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- Title: Multiple complement pathway-related proteins might regulate immunopathogenesis of Major Depressive Disorder
- Running Title: Role of complement pathway in Major Depressive Disorder
- Article Type: Original Article
- KeyWords: Major depressive disorder, Inflamamtion, Complement components, alternative pathway, Synapse elimination

Abstract:

Objectives:

Exacerbated inflammatory responses have consistently been demonstrated as a predominant etiological construct of Major Depressive Disorder (MDD). Cytokines, the critical drivers of inflammatory responses, were shown to be altered and have a significant impact on the psychopathology of MDD patients. Besides cytokines, other innate immune molecules like complement proteins induce inflammatory responses. Complement proteins also regulate key neurobiological processes, including synaptic plasticity. However, there is a dearth of literature on the impact of critical complement proteins in MDD. Herein, plasma profiling of seven complement proteins was carried out to obtain a better insight into the role of the complement pathway in MDD.

Methods:

Plasma levels of seven complement components such as C1q, C3, C3b/iC3b, C4, Factor B, Factor H and Properdin were assayed in 22 patients with MDD and 27 healthy controls by Multiplex Suspension Assay. The patients with MDD were diagnosed as per DSM IV-TR, Hamilton Depression rating scale (HAM-D), and clinical rating scales such as Montgomery Depression rating scale (MADRS) and Clinical Global Improvement (CGI) were used for clinical assessments of the patients. The plasma levels of these complement proteins were also correlated with various clinical scores and phenotypes of MDD. Analyses were done using the IBM-SPSS software (version 24.0).

Results:

The patients with MDD and healthy controls did not differ in terms of age and gender ($p>0.1$). The patients with MDD had a mean duration of illness of around 3 years, with average number of depressive episodes being 6 and the mean HAMD score was 19. Of the seven complement

components, the plasma levels of C1q, Factor B and Factor H ($p \leq 0.05$) were significantly elevated in MDD patients compared to healthy controls. However, the plasma levels of these complement proteins were not found to correlate with the clinical profile of MDD patients.

Conclusion:

Both Factor B and Factor H are crucial in the induction and regulation of the alternative pathway of complement activation. The alternative pathway also plays a critical role in inflammation. These findings suggest an important role of the alternative complement pathway in immuno-inflammation in MDD.

Keywords: Major Depressive Disorder, inflammation, complement components, alternative pathway.

Introduction:

Major depressive disorder (MDD) is a complex and debilitating disorder, widespread in the general population and a leading cause of disability worldwide. MDD is associated with a considerable functional impairment that often overlaps with symptom severity (1). The National Mental Health Survey of India during 2015-2016 showed a prevalence rate of 2.5 – 2.7% and this indicates that around 3.7 crore people are suffering from depression (2). Over the past several decades, multiple etiological models have been proposed for MDD; however, a definitive mechanistic basis remains enigmatic till to date. Compelling recent evidences from pre-clinical and clinical studies suggest a predominant role of dysregulated immune system and /or immune activation in the pathophysiology of MDD (3, 4). Among the immunological findings, altered levels of various pro-inflammatory cytokines, for example, Interleukin (IL)-1β, IL-6, and TNF-α have widely been reported in patients with MDD (5-7).

Besides cytokines, other innate immune factors such as complement components are also known to intensify inflammatory responses. Complement pathway-related proteins are emerging as critical immune molecules of pathogenic relevance in various neuropsychiatric diseases due to their regulatory role both in the immune as well as non-immune processes in the brain (8). Complement components have a variable expression in the brain and are crucially involved in the central nervous systems' development and functions (9, 10). One of the most important functions of the complement system in the central nervous system (CNS) is the regulation of synapse elimination (11). Notably, overactivation of the complement system seems to alter brain homeostasis by inducing neuroinflammation and neurodegenerative changes (12). Complement proteins reportedly accelerate the synaptic pruning process and drive the manifestation of neuropsychiatric traits (13). This notion is supported by a longitudinal study showing changes in the complement pathway at the age of 12 years are linked with psychotic experiences at age 18 years (14). A robust link between complement

proteins and major psychosis has reportedly been demonstrated by multiple studies (15, 16). However, studies examining the role of the complement system in MDD are limited and are restricted only to a few complement proteins. Increased levels of complement components like C1q and C4 in the serum and C5 in the cerebrospinal fluid (CSF) of patients with MDD were reported by earlier studies (17-19). However, the earlier studies have not examined the role of the key drivers of the classical and alternative pathways of complement activation in MDD. It is noteworthy that complement proteins functionally interact among themselves and operate in a co-ordinated way. To obtain a clear insight into the role of the complement pathway, it is essential to simultaneously analyse the profile of multiple as well as key complement proteins involved in driving both the classical and alternative pathways of complement activation in order to identify specific complement protein(s) and their subsequent contribution to the immune-inflammatory processes of MDD. To address this knowledge gap, the current study examined plasma levels of a panel of seven complement proteins in patients with MDD.

Study participants and methods:

We recruited 22 patients with MDD (DSM-IV TR) from the outpatient services of National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India. All the patients were screened using Mini International Neuropsychiatric Interview (MINI). In addition, 27 healthy, and age-matched subjects were also recruited. Subjects with any co-morbid medical disorders like asthma, cancer, chronic inflammatory diseases, atherosclerosis, diabetes, autoimmune and degenerative diseases, and also with history of any recent infection/inflammation or psychiatric disorder except nicotine dependence were excluded. Clinical assessments were done using the Hamilton Depression rating scale (HAMD), Montgomery Depression Rating Scale (MADRS) and Clinical Global Impression (CGI) by trained staff. All the recruited MDD patients had a HAMD score of ≥ 18 and were on stable

medications for at least 2 weeks. Socio-demographic and clinical proforma were used to record the details of the present and past depressive episodes. The Institute Ethics Committee of National Institute of Mental Health and Neurosciences approved the protocol and all participants provided written informed consent.

Collection of blood samples

Approximately 10 ml of peripheral blood was collected from patients and healthy controls on the same day of the clinical assessments into Ethylenediaminetetraacetic acid (EDTA)-coated vacutainers. The blood samples were collected in the morning on empty stomach for all study participants between 8:00 to 9:00 am. The blood sample was centrifuged at 4000 RPM for 10 minutes and the plasma was separated, aliquoted and stored at-80 ºC.

Measurement of plasma levels of the complement components

Plasma levels of seven complement components (C1q, C3, C3b/iC3b, C4, Factor B, Factor H and Properdin) were estimated in 22 patients with MDD and 27 healthy controls. These complement components were analyzed using MILLIPLEX Map Human Complement Panel 2 (HCMP2MAG-19K-07) in a Bio-Plex 200 analyzer (Bio-Rad, Hercules, CA, USA). The sensitivity of the complement components in this panel was high (ng/ml) and the limit of detection (LOD) for various complement proteins were as follows: C1q (0.048 ng/ml), C3 (0.120 ng/ml), C3b/iC3b (3.639 ng/ml), C4 (0.191 ng/ml), Factor B (0.024 ng/ml), Factor H (0.135 ng/ml) and properdin (0.003 ng/ml). All the plasma samples were assayed in duplicate. The standard curve of each complement component was generated using the standards provided in the kit by the manufacturer. Bioplex Manager software was used to analyse the data according to the manufacturer's instructions

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences (version-24) [SPSS Inc.,]. Univariate analysis of covariance and correlation was established using Pearson's correlation test. The complement levels were non-normally distributed and consisted of non-detects. Concentrations below the lower detection limit were considered non-detectable and were left-censored. Hence, a standard non-parametric rank-based test (Mann-Whitney test) was applied without loss of information to compare the groups and the Wilcoxon test for within-group comparison. The significance was set at $p \leq 0.05$ (two-tailed).

Results

The patients with MDD and healthy controls were comparable in age and gender (all $p>0.1$). The MDD subjects had a mean age of onset of 28.9 years, total duration of illness in months was 40 which is around 3.3 years, and the number of past depressive episodes were 6. The mean HAMD score was 19.3, MADRS 22 and CGI 4, suggesting that these patients had moderate depression. The demographic and clinical characteristics of the study participants are summarised in **Table-1.**

Significantly elevated plasma levels of complement components in MDD patients

Amongst the studied complement components, the plasma levels of three complement components significantly varied between patients with MDD and healthy controls. The plasma levels of C1q, Factor B and Factor H ($p \leq 0.05$) were significantly elevated in patients with MDD compared to healthy individuals (**Table- 2**).

Lack of correlation of plasma complement components with the clinical parameters of MDD patients

There was no correlation between the plasma complement levels and clinical parameters like age of onset, duration of illness, number of episodes, and severity of the depressive episode.

Further, the plasma complement component levels did not correlate with the clinical rating scale scores such as HAMD, MADRS, and CGI.

Discussion

The most salient finding of this study is significantly up-regulated levels of three complement proteins, C1q, Factor B and Factor H, in patients with MDD compared to the healthy controls. Our finding on the increased levels of plasma C1q coincides with the previous studies (18, 20). However, unlike the current findings, in one of the studies, elevated C1q was positively correlated with the HAMD-24 score (18). For the first time, the present study shows simultaneously elevated levels of two core components, such as Factor H and Factor B, of the alternative complement pathway in patients with MDD. Notably, there are no studies on Factor B in patients with MDD. However, the role of Factor H in depression was indicated by three other studies. In an earlier study, increased levels of Factor H were shown in individuals with geriatric depression (21). In a recent study, heightened levels of Factor H were reported in MDD patients with anhedonia (22). In addition, genetic variation within complement factor H has also been reported to confer susceptibility to MDD (23).

The patients with MDD in the current study had an age of onset of around 29 years which is in line with other epidemiological studies, where the mean age of onset was reported to be around 30 years (24). Besides, the number of female patients were comparatively more as compared to the males; this also coincides with the reported gender differences, i.e., two-fold higher prevalence MDD in women (25). The plasma levels of the complement proteins were not found to be associated with age of onset, gender and clinical rating scale scores of MDD patients. This could be due to a relatively small sample size of the current cohort.

Emerging data from both the pre-clinical and clinical studies suggest important roles of the complement system in the pathophysiology of MDD. In a post-mortem brain study on

depressed suicide patients, C3 levels were significantly up-regulated in the pre-frontal cortex (PFC) (26). In a mouse model of depression, chronic stress-induced increased expression of C3 in the PFC was shown to cause depressive-like behavior and the mice deficient in C3 were shown to be resilient to stress-induced depressive-like behavior (26). The altered levels and activities of complement components seem to have important neurobiological implications in MDD given the fact that complement proteins such as Cq1, C3 and C4 regulate synapse elimination (27). Importantly, synaptic dysfunction has been proposed as a crucial contributor to depression (28). A previous study has demonstrated that endothelial cells from human brain microvessels produce complement proteins like Factor H and Factor B under *in vitro* conditions (29). These proteins are also envisaged to play an important regulatory role in neuronal homeostasis.

In recent years, inflammation has been recognized as a predominant etiological construct of MDD. The complement proteins are essential constituents of the effector responses of the innate immune system. Contextually, complement components are also the crucial drivers of immune-inflammatory responses. C1q is the first recognition molecule of the classical pathway of complement activation. It has diverse immune and non-immune functions. C1q has wide expression in the CNS and plays a pivotal role in synaptic pruning and CNS development. Elevated levels of C1q in CNS lead to increased production of pro-inflammatory cytokines, leading to neuroinflammation and neuronal cell death. The high plasma levels of C1q in the current study suggest inflammation in MDD patients.

Factor B is a crucial component of the alternative pathway of complement activation. It is a well-established fact that alternative pathway serves as an amplification loop of the complement system and amplifies complement activation, initiated through the classical/lectin pathways. The activation of Factor B triggers the amplification loop of the complement pathway. This suggests that Factor B is a key component in complement activation and

inflammatory responses. Studies on animal models of various immune-mediated diseases have demonstrated that mice deficient in Factor B had exhibited a significant reduction in inflammation. For example, *Bf (-/-)* mice had significantly decreased mRNA levels of several complement components like C3, C4, CR2, CR3, C3aR and C5aR, as well as less inflammation in the passive transfer model of collagen-induced arthritis (30). Besides this, *fB (-/-)* mice were also shown to have reduced airway inflammation (31). Apart from Factor B, the Factor H is also a critical regulator of the alternative pathway. Factor H binds to C3b and accelerates the decay of C3 convertase. Decreased as well as increased levels of Factor H have been linked with many immune-mediated diseases of the nervous system like Multiple Sclerosis and Alzheimer's Disease (32, 33).

In the current study, the plasma levels of key initiator of classical pathway, C1q and critical activator, Factor B and a major regulator, Factor H of the alternative pathway, were found to be elevated in MDD patients. This suggests the possibility of an aberrant classical as well as alternative pathways of complement activation in MDD. Notably, previous studies have indicated alternative pathway as the major driver of inflammatory responses in multiple inflammatory disorders (34, 35). Based on our findings, it can be concluded that the activated complement pathway could serve as a key pathogenetic element in MDD. This could be mediated through microglia activation and subsequent neuroinflammation due to excessive release of inflammatory cytokines.

Complement components are involved in the regulation of inflammation and synaptic plasticity in MDD. Excess activity of the complement components is potentially detrimental for the immune as well as nervous systems. Our finding of elevated levels of three complement components, C1q, Factor H and Factor B in MDD patients suggest essential implications of the complement proteins in MDD, as these could alter brain homeostatic mechanism, especially synaptic plasticity and also mediate neuro-immune cross-talk in MDD. This is the first study

examining a panel of seven complement proteins, representing both the classical and alternative pathways of complement activation in MDD and it constitutes the major strength of this study. Notably, all MDD patients in the current study were on medications. It is important to note that the medications might interfere with the activities of the complement pathways and the blood levels of various complement proteins. The impact of medications on the plasma levels of complement proteins was not examined and this is a major limitation of the current study. Additionally, small sample size, and no correlation of plasma complement proteins with the clinical severity are also to be considered as the limitations of the current study. Further studies with a bigger sample size might help in establishing a clinical correlation with psychopathology or disability scores of MDD.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

MD: Conceptualization, Funding, Supervision and Writing original draft.

KM and GV: Conceptualization, Funding, Supervision, Review and Editing.

PVR: Data acquisition and formal analysis, Review and Editing.

PMT, MS, PHB, RS: Data acquisition and formal analysis.

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Table-1: Demographic and clinical characteristics of the study participants

Clinical Parameter	MDD (N=22) Mean ±SD	Healthy subjects (N=27) Mean ±SD	t value/$\chi 2$	p value
Age	33.31 (9.37)	29.51 (6.77)	1.594	0.12
Gender (Male: Female)	9:13	15:12	1.041	0.30
Age at onset (years)	28.90 (10.63)	-	-	-
Duration of illness (months)	40.01 (35.60)	-	-	-
No. of Past episodes	6 (2.13)	-	-	-
HAM-D score	19.31 (1.88)	-	-	-
MADRS score	22 (3.46)	-	-	-
CGI-Baseline	4.22 (0.52)	-	-	-

CGI: Clinical Global Improvement; HAM-D: Hamilton Depression Rating Scale; MDD: Major Depressive Disorder; SD: Standard Deviation; MADRS: Montgomery Depression Rating Scale.

Table-2: Differences in plasma levels of complement proteins between healthy subjects and MDD patients

Sl. no.	Complement proteins (ng/ml)	MDD (N=22) Mean ± SD	Healthy Subjects (N=27) Mean ± SD	Z	p
1	C1q	2.54 ± 0.29	2.33 ± 0.44	-1.98	**0.05**
2	C3	19.86 ± 15.58	19.83 ± 19.05	-0.55	0.58
3	C3b/iC3b	23.04 ± 21.40	25.26 ± 41.00	-0.55	0.58
4	C4	9.03 ± 2.70	8.29 ± 4.05	-1.47	0.14
5	Factor B	7.55 ± 1.89	6.19 ± 2.56	-2.17	**0.03**
6	Factor H	8.86 ± 1.40	7.91 ± 2.76	-2.21	**0.03**
7	Properdin	0.80 ± 0.12	0.77 ± 0.13	-0.96	0.34

MDD: Major Depressive Disorder; SD: Standard Deviation. $p \leq 0.05$.