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ABSTRACT

Cholesterol and its oxygenated metabolites, including oxysterols, are intensively investigated as potential players in the pathophysiology of brain disorders. Altered oxysterol levels have been described in patients with numerous neuropsychiatric disorders. Recent studies have shown that Bipolar disorder (BD) is associated with the disruption of cholesterol metabolism. The present study was aimed at investigating the profile of oxysterols in plasma, their ratio to total cholesterol and their association with clinical parameters in patients with BD. Thirty three men diagnosed with BD and forty healthy controls matched for age and sex were included in the study. Oxysterol levels were measured by isotope-dilution ultra-performance liquid chromatography-tandem mass spectrometry. Significantly higher levels were observed for cholestane-3β,5α,6β-triol, 27-hydroxycholesterol (27-OHC) and Cholestanol in patients with BD. The concentration of 24-hydroxycholesterol (24-OHC) was significantly lower in patients compared to controls. 24-OHC was also negatively correlated to MAS subscale score (r =-0.343; ***p*** = 0.049). In patients, 24-OHC was inversely correlated with age (r = -0.240; ***p*** = 0.045). Multivariate analysis found that BD acute decompensation was independently related to the rise in plasma 24-OHC (***p*** =0.002; OR = 0.966, 95% CI [0.945 – 0.987]). However, the 24-OHC assay relevance as a biomarker of this disease deserves further investigation in other studies.

Keywords

Oxysterols, 24-hydroxycholesterol, 27-hydroxycholesterol, bipolar disorder, biomarker

1. INTRODUCTION

Bipolar disorder (BD) is one of the most severe mental disorders. The World Health Organisation (WHO) ranks it as the sixth most disabling pathology[1]. Significant progress has been made in understanding its pathophysiology and treatment. However, there are still too many patients who do not benefit from it, due to the lack of early diagnosis [2]. Indeed, the prevalence of bipolar disorder would still be largely underestimated in favor of those of schizophrenia and major depression [2]. The identification of biomarkers for this disorder could be of great help for screening, treatment and prevention[3].

Recent epidemiological and experimental studies have identified many cholesterol roles, including in particular the cardiovascular, inflammatory, and immune systems as well as the central nervous system (CNS)[4,5]. Cholesterol is available in all cells primarily as a basic component of lipid bilayers. It is involved in the maturation of the CNS, signal transduction, neurotransmitter release, synaptogenesis, and membrane trafficking [6–8]. Cholesterol is also a precursor for neurosteroids, vitamins and hormones synthesis [9]. Cholesterol homeostasis is suggested to play a critical role in the pathophysiology of BD. Several studies have reported that patients with BD present perturbation in peripheral cholesterol metabolism, which is potentially associated with the development and severity of the disease [10–12]. Brain cholesterol levels are independent from those in peripheral tissues, since the Blood Brain Barrier (BBB) prevents peripheral cholesterol from entering the CNS [13]. However, high-fat diets increase the flux of oxysterols (oxidized forms of cholesterol) to the brain [14], and these particles could clarify the association between blood and brain cholesterol levels. Oxysterols represent a wide collection of bioactive molecules that can be originated from exogenous dietary intake or endogenously by cholesterol enzymatic and/or auto-oxidation mechanisms [15,16]. Moreover, Oxysterols are important regulators of many

biological processes in the cell such as the regulation of cholesterol metabolism. This function is mediated by various ways: through transcription factors (liver X receptors (LXRs)[17] or sterol-regulatory binding proteins (SREBPs))[18], by interaction with oxysterol-binding proteins (OSBPs)[19–21], or by regulation of 3-hydroxy-3 methylglutaryl CoA (HMG-CoA) reductase [22,23], a key enzyme in cholesterol metabolism. In addition, Oxysterols influence the biophysical properties of lipid membranes, such as the ordering of the bilayer and play roles in vesicular transport [24]. Therefore, maintenance of cholesterol homeostasis is essential for normal neuronal functioning and brain development. In neurons, a brain-specific enzyme cholesterol 24-hydroxylase (CYP46A1) converts the excess cholesterol into 24-hydroxycholesterol (24-OHC), which may diffuse across the BBB [25,26]. On the other hand, there is also an inflow of 27-hydroxycholesterol (27−OHC) to the brain [27]. This oxysterol is a product of peripheral metabolism of cholesterol, generated by the enzyme sterol 27-hydroxylase (CYP27A1), highly expressed in the liver and macrophages [15]. The deficiency in this enzyme was revealed to cause a metabolic disorder, known as Cerebrotendinous Xanthomatosis (CTX), characterized by increased 7α-hydroxy-4-cholesten-3-one plasma level, this accumulation is able to cross the BBB and be converted into cholestanol in the brain [28,29]. In hepatocytes, cholesterol-25-hydroxylase catalyzes the synthesis of 25-hydroxycholesterol (25-OHC) from cholesterol [30].

As a result of non-enzymatic reaction, i.e. auto- and/or oxidation induced by reactive oxygen species (ROS), 7-ketocholesterol (7-KC), and cholestane-3β, 5α, 6β-triol can be formed [27,31]. 25-OHC can also be formed by auto-oxidation pathways[32]. Several researchers confirmed that oxysterols from autooxidized cholesterol induced significant apoptosis or necrosis particularly, in vascular cells [33,34].

Altered oxysterol levels have been reported in numerous neurological disorders, including inherited metabolic disorders, such as CTX, Smith-Lemli-Opitz Syndrome, Spastic

paraplegia type 5, Niemann-Pick C, A/B diseases, Autism spectrum disorder, and neurodegeneratives diseases, such as Alzheimer's, Parkinson's, Huntington's diseases, Multiple sclerosis, and Amyotrophic Lateral Sclerosis [16,29,35–42]. All these disorders share synaptic dysfunction [43–45], which is common in BD [46–48].

In this work, we aimed at investigating the level of plasma oxysterols in drug-free male patients in acute decompensation of BD compared with healthy controls, and to assess the relationship between oxysterols profile and disease severity.

2. METHODS

2.1.Study population

The present prospective study was conducted over a period of twenty-six months, between June 2016 and July 2018. All enrolled subjects were Tunisian. Blood samples were collected from patients with BD, which were hospitalized at the "C" Psychiatry department of the University Hospital of Sfax-Tunisia which accepts only male patients. The study involved male patients who were admitted for an acute BD decompensation. The patients had not taken any psychotropic drugs for at least 3 months prior to hospitalization and/or to blood collection. The diagnosis was confirmed according to the DSM-5 criteria [49]. The positive and negative syndrome scale (PANSS) and Cognitive Assessment (MoCA) were applied for psychopathology assessment for all the patients [50,51]. The BD patients were generally subdivided into BD Manic episode and BD Depressive episode. Hence, other scores were calculated to assess the severity of mania and depression. For the severity of mania, the Bech and Rafaelsen Mania Scale (MAS) was used [52]. According to the study by Bech et al.[53], we divided the scale MAS into two categories according to the total score:moderate manic episode with MAS $\leq$21 and severe manic episode with MAS > 21. As for depression, the

evaluation was performed according to the Montgomery-Asberg Depression Rating Scale (MADRS)[54].Two trained psychiatrists independently made all these scale evaluations.

The age-matched healthy control subjects were recruited from the same geographic area. They were also diagnosed by psychiatrists and recorded to have no evidence of psychiatric illnesses at exam when the blood samples were collected. The exclusion criteria considered for all the enrolled participants were the same and included the following parameters: history of a stroke, head trauma, mental retardation or dementia, personality disorder, state of agitation of organic origin or induced by the consumption of psychoactive substances, state of extreme agitation requiring an immediate antipsychotic medication. Additional exclusion criteria were applied to the controls including personal or family history of psychosis. The Local Ethical Committee of the Protection of Persons, Sfax- Tunisia approved this study (CPP SUD N°319/2021). All parents of subjects enrolled in this study attributed a consent after a full explanation of the procedure.

2.2.Blood Sample Collection and Storage

Blood samples were collected after overnight fasting into heparin and EDTA-containing tubes, immediately placed into ice until processing. Within one hour, blood samples were centrifuged at 1000***g*** at room temperature for 10 min to separate plasma. Plasma obtained from heparin-containing tubes was immediately used for a biochemical analysis, while the one obtained from EDTA-containing tubes was added with butylated-hydroxyltoluene and stored at -80 °C until the oxysterols analysis.

2.3.Plasma biochemistry

Total cholesterol (Tchol), triglycerides, high-density lipoprotein cholesterol (HDL-C) and high sensitivity C reactive protein (hs-CRP) levels were measured by standard automated techniques. The low-density lipoprotein-cholesterol (LDL-C) level was calculated according to the Friedewald formula[55].

2.4.Oxysterols analysis

An ultra-performance liquid chromatography-tandem mass spectrometery (UPLC-MS/MS) with isotopic dilutionmethod was used to measure plasma cholestane-3β, 5α, 6β-triol, 7KC, cholestanol, 24-OHC, 25-OHC and 27-OHC. The preparation of EDTA plasma samples was performed according to a previously published method [56] with slight modifications, three plasma preparation protocols were used, the first for the quantification of free cholestane-3β, 5α, 6β-triol and 7KC, the second for total hydroxycholesterols (24-OHC, 25-OHC and 27-OHC), and the third protocol for total cholestanol. In these protocols, measurement of total 24-OHC, 25-OHC and 27-OHC and total cholestanol was performed after ethanolic KOH hydrolysis.

Briefly, mixtures of deuterated internal standards (d7-cholestane-triol and d7-7-ketocholesterol) or (d6-25-hydroxycholesterol, d6-27-hydroxycholesterol and d7-24-hydroxycholesterol) or d-5-cholestanol in methanol were added to plasma EDTA. After vortexing, hydrolysis was carried out with methanolic KOH at 80°C. Free cholestane-3β, 5α, 6β-triol, 7KC, total hydroxycholesterol, and cholestanol were extracted with *n*-hexane and evaporated to dryness under nitrogen. The residue was derivatized to the corresponding picolinyl esters by adding a derivatizating solution, consisting in 2-methyl-6-nitrobenzoic anhydrin, 4-dimethyl aminopyridine and picolinic acid and incubated for 60 min. Derivatized oxysterols were extracted with 1mL of *n*-hexane and evaporated to dryness. The residue was dissolved in methanol and injected into the UPLC-MS/MS system

The UPLC-MS / MS system consisted of a triple quadripole mass spectrometer (TQD-Waters) equipped with an electrospray ionization probe (ESI) and coupled to an UPLC Acquity system (Waters). The chromatographic separation was carried out on an RP-BEH

C18-column (50 _ 2.1 mm _ 1.7 µL, water) at 30°C. The mobile phase gradient started with 70% eluent A (Water) and 30% eluent B (acetonitrile/methanol 50:50 v/v). Linear increasing to 88% of eluent A at the end of the run after 8 min. ESI was performed in positive mode and MS/MS detection was operated in multiple-reaction monitoring mode.

The ratio of each oxysterol to Tchol correct these contents in the plasma, except for 24-OHC level because it is correlated to cholesterol levels in the brain and its presence in the plasma comes almost entirely from cerebral production through gradient-mediated diffusion [6].

2.5.*Statistical analysis*

The Statistical Package for Social Sciences software (SPSS) applied on Windows, version 23.0 (software SPSS Inc., Chicago, Illinois, USA), was used for statistical analyses. The Shapiro-Wilk test was used to test the normal distribution. Continuous variables according to Gaussian distribution were expressed as mean ± standard deviation, (SD) and compared by the Student test for unpaired samples. Continuous variables according to non-Gaussian distribution were expressed as median and median interquartile range (25-75%), compared using the Mann-Whitney ***U***-test. The evaluation of the relationships between the variables was performed by Spearman's correlation test. Categorical variables were compared using the Chi-squared test. A binary linear regression adjusted for the age and gender was used to evaluate the association between the oxysterol levels and BD risks. In all tests, $\boldsymbol{p}<0.05$ was considered statistically significant.

3. RESULTS

Thirty-three male patients diagnosed with BD and 40 healthy male controls were finally enrolled in the study. All the patients were admitted following an acute decompensation of their disorders. Table 1 presents the anthropometric and clinical

characteristics of the study population. Among our patients, three (9.1%) had a personal history of suicide attempts. Compared to controls, patients with BD showed a significantly lower BMI (***p***=0.012). The levels of Tchol, triglycerides and LDL-C were significantly lower in the patients compared to the controls (Table 2). The concentration of Tchol was significantly lower in BD patients with a manic episode (n = 27) compared to those with a depressive episode (n =6) (Tchol: 3.42 ± 0.63 mmol/L vs 4.11 ± 0.91 mmol/L, ***p***=0.033). The high sensitive C reactive protein (hs CRP) concentration was significanly higher in patients with BD compared to controls (Table 2). The profile of oxysterolsis provided in table 2.

Compared to the control group, cholestane-3β, 5α, 6β-triol and 27-OHC were significantly higher in the BD group (***p*** = 0.026 and ***p*** = 0.040, respectively). On the other hand, 7-KC and 25-OHC were significantly lower in patients with BD. The ratios of cholestane-3β,5α,6β-triol, 27-OHC and Cholestanol to Tchol ratio were also significantly higher in plasma from BD patients compared to controls (***p***< 0.001). No statistical difference was found for the plasma ratios of 7-KC and 25-OHC to Tchol in BD patients compared to controls

Moreover, patients with a personal history of suicide attempts had significantly higher cholestanol levels than those who never attempted suicide (8.38 ± 1.15 µmol/L vs 5.81 ± 2.13 µmol/L, ***p***=0.017). No statistical difference was found for cholesterol and other oxysterols tested between BD patients with personal history of suicide attempts and those who never attempted suicide.

The concentration of 24-OHC was significantly lower in patients with BD compared to controls (***p***<0.001) (Table 2), in patients with a manic episode (n = 27) compared to those with a depressive episode (n =6) (24-OHC: 89.42 ±18.29 nmol/L vs 135.05 ± 21.36nmol/L, ***p***<0.001; Figure 1A) and in patients with severe manic episode (based on MAS score> 21; n =

12) compared to those with moderate manic episode (MAS score≤ 21; n = 15) (24-OHC: 86.98 ± 11.99nmol/L vs129.44±29.20nmol/L, ***p*<** 0.001; Figure 1B). On the other hand, patients with a manic episode had significantly higher hs CRP levels compared with patients with a depressive episode (hs CRP: 2.10 mg/L vs 0.90 mg/L, ***p*** = 0.026 Figure 1C) and in patients with severe manic episode compared to those with moderate manic episode (hs CRP: 2.10 mg/L vs 0.90 mg/L, ***p*<** 0.001;Figure 1D). Using Spearman's correlation, we noticed that a negative correlation between 24-OHC levels and age was found in all patients (r =-0.240; ***p***=0.045) (Table 3). Moreover, a negative correlation was observed between 24-OHC and MAS scale (r =-0.343; ***p*** = 0.049) in patients with BD (Table 3). In addition, a positive correlation between 27-OHC levels and hs CRP was found in BD patients (r = 0.478; ***p***=0.005) (Table 3). In the other hand, we found no significant correlation neither between 24-OHC and BMI (r = -0.261; ***p*** = 0.348) nor between 24-OHC / Tchol and BMI (r = 0.071; ***p*** = 0.779). Using binary logistic regression and selecting as dependent variable the acute decompensation during BD and as covariables: age, Cholestane-3β,5α,6β-triol, 27-OHC, 24-OHC and Cholestanol, the occurrence of acute decompensation was independently linked to plasma 24-OHC (Table 4).

4. DISCUSSION

Cholesterol metabolism is under investigation in neurosciences for the potential implications in the progress of neuropsychiatric diseases [43]. The absence of specific markers for early diagnosis has inspired research into new biomarkers conceivably involving cholesterol metabolism dysregulation to neurodevelopment disorders [16]. It is reported here that, compared to the healthy control group, patients with BD showed higher levels of cholestane-3β,5α,6β-triol, 27-OHC and lower levels of Tchol, Triglycerides, LDL-C and 24-OHC. The latter further appeared as an independent risk factor for BD.

It has been previously reported that hypercholesterolemia is likely associated with psychiatric disorders [57,58]. In our BD population, we found lower plasma levels of total cholesterol, Triglycerides and LDL-C. This hypocholesterolemia could be explained by the fact that all our patients were drug-free. Indeed, antipsychotics, and especially those of second generation, are known to disturb cholesterol metabolism causing hypercholesterolemia, in patients with these disorders [59]. In addition, the decrease in cholesterol level in our patients compared to controls can be explained by the agitation and violence of the patients. Indeed, several studies have reported a relation between hypocholesterolemia and violence [60,61]. In addition, our results are in line with a more recent study which also reported lower cholesterol levels in manic BD patients compared to depressed and euthymic BD patients [62]. Moreover, an association between hypocholesterolemia and history of suicide attempts in BD patients, has been reported [63,64]. Aguglia et *al* [65] reported that only male gender, having a diagnosis of bipolar disorder, lower Tchol, and higher CRP serum levels predicted high lethal suicide attempts. In our population, such correlations were not found. However, the history of suicide attempt was associated with increased level of cholestanol in our BD population. Interestingly, psychiatric symptoms are the main clinical presentation in adults with CTX [66], an inborn error of bile acids synthesis, in which cholestanol and cholesterol accumulates in various tissues including brain [67] causing neuronal cells loss by apoptosis [68].

In line with our results, increased plasma levels of cholestane-3β,5α,6β-triol, 27-OHC and cholestanol have been reported in some neurological disorders such as Niemann-Pick type C (NPC), spastic paraplegia type 5 and CTX respectively [29,39,69]. As for CTX, atypical psychiatric disorders are among the frequent clinical symptoms associated with the NPC disease [70]. In these disorders, the increased levels of cholestane-3β,5α,6β-triol and cholestanol has been related to the alteration of the enzymatic pathways of bile acids synthesis in CTX or by altered intracellular cholesterol trafficking in NPC. Yet, this oxidative

metabolism of cholesterol might also occur through auto-oxidation and ROS oxidation, following cholesterol accumulation in cells and tissues. In our patients, we investigated whether the increase of cholestane-3β,5α,6β-triol and cholestanol is due to oxidative stress or to enzymatic alterations. In case of metabolic disorders resulting from enzymatic dysfunction, levels of these two markers are usually much higher than those of our patients [71,72]. We may deduce that their increase in our patients is probably related to oxidative stress. Inflammation is an important source of oxidative stress generating reactive oxygen species which can oxidize cholesterol in various tissues [73]. Recent studies have shown, in patients with BD, increased levels of interleukins, chemokines and cell adhesion molecules which could induce an alteration in the BBB, the renewal of glial cells and neurotransmitters and a disturbance in the transduction of the synaptic signal [74,75]. In addition, Dickerson et al. [76] found a correlation between CRP levels and the severity of the manic episode measured by the Young Mania Rating Scale (YMRS). In this context, increased level of hsCRP in BD patients could be in line with immuno-inflammatory hypothesis [77]. Moreover, Huang and Lin reported higher hsCRP in BD with manic episode than controls and patients with major depressive disorder [78]. Accordingly, in this study we found a higher level of hs CRP in BD subjects positively correlated with 27-OHC. Inflammation, even weak, but chronic, may explain the accumulation of oxysterols in BD patients.

Plasma 27-OHC level and 27OHC/Tchol ratio levels are increased, whereas, 24-OHC is decreased in BD. It has been reported that, high amounts of 27−OHC could cross the BBB, which is damaged in BD patients [79]. Excessive 27−OHC accumulation in the brain activates inflammatory responses and promotes an oxidative environment [80]. In addition, 27−OHC accentuates cholesterol biosynthesis inhibition [81]. In these circumstances the levels and activity of cholesterol 24-hydroxylase (CYP46A1) and 7α-hydroxylase (CYP7B1) enzymes appear to be reduced, thus producing little or no amounts of 24−OHC and 7α-hydroxy-3-oxo-

4-cholestenoic acid respectively [82,83]. The impairment of cholesterol and oxysterol metabolisms can affect neuronal homeostasis by different ways and mechanisms, including cytotoxicity, oxidative stress, apoptosis, and synaptic dysfunction [84–86], which are known to play a role in the BD physiopathology [87–89]. Indeed, 27—OHC is reported to impair neuronal morphology; reducing hippocampal spine density and the expression of postsynaptic protein PSD95, essential for synaptic maintenance and plasticity [90]. We found a relationship between the decrease of 24-OHC level and the severity of BD patients and a negative association between high plasma 24-OHC levels and MAS subscale score. Bretillon et al[91] reported that the blood level of 24-OHC is inversely related to body size. However, we found no significant correlation neither between 24-OHC and BMI nor between 24-OHC / Tchol and BMI. These results indicate that lower levels of 24-OHC in our bipolar patients are probably not secondary to lower BMI. Cholesterol 24-hydroxylation is the major mechanism for cholesterol removal from the brain [92]. 24-OHC is known as an activator/modulator of at least two receptors—liver X receptors (LXRs) and *N*-methyl-d-aspartate receptors (NMDARs) [15,93]. LXR activation by synthetic LXR agonists was shown to be neuroprotective and anti-inflammatory in mouse models of neurodegenerative diseases [93,94]. Moreover, 24-OHC is a positive allosteric modulator of NMDARs that mediate excitatory neurotransmission throughout the CNS and are crucial for synaptic plasticity and learning [95–97]. Decreased concentrations of 24-OHC in patients with BD, especially during manic episode could disrupt the specific endogenous enhancement of NMDA receptors, critically involved in cortical plasticity [98].

5. STRENGTHS AND WEAKNESSES

To the best of our knowledge, there are no published data on oxysterol levels in patients with BD. Therefore, our study might be the first to provide evidence of a relationship between BD and oxysterol levels, suggesting a potential role of 24-OHC as a diagnostic

marker for this disorder. Because our patients were drug-free, hence, the measured biological alterations reflect authentically the physio-pathological changes in BD. Nevertheless, this study has limitations, including the small sample size, the lack of genetic and biochemical studies of cholesterol transporter molecules, such as apolipoprotein (APOE, APO-A1, and APO-B), and the lack of assessment of magnetic resonance imaging.

6. CONCLUSION

Our study has shown that acute BD decompensation is associated with an inflammatory state and a decrease in the synthesis of plasma 24-OHC. Our finding open a new way for biomarkers for BD disease and its severity and insights to disease patho-mechanism, new therapeutic avenues. As a future perspective, we intend to measure plasma oxysterols in our patients with BD before and after they receive treatment and become in clinical remission. Data from such studies, must take into account the age of the patients and the impact of therapeutic molecules on the metabolism of cholesterol.

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Table 1: Anthropometric and clinical characteristics of study groups

Anthropometric and clinical characteristics	**HC (n =40)**	**BD (n=33)**	***p*-Value**
Median age (years)	36.50	32.00	0.264
(Interquartile range)	(31 – 40.5)	(27.75 – 41.0)	
Median BMI (Kg/m²)	24.32	22.08	**0.012**
(Interquartile range)	(22.40-25.26)	(20.76 – 23.39)	
Subtypes of Bipolar disorder			
Type I, number (%)	-	30 (90.90)	
Type II, number (%)	-	3 (9.09)	
Median age at the onset of the disease (years)	-	25.00	
(Interquartile range)	-	(20.75 –29.75)	
Mean duration of illness (years)	-	7.00	
(Interquartile range)	-	(3 - 13)	
Psychiatric family history, number (%)	-	11 (33.33)	
History of Suicide Attempts			
Attempters, number (%)	-	3 (9.09)	
Nonattempters, number (%)	-	30 (90.9)	
Mean PANSS positive subscale score± SD	-	21.85 ± 9.23	
Median PANSS negative subscale score	-	7.00	
(Interquartile range)	-	(7.00 – 8.00)	
Mean PANSS general psychopathology subscale score± SD	-	26.56 ± 8.24	
Mean PANSS total score± SD	-	56.74 ± 14.16	
Median MADRS subscale score	-	10.00	
(Interquartile range)	-	(6.50 – 14.00)	

Mean MAS subscale score± SD	-	17.89 ± 9.58
Median MOCA subscale score	-	24.00
(Interquartile range)	-	(19.50-26.00)

HC: Healthy controls; BD: Bipolar patients; SD: Standard deviation; BMI: Body Mass Index; PANSS: Positive and Negative subscale score;MADRS: Montgomery Asberg Depression Rating Scale; MAS: Mania Assessment Scale; MOCA: Montreal Cognitive Assessment; P-values less than 0.05 were considered statistically significant.

Table 2: Plasma Metabolic markers levels of study groups

Parameters	**HC (n =40)**	**BD (n=33)**	***p*-value**
TChol (mmol/L)			
Mean ±SD	4.40 ± 0.86	3.53 ± 0.74	**<0.001**
(Min-Max)	(3.10 – 6.20)	(2.40 – 4.90)	
Triglycerides (mmol/L)			
Median	1.03	0.90	**0.006**
(Interquartile range)	(0.88 – 1.60)	(0.64 – 1.24)	
HDL-C (mmol/L)			
Mean ±SD	1.09 ± 0.24	1.19 ± 0.28	0.185
(Min-Max)	(0.75 – 1.74)	(0.70 – 1.75)	
LDL-C (mmol/L)			
Mean ±SD	2.79 ± 0.74	1.91 ± 0.72	**<0.001**
(Min-Max)	(1.62 – 4.23)	(0.80 – 3.25)	
hs CRP (mg/L)			
Median	0.90	1.95	**0.020**
(Interquartile range)	(0.50 – 2.30)	(0.65 – 6.30)	
Cholestane-3β,5α,6β-triol (nmol/L)			
Median	16.20	20.95	**0.026**
(Interquartile range)	(13.70 – 20.30)	(14.90 – 24.65)	
Cholestane-3β,5α,6β-triol/TChol (nmol/L)			
Median	3.75	5.66	**<0.001**
(Interquartile range)	(2.93 – 4.63)	(4.56 – 6.92)	
7-ketocholesterol (nmol/L)			
Mean ±SD	50.41± 16.93	38.98 ± 17.50	**0.006**
(Min-Max)	(19.90 – 83.70)	(14.50 – 81.10)	
7-ketocholesterol/TChol (nmol/L)			
Mean ±SD	11.02± 4.02	11.16± 5.33	0.782
(Min-Max)	(5.24 – 22.25)	(4.83 – 31.13)	
25-OHC (nmol/L)			
Mean ±SD	43.60 ± 10.28	33.73 ± 10.91	**<0.001**
(Min-Max)	(23.40 – 66.40)	(18.40 – 57.60)	
25-OHC/TChol(nmol/L)			
Mean ±SD	9.83 ± 2.55	9.68± 2.99	0.821
(Min-Max)	(5.53 – 15.81)	(3.96 – 16.12)	
27-OHC (nmol/L)			
Median	212.00	385.70	**0.040**
(Interquartile range)	(174.90 – 244.50)	(121.20 – 910.60)	
27-OHC/TChol (nmol/L)			
Median	49.40	103.16	**<0.001**
(Interquartile range)	(40.00 – 26.69)	(54.59 – 137.29)	

24-OHC (nmol/L)			
Mean ±SD	129.44 ± 29.20	99.56±26.81	**<0.001**
(Min-Max)	(82.00 – 206.80)	(55.90 – 171.70)	
Cholestanol (µmol/L)			
Mean ±SD	5.58 ± 2.16	6.11± 2.12	0.290
(Min-Max)	(2.30 – 11.50)	(1.90 – 10.20)	
Cholestanol /TChol (µmol/L)			
Median	1.20	1.59	**<0.001**
(Interquartile range)	(0.97 – 1.67)	(1.44 – 2.08)	

SD: Standard deviation; HC: Healthy Control; BD: Bipolar Disorder patients;TChol: Total Cholesterol;HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CRP us: C reactive ultrasensibleprotein; 25-OHC: 25-hydroxycholesterol; 27-OHC: 27-hydroxycholesterol; 24-OHC: 24-hydroxycholesterol; P-values less than 0.05 were considered statistically significant.

Table 3: Correlation matrix of Metabolic Parameters, 24-OHC, 27-OHC and Different Psychometric Scales in the Study Population.

		Age (year)	BMI (kg/m²)	CRP us (mg/L)	24-OHC	PANSS	MADRS	MAS	MOCA
Age (year)	***p***								
	r								
BMI (kg/m²)	***p***	0.249							
	r	0.287							
CRP us (mg/L)	***p***	0.443	0.082						
	r	0.141	0.421						
24-OHC	***p***	**0.045**	0.348	0.525					
	r	**-0.240**	-0.261	0.121					
27-OHC	***p***	0.971	0.485	**0.005**					
	r	0.007	0.176	**0.478**					
PANSS total	***p***	0.344	0.336	0.160	0.310				
	r	0.176	-0.240	-0.259	0.199				
MADRS	***p***	0.380	0.932	0.936	0.284	0.434			
	r	-0.163	-0.022	-0.015	0.210	0.434			
MAS	***p***	0.718	0.330	0.330	**0.049**	0.811	0.229		
	r	-0.066	0.244	0.178	**-0.343**	-0.045	-0.223		
MOCA	***p***	0.108	0.776	0.543	0.607	0.763	0.342	0.812	
	r	0.310	0.077	0.108	0.108	-0.061	-0.190	-0.047	

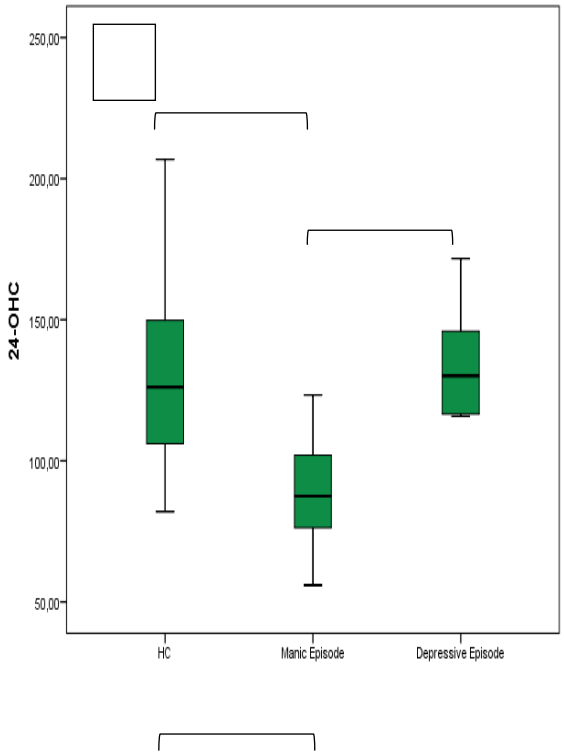
BMI: body mass index, 24-OHC: 24-Hydroxycholesterol, 27-OHC: 27-Hydroxycholesterol; CRP us: highly sensitive reactive protein C; PANSS: Positive and Negative subscale score; MADRS: Montgomery Asberg Depression Rating Scale; MAS: Mania Assessment Scale; MOCA: Montreal Cognitive Assessment;P-values less than 0.05 were considered statistically significant.

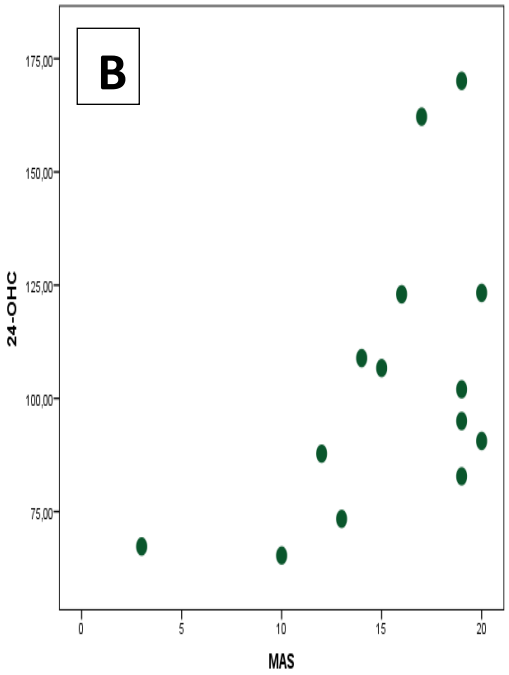
Table 4: Binary Logistic Regression presenting the Association between acute decompensation during BD and Plasma Concentration of 24-OHC.

Variable	Regression coefficient	Standard error	OR	95 % CI	*p*-Value

Age	-0.040	0.030	0.961	0.905 – 1.019	0.183
Cholestane-3β,5α,6β-triol	0.105	0.063	1.110	0.981 – 1.257	0.099
27-OHC	0.044	0.002	1.004	1.000 – 1.077	0.077
24-OHC	-0.035	0.011	0.966	0.945 – 0.987	**0.002**
Cholestanol	0.089	0.131	1.093	0.845 – 1.414	0.497

OR: odds ratio; CI: confiance interval 95%; 24-OHC: 24-Hydroxycholesterol; P-values less than 0.05 were considered statistically significant.





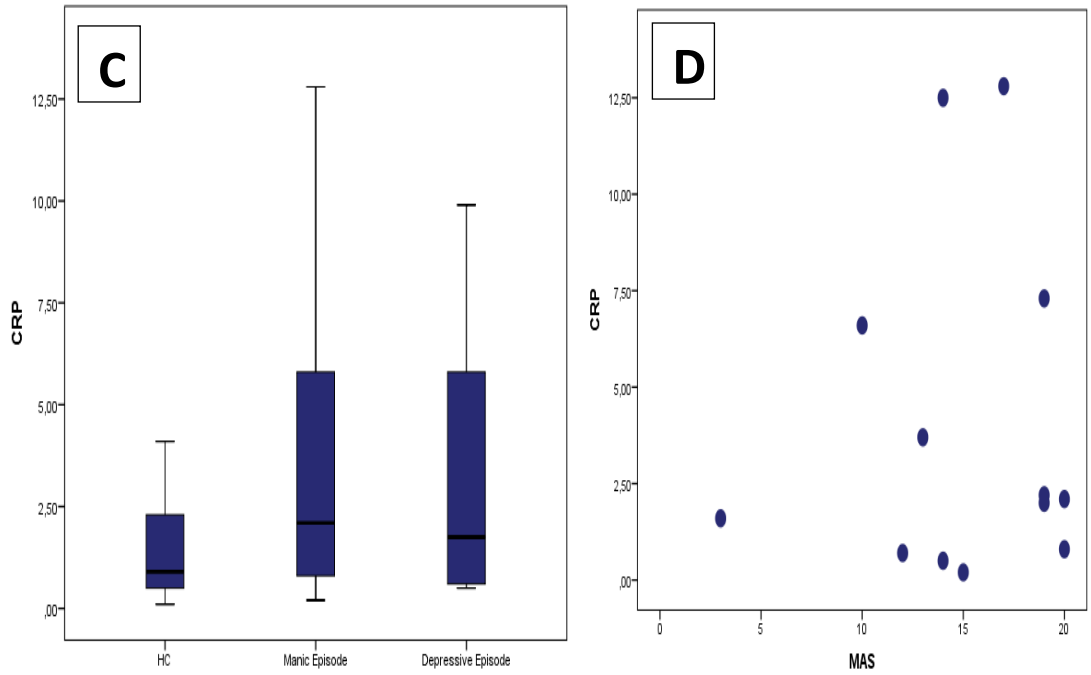


FIGURE 1:A: Variation in the concentration of 24-OHC in Bipolar patients at admission depending on the nature of the manic (n=27) or depressive (n=6) episode.**B**: Scatterplot between MAS scores and concentration of 24-OHC in Bipolar patients with moderate manic episode, severe manic episode and controls. **C**: Variation in the concentration of hs-CRP in Bipolar patients at admission depending on the nature of the manic (n=27) or depressive (n=6) episode. **D**: Scatterplot between MAS scores and concentration of hs-CRP in Bipolar patients with moderate manic episode, severe manic episode and controls.