Original Article

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Correlation of Global DNA Methylation with Symptom Severity in Drug-Naïve Bipolar Disorder Patients: A Cross-sectional Study

Psychiatry/Mental Health Section

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ABSTRACT

Introduction: Bipolar disorder has emerged as a significant health problem in India. Global Deoxyribonucleic Acid (DNA) methylation studies could provide a significant clues toward the early identification of high-risk individuals.

Aim: To estimate the levels of global DNA methylation in drug-naïve bipolar disorder patients and healthy controls and to correlate the global DNA methylation levels with the severity of the disease.

Materials and Methods: A cross-sectional study was conducted in the Department of Psychiatry at SCB Medical College, Cuttack, Odisha, India, from January 2019 to March 2020. A total of 50 bipolar disorder patients and 50 age-matched healthy adult controls were recruited from the Outpatient Departments (OPD) of the hospital, and the severity of bipolar disorder was assessed using Young's Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HAM-D). DNA methylation levels were estimated from serum samples of the subjects using a methylated DNA quantification kit. The data was analysed using Statistical Package for Social Sciences (SPSS) version 25.0. The Kruskal-Wallis test was used to compare the DNA methylation scores, and the Pearson's correlation test was used to correlate DNA methylation levels with disease severity. A p-value <0.05 was considered significant.

Results: The mean age of the study participants was 34.44±12.45 years. A total of 100 subjects were included in the study, with 50 being bipolar disorder patients and the remaining 50 in the control group. Bipolar cases, particularly depression, had higher levels of DNA methylation than controls (p=0.0001). On correlation analysis, patients with Bipolar Depression (BD) had a significant correlation with DNA methylation (p-value <0.001) compared to patients with mania and controls.

Conclusion: Bipolar patients, especially those with BD, exhibit higher levels of global DNA methylation in genes compared to healthy control groups. Global DNA methylation can serve as an important early disease marker for bipolar patients, aiding in prevention and early detection efforts.

Keywords: Depression, Deoxyribonucleic acid methylation assay, Epigenetics, Mania

INTRODUCTION

Bipolar disorder is a disabling condition characterised by severe and pervasive mood episodes, presenting with hypomanic, manic, depressive, or mixed episodes (a simultaneous mixture or rapid alternation within a few hours of depressive and manic or hypomanic manifestation) [1]. It has a multifactorial origin resulting from complex interactions between genetic susceptibility and environmental stimuli. Epigenetic mechanisms, including DNA methylation, can modulate gene expression in response to the environment and might account for part of the heritability reported for bipolar disorder [2].

Clinical symptomatology and phenomenology are still the basis for diagnosing bipolar disorder, depending on the doctor's subjective judgments. However, the diagnosis of bipolar disorder is often delayed, under-diagnosed, or over-diagnosed, with an approximate time lapse between the initial consultation and the correct diagnosis often taking more than 10 years [1,3]. It is often confused with other diagnoses, such as psychotic and cluster B personality disorders [4-6]. Additionally, it is commonly under-recognised in patients presenting with depression for the first time, creating a significant clinical problem [6]. Failing to recognise bipolar disorder in depressed patients has significant treatment and clinical implications, including the under prescription of mood stabilising medications, an increased risk of rapid cycling, and increased care costs [7]. Delay in diagnosis has also been observed to increase potential morbidity and mortality. To address this problem, different methodologies, such as molecular genetics, are now being utilised to identify at-risk populations. Recent genetic studies on affective disorders indicate that several chromosomal regions may be involved in the aetiology of bipolar affective disorder [8]. Genes with an aberrant methylation pattern can represent novel candidate factors in the aetiology and pathology of neuropsychiatric disorders like bipolar disorder and schizophrenia [9].

Previous genetic studies have suggested that bipolar disorder strongly correlates with genetic aetiology, as seen in twin studies and population-based family risk studies, which showed a heritability rate of 58% [10,11]. Environmental exposure also has an effective contribution to the onset of the illness via epigenetics, which is the effect of the environment on the genes of susceptible individuals [3]. Various epigenetic mechanisms have been implicated as a predisposing factors for the onset of bipolar disorder. These include DNA methylation, histone methylation and acetylation, and non coding Ribonucleic Acids (RNAs). Among these, DNA methylation was the most appropriate mechanism for research study as it is often the most stable form of epigenetic alteration [12-14].

The DNA methylation refers to the addition of a methyl group at the 5th carbon of cytosine residues 5-Methyl Cytosine (5mC) of CpG dinucleotides (cytosine proximal to guanine) [15]. It plays essential roles in cell dynamics, including the regulation of gene expression and the maintenance of epigenetic memory. Increased methylation levels at promoter regions can lead to decreased gene expression [16]. Epigenetic changes in bipolar disorder are supported by observations of altered methylation levels, such as 5mC, on promoter regions of specific candidate genes like Catechol-O-Methyltransferase (COMT) and Brain-derived Neurotrophic Factor (BDNF) [17-19]. Extensive investigations have been conducted on alterations in DNA methylation patterns in patients with bipolar disorder. These patterns serve as promising markers that can integrate both genotype and environmental effects

[20]. One approach to studying DNA methylation patterns is by examining global methylation patterns in peripheral tissues such as leukocytes.

Molecular genetics offers an alternative strategy for studying genetic factors involved in complex diseases, including psychiatric disorders, where the mode of inheritance is polygenic. Correlation studies in this field often compare frequencies of genetic marker alleles in patients and control populations to detect linkage disequilibrium. However, these correlations may also be spurious due to the input DNA, which can be influenced by variables such as the subject's ethnicity, gender, obesity, smoking status, and others. While previous studies have mostly focused on global methylation patterns among patients already on mood stabilisers, there is a lack of research on global DNA methylation patterns among drug-naïve bipolar patients, particularly in the Indian population.

The present study, one of the first from India, aimed to examine DNA methylation levels among drug-naïve patients with bipolar disorder. By selecting drug-naïve patients as subjects, the study eliminates the risk of confounding due to psychotropic use. Therefore, the objectives of the present study were to estimate the levels of global DNA methylation in drug-naïve bipolar disorder patients and healthy controls, compare DNA methylation between the two groups, and correlate DNA methylation with the severity of depression and mania.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Psychiatry in collaboration with the Department of Biochemistry at SCB Medical College and Hospital, Odisha, India, between January 2019 and March 2020. The study was undertaken after obtaining an Ethical clearance certificate from the Institutional Ethics Committee (IEC/IRB NO: 936/14.10.19) vide ref no-718/09-01-19 the equipment required for the present study was procured from the Multidisciplinary Research Units (MRU) fund. Each participant in both groups was informed about the usefulness and harmfulness of the study in their native language. The participants and their informant/s voluntarily gave written consent and were assured of the confidentiality of the data collected from them.

Sample size calculation: The sample calculation was done with a power of 80%, a confidence level of 95%, and after adjusting for estimated dropouts/non-consent (20%), the sample size was estimated to be around 71 age-matched pairs of cases and controls [21]. However, at the end of the study, 50 bipolar disorder cases and 50 age-matched healthy controls were recruited from the OPD of Psychiatry.

Inclusion criteria: Patients of either sex between 18 to 65 years of age, who were drug-naïve (i.e., with no previous history of psychiatric treatment in their lifetime) and diagnosed with bipolar disorder as per ICD-10 DCR criteria [22], were consecutively included in the case group. In the control group, medically stable and age-matched subjects with no history of intellectual disability, organic disorder, comorbid medical conditions, and no family history of any mental disorders were included in the study.

Exclusion criteria: Those with a history of substance abuse or drug dependence, psychosis, neurodevelopmental disorder, eating disorder, organic disorder, endocrine diseases, pregnant patients, terminal illnesses, and other psychiatric disorders other than bipolar disorder were excluded from the study.

Study Procedure

A semi-structured performa was used to obtain demographic profiles and relevant medical history. The severity of manic symptoms in bipolar disorder was assessed using YMRS [23], and the HAM-D was used to rate depressive symptoms [24]. The methylated DNA quantification Kit (colorimetric) was used to analyse DNA methylation quantitatively. This kit is suitable for detecting global DNA methylation status using DNA isolated from 2 mL of blood collected from all study subjects in Ethylenediaminetetraacetic Acid (EDTA) vials. It is optimised for quantifying methylated and hydroxymethylated DNA or quantifying methylated DNA alone [25-27]. To determine the relative methylation status (5mC) of two different DNA samples, a simple calculation of the percentage of 5mC in total DNA was made using the formula [28-31]:

$$5mC\% = \frac{(\text{sample OD-ME3 ID}) \div S}{(\text{ME4 OD-ME3 ID}) \times 2 \div P} \times 100$$

Where,'S' is the amount of input DNA in ng.

'P' is the amount of positive input control (ME4) in ng.

Two is a factor to normalise 5mC in the positive control to 100%, as the positive control holds only 50% of 5mC.

Data for quantifying DNA methylation includes the amount of input DNA in nanograms, sample OD450, average OD450 of ME3, and average OD450 of ME4. The comparison of DNA methylation was made in both the case and control groups. Additionally, the correlation of DNA methylation with the severity of depression and mania was assessed.

STATISTICAL ANALYSIS

The data was analysed using Statistical Package for Social Sciences (SPSS) version 25.0. The data were summarised using means and Standard Deviations (SD) for continuous variables and median (range) for categorical variables. Frequencies and percentages were used for categorical variables. Group differences for continuous and categorical variables were tested using the Kruskal-Wallis and Chi-square tests, respectively. The correlation between DNA methylation and psychopathology scores was assessed using Pearson's correlation. A p-value <0.05 was considered significant.

RESULTS

A total of 100 subjects were included in the present study, with 50 being bipolar disorder patients and the remaining 50 in the control group. The demographic and clinical characteristics are presented in [Table/Fig-1].

Parameters	Cases (n=50)		Controls (n=50)		
	n (%)	Mean±SD/ Median (range)	n (%)	Mean±SD	p-value
Age (in years)		34.44±12.45		29.94±9.41	0.04
Gender					
Males	33 (66)		38 (76)		0.27
Females	17 (34)		12 (24)		
Religion					
Hinduism	48 (96)		50 (100)		0.15
Islam	2 (4)		0		
Marital status					
Married	40 (80)		20 (40)		<0.001
Unmarried	10 (20)		30 (60)		
Family type					
Nuclear	3 (6)		12 (24)		0.012
Joint	47 (94)		38 (76)		
Episodes					
Manic episode (YMRS scores)	31 (62)	35 (32-42)	0	0	
Depressive episode (HAM-D)	19 (38)	25 (17-31)	0	0	

[Table/Fig-1]: Sociodemographic and clinical characteristics of study population (N=100).
Kruskal-Wallis test and Chi-square test, *p<0.05

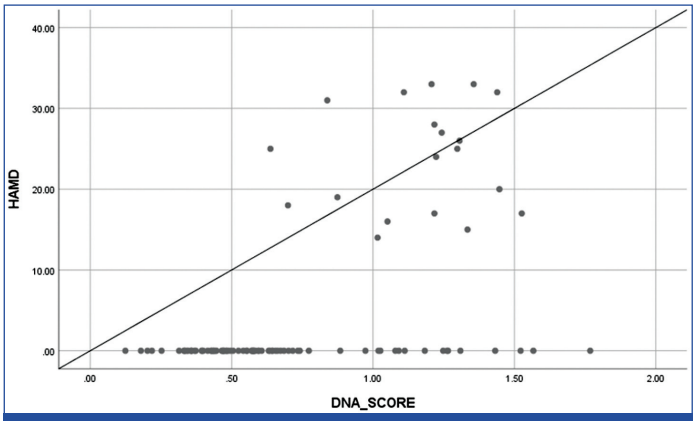
In the case group, there were 33 males and 17 females, while in the control group, there were 38 males and 12 females. The mean age

of the case group was 34.44±12.46 years, whereas in the control group, it was 29.94±9.41 years, with a statistically significant difference (p=0.04). Significant differences were also observed between the groups based on marital status and family type, with the bipolar cases in the present study being older (p=0.04), more likely to be married (p<0.001), and belonging to joint families (p=0.012). Out of the 50 cases, 31 had mania (62%), with 14 cases (45.2%) each having moderate and severe episodes [YMRS score median/range: 35/(32-42)]. Additionally, 19 cases had depressive episodes (38%), with 11 cases (57.9%) classified as having very severe depression [HAM-D score median/range: 25/(17-31)]. [Table/Fig-2] presents the mean DNA methylation scores. A significant difference in global DNA methylation levels was found when comparing bipolar disorder patients and the control group (F-value=32.67; p-value <0.001). This difference was also significant among bipolar cases of different severity, with patients experiencing severe depression showing higher DNA methylation levels compared to other cases of depression (p=0.0001).

DNA methylation levels	No of cases (%) (n=50)	Mean±SD/ median (range)	Mean±SD in controls (n=50)	p-value
Mild mania	3 (9.7)	0.66 (0.34-1.26)		0.99
Moderate mania	14 (45.2)	0.74 (0.12- 1.77)		
Severe mania	14 (45.2)	0.79 (0.33-1.57)		
Moderate depression	6 (31.6)	1.13 (0.7-1.53)		0.0001
Severe depression	2 (10.5)	1.16 (0.87-1.45)		
Very severe depression	11 (57.9)	1.12 (0.64-1.44)		
Global DNA methylation percentage (5mC %):				
1. Depression		1.16±0.25	0.53±0.16	<0.001
2. Mania		0.81±0.47		

[Table/Fig-2]: Deoxyribonucleic Acid (DNA) methylation levels with different severity of illness in the study population (N=100).
*p<0.05; Kruskal-Wallis test has been applied

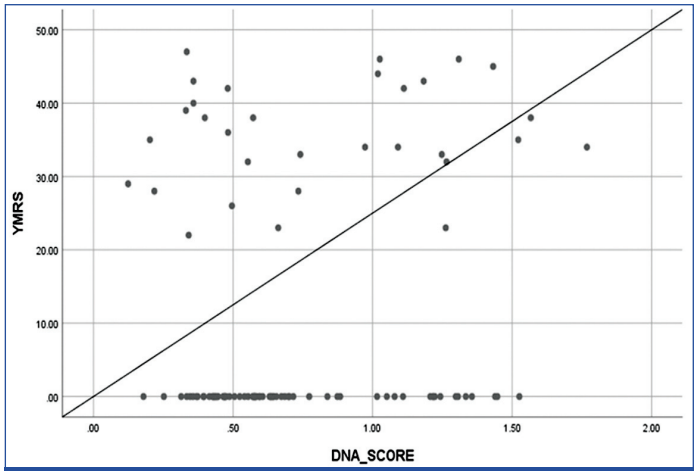
When examining the correlation between DNA methylation levels and bipolar symptoms, a significant correlation was found only with HAM-D scores (r=0.52; <0.001), while no significant correlation was observed with YMRS scores (r=0.2, p=0.12) [Table/Fig-3,4].



[Table/Fig-3]: Correlation between the severity of depression and DNA methylation.
x-axis= 5mC% score
y-axis= Hamilton depression rating scale score (n=19)

DISCUSSION

The present study aimed to examine global DNA methylation levels among bipolar patients, compare them with healthy controls, and correlate them with symptomatology in bipolar disorder patients recruited from a relatively homogeneous population. The study found significantly higher methylation patterns among bipolar disorder cases, particularly among patients with BD compared to controls. There was also a significant correlation between global DNA methylation and symptom severity among BD patients. Previous studies have reported variable levels of global DNA



[Table/Fig-4]: Correlation between the severity of mania and DNA methylation.
x-axis= 5mC% score
y-axis= Young mania rating scale score (n=31)

methylation based on age and gender [32-34]. In the present study, the cases were generally older than the controls (p=0.04), which may have influenced the findings. Therefore, it is recommended that future studies consider these factors and examine the effects of extraneous influences in longitudinal studies with age-matched groups to obtain conclusive results. No other sociodemographic factor was found to significantly influence the observed findings.

The present study observed greater methylation in cases of severe depression compared to those with moderate depression or mania, which is consistent with most previous studies [18,19,35]. Some previous studies have attempted to explain these patterns, suggesting that higher methylation levels may be linked to a hypodopaminergic state and frontal lobe hypoactivity in bipolar and schizophrenia patients. Abnormal methylation levels have been associated with abnormalities in hedonic activities, cognitive processes, working memory, and social functioning among patients with depressive disorders [2,36]. These abnormal methylation levels may lead to receptor excitation or inhibition, resulting in functional changes in neurotransmission processes [37]. However, it should be noted that these findings could also be influenced by the duration of illness, smoking status, substance use, and other lifestyle patterns that were not accounted for in the present study.

A significant correlation was observed between global DNA methylation and BD, but no correlation was found with mania. Higuchi and colleagues reported a similar correlation in their study, where they hypothesised that the findings may be state-dependent. This could also be true in the present study, as patients of varying severity were included in the sample [38].

Several previous studies have attempted to investigate the correlation between DNA methylation levels and symptoms of bipolar disorder, yielding mixed results. For instance, an earlier study found no differences in leukocytes between bipolar patients and controls. However, significant global hypermethylation was observed in the postmortem frontal cortex of affected individuals compared to controls [29]. Conversely, two other studies reported decreased global methylation in transformed lymphoblasts and whole blood samples from BD subjects [39,40]. These discrepancies could be attributed not only to the different tissues studied but also to the varying methods used, which have been shown to differ significantly [41]. Three studies assessed global methylation using Enzyme-linked Immunoassay (ELISA) [37,40,41], while others employed the cytokine-extension assay [21]. Liu C et al., found that global methylation levels mainly exhibited increases in brain samples and decreases in peripheral tissue samples. The increased methylation probes detected in Epigenome-wide Association Study (EWAS) were enriched for neuron-related pathways, whereas peripheral tissues were mainly enriched for immune-related pathways [37].

These findings, along with the results of the present study, may provide evidence for the involvement of the immune system in psychiatric disorders. Therefore, DNA studies like these have the potential to provide important insights into the pathogenesis of severe mental illnesses such as bipolar disorder. They can be utilised for early screening, prevention, and the development of novel drugs targeting specific molecular sites. Larger studies with larger sample sizes would further contribute to understanding the diagnostic and therapeutic role of DNA alterations.

Limitation(s)

Firstly, the final sample recruited was smaller than the originally estimated number of 71 pairs of cases and controls. The sample was collected from a single tertiary centre, where cases of high severity are more likely to be seen, which may limit the generalisability of the findings. Other factors that were not accounted for, such as smoking status, past subsyndromal episodes, Body Mass Index (BMI), and age of onset, could have influenced the results mentioned above. Additionally, the present study did not identify any specific candidate genes, which may have less impact on the overall findings as the causation of most major psychiatric disorders is polygenic and still being evaluated. Furthermore, only blood samples were used in the study, and testing other tissues may yield different findings compared to other studies. It is important to note that this was a one-time cross-sectional assessment, making it difficult to determine definite causation. Recruiting asymptomatic at-risk populations or individuals presenting with prodromal symptoms may provide a better understanding of the potential use of global methylation as a biomarker for bipolar disorders.

CONCLUSION(S)

Global DNA methylation levels were higher among cases of bipolar disorder, particularly among those with BD. A significant correlation was also observed between global DNA methylation and depression, which may contribute to neurochemical changes in mood disorders. Therefore, global DNA methylation could be considered a potential early marker for bipolar disorder. However, future studies should adopt a longitudinal approach and account for extraneous factors to obtain definitive answers regarding the utility of this marker.

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