46	15.8-17.6	0.001
PCT	0.22±0.007	0.22-0.24	0.30±0.06	0.14-0.61	0.001
PDW	11.75±1.80	9.25-15.38	23.60±1.10	21.4-25.9	0.001

Table 8: Descriptive Statistics of Platelet Indices in Female Participants

Total Female population					
Platelet Indices	**Control**	**Min-Max**	**Test**	**Min-Max**	**p-value**
	Mean±SD		**Mean±SD**		
PLT	2.81±0.46	1.72-3.67	1.88±0.53	0.77-2.95	0.001
MPV	9.91±0.46	8.03-11.68	16.42±0.34	15.8-17.1	0.001
PCT	0.22±0.007	022-0.24	0.28±0.06	0.15-0.48	0.001
PDW	12±1.58	9.08-14.92	23.51±1.71	16.3-27.4	0.001

4.6. Platelet Distribution Width (PDW)

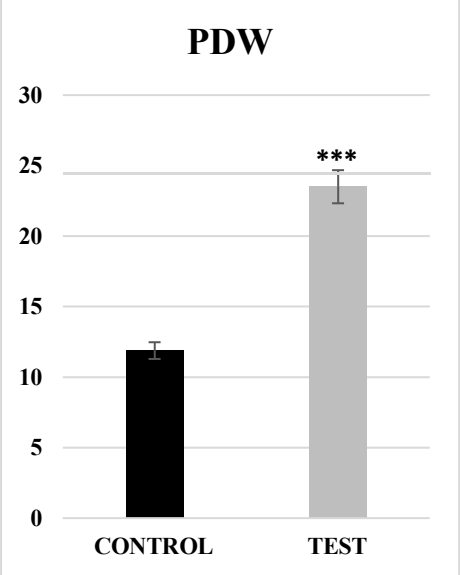
i. The biological reference range for PDW is (9.1 - 22.5%) (Table 2). The mean value for PDW of the whole sample size (including control and test groups) was recorded 19.19±5.8 i.e., within the normal range. For the control group, Mean PDW was noted below this mean (11.8±1.70) yet within normal range, and for the test group, it was found above this mean which was 23.55±1.43 (Table 3), slightly higher than the top reference range. This difference for PDW values between control and test group was found significant in given sample size.

ii. The difference in PDW distribution based on gender across the entire sample population (including both test and control groups), males exhibited a slightly lower average PDW of 19.17±5.90 compared to females' 19.21±5.80, which was not significant ($p >> 0.05$) (Table 3) which reflect that gender has no effect on PDW index for the same age group of teenage.

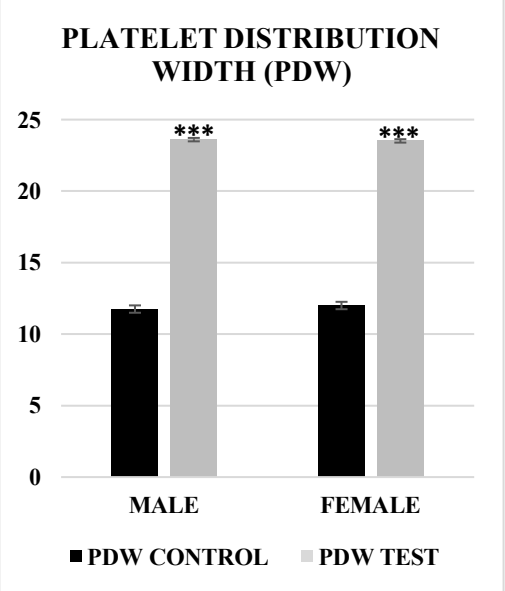
iii. The trend in point (ii) above, was continuous within control group for males with a slightly lower PDW of 11.75±1.80, than that of females, 12±1.58, which was also not significant ($p >> 0.05$) (Table 5).

iv. In contrast to above mentioned points ii & iii, within the test group, males showed a slightly higher PDW of 23.60±1.10 as compared to females, who had an PDW of 23.51±1.71, though this contrast was also not significant ($p >> 0.05$) (Table 6).

v. When comparing males between the test and control groups, the test group demonstrated a significantly higher PDW of 23.60±1.10 compared to the control group's PDW of 11.75±1.80 (Table 7). Similarly, among females, the test group displayed a significantly higher PDW of 23.51±1.71 compared to the control group's PDW of 12±1.58, with the significance of $p<0.005$ (Table 8). The increase in PDW has been noted above the top value of the biological reference range in test group for both genders.



Graph 9: Increased PDW observed in the Test subjects (from table 4)



Graph 10: Gender-Based Variations in PDW Among Test and Control Subjects

The statistical data in this study was analysed to find any significant differences in platelets indices between the two experimental groups - control and test and the comparison was also made between male and female population, in whole sample size, within the experimental groups and between the experimental groups. Considering the nature of all the results discussed above as gender neutral, the cumulative result has

been summarised as – anxiety in teenage population is associated with alterations

in physiological activities of platelets that has been recorded as changes in platelets indices. The changes in PLT and MPV were found highly significant though these differences didn't cross the biological reference range. These highly significant change in the value of PLT and MPV strongly suggest that etiology of developing anxiety disorder in teenagers is making its progress through consistent changes in platelets indices; and it may have gone unnoticed in very limited previous research because all the values fall within normal reference range. The other two indices PCT and PDW were recorded for significant changes between the two experimental groups beyond the biological reference range. PCT value as well as PDW value was found above the normal reference range with highly significant difference, recorded in test group subjects as compared with control group subjects.

The outcomes of this study strongly suggest that physiological changes which are associated with modulation in serotonergic platelets activities and vice-versa, have substantial interlinked mechanism of modulating behavioural outcomes. Further this concept is also strengthened that serotonin (stored in platelets) induced physiological modulation in periphery has sync with neurological circuits in CNS which are responsible for regulation of anxious behavioural outcome. Undesirable changes in periphery with focus on changes in serotonergic platelets activities and its measurable indices are suggested as preliminary screening tool cum early diagnostic marker for the developing anxiety traits at very early stage particularly in the age of adolescent.

Discussion

The subjects in control groups were identified as having anxiety characters dissipating when cue for any anticipated trauma gets invalidated by the sympathetic nervous system, and anxious behaviour returns to normal. On the other hand, pathological subjects in the test group have been recorded with persistent anxiety traits even after perceived threat is over with no actual trauma hence clinical concerns become valid.

The results of this study, in the teenage population, have found that all the platelet indices were significantly modulated in test group subjects while there was no actual trauma (following cue-based anticipation) for which platelet activation was required. This is collinear with previous research findings made with the adult population [21, 1,31,32]. The affected teenage population didn't show any gender bias for clinical aspects of recorded alterations in platelets' indices. which is not collinear with the adult population which has been reported to be affected by gender - adult females were reported to have significantly greater anxiety score than their male counterpart [9], while in this work it was found that among teenage both genders are affected in similar way. Platelet activation has been reported to be concomitant with anxiety, in which hyperactive platelets raise the risk of cardiovascular disease too [46,47] hence, this finding also supports our hypothesis that serotonergic platelets are important players for the development or onset of anxiety.

The blood platelet count (PLT) values were significantly down in the test subjects while other platelet parameters MPV, PDW and PCT were observed significantly higher in the test group as compared to the control group population. This shows that maintenance of homeostasis is physiologically very much similar for both genders in the teenage group. These findings validate and strongly suggest the potential of diagnostic value of platelet indices in distinguishing between control and test groups for traits of anxiety and related disorders.

Decrease in platelets' count, reported in the results of this study, is colinear with the mechanism of eliminating those activated platelets which do not find their physiological and biochemical target in absence of trauma, which is same in case of persistent anxiety, or related behavioural context, like perceived stress without any obvious cause. The low level of 5HT in the

periphery as suggested [52,45,35] to be a cause of anxious behaviour, is also, collinear with decreased PLT count in test group reported in this presented work. Hence, this decline in PLT can be suggested to have a negative effect on normal functions performed by serotonergic platelets in periphery, consequently altering serotonin

dependent behavioural outcomes, as observed in the form of persistent anxious behaviour in test subjects, and a loop of physiological events leading to alterations in platelets indices is suggested for consequent elevated anxiety.

Such modulated indices in test subjects within normal range, suggests that anxiety has started setting up its development without any noticeable clinical implication in haematological analysis.

Under decreased platelet production, existing platelets increase their size as well as activity level and contribute to increased MPV levels [13]. In the present study, the test group subjects were consistently recorded with MPV higher than control group. In healthy subjects, taken as a standard reference, platelet mass is closely regulated to keep it constant, while MPV is inversely related to platelet counts [51]. Higher MPV across the test group irrespective of gender in the teenage group has been found to be collinear with previous studies [23,13, 50,55, 3] and observed as closely associated with presence of anxiety traits as observed in test group of this study.

PCT is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT = platelet count × MPV / 10,000. As PCT depends on platelet count and MPV, it becomes an important indicator for platelet activities and the status of the hematopoietic system because, under physiological conditions, the number of platelets in the blood is maintained in an equilibrium state by regeneration and elimination. PCT values were found significantly modulated towards increment in the test subjects hence, increased PCT too along with increased MPV has been observed to be associated with presence of anxious behaviour in test subjects.

Anxiety and stress can lead to increased platelet aggregation [27,26], which could potentially affect PDW. The test subjects in this study were observed to have PDW value above the biological reference range with a significantly raised difference, with that of the control group.

These findings from presented work strongly support our hypothesis of platelet indices as potential biomarkers for the onset of anxiety. To strengthen this establishment, further use of advanced technique- based analysis of platelets' is under consideration by the research team with inclusion of a widened age range and analytical parameters. 5HT and its receptors on platelets and on nervous tissues are valuable targets for further research.

Conclusion

The present study has successfully recorded that a combinatorial modulation in PLT, MPV, PCT and PDW are associated with anxiety disorders in adolescence. Hence, early diagnosis of "onset of anxiety and/or related disorders" at its very initial stage, specifically in adolescents, would be helpful for timely diagnosis as well as for preventive and or therapeutic measures. The complexity of changes in platelet indices and their interplay with the individual variability in responses, make it challenging to draw definitive conclusions. While there is interest in understanding the relationship between anxiety and platelet indices, the clinical implications have not yet well-established before. Also, the age group under present study has not been investigated much, in available literature till date. It remains an underexplored area under ongoing research across the world, and more advanced experimental evidences are in need to strengthen the significance of association between alteration in platelets' activity parameters and existence of anxiety traits. The decrease in PLT, even under normal reference range, along with increased MPV within normal range and PCT & PDW, above the normal range, has been found significantly associated with onset of (abnormal) anxiety traits at an early age of adolescence. Hence, the aim of study, to establish a clinical parameter for early diagnosis of developing anxiety traits, is established successfully – "The combinatorial changes in biological

reference range for platelets indices, even within the range or very close to this range, in adolescent population, are an important biomarker of developing stage of anxiety traits in adolescents" and may serve as preliminary diagnostic tool towards reliable assessment of anxiety in its early stage of onset.

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