ORIGINAL ARTICLE

To Determine the Relationship of Neutrophil/Lymphocyte Ratio (NLR) and Platelet/Lymphocyte Ratio (PLR) as Inflammatory Markers with Manic Episode of Bipolar Disorder Type I

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ABSTRACT

Objective: To investigate neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) as inflammatory markers in bipolar disorder type I patients with 1st episode and recurrent mania.

Study Design: An observational, cross-sectional study.

Place and Duration of Study: The study was conducted in HBS General Hospital, the tertiary care teaching hospital of HBS Medical & dental College, a private medical college located in Islamabad. The duration of study was from 01/11/2022 to 30/04/2023 for a period of 6 months.

Materials and Methods: Forty cases were recruited by consecutive sampling, comprising of 20 1st episode mania and 20 recurrent mania and these were compared to 20 healthy controls (HC). In the patients Young Mania Rating Scale was used to assess the severity of mania. Samples for blood counts were obtained from all the participants in the morning hours.

Results: Compared to HC, both 1st episode and recurrent mania patients had significantly higher neutrophil and NLR values, and lower lymphocyte counts. When the two patient groups were compared, first episode mania cases had significantly higher neutrophil counts and NLR than patients with recurrent mania.

Conclusion: The findings of the study showed a likely inflammatory pathophysiology in the manic phase of BD. Since 1st episode, drug naive mania patients had greater inflammation as compared to recurrent mania cases, it was conceivable that psychotropic medicines exerted an anti-inflammatory effect in the latter group.

Key Words: *Bipolar Disorder, First Episode Mania, Neutrophil/Lymphocyte Ratio, Platelet/Lymphocyte Ratio, Recurrent Mania.*

Introduction

Bipolar disorder (BD), a severe and common mood disorder effects about 4% of the population globally.[1] The current classification, as envisaged in DSM-5 and ICD-11 is a spectrum condition including bipolar disorder type I (at least 1 manic episode), bipolar disorder type II (recurrent major depressive episodes [MDE] plus at least 1 hypomanic episode), cyclothymic disorder (recurrent hypomanic episodes plus depressive episodes which do not meet criteria for MDE), and bipolar disorder not otherwise specified.[2] Importantly, a cumulative amount of research incriminates inflammatory mechanisms in the pathophysiology of BD.[3] This is particularly robust with regards to manic episodes; these epitomize a severe psychological disturbance represented by elevated mood, pressure of speech, flight of ideas, over activity, grandiosity, impulsivity and psychosis.[4] In BD several original studies, meta-analyses and systematic reviews have documented increased levels of pro-inflammatory factors like cytokines, chemokines and C-reactive protein in acute episodes, particularly manic exacerbations.[5] More recently studies have looked at blood cell indices as tools to investigate inflammatory mechanisms in BD.[6]

Complete blood count is an easily done and inexpensive test and cell indices like neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are readily obtainable biomarkers. NLR and PLR are innovative and potential indicators of inflammation used in the management of several intractable systemic diseases. In this regard, some examples include coronary artery disease, cerebrovascular disease, chronic renal failure,

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inflammatory joint diseases like rheumatoid arthritis and ankylosing spondylitis.[7] In BD systemic inflammation can be measured by serum cytokine levels, but this is very expensive and requires well-equipped laboratories. Previous research has demonstrated higher NLR and PLR in BD subjects suffering from manic episodes, pointing towards intensified inflammatory processes in these patients. An important study was conducted in patients with different diagnoses, including schizophrenia, bipolar mania, bipolar depression, and major depressive disorder. It was shown that among all diagnostic categories NLR was greatest in BD type I, current episode manic signifying that inflammatory mechanisms were most activated in this group of patients.[8] However, it is not known for sure whether elevated NLR in mania actually represents a neuroinflammatory diathesis, such that specific mechanisms need to be elucidated that can relate this blood cell index with brain inflammation. Moreover, there is paucity of literature in the local population regarding the investigation of inflammatory mechanisms in psychiatric disorders.

In the current scenario, while the significance of finding reliable and easily obtainable markers of bipolar disorder is overwhelming there is a dearth of studies in our setting. Laboratory tests based on examination of peripheral blood samples can fulfill this purpose with the caveat that the purported biological markers are relatively inexpensive and within the technological reach of most laboratories. Our study which investigates NLR and PLR in manic patients is an endeavor to fill the gap in the local literature, while it is also likely to shed further light on the issue of inflammatory activation in bipolar disorder.

With this background we aimed to investigate neutrophil/lymphocyte ratio and platelet/lymphocyte ratio as inflammatory markers in bipolar disorder type I patients with 1st episode and recurrent mania.

Materials and Methods

Our study had an observational and cross-sectional design and was done in the department of psychiatry of a tertiary care hospital of a private medical college in Islamabad from 01/11/2022 to 30/04/2023 for the duration of 6 months. The Ethical Review Board of the institution gave approval of the study vide letter number Appl # EC, 3rd Sep '22. Subjects enrolled in the study included those of either gender, age 18 years or older and accepting to participate with proper consent. For 1st episode mania group and HC exclusion criteria were as following:

i) Prior history of psychiatric and chronic medical conditions,
ii) Presence of chronic inflammatory or autoimmune diseases,
iii) Systemic infection,
iv) Substance abuse,
v) Obesity defined as BMI ≥ 30 kg/m2,
vi) Abnormal laboratory test such as anemia, leukopenia/leucocytosis, thrombocytosis, etc.

Exclusion criteria were similar for BD I patients having recurrent manic episodes, however it was understood that they had prior history of mood disorders. The sample size included 20 patients in each of the 3 groups comprising of 1st episode mania, recurrent mania and control group. Patients fulfilling eligibility criteria were enrolled by consecutive sampling until the required sample size was achieved.

In the patients Young Mania Rating Scale (YMRS) was employed to measure the severity of manic symptoms. For the measurement of complete blood count blood samples from the participants were collected in the morning hours using full aseptic techniques and sent to the laboratory in vacutainer tubes containing ethylene diamine tetra acetic acid as anticoagulant. The ratio NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count and PLR by dividing the absolute platelet count by the absolute lymphocyte count.

The IBM SPSS version 23.0 was used for the sake of statistical analysis of the data. To find association between categorical variables Chi-square test was used, while for data which was not normally distributed the Kruskal–Wallis test was employed. To determine the relationship of NLR and PLR with the severity of mania Spearman's correlation co-efficient analysis was utilized. For all tests the statistical significance was standardized as $P \leq 0.05$.

Results

Table I associates patients and HC with regards to demographic variables like age, BMI and gender distribution and shows that these are mostly

comparable. It also demonstrates that the severity of mania was generally the same in 1st episode mania and recurrent mania groups as revealed by the mean YMRS scores (Table I).

With respect to blood cell indices, Table II shows that 1st episode mania cases in comparison to control subjects had a significantly higher mean value of neutrophil count and NLR and a lower number of mean lymphocyte count. When the recurrent mania group was compared to HC, it was shown that total neutrophil count and mean value of NLR was statistically significantly higher in the patients with a significantly lower mean lymphocyte count (Table II).

Table II further demonstrates that as compared to recurrent mania, 1st episode mania patients had a significantly higher mean value of neutrophils and NLR, whereas there was no difference in the mean lymphocyte count between the two groups (Table II).

Lastly, it was shown that no significant difference existed among the study groups with respect to mean platelet count and PLR (Table II).

With regards to correlation between YMRS scores and NLR and PLR in 1st episode and recurrent mania groups, the results did not reach statistical significance.

Table I: Study Participants Group-Wise Characteristics

variable	1st episode mania N = 20	Recurrent mania N = 20	Healthy control N = 20
Age (mean±SD)	27.35±6.25	30.29±8.44	28.44±7.23
BMI (mean±SD) Kg/m^2	26.25±1.55	25.99±1.46	25.76±1.39
YMRS (mean±SD)	31.74±6.59	30.77±5.89	-
Male	12 (60%)	14 (70%)	10 (50%)
Female	8 (40%)	6 (30%)	10 50%)

BMI – Body Mass Index; SD – Standard Deviation; YMRS – Young Mania Rating Scale

Table II: Complete Blood Count (Absolute Numbers) And Ratios

Variable	1st episode mania N = 20 (Mean±SD)	Recurrent mania N = 20 (Mean±SD)	Healthy control N = 20 (Mean±SD)	*P*1	*P*2	*P*3
Neutrophils ($10^3/\mu l$)	6.29±1.26	5.11±1.67	3.04±0.59	<0.001	<0.001	<0.001
Lymphocytes ($10^3/\mu l$)	2.05±0.39	2.08±0.46	2.85±0.27	<0.001	<0.001	0.053
Platelets ($10^3/\mu l$)	198. 32 ± 97.58	194. 65 ± 70.12	203. 54 ± 43.83	0.143	0.063	0.071
NLR	3.06±1.14	2.45±0.62	1.06±0.36	<0.001	<0.001	<0.001
PLR	96.58±42.63	93.26±30.89	71.22±18.99	0.127	0.385	0.096

*P*1 – 1st episode mania v/s HC; *P*2 – recurrent mania v/s HC; *P*3 – 1st episode mania v/s recurrent mania

Discussion

In the present study, compared to HC both index episode and recurrent mania groups showed a significantly greater number of neutrophils and NLR and lower lymphocyte counts. These facts link mania with sub-clinical inflammation and are in line with other published studies.[9] Overall, the findings of studies addressing the question of immune-inflammatory markers in bipolar disorder are unclear; nonetheless, mania reportedly has been associated with increased levels of proinflammatory cytokines, acute-phase reactant proteins and complement components.[10]

Innate immune responses are exemplified by neutrophils while lymphocytes mediate adaptive immunity, so that NLR can be an indicator of the balance between these two biological mechanisms. When challenged by injury or infection neutrophils

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serve as the first-line of defense in the body and initiate the protective response by secreting various cytokines, chemokines and other chemical mediators. Since lymphocytes are activated latter in the immune response, they have a regulatory function in directing bodily defenses. In this regard, absolute or relative lymphopenia may point towards increased biological vulnerability associated with poor physiologic functioning. Neutrophil to lymphocyte ratio is an incorporated variable of the two canonical immune pathways and exactly for this reason is more useful than either integer alone. Because of this value NLR may act as a potential biomarker for BD in the manic state.[11] In addition, as compared to single leukocyte parameters NLR is less influenced by such factors as physical exercise, dietary changes, autonomic arousal, etc. and remains useful in the presence of confounding variables.

In addition to NLR, in major psychiatric disorders an association between platelet parameters such as absolute platelet numbers, PLR and mean platelet volume is also documented. Platelets serve as initiators of the protective response as these have a specific function in the activation of neutrophils and macrophages along with the regulation of endothelial permeability. Therefore, PLR can be employed as a biomarker of inflammatory response in mood disorder patients. To extend this argument further, a meta-analysis demonstrated higher value of PLR in BD subjects as compared to controls.[12] Yet, in our study no significant difference in platelet count and PLR was revealed in the investigated groups. This finding is also in line with previous studies investigating the matter.[13]

Our study included drug naïve 1st episode mania subjects, ruling out an effect of psychotropic medications vis-à-vis the inflammatory status of the cases. Interestingly, a significant variance was seen between the patient groups with regards to blood cell indices as neutrophil counts and NLR were significantly higher in index mania versus recurrent mania groups, suggesting less severe inflammatory response in the latter. In this respect, it is worth stating that a recently published study had similar findings.[14] Since the recurrent mania group was on psychotropic medications, it could be surmised that these agents were exerting anti-inflammatory effect which was reflected in blood cell indices. To extend the argument further, animal and human studies are available suggesting an anti-inflammatory effect of various psychotropic medications.[15] Finally, in our study a significant association between NLR and PLR and the severity of mania (increased scores on YMRS) was not established which could be because of the presence of confounding variables like diet, exercise, smoking, etc.[16].

Limitations

i) The study had a cross-sectional design which did not permit for establishing a causative association between higher NLR values and BD.
ii) In conjunction with blood cell indices we did not evaluate other inflammatory indicators, most importantly peripheral pro-inflammatory cytokines.
iii) In the absence of other markers of inflammation it was not practicable to assess the significance of higher NLR values as a standalone marker of a pro-inflammatory state in bipolar disorder.

Conclusion

The present study suggested likely inflammatory mechanisms in the development of mania. Moreover, it showed greater value of inflammatory parameters in cases with index manic episodes as compared to BD subjects suffering from recurrent manic episodes. Since 1st episode mania patients were psychotropic drug naïve, our finding suggested that possibly these medications exerted an anti-inflammatory effect in cases with recurrent mania. Finally, it must be noted that NLR was an easily done and low-cost blood test, such that its utility as a biomarker in bipolar disorder called for further investigations.

Recommendations:

i) Future research having greater number of participants and prospective design is much needed to validate NLR and PLR as potential biomarkers of bipolar disorder.
ii) This research should preferably include multiple sites from different international locations.
iii) Blood cell indices should be combined with other biomarkers of inflammation to corroborate the diagnosis of bipolar mania.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.